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COMPUTATIONAL STUDY OF THE MECHANISMS UNDERLYING OSCILLATION IN NEURONAL LOCOMOTOR CIRCUITS

by

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A thesis submitted to Plymouth University in partial fulfilment for the degree of

DOCTOR OF PHILOSOPHY

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Computational study of the mechanisms underlying oscillation in neuronal
locomotor circuits

Robert John Merrison-Hort

In this thesis we model two very different movement-related neuronal circuits, both of which produce oscillatory patterns of activity.

In one case we study oscillatory activity in the basal ganglia under both normal and Parkinsonian conditions. First, we used a detailed Hodgkin-Huxley type spiking model to investigate the activity patterns that arise when oscillatory cortical input is transmitted to the globus pallidus via the subthalamic nucleus. Our model reproduced a result from rodent studies which shows that two anti-phase oscillatory groups of pallidal neurons appear under Parkinsonian conditions. Secondly, we used a population model of the basal ganglia to study whether oscillations could be locally generated. The basal ganglia are thought to be organised into multiple parallel channels. In our model, isolated channels could not generate oscillations, but if the lateral inhibition between channels is sufficiently strong then the network can act as a rhythm-generating “pacemaker” circuit. This was particularly true when we used a set of connection strength parameters that represent the basal ganglia under Parkinsonian conditions.

Since many things are not known about the anatomy and electrophysiology of the basal ganglia, we also studied oscillatory activity in another, much simpler, movement-related neuronal system: the spinal cord of the Xenopus tadpole. We built a computational model of the spinal cord containing approximately 1,500 biologically realistic Hodgkin-Huxley neurons, with synaptic connectivity derived from a computational model of axon growth. The model produced physiological swimming behaviour and was used to investigate which aspects of axonal growth and neuron dynamics are behaviourally important. We found that the oscillatory attractor associated with swimming was remarkably stable, which suggests that, surprisingly, many features of axonal growth and synapse formation are not necessary for swimming to emerge. We also studied how the same spinal cord network can generate a different oscillatory pattern in which neurons on both sides of the body fire synchronously. Our results here suggest that under normal conditions the synchronous state is unstable or weakly stable, but that even small increases in spike transmission delays act to stabilise it.

Finally, we found that although the basal ganglia and the tadpole spinal cord are very different systems, the underlying mechanism by which they can produce oscillations may be remarkably similar. Insights from the tadpole model allow us to predict how the basal ganglia model may be capable of producing multiple patterns of oscillatory activity.
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Author’s Declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Committee.

Work submitted for this research degree at the Plymouth University has not formed part of any other degree either at Plymouth University or at another establishment.

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Journal Publications


Oral Presentations


Posters

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- Neurodynamics: a workshop on heterogeneity, noise, delays, and plasticity in neural systems, Edinburgh, March 2012
- 2nd UK Neuroinformatics Node congress, Edinburgh, March 2012
- Towards Mathematical Modeling of Neurological Disease from Cellular Perspectives, Fields Institute, Toronto, May 2012

“The emergence of two anti-phase oscillatory neural populations in a computational model of the Parkinsonian globus pallidus”, with Roman Borisyuk. Presented at:

- Biodynamics 2013, Engineers’ House, Bristol, September 2013
# List of Abbreviations

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<th>Description</th>
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<tr>
<td>6-OHDA</td>
<td>6-hydroxydopamine (neurotoxin)</td>
</tr>
<tr>
<td>aIN</td>
<td>Ascending interneuron</td>
</tr>
<tr>
<td>A-H</td>
<td>Andronov-Hopf (bifurcation)</td>
</tr>
<tr>
<td>AHP</td>
<td>After-hyperpolarisation</td>
</tr>
<tr>
<td>AMPA</td>
<td>$\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (receptor type)</td>
</tr>
<tr>
<td>BG</td>
<td>Basal ganglia</td>
</tr>
<tr>
<td>B-T</td>
<td>Bogdanov-Takens (bifurcation)</td>
</tr>
<tr>
<td>cIN</td>
<td>Commisural interneuron</td>
</tr>
<tr>
<td>CPG</td>
<td>Central pattern generator</td>
</tr>
<tr>
<td>CUDA</td>
<td>Compute unified device architecture</td>
</tr>
<tr>
<td>DBS</td>
<td>Deep brain stimulation</td>
</tr>
<tr>
<td>dIN</td>
<td>Descending interneuron</td>
</tr>
<tr>
<td>DLA</td>
<td>Dorsolateral ascending (neuron)</td>
</tr>
<tr>
<td>DLC</td>
<td>Dorsolateral commissural (neuron)</td>
</tr>
<tr>
<td>EP</td>
<td>Entopeduncular nucleus</td>
</tr>
<tr>
<td>EPSC / EPSP</td>
<td>Excitatory post-synaptic current / potential</td>
</tr>
<tr>
<td>FFT</td>
<td>Fast Fourier transform</td>
</tr>
<tr>
<td>GABA</td>
<td>$\gamma$-aminobutyric acid (neurotransmitter)</td>
</tr>
<tr>
<td>GPe</td>
<td>Globus pallidus external segment</td>
</tr>
<tr>
<td>GPi</td>
<td>Globus pallidus internal segment</td>
</tr>
<tr>
<td>GPU</td>
<td>Graphical processing unit</td>
</tr>
<tr>
<td>HCN</td>
<td>Hyperpolarisation-activated cyclic nucleotide-gated (channel)</td>
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IPSC / IPSP . . . Inhibitory post-synaptic current / potential
JSON ............ JavaScript Object Notation
LFP ............... Local field potential
MSN ............... Medium spiny neuron
MN ............... Motoneuron
MPTP ............. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (neurotoxin)
MZ ............... Marginal zone
NMDA ............. N-methyl-D-aspartic acid (receptor type)
ODE ............... Ordinary differential equation
PD ............... Parkinson’s disease
PSC / PSP ...... Post-synaptic current / potential
RB ............... Rohon-Beard (neuron)
RHS ............... Right-hand side
SNAPPy ........ Simulation of neural activity patterns with Python
SNC ............. Substantia nigra pars compacta
SNIC .......... Saddle-node on invariant circle (bifurcation)
SNr ............. Substantia nigra pars reticulata
SWA ............ Slow-wave activity
STN ............... Subthalamic nucleus
TAN ............... Tonically active neuron
Chapter 1

Introduction

This thesis is devoted to using mathematical and computational models to study the mechanisms underlying oscillatory neuronal activity in locomotor circuits. Generating movement is one of the basic functions of all nervous systems, from the 302 neurons of the nematode worm C. Elegans to the roughly 200 billion neurons in the human brain. As with many other brain functions, oscillatory patterns of neuronal activity play an important role in locomotion. As a simple example consider how the nervous system generates the repetitive pattern of muscle contraction and relaxation associated with movements such as walking or hopping. Oscillations can also be pathological and two of the main motor symptoms of Parkinson’s disease, tremor and slowness of movement, have been linked to abnormal oscillatory firing patterns in a collection of nuclei in the vertebrate brain called the basal ganglia (BG). Thanks to increasingly biologically realistic mathematical and computational models of the nervous system, we are now able to study the mechanisms which underlie both physiological and pathological oscillations in ways which are simply not possible in traditional in vivo or in vitro experiments.

1.1 Research Questions and Thesis Structure

This thesis will use techniques from computational and mathematical neuroscience in order to investigate the following three research questions:
1. What are the processes by which normal firing patterns in the basal ganglia become pathological in Parkinson’s disease?

2. What can our understanding of non-linear dynamics tell us about how the tadpole spinal cord is able to generate multiple patterns of motor activity?

3. Is there a universal dynamical mechanism underlying the oscillatory patterns generated by the neuronal circuits in the basal ganglia and the tadpole spinal cord?

In this Introduction chapter we will review the anatomy of the basal ganglia and the changes which occur in them in Parkinson’s disease. This will be followed by a similar review of the anatomy and physiological behaviour of the *Xenopus* tadpole. We will then describe the role that oscillatory neural activity can play in motor systems, before reviewing the computational and mathematical techniques that we will use to investigate the basis for this activity. In Chapter 2 we describe the implementation of general purpose simulation software for models of single compartment Hodgkin-Huxley neurons, which was used to build the models presented in Chapters 3 and 5. This chapter also briefly presents some software we have developed for graphically exploring dynamical systems. In Chapter 3 we develop a detailed conductance-based model of the rodent globus pallidus and investigate the possibility that it can become entrained to cortical rhythms via the subthalamic nucleus (STN) under Parkinsonian conditions. In Chapter 4 we develop a population-level model of the basal ganglia based on the Wilson-Cowan equations which embodies the idea of multiple interacting “channels”. We use bifurcation analysis to study the conditions in which such a model can generate oscillations. In Chapter 5 we present a realistic conductance-based model of the neurons in the *Xenopus* spinal cord and use this model to investigate the critical features of axonal development that allow tadpoles to swim, as well as studying how this network is able to produce both anti-phase swimming motor patterns and in-phase synchronous motor patterns. Finally, Chapter 6 briefly considers the relationships between the different oscillatory mechanisms presented in the thesis and summarises its contributions.
1.2 The basal ganglia and Parkinson’s Disease

1.2.1 Terminology and structural overview of the basal ganglia

The basal ganglia are a group of nuclei found in the subcortial area of the brains of vertebrates. These nuclei include the putamen and caudate nucleus (together called the dorsal striatum, neostriatum, or here simply “the striatum”), the globus pallidus (GP), subthalamic nucleus and the substantia nigra pars compacta (SNc) and pars reticulata (SNr). In primates, the globus pallidus is divided into two segments: a medially-located “internal” segment (GPi) and more laterally-located “external” segment (GPe). In rodents, on the other hand, the globus pallidus is generally considered a single structure, with connections to other nuclei that make it similar to that of the GPe in primates. The rodent entopeduncular nucleus (EP), meanwhile, is usually considered homologous to the primate GPi. In this thesis we will typically treat the term “GP” as being equivalent to “GPe”, and “EP” as being equivalent to “GPi” — although when discussing experimental results from specific species we will use the correct terminology for that species.

The location of the basal ganglia deep within the brain makes studying their electrophysiology and functional connectivity in vivo difficult, and as a result there is still much that is not known about the detailed structure of each nucleus, and the connections between them. Here we will briefly summarise what is known about the main neuron types in each nucleus and their major synaptic inputs and outputs. Figure 1.1, from Obeso et al. (2006), shows the main synaptic projections between the different nuclei of the basal ganglia.

Striatum

The striatum is considered the main input nucleus of the basal ganglia and it receives major projections from most cortical areas (Kemp and Powell, 1970). Although the striatum is comprised of two parts, the putamen and the caudate nucleus, it is the putamen that is of most interest to us, since this is the main striatal target for projections from the motor regions of the cortex (Künzle, 1975; Nambu et al., 2002). The structure of connections from the motor cortex to the putamen is not known in detail, but the projection appears to be somewhat segregated, with axons originating in cortical regions corresponding to different body areas terminating in largely discrete regions
An estimated 95% of neurons in the rodent striatum and 77% of neurons in the monkey striatum are "medium spiny" projection neurons (MSNs) that release the inhibitory neurotransmitter \( \gamma \)-aminobutyric acid (GABA) (Graveland and Difiglia, 1985). The MSNs can be divided into two fairly evenly sized groups based on their expression of certain neuropeptides. MSNs in one group express the peptides dynorphin and substance P, while those in the other group express enkephalin. These groups are thought to differ in two other ways: their efferent targets within the basal ganglia and their dopamine receptor expression. Evidence for the former came from injecting retrograde tracers into the SNr and GPe/i and observing that the MSNs stained by the SNr and GPi injection were mostly of the dynorphin/substance P group and those stained by the GPe injection were mostly of the enkephalin group (Beckstead and Cruz, 1986; Gerfen and Young, 1988; Parent et al., 1989). To show differential dopamine receptor expression, Le Moine and Bloch (1995) used in-situ hybridisation to show that dynorphin/substance P expressing neurons contain \( D_1 \) receptor mRNA, while the enkephalin expressing class contain \( D_2 \) receptor mRNA. Since dopamine is excitatory at
1.2. The basal ganglia and Parkinson’s Disease

$D_1$ receptors and inhibitory at $D_2$ receptors, these results suggest that it acts to facilitate activity in the striatum-GPi/SNr pathway and inhibit activity in the striatum-GPe one. The situation may not be as simple as this though; Kawaguchi et al. (1990) claim that more sensitive staining shows that all MSNs in the rat striatum make collaterals in the GP(e), while Surmeier et al. (1996) found that around half of MSNs expressed mRNA for both “$D_1$-like” ($D_{1a}, D_{1b}$) and “$D_2$-like” ($D_2, D_3, D_4$) receptors.

In addition to the MSNs there are two main types of interneuron in the striatum: the cholinergic tonically active neurons (TANs) and GABAergic fast-spiking neurons. The TANs fire irregularly at around 3–10Hz, but can burst and pause in response to salient, reward-related stimuli (Kimura, 1986). It has therefore been suggested that TANs might play a role in reinforcement learning of motor actions in the striatum (Wang et al., 2006). The fast spiking neurons can generate regular spiking patterns at 200–300Hz in response to injected currents although probably display a bursting pattern in vivo (Tepper and Bolam, 2004), and may play a role in amplifying the effects of TAN pauses on MSNs, since they have a powerful inhibitory effect on the MSNs which they contact (Koós and Tepper, 2002).

GPi / Entopeduncular Nucleus and Substantia Nigra Pars Reticulata

The GPi/EP and SNr are often considered together as the “output nuclei” of the basal ganglia, since their major efferent connections are GABAergic projections to the thalamus and brainstem (DeLong, 1990; Bolam et al., 2000). Neurons in the GPi/EP and SNr receive connections from most other regions of the basal ganglia, including the subpopulation of striatal MSNs that contain $D_1$ receptors, inhibitory projection neurons in the GP/GPe, and excitatory projection neurons in the STN (Goldberg and Bergman, 2011). In animals at rest, neurons in the GPi fire tonically at a relatively high rate (DeLong, 1972), and both output nuclei are thought to exert a constant inhibitory influence on their downstream targets. According to this idea, particular muscles or body regions are allowed to move by temporary reductions in the firing rates of associated GPi/EP and SNr neurons (Alexander and Crutcher, 1990).
GPe / Globus Pallidus

The GPe is central to the basal ganglia both spatially and synaptically. Anatomically it is sandwiched between the striatum and the GPi/SNr, which are positioned laterally and medially to it respectively. The subthalamic nucleus and substantia nigra lie underneath it. All of these nuclei are synaptically connected to the globus pallidus and these connections are often reciprocal.

Strict categorisation of pallidal neurons based on electrophysiology is difficult. Brain slice studies of the rodent globus pallidus (Nambu and Llinás, 1994; Cooper and Stanford, 2000) and entopeduncular nucleus (Nakahishi et al., 1990) have suggested various categories, but it is not easy to reconcile their findings to one another (for example, the “type B” neurons of Cooper and Stanford, which were estimated to represent 32% of the GP, have no obvious correlate with any of the categories described by Nambu and Llinás). A detailed computational model (Günay et al., 2008) has suggested that the variations in electrical activity could arise simply as a result of the natural variation of channel densities that occurs across the GP, rather than a division of neurons into discrete groups. Despite these difficulties it seems that a major class of neurons exists in both GP and EP that is distinguished by transient depolarisation after release from hyperpolarisation that may lead to a transient increase in the spontaneous firing rate, high-frequency spiking without accommodation upon depolarising current injection, and strong inwardly-rectifying “anomalous” currents during hyperpolarisation (though Nambu and Llinás did not observe this latter characteristic). These neurons show regular tonic firing activity either intrinsically or in response to small membrane depolarisations.

The primary source of input to the GPe is the $D_2$-receptor expressing striatal medium spiny projection neurons. These neurons outnumber the neurons of the GP by an order of magnitude (Smith et al., 1998), such that each pallidal neuron receives input from many striatal afferents. In addition to this inhibitory input, GPe neurons also receive excitatory (glutamatergic) input from the subthalamic nucleus, and inhibitory local input from other GPe neurons (Goldberg and Bergman, 2011).

The vast majority of neurons in the external segment of the globus pallidus project to the subthalamic nucleus, but this is rarely their exclusive target. Sato et al. (2000) traced the path of individual axons from the monkey GPe and found that 86% of them projected to the STN, while
1.2. The basal ganglia and Parkinson’s Disease

the remaining 14% projected back to the striatum. All of the STN-projecting axons branched and sent collaterals to other regions: either the substantia nigra pars reticulata, the GPi, or both. In rodents the situation appears to be similar, with all GP neurons projecting to the EP and SN and a subset additionally projecting back to the striatum (Kincaid et al., 1991; Kita and Kitai, 1994). The projection from GP to striatum appears to selectively innervate the striatal interneurons, particularly the TANs (Bevan et al., 1998). Recently, convincing evidence has emerged that suggests that the GPe neurons which project back to the striatum are in fact a chemically distinct group, which are now known as arkypallidal neurons (Mallet et al., 2012). It is not yet known if these neurons are distinct from other GP projection neurons in terms of their electrophysiology or the synaptic connections which they receive, but there is some evidence from computational modelling that this may be the case (Nevado-Holgado et al., 2014).

The careful staining study of Shink et al. (1996) revealed that the projections between the GPe, GPi and STN display a great deal of selectivity, with small groups of neurons in each nucleus projecting exclusively to each other. Despite this selectivity it has been suggested that the STN may still play some role in integrating activity from different functional regions in the GP, since pallidal neurons at the borders of different functional regions often project to the same STN neuron (Bevan et al., 1997). Baufreton et al. (2009) used microstimulation and dynamic clamp techniques to demonstrate that neurons in the rat GP exert a powerful inhibitory effect on the small group of subthalamic cells that they innervate. The return projection from STN to GP appears to target roughly the same parts of the GP as the striatopallidal projection (Parent and Hazrati, 1995a).

Subthalamic Nucleus

Based on (somewhat limited) current knowledge, the constituent neurons of the STN appear to be of a single type, and are the only population of neurons in the basal ganglia that release the excitatory neurotransmitter glutamate. STN neurons have intrinsic pacemaker properties and many of them are able to fire bursts of rebound spikes upon release from hyperpolarisation. The strength of hyperpolarisation required to induce rebound bursts appears to be lower than strength of inhibitory input that STN neurons receive from their afferents in the GP (Bevan et al., 2000). As previously described, the STN receives inhibition from the GPe and sends excitation to the GPe and GPi/SNr.
Substantia Nigra Pars Compacta

Around 95% of neurons in the SNc are dopaminergic, and two thirds of these are spontaneously active at low frequencies (<10Hz) in slices (Lacey et al., 1989) and in vivo (Schultz, 1998). These neurons provide almost all of the dopaminergic input to the other nuclei of the basal ganglia, projecting mainly to the striatum, but also to the GPe, GPi and STN (Obeso et al., 2008). While transient bursts of dopaminergic neuron activity occur in response to unexpected reward and are thought to play a role in reinforcement learning (Schultz, 1998), the persistent slow spiking of SNc neurons appears to provide a constant background level of dopaminergic modulation to the basal ganglia, possibly acting as an indicator of the likelihood that movement will be required in the near future (Jenkinson and Brown, 2011).

1.2.2 Pathology and treatment of Parkinson’s disease

Parkinson’s disease is the second most common neurological disease after Alzheimer’s disease, with an estimated 1% of people in industrialised countries over the age of 60 suffering from it (de Lau and Breteler, 2006). The disease encompasses a wide range of motor and non-motor symptoms which vary from patient to patient, however it is characterised by three main motor abnormalities that are seen to some degree in nearly all patients (Jankovic, 2008):

- **Resting tremor**, in which a 4–6Hz tremor is seen when the patient is at rest, usually unilaterally.

- **Bradykinesia**, a slowness in initiating or executing movements. Voluntary (i.e. non-cued) movements are particularly affected. Around 47% of patients also experience “freezing”: a temporary inability to move certain limbs that can occur spontaneously during normal movements, such as walking.

- **Rigidity**, in which increased muscle tone causes high resistance of limb movement, postural deformities and instability.

The main pathological feature of Parkinson’s disease is the death of the dopaminergic neurons in the SNc (for review see Dauer and Przedborski, 2003). Most pharmacological treatments aim
to reverse the reduction in dopamine that results from the loss of these neurons. This is accomplished either directly, through the dopamine precursor l-DOPA (levodopa) or indirectly, through dopamine agonists or drugs that prevent the metabolism of dopamine. The UK National Institute for Health and Clinical Excellence (NICE) guidelines recognise levodopa as being the most effective drug for the reduction of the primary motor symptoms of PD (NICE, 2006) and it is therefore often the first choice for pharmacological treatment of these symptoms. Long-term levodopa use is, however, associated with a number of adverse side effects including uncontrollable abnormal movements (a form of dyskinesia) and unpredictable fluctuations in effectiveness—the so-called “on-off” phenomenon (Rascol et al., 2002). For patients with more severe symptoms, perhaps with serious side-effects from high levodopa doses, an increasingly common surgical treatment is deep-brain stimulation (DBS). In DBS, an implanted electrode provides constant high frequency (∼120Hz) electrical stimulation to either the STN or GPi (Bain et al., 2009). The precise effects of this stimulation on neuronal activity in the basal ganglia are not fully understood and are likely to be many and varied (reviewed in Kringelbach et al., 2010).

Much of the research into the causes and treatment of Parkinson’s disease makes use of animal models (see Betarbet et al. (2002) for a review). One of the most common non-human primate models involves the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which selectively kills the dopaminergic neurons of the SNc. The effect of MPTP on rodents is less reliable, therefore the use of 6-hydroxydopamine (6-OHDA) is more common for experiments involving these animals. 6-OHDA must be injected directly into the basal ganglia, which makes administration more difficult than that of MPTP but allows for comparative studies in single animals, since unilateral injection results in a unilateral lesion of the nigrostriatal pathway. It is important to remember that these animal preparations only induce Parkinson’s-like symptoms and differ from the true disease in some important ways. Tremor, for example, is absent in 6-OHDA lesioned mice and most species of MPTP lesioned monkeys.

The rate model of basal ganglia dysfunction

The “rate model”, originally described by DeLong (1990), offers a simple but persuasive explanation for the hypokinetic symptoms of Parkinson’s disease (e.g. bradykinesia). This model arose from experimental evidence showing that when a monkey is rendered Parkinsonian there is a clear
increase in the average firing rate in the GPe and STN, along with a decrease in the GPi (Miller and DeLong, 1987; Filion and Tremblay, 1991). This theory holds that the basal ganglia are organised into two parallel pathways, a pro-kinetic “direct” pathway (striatum → GPi/SNr) and an anti-kinetic “indirect” pathway (striatum → GPe → STN → GPi/SNr). The fact that direct pathway MSNs express $D_1$-type dopamine receptors whereas indirect pathway MSNs express $D_2$-type receptors (see Section 1.2.1) suggests that under Parkinsonian conditions the inhibitory output from the direct pathway becomes stronger, whereas that from the indirect pathway becomes weaker (since dopamine is excitatory at $D_1$ receptors and inhibitory at $D_2$ receptors). The direct/indirect pathway model of Parkinsonism explains the surprising result that lesioning the STN reverses the effects of MPTP poisoning (Bergman et al., 1990).

The rate model of PD remains popular and is often used to explain the motor symptoms of the disease in medical textbooks. Despite its popularity, it is clear that the model has some important limitations. Since it predicts a reduction in motor activity under Parkinsonian conditions it cannot explain how tremor arises; this appears to be related to an increase in rhythmic bursting at tremor frequencies in the STN (Bergman et al., 1994). More recent discoveries about basal ganglia connectivity suggest that the simple division into two feed-forward pathways is overly simplistic. For example, it appears that the majority of GPe neurons project to the GPi and/or SNr in addition to the STN (see Section 1.2.1). If this is the case then it is not clear what the role of the STN is within the rate model. Similarly, the model also fails to explain the functional significance of the connection back from STN to GPe, and the “hyper-direct” cortical projection to the STN. Finally, the effectiveness of high frequency electrical deep brain stimulation of the subthalamic nucleus at ameliorating the motor symptoms of Parkinson’s disease (Limousin et al., 1998; Kumar et al., 1998; Kleiner-Fisman et al., 2006) is difficult to explain using the rate model. While DBS was originally envisioned to act as a “reversible lesion”, more recent evidence has shown that high-frequency stimulation actually increases the rate of spiking in efferent STN axons (McIntyre et al., 2004); according to the rate model this should cause an exacerbation of hypokinetic symptoms.
1.3 Xenopus tadpole locomotion

Although, as we will see, oscillatory neuronal activity in the basal ganglia is worth studying due to its possible relationship with Parkinson’s disease, this is perhaps not the ideal area in which to study the fundamental mechanisms of neuronal oscillation. This is because, as discussed in the previous section, there is still much that is unknown about the anatomy and electrophysiology of the basal ganglia — especially when one considers that the experimental results which are available come from several different species (mostly rats, mice, monkeys and humans) and it is unclear how applicable findings from one species are to the others. Therefore, in addition to basal ganglia modelling work we also study the oscillatory mechanisms in an animal with a relatively well understood nervous system: the hatchling (2 day old) tadpole of the African clawed frog (*Xenopus laevis*; Figure 1.2A).

### 1.3.1 Structure of the *Xenopus* hindbrain and spinal cord

In fact, we do not study the entire *Xenopus* nervous system, only a roughly 1.5mm long segment containing part of the hindbrain and spinal cord (Figure 1.2B). This section, which contains approximately 1,500 neurons, displays robust swimming activity in response to brief sensory stimulation (Li et al., 2006), and therefore presumably includes the swimming circuitry for the animal as a whole. Remarkably, the anatomy of these neurons can be used to robustly categorise them...
into just seven main types (reviewed in Roberts et al., 2010). During development, cell type is determined by sensitivities to chemical cues that are released from a dorsal “roof plate” and ventral “floor plate” and, as a result, the cell bodies (somata) of each neuron type are arranged in a series of longitudinal columns along the body (Figure 1.2C) (Li et al., 2007b). The main features of the spinal cord, as shown in Figure 1.2B and described in Roberts et al. (2014) are (in order from top to bottom):

- A dorsal “roof plate”, which is comprised of the somata of a group of sensory neurons called Rohon-Beard (RB) cells (yellow circle in figure). These neurons generate action potentials when touch pressure is applied to them. The axons of these neurons run longitudinally along the body in a column called the dorsal tract and release the excitatory neurotransmitter glutamate.

- Below the dorsal tract is a “barrier” column made up of somata of two types of sensory pathway neurons: the dorsolateral ascending and dorsolateral commissural neurons (DLAs and DLCs; pink and red circles respectively). The dendrites of these neurons extend up into the dorsal tract, where they are contacted by the axons of RB cells. The axons of DLAs grow ventrally into a thin medial layer of axons called the marginal zone (MZ), before turning to grow in the ascending (rostral) direction through the MZ. DLC axons, on the other hand, are commissural and grow down and through the floor plate, before turning to grow into the MZ on the opposite body side. Both DLA and DLC axons release glutamate.

- Located ventrally and medially to the dorsolateral neurons are two columns of interneurons which both release the inhibitory neurotransmitter glycine. The more dorsal of the columns (light blue circle) is made up of the commissural interneurons (cINs), while the more ventral column contains ascending interneurons (aINs; purple circle). The dendrites of these neurons grow out radially into the MZ, where they are contacted by axons from all other neuron types (except RBs). As with the DLAs and DLCs, the axons of the aINs and cINs grow longitudinally through either the ipsilateral MZ (aINs) or contralateral MZ (cINs).

- Dorsal to the aINs are the somata of excitatory descending interneurons (dINs; brown circle). These are excitatory neurons which co-release the neurotransmitters glutamate and acetyl-
1.3. Xenopus tadpole locomotion

choline (Li et al., 2004). The axons of dINs, as suggested by the name, grow into the MZ and then course longitudinally through it in the descending (caudal) direction. These neurons are responsible for spreading excitation down the body of the tadpole during swimming. Their dendrites grow into the MZ and can be contacted by axons of all non-RB neurons.

- Finally, the ventral-most column is made up of the somata of motoneurons (MNs; green circle). Again, these neurons have dendrites in the MZ that receive synaptic input from the other neuron types. When driven to spike by excitatory synaptic input (from dINs), the MNs release acetylcholine into special neuromuscular synapses, which causes the contraction of nearby muscles.

1.3.2 Neuronal activity during swimming and synchrony

A very young Xenopus tadpole will mostly remain stationary, attached to the water’s surface or a solid object using its cement gland. However, when the skin on the trunk or tail of a tadpole is briefly touched or stimulated with an electric current, the tadpole begins swimming. This swimming behaviour, which can potentially last for minutes if the tadpole doesn’t bump into anything, consists of a repeating pattern of left-right flexions of the body with a frequency of 10–25Hz (Roberts et al., 2010).

In order to study the neuronal activity during swimming, tadpoles are immobilised by applying a chemical, such as α-bungarotoxin, to block the neuromuscular synapses between MNs and muscles. Many years of electrophysiological studies using this preparation mean that we have a good idea of the electrical properties of each neuron type, and their activity during swimming. Immediately following skin stimulation there is a period of spiking in the sensory pathway neurons (RBs, DLAs and DLCs), and after approximately 45ms (Roberts et al., 2014) the first motoneuron spikes occur and the main swimming pattern appears. During each cycle of swimming most dINs along one side of the body fire single spikes in a synchronised fashion, and the resulting excitation causes subsequent firing in most ipsilateral cINs, aINs and MNs shortly afterwards; it is this MN activity that causes the body to flex to one side. Simultaneously with this happening, contralateral dINs receive large inhibitory post-synaptic currents (IPSCs) due to the commissural inhibition from the cINs. Half a cycle later these contralateral dINs will fire spikes that trigger a burst of activity on
Several unique features of dINs appear to be necessary to facilitate the generation of swimming patterns. Firstly, they are “Class 3” excitable neurons (Izhikevich, 2007, p.15), meaning they only fire single spikes in response to depolarising current injections (Li et al., 2006) — a property that appears to be important for the generation of stable swimming patterns (Sautois et al., 2007). Secondly, dINs are coupled to each other both electrically and synaptically. This electrical coupling acts to synchronise the firing of dINs during a burst of swimming activity and increase the number of dINs which fire (Li et al., 2009), while the excitatory synaptic coupling (which activates fast AMPA-type and long-duration NMDA-type receptors) causes a persistent background level of depolarisation in the dIN population following spiking (Dale and Roberts, 1985). Finally, dINs are able to fire single “rebound” spikes following the removal of inhibition, although only when the inhibition arrives against a background of membrane depolarisation (Soffe et al., 2009), such as that which is seen during NMDA receptor activation. This mechanism appears to be standard anode break excitation, where slow channel gating dynamics during hyperpolarization cause a temporary reduction in the firing threshold that persists for longer than the inhibition — although some excitatory drive is required in order for the membrane potential to reach the reduced threshold. As we shall see in Chapter 5, this rebound mechanism appears to be the fundamental way in which anti-phase swimming oscillations persist.

An additional interesting phenomenon is that occasionally the activity on both sides of the tadpole’s body can show transient synchrony, with both sides spiking in-phase (Khan and Roberts, 1982). This synchronous firing, which has double the frequency of swimming, occurs most frequently shortly after swimming initiation. Interestingly, some individual animals appear to be
1.4 Oscillations in neuronal circuits

Rhythmic patterns are seen at a wide range of temporal and spatial scales in neuroscience. Probably the simplest example of this is neurons which generate action potentials (spikes) repetitively. Many neurons display so-called “Class 1” or “Class 2” excitability (see Izhikevich, 2007, pp.14–15), and spike tonically in response to depolarising currents at either a current-dependent or constant rate. Other neurons, such as those found in the globus pallidus of rodents (Chan et al., 2004), are intrinsic pacemakers that spike repetitively even in the absence of external currents. Human motoneurons, the communication interface between our nervous system and our muscles, spike repetitively when muscle contraction is required with a frequency that is proportional to the force of contraction (De Luca, 1985). This “rate coding” is perhaps the most direct way in which neuronal oscillations can be related to movement control.

Repetitive bursting (sometimes called “chattering”) is a more complex firing pattern that individual neurons can display, in which there are two frequencies of oscillation: the rate at which spikes occur within bursts (the intra-burst interval) and the (slower) rate at which the bursts themselves occur (the inter-burst interval). Activity of this kind is seen in some neurons under healthy conditions, such as the cortical chattering neurons (Gray and McCormick, 1996) and the neurons of the rodent subthalamic nucleus under anaesthetised conditions (Magill et al., 2000), but what is more relevant for this thesis is the increase in repetitive bursting that occurs in animal models of Parkinson’s disease. In non-human primate models of the disease, STN neurons show rhythmic bursts that are synchronised with the muscle tremors that are associated with PD (Bergman et al., 1994), while in rats that have been rendered Parkinsonian (which typically don’t display tremor), neurons in the globus pallidus begin bursting in oscillatory patterns that are synchronised with the cortex (Mallet et al., 2008a). In Chapter 3, we will investigate a possible mechanism for this increase in pallidal bursting.
In Section 1.3.1 we mentioned that tadpoles can still generate normal movements even when all but the caudal hindbrain and spinal cord is removed, but tadpoles are by no means unique in this regard. The cat spinal cord, for example, is able to produce fictive walking behaviour without any input from the brain (Pearson and Rossignol, 1991). Such results have led to the idea that the spinal cord can act as a central pattern generator (CPG), able to produce rhythmic patterns of activity without any rhythmic or sensory input. One way in which a CPG can be organized is as a collection of independent half-centre oscillators. A half-centre oscillator is a type of neuronal circuit that can produce rhythmic activity through the interaction of two non-oscillatory sub-networks. Brown (1914) described a very simple mechanism for a half-centre oscillator based on the neuronal circuitry of the spinal cord. In this scheme, each oscillator controls the movement in one flexor/extensor muscle pair, where one half-centre corresponds to the flexor muscle and the other corresponds to the extensor muscle. Neurons in each half provide excitation to motoneurons for the associated muscle and to inhibitory interneurons, which inhibit the opposite half. The inhibitory coupling ensures that only one half is active at once, whilst an (unspecified) fatigue mechanism causes the active side to switch periodically in an anti-phase pattern.

In their review of CPG mechanisms, McCrea and Rybak (2008) point out that although the “classic” half-centre oscillator circuit has the advantage of simplicity and some biological support, it cannot produce more complicated patterns of movement that the spinal cord is known to be able to produce. The salamander spinal cord, for example, is capable of generating two patterns of activity: low levels of stimulation generate low frequency walking behaviour (“standing wave” left-right trunk flexions), but once this drive crosses a critical threshold the frequency spontaneously increases and the behaviour switches to swimming (“travelling wave” left-right flexions that propagate from head to tail) (Cabelguen et al., 2003). A robotic salamander model was able to reproduce this behaviour by employing a pair of CPGs, each of which is arranged as a set of half-centre oscillators. One CPG produces the swimming pattern and the other produces the walking pattern; this latter CPG makes strong connections onto the former CPG and is able to “override” swimming activity when it is active. In this model the transition from walking to swimming occurs because the walking CPG stops generating oscillations when the driving input is strong, and when this happens the swimming CPG is able to produce its natural behaviour (Ijspeert, 2008). In Chapter 5 we will investigate the CPG mechanism responsible for *Xenopus* tadpole swimming and investigate a possible way in
which this single CPG is able to produce both swimming and synchronous activity.

As well as the firing patterns of individual neurons, oscillations are also seen at larger spatial scales. Local field potential (LFP) recordings are made using relatively large electrodes, which sample the electrical field from a wide area that encompasses many neurons. In the posterior parietal cortex, an area that is known to be involved in movement planning and preparation, oscillatory LFP signals are better able to predict the direction of planned movements than recordings of spiking activity from individual neurons (Scherberger et al., 2005). Specifically, during movement planning spectral power in frequencies below 20Hz drops consistently across different LFP recording sites, but power above 20Hz shows a large increase in certain recording sites that are tuned for particular movement directions. This drop in power below 20Hz prior to movement is so widespread that it can be seen even in measurements of neuronal activity with very coarse spatial resolution, such as scalp electroencephalograph (EEG) recordings taken from above the motor cortex (Klostermann et al., 2007). LFP recordings from within the motor areas of the basal ganglia also show this characteristic drop in low-frequency power prior to movement (Kühn et al., 2004). These results have led to the suggestion that oscillations in the β frequency band (roughly 12–30Hz) may act as a global signal in the motor system which promotes maintenance of the current state and prevents switching to new motor programs (Gilbertson et al., 2005; Engel and Fries, 2010).

The function of β oscillations is currently under much investigation in the context of Parkinson’s disease. This is due to the finding that there is a prominent peak in β power in LFP recordings taken from patients with the disease when they are off medication, and that the reduction of this peak after medication is given correlates with the associated improvement in bradykinesia and rigidity (Kühn et al., 2006). Although straightforward comparisons of absolute β band power between different patients do not show a similar correlation with symptom severity, other measures such as signal coherence (Pogosyan et al., 2010) and complexity (Chen et al., 2010) do. These results have led to the hypothesis that dopamine modulates the strength of β oscillations in the basal ganglia, and the lack of dopamine under Parkinsonian conditions causes excessive hypokinetic β activity (Jenkinson and Brown, 2011).

In this thesis we make the assumption that excessive β activity plays a causative role in the hypokinetic symptoms of Parkinson’s disease, but some evidence suggests that it is merely a
correlative epiphenomenon. When the progression of Parkinson’s disease is simulated in monkeys by the selective lesioning of dopaminergic SNc neurons over the course of many days, oscillatory activity is not observed in the firing rate of individual GPi neurons until long after motor symptoms have appeared (Leblois et al., 2007). It is not clear, however, whether or not LFP signals (where the \( \beta \) peak is usually seen) in the GPi are related to unit activity in that nucleus (Boraud et al., 2005). Other studies with rats have compared the effects of chronic SNc lesioning with acute dopamine blockade and found that only the chronic condition results in a peak in \( \beta \) power in STN LFP (Mallet et al., 2008b) and motor cortex electrocorticogram (Degos et al., 2009), even though both chronic and acute dopamine depletion/blockade induce akinesia. Such evidence does not necessarily rule out the possibility of \( \beta \) oscillations having an anti-kinetic effect, however, since acute dopamine blockade may disrupt motor pathways in a way which is different to the mechanism by which \( \beta \) oscillations act to prevent movement. Even if excessive \( \beta \) activity is simply a side-effect of chronic loss of dopaminergic input to the basal ganglia that does not directly cause Parkinsonian motor symptoms it may still serve as a marker for this neuronal damage that is useful experimentally (Eusebio and Brown, 2009). Furthermore, it has been proposed that elevated \( \beta \) LFP power could be used as a trigger for a new generation of “on-demand” devices for DBS (Tass, 2003; Rosin et al., 2011). Whether the relationship between abnormal \( \beta \) synchronisation and the hypokinetic symptoms of Parkinson’s disease is causative or merely correlative, it is clearly a significant characteristic of the Parkinsonian basal ganglia that should be properly understood.

In Chapters 3 and 4 we will use computational and mathematical models to investigate two possible ways in which excessive oscillations may appear in the Parkinsonian basal ganglia: entrainment to cortical rhythms and “pacemaker” activity in the interconnected STN and GPe.

Of course, oscillations arise in virtually all physical systems and are central to many concepts in the natural sciences, from the subatomic to the astronomical. The field of non-linear systems studies the mathematical principles that underlie complex dynamical behaviours such as oscillation; principles which are remarkably universal across a wide range of physical phenomena. A main aim of this thesis is to use ideas and techniques from non-linear systems to understand the mechanisms behind oscillations that are seen in movement-related neuronal circuits, such as the Xenopus tadpole and mammalian basal ganglia.
1.5 Mathematical and Computational Modelling

In order to use non-linear dynamics to make hypotheses about how certain phenomena arise, it is necessary to have, on some level, a mathematical model of the neuronal system in which the phenomena are found. This model may be explicitly specified as a set of written equations that can be formally analysed; this is the subject of the field of mathematical neuroscience. However, it is also possible that the model is only implicitly defined, and exists solely as an intuitive understanding of the system in the minds of the people studying it. As a simple example of this latter situation, consider an electrophysiologist who knows that injecting increasingly large depolarising currents into a particular neuron will cause it to spike repetitively at a faster and faster rate; without needing to write any equations she or he understands that there is a variable which changes with time (the membrane potential), and that some aspect of this variation (its frequency) depends upon a parameter (the level of injected current).

Once one has, in some form, a mathematical model of the system of interest, this model can be reasoned about in order to form hypotheses about why it behaves in the way that it does, and about how to modify its behaviour. This latter point is particularly important, because if we can understand the fundamental mechanisms underlying pathological neuronal activity (such as that seen in Parkinson’s disease or epilepsy) then the hope is that we will be able to devise new ways to prevent or counteract such activity. When we have a hypothesis about some aspect of neuronal activity, we should test and refine it through experiments. In ideal circumstances these experiments can be performed using the actual neuronal tissue concerned, either in vivo or in vitro, but unfortunately (and despite the ever-increasing sophistication of neuroscience techniques) in many cases this is not practically possible. This is where techniques from the field of computational neuroscience can be applied.

Computational neuroscience is concerned with building models that are typically too complex for formal mathematical analysis, but which allow us to perform so-called in silico experiments in an attempt to understand how they work. Such experiments are appealing because, with a computer simulation, the experimenter is omniscient and omnipotent, and is not limited by the constraints which an electrophysiologist or neurobiologist faces. However, in order for the results of these experiments to be useful we have to assume the hypothesis that the computer model captures
the biological system in sufficient detail to accurately represent it. We can test this hypothesis in two ways: by repeating previous in vivo or in vitro experiments using the model, or by designing new experiments that can be carried out in parallel on real neurons and on the computer. In both cases any significant inconsistencies between the model and reality should be addressed by refining the model.

1.5.1 Spiking models

One of the most common approaches to building an in silico simulation of a neuronal network is to use a spiking model. The equations in these models represent the state of individual neurons and, often, the synapses between them.

The Hodgkin-Huxley Model

The basis for most spiking models is the pioneering work of Alan Hodgkin and Andrew Huxley that was undertaken at the University of Cambridge and the Marine Biological Association Laboratory in Plymouth. Hodgkin and Huxley studied the electrical properties of the giant axon that runs along the length of the body of the squid. They produced a set of non-linear ordinary differential equations that accurately describe how the excitable membrane of the cell produces all-or-nothing voltage spikes in response to current stimulation. The equations that they determined were based on approximating the membrane of the cell as an electronic circuit and then writing equations for the change of membrane potential (V) over time (Hodgkin and Huxley, 1952).

The membrane-equivalent circuit, shown in Figure 1.4, consists of a membrane capacitance (C) in parallel with a number of transmembrane “channels” (na, k, lk), each of which is approximated as a battery in series with a resistor. The currents that flow across these channels represent charged ions moving across the membrane of the cell. Two of these channel types (na and k) correspond to pores in the membrane that are selective for specific ion types: sodium (Na+) and potassium (K+) respectively, while the lk channel represents the natural permeability of the membrane to certain (non-specific) ions. Depending of the charge of the ions and the voltage of the cell’s interior relative to its exterior, an electrical force exists that either attracts ions into the cell or repels them out of it. For example, the Na+ and K+ ions are both positively charged, and so
when the neuron’s membrane potential is below 0mV ions of these types are electrically attracted into the cell. However, there is also an imbalance in the concentration of each ion type between the interior and the exterior of the cell which is maintained by cellular pump mechanisms, and this chemical gradient is a second force which either attracts ions to or repels ions from the cell. The battery voltages (\(E_{\text{na}}, E_{\text{k}}, E_{\text{lk}}\)) represent the equilibrium (or reversal) potentials at which these two forces are balanced for a particular ion type — when the membrane potential is equal to the equilibrium potential then the current due to that channel type is zero. The concentration of sodium ions is typically much lower inside the cell than outside it, meaning that these ions are attracted into the cell by the chemical gradient. This means that in order to be in equilibrium the electrical force must repel sodium ions, therefore \(E_{\text{na}}\) is positive (around 60–90mV). On the other hand, potassium is much more concentrated inside the cell, so \(E_{\text{k}}\) is negative (around -90mV). The reversal potential of leak channels is typically around -50 to -60mV.

While the conductance of the leak channel (\(g_{\text{lk}}\)) is a fixed parameter of the system, the sodium and potassium channels are active (voltage gated), meaning their conductances (\(g_{\text{na}}\) and \(g_{\text{k}}\)) vary with time according to the movements of “gate” molecules. Depending on their location, gate molecules can either block or allow the flow of ions through the channel that they are associated
with. The canonical Hodgkin-Huxley equation for evolution of the membrane potential in time is:

\[ C \frac{dV}{dt} = g_{l}(E_{l} - V) + m^{3}h\tilde{g}_{na}(E_{na} - V) + n^{4}\tilde{g}_{k}(E_{k} - V) \]  \hspace{1cm} (1.1)

In this equation \( \tilde{g}_{na} \) and \( \tilde{g}_{k} \) are parameters which specify the maximum possible Na\(^{+} \) and K\(^{+} \) conductances, while \( m, h \) and \( n \) are variables which represent gating of the sodium and potassium channels. Specifically, each of these gating variables gives the proportion (from 0 to 1) of gating molecules that are currently in the configuration which allows ions to pass through the channel. The dynamics of these variables is given by:

\[
\begin{align*}
\frac{dm}{dt} &= \alpha_{m}(V)(1 - m) - \beta_{m}(V)m \\
\frac{dh}{dt} &= \alpha_{h}(V)(1 - h) - \beta_{h}(V)h \\
\frac{dn}{dt} &= \alpha_{n}(V)(1 - n) - \beta_{n}(V)n
\end{align*}
\]  \hspace{1cm} (1.2–1.4)

Here \( \alpha_{m,h,n}(V) \) are the forward rate functions, each of which specifies the rate (in msec\(^{-1} \)) at which its corresponding gating molecule moves from its configuration where ions are blocked into its configuration where ions can flow. Conversely, \( \beta_{m,h,n}(V) \) are the backward rate functions, which determine how quickly gating molecules move from the unblocked to the blocked configuration. Hodgkin and Huxley determined various \( \alpha \) and \( \beta \) functions that fitted the particular channels they were studying, but all follow the form:

\[ f(V) = \frac{A + BV}{C + e^{BP(V)}} \]  \hspace{1cm} (1.5)

Where \( A, B, C, D \) and \( E \) are parameters that must be chosen based on experimental data. Each of the Equations 1.2–1.4 can be rewritten in a more intuitive form by making the following substitutions:
\[ \gamma_\infty(V) = \frac{\alpha_\gamma(V)}{\alpha_\gamma(V) + \beta_\gamma(V)} \]  
(1.6)

\[ \tau_\gamma(V) = \frac{1}{\alpha_\gamma(V) + \beta_\gamma(V)} \]  
(1.7)

Here \( \gamma \) can be \( m, h \) or \( n \). Using these substitutions, Equations 1.2–1.4 can be rearranged into the form:

\[ \frac{d\gamma}{dt} = \frac{\gamma_\infty(V) - \gamma}{\tau_\gamma(V)} \]  
(1.8)

We can see from Equation 1.8 that \( \gamma_\infty(V) \) and \( \tau_\gamma(V) \) have intuitive interpretations in terms of the model’s dynamics: \( \gamma_\infty(V) \) represents the steady-state value that the gating variable will tend to for a given value of \( V \), while \( \tau_\gamma(V) \) gives the characteristic time at which the steady-state value is reached. Physiological curves for \( \gamma_\infty(V) \) often have sigmoidal shapes, so some models use the following simplification:

\[ \gamma_\infty(V) = \gamma_0 + \frac{\gamma_1 - \gamma_0}{1 + \exp((V - V_{0.5})k_1^{-1})} \]  
(1.9)

Here \( \gamma_0 \) and \( \gamma_1 \) are the minimum and maximum values of the gating variable respectively (usually 0.0 and 1.0), \( V_{0.5} \) is the midpoint and \( k \) is the slope. If \( k > 0 \) then the sigmoid is an increasing function of the membrane potential, meaning the gate activates as the neuron becomes depolarised. On the other hand, if \( k < 0 \) then the sigmoid is a decreasing function, and the gate inactivates as the neuron becomes depolarised.

Likewise, real curves for \( \tau_\gamma(V) \) often have a Gaussian shape, and can be approximated by functions such as that given in Equation 1.10:

\[ \tau_\gamma(V) = \tau_0^\gamma + \frac{\tau_1^\gamma - \tau_0^\gamma}{\exp((V - V)k_1^{-1}) + \exp((V - V)k_2^{-1})} \]  
(1.10)

Here \( k_1 > 0 \) and \( k_2 < 0 \). The function behaves similarly to the Gaussian function: as \( V \) increases \( \tau_\gamma(V) \) increases with a slope that is inversely proportional to \( k_1 \), before reaching a maximum near to \( V = \bar{V} \) and then decreasing with a rate inversely proportional to \( (-k_2) \). Using the approximations
given in Equations 1.9 and 1.10 and assuming $\gamma_0 = 0$ and $\gamma_1 = 1$ means that only seven parameters are required to define the dynamics of a gate, rather than the ten that are required for the form given in Equation 1.5.

The importance of Hodgkin and Huxley’s work goes far beyond the specific equations and parameters that they defined for the squid giant axon. The general form of their equations for membrane potential and gating variables provide a framework for electrophysiological modelling that can be applied to other types of neurons with different ion channels and gating dynamics. In Chapter 2 we will describe our software for building spiking models that is based on this framework.

**Simplified Spiking Models**

Although the Hodgkin-Huxley equations represent a “gold standard” for biologically realistic modelling of neurons, in practice their relative complexity can be problematic. With the classic Na$^+$ and K$^+$ channels, the equations define a four dimensional system, with non-trivial functions for the time derivatives of the gating variables. This makes formal mathematical analysis of the equations’ dynamics very difficult and increases the computational power required to perform simulations. Models based on the Hodgkin-Huxley equations also include very large numbers of parameters: three for the passive membrane properties, two for each channel and eleven for each gate (if using $\alpha$ and $\beta$ functions of the form given in Equation 1.5). Not all of these parameters can be found directly through experiments, and when fitting so many parameters to limited experimental data there are likely to be multiple best solutions. Having to arbitrarily choose from several possible sets of parameters somewhat negates the advantage of having a biologically realistic model. For these reasons, several simplified models have become popular.

The Morris-Lecar equations (Morris and Lecar, 1981) are a two dimensional system of ordinary differential equations (ODEs) that were derived from a three dimensional version of the Hodgkin-Huxley model with activating inward (Ca$^{2+}$) and outward (K$^+$) currents, by assuming instantaneous dynamics for the Ca$^{2+}$ channel in order to eliminate its gating variable. Despite this simplification, model neurons based on the Morris-Lecar equations demonstrate many of the same basic dynamical features as their biological counterparts, such as all-or-nothing spikes and repetitive spiking in response to depolarising current injections. FitzHugh (1961) produced a similar two dimensional reduced set of equations by following a more abstract mathematical approach. In
1.5. Mathematical and Computational Modelling

these equations, one is non-linear and the other linear. The variable with the non-linear RHS oper-
erates on a fast time scale and can be thought of as being equivalent to membrane potential, while
the other variable operates on a slower time scale and is responsible for the repolarisation phase
of a spike (equivalent to Na\(^+\) inactivation and/or K\(^+\) activation). Unlike the Hodgkin-Huxley and
Morris-Lecar equations, the FitzHugh-Nagumo model was not designed to specifically represent
biological processes, meaning that selecting parameter values on the basis of experimental evidence
can be difficult. However, the equations can reproduce many of the same dynamical phenomena
as those of Morris and Lecar. Neither of the reduced models discussed so far can produce more
complex oscillatory spiking patterns such as bursting and chaotic behaviour. In order to obtain
a conductance-based model that does exhibit these features it is necessary to use a three dimen-
sional system, such as that of Hindmarsh and Rose (1984). These equations are very similar to the
FitzHugh-Nagumo equations but with two modifications: non-linearity of the ODE for the second
variable (which allows both rest and repetitively spiking states to be stable) and the addition
of a third variable which represents a slowly activating outward current (which allows periodic
bursting).

Another class of simplified spiking models are based on resetting. In these models, the value
of the state variable which corresponds to membrane potential is monitored and when it crosses
a threshold value it is reset (possibly along with the other state variables) to a new value. The
simplest of these models is the one dimensional linear “leaky integrate and fire” (Lapicque, 1907;
Izhikevich, 2007) neuron. This model gives Class 1 excitability in a mathematically and computa-
tionally straightforward way, but its state variable cannot really be thought of as membrane
potential as it does not increase sharply towards the threshold when “spiking”. Also, this model
transitions to regular spiking in a non-standard way and displays a frequency-current curve that
is not biologically realistic (Izhikevich, 2007, p.269). These drawbacks can be addressed by mak-
ing the system non-linear, for example with an exponential (Fourcaud-Trocmé et al., 2003) or
quadratic (Latham et al., 2000) function. Two popular models, the Izhikevich neuron (Izhikevich,
2003) and the adaptive leaky integrate and fire (Brette and Gerstner, 2005) neuron, extend non-
linear one dimensional models by adding a second dimension for slow adaptation dynamics and are
able to produce an extremely large range of physiological spiking patterns. Despite the dynamical
capabilities and computational and mathematical tractability of resetting models, they are not
suitable for cases where the shape of spikes is important. This is because the simplification that all these models make is to disregard the mechanisms that terminate the rising phase of a spike and repolarise the neuron, instead replacing these with instantaneous resetting.

1.5.2 Population-level modelling

Reduced spiking models such as those discussed in the previous section are simple enough to allow mathematical analysis of the behaviour of individual neurons. In certain special circumstances, small populations of very abstract neuron models, e.g. networks of phase oscillators, can be studied using mathematical approaches such as bifurcation analysis (Burylko et al., 2012). However, even relatively small regions in relatively small brains contain huge numbers of neurons and synapses: the rat STN and GP contain an estimated 14,000 and 46,000 neurons respectively (Oorschot, 1996), with approximately 12 million GP-STN synapses (Baufreton et al., 2009). While the ever-increasing availability of cheap computing power means that spiking models of networks of this scale are now becoming computationally possible, mathematical analysis of such systems is clearly impractical. An alternative to “microscopic-scale” spiking models is to instead consider activity on a larger “mesoscopic” scale, by considering the average activity in one or more interconnected populations of neurons.

Rigorously deriving equations that describe large scale population dynamics from the behaviour of individual neurons is a very difficult task, and many attempts have been made — for reviews see Ermentrout (1998) and Coombes (2006). The behaviour of the averaged system depends strongly on what the measure of activity is; for example this may be the proportion of neurons that have recently spiked (Wilson and Cowan, 1972; Amari et al., 1977), the average membrane potential (Gerstner and Kistler, 2002, p.207), or the average dendritic current (Coombes, 2006). One of the most successful approaches to population level modelling is based on the equations derived by Wilson and Cowan (1972), which describe the proportion of active neurons in a pair of neuronal subpopulations. A fundamental feature of the model is the idea of “subpopulation response functions”, which determine the proportion of cells in a subpopulation that will become active (i.e. fire a spike) in response to a given level of excitation. Although different neurons may have different firing thresholds or afferent synapse counts, across the population these differences
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can be averaged away, giving a subpopulation response that is a monotonically increasing function of excitation with range (0,1). An example of such a function is the (vertically shifted) logistic curve:

\[
Z(x) = \frac{1}{1 + \exp(-a(x-\theta))} - \frac{1}{1 + \exp(a\theta)}
\]  

Here the parameters \(a\) and \(\theta\) represent the maximum slope of the sigmoid and its position on the horizontal axis respectively. Assuming all neurons in a population receive a sufficiently high number of afferent synapses, the level of excitation that each population receives can be represented as a linear function of the population activity levels, leading to the Wilson-Cowan equations:

\[
\tau_1 \frac{dx_1}{dt} = -x_1 + (k_1 - r_1 x_1)Z_1(w_{11}x_1 + w_{21}x_2 + I_1) \\
\tau_2 \frac{dx_2}{dt} = -x_2 + (k_2 - r_2 x_2)Z_2(w_{22}x_2 + w_{12}x_1 + I_2)
\]  

Here the state variables \(x_1\) and \(x_2\) are the proportions of active neurons in the two populations, parameters \(\tau_{1,2}\) roughly correspond to the average membrane time constants of the neurons in the populations and \(I_{1,2}\) represent external currents applied to the populations. The weight parameters \(w_{ab}\), where \(a, b \in \{1, 2\}\), represent the strength of connection from population \(a\) to population \(b\). The term involving the parameters \(k_{1,2}\) and \(r_{1,2}\) arises from the theoretical derivation of the equations from a neuron model that includes a post-spike refractory period. Removing this term by setting \(k_1 = k_2 = 1\) and \(r_1 = r_2 = 0\) does not have a major impact on the dynamics of the equations (Ermentrout and Terman, 2010, pp.344–5) and therefore we will use this simplification in this thesis.

Wilson and Cowan based their model on the idea that “all nervous processes of any complexity are dependent upon the interaction of excitatory and inhibitory cells” (Wilson and Cowan, 1972), and therefore considered the population given in Equation 1.12 to be an excitatory subpopulation \((w_{11,12} > 0)\) and the population in Equation 1.13 to be an inhibitory one \((w_{22,21} < 0)\); in fact it can be shown that limit cycles cannot exist unless these conditions are true. Borisyuk and Kirillov (1992) used bifurcation analysis in two dimensions in order to understand the dynamical regimes.
that are possible for different values of $I_1$ and $w_{12}$. This approach elucidated the way in which the different dynamics described by Wilson and Cowan (steady state, bi-stability and oscillations) arise as the parameters are varied and revealed an interesting new regime in which there is bi-stability between a stable fixed point and a stable limit cycle. We will use a similar approach to analyse a population-level model of the basal ganglia in Chapter 4.
Chapter 2

Building Software Tools for Modelling

2.1 A general-purpose simulator for single compartment Hodgkin-Huxley models

We developed our own software, tentatively named Simulation of Neural Activity Patterns with Python (SNAPPy), for implementing the spiking neuron models that are described in this thesis. Although there are several existing software packages for building conductance-based models of neuronal networks, such as NEURON (Carnevale and Hines, 2006), GENESIS (Bower and Beeman, 1995) and NEST (Gewaltig and Diesmann, 2007), we required a specific set of features (including synaptic delays, gap junctions, and ion channel down-regulation) that appeared to be either difficult to use or missing from other software. As well as being a valuable learning experience, writing our own simulator would give us an understanding of the code that would mean we could easily implement new features and test new ideas. We kept three (somewhat contradictory) main aims in mind when developing SNAPPy:

- **Simplicity**, with regard to keeping the code straightforward and making the software relatively easy for people without a computer science background to use. We tried to achieve this by...
by not including extraneous features and not trying to make a completely general simulator that was suitable for every possible modelling task. However:

- **Flexibility** was also a key aim, within the limited scope of single-compartment Hodgkin-Huxley type models. Although specialised for this particular domain, we wanted the software to support building a wide range of neuronal models of this type.

- **Speed**, generally in terms of allowing us to quickly build and investigate new models using SNAPPy, but also specifically in the performance of the software itself. Simulations containing several thousand neurons with tens of thousands of synapses needed to execute quickly enough for us to interactively observe them running.

It was also important for us that the software be cross-platform, supporting the Linux, Windows and Mac operating systems. With this in mind, we chose to implement the main graphical front-end of SNAPPy using the Python programming language. Python is a multi-platform interpreted language with support for object-oriented programming, as well as modern functional programming-type features. Importantly, Python also has an extensive built-in library of packages that support many common tasks. Many excellent third party, cross-platform, open source, libraries are also available. From past experience, however, we were not confident that implementing the core simulation in Python would give us acceptable performance in large models, due its interpreted nature. We therefore chose to implement the simulation part of the software in a separate program, written in the C programming language. Although missing many of the niceties of Python, C code is compiled to machine language and can be automatically optimised by the compiler to a very high degree.

Figure 2.1 shows a high-level overview of the software. The user prepares a number of input files that describe their model and specify how the simulation should run, as well as how the results should be displayed. These input files are plain text and most of them (apart from the cell list and connectome files) are formatted as JavaScript Object Notation (JSON) structures. JSON provides a convention for representing objects as text — an object here being a set of name-value pairs, where a value can be a simple type (string, integer, real), another object, or a list of values. Figure 2.2 shows the different types of objects that are used to define a model in SNAPPy and which configuration files they are specified in. Appendix A gives examples of each of the different types
2.1. A general-purpose simulator for single compartment Hodgkin-Huxley models

Figure 2.1: High-level overview of SNAPPy. To run a simulation, configuration files are passed to the graphical front-end, which starts a sub-process containing the main simulation kernel. Results are saved as data files containing state variable values at regular time steps and a list of spikes. The user can also use the graphical front-end to save figures in various file formats.

These files are passed as arguments to the Python graphical front-end, which then spawns a sub-process containing the simulation kernel. The simulation kernel communicates with the main program via a (local) network socket, and as the simulation runs the kernel sends back results for the front-end to display. While running, the kernel also generates two output files for permanent storage of the results: one logging the state variables for each neuron at regular time steps and one containing a list of spikes that occur during the simulation. If an interactive graphical display is not needed the kernel can be launched on its own, in which case it will run the simulation for the specified time and generate the results files for later display or processing. Similarly, the interactive program can load and display the results of a previous simulation rather than launching a new kernel.

During a simulation the current state of the system is represented by a set of state variables. The state variables are 64-bit floating-point values, and can include the membrane potential, calcium concentration and ion channel gating variables for each neuron, as well as the activation level of each synapse. Each time step of the simulation involves performing three sub-steps:
Figure 2.2: Class diagram showing the objects that make up a simulation configuration in SNAPPy, along with their main properties and the relations between them. The connections between classes are annotated with the “multiplicity” of the connection, according to Unified Modelling Language (UML) conventions, where the number indicates how many of the child objects are contained by each parent object (“0..*” indicates zero or more, “1..*” indicates one or more). The objects are colour-coded based on which configuration file they are specified in.
1. Using a numerical method to advance the current values of the state variables forwards in time. SNAPPy calculates the instantaneous time derivative, or “right hand side” (RHS), for the state variables and then uses the ODE solver routines from the GNU Scientific Library (version 1.15) (Galassi et al., 2009) in order to calculate the new state values (the details of the exact routine used are configurable).

2. Spike detection. All neurons’ new membrane potential values are checked to see if they have crossed a (configurable) spike threshold (typically 0mV). If a spike has occurred, instantaneous changes are made to the state variables representing synaptic activation in the spiking neuron, which will result in synaptic currents arising in any neurons that are post-synaptic to it. Also, information about any spikes that occurred is sent to the front-end and is written to the spike file.

3. Results transmission and logging. The current value of all state variables, as well as various other values such as synaptic currents, is sent to the front-end for display, provided a given (configurable) period of simulation time has elapsed since results were last sent. The values are also written to the time step file.

The first sub-step in this list takes up the vast majority of computation time during a simulation and is where most of the software’s complexity is found. The following sections describe how the RHS is computed for each of the state variable types: membrane potential, gating variable, synaptic activation, and intracellular calcium concentration.

### 2.1.1 Calculating the RHS of the membrane potential

Recall from Section 1.5.1 that the Hodgkinson-Huxley model considers the membrane of a neuron as an electric circuit with capacitance $C$, and various currents, summed as $I$, flowing across it. According to this scheme the time derivative of the membrane potential is simply given by:

$$ \frac{dV}{dt} = \frac{I}{C} $$

(2.1)

The currents that flow across the membrane can be of many different types, and in SNAPPy these correspond to many of the model objects shown in Figure 2.2. Breaking $I$ down into the sum
of these different current types gives:

\[ I = I_{lk} + I_{ch} + I_{syn} + I_e + I_{inj} \]  

(2.2)

Here \( I_{lk} \) is current due to the passive “leak” channel; \( I_{ch} \) is the total current due to all active ionic channels; \( I_{syn} \) is the total current due to all synapses that the neuron receives from other neurons; \( I_e \) is the current from any gap junctions (electrical synapses) that the neuron has, and \( I_{inj} \) represents any applied injected currents that may be active as part of the experimental paradigm.

We will now describe the calculation of each of these current in turn.

**Leak Channel Current**

This current represents the non-gated flow of ions of non-specific type across the neuronal membrane as a result of its natural permeability to certain ions. It is a simple ohmic current that is calculated according to:

\[ I_{lk} = g_{lk}(E_{lk} - V) \]  

(2.3)

The two parameters \( g_{lk} \) and \( E_{lk} \) represent the conductance and equilibrium (reversal) potential of the leak channels. These parameters are specified individually for each neuron type in the corresponding cell type specification file.

**Active Channel Currents**

Model neurons in SNAPPy can include arbitrarily many types of active channel. The total active channel current \( I_{ch} \) for neuron \( i \) is given by the sum of the individual channel currents, each of which is the product of a “base” current with a set of gating variables:

\[ I_{ch} = \sum_{c \in \chi_i} \hat{I}_c \prod_{\mu \in \Gamma_c} (\gamma_\mu)^{p_\mu} \]  

(2.4)

Here \( \chi_i \) is the set of all active channel types that neuron \( i \) contains, \( \hat{I}_c \) is the base current for channel \( c \), and \( \Gamma_c \) is the set of gates that channel \( c \) has. For each gate \( \mu \) we define the pair \( (\gamma_\mu, p_\mu) \), where \( \gamma_\mu \) is the corresponding gating state variable (e.g. the variables \( m, n, h \) in the standard
SNAPPy supports two different equations for calculating the base current across a channel. In the standard Hodgkin-Huxley model, the base current is defined by two parameters: the equilibrium (or reversal) potential $E_c$ (mV) and maximum conductance $g_c$ (nS), and is calculated according to:

$$I_c = g_c(E_c - V)$$  \hspace{1cm} (2.5)

For some ionic currents, it may be necessary to use a more accurate equation for the base current in order to fit experimental data well. The second equation for base channel current that SNAPPy supports is the Goldman-Hodgkin-Katz equation (Hille, 2001, pp.445–446):

$$I_c = -\hat{p}kzF(S_{in} - S_{out} \times \exp(k)) \frac{1}{1 - \exp(k)}$$  \hspace{1cm} (2.6)

$$k = \frac{zFV}{RT}$$  \hspace{1cm} (2.7)

This equation introduces five parameters that must be specified for the channel: $\hat{p}$ is the maximum permeability of the channel to the ion it is selective for (cm$^3$/s), $z$ is the valence of the ion, $T$ is the temperature of the cell (Kelvin), and $S_{in}$ and $S_{out}$ are the intracellular and extracellular concentrations of the ion (M). Equations 2.6 and 2.7 also include two physical constants: $F$ is Faraday’s constant and $R$ is the ideal gas constant.

### Synaptic Currents

When a neuron generates an action potential (spike), a transient post-synaptic current (PSC) arises in all of the neurons with which it makes synaptic contact. In SNAPPy, the user specifies the different synapse types that can exist between neurons in the Simulation Parameters file, where each synapse type is defined by its equilibrium (reversal) potential and various parameters which control the dynamics of its post-synaptic currents. Each model neuron contains two state variables, $o(t)$ and $c(t)$, for each of the synapse types in the model. These represent the average opening and closing dynamics of all of the synapses of the given type which that neuron makes onto other
neurons. Section 2.1.3 will describe how these variables evolve in time and how delayed values are calculated, but for now we assume that for a given neuron $i$ and synapse type $T$ we can find the values $o_{i,T}(t)$ and $c_{i,T}(t)$ at any given time $t$ prior to the current simulation time.

Let an individual synapse be defined by the tuple $(p, q, T, g, d)$, where $p$ and $q$ are the indexes of the pre- and post-synaptic neurons respectively, $T$ is the index of the synapse type (referencing the list of synapse types given in the Simulation Parameters file), $g$ is the maximum conductance of the synapse, and $d$ is the time delay associated with the synapse. If $S$ is the set of all such synapses in the model, let $S_i$ be the subset of synapses that have neuron $i$ as the post-synaptic neuron (i.e. $S_i = \{(p, q, T, g, d) \in S : q = i\}$). The total synaptic current that neuron $i$ is subject to is the sum of the currents at each of the synapses it receives:

$$I_{syn} = \sum_{s \in S_i} \hat{I}_{syn}^s$$  \hspace{1cm} (2.8)

where

$$\hat{I}_{syn}^s = g \times (E_T - V(t)) \times (c_{p,T}(t-d) - o_{p,T}(t-d))$$  \hspace{1cm} (2.9)

Here $E_T$ is the reversal potential of synapse type $T$ (mV) and $V(t)$ is the membrane potential of the neuron $i$ (mV).

In many models which contain synapses with NMDA receptors an important property is the non-linear voltage dependence of these receptors (Hille, 2001, pp.195–199). When the neuron’s membrane potential is at its hyperpolarised resting state level, extracellular Mg$^{2+}$ ions are attracted to the membrane and act to block NMDA receptors. This means that the receptors are only sensitive to neurotransmitter when the post-synaptic neuron is already depolarised and the Mg$^{2+}$ block has been removed — a property that may play a “coincidence detection” role. SNAPPy allows this phenomenon to be modelled using an approach from Lisman et al. (1998), whereby the current due to a synapse is multiplied by a voltage-dependent sigmoidal function $f(V)$, defined as:

$$f(V) = \frac{1}{1 + c_1 \exp(c_2 V)}$$  \hspace{1cm} (2.10)

This introduces two new parameters for the synapse type, $c_1$ and $c_2$, where $c_1 > 0$. If $c_2 < 0$
2.1. A general-purpose simulator for single compartment Hodgkin-Huxley models

2.1.1. Hodgkin-Huxley Models

A Hodgkin-Huxley model is a mathematical model that describes the ionic currents and voltage-gated channels in a neuron's membrane. It is based on the experimental observations of Hodgkin and Huxley (1952), who recorded the voltage changes in a giant axon of the giant squid. The model consists of four differential equations that describe the dynamics of the membrane potential $V$ as a function of time $t$, and the activation and inactivation of sodium and potassium channels.

$$
\begin{align*}
\frac{dV}{dt} & = \frac{1}{C_m} \left( I_{\text{Na}} + I_{\text{K}} - I_{\text{Cl}} - I_{\text{leak}} \right) \\
I_{\text{Na}} & = g_{\text{Na}} m^3 h (V - V_{\text{Na}}) \\
I_{\text{K}} & = g_{\text{K}} n^4 (V - V_{\text{K}}) \\
I_{\text{Cl}} & = g_{\text{Cl}} (V - V_{\text{Cl}}) \\
I_{\text{leak}} & = g_{\text{leak}} (V - V_{\text{leak}})
\end{align*}
$$

where $V$ is the membrane potential, $C_m$ is the membrane capacitance, $g_{\text{Na}}, g_{\text{K}}, g_{\text{Cl}}$ are the conductances of sodium, potassium, and chloride channels, respectively, and $V_{\text{Na}}, V_{\text{K}}, V_{\text{Cl}}, V_{\text{leak}}$ are the reversal potentials.

The equations describe the time course of the membrane potential $V$, which is governed by the net ionic currents $I_{\text{Na}}, I_{\text{K}}, I_{\text{Cl}}, I_{\text{leak}}$. Each current depends on the state variables $m, n, h$, which represent the fraction of open sodium, potassium, and potassium channels, respectively.

**Figure 2.3**: An example of non-linear voltage dependence of synapses, shown here in the case of the Mg$^{2+}$ block of NMDA synapses in the *Xenopus* tadpole model described in Chapter 5. A: Plot of the sigmoidal function $f(V)$ (Equation 2.10), which is used to scale synaptic currents based on the membrane potential of the post-synaptic neuron. In this case, with $c_1 = 0.05$ and $c_2 = -0.08$, the synapse is blocked when the neuron is hyperpolarised. B: Excitatory post-synaptic potentials evoked by a single spike in neurons at different rest potentials, demonstrating the voltage dependence shown in (A).

then the slope of the sigmoid is positive and receptors will become unblocked as the membrane potential increases, as is the case for Mg$^{2+}$ block of NMDA synapses. Alternatively, if $c_2 > 0$ then the slope of the sigmoid is negative, and receptors become blocked as the membrane potential increases. Receptors are half-blocked ($f(V) = 0.5$) and the sigmoid slope is vertical when $V = V_{0.5} = -c_2^{-1} \ln(c_1)$. In the model of the *Xenopus* tadpole spinal cord that we will describe in Chapter 5 we use $c_1 = 0.05$ and $c_2 = -0.08$ to model the voltage dependence of NMDA receptors. Figure 2.3 shows $f(x)$ for these parameter values and the NMDA excitatory post-synaptic potentials (EPSPs) evoked in neurons at different levels of hyperpolarization.

**Gap junction currents**

When the outer membranes of pairs of certain types of neuron are located very close to each other, gap junctions (or electrical synapses) may form between the two neurons. Gap junctions act to electrically couple the intracellular space of the neurons, allowing currents to flow directly between them. In SNAPPy, gap junctions are always symmetric: current flowing into one neuron is exactly matched by current flowing out of the other neuron, although this is not always the case in real neurons (Hille, 2001, pp.302–304). The user can specify, using the Simulation Parameters file, that...
gap junctions with a specific conductance \( (g_e) \) should exist between pairs of neurons of certain types whenever two neurons are located within a certain distance of each other. If \( GJ_i \) is the set of all neurons that neuron \( i \) makes gap junctions with, then the total gap junction current for neuron \( i \) is given by:

\[
I_e = \sum_{j \in GJ_i} g_e (V_j - V_i)
\]  

(2.11)

Where \( g_e \) is the gap junction conductance specified in the simulation parameters file, and \( V_i \) and \( V_j \) are the membrane potentials of neurons \( i \) and \( j \) respectively.

### Injected currents

Current clamping is a very common electrophysiological technique where additional electrical currents are “injected” into a neuron through an electrode. Many of the properties that are used to classify the dynamic behaviour of neurons are based on responses to different current injections. The ability to inject currents into model neurons is therefore important in order to compare their behaviour with their real-life counterparts. In SNAPPy, users can specify zero or more current injections using the Simulation Parameters file. For each injection the user must specify the start and end times \( (t_{start}, t_{end}) \), and the neuron (or range of neurons) that the injection should apply to. If multiple current injections apply to a neuron at once, the currents are summed. Three different current injection functions are available, the simplest of which provides a constant current for its duration:

\[
f_{\text{cnst}}(t) = \begin{cases} 
    i_{\text{inj}} & t_{\text{start}} < t \leq t_{\text{end}} \\
    0 & \text{otherwise}
\end{cases}
\]  

(2.12)

Where \( i_{\text{inj}} \) is the constant level of current to inject.

Alternatively, a repetitive square-wave current can be used, which alternates between two levels of current injection:
2.1. A general-purpose simulator for single compartment Hodgkin-Huxley models

\[
f_{sq}(t) = \begin{cases} 
  i_{hi} & t_{start} < t \leq t_{end} \land \omega(t) < D \\
  i_{lo} & t_{start} < t \leq t_{end} \land \omega(t) \geq D \\
  0 & \text{otherwise}
\end{cases}
\]

\[
\omega(t) = \frac{(t - t_{start})}{p} - \lfloor \frac{(t - t_{start})}{p} \rfloor
\]  

(2.13)

Where \(i_{hi}\) and \(i_{lo}\) are the two injection levels, \(p\) is the period of the square wave, and \(D\) is the proportion of each cycle for which the high current level should be active (duty cycle), where \(0 < D < 1\). \([x]\) represents the integer part (floor) of \(x\) and \(\land\) represents logical “and”.

Finally, injected currents can follow a sine wave function:

\[
f_{sin}(t) = \begin{cases} 
  i_{lo} + \frac{1}{2}(i_{hi} - i_{lo})(1 + \sin(2\pi(t + \phi - t_{start})p^{-1})) & t_{start} < t \leq t_{end} \\
  0 & \text{otherwise}
\end{cases}
\]

(2.14)

Where \(i_{hi}\) and \(i_{lo}\) are the two injection levels, \(p\) is the period of oscillation and \(\phi\) is a constant phase shift.

2.1.2 Calculating the RHS of channel gating variables

Recall from Section 2.1.1 that in the Hodgkin-Huxley model each active channel current is scaled by one or more gating variables. In SNAPPy, a generic gating variable \(\gamma(t)\) evolves in time according to the following equation:

\[
\frac{d\gamma}{dt} = \frac{1}{\tau_{\gamma}(V)}(\gamma_{\infty}(V) - \gamma)
\]

(2.15)

The two voltage-dependent functions \(\tau_{\gamma}(V)\) and \(\gamma_{\infty}(V)\) represent the characteristic time and steady-state activation level of the gate respectively. Each gating variable will tend exponentially towards its steady-state level according to its characteristic time. In SNAPPy several different forms can be used for these functions. In the simplest case, the steady-state activation and/or
characteristic time can take a constant value which doesn’t depend on membrane potential. In most cases, of course, the functions depend on voltage and SNAPPy supports both the forward/backward rate function \((\alpha(V), \beta(V))\) format (Equations 1.5–1.7) or the sigmoid/Gaussian format (Equations 1.9–1.10).

### 2.1.3 Calculating the RHS of synaptic variables

In SNAPPy there are two state variables associated with every neuron / synapse type pair: \(o(t)\) and \(c(t)\), representing synaptic opening and closing respectively. Post-synaptic currents arise when there is a difference between the values of these state variables (Equation 2.9). In this section we show how this difference arises following a spike.

The post-spike synaptic rise and decay is based on a difference of exponentials scheme. The opening and closing variables obey the following linear differential equations:

\[
\frac{do}{dt} = -\frac{o}{\tau_o} \tag{2.16}
\]
\[
\frac{dc}{dt} = -\frac{c}{\tau_c} \tag{2.17}
\]

Here \(\tau_o\) and \(\tau_c\) are the opening and closing time constants respectively, with \(\tau_c > \tau_o > 0\). The time constants, which have units of milliseconds, are specified for each synapse type in the Simulation Parameters file and determine how quickly the post-synaptic current rises \((\tau_o)\) and then decays \((\tau_c)\) following a spike. When a spike is detected in a neuron, each pair of synaptic opening/closing variables associated with that neuron is immediately increased by a value \(\Delta s\), specified as part of the synapse type. Since pairs are increased by the same value, \(c(t) - o(t) = 0\) immediately following an isolated spike, but because \(\tau_c > \tau_o\) the opening variable decays to 0 more quickly than the closing one. This means that, following a spike, \(c(t) - o(t)\) quickly increases and then decays more slowly back to 0. Figure 2.4 shows plots of \(o(t)\), \(c(t)\) and \(c(t) - o(t)\) for an AMPA synapse following a spike.
2.1. A general-purpose simulator for single compartment Hodgkin-Huxley models

Figure 2.4: The gating of each synapse in SNAPPy is controlled by two state variables, \( o(t) \) and \( c(t) \), where synaptic activation is calculated as \( c(t) - o(t) \). This plot shows \( c(t) \), \( o(t) \) and \( c(t) - o(t) \) for a typical AMPA synapse (from the Xenopus model in Chapter 5) following a spike at approximately 55ms. The synaptic activation (red line) rises quickly following the spike, and then decays away more slowly. The synaptic time constants here are \( \tau_o = 0.2 \text{ms} \) and \( \tau_c = 3.0 \text{ms} \).

Calculating delayed synaptic values

Each individual synapse in SNAPPy can (optionally) have a delay associated with it. These delays are calculated as a combination of a constant part and a part that depends linearly on the distance between the pre- and post-synaptic neurons, intended to approximate the axonal conductance delay. Recall that Equation 2.9 calculates the current due to a synapse using \( o(t - d) \) and \( c(t - d) \), where \( d \) is the delay associated with that synapse.

SNAPPy allows past values of the synaptic opening and closing variables (and, in fact, any state variable) to be calculated by maintaining a circular buffer of past state variable values. After each simulation step is completed the current values of all state variables are copied into this buffer if a certain interval of simulation time has passed since the values were last stored. This interval is defined as \( d_{\text{max}}/N \), where \( d_{\text{max}} \) is the longest synaptic delay in the model and \( N \) is the (configurable) size of the history buffer. To calculate the past value of a state variable, SNAPPy finds the two history buffer entries either side of the required time and then linearly interpolates the state variable values from the two entries.
Figure 2.5: The accumulation of synaptic activation and the effects of our pre-synaptic saturation mechanism. The main plot shows the membrane potential of a passive neuron relative to its rest potential. The neuron receives synaptic input from a second neuron, which spikes repetitively (bottom plot) and activates NMDA receptors. Because these NMDA synapses are slow ($\tau_o = 5\text{ms}, \tau_c = 80\text{ms}$), the EPSPs from the spikes accumulate over time (green line). When synaptic saturation is enabled (blue and red lines), the level to which EPSPs can accumulate is limited.

Synaptic saturation

If a model neuron spikes at a rate that is faster than that at which its synaptic activation ($c(t) - o(t)$) decays then the activation will sum over time, potentially to very high levels. At real synapses this summation is limited by various factors, such as neurotransmitter depletion and so we included a simple model for pre-synaptic saturation in SNAPPy (Dayan and Abbott, 2001, pp.184–185). For each synapse type an optional parameter, $\Delta s_{\text{sat}}$ can be specified. If this parameter is given, then the value by which $o(t)$ and $c(t)$ are incremented following a spike is scaled by a factor $\lambda(t)$, where:

$$
\lambda(t) = 1 - \frac{c(t) - o(t)}{\Delta s_{\text{sat}}}
$$

(2.18)

Figure 2.5 shows the activation of a slow NMDA synapse in a repetitively firing neuron, both with and without the synaptic saturation mechanism enabled.
2.1.4 Calculating the RHS of calcium concentration

Many neurons contain channels which allow calcium ions to flow into the cell when the membrane potential becomes relatively high, such as during spiking. This calcium influx can affect the dynamics of calcium gated channels, and it plays an important role in many models, for example in burst generation through low frequency oscillations in calcium concentration (Bertram and Sherman, 2005). SNAPPy can, optionally, track the concentration of intracellular calcium in neurons. When this feature is enabled each neuron of that type has an additional state variable, \([Ca](t)\), with dynamics governed by the following equation, which is based on the model of Terman et al. (2002):

\[
\frac{d[Ca]}{dt} = \epsilon(I_{Ca} - k_{Ca}[Ca]) \tag{2.19}
\]

Here \(\epsilon\) represents calcium buffering (with units \(\text{mole sec}^{-1}\)), and \(k_{Ca}\) is the rate at which calcium is pumped back out of the cell (\(\text{coulomb litre}^{-1}\)). \(I_{Ca}\) is not a parameter: it represents the sum of the currents due to any channels that are permeable to calcium ions (SNAPPy has a way to indicate that a particular channel is a calcium channel).

Another likely role of intracellular calcium is for homeostatic regulation of neuronal excitability (Chan et al., 2011). This mechanism is thought to be based on down-regulation of certain ionic channels in response to elevated firing rates (indicated by calcium influx). Channel down-regulation is currently the only way in which the value \([Ca](t)\) is used in SNAPPy. Our novel implementation of this process is based on adjusting the maximum conductance of certain channels between steps when \([Ca] > T_{dr}\), where \(T_{dr}\) is the calcium concentration threshold above which down-regulation occurs. Any channels which the user has specified should be affected by down-regulation have their maximum conductance parameters adjusted after each step. The amount by which the channels’ conductances are changed takes the form of a sigmoid curve and is given by:

\[
g_c(t + \Delta t) = \max \left[ 0, g_c(t) - \frac{k_c \Delta t}{1 + \exp\left(\frac{\theta - [Ca](t + \Delta t)}{\sigma}\right)} \right] \tag{2.20}
\]

This formula calculates the new maximum conductance of an affected channel at the end of a
simulation step, where $g_c(t)$ was the maximum conductance at the beginning of the step and $\Delta t$ is the size of the step that was taken. $k_c$ is the maximum conductance change that can occur in one step, $\theta$ is the level of intracellular calcium that gives half the maximum change, and $\sigma$ is the slope of the sigmoid.

### 2.1.5 Numerical methods

Once the RHS of all the state variables has been calculated using the methods described above, SNAPPy uses the ODE solver routines from the GNU Scientific Library (Galassi et al., 2009) in order to calculate the new state of the system. Specifically, the explicit Runge-Kutta-Fehlberg routine is used, which performs 4th and 5th order steps in order to efficiently produce an estimate of the error at each step. Absolute and relative error tolerances must be specified by the user in the Simulation Parameters file, and the user can select whether to use a fixed or adaptive step size. If the step size is fixed then SNAPPy will abort the simulation if the estimated error for any step is outside the given bounds. Using an adaptive step size has a hugely positive effect on performance, although care must be taken to select a suitable maximum step size to ensure that spike detection will be accurate. We typically use a maximum step size of 0.5ms.

### 2.1.6 Adding parameter noise

In order to introduce heterogeneity to models, SNAPPy allows random Gaussian noise to be added to many of a model’s parameters at the beginning of a simulation. Noise can be applied to the capacitance, leak conductance, and channel conductance of neurons, as well as the conductance and delays of individual synapses. For each parameter that should have noise applied the user specifies the standard deviation of the Gaussian distribution that parameter values should be drawn from, where the specified parameter value is the distribution mean. SNAPPy uses the gsl_ran_gaussian routine from the GNU Scientific Library (Galassi et al., 2009) to generate random numbers, which is based on the Box-Muller algorithm (Box and Muller, 1958). If the noise added to a particular parameter value would result in a negative value, the parameter is set to zero.
2.1. A general-purpose simulator for single compartment Hodgkin-Huxley models

Figure 2.6: A screenshot of the Python front-end during a simulation. Here SNAPPy has been configured to show four sub-plots: two “traces” type and two “raster” type. The buttons at the bottom left and plot configuration window (both standard tools included with Matplotlib) allow the user to zoom and pan around the diagram, adjust spacing, and save plots.
2.1.7 The graphical interface

The graphical Python front-end to SNAPPy allows the user to view trace graphs and raster plots showing the simulation as it progresses. This functionality is built using the Matplotlib library for Python (Hunter, 2007), which provides an extremely flexible framework for generating charts and graphs. Figure 2.6 shows a screenshot of the graphical front-end, showing trace and raster plots. Thanks to Matplotlib, the window in which plots are shown includes a number of standard buttons, which allow the user to zoom and pan around the plots, and save the current view in a wide range of file formats, such as PNG, PDF and EPS.

When calling the SNAPPy front-end the user must supply a Plot Specification file, which is a JSON file that tells SNAPPy what information to display. Multiple subplots can be included, each of which is either a traces or a raster plot:

- A traces plot plots the value of one or more expressions against time. Any expression is a formula (written in Python) which can make use of the state variables associated with a particular neuron using the $ symbol. For example, a simple expression that will plot the membrane potential of the selected neuron(s) would be $v$. A slightly more complicated expression is one to show the current AMPA synaptic activation of a neuron: $ampa\_c -ampa\_o$. Arbitrary Python statements can be used, allowing practically any mathematical formula. Traces can show expressions evaluated for single neurons or ranges of neurons with a vertical offset between them.

- A raster plot shows the spiking activity of a set of neurons against time. Multiple ranges of neurons can be shown (in different colours for example).

If running a new simulation, the front-end spawns two sub-processes when it starts: one containing the simulation kernel and another Python process which listens for results data coming in from the kernel on a local network socket. When new data is received, this sub-process parses it into suitable Python objects and then transmits it to the main user interface process, using Python’s multiprocessing.Queue class. The main user interface process checks its end of this queue periodically, and when new results objects are received they are passed to the various sub-plots, which may or may not update themselves based on the new data. The code is written
2.2. A GPU-powered tool for exploring dynamical systems

following object-oriented design principles, and should therefore be reasonably easy to extend with new plot types and features.

2.1.8 Conclusions

Despite the availability of existing simulation software, developing our own software was a valuable learning experience and allowed us to ensure that the specific features required by our modelling were available, whilst keeping overall complexity low. SNAPPy makes no attempt to be “all things to all people”, and as a consequence is relatively lightweight and simple to use, with clean and low-maintenance code. That said, in the domain of modelling networks of single compartment Hodgkin-Huxley neurons SNAPPy is quite flexible, and we have used it successfully to model two quite different systems: the rodent basal ganglia and the *Xenopus* tadpole spinal cord. Our focus for the future will be on improving the performance of the software, possibly by porting it to new hardware such as graphical processing units (GPUs) or the SpiNNaker system (Khan et al., 2008). Along these lines, SNAPPy has been adapted by another group to run on dedicated field programmable gate array (FPGA) hardware.

2.2 A GPU-powered tool for exploring dynamical systems

A graphical processing unit (GPU) is a component of all modern computers that is designed for massively parallel computation. Whereas a typical central processing unit (CPU) contains a small number (generally 2–8) of highly flexible execution cores which can all run separate processes independently, a GPU can contain thousands of cores which execute in parallel but which all share the same instructions. The GPU architecture is extremely powerful when the same set of instructions must be carried out on many data items with little communication between processes. This is exactly what is required in the application that GPUs were originally developed for, namely video games; rendering a 3D scene in a computer game involves applying the same set of linear transformations to a huge number of graphical primitives in order to transform from 3D “world” space to 2D “screen” space. Due to the size of the gaming market GPUs are mass produced and are therefore an extremely cheap form of computing power, and for this reason they are becoming increasingly popular for scientific applications. A relevant example of this is the GPU Enhanced
Neural Network Simulator (GeNN)\(^1\), which takes advantage of GPU acceleration in order to simulate spiking neural networks. There are two main libraries available for GPU computing: NVidia’s proprietary Compute Unified Device Architecture (CUDA) and the Open Computing Language, or OpenCL (Kirk and Hwu, 2013).

We developed a piece of software called DSvis that uses the GPU (via the OpenCL library) to allow users to graphically explore the n-dimensional state space of arbitrary ODE systems. This was mostly an experiment to see if such a visualisation is useful in understanding how a system behaves. DSvis uses the GPU to simulate a large number (typically hundreds of thousands) of trajectories through state space simultaneously. The current positions of each trajectory is displayed as a small coloured particle according to either a 2D or 3D projection. In the case of the 3D projection, the user can “fly around” the state space of their system using the mouse and keyboard, similar to controlling a computer game. The parameters of the system can be modified in real time with a set of sliders, allowing the user to observe in real time the effect that parameter variation has on the motion of the particles.

Figure 2.7 shows a screenshot of the DSvis window. Typically a simulation is first configured using the “Define” tab, after which the “Interact” tab is used to control the simulation in real time. In order to set up a DSvis simulation, the user must specify the following objects:

1. The state variables and their time derivatives (right hand sides), as well as a set of bounds for each variable. The right hand side is entered as a standard “C” language expression, where placeholders can be used to represent state variables and parameters.

2. The parameters of the system, along with their default, minimum and maximum values.

3. A set of one or more “render techniques”. A render technique specifies how the position of a trajectory is shown on the screen, and includes whether the projection should be 2D or 3D, the colour to render particles in (can be “auto” if the particles should be coloured according to their position), and the state variable which corresponds to the first co-ordinate of the project. A current technical limitation is that particle positions in space must correspond to two or three (depending on the projection) contiguous state variables.

\(^1\)http://sourceforge.net/projects/genn/
2.2. A GPU-powered tool for exploring dynamical systems

Figure 2.7: Screenshot of the DSvis window while exploring the strange attractor found in the Lorenz equations. The screenshot shows the viewport where the system is visualised (A), the tab in which the user defines the system (B) and the tab that can be used to adjust simulation settings in real time (C).
4. One or more “particle groups”. Each particle group contains a user-defined number of particles that can be simulated either forwards or backwards in time. The user specifies which render technique is used to render each particle group. This allows one group of particles to be simulated forwards in time and shown in one colour and another group of particles to be simulated backwards and shown in a different colour.

Once the simulation has been configured (or any time the simulation is reset), DSvis performs a number of initialisation tasks to set up the simulation on the GPU. Once this is complete, the main update/render cycle begins. The following list describes briefly what is involved in the initialisation, update and render phases.

- The **Initialisation** phase involves two tasks: compiling the executable kernel that will run on the GPU during the **Update** phase, and setting up a buffer in GPU memory that contains the state of all the particles. Kernel code is generated automatically by combining the user-defined state variables and parameters with a “template” kernel included with DSvis. This code is then compiled by OpenCL and copied into the GPU’s instruction memory. The buffer is allocated in GPU memory according to the dimensionality of the system and the number of particles. Initially the state of each particle in the buffer is set randomly, subject to the constraints of the state variables.

- The **Update** phase involves executing the OpenCL kernel on the GPU. The template kernel included with DSvis contains a 4th order Runge-Kutta method with a fixed step size that is specified by the user. The GPU is instructed to execute the kernel on every particle in the state buffer, and it intelligently splits the workload up according to its number of cores.

- The **Render** phase involves passing each particle group through a set of programmable “shaders” on the GPU in order to draw the particles onto the screen. Briefly:

  - The *vertex shader* applies a set of linear transformations to convert the particles’ positions in state space into positions relative to the camera. In the case of a 2D projection this is relatively straightforward, but for a 3D projection it involves translation and several rotations.
2.2. A GPU-powered tool for exploring dynamical systems

- The geometry shader converts the vertex corresponding to a particle’s position into four vertices arranged in a square, representing the corners of an image of an individual particle that will be rendered to the screen. For 3D projection another set of linear transformations are applied which convert from 3D positions relative to the camera to 2D screen positions.

- The fragment shader actually draws each particle square to the screen. If multiple particles are drawn to the same location on the screen the pixel colours sum, leading to a saturation effect.

Figure 2.8 shows DSvis being used to explore the dynamics of a model neuron based on the Hindmarsh-Rose equations (Hindmarsh and Rose, 1984) with the parameters set so that the model exhibits periodic bursting. The concentric solid bands represent the active bursting phase of the system’s stable attractor, which all particles will eventually tend towards. The thin narrow band which extends off of the bottom of the figure is the inter-burst interval; particles move very slowly along this part before returning to the start of the bursting region. DSvis allows the user to move around this structure and adjust the model’s parameters in real-time. Figure 4.8 in Chapter 4 shows a 2D projection in DSvis with two sets of particles: one moving forwards in time and one moving backwards. These two groups reveal the stable and unstable attractors of the system respectively.

DSvis is written in Python using the PySide\(^2\) library for the user interface, the PyOpenCL\(^3\) and PyOpenGL\(^4\) libraries for interfacing with the GPU, and the Mako\(^5\) library for generating OpenCL code from a template. The software is an ongoing experiment to see if this way of exploring dynamical systems is useful for people who are trying to understand them. So far it has been useful for teaching and for understanding the 2D system in Chapter 4, but it probably requires some additional features such as axis lines and improved movement before it will be useful for exploring higher dimensional systems.

\(^2\)http://qt-project.org/wiki/pyside
\(^3\)http://mathema.tician.de/software/pyopencl/
\(^4\)http://pyopengl.sourceforge.net/
\(^5\)http://www.makotemplates.org/
Figure 2.8: DSvis being used to explore the Hindmarsh-Rose reduced neuron model in the periodic bursting regime.
Chapter 3

Investigating the Possibility of Cortex-Driven Oscillations in the Globus Pallidus in a Spiking Model

3.1 Parkinsonian basal ganglia oscillations may be generated in the cortex

One possible explanation for exaggerated basal ganglia $\beta$ oscillations in Parkinson’s disease is that dopamine acts to modulate the effects of rhythmic cortical activity on cortical-basal ganglia pathways, such that in conditions of reduced dopamine this network becomes pathologically entrained to cortical rhythms. Evidence for this comes from studies that have used signal processing techniques to attempt determine whether $\beta$ band coherence between the cortex and basal ganglia is directed from cortex to STN or vice versa. Such studies have shown that, in patients with Parkinson’s disease (Litvak et al., 2011) or Parkinsonian rodents (Sharott et al., 2005), the oscillations arise
in the cortex and drive STN activity. Computational studies that investigate the synchronisation of basal ganglia neurons in Parkinson’s disease often consider the neurons to be phase oscillators, which either synchronise themselves (Popovych and Tass, 2012) or become synchronised through common external inputs (Wilson et al., 2011).

Experiments using rodent models of Parkinson’s disease provide compelling evidence that under Parkinsonian conditions the activity of neurons in the GP are much more susceptible to entrainment by cortical rhythms than in the healthy case. Under conditions of urethane anaesthesia, neurons in the GP of healthy rodents show uncorrelated tonic firing. However, in animals where Parkinsonism has been induced, either through chronic lesioning of the SNc with 6-OHDA (Ni et al., 2000; Magill et al., 2001) or acute inactivation of SNC projection fibres (Galati et al., 2009), the spiking activity of the majority of GP neurons becomes significantly correlated with cortical “slow wave activity” (SWA); this is the major cortical rhythm in the anaesthetised state and has a frequency of approximately 1Hz. These experiments also reveal that, in the chronic lesioned case at least, the neurons in GP are split into two major groups, distinguished by whether they preferentially fire during the active phase (ECoG peaks) or inactive phase (ECoG troughs) of SWA in dopamine-deprived conditions. These will be referred to as the TA and TI groups respectively. The underlying basis for this division is unknown, but the same division is seen in respect to cortico-pallidal synchronisation that occurs transiently at β frequencies in response to sensory stimulation in OHDA lesioned rodents (Mallet et al., 2008a), which suggests that the same mechanism may be responsible for pathological entrainment in both behavioural states / frequency bands. If this is the case, then understanding this mechanism may lead to improved treatments for Parkinson’s disease. Unfortunately we do not currently know the route through which oscillatory cortical input entrains the GP, although it is likely to involve the two major sources of synaptic input to GP neurons: the inhibitory medium spiny projection neurons of the striatum and excitatory STN neurons. Both receive widespread cortical inputs and both show increased firing during the peaks of SWA under Parkinsonian conditions in rodents (Tseng et al., 2001; Magill et al., 2001). Given that the majority of GP neurons belong to the TI group it has been suggested that cortical oscillations are most effectively relayed via the inhibitory striatum (Walters et al., 2007), but this view is challenged by the fact that the entrainment of GP neurons to SWA appears to be critically dependent on a functioning STN (Ni et al., 2000; Galati et al., 2009).
3.2 Model description

Figure 3.1 shows a simple representation of the neural network model which includes a population of 100 interconnected GP neurons (right panel, blue) which receive excitatory synaptic input from 50 STN neurons (left panel, red). Each GP neuron is described by a detailed one compartmental conductance based model of the Hodgkin-Huxley type with inhibitory connections from other GP
neurons. The STN neurons are described by a simple enhanced leaky integrate-and-fire model. Neurons in the STN population are not connected to each other but they are modulated by a common cortical slow-wave rhythm and make excitatory synapses onto GP neurons.

We implemented the model using the SNAPPy software described in Section 2.1. The absolute and relative error tolerances were $10^{-5}$ and the maximum step size was $1ms$ for all simulations. To analyse the results we used scripts written in Python with routines from the NumPy and SciPy (Jones et al., 2001) libraries. For each set of parameters twelve simulations were performed, with different random neural connectivity, STN spike trains and parameter noise in each simulation.

3.2.1 Model GP neurons

The model GP neurons are of Hodgkin-Huxley type, with a single compartment per neuron. We included ten voltage-gated ionic channels as in the multicompartmental modelling work of Güney et al. (2008): fast and slow delayed rectifying $K^+$ (Kv3 and Kv2 respectively), fast and slow A-type $K^+$ ($K_{V4_{fast}}$, $K_{V4_{slow}}$), M-type $K^+$ ($K_{CNQ}$), fast spike-producing $Na^+$ ($Na_{F}$), persistent pacemaking $Na^+$ ($Na_{P}$), hyper-polarisation activated $Ca^{2+}$ ($HVA$), and fast and slow mixed-conductance hyperpolarization-activated channels ($HCN_{fast}$, $HCN_{slow}$). For simplicity our model does not include calcium-gated potassium “SK” channels, as these channels’ most significant effect on the activity of GP neurons appears to be a lengthening of spike after-hyperpolarization (AHP) (Deister et al., 2009), and we were able to achieve physiologically realistic AHPs without this channel.

The model was implemented using the SNAPPy software described in Section 2.1. Values for the passive membrane properties are given in Table 3.1. The parameters governing the dynamics of each gate vary from channel to channel. Since we use the same equations and parameters for channel gates as Güney et al. (2008) we do not reproduce these here and instead refer to the supplementary material of that paper where they are listed.

We could not directly use the channel maximum conductance parameters from Güney et al. (2008), since this was a multicompartmental model and the conductances varied widely between the different compartments. Instead we adjusted the channel conductances so that our model neurons exhibited intrinsic pacemaking and displayed realistic responses to depolarizing and hyperpolarising current injections. The chosen conductances are shown in Table 3.1. In order to generate a range
3.2. Model description

Table 3.1: Passive membrane parameters and channel conductances for the model GP neurons.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>141.6</td>
<td>pF</td>
</tr>
<tr>
<td>$g_{lk}$</td>
<td>4.012</td>
<td>nS</td>
</tr>
<tr>
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<tr>
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<td>nS</td>
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<tr>
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<tr>
<td>$E_{hcn}$</td>
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Table 3.1: Passive membrane parameters and channel conductances for the model GP neurons.

of intrinsic pacemaking frequencies we applied Gaussian noise to the maximum conductances of the persistent sodium and HCN channels ($g_{nap}$, $g_{hcnf}$ and $g_{hcns}$). The mean values are given in Table 3.1 and the standard deviation was 50% for NaP and 30% for both HCN channels.

It has been proposed (Chan et al., 2011) that a homeostatic mechanism may reduce the intrinsic firing rate of GP neurons via downregulation of HCN channels in response to burst firing. Our model includes a possible mechanism for this downregulation by allowing the maximum conductance of HCN channels to decrease. This occurs during periods of elevated firing rate, which are indicated by high intracellular calcium concentration ([Ca]). Section 2.1.4 described how the dynamics of intracellular calcium operate and how channel conductances are modified after each time-step in response to calcium influx. For the parameters of the calcium concentration equation (2.19) we used the values from Rubin and Terman (2004): $10^{-4}$ mole sec coulomb litre for calcium buffering ($\epsilon$), and $15.0$ coulomb litre mole sec for the calcium pump rate ($k_{Ca}$). The only ionic channel that contributes to the calcium dynamics is that from the HVA channels, since these are the only Ca$^+$ channels in the model.

We do not have any data from biological experiments to suggest values for the parameters of the equation that determines how HCN channel conductances are modified after each time step (2.20). Since we hypothesise that downregulation mostly only occurs during fast burst firing under Parkinsonian conditions, we chose parameters such that, in healthy conditions, downregulation only occurs in the very fastest firing GP neurons. For the maximum conductance change that can
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occur in one step \( (k_c) \) we used a value of \( 6 \times 10^{-4} \), for the level of intracellular calcium that gives half the maximum change \( (\theta) \) we used 0.5 and for the slope of the sigmoid \( (\sigma) \) we used 0.1. The concentration threshold at which downregulation occurred \( (T_{dr}) \) was set at 0.2.

Note that our model GP neurons are supposed to represent those in the rodent GP (usually considered to be equivalent to the primate GPe).

3.2.2 Model STN neurons

Since the aim of this study is to investigate the effects of rhythmic STN input upon the neurons of the GP, we did not model STN neurons to the same level of biological detail as GP neurons. Instead, to simulate the SWA-modulated bursting of STN neurons that occurs under urethane anaesthesia we use an enhanced integrate-and-fire generator of neural activity, as described in Borisuyk (2002). The STN neurons include an exponentially decaying threshold, accumulation of membrane potential, stochastic noise and an absolute refractory period. Aside from approximating the SWA modulation of STN activity our model does not include any synaptic inputs to the STN. In particular we do not include the GP-STN projection because experiments in urethane-anaesthetised rats have shown that the changes in firing rate and pattern that occur in the STN following OHDA lesion are not dependent on synaptic input from the GP (Hassani et al., 1996).

During urethane anaesthesia STN neurons display uncorrelated bursting activity that is modulated by a slow rhythm (Magill et al., 2001) which here we assume arises from excitatory cortical inputs. Since firing is uncorrelated, each STN spike train is generated independently using a procedure that results in spiking activity that is similar to the spiking activity of real neurons. The generated spike trains contain activity that is oscillatory with a period of 1300ms \( (\approx 0.8Hz) \), where each cycle is made up of an 800ms “inactive” phase and a 500ms “active” phase. The spike trains are constructed by alternately sampling from two intermediate spike trains, one with slow irregular firing and one with fast irregular firing (for the inactive and active periods respectively). The average firing rates are 0.5Hz for the inactive period and 30Hz for the active period; under simulated Parkinsonian conditions the firing frequency during the active phase is increased to 60Hz. Figure 3.2 shows cross-correlations between two STN spike trains which demonstrate that within the active bursting period activity is not correlated (A), but that there is strong common
3.2. Model description

modulation at 0.8Hz (B). Figure 3.2C and D are raster plots of the generated spiking activity of 50 STN neurons in healthy and Parkinsonian conditions respectively, demonstrating a clear increase in firing frequency during the active phase in the Parkinsonian case.

3.2.3 Synaptic parameters and connectivity

As described in Sections 2.1.1 and 2.1.3, synapses in SNAPPy are defined by five parameters: \( E_{rev} \) (reversal potential), \( \tau_c \) and \( \tau_o \) (closing and opening time constants), \( \Delta s \) (post-spike step increment), and \( g \) (maximum unitary conductance). For convenience, here we let \( g = g_{SG} \) for STN-GP synapses and \( g = g_{GG} \) for intra-GP synapses. Random Gaussian noise was added to the conductance values of all synapses with a standard deviation of 30% of the mean. We set \( \Delta s = 1 \) and did not use the synaptic delay and saturation features of SNAPPy.

For the intra-GP inhibitory synapses we used synaptic time constants \( \tau_o = 5ms \) and \( \tau_c = 40ms \) from unpublished current-clamp recordings of GP-GP IPSPs taken from slices containing rat GP, cortex and striatum [Alon Korngreen, personal communication]. Similarly, we chose \( g_{GG} = 0.5nS \) which gives a peak IPSP of 0.5mV (measured as deflection away from a holding potential of \(-65mV\)) to match the same experimental recordings. We used a standard GABA reversal potential of \(-80mV\). Connectivity between GP neurons was random: each neuron made inhibitory connections onto 20 other, randomly selected, GP neurons (no self-connections).

Anatomical data regarding the structure of the STN-GP projection is currently lacking. However, it is clear that there are many fewer STN neurons than GP neurons and that each GP neuron only samples the activity of a small proportion of STN neurons (Jaeger and Kita, 2011). We therefore arbitrarily chose to model 50 STN neurons, each of which makes excitatory synapses onto two randomly selected GP neurons. The time constants of STN-GP synapses in the model are \( \tau_o = 0.2ms \) and \( \tau_c = 60ms \), based on the recordings shown in Loucif et al. (2005). The average maximum synaptic conductance \( (g_{SG}) \) used for the healthy case was chosen to be just low enough such that the majority of GP neurons didn’t show significant entrainment to the SWA rhythm and we investigated the effects of increasing the value in the Parkinsonian case.
Figure 3.2: Properties and spiking activity of STN neurons. A: Example cross-correlation between two STN neuron spike trains on a short time window (30 bins, 3ms each) shows independent firing. B: Longer time window reveals slow (1300ms) oscillations (400 bins, 20ms each). C: Spiking activity of STN neurons under healthy conditions. D: Increased intensity of active-phase firing under Parkinsonian conditions. In C and D the background is shaded to show the active (pink) and inactive (blue) phases of the SWA cycles. A and B are normalised using the procedure described in Brillinger (1976): if $X_i$ is the unscaled spike count in histogram bin $i$ then the scaled value $X'_i$ is given by: $X'_i = \frac{TX}{2hN_A N_B}$, where $T$ is the total simulation time, $h$ is half the width of a cross-correlation bin and $N_A$ and $N_B$ are the total spike counts for each spike train. A normalised value of 1 indicates that there is no correlation at a particular delay, while deviations from 1 indicate positive or negative correlations. The horizontal bars show the 95% confidence interval for significant correlations (Brillinger, 1976).
3.2.4 Modelling of Parkinsonism

We simulate the OHDA lesioned (Parkinsonian) rat basal ganglia by making three changes to the model’s parameters: i) faster STN firing during the active phase of SWA (Figure 3.2D) ii) increased STN-GP synapse strength and iii) increased intra-GP inhibition strength. Although the changes that occur to functional connectivity in the basal ganglia in Parkinson’s disease are currently under active investigation, there is experimental support for facilitation of both GP-GP (Johnson and Napier, 1997) and STN-GP (Johnson and Napier, 1997; Hernández et al., 2006) synapses. Similarly, under urethane anaesthesia it has been shown that spiking in the STN continues to be modulated by the cortical SWA rhythm, but that its firing becomes more intense during the active period (Magill et al., 2001; Galati et al., 2009).

3.2.5 Categorisation of neurons based on spiking activity

We used a simple method to categorise GP neurons as being of type TA (in-phase with active SWA), TI (in-phase with inactive SWA), NM (not modulated by SWA) or QU (quiet). Each spike fired by the neuron to be categorised is represented by a complex number that indicates its phase in relation to SWA. The sum of these complex numbers then gives an indication of the average phase, $\omega$, as shown in Eq. 3.1.

$$\omega_k = \sum_{s \in S_k} e^{i\theta(s)}$$

(3.1)

Here $S_k$ is the set of spike times for neuron $k$ ($0 \leq k < 100$) and $\theta(s)$ is the phase of SWA at time $s$ ($0 \leq \theta(s) < 2\pi$). The argument of the complex number $\omega_k$ indicates the average SWA phase at which neuron $k$ fires, while its modulus provides an indication of how strongly SWA-modulated the firing is. Normalizing the modulus by the number of spikes gives a confidence measure $c_k = \frac{|\omega_k|}{|S_k|}$, where $c_k = 0$ indicates that spikes did not fire preferentially at any one phase and $c_k = 1$ indicates that every spike occurred at exactly the same phase. After visual inspection of spike trains, we decided to categorise neurons with confidence $c < 0.1$ as NM. We categorised neurons with $c \geq 0.1$ as either TA or TI based on whether the average phase was during the active or inactive part of the SWA cycle. Neurons that fired fewer than one spike every SWA cycle on
Figure 3.3: Responses of a typical isolated model GP neuron to different current injections. A: Neuron with normal HCN channel density. Depolarizing current causes fast, regular spiking (green trace), while hyperpolarising current reveals a sag in membrane potential and a fast return to pacemaker firing following its removal (red, cyan and purple traces). With no current injection the neuron fires regularly at approximately 22Hz (blue trace). B: Neuron with HCN channels removed. Sag in membrane potential is lost and pacemaking is slowed. Note the difference in scale for the injection currents between A and B.

average were categorised as QU.

3.3 Results

3.3.1 Model GP neurons behave realistically under healthy conditions

The characteristics of the model GP neurons qualitatively match those of real rodent GP neurons in a number of key ways and are illustrated in Figure 3.3A. When no synaptic or injected currents are present (dark blue trace in Figure 3.3A), most model neurons (96%; 481/500) pacemake at a range of frequencies (23.6 ± 4.0Hz). Depolarizing current injections increase the frequency of firing (green trace), with very high frequencies possible (up to approximately 200Hz). Hyperpolarizing current injections result in a prominent and transient “sag” in membrane potential (red, cyan and pink traces). The first spike after hyperpolarizing current is removed occurs after a similar delay regardless of the size of the injected current. These properties match those seen in experiments with slices of rodent GP (Chan et al., 2004; Bugaysen et al., 2010).

The mixed-conductance HCN channels play an important role in the activity of the model GP neurons and their response to hyperpolarizing input. The combination of a reasonably depolarized
reversal potential (−30mV) (Lüthi and McCormick, 1998) and activation at hyperpolarized membrane potentials (lower than −60mV) means that these channels act to return neurons to spiking threshold faster after hyperpolarizing current (or inhibitory synaptic input) is removed. Figure 3.3B shows how the simulated blockade of HCN channels affects the activity of a model GP neuron. When HCN channels are removed ($g_{hcnf,s} = 0$), the average pacemaking frequency decreases to 15.8 ± 2.5Hz and 12% (58/500) of neurons do not pacemake. Without HCN channels the membrane potential sag is no longer seen, and hyperpolarizing current has a much stronger effect on membrane potential. The time between the removal of hyperpolarizing current and the return of spiking is also much longer, and much more sensitive to the hyperpolarization level. These results agree with previous work that has investigated the role of HCN channels using mouse GP slices and multicompartmental simulations (Chan et al., 2004).

3.3.2 Network activity in healthy conditions

Whilst we were able to base STN firing rate and the conductance of GP-GP synapses directly on experimental evidence, we could only do this indirectly with the STN-GP synaptic conductance ($g_{SG}$). We chose a value of 0.1nS for this parameter in the healthy case as this gives similar proportions of neurons in the TI, TA and NM groups as seen in experiments. Figure 3.4A shows this distribution (c.f. Fig 2A in Mallet et al. (2008b)) and Figure 3.4B shows the spiking activity of the TI, TA and NM neurons in one trial. A small proportion of neurons (9.9% ± 2.1) are categorised as QU because they fire spikes rarely or not at all; we are not sure if this is a biologically accurate result as such neurons may have been excluded from the results of electrophysiological studies. The majority of neurons (68.3% ± 3.9) are categorised as NM and neurons in this group spike with an average firing rate of 12.3 ± 3.3Hz and coefficient of variation (CV) of 0.12 ± 0.04. These statistics are in good agreement with those of neurons recorded from mice GP slices by Chan et al. (2004) (firing rate 12.5, CV 0.18). However, in contrast to these experimental results, which found no effect on firing rate or CV after blocking GABA_A receptors, we would expect the average firing rate of the neurons in our model to increase slightly with inhibition blocked, as the average firing rate in the network is lower than the average pacemaking frequency of isolated model neurons. The average firing frequencies in the (small) TI and TA groups were 3.7 ± 1.8Hz and 10.7 ± 4.7Hz.
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Figure 3.4: Categorisation and activity of neurons under healthy conditions. A: The average number of GP neurons in each category across all trials (n = 12), showing that most cells do not display prominent modulation by SWA (error bars show standard deviation). B: Raster plot showing the spiking activity of TI, TA and NM neurons in one representative trial. The spike trains above the solid grey line are from neurons whose average spike phase is in the active part of the SWA cycle (light pink shaded background), whilst those below the line have average phases in the inactive part of SWA (light blue shaded background). The spike trains are ordered such that those closer to the solid grey line have lower confidence measures than those further away. The dashed grey lines show the confidence measure boundaries that divide neurons classified as NM from those classified as TI or TA (this boundary is set at 0.1).
3.3. Results

respectively.

3.3.3 Network activity in Parkinsonian conditions

The effects of dopamine lesion were simulated in the model by an increased intensity of STN firing and increased strength of STN-GP excitation and intra-GP inhibition. These changes have a profound effect on activity in the model GP that is similar to what is seen in experiments. As Figure 3.5 shows, most neurons begin to preferentially fire during either the active or inactive phase of the SWA. In order to see proportions of TA and TI neurons that were similar to in vivo results it was necessary to double the strength of STN-GP and GP-GP synapses ($g_{SG} = 0.2nS$, $g_{GG} = 1.0ns$).

The average firing frequency of NM neurons decreased slightly under Parkinsonian conditions while the average firing rates of the TI and TA groups increased to $6.3 \pm 3.6Hz$ and $11.1 \pm 5.1Hz$ respectively (Figure 3.6A). Although the variance of these statistics is fairly large, there does appear to be a trend for different firing rates between the different groups that is not seen in vivo (Magill et al., 2001). This difference is perhaps not too surprising given our simplistic and somewhat arbitrary choices for STN-GP and GP-GP connectivity.

In order to investigate the factors that determine whether a neuron becomes TA, TI, NM or QU we examined the following statistics of each neuron: maximum conductance of the NaP persistent sodium channel; initial (before downregulation) maximum conductance of fast and slow HCN channels; total maximum conductance of all excitatory (AMPA) synapses from STN neurons; total maximum conductance of all inhibitory synapses from other GP neurons. In general these statistics were remarkably similar between each of the groups, with two exceptions. Firstly, quiet (QU) neurons have, on average, much lower maximum conductances for their NaP channel (Figure 3.6B). These channels underlie autonomous pacemaking (Mercer et al., 2007) and the intrinsic pacemaking frequency is strongly dependent on the value of the NaP maximum conductance. Since QU neurons have low NaP conductance they are likely to pacemake very slowly or not at all, and it appears (from examination of voltage traces) that the incoming inhibition from other GP neurons is sufficient to prevent them from ever firing. Secondly, TA neurons receive on average more excitatory synaptic input from the STN than the other groups (Figure 3.6C). This result was
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Figure 3.5: Categorisation and activity of neurons under Parkinsonian conditions. A: The average number of GP neurons in each category across all trials ($n = 12$), showing that most neurons start to display SWA-modulated firing patterns, either in-phase (TA) or anti-phase (TI). B: Raster plot of Parkinsonian GP neuron activity (description as in Figure 3.4B).
3.3. Results

Figure 3.6: Average cell properties by categorisation across all GP neurons from all trials ($n = 1200$). A: Average firing rate is rather variable but is in general slightly lower for TI neurons. B: Maximum conductance of the persistent sodium channel (NaP) which underlies pacemaking. Quiet neurons can be easily categorised as those with very low NaP conductance. C: Average total maximum conductance due to excitatory synapses. TA neurons receive more excitation on average, but it is highly variable.
expected, since STN firing occurs during the active phase of SWA and so it is not surprising that those GP neurons that receive more STN input also fire preferentially during the active phase. In general, however, the simple statistics we examined about membrane properties and synaptic connectivity are not enough to determine which group a particular neuron will fall into. Predicting the classification of a neuron involves knowing the classification of the other GP neurons that it receives inhibition from. This makes the problem complex and extremely difficult to resolve a priori. Instead, when the network is simulated, a dynamic process takes place in which the network self-organises its activity into the different groups of neurons.

HCN channel downregulation plays a significant, but not essential, role in the emergence of the different groups of neurons in our model. Without this mechanism, many neurons in the TA group continue to fire during the inactive phase due to their intrinsic pacemaker properties. Although this inactive-phase firing is slower than their active-phase firing (due to reduced excitatory input), it is still a source of inhibitory input to other GP neurons and may silence or slow the firing of some which may otherwise be categorised as TI. With the HCN downregulation mechanism those neurons that receive the most excitatory STN input, and therefore fire at the fastest rate during the active period, will have their maximum HCN conductances reduced. This reduction has the effect of decreasing the intrinsic pacemaking frequency and increasing the hyperpolarization that occurs in response to inhibitory input. To quantify the effect on pacemaking frequency, we ran four simulations using the Parkinsonian parameter values for a period of time long enough for downregulation to take effect (6.5s) and then removed all synapses and recorded firing rates. The average pacemaker frequency after downregulation was $16.9 \pm 2.1 \text{Hz}$, a clear reduction from the normal pacemaker frequency of our model neurons ($23.6 \pm 4.0 \text{Hz}$). The proportion of QU neurons after downregulation was 2%, lower than the 4% that we would expect based on the normal pacemaker properties, but we attribute this to statistical noise due to the rather small sample sizes.

These changes to pacemaking affect the competition dynamics during the inactive phase and mean that most TA neurons are no longer able to fire at all during this phase. Although the proportion of neurons classified as either TA or TI is similar with or without HCN downregulation ($72.7\% \pm 5.4$ normally, $68.7\% \pm 5.0$ without downregulation), TA neuron firing is much more clearly restricted to the active phase with downregulation. This is seen in the average confidence measure
3.3. Results

(\(\omega\)) of TA neurons, which is \(0.44 \pm 0.22\) with HCN downregulation and \(0.32 \pm 0.18\) without. The effect is also shown by phase diagrams showing the spiking activity of typical neurons (Figure 3.7). In these plots the background is shaded to show the active (pink) and inactive (blue) parts of the SWA cycle, showing that TI neurons fire preferentially in the inactive part and TA neurons fire preferentially in the active part. The red bars show the average spike phase and their length indicates the confidence measure as a proportion of the total radius.

3.3.4 Larger Networks

The results described above were from simulations with 100 GP neurons, each of which made 20 inhibitory synapses onto 20 other (randomly chosen) GP neurons. This level of coupling (\(\approx 20\%\)) is probably much higher than what is seen in the real GP (Sadek et al., 2007). We ran several simulations, using Parkinsonian parameter settings, where the coupling proportion was decreased by scaling up the number of GP and STN neurons but keeping the number of synapses that each neuron made constant. In the first set of simulations we used 200 GP neurons and 100 STN neurons and in the second set we used 300 GP neurons and 150 STN. These give GP-GP coupling levels of \(20/199 \approx 10\%\) and \(20/299 \approx 7\%\) respectively. In each case we ran three simulations. For the simulations with 200 GP neurons there were an average of \(71 \pm 2.8\) TI neurons and \(73.7 \pm 1.7\) TA neurons. For the simulations with 300 GP neurons there were an average of \(122 \pm 4.1\) TI neurons and \(101.3 \pm 2.5\) TA neurons. These proportions (particularly in the latter case) are similar to the proportions in the smaller network (see Figure 3.5).

3.3.5 Other Frequency Bands

We briefly investigated to see if the activity of GP neurons could become entrained to higher frequency cortical rhythms, specifically those in the \(\beta\) band. To do this we generated STN spike trains that were modulated at approximately 14Hz (70ms period: 40ms inactive phase, 30ms active phase). Although biological experiments on OHDA lesioned rodents find that most neurons fall under the same TI or TA category regardless of whether the cortical rhythm is SWA or \(\beta\), it was difficult and not effective to use our normal method to categorize neurons because the number of spikes fired by GP neurons in each \(\beta\) cycle was very low. However, examining spike
Figure 3.7: Phase histograms showing the distribution of spikes relative to SWA phase grouped by categorisation (top: TI, middle: TA, bottom: NM), demonstrating the effects of HCN down-regulation. The SWA cycle was divided into 20 bins (65ms bin width) and the spikes’ phases within cycles were used to construct polar histograms. Numbers indicate the number of spikes in each bin. Data are shown from two sets of 12 simulations, one with the HCN downregulation mechanism enabled (left) and one with it disabled (right). The background of each diagram is shaded to illustrate the active (pink, 500ms) and inactive (blue, 800ms) parts of the SWA cycle. Downregulation reduces TA neuron firing during the inactive phase (middle), increases TI firing during the inactive phase (top) and decreases NM firing (bottom). Spikes from the latter half of simulations (6.5s out of 13s) from all neurons (except those categorised as QU) were used to generate the diagrams. The orientation of the red bars shows the average spike phase and their length shows the phase confidence measure ($\omega$) as a proportion of the total radius.
cross-correlations between STN and GP neurons showed that the majority of GP neurons did show oscillatory firing that was in-phase with the STN input (Figure 3.8A). We examined autocorrelations for individual GP neurons and found that the frequency of these neurons’ oscillations varied somewhat from neuron to neuron, which suggests that their firing becomes synchronized to some intermediate frequency between the 14Hz input and their intrinsic pacemaking frequency. We did not see any GP neurons that showed anti-phase oscillations when using our standard Parkinsonian parameters. However, when the degree of intra-GP inhibition is dramatically increased (40% coupling, $g_{GG} = 3.0mS$) then a few neurons do begin to show a preference for anti-phase firing (Figure 3.8B), albeit at a very low rate. It is possible that with different synaptic parameters or connection topology (for example, STN input that preferentially makes contact with a particular group of GP neurons), the synchronized activity of one group could cause a second group to become synchronized in anti-phase.
3.4 Discussion

Our results demonstrate a mechanism whereby local inhibitory connections allow two anti-phase oscillatory subpopulations of GP neurons to emerge in response to rhythmic excitatory input from the STN. The two subpopulations appear due to a complex self-organization process and despite the homogeneity of the overall population. This effect is only seen when both the STN input and inhibitory GP-GP coupling are sufficiently strong, and there is good experimental evidence that both STN input to the GP and intra-GP coupling increase in rodent models of Parkinson’s disease. We therefore claim that our model shows a plausible mechanism for those experimental results which show a prominent increase in the number of TA and TI neurons that occurs in the rodent GP after dopamine lesioning (Magill et al., 2001). In our model, HCN channel downregulation makes oscillatory entrainment of the in-phase group of neurons more prominent but is not essential for the two groups to appear. This may explain the result of Chan et al. (2011) whereby artificial up-regulation of HCN channels via viral transfection restored the cells’ ability to autonomously pacemake but did not give any significant improvement to Parkinsonian motor impairment. The fact that we did not see an increase in the number of neurons that were unable to autonomously pacemake following simulations of the Parkinsonian network may indicate that we didn’t set the threshold for HCN downregulation low enough.

The main source of synaptic input to the GP is the inhibitory medium spiny neurons of the striatum, which we did not include in our model. Galati et al. (2009) demonstrated the delivery of a GABAA antagonist into the GP also causes cortical entrainment of the neurons there and this effect is dependent on a functioning STN. They suggest that this demonstrates that inhibitory striatal input is also involved in oscillatory entrainment. This result is more difficult to explain in our model, since it is unlikely that decreasing the level of GP-GP inhibition would cause oscillations to appear in the (otherwise) healthy case. However, if the effect of GABA antagonism is to remove tonic background inhibition (probably from the striatum) then we could include this in our model as a depolarizing current injection to all GP neurons. This would move their membrane potentials closer to the spike threshold and make them more sensitive to the (weak) rhythmic STN input that is present in the healthy case.
3.4. Discussion

3.4.1 Relationship to previous studies on coupled oscillators.

Networks of coupled oscillators are found in many areas of science and the dynamics of such networks have therefore been widely studied from a theoretical standpoint. Our model can be thought of as a network of inhibitory coupled oscillators that receive random, sparse excitatory input with a particular global frequency. Although many theoretical studies of similar systems use reduced models, they may still provide insights into the different dynamical behaviours that our model is likely to exhibit.

Most previous theoretical studies do not include common external input to the coupled oscillators, although some may consider the effects of input that is constant in time. Chow (1998) describes the analysis of a neuronal network that consists of a number of oscillators with heterogeneous spiking frequencies that are all-to-all coupled by inhibitory connections. Such networks are capable of producing a range of dynamics, including almost-synchronous phase-locking, harmonic locking, and suppression. The stability of these states strongly depends on the details of the neurons’ response to synaptic input. This network is similar to our model in the case where STN input is made constant in time, although inhibitory coupling in the GP network is random and relatively sparse rather than all-to-all. Since SWA oscillations are much slower than the GP neurons’ intrinsic pacemaking, we can consider the GP network during the active and inactive phases separately, with constant STN input within each phase. Using the terminology of Chow (1998), during the active phase of SWA (high STN input) the TI neurons are in the suppressed state while in the inactive phase the TA neurons are suppressed. We have not observed synchrony between neurons during active or inactive phases, although it’s possible that the system would converge to these states after a long period of time with constant input.

As the frequency of the cortical modulation is increased to be closer to the GP neurons’ pacemaker frequencies (e.g. into the β band) it no longer makes sense to consider the scenario of constant STN input. In this scenario, theoretical results from oscillator models may be useful for suggesting conditions that support the emergence of in-phase and anti-phase groups. Golomb et al. (1992) describe a network of phase oscillators that all receive common global input that is a function of the phases of the oscillators. This is not explicitly the case in our model, but nevertheless their findings regarding the stability of solutions with clustered phase distributions may be rele-
vant. In particular they show that the fewer clusters a state has, the more stable it is (larger basin of attraction). This could explain why the GP network under $\beta$ modulation organises into just two anti-phase clusters. Kilpatrick and Ermentrout (2011) study a more biologically realistic model for the emergence of gamma rhythms in a network containing a large population of excitatory neurons with a smaller subpopulation of inhibitory interneurons. Interestingly, they show that the number of clusters that emerge in their model depends on the level of spike frequency adaptation in the excitatory neurons, which arises due to a calcium current. Our model contains a calcium channel that activates during fast firing and causes some degree of spike frequency adaptation, which raises the possibility that using different conductances for this channel may result in patterns with more than two clusters. It has also been shown that networks of neurons that have heterogenous synaptic interconnectivity may display clustered dynamics if the connectivity structure satisfies certain conditions (Li et al., 2003) - although this has only been shown for excitatory synapses and so it is not clear whether the same would apply to the GP network.

3.4.2 Stimulation of the STN may reduce oscillations in the hyper-direct pathway

The hypothesis that basal ganglia activity is entrained to cortical rhythms via the hyper-direct pathway in Parkinson’s disease offers some explanation of the possible mechanism(s) underlying the clinical effectiveness of STN deep-brain stimulation (DBS). The precise effects of this stimulation on neuronal activity in the basal ganglia are not fully understood and are likely to be many and varied (Kringelbach et al., 2010). The computational model of the basal ganglia of Kumar et al. (2011) included the effects of DBS through either a reduction of strength of cortex-STN synapses or inhibitory input onto STN and in both cases DBS was found to reduce oscillatory firing. In this model oscillations appear because the STN and GPe act as a pacemaker circuit due to the excitatory connections between STN neurons. It is clear that if DBS were added to our model in a similar manner then oscillations in the GP would be much reduced, as they are dependent on reasonably strong input from the STN. Another possible mechanism of DBS that has good experimental support is that it antidromically activates the fibres that project from the cortex to the STN (Li et al., 2007a). The effect of this antidromic activation is a reduction of oscillatory
3.4. Discussion

activity in the cortical regions that project to the STN. Since our model only includes STN input to the GP, clearly a reduction of oscillatory STN activity would reduce GP oscillations as well. Wilson et al. (2011) found that in a relatively abstract model of the GP consisting of uncoupled phase oscillators synchronized to a common input, chaotic dynamics served to desynchronize the population at frequencies and intensities similar to those that are clinically effective for DBS. The same may also be true of our model when the GP neurons are entrained to β-frequency STN input but further investigation is required. Our model could also help to test and improve the effectiveness of new forms of DBS, such as that proposed by Popovych and Tass (2012) which involves using multiple electrodes to desynchronize groups of neurons that have become entrained to particular rhythms.

3.4.3 The emergence of TI/TA groups depends on strong inhibitory coupling

One possible weakness of our model is that it relies on intra-GP inhibition being much denser than is currently supported by experimental evidence. It has been estimated that the probability of a given GP neuron synapsing onto any other, randomly-chosen, GP neuron is less than 1% (Sadek et al., 2007) but in most of our simulations this value is 20%. Our experiments with larger networks have suggested that the level of intra-GP coupling can be reduced while preserving the division into TI and TA groups by increasing the size of the network. Tseng et al. (2001) showed that the activity of striatal projection neurons is modulated by cortical SWA and increases after OHDA lesion. Including the effects of this in our model would probably increase the number of TI neurons and may allow us to reduce the amount of intra-GP inhibition to a more realistic level.

If further increases in network size cannot generate realistic activity with physiological levels of GP-GP coupling, there are several other possible reasons why the connectivity may be greater than has so far been measured experimentally. In Chapter 4 we will develop a model that is based on the idea that the basal ganglia are organized into a series of partially overlapping “channels” (Alexander and Crutcher, 1990), where neurons preferentially synapse onto other neurons in the same channel. If the small population of GP neurons that we modelled in this chapter were taken to represent part of a single channel, the proportion of coupled neurons might be much higher.
than would be seen from picking pairs of neurons from across the whole GP at random. It is also possible that the projection from GP to STN, which is not included in our model, may contribute to the effect of lateral inhibition since the tri-synaptic GP-STN-GP pathway is a route by which GP neurons inhibit other GP neurons, and there is experimental evidence to suggest the strength of this pathway may be increased under Parkinsonian conditions (Johnson and Napier, 1997). However, it is hard to say whether or not this explanation is plausible without more detailed information about the topology of the STN-GP and GP-STN projections.

3.4.4 Other considerations

Although our model has demonstrated that discrete groups of neurons can emerge from a population of GP neurons with homogeneous (unimodally distributed) membrane properties, there is now some evidence that the neurons in the TA and TI groups are distinct in some ways, including the nature of local inhibitory connectivity, the basal ganglia nuclei that they project to, and in their chemistry (Mallet et al., 2012). Similarly, several studies (Nambu and Llinás, 1997; Cooper and Stanford, 2000; Bugaysen et al., 2010) have attempted to categorise GP neurons based on their electrophysiological properties, and their results seem to suggest that several distinct groups may exist (although the boundaries remain fuzzy). Nevado-Holgado et al. (2014) built a population-level computational model of the basal ganglia which included two distinct groups of GP neurons: one TI and one TA. When they ran an optimization process to find the inter-population connection strengths that best matched experimental recordings, they found that the best fit was achieved when the STN→GP-TA, striatum→GP-TI and GP-TI→GP-TA connections were relatively strong. The first of these results is in line with our finding that the TA neurons in our model are those which receive the most STN input (Figure 3.6). In our model each of the GP populations is responsible for silencing the other during its active phase, but the latter two findings from Nevado-Holgado et al. (2014) suggest that it may be inhibitory input from the striatum that silences TI neurons during the active phase, while strong TI-TA inhibition silences TA neurons during the inactive phase. We could incorporate these ideas into our model by making the parameter noise for the NaP or HCN channels bi- or tri-modal and by giving one group of neurons a higher degree of local connectivity than another. This should promote the emergence of the TI, TA and NM groups and
reduce the degree of GP-GP connectivity that is required in order to obtain results that are similar
to experiments.

Another possible weakness is that the increase in GP-GP synaptic conductance that occurs
under Parkinsonian conditions in our model may be larger than in reality. Although we have used
data from paired-pulse experiments to choose the conductance of GP-GP synapses in the healthy
case, it is not clear how much this increases by following loss of dopaminergic input. Miguelez
et al. (2012) used an optogenetic technique to stimulate a number of GP neurons whilst recording
IPSCs and found an increase of approximately 67% after dopamine lesioning. This is considerably
smaller than the increase we use under Parkinsonian conditions, where the GP-GP conductance
is doubled. However, the increase seen by Miguelez et al. (2012) may be lower than the increase
at a single GP-GP synapse, since their method simultaneously activates many pre-synaptic GP
neurons and the summation of the resulting IPSCs may not be linear.

We did not include the projection from the GP back to the STN for reasons of simplicity,
and because experimental evidence suggests that this is not important for the entrainment of
GP neurons to SWA (Hassani et al., 1996). However, as we described in the previous chapter,
computational models of networks that include this connection have shown that the STN and GP
can work together to act as a pacemaker circuit (Terman et al., 2002; Gillies et al., 2002; Holgado
et al., 2010). In particular, Terman et al. (2002) describes the results of simulating a spiking
model of the interconnected STN-GPe network in which the tonic activity of both populations can
become bursty with a regular bursting rhythm. In fact, the neurons in this model can self-organise
into different sized clusters, which allows for the possibility of two anti-phase groups under some
conditions. We expect that our model could support similar regimes if the GP-STN connection
was added, provided that we also introduced a more realistic STN neuron model.

3.5 Conclusions

In this chapter we have shown, using a detailed computational model, how the changes that occur
under Parkinsonian conditions may allow neurons in the globus pallidus to become entrained to
cortical rhythms. While we were not able to show that this is possible for oscillations in the β
frequency band, simulated low frequency cortical modulation drives the emergence of two groups of
GP neurons with oscillatory anti-phase firing patterns. This mirrors the results seen in experiments with healthy and Parkinsonian rodents in the anaesthetised state. Unfortunately, due to the lack of experimental evidence regarding the types of neurons that are present in the globus pallidus and the way in which they are connected to each other, we have had to make many assumptions when choosing values for the large number of parameters that are associated with modelling a network of neurons using the Hodgkin-Huxley equations. In the next chapter we will somewhat deal with this problem by modelling activity in the interconnected STN/GPe network at a much higher level, in order to study the possibility that oscillatory activity is actually generated within the basal ganglia under Parkinsonian conditions.
Chapter 4

Oscillations in a “Multi-Channel” Model of the Basal Ganglia

4.1 The interconnected STN-GPe is a candidate pacemaker circuit

It is possible that abnormal synchronous oscillatory neuronal activity is generated within the basal ganglia under Parkinsonian conditions. This chapter will study one possible mechanism for this, namely interactions between the excitatory neurons in the STN and the inhibitory neurons in the GPe. The possibility that these two nuclei may act as an oscillation generating “pacemaker” circuit comes from evidence that connectivity between neurons in the two areas appears to be reciprocal and selective (Shink et al., 1996), and that STN neurons are able to fire bursts of post-inhibitory spikes following the removal of hyper-polarising current (Bevan et al., 2000). There is some biological evidence that this is a plausible mechanism: in vitro slices containing reciprocally connected STN and GPe neurons display transient oscillations at low frequencies (Plenz and Kital, 1999) and in vivo evidence from primate models of Parkinson’s disease suggest that reciprocal STN-GPe connections are necessary for the generation of oscillatory firing patterns in the STN (Tachibana et al., 2011).
Previous computational and mathematical modelling studies have examined the possibility of an STN-GPe pacemaker. Terman et al. (2002) used small networks of single compartment conductance based model STN and GPe neurons to show that, depending on the precise structure of connections between the two nuclei, such networks are able to generate a range of patterns including transient synchronous oscillations. In line with the hypothesised pacemaker mechanism, the way in which oscillations are generated in this model appears to be critically dependent on the ability of STN neurons to fire bursts in response to the removal of inhibitory synaptic currents. As discussed in Section 1.5.2, a disadvantage of conductance based modelling is that the complexity of the equations makes mathematical analysis intractable. Gillies et al. (2002) use a firing rate model to analyse the conditions for oscillatory activity to occur in a simple model containing two equations, representing the average firing rate in the STN and GP respectively. They show that self-excitation within the STN is a necessary condition for oscillations in such a model but unfortunately there is little biological evidence for this (see Section 1.2.1). However, firing rate models of the STN/GPe circuit that include the additional biological realism (and corresponding mathematical complexity) of synaptic delays between the two populations are able to produce oscillatory activity in the $\beta$ band without STN self-excitation (Holgado et al., 2010; Pavlides et al., 2012).

4.2 A Multiple-Channel STN/GPe Model

Although it is not yet known what the precise organisation and function of the motor areas of the basal ganglia are, the overall architecture may be that of multiple parallel, largely separate, pathways flowing through the various nuclei (DeLong et al., 1985; Alexander and Crutcher, 1990; Parent and Hazrati, 1995b). These pathways may correspond to individual muscles, body regions, motor actions, or some hierarchical combination of these. This idea can be represented in a population level model of the basal ganglia, with $N$ interconnected pairs of STN/GPe populations representing $N$ parallel channels. Figure 4.1 shows an overview of this model. Within an individual channel, the STN population excites the GPe population and the GPe population inhibits the STN population. The populations also have self connections, such that they inhibit and excite themselves (this is for generality - we will later set the strength of STN self-excitation to zero in line with
4.2. A Multiple-Channel STN/GPe Model

Figure 4.1: Schematic diagram showing the system arranged in a line topography, including the excitatory STN sub-populations, the inhibitory GPe sub-populations, and the connections between them. Red represents excitatory sub-populations and connections; blue represents inhibitory sub-populations and connections.

Biological evidence). Since GP neurons possess local axon collaterals that inhibit neighbouring GP neurons, the model includes some inhibition between GP populations in neighbouring channels - although we assume due to the specificity of connections that this inhibition is weaker than the self-inhibition within a single GP population. The cortex is not included as a population in this model — it simply provides a constant level of excitatory input to each STN subpopulation (this corresponds to the hyper-direct pathway).

The equations of the model are based on those of Wilson and Cowan (1972) and are as follows:

\[
\tau_s \dot{x}_i = -x_i + Z_s(w_{ss}x_i - w_{gs}y_i + I) \tag{4.1}
\]

\[
\tau_g \dot{y}_i = -y_i + Z_g \left( -w_{gg}y_i + w_{sg}x_i - \alpha w_{gg} \sum_{j \in L_i} y_j \right) \tag{4.2}
\]

\[i = 1, 2, ..., N\]

The time-dependent variables \(x_i\) and \(y_i\) represent the mean field activity of the excitatory STN subpopulation and inhibitory GPe subpopulation of channel \(i\), respectively. The connection
strength parameters \((w_{ss}, w_{sg}, w_{gg} \text{ and } w_{gs})\) are non-negative and represent the strength of synaptic connectivity within and between the populations, where \(w_{pq}\) is the connection strength from population \(p\) to population \(q\) (e.g. \(w_{sg}\) is the synaptic connectivity from STN to GPe). \(\tau_s\) and \(\tau_g\) represent the average membrane time constants of neurons in the two populations, while \(I\) specifies the level of cortical excitation of the STN. For simplicity this study is restricted to the case when there is the same degree of constant cortical input to each of the channels. The functions \(Z_s\) and \(Z_g\) are sigmoid functions as described in Section 1.5.2, with corresponding parameters \(a_s, a_g, \theta_s\) and \(\theta_g\).

The strength of the lateral inhibition between channels is taken to be a proportion \(\alpha\) of the coupling strength within GPe subpopulations \((w_{gg})\), where \(0 \leq \alpha \leq 1\). Different connection schemes are possible and are specified by the term \(L_i\) in Equation (4.2). For a given channel \(i\), \(L_i\) is a set of indexes that specifies which channels the GPe subpopulation receives inhibition from. In this study we consider only local connections to immediate neighbours, with two different arrangements of channels: on a line (Equation 4.3) and on a circle (Equation 4.4).

\[
L_i = \begin{cases} 
{i - 1, i + 1} & 1 < i < N \\
{i + 1} & i = 1 \\
{i - 1} & i = N
\end{cases}
\quad (4.3)
\]

\[
L_i = \begin{cases} 
{i - 1, i + 1} & 1 < i < N \\
{i + 1, N} & i = 1 \\
{i - 1, 1} & i = N
\end{cases}
\quad (4.4)
\]

### 4.3 Choice of parameter values

Wilson and Cowan (1972) give “typical” values for the parameters controlling the shape of the sigmoid functions in the model for inhibitory and excitatory subpopulations. We used these typical values for the parameters \(a_s, a_g, \theta_s\) and \(\theta_g\). For the remaining fixed parameters we used the values calculated in Holgado et al. (2010). There are two sets of values for the connection strength


### 4.3. Choice of parameter values

<table>
<thead>
<tr>
<th>Healthy</th>
<th>Parkinsonian</th>
</tr>
</thead>
<tbody>
<tr>
<td>$w_{gg}$</td>
<td>6.6</td>
</tr>
<tr>
<td>$w_{gs}$</td>
<td>1.12</td>
</tr>
<tr>
<td>$w_{sg}$</td>
<td>19.0</td>
</tr>
<tr>
<td>$\tau_s$</td>
<td>6ms</td>
</tr>
<tr>
<td>$\tau_g$</td>
<td>14ms</td>
</tr>
<tr>
<td>$a_s$</td>
<td>4</td>
</tr>
<tr>
<td>$\theta_s$</td>
<td>1.3</td>
</tr>
<tr>
<td>$a_g$</td>
<td>3.7</td>
</tr>
<tr>
<td>$\theta_g$</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 4.1: Fixed parameter values for the healthy and Parkinsonian conditions.

parameters: “healthy” and “Parkinsonian”. Holgado et al. determined these parameters on the basis of previously published experimental recordings of unit activity from the STN and GPe of monkeys. They used recordings from both healthy animals and animals that were rendered Parkinsonian via MPTP lesioning. In each case, the recorded firing rate under a variety of conditions (normal, transmitter block, current injection) was compared to the firing rate predicted by their model under the same (simulated) conditions, and a genetic algorithm was used in order to find two sets of connection strengths that best fit the data. The parameter fitting that Holgado et al. performed suggested that all connections became stronger under Parkinsonian conditions, and they cite several experimental results that support this increase, including the presence of $D_2$ receptors in the STN (Shen and Johnson, 2000) and GPe (Hoover and Marshall, 2004; Kita, 2007) and the enhanced effect of GABA on STN neurons (Cragg et al., 2004; Shen and Johnson, 2005) and glutamate on GPe neurons (Johnson and Napier, 1997; Kita, 2007) when dopamine is reduced. MPTP lesioning represents chronic dopamine depletion, which is the condition under which synchronised $\beta$ activity is seen in experiments. Table 4.1 lists the fixed parameter values for the healthy and Parkinsonian conditions. The equations in Holgado et al. (2010) directly represent the average unit firing rate of the populations and each weight parameter has a direct physical meaning: it represents how many spikes/sec the target population increases (or decreases) by when the source population’s firing rate increases (or decreases) by 1 spike/sec. The Wilson-Cowan equations do not directly represent the firing rates of populations, and so in our model the parameters should therefore be interpreted as representing only the general relative strengths of synaptic connections.
4.4 Bifurcation analysis of isolated channels

We initially investigated the behaviour of the system when the channels were completely independent of each other. This corresponds to the condition $\alpha = 0$, but for convenience we simply set $N = 1$ and studied the 2-dimensional model representing a single independent channel. To begin to understand the dynamics of this system, the software XPPAUT (Ermentrout, 2002) was used to plot the nullclines of the system and a range of phase space trajectories starting from different initial conditions. XPPAUT allowed us to interactively change the strength of cortical input ($I$) and STN self-excitation ($w_{ss}$) and observe the effect that these changes had on the nullclines and trajectories. While this is not a rigorous approach, the number of fixed points can be seen (from nullclines) and the sample trajectories are likely to reveal most of the stable limit cycles.

4.4.1 Isolated channels cannot oscillate under “healthy” conditions

When the “healthy” parameter values are used, isolated channels do not appear to be able to generate oscillations. Figure 4.2A shows the nullclines for a particular pair of values for $w_{ss}$ and $I$. 

Figure 4.2: Behaviour of the isolated channel system under healthy conditions with $w_{ss} = 3.4, I = 0$. A: The nullclines and fixed points of the system. B: Fixed points, stable and unstable manifolds of the saddle point, and example trajectories.
4.4. Bifurcation analysis of isolated channels

With these parameters the system is bistable, such that all trajectories in state space tend towards either a high or low level of activity in both nuclei depending on initial conditions. 4.2B shows the stable and unstable manifolds of the saddle point. These manifolds divide the phase space into two regions, where the fixed point that a given trajectory ends up in depends on which of these regions contains its initial position. Adjusting the parameters changes the \( \dot{x}_s = 0 \) nullcline (the red line in Figure 4.2A): increasing \( w_{ss} \) makes the slope of the middle branch steeper, while increasing \( I \) shifts the nullcline upwards. Both of these changes increase the proportion of initial conditions that give trajectories tending to the high activity state, as would be expected from increased STN self-excitation or cortical input. If the parameters are raised past a critical point, the system undergoes a saddle-node bifurcation whereby the low activity stable fixed point and the saddle meet and annihilate, leaving the high activity state as the only fixed point of the system. Alternatively, if the parameters are lowered past a critical point then the high activity stable state disappears in a saddle-node bifurcation instead, leaving only the low activity state. Since these saddle-node bifurcations are the only bifurcations that the fixed points of the system undergo, it is unlikely that any stable limit cycles exist with the healthy fixed parameter values. While we can’t completely rule out the possibility of oscillations appearing via global bifurcations, these seem unlikely given that none of the trajectories we plotted during parameter variation showed oscillatory activity. The system displays hysteresis because increasing the parameter passes a critical value can cause trajectories to “jump” from one stable point to another, and reducing the parameter back past this critical value does not cause a jump back to the original fixed point.

4.4.2 Oscillations under Parkinsonian conditions require STN self-excitation

Applying the qualitative methods in the previous section to the system using the Parkinsonian set of fixed parameter values revealed a much richer array of possible dynamics and also suggested a parameter range within which bifurcations were present. We used bifurcation analysis to fully understand the different dynamical regimes that exist within this parameter range. This was first performed in one dimension by starting at a fixed point and varying a single parameter (as before, either \( w_{ss} \) or \( I \)) and then in two dimensions by starting at a bifurcation point and allowing both parameters to change. Figure 4.3 shows the complete 2D bifurcation diagram of the system under
Figure 4.3: A: 2D bifurcation diagram showing the bifurcations that the isolated channel system undergoes under variation of $I$ and $w_{ss}$ in the Parkinsonian case. B: Zoom of the area inside the small rectangle in the lower right-hand corner of A).
4.4. Bifurcation analysis of isolated channels

<table>
<thead>
<tr>
<th>Region</th>
<th>( I )</th>
<th>( w_{ss} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Low)</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>A (High)</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>11.8</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>D</td>
<td>3.5</td>
<td>5</td>
</tr>
<tr>
<td>E</td>
<td>10.45</td>
<td>2.345</td>
</tr>
<tr>
<td>F</td>
<td>10.495</td>
<td>2.29</td>
</tr>
</tbody>
</table>

Table 4.2: The parameter values that were used for each of the regions in Figure 4.4.

Parkinsonian conditions. We mostly performed this analysis using the numerical continuation software LOCBIF (Khibnik et al., 1993), although we were not able to use this software to plot the curves of the two of the bifurcations: the fold of limit cycles and the homoclinic bifurcation. In these cases, we performed the analysis by hand, finding for each value of \( I \) (in sensible steps) the corresponding value of \( w_{ss} \) where the stable limit cycle disappeared, using XPPAUT for numerical simulation.

The bifurcation curves divide the parameter space up into six regions. Within each region the phase portraits of the system are topologically equivalent, having the same number of stable and unstable fixed points and limit cycles. The characteristics of these features (such as frequency and amplitude of oscillation) may vary within regions. Figure 4.4 shows example phase portraits that are representative of the system’s behaviour in each of the regions. The parameters corresponding to each region in the figure are given in Table 4.2.

Region A makes up the majority of the parameter space. Within this region the system possesses a single, stable, fixed point. The location of this fixed point in both dimensions increases with \( I \) and \( w_{ss} \), as is expected from increased external stimulation or self-excitation. The behaviour of the system is more interesting in the other five regions (B-F), which together make up the large wedge-shaped area in the middle of the bifurcation diagram.

As one or both of the parameters is reduced from values that give a constant high rate of firing (the area above the wedge in the bifurcation diagram), they move towards and eventually pass through the saddle-node bifurcation curve and into region B. Two additional fixed points appear at this point, both unstable. Although in region B all trajectories still tend to the single stable fixed point, the effects of the saddle point’s manifolds causes some trajectories to take long
Figure 4.4: Example phase portraits showing the behaviour of the Parkinsonian isolated channel system within each of the regions of parameter space.
4.4. Bifurcation analysis of isolated channels

paths around the phase space first. At the point where the parameters cross the saddle-node on invariant circle (SNIC) bifurcation curve the stable node and the saddle point join together and the stable and unstable manifolds of the saddle point form a loop (a homoclinic orbit). Beyond the bifurcation, in region C, the saddle and the stable node have disappeared leaving the unstable spiral as the only fixed point. The homoclinic orbit has now become a stable limit cycle and so in this region all trajectories are attracted to the limit cycle, so the system is universally oscillatory.

Both the frequency and amplitude of the stable oscillations in region C vary as the parameters move around within it. Close to the SNIC bifurcation line the frequency is extremely low, since the effects of the “ghost” saddle point cause trajectories to pass very slowly through the part of the limit cycle that is close to where the saddle was located\(^1\). When the parameters are within region C the activity of the subpopulations may show either low activity with short pulses of high activity, or the opposite, or something in between. Figure 4.5 illustrates this by showing a number of plots of population activity against time from within region C.

The lower border of region C is, for the most part, an Andronov-Hopf (A-H) bifurcation curve. This curve is divided into three segments - two supercritical parts that are separated by a long sub-critical A-H curve. The points where the criticality of the bifurcation changes are the codimension-2 Bautin bifurcation points. The change in behaviour of the system as its parameters pass through the lower border of region C depends on whether they cross a sub- or super-critical A-H curve. In the case of two supercritical curves this change is simple: the limit cycle shrinks around the unstable spiral until, at the bifurcation point, its amplitude becomes zero. At this point the limit cycle disappears and the spiral becomes stable: the system has returned to region A.

The situation when the system leaves region C across the sub-critical A-H curve is more interesting. In this case the spiral becomes stable before the limit cycle has shrunk to zero amplitude. An expanded phase portrait of the system in this region is shown in Figure 4.6. Since both the stable fixed point and the stable limit cycle have local basins of attraction, the region inside the stable limit cycle is divided into two concentric areas. Trajectories that begin within the inner area tend to the fixed point and trajectories that start within the outer area tend to the stable

\(^1\)The saddle's ghost also has an effect on the amplitude of oscillations in this case (though this is not generally true of SNIC bifurcations). This is because the shape of the limit cycle is defined by the position of both the unstable spiral and the saddle's ghost. As the parameters are varied in region C these two points move in relation to each other.
Figure 4.5: Population activity over time for three points in region C, showing periodic pauses (top), bursts of high activity (bottom) and roughly even oscillation between high and low activity (middle). The red and blue lines represent the activity of the STN ($x(t)$) and GPe ($y(t)$) respectively.
4.4. Bifurcation analysis of isolated channels

Figure 4.6: Enlarged portion of Figure 4.4 showing the nullclines and phase portrait of the isolated channel system in region D.

cycle. The border between these two areas is a new unstable limit cycle that appears at the point of sub-critical bifurcation. The behaviour of the system within region D is therefore bistable and, depending on initial conditions, may show either steady-state or oscillatory activity levels. As the parameters move from the top to the bottom of region D, the stable limit cycle continues to shrink while the unstable limit cycle grows. The point at which the cycles meet and annihilate lies on the fold of cycles bifurcation curve. This leaves just one stable fixed point, returning the system to region A.

While regions A-D make up the majority of the parameter space, there are two small additional regions (shown in detail in Figure 4.3B). The point at which the A-H curve terminates on the saddle-node curve is a codimension-2 Bogdanov-Takens (B-T) bifurcation point. Due to the normal form of the B-T bifurcation, this point must also be one end of a homoclinic bifurcation curve. The other end of the homoclinic curve is also located on the saddle-node curve at the saddle-node/homoclinic
point; here the two curves merge and the saddle-node curve becomes a SNIC curve. At the point where the parameters cross the homoclinic curve from region B (unstable spiral, saddle, stable node) to region E the stable and unstable manifolds of the saddle form a closed loop with each other. Beyond this bifurcation point the two manifolds have crossed one another and a stable limit cycle appears between the saddle’s unstable manifold and the unstable spiral (see Izhikevich (2007) pp. 185-190). Like region D, region E has a bi-stability between steady-state and oscillatory behaviour that depends on initial conditions. The set of initial conditions that leads to oscillations is very small, however, due to the shape of the saddle point’s manifolds.

If the system’s parameters leave region E through the supercritical A-H curve then the unstable spiral becomes stable and the limit cycle is destroyed. Behaviour in this region (region F) is still bistable, but the two stable states are both fixed points so there can be no oscillation. Furthermore these two states are extremely close to each other in phase space, both being regions of high activity. The parameters can leave region F through one of two parts of the saddle-node curve. Crossing either of these parts results in the loss of one of the stable fixed points and the saddle, leaving just one fixed point, which is stable.

Since bifurcation analysis revealed a number of oscillatory regions in the parameter space a further numerical experiment was performed to investigate the characteristics of these regions. Specifically, a large scale set of numerical simulations were performed to determine how the frequency and amplitude of the limit cycles varied with the parameters. The parameter space was divided up into a uniform grid and, for each pair of parameter values, the system was simulated for a period of time. The power spectrum of the resulting activity was computed using a Fast Fourier Transform (FFT) and the frequency of the strongest oscillation visualised. These experiments were implemented in the Python programming language, with numerical integration from XPPAUT via the XPPy library (Nowacki, 2011), using the FFT routine from the SciPy library (Jones et al., 2001). Figure 4.7 shows the results of these computations. The same simulations were performed using the healthy fixed parameters, but as expected no oscillations were seen and so the results are not shown.

These results are what would be expected based on the bifurcation analysis. Only regions C and D contain oscillatory activity (the only other oscillatory area in Figure 4.3, region E, is too small to be shown here). The frequency of oscillations decreases to zero as the parameters move
4.4. Bifurcation analysis of isolated channels

Figure 4.7: The frequency and amplitude of the strongest oscillation present for each pair of parameter values for the isolated channel system under Parkinsonian conditions. The bifurcation curves and regions are shown for ease of comparison with Figure 4.3.

towards the SNIC bifurcation curve (the boundary between regions B and C) and increases as the parameters are decreased away from this curve. As previously discussed, the amplitude of the oscillations is greater when the parameters are close to the SNIC bifurcation line, since the unstable spiral and “ghost” saddle point are far apart here. The frequency in much of region C is in the $\beta$ band, but region D contains some areas of higher frequency oscillation (up to about 50Hz, which falls within the low part of what is termed the $\gamma$ band). These frequencies can only be considered as some approximation of rhythms that would be found in the real STN-GPe network.

To further confirm our findings about the behaviour of isolated channels we used our DSvis software (described in Section 2.2) to interactively explore the dynamics of the equations. DSvis uses the GPU to simulate, in parallel, trajectories starting from a million sets of random initial conditions both forwards and backwards in time. This reveals the stable and unstable attractors of the system, and shows the results of interactively changing parameter values in real time. The results of this investigation were in agreement with what was expected from the bifurcation analysis and parameter grid simulations. Figure 4.8 is a screenshot of the DSvis software when the parameters are within the bistable region D, showing the stable fixed point and limit cycle (green particles) and unstable limit cycle (pink particles).
Figure 4.8: Screenshot of DSvis software, visualising the activity of a single isolated channel under Parkinsonian conditions. The parameter values for the simulation shown are within the bistable region D of Figure 4.3. Each particle shows the state of one trajectory, with the horizontal and vertical axes representing STN and GPe activity respectively. The green particles are integrated forwards in time and show the stable limit cycle (big closed orbit) and fixed point (centre dot). The pink particles are integrated backwards in time and show the unstable limit cycle (smaller closed orbit).
4.4.3 Oscillations under Parkinsonian conditions do not require GPe self-inhibition

In our model the strength of GPe self-inhibition \((w_{gg})\) is increased under Parkinsonian conditions. In contrast, some models — notably that of Terman et al. (2002) — find that a reduction in pallidal self-inhibition may facilitate increased rhythmic activity in the STN-GPe network. There is some evidence to suggest that this decrease of \(w_{gg}\) in the Parkinsonian case is more appropriate, based on the effects of increased striatal-pallidal activity on GABA release in the the GPe (Ogura and Kita, 2000; Stanford and Cooper, 1999). We briefly investigated how reducing \(w_{gg}\) affected the ability of the isolated channel model to generate oscillations. This revealed that, unlike STN self-excitation, GPe self-inhibition is not essential for oscillation. Specifically, we found that under Parkinsonian conditions there are a wide range of values for \(I\) and \(w_{ss}\) that generate oscillations even when \(w_{gg} = 0\).

4.4.4 A possible physiological interpretation

Our analysis of the isolated channel model demonstrates that, when the Parkinsonian connection strengths are used, a simple model of a coupled pair of STN and GPe subpopulations can generate dynamic behaviour that is either steady-state (regions A and B), oscillatory (region C), or bistable between a steady and oscillating state (region D). The oscillatory and bistable regimes rely on a non-zero degree of STN self-excitation. This section will describe one possible model of basal ganglia movement processing that these dynamics could represent. Here we do not mention regions E and F as they are extremely small and are therefore unlikely to correlate with observed features of basal ganglia (dys)function.

We consider a system that consists of multiple isolated channels which all have parameters such that they are in region D (see Figure 4.6). Each channel can be switched between oscillation and steady-state activity by a short transient external perturbation of the activity in either STN or GPe. To take a channel from steady-state to oscillatory activity, this perturbation must be sufficient to move the system outside the basin of attraction of the fixed point (this is the region enclosed by the unstable limit cycle). Transferring the system to the steady-state is more difficult. The perturbation must arrive at the correct time in the oscillatory cycle in order to move the
current position in phase space towards the unstable cycle. The correct time depends on whether the short external perturbation affects the STN or GPe, and whether it has an excitatory or inhibitory effect. For example, an inhibitory perturbation applied to a GPe subpopulation must occur during the high activity phase of oscillation as this will move the trajectory down in phase space and, if the perturbation is of the correct amplitude, bring the trajectory inside of the unstable limit cycle where it will be attracted into the stable spiral.

LFP recordings reveal a drop in synchronous $\beta$ oscillations in the basal ganglia prior to and during movement (Cassidy et al., 2002) and, according to our interpretation, this corresponds to one or more channels transferring from a limit cycle to a stable fixed point’s basin of attraction - which requires a precisely timed perturbation. One possible source for this perturbation is the inhibitory input that the GPe receives from the striatal medium spiny neurons. This projection is organised in a segmented manner, which suggests that each of our channels receives striatal input from a different set of MSNs (Flaherty and Graybiel, 1994). Recordings in monkeys have found that a subset of these neurons, the phasically active neurons (PANs), are normally silent but show short bursts of activity just prior to movement (Kimura et al., 1990). Simultaneous LFP and unit activity recordings from the striatum of healthy behaving monkeys reveals that there is a transient $\beta$ rhythm in the striatal LFP and, furthermore, that the firing of PANs occurs at a particular point in the cycle of this oscillation (Courtemanche et al., 2003). If the striatal and pallidal $\beta$ LFP oscillations are synchronised to some degree (this is currently unknown) then it is possible that the PAN bursts arrive during the correct part of the STN-GPe oscillation cycle to push a channel into the stable state. After a movement has been completed the channel can easily be switched back to its $\beta$ oscillatory mode by an excitatory or inhibitory perturbation of its STN or GPe subpopulation. Each channel that is in region D therefore acts as a switch, or filter. Assuming each channel corresponds to a movement or body region, synchronised oscillatory activity in the circuit prevents movement either by reducing information transfer or acting as a global “anti-kinetic” signal. When movement is required, precisely timed striatal input effectively switches the oscillations off temporarily.

If, due to some modulation of cortical input or STN self-excitation ($I, w_{ss}$), the system moves close towards region C then the basin of attraction for the stable fixed point becomes smaller. When this happens the external perturbation required to escape the oscillatory region must be of
larger amplitude and timed more precisely. Finally, when the parameters pass into region C, the fixed point loses stability and no external perturbation of trajectories would be able to stop the system oscillating. The severity of the hypokinetic symptoms of Parkinson’s disease fluctuate from day to day and with disease progression, and if this interpretation is true, it is possible that these fluctuations could correspond to changes in the size of fixed point’s basin of attraction. According to this hypothesis, region C would correspond to a completely akinetic state where movement cannot be initiated at all.

The physiological plausibility of this mechanism for activating and deactivating different movement channels is limited by the fact that the bistable region only exists when the Parkinsonian connection strengths are used and STN self-excitation is non-zero. However, we shall show in the next section that introducing a degree of coupling between channels unlocks much more interesting dynamics within each channel, even in the healthy case. Introducing heterogeneity to the level of cortical input that each channel receives will make the possible dynamics richer still. It is possible that under these more realistic conditions there are regions of parameter space where channels can exhibit similar bistable behaviour to what is described here.

4.5 Investigation of the full coupled channels model

The coupled channels model is a $2N$ dimensional system (with $N > 1$), which means that bifurcation analysis is much more difficult than for the isolated channel model. We will begin by discussing the parameter values that were selected before presenting some general results that show that the coupled channels model has an oscillatory regime that is very robust and exists for a wide range of parameters. Finally section 4.5.3 will briefly describe the detailed structure of the attractors that the system has.

4.5.1 Parameter choice

When studying the coupled channels model we used the same values for the fixed parameters as were used in the isolated channel model (see Table 1). As before, the connection strengths were divided into a healthy set and a Parkinsonian set. However, since there is no known mechanism whereby STN neurons can excite other STN neurons, we chose to fix $w_{ss} = 0$. Although the analysis of the
isolated channel model found $w_{ss} > 0$ to be a necessary condition for oscillations, we hypothesised that the coupled channels model might be able to oscillate with $w_{ss} = 0$, since the path from an STN subpopulation to its neighbouring STN subpopulation and back again will have the effect of indirect delayed self-excitation. The coupled channels model has an additional parameter that can be varied ($\alpha$), which controls the strength of inhibition between neighbouring GPe subpopulations as a proportion of the self-inhibition within GPe subpopulations ($w_{gg}$). Since we are basing our model on the idea that sensorimotor channels remain largely segregated throughout the STN/GPe network (DeLong et al., 1985; Alexander and Crutcher, 1990; Parent and Hazrati, 1995b), we argue that $\alpha < 1$ is the physiological range for this parameter. We studied the system under variation of $\alpha$ and $I$, using the results of our analysis of the isolated channel model to guide the selection of a reasonable range of values for $I$.

4.5.2 Oscillations with $w_{ss} = 0$ require strong coupling, particularly under healthy conditions

We began by manually carrying out many numerical simulations of different coupled systems, varying the number of channels, connection topology, $\alpha$ and $I$, and whether the healthy or Parkinsonian fixed connection strengths were used. Each simulation was started from random initial conditions. During this experimental work we found that for relatively low lateral coupling (e.g. when $\alpha < 0.5$) the systems always converged to a single fixed point attractor. None of our experiments with low $\alpha$ found oscillatory regimes or multi-stability. We found that it is possible (for some values of $I$) for the fixed point attractor to undergo a supercritical Andronov-Hopf bifurcation as the parameter $\alpha$ is increased towards 1. This bifurcation causes a stable limit cycle of small amplitude to appear. This limit cycle appears to be a global attractor. The range of values of $I$ for which this bifurcation exists depends upon whether the healthy or Parkinsonian connection strengths are used: it is much wider in the Parkinsonian case than in the healthy case.

To make this investigation more rigorous we ran similar large-scale simulations to the one which was used to generate Figure 4.7, for a range of different connection topologies and channel counts. Figure 4.9 shows one such result for five channels coupled in a line topology, under both healthy and Parkinsonian conditions. It can be seen that this appears to confirm our finding that oscillations with $w_{ss} = 0$ require strong coupling, particularly under healthy conditions.
4.5. Investigation of the full coupled channels model

Figure 4.9: The frequency (left) and amplitude (right) of the strongest FFT bin encountered during numerical simulation from random initial conditions across a range of parameter values. The top row shows the system under healthy conditions and the bottom row shows Parkinsonian conditions. The system here has 5 channels arranged in a line topology.

Oscillations require reasonably strong lateral coupling and are much more prevalent under Parkinsonian conditions. This fact appears to be generally true regardless of the connection topology used or number of channels (up to 100 channels were used).

As in the isolated channels case, we briefly studied whether oscillations could arise in coupled channels if the strength of GPe self-inhibition is reduced, rather than increased, under Parkinsonian conditions. To do this we used XPPAUT to integrate, from various initial conditions, the equations corresponding to the five and six channel models in both line and circle topologies, while varying $\alpha$ and $I$ in order to try to find stable limit cycles. Initially we kept $w_{gg}$ at its healthy value (6.6) rather than increasing it, and in this case were able to see stable oscillations for a wide range of values of $I$ when lateral coupling was strong ($\alpha > 0.95$) in all channel configurations. However, decreasing $w_{gg}$ rapidly shrunk the parameter region containing oscillations. The five and six channel models with line topologies could still produce oscillations when $w_{gg}$ was reduced by a sixth (to 5.5), while the six channel model with a circle topology could oscillate with $w_{gg}$ reduced by two sixths (to 4.4). Reducing $w_{gg}$ further than these limits meant that the oscillatory regime either ceased to exist or was too small for us to find. We demonstrated in Section 4.4.3 that GPe self-inhibition is not required for oscillations in isolated channels, and hypothesised that
lateral inhibition between channels may play a similar role as STN self-excitation. Taken together, these two ideas would suggest that the coupled channels model could generate oscillations even if $w_{gg} = 0$ as long as lateral inhibition was present. We again used XPPAUT to investigate this possibility and found that it is indeed the case - although note that this condition violates our assumption that intra-channel inhibition should be weaker than inter-channel inhibition.

4.5.3 Detailed attractor structure depends on channel count and topology

Qualitative investigation of the coupled channels system revealed that the stable attractors are structured in a way that depends on the coupling topography (i.e. circle or line) and whether the number of channels is odd or even. This section will briefly illustrate the different attractor structures that our model can have in order to demonstrate the range of possibilities.

We first consider the effect of gradually raising the value of $\alpha$ up from zero while keeping $I$ constant. When $\alpha = 0$ we know from analysis of the isolated channel model that all of the STN subpopulations will converge to some fixed activity level (determined by $I$) and all the GPe subpopulations will converge to some other fixed level (i.e. there is a single fixed point where $x_1 = x_2 = \ldots = x_N$ and $y_1 = y_2 = \ldots = y_N$). Increasing $\alpha$ changes the co-ordinates of this single steady-state in phase space in a way that depends on whether the system is coupled as a line or a circle. In the case of channels arranged on a circle there continues to be a single activity level for all STN subpopulations and another level for GPe subpopulations and increasing $\alpha$ decreases the GPe level while increasing the STN one. When the channels are arranged in a line their steady-state activity levels become paired symmetrically (i.e. $(x_i, y_i) = (x_{N-(i-1)}, y_{N-(i-1)})$). When $N$ is odd the centre channel has its own unique activity level. Increasing $\alpha$ causes the activity levels associated with the different channel pairs to spread out in phase space. Figure 4.10 shows the steady-state activity for a number of topologies (circle, line with $N$ even, line with $N$ odd).

The system begins to oscillate when $\alpha$ passes some critical value $\alpha_{crit}$. The precise value of $\alpha_{crit}$ depends on $I$, $N$ and the coupling topography / strengths, but in every case the stable fixed point becomes unstable and a new stable oscillatory attractor appears. The amplitude of the associated oscillations is small near the bifurcation and increases as $\alpha$ moves further away from its critical
4.5. Investigation of the full coupled channels model

Figure 4.10: Steady-state activity of the healthy coupled channels model with $\alpha = 0.5$ and $I = 2.5$ under three different configurations. The activity of the STN subpopulations is shown in shades of red and the activity of the GPe subpopulations is shown in shades of blue. A: Circular topology with four channels (same activity level across all channels). B: line topology with four channels (pairs of channels with same activity). C: line topology with five channels (pairs of channels with same activity plus middle channel).

value. Close to the bifurcation the oscillatory activity can take four different forms depending on the coupling topography and whether $N$ is odd or even. In the case of the line topography, each of the pairs of channels begin oscillating together either anti-phase ($N$ even) or in-phase ($N$ odd). With the circle topography the channels all oscillate identically but in either 2 anti-phase groups ($N$ even) or in a “splay state” with a constant phase-shift between channels such that they span the oscillation period ($N$ odd). Figure 4.11 shows these four patterns of oscillatory activity.

The patterns shown in Figure 4.11 are the possible oscillatory patterns seen close to the bifurcation where oscillations appear as $\alpha$ is increased, when initial conditions are chosen randomly and have no particular structure. Further away from the bifurcation, however, the system can display much more complex dynamics, potentially consisting of many stable oscillatory attractors. Due to the high dimensionality of the system and the fact that the attractor structure depends on coupling topology, $N$, $\alpha$, $I$ and $\mu$ meant that we could not hope to understand the full dynamics in every case. However, we found that in most configurations there appear to be only a small number of strongly stable attractors; trajectories starting from random initial conditions will converge to one of these. By imposing additional constraints on initial conditions, we were also able to identify several “degenerate” attractors that presumably have stable manifolds which represent a very small area of state space (including, but perhaps not limited to, the region where the constraints
Chapter 4. Oscillations in a “Multi-Channel” Model of the Basal Ganglia

Figure 4.11: Oscillatory activity of the coupled channels model with $\alpha = 0.95, I = 2.5$ and healthy connection strengths, under four different configurations. The activity of the STN subpopulations is shown in shades of red and the activity of the GPe subpopulations is shown in shades of blue. A: Line topology with ten channels (five anti-phase pairs). B: Line topology with eleven channels (five in-phase pairs plus middle channel). C: Circle topology with ten channels (two anti-phase groups). D: Circle topology with eleven channels (splay state).

we imposed on the initial conditions hold). These attractors are assumed to be unstable, since a trajectory with initial conditions which very nearly satisfy the constraints, but with some small perturbation, will not converge to the attractor. The attractors we discovered for different values of $N$ and coupling topographies are briefly summarised below, but we do not claim that this is an exhaustive list of all possible dynamics.

- **Line, $N$ even**: Close to the bifurcation where oscillations appear, symmetric pairs of channels oscillate together in anti-phase with the same shape and amplitude, as shown in Figure 4.11A. However, for parameters further away from the bifurcation, it is also possible to find another pair of stable oscillatory attractors. In both of these new attractors, every channel oscillates with a unique shape and amplitude. The channels’ activity on one attractor is the mirror image (about the centre of the network) of their activity on the other attractor. For some parameter values these two attractors are stable at the same time as the original oscillatory attractor (giving tri-stability), while for other parameter values only the paired attractors are stable (bi-stability). **Degenerate attractors**: If the initial channel activity levels are chosen so that the activity in each symmetric pair is identical, the system converges to a special oscillatory attractor in which each symmetric pair of channels oscillates around a particular activity level (like in Figure 4.11A), but the channels in each pair are in-phase, rather than anti-phase.
• **Line, N odd:** All sets of initial conditions that we tried led to the same oscillatory attractor, where symmetric pairs of channels oscillate together in-phase (as in Figure 4.11B). This was the case even when the initial conditions were chosen such that the initial activity in each channel was identical.

• **Circle, N even:** Channels oscillate together in two anti-phase groups, as in Figure 4.11C.  
  *Degenerate attractors:* Identical initial activity in all channels leads to a fixed point attractor where all channels have the same level of activity.

• **Circle, N odd:** As noted above, close to the bifurcation where oscillations first appear there is a single stable “splay-state” oscillatory attractor. However, as the system moves away from the bifurcation, many more stable oscillatory attractors appear, which may co-exist with the splay state (although past a certain point the splay state disappears or becomes unstable). It’s unclear what the structure of these attractors is, or how the choice of initial conditions affects which attractor a trajectory will converge to. *Degenerate attractors:* Identical initial activity in all channels leads to a fixed point attractor where all channels have the same level of activity. Picking one channel as the “centre” and making the initial activity levels symmetric about this channel results in oscillatory activity where the symmetric pairs oscillate together with the same magnitude and phase, similar to Figure 4.11A.

We used a specially developed CUDA program to visualise the oscillatory activity of the coupled channels model. Figure 4.12A shows a snapshot of the activity of five parallel channels under Parkinsonian conditions with \( I = 5 \). Many sets of initial conditions are chosen uniformly from across the 10-dimensional phase space and each set of initial conditions is integrated in parallel using the computer’s graphical processing unit, with random resetting. For each set of initial conditions being integrated, the level of STN and GPe activity is projected onto a different part of the screen (and in a different colour) for each channel, where the STN and GPe activity levels are represented by the horizontal and vertical axes respectively. A white line is used to link the particles corresponding to the first set of initial conditions (which are never randomly reset). The shape of the limit cycle is clearly visible on each projection, and the white line shows the anti-phase relationship between neighbouring channels and in-phase relationship between symmetrically paired channels. Figure 4.12B shows three snapshots from a similar visualisation of 799 channels.
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Figure 4.12: Visualisation of the coupled channels model using the GPU. A: Parallel simulation of trajectories from random initial conditions for five channels coupled according to a line topology. Parkinsonian connection strengths are used, with $I = 5$. Each particle represents the activity of one channel in one trajectory, where the different channels’ activities are projected onto different planes in different colours. The white line links the channel activities for the first simulated trajectory. B: Simulation of one trajectory for 799 channels coupled in a line, with the same parameters as in A. The vertical displacement of each particle shows the activity of the GPe subpopulation for the channel at the corresponding horizontal position. The sub-figures show: i. oscillations spreading from the sides during an initial period; ii. a period where the complex structure of the limit cycle makes the activity appear random; iii. a later (transient) period where more regular activity is seen. Note that due to symmetry of the system and initial conditions, the activity each of the sub-figures in B is symmetric about the middle channel.
4.5. Investigation of the full coupled channels model

arranged in a line; although due to screen space limits only the GPe activity in each channel is shown (as vertical displacement), and only one set of initial conditions is used. When the steady state becomes unstable, oscillations begin at the ends of the line, where lateral inhibition is weaker, and spreads towards the middle (Figure 4.12Bi). Due to the large number of channels the long-term behaviour is very complex, with periods of seemingly random activity (Figure 4.12Bii) interspersed with periods of more regular structure (Figure 4.12Biii).

In order to confirm that the general behaviour of the system was independent of \( N \) and coupling topography, we generated diagrams similar to Figure 4.9 for values of \( N \) from 3 to 30, under both coupling schemes. Qualitative inspection showed that all the diagrams were similar, as expected. For a more objective measure we computed, from each diagram: the fraction of nodes in the \((I, \alpha)\) parameter grid that gave oscillatory activity, the minimum coupling strength \((\alpha)\) that gave oscillations, and the average frequency of oscillation. These calculations confirmed that oscillations are present for a much greater range of parameter values under Parkinsonian conditions (Figure 4.13) and, similarly, that the minimum value of \( \alpha \) required for oscillations was always much higher in the healthy case than in the Parkinsonian one (0.8 vs. 0.65). As expected, these measures tended to a constant level (which did not vary with coupling topography) as \( N \) was increased, showing that the general behaviour (oscillatory versus steady-state) was independent of channel count and topography. The calculations also found that the average oscillation frequency did not vary much with \( N \) or coupling topology, but that this average frequency was consistently much lower under healthy conditions than Parkinsonian ones (55Hz vs. 130Hz).

4.5.4 Physiological plausibility of the coupled channels result

The analysis of the coupled channels model above demonstrates that the range of parameters that cause oscillations in a system of \( N \) STN/GPe subpopulation pairs, laterally coupled at the GPe level, is relatively independent of the coupling topology used and the value of \( N \) (as long as \( N > 3 \)). In all cases the subpopulations all tend to a constant level of activity when the strength of lateral inhibition is weak compared to inhibition within GPe subpopulations. When lateral inhibition is made almost as strong as the inhibition within GPe subpopulations then the network as a whole can begin to generate oscillations when the level of cortical input received by each channel is within
Figure 4.13: The area of the oscillatory region in diagrams similar to those in Figure 4.9, for different values of $N$ and coupling topographies. In every case the area is much larger under Parkinsonian conditions than healthy ones and is not significantly affected by $N$ or the coupling topography, for values of $N$ greater than approximately 14.
4.5. Investigation of the full coupled channels model

a certain range; this range is much wider when the remaining connection strengths are set at values representing the Parkinsonian basal ganglia.

There is some experimental evidence that suggests that this result could represent what happens in the real basal ganglia. LFPs recorded simultaneously from multiple sites within the (healthy) rat globus pallidus display a degree of coherence that varies with global brain state: under anaesthetised slow wave activity conditions the LFP signals have little coherence, but when the brain state becomes “globally activated” the signals become much more coherent with one another suggesting an increased level of lateral coupling (Magill et al., 2006). In terms of our model this would correspond to the value of $\alpha$ varying with brain state - low during SWA and higher during global activation. Interestingly, a similar study using rats that were chronically dopamine depleted via OHDA lesion found that the characteristic $\beta$ LFP peak in the STN was present only in the globally activated brain state, not during SWA (Mallet et al., 2008b). Our model suggests that this oscillatory activity may be generated locally by the STN/GPe circuit as a result of the increased lateral coupling between GPe subpopulations that is seen during global activation.

The frequency of oscillations generated by our model is generally much higher than the 15–30Hz $\beta$ band - although it is interesting to note that the parameter values that resulted in the largest amplitude of oscillation were those that gave the lower frequency oscillations, including the $\beta$ band (Figure 4.9). Although we found that shifting the fixed connection strengths towards their healthy values reduced this average frequency (whilst shrinking the oscillatory region of parameter space), we did not find a simple relationship between the frequency of oscillation and any one individual connection strength. It is possible that more complex coupling topologies (for example, linking each GPe subpopulation with more of its neighbours, with a strength that decreased with distance) could have the effect of reducing oscillation frequency. Our definition of the frequency of oscillation was also very simple: we considered only the frequency of the highest peak that was found across the power spectra of all of the subpopulations’ activity. A more thorough study should examine the entire spectrum in each case and check for a peak at $\beta$, and could consider a measure that would more accurately correspond to a simulated LFP recording (such as the summation of activity across all channels). Finally, it is possible that the time constants that were used (particularly for the GPe subpopulations) were significantly different to the typical cell membrane time constants of the populations we are modelling. Experiments have reported a wide range of possible values for
membrane properties of GPe neurons (Günay et al., 2008). At present our model only demonstrates that some oscillatory activity is possible in the Parkinsonian STN-GPe when the level of lateral coupling in the GPe is sufficiently strong.

4.6 Comparison with other models

The results of our analysis of a single isolated channel agrees, to a large extent, with the results of the study of Holgado et al. (2010), which considered the entire STN and GPe each as single populations and from which our parameters were taken. As in Holgado et al. (2010), stable β oscillations occur only when the parameter values corresponding to the Parkinsonian state are used. The model presented here is simpler than that of Holgado et al. (2010) as it does not attempt to model the synaptic transmission delay between subpopulations. This simplicity made bifurcation analysis possible, which revealed a region of interesting behaviour that is bistable between oscillatory and steady-state activity. Such behaviour was not seen in the model presented in Holgado et al. (2010), presumably as it only occurs when the degree of STN self-excitation is non-zero and this was not the case in the model of Holgado et al.

Another previous modelling study, by Gillies et al. (2002), considered a population-level model of the STN-GPe circuit that is also very similar to our isolated channel model. They described three different states for the system: a single fixed point, an oscillatory state that showed low frequency short periods of high activity, and a state that was bistable between two stable fixed points. All of these states are also present in the model presented in this paper. The single fixed point state corresponds to the system when healthy values of the fixed parameters are used or when the Parkinsonian values are used and the system is in region A. The oscillatory state corresponds to region C of the Parkinsonian parameter space. Finally, the parameter values that give bi-stability between two fixed points are found in region F. Gillies et al. hypothesised that this could represent the physiological mode of operation of the STN-GPe circuit but our model suggests that this is unlikely as region F represents an extremely small part of the parameter space. This means that the fixed point bistable state is very fragile and small changes in cortical input would move the system out of it. Furthermore, within region F the two stable fixed points are very close together in phase space and so the bi-stability would only switch between two very similar levels of activity.
Instead, our model suggests that the physiological state is in fact bistable between a fixed point and a limit cycle.

Berns and Sejnowski developed a population-level model of action selection in the basal ganglia that embodies the idea of multiple sensorimotor pathways (Berns and Sejnowski, 1996). Each channel in this model contains subpopulations for the cortex, striatum, GPi/e and thalamus, however the STN is modelled as a single global subpopulation that is the only link between channels. The authors consider how Parkinsonian conditions affect the ability of the model to select actions, but they do not investigate its ability to generate oscillatory behaviour in this case. This model does not contain the projection from STN back to GPe, and so cannot be used to study the possible pacemaker role of this circuit. A similar model (Gurney et al., 2001a,b) also considers the effect of dopamine depletion in terms of the failure of action selection and again does not examine the possibility of oscillations emerging. A more refined version of this model that used the same functional connectivity but with computational current-based modelling of the individual neurons within each sensorimotor channel exhibits several features that are found in experimental recordings under both healthy and Parkinsonian conditions (Humphries et al., 2006), including oscillations (although in this case only the γ band is considered). Since the mathematical complexity of this model is much greater than population-based models, mathematical analysis (such as considering the dynamical capabilities of individual channels) becomes intractable.

## 4.7 Conclusions

Our analysis in Section 4.4 revealed that isolated channels can display oscillatory activity when a set of connection strength parameters corresponding to the Parkinsonian basal ganglia are used. This result, which is in line with previous modelling studies, supports the idea that the neurons in the STN/GPe circuit may work together to generate oscillatory neuronal activity in Parkinson’s disease. Furthermore, by using bifurcation analysis we found that certain ranges of parameter values give more interesting dynamics, where stable steady state and oscillatory attractors co-exist. We described a hypothetical mechanism where this bi-stability could be exploited such that individual channels could be switched “on” and “off” and this could form part of a system for action selection. However, in the case of isolated channels, no oscillatory activity is possible unless
some degree of STN self-excitation is included, which does not appear to be a biologically realistic condition.

The inclusion of lateral coupling between the GPe populations of neighbouring channels allows the network as a whole to generate oscillations without STN self-excitation. This is true for both healthy and Parkinsonian connection strengths, although oscillations occur for a much wider range of values for cortical input and lateral connection strength in the Parkinsonian case. In most cases the coupled channels model can only produce dynamics that are common across all channels, which is clearly not useful for action selection. An exception to this is when the channels are arranged in a circle topology with an odd number of channels and strong lateral coupling - in this case multiple stable oscillatory attractors exist. It is also possible to break the symmetry of the model by providing different levels of cortical input to each STN subpopulation.

The difficulty with attempting to use mathematical and computational modelling to understand pathological oscillations in the Parkinsonian basal ganglia is that our understanding of the electrophysiology and connectivity of neurons in these regions is currently quite limited, even in relatively simple mammals such as rats. We have largely dealt with this problem in this chapter (and in Chapter 3) by adapting previously developed models, but we have still had to make numerous assumptions, particularly in regard to details of synaptic dynamics and connectivity. In the next chapter we will study the role of oscillations in a computational model of a much simpler (and better understood) neuronal locomotor circuit.
Chapter 5

Studying the Mechanisms Underlying Oscillatory Motor Pattern Generation in *Xenopus* Tadpoles

In Chapters 3 and 4 we investigated oscillations in the motor areas of the mammalian basal ganglia. Until now our focus has been on the pathological role of oscillations in Parkinson’s disease. In this chapter, however, we will primarily consider the functional role that oscillations play in generating movements that involve rhythmic left-right motor activity. One simple way of classifying such movements is whether the two body sides move with an anti-phase (walking, swimming front crawl) or in-phase (hopping, swimming breast stroke) pattern. Using a biologically realistic computational model of a section of *Xenopus* tadpole hindbrain and spinal cord, we will show how both types of movement can be generated by a single mechanism.

The chapter begins with a description of the development of the computational model and demonstrates how simulations of the model agree well with what is known about the neuronal activity in real tadpoles. We will then describe how we used the model to investigate the essential
features of neuronal development that allow anti-phase “swimming” behaviour to occur. The latter part of the chapter will then show how the same network is capable of producing in-phase (synchronous) motor activity under certain circumstances.

5.1 Building the computational model

Short sections of *Xenopus* hindbrain and spinal cord are able to produce a sustained pattern of alternating left-right motoneuron (MN) activity (“swimming”) in response to brief skin stimulation (see Section 1.3 or Roberts et al. (2010) for review). In addition to MNs, two other interneuron populations are active during swimming: the descending and commissural interneurons (dINs and cINs respectively). The dINs and cINs fire reliably at a similar point in the cycle to the MNs on the same body side. Three other neuronal populations are only active immediately following skin stimulation: the mechanoreceptive Rohon-Beard (RB) cells and the dorsolateral ascending (DLA) and dorsolateral commissural (DLC) neurons. A seventh type of neuron, the ascending interneurons (aINs), only fire reliably during initiation and the first few cycles of swimming.

It has long been hypothesised that many locomotor CPGs are organized as a set of half-centre oscillators (see Section 1.4). However, experimental evidence from the tadpole spinal cord suggests a different CPG organisation, with continuous populations of neurons along each side of the body that are coupled to each other, rather than discrete half-centres (Roberts et al., 2010). According to this organisation, dINs excite ipsilateral cINs, which in turn inhibit contralateral dINs, causing them to generate single anode-break excitation type rebound spikes and continue the swimming pattern. Long duration NMDA receptor activation on dINs provides the background level of depolarisation that is required for rebound firing, and this is supplied by glutamatergic synaptic drive from the DLA, DLC, and other dINs. Figure 5.1 shows the main synaptic pathways involved in the proposed CPG mechanism, along with an event diagram showing an idealised version of how swimming is initiated and maintained.

In Sautois et al. (2007) the viability of a rebound-based CPG for *Xenopus* swimming was demonstrated using a computational model. This model featured conductance-based (Hodgkin-Huxley) neurons of each of the seven types described above, with the parameters of each type chosen to match physiological properties. When the neurons were synaptically coupled together
5.1. Building the computational model

in a network similar to that shown in Figure 5.1A, RB cell stimulation reliably initiated a stable
swimming pattern of MN firing at approximately 20Hz. Although the neuron types in the model
were biologically realistic and it demonstrated that the pacemaker mechanism is viable in principle,
only one neuron of each type was included on each side of the body — limiting its biological
plausibility and utility. The model that we describe in this chapter is based on that of Sautois et al.,
but features up to 2,000 neurons and a realistic network of connections between them, representing
almost the entire spinal cord. Our model, which was implemented in SNAPPy (Section 2.1), also
features several other functionally significant improvements, such as electrical coupling between
dINs and synaptic/axonal delays.

5.1.1 Neuronal connectivity

In order to simulate the full tadpole spinal cord it is necessary to have a connectome: a complete
set of the connections—synaptic and electrical—that exist between neurons. For some animals
with relatively few neurons, such as the nematode worm C. Elegans, such connectomes have been
found entirely through experimental work (Varshney et al., 2011), but unfortunately this is not yet
possible for Xenopus tadpoles; while paired-pulse experiments can determine whether a given pair
of neurons are connected, it is clearly impractical to use this method to determine the connections
between 2,000 neurons.

Our solution is to use “virtual” connectomes which are generated using a pioneering develop-
mental approach that simulates neuronal growth within the developing nervous system in order
to determine connectivity. The connectomes are generated using a computational model of axon
growth that generalises from limited anatomical data (Borisyuk et al., 2014). This model generates
soma, dendrite and axon positions on a 2D plane, where one direction corresponds to the anatom-
ical rostro-caudal direction and the other to the dorso-ventral one. The transformation from 3D
anatomy to the 2D plane can be imagined as slicing from head to tail along the dorsal edge and
then opening up the nervous system like a book (see Figure 5.2), and is justified on the basis that
the marginal zone, where the majority of axons are located, is a very thin (∼ 10µm) sheet. The
growth model generates virtual connectomes according to the following steps (some minor details
omitted for clarity):
Chapter 5. Studying the Mechanisms Underlying Oscillatory Motor Pattern Generation in *Xenopus* Tadpoles

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(A) Schematic overview of the presumed CPG network. Note that the neuronal populations that are only active during initiation (RBs, DLCs and DLAs) on the left hand side are omitted here, as are all aINs. This diagram shows the minimum set of connections that are required for the proposed pattern generation mechanism; in reality most of the neuron types synapse widely. The dotted glutamatergic connections onto dINs activate slow NMDA-Rs which may be insufficient for post-synaptic firing, but provide the long depolarisation required for dIN post-inhibitory spiking.

(B) Schematic raster plot / event diagram showing how swimming is initiated and maintained in the proposed CPG. The dots represent spiking in the corresponding populations, and the lines indicate synaptic drive that leads to spiking. Arrowheads as in (A). Time not shown to scale. At $t_a$, skin touch on the right side of the tadpole causes RB cells to spike, which in turn cause the sensory pathway neurons to fire at $t_b$. The DLAs activate NMDA-Rs on dINs on the right leading to long duration sub-threshold depolarisation (not shown). The DLCs, however, provide sufficient excitatory drive for the dINs on the left to spike at $t_c$. These dINs then lead to MN and cIN firing at $t_d$, and the inhibition from the cINs crosses the body and acts to hyperpolarise the right-side dINs. These dINs are still depolarised from their DLA input, and this depolarisation drives them above their firing threshold (lowered due to inhibition), resulting in spikes at $t_e$. This excitatory dIN input causes right-side MNs and cINs to fire, and when the inhibition crosses back to the left side the cycle repeats.

Figure 5.1: Diagrams illustrating the key synaptic connections that comprise the proposed CPG, and the idealised spiking activity during initiation and swimming.
Figure 5.2: The 3D anatomy of the tadpole (A) is mapped onto a 2D plane (B) by opening the nervous system up like a book. The columns of DLC and DLA somata are shown together as the red line labelled “dli column”. Modified, with permission, from Borisyuk et al. (2014).
1. For each neuron type, generate distribute soma positions along the body.

2. Generate a straight-line dendrite for each neuron, centred on the soma position and oriented in the dorso-ventral direction.

3. Grow axons using a set of three non-linear difference equations for position and growth angle. The growth angle is updated according to neuron type dependent sensitivities to predefined molecular gradients, in addition to a random component.

4. Where the axon of one neuron crosses the dendrite of another, potentially create a synapse between the two neurons according to a synapse formation probability parameter.

Many stages of this procedure involve sampling from random distributions, for example when choosing soma positions and dendrite and axon lengths; these random distributions are generated using the limited anatomical data. The parameters which control the main stage of axon growth (growth cue sensitivities and magnitude of random variation) for each neuron type are chosen using a stochastic optimisation method, with a cost function that compares generated axons with anatomical ones. The procedure generates axon trajectories that are statistically indistinguishable from those of real neurons. We restricted our model to the 1.5mm section of spinal cord and rostral hindbrain where most anatomical data is available. The models typically contained approximately 1,400 neurons and 90,000 synapses.

5.1.2 Physiological properties of model neurons

Model dINs

Recall from Section 1.3 that dINs have a number of interesting electrophysiological properties, including only firing single spikes in response to sustained depolarising current, and the ability to fire rebound spikes upon removal of inhibition. Since these features appear to play a critical role in the swimming network (Sautois et al., 2007) it is especially important that they are present in our model dINs.

It is not known why dINs do not spike repeatedly when depolarised. Sautois et al. (2007) assume that this is an innate property that arises from the dynamics of the neurons’ active channels and choose their model neuron parameters accordingly. However, Hull (2013) showed that in a realistic
5.1. Building the computational model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>dINs</th>
<th>Others</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leak reversal potential</td>
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<td>-61</td>
<td>mV</td>
</tr>
<tr>
<td>Leak conductance</td>
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<td>2.47</td>
<td>nS</td>
</tr>
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<td>mV</td>
</tr>
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<td>Sodium max. conductance</td>
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<td>110</td>
<td>nS</td>
</tr>
<tr>
<td>Potassium reversal potential</td>
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<td>-80</td>
<td>mV</td>
</tr>
<tr>
<td>Fast potassium max. conductance</td>
<td>12</td>
<td>8</td>
<td>nS</td>
</tr>
<tr>
<td>Slow potassium max. conductance</td>
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<td>nS</td>
</tr>
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</tr>
<tr>
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</tr>
<tr>
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<td>-</td>
<td>mM</td>
</tr>
<tr>
<td>Temperature</td>
<td>300</td>
<td>-</td>
<td>K</td>
</tr>
</tbody>
</table>

Table 5.1: Membrane parameters for model dINs and non-dINs.

multi-compartmental model of a network of electrically coupled dINs, isolated dINs are able to spike repetitively and the single firing property is a result of electrical coupling. Experiments cannot yet determine which of these hypotheses is true, since it is very hard to obtain electrically isolated dINs in real tadpoles. In the absence of experimental data, we follow the work of Hull (2013) and assume that single spiking is a result of electrical coupling, since the Hull dIN model is considerably more biologically realistic than that of Sautois et al.

The equations and parameters for our model dINs mostly match those of Hull (2013), with the notable difference that in our model all neurons are modelled as single compartment point neurons. The dIN membrane parameter values are listed in Table 5.1. Each dIN is subject to four voltage gated ionic currents: sodium ($i_{na}$), fast- and slow-activating potassium ($i_{kf}$, $i_{ks}$) and calcium ($i_{ca}$). The currents across the sodium and potassium channels follow the standard Hodgkin-Huxley equations given in Section 1.5.1, while the calcium current is governed by the Goldman-Hodgkin-Katz equation (Section 2.1.1). All currents have activation gates, and the sodium current also includes an inactivation gate. The gating dynamics are given by the “alpha-beta” equations that are described in Section 1.5.1 and Table B.1 in Appendix B lists the parameters used for each gate. Note that in line with Dale (1995), one of two sets of gate parameters is used for the calcium activation gate, based on the membrane voltage.

We are assuming that the single spiking property of dINs is a result of electrical coupling, so our model clearly requires an accurate representation of gap junctions. Around 90% of randomly
selected ipsilateral pairs of dINs show some degree of electrical coupling and this property appears to be unique to dINs, since coupling is rarely seen in pairs containing any other interneuron types (Li et al., 2009). Gap junctions in SNAPPy are implemented as simple ohmic currents (Section 2.1.1) between pairs of neurons and are controlled by two parameters: conductance $g_{\text{j}}$ and distance threshold $d_{\text{j}}$; pairs of ipsilateral dINs with rostro-caudal positions within $d_{\text{j}}$ of each other are directly electrically coupled. The degree of electrical coupling between a pair of neurons can be quantified by their “coupling coefficient”: the ratio of the membrane potential changes that occur in the two neurons when a hyperpolarising current is injected into one of them. In real tadpoles this measurement is around 5–10% and decreases as the distance between neurons increases (Li et al., 2009). The passive input resistance of dINs, which should be around $272M\Omega$ (Sautois et al., 2007), is inversely proportional to gap junction conductance, since gap junctions allow injected current to spread to neighbouring neurons. We therefore had to choose values for $g_{\text{j}}, d_{\text{j}}$, and the passive leak channel conductance $g_{\text{l}}$, which gave realistic coupling coefficients and input resistance. The values we chose were: $g_{\text{j}} = 0.2nS$, $d_{\text{j}} = 100\mu m$ and $g_{\text{l}} = 1.41nS$. Figure 5.3A shows how the coupling coefficient was measured and its dependence on separation distance.

Figures 5.3B and 5.3C show how model dINs display realistic physiological features. Depolarising current injections into individual dINs elicit single spikes, while a brief hyperpolarising current during background depolarisation generates a rebound spike. Injecting depolarising current into all dINs simultaneously, to mimic the effect of bath NMDA application as in Li et al. (2010), causes rhythmic firing.

5.1.3 Other neuron types

The other neurons involved in swimming do not appear to have the same degree of specialization as dINs. We therefore chose, for the sake of simplicity, to use one set of parameters for all non-dIN neuron types. We use the parameters from model MNs of Sautois et al. (2007) as these model neurons had fairly typical properties. These model neurons have sodium and fast and slow potassium channels, but lack the calcium channels of the model dINs. Tables 5.1 and B.1 show the parameters that were used, and Figure 5.4 shows typical responses to current injections.
5.1. Building the computational model

Figure 5.3: Current injections into model dINs show their physiological properties. All synaptic conductances were set to 0nS for these experiments and only dINs on one side of the body are shown. Ai.: No spiking on removal of a hyper-polarising current injection into a dIN (dark brown trace) when it is at its rest potential. Injected current spreads to neighbouring dINs (faint brown lines, offset vertically). ii.: Coupling coefficient as a function of distance from the injected dIN. B: Sustained depolarising current only elicits a single spike, but brief inhibitory currents during depolarisation cause single rebound spikes to be fired. C: Injecting a depolarising current into the entire dIN population causes synchronised pacemaker activity. The dark brown line shows the rostral-most dIN, faint lines show dINs from along the body offset vertically.
Chapter 5. Studying the Mechanisms Underlying Oscillatory Motor Pattern Generation in Xenopus Tadpoles

Figure 5.4: Current injections into model MNs show the physiological properties of all non-dIN neurons. All synaptic conductances were set to 0nS for these experiments. A: Relatively large depolarising currents can cause repetitive spiking with wide, low amplitude, spikes. B: Smaller depolarising currents result in single spiking. Unlike dINs, there is no rebound spiking following a brief reduction in current.

Table 5.2 gives the parameters that we used for most of the synapses in the model. These match the standard parameters for AMPA, NMDA and glycinergic synapses given in Sautois et al. (2007), although we adjusted the inhibitory reversal potential, and the post-spike increment parameter ($\Delta s$) for each type from the value of 1.0 used by Sautois et al. in order to give unitary PSPs that agreed more closely with experimental results. In general each neuron type only made synapses of a single type onto other neurons, with DLCs and DLAs making NMDA synapses, RBs, dINs and MNs making AMPA synapses, and aINs and cINs making glycinergic synapses. However, dIN→dIN synapses were a mixture of AMPA and NMDA conductances, since the background NMDA excitation that dINs provide to each other appears to be important for the swimming

5.1.4 Synaptic dynamics and conductances

Table 5.2: Standard synaptic parameters for the *Xenopus* model.
5.1. Building the computational model

mechanism. In four specific cases we used different maximum synaptic conductances to those given in Table 5.2:

- RB→DLC/DLA AMPA: increased to 8nS as this was required for single RB spikes to cause post-synaptic spiking.

- DLC→dIN NMDA: increased to 1nS to cause a greater left-right asymmetry during initiation leading to a faster transition to swimming.

- dIN→dIN NMDA: decreased to 0.15nS so that the background level of dIN depolarisation during swimming better matched experimental results.

- dIN→aIN AMPA: decreased to 0.1nS so that aINs mostly only fired during the first few cycles of swimming.

5.1.5 Experimental details

We performed the main set of swimming experiments using a set of twelve generated connectomes. At the start of each simulation random Gaussian noise was added to the parameters controlling the neurons’ membrane properties (capacitance and channel conductances/permeabilities) and the strengths of synaptic connections. For the membrane properties the standard deviation of this noise was 2% of the mean values, while for synapse conductances it was 5% of the mean.

In each simulation we stimulated two RB neurons in the middle of one side of the body, which is analogous to briefly touching the skin on the tadpole’s trunk. We classified the long-term motoneuron output that emerged after this stimulation visually as either: swimming (alternating bursts of left-right activity), synchrony (repetitive MN bursts that occur together on both sides), single-side (repetitive firing on just one side of the body), and failure (no long term activity). When swimming, synchrony or single-side activity was seen, the corresponding frequency was calculated by finding the peak of the spike cross-correlation between all MNs on one side. Additionally, for swimming and synchrony cases we also calculated the left-right phase by dividing the peak of the cross-correlation between MNs on the left and right sides by the period of swimming.
Chapter 5. Studying the Mechanisms Underlying Oscillatory Motor Pattern Generation in Xenopus Tadpoles

Figure 5.5: The emergence of stable swimming activity in the model spinal cord following stimulation of two RB neurons at t=50ms, shown as: (A) membrane potential traces from example neurons; (B) spike raster diagrams. The two bursts of MN activity on the left-side immediately following stimulation are not typically seen in real experiments, but the pattern of swimming that follows matches experiments quite well. The voltage traces clearly show where dINs receive mid-cycle IPSPs (marked * on the right-side trace), leading to post-inhibitory rebound firing.

5.2 Results

5.2.1 The physiological model “swims” when stimulated

It was clear from our initial qualitative experiments that the swimming state is stable, since RB stimulation always resulted in swimming emerging. Figure 5.5 shows swimming activity in a typical simulation. The post-inhibitory rebound rhythm generation mechanism can be seen clearly, with dINs (brown) remaining relatively depolarised following a swim burst and then receiving mid-cycle inhibition when the contralateral cINs (light blue) fire. In order to compare the model with data from real tadpoles more quantitatively, we generated 100 different virtual connectomes and simulated each one with the physiological model. In 99/100 cases the resulting MN activity was classified as swimming. In a single case a failure occurred and there was no long-term activity — on investigation we discovered that in this case the two RB neurons which were stimulated made relatively few synapses onto sensory pathway neurons; stimulating an additional RB neuron or
5.2. Results

Figure 5.6: Raster plots showing dIN activity during two cycles of swimming, with (left) and without (right) gap junctions between dINs. Gap junctions act to synchronise the firing of dINs, such that their spikes occur in tighter bursts. Note that the time scale is the same in both halves of the figure.

picking a different position to stimulate did give stable swimming. The swimming frequency (17.7 ± 0.55Hz) did not vary much between trials and was well within the range seen in real tadpoles (10–25Hz; Roberts et al. (2010)). The left-right phase (0.5 ± 0.01) indicated clear anti-phase activity with little variation. In most trials a small number of dINs (median 6, range 0–22) fire twice per cycle: once during the normal swimming burst and once mid-cycle. This behaviour appears to be caused by higher than average NMDA excitation from other dINs. This extra excitation means that the affected dINs are able to reach spiking threshold before the commisural inhibition arrives.

Experiments with the physiological model suggest that gap junctions, delays, and dIN-specific properties all play important roles in swimming. When we removed all gap junctions from the model it still generated swimming activity, albeit at a slightly higher frequency (20.0 ± 0.64 Hz, n=12), but spiking activity was very “messy” (Figure 5.6). This is in line with the results of previous experimental (Li et al., 2009) and modelling (Hull, 2013) studies, which have shown that gap junctions act to synchronize dIN firing. Since removing gap junctions increases the input resistance of dINs to non-realistic values, we tried increasing the leak channel conductance (from 1.41 to 3.96nS) to compensate, but we were not able to get any stable swimming activity under these conditions. If synaptic delays were removed then the model still swam (12/12 trials), but at a frequency that was higher than is seen in real tadpoles (27.6 ± 1.25 Hz). That the network can swim without delays is perhaps unsurprising, since the theoretical rhythm generation mechanism does
not rely on delays between neurons. Finally, if we disregarded the special properties that our model dINs have and gave them the same parameters all other types, we were unable to start swimming in any simulations as expected. Conversely, if all neurons were given the same parameters as dINs then a stable pattern emerged (12/12 trials) where the neurons on the non-stimulated side fired repetitively while those on the stimulated side were quiescent.

While the characteristics of swimming in the model quite accurately matched those of real tadpole swimming activity, some aspects of model’s activity during the transient “initiation” period after stimulation are not seen in real animals. There was often a period of synchrony (median 2 cycles, range 0–4, n=12), where some dINs and MNs on both sides would fire in-phase at roughly double the swimming frequency. Although some synchronous activity is sometimes seen before swimming in dINs in vivo, this is very rarely seen in MNs. This may indicate that the level of NMDA input that dINs receive from the DLCs and DLAs following sensory input may be too high, triggering pacemaker firing; however we were unable to improve the situation via simple changes to the sensory pathway synaptic strengths. Another difference between initiation in our model and real animals is that in vivo swimming can start on either side of the body, whereas in the model it always starts on the opposite side to stimulation. The model’s behaviour is caused by the difference in maximum conductance between DLA→dIN and DLC→dIN synapses (0.29 vs. 1.0nS), since reversing the asymmetry caused swimming to always begin on the stimulated side. Removing the asymmetry and making both sets of synapses equally strong still usually gave stable swimming (11/12 trials), but introduced a very long silent period (216 ± 76ms) between the initial activity following stimulation and the first burst of MN swimming activity. A final clue that the initiation pathway in our model is overly simplistic is that the first MN spikes occur after only 19.6 ± 0.49ms (n=12) in the model, versus approximately 44ms in real tadpoles. Many of these differences in initiation are likely to be because we have crudely used identical neuronal properties for all non-dIN neurons, and many of these neurons play important roles in the sensory pathway and swimming initiation.

The physiological model effectively allowed us to “test” virtual connectomes to see if they were capable of producing swimming behaviour. We could therefore investigate which aspects of neuronal development are important for swimming, by modifying the axon growth and connectome generation model and testing the resulting connectomes. The standard growth model is already
reasonably unconstrained, since, for example, when an axon meets a dendrite the probability that a synapse will form is completely independent of the post-synaptic neuron type. However, we found that we could relax the constraints considerably further and the model would still swim. Increasing the synapse formation probability to 1, such that a synapse always formed when an axon met a dendrite, increased the total number of synapses from approximately 90,000 to around 135,000 but did not affect the model tadpole’s ability to swim (12/12 trials), though the swimming frequency did increase (23.2 ± 0.6Hz). In another experiment the dendrites of all the CPG neurons were made to stretch from the dorsal edge of the marginal zone down to the floor plate, effectively disregarding all anatomical data about the dorsoventral position of these neurons and the length of their dendrites. Due to the much larger dendritic area, huge numbers of synapses (~150,000) were generated in this case, and the resulting connectomes could not swim. However, if the synapse formation probability was reduced such that a more typical number of synapses were generated (~85,000), swimming was once again stable.

The fact that many of the features of tadpole anatomy that were included in the growth model do not appear to be necessary for swimming was somewhat unexpected. In terms of evolution, however, it makes some sense: a hatchling tadpole faces many threats and must have a robust mechanism for avoiding them if it is to survive. From this perspective it would be surprising if there was a very precise set of developmental conditions required in order for the tadpole to swim. We also found during our initial qualitative experiments that the swimming state was extremely stable, and it is therefore perhaps not too surprising that making changes to the network largely do not result in swimming becoming unstable, as long as the changes preserve commisural inhibition and the ability of dINs to fire post-inhibitory spikes.

5.3 The multi-stable dynamics of the virtual spinal cord

We worked with another group to investigate the mechanism responsible for transient periods of synchronous left-right motoneuron activity that is sometimes seen during experimental recordings from Xenopus tadpoles. These synchrony bouts, which serve no currently known functional purpose, consist of several (14–62; Li et al. (2014)) bursts of motoneuron activity that occur at the same time on both sides of the body, with a frequency that is double that of swimming. Our
collaborators hypothesized that synchrony bouts are triggered by occasional mid-cycle spiking by dINs, but cannot test this hypothesis in real tadpoles due to technical limitations. We therefore wanted to use our computational model to investigate the dynamics of synchrony.

### 5.3.1 Changes to model parameters

It is important to remember that no model can be 100% correct, since different experimental groups can have different interpretations of limited experimental data and therefore have different opinions on what “correct” is. Before beginning our investigation of synchrony we made a number of minor changes to the model’s parameters so that it better matched the new group’s interpretation of the biological evidence. Four main changes were requested:

- The mechanism by which dINs fire single spikes in response to depolarising current should be intrinsic, not reliant on gap junctions.
- dINs should not include a fast potassium current, as voltage clamp measurements do not indicate a fast component.
- Saturation at NMDA synapses should be included, as this is potentially important for sustaining synchrony.
- In our original model, aINs only fired during the first few cycles of swimming. In the tadpoles that were used for synchrony experiments, however, the firing reliability\(^1\) for aINs during swimming was 64%.

We achieved the first two items on this list by removing the fast potassium current from dINs and increasing the maximum conductance of the slow potassium current to compensate. The synaptic saturation feature (see Section 2.1.3) was added to SNPPy in order to satisfy the third requirement, with the saturation level set to 5.0. We made various changes to maximum synaptic conductances in order to increase aIN firing reliability whilst still preserving stable swimming, including reducing the maximum conductance of all inhibitory synapses made by aINs to 10% of the corresponding synapses made by cINs, in line with biological data. Table 5.3 shows the firing reliability\(^1\) for aINs during swimming was 64%.

\(^1\)Here we define the firing reliability of a neuron as the percentage of swimming or synchrony cycles in which it fires a spike.
5.3. The multi-stable dynamics of the virtual spinal cord

<table>
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<tr>
<th>Neuron Type</th>
<th>Original Model</th>
<th>Modified Model</th>
<th>Experimental</th>
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<td>98%</td>
<td>99%</td>
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<tr>
<td>cINs</td>
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<tr>
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<td>86%</td>
</tr>
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</table>

Table 5.3: Swimming reliability measures for each neuron type in the original and modified models, and from experiments.

Table B.2 in Appendix B lists all changes that were made between the two versions of the tadpole model. It is important to note that these changes did not make a big difference to the overall dynamics of the system, and we believe that all of the results described in the previous section would be equally true in the new model.

5.3.2 Inducing synchrony with mid-cycle dIN spiking

The idealized swimming CPG circuit shown in Figure 5.1A suggests a possible theoretical mechanism by which synchronous activity patterns can be generated. Assume that both sides of the network are identical and start in the same state. If the dINs on both sides are driven to spike at precisely the same time then shortly afterwards both dIN populations will receive cIN inhibition simultaneously. Some time later all dINs will together fire again due to the rebound mechanism, and another cycle of synchrony will begin. According to this mechanism the frequency of synchrony must necessarily be double that of swimming, since a swimming cycle lasts for twice the length of time it takes a dIN to fire a rebound spike following inhibition (∼25ms). Could the occasional mid-cycle firing of individual dINs that is sometimes seen in experiment recordings cause the onset of transient synchrony bouts? Attempts to show this experimentally by using injected currents to elicit mid-cycle dIN spikes failed, but it is possible that this was because mid-cycle spiking could only be induced in a single dIN at a time due to technical restrictions. Our computational model does not have this limitation of course, and so we investigated whether injecting depolarising currents to multiple dINs mid-cycle could trigger synchrony bouts.

After much trial and error we arrived at a protocol that could reliably induce periods of synchronous activity through mid-cycle dIN stimulation. First, swimming was initiated with depo-
Figure 5.7: Firing reliability for CPG neuron types during swimming and synchrony, in both our computational model (left) and experiments performed by our collaborators (right). There is a clear decrease in firing reliability in all neuron types during synchrony.

larising current stimulation (5ms, 150pA) of four RB cells on the right side of the body. Then, at the time of the first left hand side dIN spike that occurred at least 400ms (eight swim cycles) after the swim-initiating current, further depolarising currents (5ms, 150pA) were injected to a set of right-side dINs. This stimulation caused the injected right-side dINs to fire approximately in the middle of the normal left-side dIN burst. Although stimulating only small clusters of dINs mid-cycle could induce short synchrony periods, the longest and most reliable synchrony bouts occurred when the injection was to all right-side dINs (118 neurons), so this is what we chose to do in all subsequent experiments. Only one connectome was used for all synchrony experiments, but for each experiment we still ran twelve simulations with different membrane and synapse parameters, as described above.

The procedure above always produced transient bouts of synchronous motoneuron activity (12/12 trials). Like the spontaneous synchrony seen in real tadpoles, these bouts had a wide range of durations (model: 6–47 cycles; in vivo: 14–62). Model synchrony bouts were, however, considerably shorter on average than the spontaneous bouts seen in real tadpoles (model: 16 ± 11 cycles; in vivo: 30 ± 4). The frequency of synchronous firing, calculated as the peak of the spike auto-correlation function, was 43.7 ± 0.5Hz — very close to double that of swimming (20.4 ± 0.1Hz) as expected — and the average phase of right-side MN spikes relative to left-side ones was 1. As is the case in vivo, the firing reliability of all CPG neuron types was lower decreased during synchrony, although the reduction in aIN reliability that occurs in the model is particularly
5.3. The multi-stable dynamics of the virtual spinal cord

Figure 5.8: Membrane potential traces (A) and spike raster plots (B) showing transient synchronous motoneuron activity. The synchrony is induced by a 5ms 150pA depolarising current injection to all right-side dINs at t=450ms, shown as the shaded region in (A). Synchrony persists for eleven cycles and is double the frequency of swimming.

dramatic (see Figure 5.7). In all cases synchrony terminated and swimming returned suddenly, with little or no drifting of phase or frequency, as expected. Figure 5.8 shows voltage traces and raster plots for a typical bout of synchrony in the model.

5.3.3 Commissural delays stabilise synchrony

The fact that the length of synchrony bouts varied greatly despite a very rigid protocol for the initiation of synchrony suggests that the synchronous state is very unstable. According to the proposed synchrony mechanism, dINs on both sides of the body must spike at very similar moments in time every cycle for synchrony to persist. If the dINs on one side fire slightly earlier than those on the other then the resulting inhibition (from cINs) may prevent the later side dINs from firing; in fact these neurons will fire some time later as a result of post-inhibitory rebound and the system will switch back to stable swimming. We jittered dIN spike timing on one side by injecting a small depolarising current (3ms, 100pA) into all left-side dINs shortly before they fired at the end of the fifth synchrony cycle — this brought forward spike times by 2.3 ± 0.5ms and reliably switched synchrony back to swimming within a few cycles (Figure 5.9). The average total length of
Figure 5.9: Spike raster plots of left-side spiking activity showing termination of synchrony. (A) shows a period of synchronous activity that was induced according to the procedure described in the text. (B) shows a simulation using identical parameters and initial conditions, but with an additional 3ms 100pA depolarising current injection to all left-side dINs at t=561ms (shaded area). This current injection causes the left-side dINs to fire slightly earlier than normal, and results in a rapid termination of synchrony. The right-side activity is not shown, but swimming and synchrony can be distinguished by burst frequency.

synchrony when jitter was applied after five cycles was 6 ± 1 cycles (range: 5–9). This experiment illustrates the fragile nature of synchrony and how it depends on very precise spike timing in CPG neurons.

Increasing synaptic and axonal delays along the comissural dIN→cIN→dIN pathway should act to make the synchronous state more stable. If dINs on one side fire earlier than those on the other, a delay means that the dINs on the later side still have some time in which to spike before the inhibition arrives. We increased the fixed delay at dIN→cIN synapses from 1ms to 1.25ms and induced synchrony according to our usual protocol. In 10 out of 12 trials this resulted in a transient period of synchrony that was, surprisingly, shorter on average (11.7 ± 4.8 cycles, range 7–24, n=10) than with the original delay, although given the large range of values and small number of trials this may not be significant. In two cases, however, once initiated synchrony persisted for the entire simulation time (>100 cycles) and appeared to be a stable regime. Progressively lengthening the delay to 1.375 and then 1.5ms increased the proportion of trials in which the induced synchrony was apparently stable in each case, and with delays of 2 or 5ms every trial showed “stable” synchrony. Note that even with the longest delays the model swam normally if we didn’t induce synchrony, suggesting that both swimming and synchrony are stable in these regimes.
5.4 Conclusions

In this chapter we developed a biologically realistic model of spiking activity in the tadpole spinal cord based on detailed experimental evidence. Tadpole swimming is hypothesised to rely on two key features of the spinal network: ipsilateral excitation between dINs from slow NMDA synapses, which provides a background level of depolarisation, and commissural inhibition from cINs which causes dINs to fire delayed post-inhibitory rebound spikes. Our model confirmed that these two features can indeed work together to produce physiological swimming activity in a large spiking model of almost the entire spinal cord, with realistic neuronal properties and synaptic connectivity. We found that the swimming state is extremely robust to dynamic perturbations (e.g. current injections), parameter noise, and even some changes to the connection architecture. Other biological features were found to enhance the realism of swimming activity but were not critical for stability. Gap junctions, for example, caused tighter motor activity bursts, while including synaptic delays slowed down the swimming frequency to a realistic level.

We also used our model to investigate a proposed mechanism for the experimental observation that the same spinal cord network can produce transient periods of synchronous left-right motoneuron activity at double the swimming frequency. We confirmed that with suitable perturbations (mid-cycle current injection) the model could generate transient synchrony. The basin of attraction for synchrony is extremely small (or non-existent) when the standard parameters are used. However, small increases to the delay associated with inhibitory commissural axons dramatically increases the size of this basin of attraction. With longer commissural delays, the system can generate two stable oscillatory patterns: in-phase and anti-phase. We tentatively suggest that this may correspond to the switch from anti-phase to in-phase swimming that occurs as tadpoles develop into adult frogs. This type of bistable CPG based on reciprocal delayed inhibition could underlie the ability of animals, such as the salamander (Ijspeert, 2008), to produce both anti-phase and (faster) in-phase oscillatory motor patterns.
Chapter 6

Conclusions

6.1 A surprising link

In Chapter 4 we presented a population level model of the multi-channel basal ganglia and demonstrated that this model is able to produce oscillatory patterns of neuronal activity when lateral inhibition between neighbouring channels is sufficiently strong. In Chapter 5 we built a detailed conductance-based model of the *Xenopus* tadpole spinal cord and demonstrated how this model is able to produce stable left-right anti-phase patterns of motoneuron output. While these two models are clearly very different in many ways, in this section we will attempt to demonstrate that the same fundamental mechanism underlies rhythm generation in both models.

To begin, consider an isolated pair of STN and GPe subpopulations from the basal ganglia model in Chapter 4. Figure 6.1 shows the activity in these two subpopulations following a transient external inhibitory input to the GPe subpopulation. The external inhibition causes a decrease in GPe activity, which in turn causes an increase in STN activity that persists for longer than the external inhibition. When the inhibition ceases, the increased excitatory drive from the STN causes a temporary increase in GPe activity which may be greater in magnitude than the decrease that occurred during inhibition; in other words a transient inhibitory input to the pair causes a delayed inhibitory output. At a very abstract level, this is similar to what happens in the tadpole when the excitatory dIN neurons on one side of the body receive inhibitory input from the opposite side:
Figure 6.1: Behaviour of an isolated STN/GPe channel in response to a transient negative input to the GPe. Parkinsonian parameters are used for the coupling strengths and there is no STN self-excitation. A transient negative external input with magnitude 25.0 is applied to the GPe subpopulation between \( t = 100 \) and \( t = 150 \text{ms} \) (shaded area). There is a large rebound in GPe activity after the external input is removed.

in this case the dINs fire post-inhibitory rebound spikes (after some delay that is presumably a combination of the synaptic delay and hyperpolarisation block of NMDA receptors), which excites ipsilateral cINs and generates inhibitory output. In both models, inhibitory input generates delayed inhibitory output, although it must be stressed that the mechanisms which underlie this are very different between the two models.

The STN/GPe and dIN/cIN circuits can generate anti-phase rhythmic activity and can be thought of as chains of half-centre oscillators. In the multi-channel STN/GPe case (with \( N \) even and line topology), we consider consecutive pairs of channels as half-centre oscillators, such that each oscillator contains one odd and one even numbered channel. Each oscillator pair is also coupled to its neighbours by inhibitory connections. The real tadpole spinal cord cannot be divided into discrete oscillatory units, but it is nevertheless useful to consider it as a chain of half-centre dIN/cIN oscillators that have longitudinal coupling. According to this scheme, the dINs and cINs on the left side are one half-centre of each oscillator and those on the right side are the other half. Both models are composed of pairs of half-centre oscillators, each of which includes cross-centre inhibition: inter-channel lateral coupling in the STN/GPe case and commissural cIN→dIN axons.
6.2 Thesis contributions

in the tadpole. Since each half-centre produces delayed inhibitory output in response to transient inhibitory input, provided the cross-centre inhibition is strong enough both networks are able to produce stable anti-phase oscillations. In the STN/GPe model the oscillatory activity in the odd and the even numbered channels is in anti-phase, while in the tadpole model the half-centres on the left and right sides of the body are anti-phase.

Insights from the tadpole model suggest how it may be possible to produce widespread synchronised oscillations in the basal ganglia model. In Section 4.5.3 we saw that the multi-channel BG model (arranged as line with \( N \) even) could produce oscillatory activity that was synchronous across all channels, provided the initial conditions were chosen to be symmetric about the middle of the network. However, any slight breaking of symmetry in the initial conditions would cause the system to converge to the standard anti-phase attractor, suggesting that in this case a synchronous limit cycle exists but is unstable. In Section 5.3 we showed how, in the tadpole model, increasing the delay associated with commissural cIN→dIN connections had the effect of stabilising a synchronous state in which the neurons on both sides of the body spike synchronously at double the swimming frequency. It is not clear if introducing a similar delay in the lateral coupling between STN/GPe channels would have a similar effect, but if so then it may be possible to find a dynamical regime in which the basal ganglia model is bi-stable between anti-phase and (double frequency) synchronous oscillations.

6.2 Thesis contributions

A new, biologically detailed, model for studying GP network dynamics. In Chapter 3 we presented a novel model of GP neurons that features much of the biological realism of previous detailed multi-compartmental models but considerably reduced complexity (both computationally and in terms of model construction). This makes our model well suited to detailed modelling of the dynamics of networks of GP neurons and their connections with other nuclei. We have also introduced a possible computational mechanism for simulating the down-regulation of HCN channels.

Uncovering a possible mechanism for the emergence of anti-phase groups of GP neurons. We used our GP neuron model to investigate the possibility that the neurons in the
GP could become organised into two groups (TI and TA) as a result of rhythmic input from the cortex via the STN and local inhibition between GP neurons. Our results showed that this was a plausible mechanism and that the changes that occur in the basal ganglia under Parkinsonian conditions make its effects stronger, as expected from experiments. This work was published as Merrison-Hort and Borisyuk (2013).

**An oscillatory “multi-channel” model of the basal ganglia.** In Chapter 4 we developed a novel population-level model of the basal ganglia that reflected its hypothesised division into multiple information channels. By using two-dimensional bifurcation analysis we were able to understand the dynamics that a single channel is capable of, including an interesting regime in which a channel is bistable between oscillatory and steady-state activity — although oscillations in single channels were dependent on STN self-excitation. When coupling was introduced we showed that the network could produce oscillations without self-excitation. Oscillations were fairly independent of the number of channels and coupling topography, but were far more common when Parkinsonian inter-population connection strengths were used. This work was published as Merrison-Hort et al. (2013).

**Insights into the generation of swimming patterns by the tadpole spinal cord.** Working closely with experimental collaborators and using the developmental model of Borisyuk et al. (2014), we built a detailed and biologically realistic conductance based model of the network of 1,500-2,000 neurons in the *Xenopus* tadpole spinal cord. This model allowed us to study the critical physiological and developmental features of the spinal cord that allow tadpoles to swim. Our results suggested that during development many of the anatomical features that influence axon growth and synapse formation may not be as important as was previously assumed. This work was published as Roberts et al. (2014).

**Demonstrating a biological mechanism for a simple CPG to generate multiple activity patterns.** Again working closely with experimental collaborators, we were able to use our computational model of the tadpole spinal cord to test a hypothesis about how the tadpole spinal cord can sometimes produce synchronous patterns of motor activation. Our model showed that a single simple network based on commissural inhibition and post-inhibitory rebound can generate both anti-phase and in-phase oscillations. Specifically, we found that the stability of the in-phase limit cycle was critically dependent on the size of commissural axonal delays. This
work was published as Li et al. (2014). We plan to continue to use the *Xenopus* model to further understand the neuronal mechanisms of the animal’s behaviour.

**New software for simulation of large neuronal networks and exploration of dynamical systems.** We developed a piece of software named SNAPPy which is designed for simulating large networks of single-compartment Hodgkin-Huxley neurons. This software is simple yet flexible enough to let us very quickly try out new modelling ideas, and we will continue to use it for our future work. We also developed DSvis, which is an exciting tool that harnesses the massively parallel hardware found in GPUs to allow visual exploration of arbitrary systems of ODEs. Several people are already starting to use this software to explore their models, and we are working on adding features to make it easier to use. Both SNAPPy and DSvis are available to anyone through an open source licence.
Appendix A

Sample JSON SNAPPy

Configuration Files

Listing A.1: Simulation parameters file for a Xenopus simulation in which swimming and synchrony are induced through current injections.

```json
{
    "note_" : "The connectome and cell list files specify the type of each cell as an index, from 1 to 7.",
    "note_" : "This list (‘types’) specifies which file contains the parameters of each of these indices.",
    "note_" : "e.g. index 1 in the connectome/cell list files corresponds to RB cells. We use",
    "note_" : "MN parameters for non-dIN cells",
    "types" : [
        {"file" : "CellTypes/MN/MN.json", "note_" : "1: RB"},
        {"file" : "CellTypes/MN/MN.json", "note_" : "2: dIC"},
        {"file" : "CellTypes/MN/MN.json", "note_" : "3: aIN"},
        {"file" : "CellTypes/MN/MN.json", "note_" : "4: cIN"},
        {"file" : "CellTypes/dIN-Wenchang/dIN-Wenchang.json", "note_" : "5: dIN"},
        {"file" : "CellTypes/MN/MN.json", "note_" : "6: N5"},
        {"file" : "CellTypes/MN/MN.json", "note_" : "7: dIA"}
    ],
    "note_" : "The ‘gap_junctions’ property is a list of the different types of gap junction",
    "note_" : "that exist (only dIN to dIN at the moment).",
    "gap_junctions" : [
        {
            "type1" : 5,
            "note_" : "‘type1’ and ‘type2’ specify the indices of the two cell types that",
            "type2" : 5,
            "note_" : "should have gap junctions between them (e.g. dIN-dIN here)."
        },
        {
            "note_" : "The simulator creates gap junctions between cells that are close enough",
            "note_" : "together, according to this threshold. Units are micrometres.",
            "dist_thresh" : 100.0
        }
    ]
}
```
Appendix A. Sample JSON SNAPPy Configuration Files

```
"g" : 0.2,  "note_" : "g specifies the maximum conductance of a gap junction, in nS."

"synapses_types" : 
  { 
    "name" : "ampa",  "syn_type" : "standard",  "erev" : 0.0,  "tau_o" : 0.2,  "tau_c" : 3.0,  
    "step" : 1.25,  "sat" : 1.0 
  },

  { 
    "name" : "nmda",  "syn_type" : "standard",  "erev" : 0.0,  "tau_o" : 5.0,  "tau_c" : 80.0,  
    "step" : 1.25,  "sat" : 5.0,  "ele" : 0.05,  "e2" : -0.08 
  },

  { 
    "name" : "isa",  "syn_type" : "standard",  "erev" : -75.0,  "tau_o" : 1.0,  "tau_c" : 4.0,  
    "step" : 3.0,  "sat" : 1.0 
  },

"note_" : "The 'synaptic_conductances' field is a list of the synaptic connections that can exist between cells. Each pair of cells that are connected in the connectome file, 'note_" : "must have at least one synaptic conductance listed here (there can be more than one 'note_" : "e.g. BMDA and AMPA for dIN-dIN synapses).'.

"synaptic_conductances" : 
  { 
    "note_" : "RB -> dla",  "pre_type" : 1,  "post_type" : 2,  "syn_type" : "ampa",  "g" : 8.0,  "g_stdev" : 0.4,  "fixed_delay" : 1.0,  " 
    dist_delay" : 0.0035 
  },

  { 
    "note_" : "RB -> dla",  "pre_type" : 1,  "post_type" : 7,  "syn_type" : "ampa",  "g" : 8.0,  "g_stdev" : 0.4,  "fixed_delay" : 1.0,  " 
    dist_delay" : 0.0035 
  },

  { 
    "note_" : "dla -> dIN",  "pre_type" : 2,  "post_type" : 5,  "syn_type" : "nmda",  "g" : 1.0,  "g_stdev" : 0.05,  
    "fixed_delay" : 1.0,  " 
    dist_delay" : 0.0035 
  },

  { 
    "note_" : "dla -> dIN",  "pre_type" : 7,  "post_type" : 5,  "syn_type" : "nmda",  "g" : 0.20,  
    "g_stdev" : 0.015,  "fixed_delay" : 1.0,  " 
    dist_delay" : 0.0035 
  },

  { 
    "note_" : "Most synaptic conductances omitted here for brevity..." 
  },

"note_" : "The 'current_injections' property is a list of temporary current injections, 'note_" : "that are to be simulated. If multiple current injections for the same cell, 'note_" : "overlap in time, the currents will sum."

"current_injections" : 
  { 
    "note_" : "Times at which injection should begin and end (in ms).",
    "start_time" : 50.0,  
    "end_time" : 55.0,  

    "note_" : "The type of the cells to be stimulated.",
    "cell_type" : 1,  

    "note_" : "The side of the body of the cells to be stimulated.",
    "body_side" : "right",  

    "note_" : "(Inclusive) range of cells to stimulate. Note that these are relative to the",
    "note_" : "cell type and body side, so a value of 5 with cell_type=5 and side=right",
    "note_" : "corresponds to the fifth dIN on the right hand side of the body.",
    "first_cell" : 30,  
    "last_cell" : 33,  

    "note_" : "Amount of current to inject to each cell (pA).",
    "current" : 150.0 
  }
```
Listing A.2: Neuron type file for dINs in the model we used to investigate synchrony.

```json
{
  "cell_type" : "dIN",
  "note_" : "Surface area of the cell in cm^2. Capacitance, conductances and permeabilities are multiplied by this value when the file is loaded to get the 'whole cell' values that are used during simulation.",
  "sa" : 1e-5,
  "note_" : "Add some noise to the surface area parameter."
}
```

```json
{
  "cell_type" : "dIN",
  "note_" : "Surface area of the cell in cm^2. Capacitance, conductances and permeabilities are multiplied by this value when the file is loaded to get the 'whole cell' values that are used during simulation."
}
```
Appendix A. Sample JSON SNAPpy Configuration Files

"note_": "Leakage reversal potential, in mV."
"erev_lk": -52.0,

"note_": "This specifies a list of channels to load into the cell."
"channels": [  
  
  
  
  
  "note_": "File to load channel parameters from."
  "file": "Channels/na.json",

  "note_": "Maximum channel conductance, in nS/cm^2."
  "gmax": 240.5e5,

  "note_": "Channel reversal potential, in mV."
  "erev": 50.0
},

  "file": "Channels/kSlow.json",  
  "gmax": 150.0e5,  
  "erev": -81.5
},

  "file": "Channels/ca.json",  
  "pmax": 0.0,  
  "pmax": 1.425e-5,  
  "temp": 300.0
]

Listing A.3: Ionic channel specification file for the Na^+ channel in our model dINs.

{  
  "note_": "Sodium channel",
  "name": "na",

  "note_": "The equation type that should be used for calculating the maximum current."
  "i_eqn": "standard",

  "note_": "This specifies a list of the channel’s gates. The maximum current is multiplied by the value of each gating variable, to the power specified here."
  "gates": [  
  "name": "h",
  "power": 1,
  "equations": [  

  "note_": "Steadystate expression for each gate.",
  "name": "h",
  "power": 1,
  "equations": [  
    "steady_state": [  
      "eqn": "alpha*beta*",  
      "tau": [  
        "eqn": "alpha*beta*",  
        "alpha_coeffs": "0.08,0.0,0.0,0.0,0.0,0.0",  
        "beta_coeffs": "0.08,0.0,0.0,0.0,0.0,0.0"
      ]  
    ]  
  ]  
  
  "note_": "Steadystate expression for each gate.",
  "name": "m",
  "power": 3,
  "equations": [  
    "steady_state": [  
      "eqn": "alpha*beta*",  
      "tau": [  
        "eqn": "alpha*beta*",  
        "alpha_coeffs": "4.08,0.0,0.0,0.0,0.0,0.0",  
        "beta_coeffs": "0.08,0.0,0.0,0.0,0.0,0.0"
      ]  
    ]  
  ]  
}
Listing A.4: Plotting parameters for a plot of membrane potentials, spikes, and NMDA synaptic activation in the tadpole model.

```
"note_": "The colours to use for plotting spikes of each neuron type ".
"cell_colours": ["#ff3232", "#ff0000", "#f1f5fa", "#5ba9da", "#66c19e", "#00998c", "#ffaa8c"],

"note_": "Plots with the given time ranges will be automatically generated.",
"time_windows": [[0, 2000], [0, 250], [800, 1000]],

"plots": [*

"note_": "The first plot shows membrane potential traces for a cIN and dIN on the right-hand side of the body.",
"type": "traces",
"title": "cIN, dIN (Right)",
"y_min": -80, "y_max": 40,
"y_axis_label": "mV",
"traces": [
  {
    "comment": "cIN",
    "expression": "\t v",
    "cell_range": {"start": 241, "end": 241, "step": 4, "type": 4 },
    "colour": "#00aadc"
  },
  {
    "comment": "dIN",
    "expression": "\t v",
    "cell_range": {"start": 158, "end": 158, "step": 4, "type": 5 },
    "colour": "#96501e"
  }
]

"note_": "The second plot shows spikes from all neurons on the right-hand side of the body.",
"title": "Spikes (Right)",
"y_axis_label": "Position (\t \mu m \t)",
"y_min": 500, "y_max": 2000,
"note_": "Raster plot, so draw spikes rather than variable values.",
"type": "raster",
"cell_range": {"start": 692, "end": 1382, "step": 1 },

"note_": "The third plot shows spikes from all neurons on the left-hand side of the body.",
"title": "Spikes (Left)",
"y_axis_label": "Position (\t \mu m \t)",
"y_min": 500, "y_max": 2000,
"type": "raster",
"cell_range": {"start": 1, "end": 691, "step": 1 },

"note_": "The fourth plot shows membrane potential traces for a cIN and dIN on the left-hand side of the body.",
"type": "traces",
"title": "cIN, dIN (Left)",
```
Appendix A. Sample JSON SNAPPy Configuration Files

```
"y_min": -80, "y_max": 40,
"y_axis_label": "mV",
"traces": [
  { "comment": "cIN",
    "expression": "$v",
    "cell_range": { "start": 49, "end": 49, "step": 4, "type": 4 },
    "colour": "#00aadc"
  },
  { "comment": "dIN",
    "expression": "$v",
    "cell_range": { "start": 217, "end": 217, "step": 4 },
    "colour": "#96501e"
  }
],

"note": "The fifth plot shows the activation of NMDA synapses that are"

"note": "made by a particular neuron.",
"type": "traces",
"title": "Example LHS dIN -> dIN NMDA Activation",
"y_min": -0.01, "y_max": 2.0,
"y_axis_label": "nS",
"traces": [
  { "expression": "($nmda_c - $nmda_o)",
    "cell_range": { "start": 236, "end": 236, "step": 4 }
  }
]
```
Appendix B

Parameters for Xenopus

Physiological Model
Appendix B. Parameters for Xenopus Physiological Model

### I Gate Power Equation A B C D E

<table>
<thead>
<tr>
<th>I Gate</th>
<th>Power</th>
<th>Equation</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
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<tr>
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<td>α</td>
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<td>β</td>
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<td>-12.56</td>
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<tr>
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<td>β</td>
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<td></td>
<td>5.73</td>
<td>0.0</td>
<td>5.01</td>
<td>9.69</td>
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Table B.1: Channel gating parameters for model dINs and non-dINs.

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<thead>
<tr>
<th>Parameter</th>
<th>Original</th>
<th>New</th>
</tr>
</thead>
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<tr>
<td>dIN fast potassium max. conductance (nS)</td>
<td>12.0</td>
<td>-</td>
</tr>
<tr>
<td>dIN slow potassium max. conductance (nS)</td>
<td>9.6</td>
<td>150.0</td>
</tr>
<tr>
<td>AMPA synapse saturation $\Delta s_{sat}$</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>NMDA synapse saturation $\Delta s_{sat}$</td>
<td>-</td>
<td>5.0</td>
</tr>
<tr>
<td>Glycine synapse saturation $\Delta s_{sat}$</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>dIN→dIN NMDA synapse max. conductance (nS)</td>
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<td>0.29</td>
</tr>
<tr>
<td>dIN→cIN AMPA synapse max. conductance (nS)</td>
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<td>0.65</td>
</tr>
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<td>dIN→aIN AMPA synapse max. conductance (nS)</td>
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<td>0.3</td>
</tr>
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<td>dIN→MN AMPA synapse max. conductance (nS)</td>
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<td>aIN→All Glycine synapse max. conductance (nS)</td>
<td>0.435</td>
<td>0.0435</td>
</tr>
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Table B.2: Changes made to the Xenopus model when studying synchrony mechanisms.


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Bibliography


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An Interactive Channel Model of the Basal Ganglia: Bifurcation Analysis Under Healthy and Parkinsonian Conditions

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Abstract Oscillations in the basal ganglia are an active area of research and have been shown to relate to the hypokinetic motor symptoms of Parkinson’s disease. We study oscillations in a multi-channel mean field model, where each channel consists of an interconnected pair of subthalamic nucleus and globus pallidus sub-populations.

To study how the channels interact, we perform two-dimensional bifurcation analysis of a model of an individual channel, which reveals the critical boundaries in pa-
rameter space that separate different dynamical modes; these modes include steady-
state, oscillatory, and bi-stable behaviour. Without self-excitation in the subthalamic
nucleus a single channel cannot generate oscillations, yet there is little experimental
evidence for such self-excitation. Our results show that the interactive channel model
with coupling via pallidal sub-populations demonstrates robust oscillatory behaviour
without subthalamic self-excitation, provided the coupling is sufficiently strong. We
study the model under healthy and Parkinsonian conditions and demonstrate that it
exhibits oscillations for a much wider range of parameters in the Parkinsonian case. In
the discussion, we show how our results compare with experimental findings and dis-
cuss their possible physiological interpretation. For example, experiments have found
that increased lateral coupling in the rat basal ganglia is correlated with oscillations
under Parkinsonian conditions.

**Keywords** Parkinson’s disease · Mean-field model · Bifurcation analysis · Beta
oscillations · Subthalamic nucleus · Globus pallidus · Wilson–Cowan equations

1 Introduction

The basal ganglia are a group of densely interconnected subcortical nuclei comprising
of the striatum, globus pallidus, subthalamic nucleus (STN), and substantia nigra.
Cortical projections to the ventral striatum and STN provide input to the basal ganglia
from almost all areas of the cortex [1–3]. In primates efferent output from the basal
ganglia innervates ascending and descending neurons in the thalamus and brainstem,
via the internal segment of the globus pallidus (GPI) and the substantia nigra pars reticulata (SNr) [4, 5]. The basal ganglia therefore appear to be in a key position to
modulate the flow of information along motor and sensory pathways.

Parkinson’s disease is primarily a disease of the basal ganglia. Its main pathophys-
iological feature is the death of the neurons in the substantia nigra pars compacta
(SNc) that provide widespread dopaminergic innervation to the other basal ganglia
nuclei [6]. Electrical recordings from animal models of Parkinson’s disease and pa-
tients undergoing functional neurosurgery have revealed several characteristics of the
electrical activity in the Parkinsonian basal ganglia that presumably arise as a result
of this loss of dopaminergic input. Perhaps the most well studied of these pathologi-
cal features is a marked increase in the degree of widespread synchronised oscillatory
activity within the STN and GPi. This increased synchrony is shown by an increase
in spectral power of the local field potential (LFP) signal recorded from these nu-
clei, particularly within the so-called β frequency band (8–30 Hz) [7]. LFP power
in this range decreases when patients are taking the dopamine prodrug L-DOPA and
has been shown to be positively correlated with the severity of the main hypokinetic
motor symptoms of Parkinson’s disease: bradykinesia and rigidity [8]. Although in
general LFPs appear to better represent subthreshold synaptic currents rather than
widespread spiking activity [9], several studies have found that (in the STN at least)
the LFP signal is indeed linked to the activity of local neurons [10–12].

There is some experimental evidence that supports the idea that excessive levels
of synchronous β activity are the causal basis for bradykinesia and rigidity [13].
Macro-electrode stimulation of the STN at 20 Hz reduces the speed of movement
during a finger tapping task [14] and slows force development in a grip task [15]. Exactly how synchronous \( \beta \) activity could have an anti-kinetic effect remains to be seen. One theory considers the basal ganglia as one or more information channels in which the available bandwidth corresponds to the degree of independence between neurons [16]. In the pathological case where many neurons have become entrained to fire synchronously in time with a particular rhythm then the ability for the basal ganglia to convey meaningful information would clearly be limited. An alternative hypothesis holds that \( \beta \) oscillations are a globally coherent signal that correspond to tonic maintenance of the current pose [17]. This is supported by evidence from monkeys [18] and humans [19] that shows that during tonic muscle activity there are widespread synchronous \( \beta \) oscillations in both the central and peripheral nervous system. It is suggested that this oscillatory activity may be subject to modulation in the basal ganglia, with dopamine acting as an indicator of movement-related stimuli that reduces the level of synchronous \( \beta \) activity [20].

Understanding the nature of abnormal \( \beta \) oscillations in Parkinson’s disease may lead to new treatments and, more generally, insights into the motor functions of the basal ganglia. Two important questions are where the oscillations arise and the mechanism by which they are generated. While it is possible that they are of cortical origin [21], Parkinson’s disease primarily affects the nuclei of the basal ganglia so it seems plausible that these nuclei are somehow involved in the generation of \( \beta \) rhythms. Two potential sources within the basal ganglia that have been suggested by experimental and theoretical work are the striatum and the reciprocally connected neurons of the STN and external segment of the globus pallidus (GPe). LFP recordings from healthy monkeys show transient \( \beta \) oscillations that are synchronous across large areas of the striatum [22]. In [23], McCarthy et al. develop a computational model of a network of striatal medium spiny neurons that shows a peak in the \( \beta \) power of a simulated LFP that increases under reduced dopamine conditions. The basis for these oscillations in their model is a non-inactivating potassium current known as “M-current”. A key prediction of the model, that increased striatal acetylcholine levels will increase \( \beta \) power, was verified in rodent experiments as part of the same study.

The reciprocally connected neurons of the GPe and STN have been more extensively studied as a possible source of \( \beta \) oscillations than the striatum. Intra-cellular tracing studies suggest that both the inhibitory GABAergic projection from GPe to STN and the excitatory glutamatergic projection from STN to GPe show a great degree of spatial selectivity, with individual groups of pallidal neurons projecting to individual groups of subthalamic neurons, which in turn project back to their afferent pallidal neurons [24]. Since STN neurons are capable of rebound firing upon release from GABA-mediated hyperpolarization [25], this arrangement suggests that reciprocally connected groups of STN-GPe neurons may be able to act as pacemaker circuits. In vitro co-cultures of cortical, striatal, pallidal, and subthalamic cells show that neurons in the GPe-STN circuits are indeed capable of generating oscillatory firing patterns in the absence of rhythmic inputs [26]. Experiments in Parkinsonian primates in which synaptic connections in the basal ganglia were selectively blocked demonstrated that \( \beta \) oscillations were dependent on glutamatergic input to the STN and the reciprocal connections between the STN and GPe [27].

Computational and mathematical modelling has also demonstrated that the GPe and STN are capable of acting as a pacemaker circuit. The detailed conductance-
based models of Terman et al. [28] show that GPe-STN networks with various topologies are capable of producing a wide range of different activity patterns, including transient oscillations. An average firing rate model of a coupled pair of GPe and STN populations suggests that robust β-band oscillatory activity is possible provided that self-excitation within the STN population exceeds a certain level [29]. It seems unlikely, however, that STN neurons exert any excitatory influence over other STN neurons since there is no evidence of local axon collaterals or gap junctions within the nucleus [30, 31]. A similar modelling study has demonstrated that with the addition of synaptic delays, coupled GPe-STN populations can generate β oscillations without any STN self-excitation [32].

The aim of this work is to extend the population-level models of [29] and [32] to investigate the behaviour when multiple interactive groups (or “channels”) of GPe and STN neurons are present. This is based on the idea that information flows through the basal ganglia in circuits that remain largely segregated [3, 33, 34]. It should be noted that it is unclear from the current biological data to what level of representation this segregation is maintained and, therefore, whether channels correspond to body regions, limbs, individual muscles or even particular motor actions. In general, we do not assume any level of representation and simply seek to identify what dynamics are possible in a system of coupled parallel channels. We initially study a single isolated channel and use two-parametric bifurcation analysis to find the critical boundaries in parameter space that separate regions of different dynamics. This bifurcation analysis provides useful guidance for the study of the collective behaviour of locally coupled channels (arranged in either a circle or line topology); in particular it suggests parameter values that correspond to oscillatory dynamics. Additionally, while [29] studied the dynamics of the system under changing levels of excitatory and inhibitory striatal GPe input, the neurons that project from striatum to GPe are usually silent [35]. We investigate the possibility that the direct cortical projection to the STN (the “hyper-direct” pathway) plays an important role in modulating pallidal and subthalamic activity [36].

Section 2.1 will describe the model and introduce its equations and parameters. In Sect. 3, we will present the results of bifurcation analysis and numerical simulations of the model in the case of a single uncoupled channel. These results are used to inform the analysis of the locally coupled model, which we will present in Sect. 4. Section 5 will discuss a possible physiological interpretation of our results and compare them with previous experimental and theoretical studies.

2 Methods

2.1 Model Description

The model consists of 2N coupled non-linear differential equations, where N is the number of channels being modelled:

\[
\tau_s \ddot{x}_i = -x_i + Z_s (w_{ss} x_i - w_{gs} y_i + I) \quad (1)
\]

\[
\tau_g \ddot{y}_i = -y_i + Z_g \left( -w_{gg} y_i + w_{gs} x_i - \alpha w_{gg} \sum_{j \in L_i} y_j \right), \quad i = 1, 2, \ldots, N \quad (2)
\]
These equations are based on those developed by Wilson and Cowan [37]. The time-dependent variables $x_i$ and $y_i$ represent the mean field activity of the excitatory STN subpopulation and inhibitory GPe subpopulation of channel $i$, respectively. Taken together the equations represent a pair of reciprocally connected STN-GPe sub-populations corresponding to one of many hypothesised basal ganglia information channels [34]. The connection strength parameters ($w_{ss}$, $w_{sg}$, $w_{gg}$, and $w_{gs}$) are non-negative and represent the strength of synaptic connectivity within and between the populations, where $w_{pq}$ is the connection strength from population $p$ to population $q$ (e.g. $w_{sg}$ is the synaptic connectivity from STN to GPe). $\tau_s$ and $\tau_g$ represent the average membrane time constants of neurons in the two populations, while $I$ represents a constant level of cortical excitation of the STN (the hyper-direct pathway). For simplicity, this study is restricted to the case when there is the same degree of constant cortical input to each of the channels.

Connections between the channels take the form of lateral inhibition between GPe sub-populations. The strength of this lateral coupling is taken to be a proportion $\alpha$ of the coupling strength within GPe sub-populations ($w_{gg}$), where $\alpha \geq 0$. Different connection schemes are possible and are specified by the term $L_i$ in Eq. (2). For a given channel $i$, $L_i$ is a set of indexes that specifies which channels the GPe subpopulation receives inhibition from. In this study, we consider only local connections to immediate neighbours, with two different arrangements of channels: on a line (Eq. (3)) and on a circle (Eq. (4)).

$$L_i = \begin{cases} 
\{i - 1, i + 1\} & 1 < i < N \\
\{i + 1\} & i = 1 \\
\{i - 1\} & i = N
\end{cases} \quad (3)$$

$$L_i = \begin{cases} 
\{i - 1, i + 1\} & 1 < i < N \\
\{i + 1, N\} & i = 1 \\
\{i - 1, 1\} & i = N
\end{cases} \quad (4)$$

The system is non-linear due to the functions $Z_s(\cdot)$ and $Z_g(\cdot)$, which represent how different levels of synaptic input influence the activity of the population. The functions are sigmoidal in shape and are described by Eq. (5):

$$Z_j(x) = \frac{1}{1 + \exp(-a_j(x - \theta_j))} - \frac{1}{1 + \exp(a_j\theta_j)} \quad (5)$$

Here, $j = s$ or $g$. This adds four new parameters, $a_s$, $a_g$, $\theta_s$, and $\theta_g$, which represent the maximum slope of the sigmoid and its position on the horizontal axis respectively, for STN and GPe sub-populations. The constant term that is subtracted in Eq. (5) is used in the Wilson–Cowan formalism to ensure that $Z_j(0) = 0$, which means that when a subpopulation receives no inputs its activity tends to a single stable fixed point [37]. The model is summarised in Fig. 1. Note that the cortex is not modelled as a population, it simply provides a constant level of input to each STN subpopulation.
2.2 Parameter Values

For the values of $a_s$, $a_g$, $\theta_s$, and $\theta_g$ we use the typical values for excitatory and inhibitory sub-populations specified by Wilson and Cowan [37]. For the remaining fixed parameters, the values determined by Holgado et al. [32] are used. Due to a lack of experimentally determined electrical characteristics of neurons in the primate basal ganglia, the membrane time constants used are those from rodent studies. It should be noted, however, that neurons in the rodent globus pallidus appear to vary widely in their electrical characteristics, and the value for $\tau_g$ used here (from [32]) lies below the range of values estimated by some experimental studies (see, e.g. [38]).

Two sets of values for the connection strengths are used, which will be termed the “healthy” and “Parkinsonian” parameters. Holgado et al. determined these parameters on the basis of previously published experimental recordings of unit activity from the STN and GPe of monkeys. Recordings were used from both healthy animals and animals that were rendered Parkinsonian via 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesioning. In each case, the recorded firing rate under a variety of conditions (normal, transmitter block, current injection) was compared to the firing rate predicted by their model under the same (simulated) conditions, and a genetic algorithm was used in order to find two sets of connection strengths that best fit the data. The parameter fitting that Holgado et al. performed suggested that all connections became stronger under Parkinsonian conditions, and they cite several experimental results that support this increase, including the presence of $D_2$ receptors in the STN [39] and GPe [40, 41] and the enhanced effect of GABA on STN neurons [42, 43] and glutamate on GPe neurons [41, 44] when dopamine is reduced. We note that MPTP lesioning represents chronic dopamine depletion, which is the condition under which synchronised $\beta$ activity is seen in experiments. In Holgado et al.’s model, the system has only steady-state behaviour when the healthy parameters are used, but linearly scaling the parameters towards the Parkinsonian values causes stable $\beta$ oscillations to appear. See Table 1 for fixed parameter values. It should be noted that...
Table 1

Fixed parameter values for the healthy and Parkinsonian conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy</th>
<th>Parkinsonian</th>
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<tr>
<td>(w_{gg})</td>
<td>6.6</td>
<td>12.3</td>
</tr>
<tr>
<td>(w_{gs})</td>
<td>1.12</td>
<td>10.7</td>
</tr>
<tr>
<td>(w_{sg})</td>
<td>19.0</td>
<td>20.0</td>
</tr>
<tr>
<td>(\tau_s)</td>
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</tr>
<tr>
<td>(\tau_g)</td>
<td>14 ms</td>
<td></td>
</tr>
<tr>
<td>(a_s)</td>
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<td></td>
</tr>
<tr>
<td>(a_g)</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>(\theta_s)</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>(\theta_g)</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

the equations in Holgado et al.’s model directly represent the average unit firing rate of the populations. In this regime, each weight parameter has a direct physical meaning: it represents how many spikes/s the target population increases (or decreases) by when the source population’s firing rate increases (or decreases) by 1 spike/s. Our model does not directly represent the firing rates of populations, and so the parameters should therefore be interpreted as representing the general relative strengths of synaptic connections.

2.3 Technical Details

For qualitative investigation of the isolated channel model, we used the software package XPPAUT [45] with the default integrator, a fourth-order Runge–Kutta method and a fixed step size of 0.5 ms. Numerical continuation in the isolated channel model was carried out using XPPAUT and LOCBIF [46]. In a few cases, numerical continuation failed to compute some parts of the 2D bifurcation diagram and in these cases the analysis was performed by fixing one parameter and observing the changing dynamics as the other was carefully varied. Qualitative investigation of the coupled channels model was done using XPPAUT and associated XPPy Python interface [47]. Numerical continuation of this system was carried out using CONTENT [48].

The frequency visualisation plots were computed using XPPAUT and XPPy for the numerical simulation and the FFT routine from the SciPy library [49]. When calculating the FFT the total integration time was 2.048 s, but only the second half of the integration output was passed to the FFT routine to try to ensure that the trajectory was close enough to the stable limit cycle for only truly oscillatory activity to be included. This gave the FFT output a range of 0–1024 Hz across 1024 bins. This was repeated five times for each parameter pair, with random initial conditions. The frequency and amplitude of the most powerful FFT bin over the ten runs were recorded and plotted.

3 Isolated Channel Model

This section will consider the simplified system that is obtained by setting \(\alpha = 0\). This condition corresponds to the case where neurons from each STN and GPe subpopulation never make synapses onto neurons outside their own channel. The detailed study
of a single element enables us to understand some aspects of the dynamics in the system of interactive channels. For example, the boundaries of oscillatory regimes in the 2D bifurcation diagram allow estimation of the level of input channels must receive from the cortex and their neighbours in order to give oscillatory dynamics.

Since it is only necessary to consider two equations in this reduced model, bifurcation analysis can be used to completely understand the different dynamical regimes that are possible within a single channel. We consider the bifurcations of the system under variation of the following two parameters:

- The level of cortical input to the STN ($I$). There are two major pathways by which cortical input reaches the basal ganglia: one via the striatum and one projecting directly to the STN. Striatal projection neurons fire very infrequently during periods of rest, so the system’s behaviour in response to varying levels of steady-state input via the cortico-subthalamic “hyper-direct” pathway is studied.
- The amount of self-excitation within the STN ($w_{ss}$). The work of Gillies et al. [29] suggests that there must be some ability for STN neurons to provide excitation to other STN neurons in order for the STN-GPe network to exhibit oscillations. Since the biological plausibility of this is contentious, bifurcation analysis is used to determine how much STN self-excitation is required for oscillations and how this depends on the level of hyper-direct input. It is also useful to study the behaviour of the isolated channel model under variation of $w_{ss}$ because the laterally coupled GPe sub-populations in the full coupled model introduce a similar effect.

3.1 There Are no Globally Stable Limit Cycles when $w_{ss} = 0$

When there is no self-excitation within the STN (i.e. $w_{ss} = 0$) then it can be seen from the equations of the isolated channel system that there cannot be a globally stable limit cycle. Under these conditions, the Jacobian matrix at any fixed point has a negative trace and positive determinant, therefore, the fixed point must be stable. Let $q = (1 + \exp(a_j/\theta_j))^{-1}$ (i.e. the constant term in Eq. (5)) and consider the box in phase space bounded by $x = -q$, $y = -q$, $x = 1 - q$, $y = 1 - q$; note that in general $q$ is very small and so this box covers almost all of the phase space. It can be seen that the vector field around the edges of the box must point inwards. The box must therefore contain just one fixed point, which is stable. This means that globally stable oscillations are not possible. This analysis does not rule out the existence of pairs of stable and unstable limit cycles surrounding the fixed point, however, and so we will use qualitative analysis to investigate this possibility.

3.2 An Isolated Channel Cannot Oscillate Under Healthy Conditions

The dynamics of the system when the healthy set of values for the fixed parameters were used can be understood qualitatively by examining the stability of the system’s fixed points and the shape of its nullclines for different values of the bifurcation parameters. Figure 2 shows the nullclines for a particular pair of values for $w_{ss}$ and $I$. With these parameters, the system is bi-stable, such that all trajectories in state space tend toward either a high or low level of activity in both nuclei depending on initial conditions. Also shown in Fig. 2 are the stable and unstable manifolds of the saddle
Fig. 2  Isolated channel phase space under healthy conditions. Behaviour of the isolated channel system under healthy conditions with $w_{ss} = 3.4$, $I = 0$. **Left:** The nullclines and fixed points of the system. **Right:** Fixed points, stable and unstable manifolds of the saddle point, and example trajectories point. Trajectories cannot cross these manifolds and the stable fixed point that any given trajectory tends toward depends on which side of the stable manifold its initial conditions lie upon.

Adjusting the two parameters changes the $\dot{x} = 0$ nullcline (the red line in Fig. 2): increasing $w_{ss}$ makes the slope of the middle branch steeper, while increasing $I$ shifts the nullcline upward. Both of these changes increase the proportion of initial conditions that give trajectories tending to the high activity state, as would be expected from increased STN self-excitation or cortical input. If the parameters are raised past a critical point, the system undergoes a saddle-node bifurcation whereby the low activity stable fixed point and the saddle meet and annihilate, leaving the high activity state as the only fixed point of the system. Alternatively, if the parameters are lowered past a critical point then the high activity stable state disappears in the saddle-node bifurcation instead, leaving only the low activity state. Since these saddle-node bifurcations are the only bifurcations that the system undergoes, there is no possibility for limit cycles to arise when using the healthy fixed parameter values. The system displays hysteresis because increasing the parameter passes a critical value can cause trajectories to “jump” from one stable point to another, and reducing the parameter back past this critical value does not cause a jump back to the original fixed point.

3.3 Oscillatory Regimes Are Possible in Isolated Channels Under Parkinsonian Conditions

Applying the qualitative methods in the previous section to the system using the Parkinsonian set of fixed parameter values revealed a much richer array of possible dynamics and also suggested a parameter range within which bifurcations could be present. To fully understand the different dynamical regimes, numerical continuation was used. Continuation was first performed in one dimension by starting at a
Fig. 3 2D bifurcation diagram for isolated channel under Parkinsonian conditions. 2D bifurcation diagram showing the bifurcations that the isolated channel system undergoes under variation of $I$ and $w_{ss}$ in the Parkinsonian case. A zoom of the area inside the small rectangle in the lower right-hand corner is shown in Fig. 4.

Fig. 4 2D bifurcation diagram for isolated channel under Parkinsonian conditions (zoom). Zoom of the part of the diagram inside the black rectangle in Fig. 3.

fixed point and varying a single parameter and then in two dimensions by starting at a bifurcation point and allowing both parameters to change.

Figures 3 and 4 show the complete 2D bifurcation diagram of the system under Parkinsonian conditions.

The bifurcation curves divide the parameter space up into six regions. Within each region the phase portraits of the system are topologically equivalent, having the same number of stable and unstable fixed points and limit cycles. The characteristics of these features (such as frequency and amplitude of oscillation) may vary within regions. Figure 5 shows example phase portraits that are representative of the system’s behaviour in each of the regions. The parameters corresponding to each region in the figure are given in Table 2.

Region A makes up the majority of the parameter space. Within this region, the system possesses a single, stable, fixed point. The location of this fixed point in both dimensions increases with $I$ and $w_{ss}$, as is expected from increased external stimulation or self-excitation. The behaviour of the system is more interesting in the other
Fig. 5 Phase portraits of isolated channel system under Parkinsonian conditions. Example phase portraits showing the behaviour of the isolated channel system within each of the regions of parameter space.

Table 2 The parameter values that were used for each of the regions in Fig. 5

<table>
<thead>
<tr>
<th>Region</th>
<th>$l$</th>
<th>$v_{53}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (low)</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>A (high)</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>11.8</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>D</td>
<td>3.5</td>
<td>5</td>
</tr>
<tr>
<td>E</td>
<td>10.45</td>
<td>2.345</td>
</tr>
<tr>
<td>F</td>
<td>10.495</td>
<td>2.29</td>
</tr>
</tbody>
</table>

five regions (B–F), which together make up the large wedge-shaped area in the middle of the bifurcation diagram.

As one or both of the parameters is reduced from values that give a constant high rate of firing (the area above the wedge in the bifurcation diagram), they move toward and eventually pass through the saddle-node bifurcation curve and into region B. Two
Fig. 6 Oscillatory population activity in isolated channel model. Population activity over time for three points in region C, showing periodic pauses (top), bursts of high activity (bottom) and roughly even oscillation between high and low activity (middle). As in previous figures, the red and blue lines represent the activity of the STN ($x(t)$) and GPe ($y(t)$), respectively.

Additional fixed points appear at this point, both unstable. Although in region B all trajectories still tend to the single stable fixed point, the effects of the saddle point’s manifolds causes some trajectories to take long paths around the phase space first. At the point where the parameters cross the saddle-node on invariant circle (SNIC) bifurcation curve, the stable node and the saddle point join together and the stable and unstable manifolds of the saddle point form a loop (a homoclinic orbit). Beyond the bifurcation, in region C, the saddle and the stable node have disappeared leaving the unstable spiral as the only fixed point. The homoclinic orbit has now become a stable limit cycle and so in this region all trajectories are attracted to the limit cycle and the system displays robust oscillations no matter what the initial conditions.

Both the frequency and amplitude of the stable oscillations in region C vary as the parameters move around within it. Close to the SNIC bifurcation line the frequency is extremely low, since the effects of the “ghost” saddle point cause trajectories to pass very slowly through the part of the limit cycle that is close to where the saddle was located. When the parameters are within region C the activity of the sub-populations may show either low activity with short pulses of high activity, or the opposite, or something in between. Figure 6 illustrates this by showing a number of plots of population activity against time from within region C.

The lower border of region C is, for the most part, an Andronov–Hopf bifurcation curve. This curve is divided into three segments—two supercritical parts that are separated by a long subcritical A-H curve. The points where the criticality of the bifurcation changes are the co-dimension-2 Bautin bifurcation points. The change in behaviour of the system as its parameters pass through the lower border of region C depends on whether they cross a sub- or super-critical A-H curve. In the case of two supercritical curves, this change is simple: the limit cycle shrinks around the unstable spiral until, at the bifurcation point, its amplitude becomes zero. At this point, the limit cycle disappears and the spiral becomes stable: the system has returned to region A.

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1The saddle’s ghost also has an effect on the amplitude of oscillations in this case (though this is not generally true of SNIC bifurcations). This is because the shape of the limit cycle is defined by the position of both the unstable spiral and the saddle’s ghost. As the parameters are varied in region C, these two points move in relation to each other.
The situation when the system leaves region C across the subcritical A-H curve is more interesting. In this case, the spiral becomes stable before the limit cycle has shrunk to zero amplitude. An expanded phase portrait of the system in this region is shown in Fig. 7. Since both the stable fixed point and the stable limit cycle have local basins of attraction, the region inside the stable limit cycle is divided into two concentric areas. Trajectories that begin within the inner area tend to the fixed point and trajectories that start within the outer area tend to the stable cycle. The border between these two areas is a new unstable limit cycle that appears at the point of subcritical bifurcation. The behaviour of the system within region D is therefore bi-stable and, depending on initial conditions, may show either steady-state or oscillatory activity levels. As the parameters move from the top to the bottom of region D, the stable limit cycle continues to shrink while the unstable limit cycle grows. The point at which the cycles meet and annihilate lies on the fold of cycles bifurcation curve. This leaves just one stable fixed point, returning the system to region A.

While regions A–D make up the majority of the parameter space, there are two small additional regions (shown in detail in Fig. 4). The point at which the A-H curve terminates on the saddle-node curve is a co-dimension-2 Bogdanov–Takens (B-T) bifurcation point. Due to the normal form of the B-T bifurcation, this point must also be one end of a homoclinic bifurcation curve. The other end of the homoclinic curve is also located on the saddle-node curve at the saddle-node/homoclinic point; here the two curves merge and the saddle-node curve becomes a SNIC curve. At the point where the parameters cross the homoclinic curve from region B (unstable spiral, saddle, stable node) to region E, the stable and unstable manifolds of the saddle form a closed loop with each other. Beyond this bifurcation point, the two manifolds have crossed one another and a stable limit cycle appears between the saddle’s unstable manifold and the unstable spiral (see pp. 185–190 in [50]). Like region D, region E has a bi-stability between steady-state and oscillatory behaviour that depends on initial conditions. The set of initial conditions that leads to oscillations is very small, however, due to the shape of the saddle point’s manifolds.

If the system’s parameters leave region E through the supercritical A-H curve, then the unstable spiral becomes stable and the limit cycle is destroyed. Behaviour in this
region (region F) is still bi-stable, but the two stable states are both fixed points so there can be no oscillation. Furthermore, these two states are extremely close to each other in phase space, both being regions of high activity. The parameters can leave region F through one of two parts of the saddle-node curve. Crossing either of these parts results in the loss of one of the stable fixed points and the saddle, leaving just one fixed point, which is stable.

Since bifurcation analysis revealed a number of oscillatory regions in the parameter space a further numerical experiment was performed to investigate the characteristics of these regions. Specifically, a large scale set of numerical simulations were performed to determine how the frequency and amplitude of the limit cycles varied with the parameters. The parameter space was divided up into a uniform grid and, for each pair of parameter values, the system was simulated for a period of time. The power spectrum of the resulting activity was computed using a fast Fourier transform (FFT) and the frequency of the strongest oscillation visualised. Figure 8 shows the results of these computations. The same simulations were performed using the healthy fixed parameters, but as expected no oscillations were seen and so the results are not shown.

These results are what would be expected based on the bifurcation analysis. Only regions C and D contain oscillatory activity (the only other oscillatory area in Fig. 4, region E, is too small to be shown here). The frequency of oscillations decreases to zero as the parameters move toward the SNIC bifurcation curve (the boundary between regions B and C) and increases as the parameters are decreased away from this curve. As previously discussed, the amplitude of the oscillations is greater when the parameters are close to the SNIC bifurcation line, since the unstable spiral and “ghost” saddle point are far apart here. The frequency in much of region C is in the \( \beta \) band, but region D contains some areas of higher frequency oscillation (up to about 50 Hz, which falls within the low part of what is termed the \( \gamma \) band). These
frequencies can only be considered as some approximation of rhythms that would be found in the real STN-GPe network.

We will now use the results of our analysis of the isolated channel model to investigate how the dynamics of the system change as coupling between channels is introduced.

4 Analysis of the Coupled Channels Model

The coupled channels model is a $2^N$ dimensional system (with $N > 1$), which means that analysis is much more difficult than for the isolated channel model. We will begin by discussing the parameter values that were selected before presenting some general results that show that the coupled channels model has an oscillatory regime that is very robust and exists for a wide range of parameters. Finally, Sect. 4.3 will briefly describe the detailed structure of the attractors that the system has.

4.1 Parameter Choice

When studying the coupled channels model, we used the same values for the fixed parameters as were used in the isolated channel model (see Table 1). As before, the connection strengths were divided into a healthy set and a Parkinsonian set. However, since there is no known mechanism whereby STN neurons can excite other STN neurons, we chose to fix $w_{ss} = 0$. Although the analysis of the isolated channel model found $w_{ss} > 0$ to be a necessary condition for oscillations, we hypothesised that the coupled channels model might be able to oscillate with $w_{ss} = 0$, since the path from an STN subpopulation to its neighbouring STN subpopulation and back again will have the effect of indirect delayed self-excitation. The coupled channels model has an additional parameter that can be varied ($\alpha$), which controls the strength of inhibition between neighbouring GPe sub-populations as a proportion of the self-inhibition within GPe sub-populations ($w_{gg}$). Since we are basing our model on the idea that sensorimotor channels remain largely segregated throughout the STN/GPe network [3, 33, 34], we argue that $\alpha < 1$ is the physiological range for this parameter. We studied the system under variation of $\alpha$ and $I$, using the results of our analysis of the isolated channel model to guide the selection of a reasonable range of values for $I$.

4.2 Oscillations Require Strong Coupling, Particularly Under Healthy Conditions

We began by manually carrying out many numerical simulations of different coupled systems, varying the number of channels, connection topology, $\alpha$ and $I$, and whether the healthy or Parkinsonian fixed connection strengths were used. Each simulation was started from random initial conditions. During this experimental work, we found that for relatively low lateral coupling (e.g. when $\alpha < 0.5$) the systems always converged to a single fixed-point attractor. None of our experiments with low $\alpha$ found oscillatory regimes or multi-stability. We found that it is possible (for some values of $I$) for the fixed-point attractor to undergo a supercritical Andronov–Hopf bifurcation as the parameter $\alpha$ is increased toward 1. This bifurcation causes a stable limit
Effect of parameters on oscillation frequency and amplitude in coupled channels. The frequency (left) and amplitude (right) of the strongest FFT bin encountered during numerical simulation from random initial conditions across a range of parameter values. The top row shows the system under healthy conditions and the bottom row shows Parkinsonian conditions. The system here has 5 channels arranged in a line topology.

cycle of small amplitude to appear. This limit cycle is a global attractor. The range of values of $I$ for which this bifurcation exists depends upon whether the healthy or Parkinsonian connection strengths are used: It is much wider in the Parkinsonian case than in the healthy case.

To make this investigation more rigorous, we ran similar large-scale simulations to the one which was used to generate Fig. 8, for a range of different connection topologies and channel counts. Figure 9 shows one such result for 5 channels coupled in a line topology, under both healthy and Parkinsonian conditions. It can be seen that this appears to confirm our finding that oscillations require reasonably strong lateral coupling and are much more prevalent under Parkinsonian conditions. This fact appears to be generally true regardless of the connection topology used or number of channels (up to 100 channels were used).

To further confirm these results, we used numerical continuation software to plot the curve of the A-H bifurcation in parameter space. Figure 10 shows the results of these computations for both the healthy and Parkinsonian cases, using five channels arranged on a line. In the case of five channels on a circle, the bifurcation is more complex because a symmetry means that two pairs of complex conjugate eigenvalues simultaneously cross the imaginary axis (Hopf–Hopf bifurcation with equal pairs of eigenvalues).

4.3 Detailed Attractor Structure Depends on Channel Count and Topology

Qualitative investigation of the coupled channels system revealed that the attractors of each system are structured in a way that depends on the coupling topography (i.e. circle or line) and whether the number of channels was odd or even. This section will
briefly illustrate the different attractor structures that our model can have in order to demonstrate the range of possibilities.

We first consider the effect of gradually raising the value of $\alpha$ up from zero while keeping $I$ constant. When $\alpha = 0$, we know from analysis of the isolated channel model that all of the STN sub-populations will converge to some fixed activity level (determined by $I$) and all the GPe sub-populations will converge to some other fixed level (i.e. there is a single fixed point where $x_1 = x_2 = \cdots = x_N$ and $y_1 = y_2 = \cdots = y_N$). Increasing $\alpha$ changes the co-ordinates of this single steady-state in phase space in a way that depends on whether the system is coupled as a line or a circle. In the case of channels arranged on a circle, there continues to be a single activity level for all STN sub-populations and another level for GPe sub-populations, but increasing $\alpha$ decreases the GPe level and increases the STN one. When the channels are arranged on the line, their steady-state activity levels become paired symmetrically (i.e. $(x_i, y_i) = (x_{N-(i-1)}, y_{N-(i-1)})$). When $N$ is odd, the centre channel has its own unique activity level. Increasing $\alpha$ causes the activity levels associated with the different channel pairs to spread out in phase space. Figure 11 shows the steady-state activity for a number of topologies (circle, line with $N$ even, line with $N$ odd).

The system begins to oscillate when $\alpha$ passes some critical value $\alpha_{\text{crit}}$. The precise value of $\alpha_{\text{crit}}$ depends on $I$, $N$ and the coupling topography/strengths, but in every case the stable attractor becomes unstable and a new stable oscillatory attractor appears. The amplitude of the associated oscillations is small near the bifurcation and increases as $\alpha$ moves further away from its critical value. The oscillatory activity can take four different forms depending on the coupling topography and whether $N$ is odd or even. In the case of the line topography, each of the pairs of channels begin oscillating together either anti-phase ($N$ even) or in-phase ($N$ odd). With the circle topography, the channels all oscillate identically, but in either 2 anti-phase groups ($N$ even) or in a “splay state” with a constant phase-shift between channels such that they span the oscillation period ($N$ odd). Additionally, for the circle with $N$ odd, it appears that additional bifurcations can occur as $\alpha$ is increased further that result in
Steady state activity of coupled channels model. Steady-state activity of the healthy coupled channels model with $\alpha = 0.5$ and $I = 2.5$ under three different configurations. The activity of the STN sub-populations is shown in shades of red and the activity of the GPe sub-populations is shown in shades of blue. **Left:** circular topology with four channels (same activity level across all channels); **middle:** line topology with four channels (pairs of channels with same activity); **right:** line topology with five channels (pairs of channels with same activity plus middle channel).

In order to confirm that the general behaviour of the system was independent of $N$ and coupling topography, we generated diagrams similar to Fig. 9 for values of $N$ from 3 to 30, under both coupling schemes. Qualitative inspection showed that all the diagrams were similar, as expected. For a more objective measure, we computed, from each diagram: the fraction of nodes in the $(I, \alpha)$ parameter grid that gave oscillatory activity, the minimum coupling strength ($\alpha$) that gave oscillations, and the average frequency of oscillation. These calculations confirmed that oscillations are present for a much greater range of parameter values under Parkinsonian conditions (Fig. 13), and similarly that the minimum value of $\alpha$ required for oscillations was always much higher in the healthy case than in the Parkinsonian one (0.8 vs. 0.65, not shown). As expected, these measures tended to a constant level (which did not vary with coupling topography) as $N$ was increased, showing that the general behaviour (oscillatory versus steady-state) was independent of channel count and topology. The calculations also found that the average oscillation frequency did not vary much with $N$ or coupling topology, but that this average frequency was consistently much lower under healthy conditions than Parkinsonian ones (55 Hz vs. 130 Hz, not shown).

5 Discussion

5.1 The STN and GPe May Generate Oscillations when Lateral Coupling Is Strong

The analysis of the coupled channels model above demonstrates that the range of parameters that cause oscillations in a system of $N$ STN/GPe subpopulation pairs, laterally coupled at the GPe level, is relatively independent of the coupling topology used and the value of $N$ (as long as $N > 3$). In all cases, the sub-populations all
Fig. 12 Oscillatory activity of coupled channels model. Oscillatory activity of the coupled channels model with $\alpha = 0.95$, $I = 2.5$ and healthy connection strengths, under four different configurations. The activity of the STN sub-populations is shown in shades of red and the activity of the GPe sub-populations is shown in shades of blue. Top left: line topology with ten channels (five anti-phase pairs); top right: line topology with eleven channels (five in-phase pairs plus middle channel); bottom left: circle topology with ten channels (two anti-phase groups); bottom right: circle topology with eleven channels (splay state)

tend to a constant level of activity when the strength of lateral inhibition is weak compared to inhibition within GPe sub-populations. When lateral inhibition is made almost as strong as the inhibition within GPe sub-populations then the network as a whole can begin to generate oscillations when the level of cortical input received by each channel is within a certain range; this range is much wider when the remaining connection strengths are set at values representing the Parkinsonian basal ganglia.

There is some experimental evidence that suggests that this result could represent what happens in the real basal ganglia. LFPs recorded simultaneously from multiple sites within the rat globus pallidus (homologous to the human GPe) display a degree of coherence that varies with global brain state: Under anaesthetised slow wave activity (SWA) conditions, the LFP signals have little coherence, but when the brain state becomes “globally activated” the signals become much more coherent with one another suggesting an increased level of lateral coupling [51]. In terms of our model, this would correspond to the value of $\alpha$ varying with brain state—low during SWA and higher during global activation. Interestingly, a similar study using rats that were chronically dopamine depleted via 6-hydroxydopamine (6-OHDA) lesion found that
Fig. 13 Area of oscillatory region in parameter space. The area of the oscillatory region in diagrams similar to those in Fig. 9, for different values of $N$ and coupling topographies. In every case, the area is much larger under Parkinsonian conditions than healthy ones and is not significantly affected by $N$ or the coupling topography, for values of $N$ greater than approximately 14.

The characteristic $\beta$ LFP peak in the STN was present only in the globally activated brain state, not during SWA [52]. Our model suggests that this oscillatory activity may be generated locally by the STN/GPe circuit as a result of the increased lateral coupling between GPe sub-populations that is seen during global activation.

The frequency of oscillations generated by our model is generally much higher than the 15–30 Hz $\beta$ band—although it is interesting to note that the parameter values that resulted in the largest amplitude of oscillation were those that gave the lower frequency oscillations, including the $\beta$ band (Fig. 9). Although we found that shifting the fixed connection strengths toward their healthy values reduced this average frequency (whilst shrinking the oscillatory region of parameter space), we did not find a simple relationship between the frequency of oscillation and any one individual connection strength. It is possible that more complex coupling topologies (for example, linking each GPe subpopulation with more of its neighbours, with a strength that decreases with distance) could have the effect of reducing oscillation frequency. Our definition of the frequency of oscillation was also very simple: we considered only the frequency of the highest peak that was found across the power spectra of all of the sub-populations’ activity. A more thorough study should examine the entire spectrum in each case and check for a peak at $\beta$, and could consider a measure that would more accurately correspond to a simulated LFP recording (such as the summation of activity across all channels). Finally, it is possible that the time constants that were used (particularly for the GPe sub-populations) were significantly different to the typical cell membrane time constants of the populations we are modelling. Experiments have reported a wide range of possible values for membrane properties of GPe neu-
rons [53]. At present, our model only demonstrates that some oscillatory activity is possible in the Parkinsonian STN-GPe when the level of lateral coupling in the GPe is sufficiently strong.

5.2 Individual Channels Are Capable of Complex Dynamics

Our analysis of the isolated channel model demonstrates that, when the Parkinsonian connection strengths are used, a simple model of a coupled pair of STN and GPe sub-populations can generate dynamic behaviour that is either steady-state (regions A and B), oscillatory (region C), or bi-stable between a steady and oscillating state (region D). The oscillatory and bi-stable regimes rely on a non-zero degree of STN self-excitation. This section will describe one possible model of basal ganglia movement processing that these dynamics could represent. Here, we do not mention regions E and F as they are extremely small and are therefore unlikely to correlate with observed features of basal ganglia (dys)function.

We consider a system that consists of multiple isolated channels which all have parameters such that they are in region D (see Fig. 7). Each channel can be switched between oscillation and steady-state activity by a short transient external perturbation of the activity in either STN or GPe. To take a channel from steady-state to oscillatory activity, this perturbation must be sufficient to move the system outside the basin of attraction of the fixed point (this is the region enclosed by the unstable limit cycle). Transferring the system to the steady-state is more difficult. The perturbation must arrive at the correct time in the oscillatory cycle in order to move the current position in phase space toward the unstable cycle. The correct time depends on whether the short external perturbation affects the STN or GPe, and whether it has an excitatory or inhibitory effect. For example, an inhibitory perturbation applied to a GPe sub-population must occur during the high activity phase of oscillation as this will move the trajectory down in phase space and, if the perturbation is of the correct amplitude, bring the trajectory inside of the unstable limit cycle where it will be attracted in to the stable spiral.

LFP recordings reveal a drop in synchronous $\beta$ oscillations in the basal ganglia prior to and during movement [54] and, according to our interpretation, this corresponds to one or more channels transferring from a limit cycle to a stable fixed-point’s basin of attraction. This transfer requires precisely timed perturbation. One possible source for this perturbation is the inhibitory input that the GPe receives from the striatal medium spiny neurons (MSNs). This projection is organised in a segmented manner, which suggests that each of our channels receives striatal input from a different set of MSNs [55]. Recordings in monkeys have found that a sub-set of these neurons, the phasically active neurons (PANs), are normally silent but show short bursts of activity just prior to movement [35]. Simultaneous LFP and unit activity recordings from the striatum of healthy behaving monkeys reveals that there is a transient $\beta$ rhythm in the striatal LFP and, furthermore, that the firing of PANs occurs at a particular point in the cycle of this oscillation [22]. If the striatal and pallidal $\beta$ LFP oscillations are synchronised to some degree (this is currently unknown), then it is possible that the PAN bursts arrive during the correct part of the STN-GPe oscillation cycle to push a channel into the stable state. After a movement has been completed,
the channel can easily be switched back to its β oscillatory mode by an excitatory or inhibitory perturbation of its STN or GPe sub-population. Each channel that is in region D therefore acts as a switch or filter. Assuming each channel corresponds to a movement or body region, synchronised oscillatory activity in the circuit prevents movement either by reducing information transfer or acting as a global “anti-kinetic” signal. When movement is required, precisely timed striatal input effectively switches the oscillations off temporarily.

If, due to some modulation of cortical input or STN self-excitation \((I, w_{ss})\), the system moves close toward region C then the basin of attraction for the stable fixed point becomes smaller. When this happens, the external perturbation required to escape the oscillatory region must be of larger amplitude and timed more precisely. Finally, when the parameters pass into region C, the fixed point loses stability and no external perturbation of trajectories would be able to stop the system oscillating. We claim that these changes may correspond to the daily fluctuations in the severity of the hypo-kinetic motor symptoms of Parkinson’s disease, with region C corresponding to the akinetic state where movement cannot be initiated at all.

An alternative biological interpretation of the bifurcation diagram does not involve external perturbations, but instead relies on the fact that when the system is close to one of the bifurcation curves its behaviour depends very sensitively on the parameters. For example, close to the SNIC and fold cycle curves small changes in cortical input can switch the system between oscillatory and steady-state behaviour. Under Parkinsonian conditions where there is a large oscillatory region, a greater value of \(I\) may be needed to escape this region.

The physiological plausibility of this mechanism for activating and deactivating different movement channels is limited by the fact that the bi-stable region only exists when the Parkinsonian strengths are used and STN self-excitation is non-zero. However, our results have shown that introducing a degree of coupling between channels unlocks much more interesting dynamics within each channel, even in the healthy case. Further preliminary work (not shown here) suggests that introducing heterogeneity to the level of cortical input that each channel receives makes the possible dynamics richer still. It is possible that under these more realistic conditions, there are regions of parameter space where channels can exhibit similar bi-stable behaviour to what is described here.

Our analysis of the isolated channel model could help to identify parameter values that give interesting dynamics (such as oscillations and bi-stability) in the coupled model. A possible approach is to use numerical continuation to smoothly move the system from its oscillating (or bi-stable) isolated channel state (with non-zero \(w_{ss}\)) to a similar state with GPe coupling and \(w_{ss} = 0\). To do this, we could take \(w_{ss} = (1 - \alpha)w_{ss}'\), where \(w_{ss}'\) is an STN self-excitation strength that was found to give oscillations or bi-stability in the isolated model. In this modified model, \(\alpha = 0\) would correspond to isolated channels with STN self-excitation and \(\alpha = 1\) would correspond to coupled channels without STN self-excitation. If our hypothesis that lateral inhibition between neighbouring GPe sub-populations has a similar effect to STN self-excitation is correct, it should be possible to examine what happens to the different dynamic regimes as one mechanism replaces the other.
5.3 Comparison with Other Models

The results of our analysis of a single isolated channel agrees, to a large extent, with the results of the study of Holgado et al. [32], which considered the entire STN and GPe each as single populations and from which our parameters were taken. As in [32], stable β oscillations occur only when the parameter values corresponding to the Parkinsonian state are used. The model presented here is simpler than that of [32] as it does not attempt to model the synaptic transmission delay between sub-populations. This simplicity made bifurcation analysis possible, which revealed a region of interesting behaviour that is bi-stable between oscillatory and steady-state activity. Such behaviour was not seen in the model presented in [32], presumably as it only occurs when the degree of STN self-excitation is non-zero and this was not the case in the model of Holgado et al.

Another previous modelling study, by Gillies et al. [29], considered a population-level model of the STN-GPe circuit that is also very similar to our isolated channel model. They described three different states for the system: a single fixed point, an oscillatory state that showed low frequency short periods of high activity, and a state that was bi-stable between two stable fixed points. All of these states are also present in the model presented in this paper. The single fixed-point state corresponds to the system when healthy values of the fixed parameters are used or when the Parkinsonian values are used and the system is in region A. The oscillatory state corresponds to region C of the Parkinsonian parameter space. Finally, the parameter values that give bi-stability between two fixed points are found in region F. Gillies et al. hypothesised that this could represent the physiological mode of operation of the STN-GPe circuit, but our model suggests that this is unlikely as region F represents an extremely small part of the parameter space. This means that the fixed-point bi-stable state is very fragile and small changes in cortical input would move the system out of it. Furthermore, within region F the two stable fixed points are very close together in phase space and so the bi-stability would only switch between two very similar levels of activity. Instead, our model suggests that the physiological state is in fact bi-stable between a fixed point and a limit cycle.

Berns and Sejnowski developed a population-level model of action selection in the basal ganglia that embodies the idea of multiple sensorimotor pathways [56]. Each channel in this model contains sub-populations for the cortex, striatum, GPi/e, and thalamus; however, the STN is modelled as a single global sub-population that is the only link between channels. The authors consider how Parkinsonian conditions affect the ability of the model to select actions, but they do not investigate its ability to generate oscillatory behaviour in this case. This model does not contain the projection from STN back to GPe, and so cannot be used to study the possible pacemaker role of this circuit. A very similar model by Gurney et al. [57, 58] also considers the effect of dopamine depletion in terms of the failure of action selection and again does not examine the possibility of oscillations emerging. A more refined version of this model that used the same functional connectivity but with computational current-based modelling of the individual neurons within each sensorimotor channel exhibits several features that are found in experimental recordings under both healthy and Parkinsonian conditions [59], including oscillations (although in this case only the
\( \gamma \) band is considered). Since the mathematical complexity of this model is much greater than population-based models, mathematical analysis (such as considering the dynamical capabilities of individual channels) becomes intractable.

In our model, the strength of GPe self-inhibition \((w_{gg})\) is increased under Parkinsonian conditions. In contrast, some models (notably that of Terman et al. \([28]\)) find that a reduction in pallidal self-inhibition may facilitate increased rhythmic activity in the STN-GPe network. There is some evidence to suggest that this decrease of \(w_{gg}\) in the Parkinsonian case is more appropriate, based on the effects of increased striatal-pallidal activity on GABA release in the GPe \([60, 61]\). It would be interesting to see how our results would differ with decreased \(w_{gg}\) under Parkinsonian conditions.

5.4 Further Work

The models described in the previous section raise the interesting question of whether or not our model is capable of performing action selection. When analysing the dynamics of an individual channel in Sect. 3.3, we found a hypothetical mechanism by which channels could be switched on and off and this could form part of a system for action selection. As a result of its symmetry our coupled channels model could only produce dynamics that were common across all channels, which is clearly not useful for action selection, and so the first step will be to break this symmetry. One way to do this is to provide a heterogeneous level of cortical input to each STN subpopulation.

It may also be possible to use this model to investigate the basis for the remarkable improvement in symptoms that can be achieved through high-frequency electrical stimulation of the STN \([62]\). One potential way to incorporate the effects of deep-brain stimulation (DBS) into the model is to add an external periodic input to the equation for activity in one or more STN sub-populations. When investigating the isolated channel model, we observed that with parameters set such that it is in a region with stable \(\beta\) oscillations, there exists a range of frequencies for the external input that cause the oscillations to become chaotic, flattening the power spectrum. This range of frequencies appears similar to the range of clinically effective DBS frequencies. This interesting result requires further investigation.

This paper is based on the assumption that excessive \(\beta\) activity plays a causative role in the hypo-kinetic symptoms of Parkinson’s disease, but some evidence suggests that it is merely a correlative epiphenomenon. When the progression of Parkinson’s disease is simulated in monkeys by the selective lesioning of dopaminergic SNc neurons over the course of many days, oscillatory activity is not observed in the firing rate of individual GPi neurons until long after motor symptoms have appeared \([63]\). It is not clear, however, whether or not LFP signals (where the \(\beta\) peak is usually seen) in the GPi are related to unit activity in that nucleus \([9]\). Other studies with rats have compared the effects of chronic SNc lesioning with acute dopamine blockade and found that only the chronic condition results in a peak in \(\beta\) power in STN LFP \([52]\) and motor cortex ECoG \([64]\), even though both chronic and acute dopamine depletion/blockade induce akinesia. Such evidence does not necessarily rule out the possibility of \(\beta\) oscillations having an anti-kinetic effect, however, since acute dopamine blockade may disrupt motor pathways in a way which is different to the mechanism by which \(\beta\) oscillations act to prevent movement. Even if excessive
$\beta$ activity is simply a side-effect of chronic loss of dopaminergic input to the basal ganglia that does not directly cause Parkinsonian motor symptoms it may still serve as a marker for this neuronal damage that is useful experimentally [13]. Furthermore, it has been proposed that elevated $\beta$ LFP power could be used as a trigger for a new generation of “on-demand” devices for DBS [65, 66]. Whether the relationship between abnormal $\beta$ synchronisation and the hypokinetic symptoms of Parkinson’s disease is causative or merely correlative, it is clearly a significant characteristic of the Parkinsonian basal ganglia that should be properly understood. Further modelling work will help to achieve this understanding.

**Competing Interests**

The authors declare that they have no competing interests.

**Authors’ Contributions**

RM, NY, FN and RB designed the study, performed the experiments, and analysed the results. OB assisted with simulation and bifurcation analysis of the multiple channels model. All authors contributed to the writing of the manuscript. All authors read and approved the final manuscript.

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**Appendix**

**Video 1:** [http://www.youtube.com/watch?v=sT0_Ognnsns](http://www.youtube.com/watch?v=sT0_Ognnsns)

This video shows the activity in a single pair of coupled STN-GPe subpopulations under Parkinsonian conditions. Each particle is a 2-dimensional vector representing a point in state space and the particles are initialized to have random positions. As the simulation runs, each particle’s position evolves according to the equations of the system in either forward time (red particles) or backward time (blue particles). Each frame a random sub-set of particles are reset to a new random position. The level of STN self-excitation ($w_{ss}$) is gradually increased, showing a range of dynamical regimes: globally single stable fixed point, bistability between fixed point and stable oscillations, globally stable oscillations, and back to a globally stable fixed point.
The first part of this video shows the activity of five parallel channels under Parkinsonian conditions, each of which is made up of a coupled STN-GPe subpopulation pair. As in Video 1, many sets of initial conditions are chosen uniformly from across the 10-dimensional phase space and each set of initial conditions is integrated in parallel using the computer’s graphical processing unit (GPU), with random resetting. For each set of initial conditions being integrated, the level of STN and GPe activity is projected onto a different part of the screen (and in a different colour) for each channel. A white line is used to link the particles corresponding to the first set of initial conditions (which are never randomly reset). As the strength of coupling between the channels (\(\alpha\)) is increased, oscillatory activity appears that is anti-phase between neighbouring channels.

The second part of this video is similar to the first part except that 799 channels are shown. Only one set of initial conditions is used here and there is no resetting. The vertical position of each dot indicates the level of GPe activity in each channel. As before, oscillations appear as lateral coupling is increased and the shape of the oscillatory attractor appears to be very non-regular.

In both parts of the video the level of cortical input is fixed across all channels at 5.0.

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The emergence of two anti-phase oscillatory neural populations in a computational model of the Parkinsonian globus pallidus

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Keywords: Parkinson's disease, globus pallidus, oscillation, synchronization, HCN, downregulation, deep-brain stimulation

1. INTRODUCTION

Parkinson's disease is a neurodegenerative disorder which ( amongst other symptoms) causes a range of movement-related disturbances such as tremor and slowness (bradykinesia). The primary pathology of the disease is the death of dopaminergic neurons in the basal ganglia (BG), specifically those in the substantia nigra pars compacta (SNc). Since the dopaminergic neurons in the SNc provide widespread innervation to the other regions of the basal ganglia, it is not surprising that their loss results in profound changes to neuronal activity in these regions. What is not yet understood is the precise mechanism by which abnormal neuronal activity arises as a result of dopamine loss—and how this activity relates to motor symptoms. One very successful hypothesis for this was the so-called “rate” hypothesis (DeLong, 1990), which held that motor areas of the basal ganglia are divided into two feed-forward pathways that transfer information from the cortex to the thalamus: a pro-kinetic “direct” and an anti-kinetic “indirect” pathway. According to this model, loss of dopamine input to the striatum upsets the balance of activity in these two pathways, resulting in movement abnormalities. While the rate hypothesis makes predictions that have resulted in successful treatments, such as lesioning of hyperactive nuclei on the indirect pathway (Lozano et al., 1995; Gill and Heywood, 1997), more recent electrophysiological studies have demonstrated that the changes in neuronal activity that underlie Parkinsonian motor impairment are likely to be considerably more complex than those implied by the rate model [see Rubin et al. (2012) for review].

One aspect of pathological activity in the Parkinsonian basal ganglia that is under active investigation is the increase in synchronous oscillatory firing. Local field potential (LFP) recordings from the subthalamic nucleus (STN) of patients with Parkinson’s disease show a clear increase in power in the β frequency band (10–30 Hz) when patients are off medication [reviewed in Eusebio and Brown (2009)], and the size of the reduction in β power that occurs with dopamine-replacement medication is positively correlated with the concomitant improvement in severity of anti-kinetic symptoms (Kühn et al., 2006). There are a number of reasons why widespread pathological oscillations may cause motor deficits, for example they may impair the ability to relay information (Mallet et al., 2008b). It has also been proposed that, in health, sporadic β oscillations act as a global signal for maintenance of the current motor activity (Jenkinson and Brown, 2011).

Experiments in rodent models of Parkinson's disease have demonstrated a prominent increase of oscillatory firing patterns in neurons within the Parkinsonian globus pallidus (GP) which may underlie some of the motor symptoms of the disease. There are two main pathways from the cortex to GP: via the striatum and via the subthalamic nucleus (STN), but it is not known how these inputs sculpt the pathological pallidal firing patterns. To study this we developed a novel neural network model of conductance-based spiking pallidal neurons with cortex-modulated input from STN neurons. Our results support the hypothesis that entrainment occurs primarily via the subthalamic pathway. We find that as a result of the interplay between excitatory input from the STN and mutual inhibitory coupling between GP neurons, a homogeneous population of GP neurons demonstrates a self-organizing dynamical behavior where two groups of neurons emerge: one spiking in-phase with the cortical rhythm and the other in anti-phase. This finding mirrors what is seen in recordings from the GP of rodents that have had Parkinsonism induced via brain lesions. Our model also includes downregulation of Hyperpolarization-activated Cyclic Nucleotide-gated (HCN) channels in response to burst firing of GP neurons, since this has been suggested as a possible mechanism for the emergence of Parkinsonian activity. We found that the downregulation of HCN channels provides even better correspondence with experimental data but that it is not essential in order for the two groups of oscillatory neurons to appear. We discuss how the influence of inhibitory striatal input will strengthen our results.

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What is the neural basis for the exaggerated oscillatory activity of the Parkinsonian basal ganglia? One possibility is that the reciprocally-connected neurons of the excitatory STN and inhibitory globus pallidus (GP; homologous to the external globus pallidus in primates) act as a neural oscillator. Several computational studies have suggested that this is a plausible mechanism (Gillies et al., 2002; Terman et al., 2002; Holgado et al., 2010). In vitro work in slices containing only STN and GP neurons have also shown that oscillatory firing is indeed possible (Plenz and Kital, 1999), though only at frequencies much lower than the β band.

Another possible explanation for exaggerated BG β oscillations in Parkinson's disease is that dopamine acts to modulate the effects of rhythmic cortical activity on cortical-basal ganglia pathways, such that in conditions of reduced dopamine this network becomes pathologically entrained to cortical rhythms. Evidence for this comes from studies that have used signal processing techniques to attempt determine whether β band coherence between the cortex and basal ganglia is directed from cortex to STN or vice versa. Such studies have shown that, in patients with Parkinson's disease (Litvak et al., 2011) or Parkinsonian rodents (Sharott et al., 2005), the oscillations arise in the cortex and drive STN activity. Computational studies that investigate the synchronization of basal ganglia neurons in Parkinson's disease often consider the neurons to be phase oscillators, which either synchronize themselves Popovych and Tass (2012) or become synchronized through common external inputs Wilson et al. (2011).

Experiments using rodent models of Parkinson's disease provide compelling evidence that under Parkinsonian conditions the activity of neurons in the GP are much more susceptible to entrainment by cortical rhythms than in the healthy case. Under conditions of urethane anaesthesia, neurons in the GP of healthy rodents show uncorrelated tonic firing. However, in animals where Parkinsonism has been induced, either through chronic lesioning of the SNc with 6-hydroxydopamine (OHDA) (Ni et al., 2000; Magill et al., 2001) or acute inactivation of SNc projection fibers (Galati et al., 2009), the spiking activity of the majority of GP neurons becomes significantly correlated with cortical “slow wave activity” (SWA); this is the major cortical rhythm in the anaesthetized state and has a frequency of approximately 1 Hz. These experiments also reveal that, in the chronic lesioned case at least, the neurons in GP are split into two major groups, distinguished by whether they preferentially fire during the active phase (ECoG peaks) or inactive phase (ECoG troughs) of SWA in dopamine-deprived conditions. These will be referred to as the TA and TI groups, respectively. The underlying basis for this division is unknown, but the same division is seen in respect to cortico-pallidal synchronization that occurs transiently at β frequencies in response to sensory stimulation in OHDA lesioned rodents (Mallet et al., 2008a), which suggests that the same mechanism may be responsible for pathological entrainment in both behavioral states/frequency bands. If this is the case, then understanding this mechanism may lead to improved treatments for Parkinson's disease. Unfortunately we do not currently know the route through which oscillatory cortical input entrains the GP, although it is likely to involve the two major sources of synaptic input to GP neurons: the inhibitory medium spiny projection neurons of the striatum and excitatory STN neurons. Both receive widespread cortical inputs and both show increased firing during the peaks of SWA under Parkinsonian conditions in rodents (Magill et al., 2001; Tseng et al., 2001). Given that the majority of GP neurons belong to the TI group it has been suggested that cortical oscillations are most effectively relayed via the inhibitory striatum (Walters et al., 2007), but this view is challenged by the fact that the entrainment of GP neurons to SWA appears to be critically dependent on a functioning STN (Ni et al., 2000; Galati et al., 2009).

In this paper we test the hypothesis that the inhibitory network of GP neurons allows two anti-phase groups of oscillatory neurons to appear in response to rhythmic excitatory STN input only. To do this we consider a small neural network model of interconnected conductance-based GP neurons. Although the parameters of the neurons in this population are homogeneous, our simulations reveal a mechanism by which the two oscillatory groups can appear. This collective behavior is the result of a self-organization process that depends on the GP neurons’ inhibitory dynamics and rhythmic STN modulation. We study the neural network model under healthy and Parkinsonian conditions and demonstrate a good correspondence between simulation results and experimental recordings. Special attention has been paid to studying the possible role of downregulation of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels in this network, based on the effect that these channels appear to have on GP neurons’ responses to synaptic input (Chan et al., 2004; Boyes et al., 2007) and the suggestion that oscillatory activity may not appear immediately after dopamine lesion and might instead depend on slower adaptive processes (Degos et al., 2009).

The structure of this paper is as follows. Section 2 describes our model including the cellular properties of model GP neurons, how STN neuron activity was generated, the nature of synaptic connectivity and our proposed model for HCN downregulation. Section 3 describes the results of simulations and demonstrates that model GP neurons behave realistically both in isolation and as part of a network. It also shows how the network’s activity changes under simulated Parkinsonian conditions in a way that is similar to the results of previous biological experiments. Section 4 examines results in the context of what has been seen in animal models of Parkinson’s disease and discusses what the results might mean in terms of potential improvements to treatments for the disease.

2. MATERIALS AND METHODS

Figure 1 shows a simple representation of the neural network model which includes a population of 100 interconnected GP neurons (right panel, blue) which receive excitatory synaptic input from 50 STN neurons (left panel, red). Each GP neuron is described by a detailed single compartment conductance based model of the Hodgkin–Huxley type with inhibitory connections from other GP neurons. The STN neurons are described by a simple enhanced leaky integrate-and-fire model. Neurons in the STN population are not connected to each other but they are modulated by a common cortical slow-wave rhythm and make excitatory synapses onto GP neurons.
2.1. MODEL GP NEURONS
The model GP neurons are of standard Hodgkin–Huxley type, with a single compartment per neuron. We included ten voltage-gated ionic channels as in the multicompartmental modeling work of Günay et al. (2008): fast and slow delayed rectifying K⁺ (Kv3 and Kv2, respectively), fast and slow A-type K⁺ (Kv4 fast, Kv4 slow), M-type K⁺ (KCNQ), fast spike-producing Na⁺ (NaF), persistent pacemaking Na⁺ (NaP), hyper-polarization activated Ca²⁺ (HVA), and fast and slow mixed-conductance hyperpolarization-activated channels (HCN fast, HCN slow). For simplicity our model does not include calcium-gated potassium “SK” channels, as these channels’ most significant effect on the activity of GP neurons appears to be a lengthening of spike afterhyperpolarization (AHP) (Deister et al., 2009), and we were able achieve physiologically realistic AHPs without this channel. Equation 1 describes how the membrane potential (V) of a model GP neuron evolves in time.

\[
\frac{dV}{dt} = g_{\text{leak}}(E_{\text{leak}} - V) + I_{\text{NaF}} + I_{\text{KCNQ}} + I_{\text{NaP}} + I_{\text{HCN}} + I_{\text{syn}} + I_{\text{ext}}
\]

Here \(C\) and \(g_{\text{leak}}\) are the total membrane capacitance and leak conductance and \(E_{\text{leak}}\) is the reversal potential of the leak channels. Values for these parameters are given in Table 1. \(I_{\text{syn}}\) is the total synaptic current received by the neuron (see below). \(I_{\text{ext}}\) is an externally applied current that was only non-zero when testing the response of individual GP neurons to current injections. The remaining currents correspond to the voltage-dependent channels, each of which has an activation gate (represented by the state variable \(m\)) and, for most channels, an inactivation gate (state variable \(h\)). Two channels have slow inactivation gates (\(s\)) in addition to their activation and inactivation gates. Equation 2 shows the current due to a channel with all three gates:

\[
I = m^\mu h^\rho s^\phi g_X(E_X - V)
\]

Here \(E_X\) is the reversal potential of the channel, \(g_X\) is the maximum conductance of the channel, and \(\mu\), \(\rho\) and \(\phi\) are integers that give the relative numbers of gating molecules. Table 2 shows which channels contain each gate type and the corresponding values of \(\mu\), \(\rho\) and \(\phi\). Note that the parameters governing the dynamics of each gate vary from channel to channel. Since we use the same equations and parameters for channel gates as Günay et al. (2008) we do not reproduce these here and instead refer to the supplementary material of that paper where they are listed.

We could not directly use the channel maximum conductance parameters from Günay et al. (2008), since this was a multicompartmental model and the conductances varied widely between the different compartments. Instead we adjusted the channel conductances so that our model neurons exhibited intrinsic pacemaking and displayed realistic responses to depolarizing and hyperpolarizing current injections. The chosen conductances are shown in Table 1. In order to generate a range of intrinsic pacemaking frequencies we applied Gaussian noise to

<table>
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<th>Value</th>
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<td>(g_{\text{Kv2,3,4fast}})</td>
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<td>(g_{\text{KCNQ}})</td>
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<td>(\text{nS})</td>
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<td>(g_{\text{HVA}})</td>
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<td>(g_{\text{HCNfast, HCNslow}})</td>
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<th>(\rho)</th>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>NaP</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Kv2.3,4fast, 4slow</td>
<td>4</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>KCNQ</td>
<td>4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HVA</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HCNfast, HCNslow</td>
<td>1</td>
<td>–</td>
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</table>
the maximum conductances of the persistent sodium and HCN channels ($g_{NaP}$, $g_{HCN}$, and $g_{GG}$). The mean values are given in Table 1 and the standard deviation was 50% of the mean value for NaP and 30% for both HCN channels.

It has been proposed (Chan et al., 2011) that a homeostatic mechanism may reduce the intrinsic firing rate of GP neurons via downregulation of HCN channels in response to burst firing. Our model includes a possible mechanism for this downregulation by allowing the maximum conductance of HCN channels to decrease. This occurs during periods of elevated firing rate, which are indicated by high intracellular calcium concentration ($\left[Ca^{2+}\right]$).

In order to model the dynamics of this calcium concentration we use the equations from Terman et al. (2002). The rate of change of intracellular calcium is given by Equation 3:

$$\frac{d[Ca]}{dt} = \epsilon(I_{ina} - k_{Ca}[Ca])$$

Here $\epsilon$ represents calcium buffering and has the value $10^{-4} M_0^{-1}$, while $k_{Ca}$ is the calcium pump rate and has the value $15.0 M_0^{-1}$ (parameter values from Rubin and Terman (2004)). $I_{ina}$ represents the instantaneous current due to HVA channels (these are the only Ca+ channels in the model). The maximum conductance of HCN channels remains constant during time steps and is adjusted between steps when $[Ca] > T_{HCN}$, where $T_{HCN}$ is the threshold above which downregulation occurs. The amount by which the conductance is adjusted takes the form of a sigmoid curve and is given in Equation 4.

$$g_{HCN}(t + \Delta t) = \max\left(0, g_{HCN}(t) - \frac{k_{HCN}\Delta t}{1 + \exp\left(\frac{\left[Ca\right](t + \Delta t)}{\sigma}\right)}\right)$$

Here $k_{HCN}$ is the maximum conductance change that can occur in one step, $\theta$ is the level of intracellular calcium that gives half the maximum change, and $\sigma$ is the slope of the sigmoid. We do not have any data from biological experiments to suggest values for the downregulation parameters. Since we hypothesize that downregulation mostly only occurs during fast burst firing under Parkinsonian conditions, we chose parameters such that, in healthy conditions, downregulation only occurred in the very fastest firing GP neurons. The values of $k_{HCN}$ and $\sigma$ that we chose give a fairly rapid reduction in maximum HCN conductance in response to elevated firing rates. The downregulation parameters that we chose are: $T_{HCN} = 0.2$, $k_{HCN} = 6 \times 10^{-3}$, $\sigma = 0.1$, $\theta = 0.5$.

Note that our model GP neurons are supposed to represent those in the rodent GP. This nucleus is usually considered to be equivalent to the so-called “external” pallidus (GPe) in primates.

2.2. MODEL STN NEURONS

Since the aim of this study is to investigate the effects of rhythmic STN input upon the neurons of the GP, we did not model STN neurons to the same level of biological detail as GP neurons. Instead, to simulate the SWA-modulated bursting of STN neurons that occurs under urethane anaesthesia we use an enhanced integrate-and-fire generator of neural activity, as described in Borisyuk (2002). The STN neurons include an exponentially decaying threshold, accumulation of membrane potential, stochastic noise and an absolute refractory period. Aside from approximating the SWA modulation of STN activity our model does not include any synaptic inputs to the STN. In particular we do not include the GP-STN projection because experiments in urethane-anaesthetized rats have shown that the changes in firing rate and pattern that occur in the STN following OHDA-lesion are not dependent on synaptic input from the GP (Hassani et al., 1996).

During urethane anaesthesia STN neurons display uncorrelated bursting activity that is modulated by a slow rhythm (Magill et al., 2001) which, for the purposes of this paper, we assume arises from excitatory cortical inputs. Since firing is uncorrelated, each STN spike train is generated independently using a procedure that results in spiking activity that is similar to the spiking activity of real neurons. The generated spike trains contain activity that is oscillatory with a period of 1300 ms ($\approx 0.8$ Hz), where cycle is made up of an 800 ms “inactive” phase and a 500 ms “active” phase. The spike trains are constructed by alternately sampling from two intermediate spike trains, one with slow irregular firing and one with fast irregular firing (for the inactive and active periods, respectively). The average firing rates are 0.5 Hz for the inactive period and 30 Hz for the active period; under simulated Parkinsonian conditions the firing frequency during the active phase is increased to 60 Hz. Figure 2 shows cross-correlations between two STN spike trains which demonstrate that within the active bursting period activity is not correlated (A), but that there is strong common modulation at $0.8$ Hz (B). Figures 2C,D are raster plots of the generated spiking activity of 50 STN neurons in healthy and Parkinsonian conditions, respectively, demonstrating a clear increase in firing frequency during the active phase in the Parkinsonian case.

2.3. SYNAPTIC CONNECTIVITY

Each model neuron contains two variables, $o(t)$ and $c(t)$, which represent synaptic opening and closing, respectively. When a neuron spikes (defined by the membrane potential crossing 0 mV in the positive direction), both variables are step-increased by 1. Following this, the variables decay exponentially to zero according to different time constants $\tau_o$ and $\tau_c$ respectively (Eq. 5). Since $\tau_o < \tau_c$, a transient synaptic current arises in all post-synaptic neurons following a spike, according to Eq. 6:

$$\frac{do}{dt} = -\frac{1}{\tau_o} o$$

$$\frac{dc}{dt} = -\frac{1}{\tau_c} c$$

$$I_{syn} = (c - o) g_{Syn}(e_{rev} - V)$$

Here $e_{rev}$ is the reversal potential of the synapse (mV) and $V$ is the membrane potential of the post-synaptic neuron (mV). The value of parameters $e_{rev}$, $\tau_o$, and $\tau_c$ vary based on the type of the neuron (glutamatergic STN or GABAergic GP). $g_{Syn}$ denotes the maximum unitary conductance of a synapse ($nS$) and its value for a particular synapse is drawn from a Gaussian distribution. The mean of this distribution was $g_{SG}$ for STN-GP synapses and $g_{GG}$ for intra-GP synapses and the standard deviation was 30% of the mean in both cases.
For the intra-GP inhibitory synapses we used synaptic time constants $\tau_e = 5$ ms and $\tau_c = 40$ ms from unpublished current-clamp recordings of GP-GP IPSPs taken from slices containing rat GP, cortex and striatum [Alon Korngreen, personal communication]. Similarly, we chose $g_{GG} = 0.5 \text{nS}$ which gives a peak IPSP of 0.5 mV (measured as deflection away from a holding potential of −85 mV during injection of hyperpolarizing current) to match the same experimental recordings. We used a standard GABA reversal potential of −80 mV. Connectivity between GP neurons was uniformly random, with each neuron inhibiting 20 others (no self-connections).

Anatomical data regarding the structure of the STN-GP projection is currently lacking. However, it is clear that there are many fewer STN neurons than GP neurons and that each GP neuron only samples the activity of a small proportion of STN neurons (Jaeger and Kita, 2011). We therefore arbitrarily chose to model 50 STN neurons, each of which makes excitatory synapses onto two randomly selected GP neurons. The time constants of STN-GP synapses in the model are $\tau_o = 0.2$ ms and $\tau_c = 60$ ms, based on the recordings shown in Loucif et al. (2005). The average maximum synaptic conductance ($g_{GG}$) used for the healthy case was chosen to be just low enough such that the majority of GP neurons didn’t show significant entrainment to the SWA rhythm and we investigated the effects of increasing the value in the Parkinsonian case.

### 2.4. Modeling of Parkinsonism

We simulate the OHDA-lesioned (Parkinsonian) rat basal ganglia by making three changes to the model’s parameters: (i) faster STN firing during the active phase of SWA (Figure 2D) (ii) increased STN-GP synapse strength and (iii) increased intra-GP inhibition strength. Although the changes that occur to functional connectivity in the basal ganglia in Parkinson’s disease are currently under active investigation, there is experimental support for facilitation of both GP-GP (Johnson and Napier, 1997) and STN-GP (Johnson and Napier, 1997; Hernández et al., 2006) synapses. Similarly, under urethane anaesthesia it has been shown that spiking in the STN continues to be modulated by the cortical SWA rhythm, but that its firing becomes more intense during the active period (Magill et al., 2001; Galati et al., 2009).

### 2.5. Categorization of Neuron Activity

We used a simple method to categorize GP neurons as being of type TA (in-phase with active SWA), TI (in-phase with inactive SWA), NM (not modulated by SWA) or QU (quiet). Each spike fired by the neuron to be categorized is represented by a complex number that indicates its phase in relation to SWA. The sum of these complex numbers then gives an indication of the average phase, $\omega$, as shown in Eq. 7.

$$\omega_k = \sum_{s \in S_k} e^{\theta(s)}$$

Here $S_k$ is the set of spike times for neuron $k$ (0 ≤ $k$ < 100) and $\theta(s)$ is the phase of SWA at time $s$ (0 ≤ $\theta(s)$ < 2$\pi$). The argument of the complex number $\omega_k$ indicates the average SWA phase at which neuron $k$ fires, while its modulus provides an indication of how strongly SWA-modulated the firing is. Normalizing the
modulus by the number of spikes gives a confidence measure $c_k = \frac{\omega_k}{\omega}$, where $c_k = 0$ indicates that spikes did not fire preferentially at any one phase and $c_k = 1$ indicates that every spike occurred at exactly the same phase. After visual inspection of spike trains, we decided to categorize neurons with confidence $c < 0.1$ as NM. We categorized neurons with $c \geq 0.1$ as either TA or TI based on whether the average phase was during the active or inactive part of the SWA cycle. Neurons that fired fewer than one spike every SWA cycle on average were categorized as QU.

2.6. IMPLEMENTATION DETAILS

We simulated the model using custom written software developed by R Merrison-Hort. This software is written in C and uses the adaptive Runge-Kutta-Felberg ODE solver routine from the GNU Scientific Library (version 1.15). Absolute and relative error tolerances of $10^{-5}$ and a maximum step size of 1 ms were used for all simulations. To analyse the results we used scripts written in the Python (2.7) programming language with routines from the NumPy (1.6.2) and SciPy (0.11.0rc1) libraries. For each set in the Python (2.7) programming language with routines from the NumPy (1.6.2) and SciPy (0.11.0rc1) libraries. For each set in the Python (2.7) programming language with routines from the NumPy (1.6.2) and SciPy (0.11.0rc1) libraries.

All reported mean values are given with their standard deviation.

3. RESULTS

3.1. MODEL GP NEURONS BEHAVE REALISTICALLY UNDER HEALTHY CONDITIONS

The characteristics of the model GP neurons qualitatively match those of real rodent GP neurons in a number of key ways and are illustrated in Figure 3A. When no synaptic or injected currents are present (dark blue trace in Figure 3A), most model neurons (96%; 481/500) pacemake at a range of frequencies (23.6 Hz ± 4.0). Depolarizing current injections increase the frequency of firing (green trace), with very high frequencies possible (up to approximately 200 Hz). Hyperpolarizing current injections result in a prominent and transient “sag” in membrane potential (red, cyan and pink traces). The first spike after hyperpolarizing current is removed occurs after a similar delay regardless of the size of the injected current. These properties match those seen in experiments with slices of rodent GP (Chan et al., 2004; Bugaysen et al., 2010).

The mixed-conductance HCN channels play an important role in the activity of the model GP neurons and their response to hyperpolarizing input. The combination of a reasonably depolarized reversal potential (~30 mV) (Lüthi and McCormick, 1998) and activation at hyperpolarized membrane potentials (lower than −60 mV) means that these channels act to return neurons to spiking threshold faster after hyperpolarizing current (or inhibitory synaptic input) is removed. Figure 3B shows how the simulated blockade of HCN channels affects the activity of a model GP neuron. When HCN channels are removed ($g_{hcnf} = 0$), the average pacemaking frequency decreases to 15.8 Hz ± 2.5 and 12% (58/500) of neurons do not pacemake. Without HCN channels the membrane potential sag is no longer seen, and hyperpolarizing current has a much stronger effect on membrane potential. The time between the removal of hyperpolarizing current and the return of spiking is also much longer, and much more sensitive to the hyperpolarization level. These results agree with previous work that has investigated the role of HCN channels using mouse GP slices and multicompartmental simulations (Chan et al., 2004).

3.2. HEALTHY NETWORK ACTIVITY

Whilst we were able to base STN firing rate and the conductance of GP-GP synapses directly on experimental evidence, we could only do this indirectly with the STN-GP synaptic conductance ($g_{SG}$). We chose a value of 0.1 nS for this parameter in the healthy case as this gives similar proportions of neurons in the TI, TA and NM groups as seen in experiments. Figure 4A shows this distribution [cf. Figure 2A in Mallet et al. (2008b)] and Figure 4B shows the spiking activity of the TI, TA and NM neurons in one trial. A small proportion of neurons (9.9% ± 2.1) are categorized as QU because they fire spikes rarely or not at all; we are not sure if this is a biologically accurate result as such neurons may have been excluded from the results of electrophysiological studies. The majority of neurons (68.3% ± 3.9) are categorized as NM and neurons in this group spike with an average firing rate.
of 12.3 Hz ± 3.3 and coefficient of variation (CV) of 0.12 ± 0.04. These statistics are in good agreement with those of neurons recorded from mice GP slices by Chan et al. (2004) (firing rate 12.5, CV 0.18). However, in contrast to these experimental results, which found no effect on firing rate or CV after blocking GABA_A receptors, we would expect the average firing rate of the neurons in our model to increase slightly with inhibition blocked, as the average firing rate in the network is lower than the average pacemaking frequency of isolated model neurons. The average firing frequencies in the (small) TI and TA groups were 3.7 Hz ± 1.8 and 10.7 Hz ± 4.7, respectively.

3.3. PARKINSONIAN NETWORK ACTIVITY

The effects of dopamine lesion were simulated in the model by an increased intensity of STN firing and increased strength of STN-GP excitation and intra-GP inhibition. These changes have a profound effect on activity in the model GP that is similar to what is seen in experiments. As Figure 5 shows, most neurons begin to preferentially fire during either the active or inactive phase of the SWA. In order to see proportions of TA and TI neurons that were similar to in vivo results it was necessary to double the strength of STN-GP and GP-GP synapses (g_{SG} = 0.2 nS, g_{GG} = 1.0 nS).

The average firing frequency of NM neurons decreased slightly under Parkinsonian conditions while the average firing rates of the TI and TA groups increased to 6.3 Hz ± 3.6 and 11.1 Hz ± 5.1, respectively (Figure 6A). Although the variance of these statistics is fairly large, there does appear to be a trend for different firing rates between the different groups that is not seen in vivo (Magill et al., 2001). This difference is perhaps not too surprising given our simplistic and somewhat arbitrary choices for STN-GP and GP-GP connectivity.

In order to investigate the factors that determine whether a neuron becomes TA, TI, NM, or QU we examined the following statistics of each neuron: maximum conductance of the NaP persistent sodium channel; initial (before downregulation) maximum conductance of fast and slow HCN channels; total maximum conductance of all excitatory (AMPA) synapses from STN neurons; total maximum conductance of all inhibitory synapses from other GP neurons. In general these statistics were remarkably similar between each of the groups, with two exceptions. Firstly, quiet (QU) neurons have, on average, much lower maximum conductances for their NaP channel (Figure 6B). These channels underlie autonomous pacemaking (Mercer et al., 2007) and the intrinsic pacemaking frequency is strongly dependent on the value of the NaP maximum conductance. Since QU neurons have low NaP conductance they are likely to pacemake very slowly...
neurons. The network self-organizes its activity into the different groups of neurons and in the second set we used 300 GP neurons and 150 STN neurons. This level of coupling (≈ 20%) is probably much higher than what is seen in the real GP (Sadek et al., 2007). We ran several simulations, using Parkinsonian parameter settings, where the coupling proportion was decreased by scaling up the number of GP and STN neurons but keeping the number of synapses that each neuron made constant. In the first set of simulations we used 200 GP neurons and 100 STN neurons and in the second set we used 300 GP neurons and 150 STN. These give GP-GP coupling levels of 20/199 ≈ 10% and 20/299 ≈ 7%, respectively. In each case we ran three simulations. For the simulations with 200 GP neurons there were an average of 3.4 ± 0.2 spikes per second for regular firing, and 16.5 ± 0.8 spikes per second for burst firing. The average pacemaker frequency after downregulation was 16.9 Hz ± 2.1, a clear reduction from the normal pacemaker frequency of our model neurons (23.6 Hz ± 4.0). The proportion of QU neurons after downregulation was 2%, lower than the 4% that we would expect based on the normal pacemaker properties, but we attribute this to statistical noise due to the rather small sample sizes. The changes to pacemaking affect the competition dynamics during the inactive phase and mean that most TA neurons are no longer able to fire at all during this phase. Although the proportion of neurons classified as either TA or TI is similar with or without HCN downregulation (72.7% ± 5.4 normally, 68.7% ± 5.0 without downregulation), TA neuron firing is much more clearly restricted to the active phase with downregulation. This is seen in the average confidence measure (ω) of TA neurons, which is 0.44 ± 0.22 with HCN downregulation and 0.32 ± 0.18 without. The effect is also shown by phase diagrams showing the spiking activity of typical neurons (Figure 7). In these plots the background is shaded to show the active (pink) and inactive (blue) parts of the SWA cycle, showing that TI neurons fire preferentially in the inactive part and TA neurons fire preferentially in the active part. The red bars show the average spike phase and their length indicates the confidence measure as a proportion of the total radius.

3.4. LARGER NETWORKS

The results described above were from simulations with 100 GP neurons, each of which made 20 inhibitory synapses onto 20 other (randomly chosen) GP neurons. This level of coupling (≈ 20%) is probably much higher than what is seen in the real GP (Sadek et al., 2007). We ran several simulations, using Parkinsonian parameter settings, where the coupling proportion was decreased by scaling up the number of GP and STN neurons but keeping the number of synapses that each neuron made constant. In the first set of simulations we used 200 GP neurons and 100 STN neurons and in the second set we used 300 GP neurons and 150 STN. These give GP-GP coupling levels of 20/199 ≈ 10% and 20/299 ≈ 7%, respectively. In each case we ran three simulations. For the simulations with 200 GP neurons there were an average

HCN channel downregulation plays a significant, but not essential, role in the emergence of the different groups of neurons in our model. Without this mechanism, many neurons in the TA group continue to fire during the inactive phase due to their intrinsic pacemaker properties. Although this inactive-phase firing is slower than their active-phase firing (due to reduced excitatory input), it is still a source of inhibitory input to other GP neurons and may silence or slow the firing of some which may otherwise be categorized as TI. With the HCN downregulation mechanism those neurons that receive the most excitatory STN input, and therefore fire at the fastest rate during the active period, will have their maximum HCN conductances reduced. This reduction has the effect of decreasing the intrinsic pacemaking frequency and increasing the hyperpolarization that occurs in response to inhibitory input. To quantify the effect on pacemaking frequency, we ran four simulations using the Parkinsonian parameter values for a period of time long enough for downregulation to take effect (6.5 s) and then removed all synapses and recorded firing rates. The average pacemaker frequency after downregulation was 16.9 Hz ± 2.1, a clear reduction from the normal pacemaker frequency of our model neurons (23.6 Hz ± 4.0). The proportion of QU neurons after downregulation was 2%, lower than the 4% that we would expect based on the normal pacemaker properties, but we attribute this to statistical noise due to the rather small sample sizes.

or not at all, and it appears (from examination of voltage traces) that the incoming inhibition from other GP neurons is sufficient to prevent them from ever firing. Secondly, TA neurons receive on average more excitatory synaptic input from the STN than the other groups (Figure 6C). This result was expected, since STN firing occurs during the active phase of SWA and so it is not surprising that those GP neurons that receive more STN input also fire preferentially during the active phase. In general, however, the simple statistics we examined about membrane properties and synaptic connectivity are not enough to determine which group a particular neuron will fall into. Predicting the classification of a neuron involves knowing the classification of the other GP neurons that it receives inhibition from. This makes the problem complex and extremely difficult to resolve a priori. Instead, when the network is simulated, a dynamic process takes place in which the network self-organizes its activity into the different groups of neurons.

Figure 6 | Average cell properties by categorization across all GP neurons from all trials (n = 1200). (A) Average firing rate is rather variable but is in general slightly lower for TI neurons. (B) Maximum conductance of the persistent sodium channel (NaP) which underlies pacemaking. Quiet neurons can be easily categorized as those with very low NaP conductance. (C) Average total maximum conductance due to excitatory synapses. TA neurons receive more excitation on average, but it is highly variable.
of 71 ± 2.8 TI neurons and 73.7 ± 1.7 TA neurons. For the simulations (n = 3) with 300 GP neurons there was an average of 122 ± 4.1 TI neurons and 101.3 ± 2.5 TA neurons. These proportions (particularly in the latter case) are similar to the proportions in the smaller network (see Figure 5).

### 3.5. OTHER FREQUENCY BANDS

We briefly investigated to see if the activity of GP neurons could become entrained to higher frequency cortical rhythms, specifically those in the β band. To do this we generated STN spike trains that were modulated at approximately 14 Hz (70 ms period: 40 ms inactive phase, 30 ms active phase). Although biological experiments on OHDA lesioned rodents find that most neurons fall under the same TI or TA category regardless of whether the cortical rhythm is SWA or β, it was difficult and not effective to use our normal method to categorize neurons because the number of spikes fired by GP neurons in each β cycle was very low. However, examining spike cross-correlations between STN and GP neurons showed that the majority of GP neurons did show oscillatory firing that was in-phase with the STN input (Figure 8A). We examined auto-correlations for individual GP neurons and found that the frequency of these neurons’ oscillations varied somewhat from neuron to neuron, which suggests that their firing becomes synchronized to some intermediate frequency between the 14 Hz input and their intrinsic pacemaking frequency. We did not see any GP neurons that showed anti-phase oscillations when using our standard Parkinsonian parameters. However, when the degree of intra-GP inhibition is dramatically increased (40% coupling, \( g_{GG} = 3.0 \text{ nS} \)) then a few neurons do begin to show a preference for anti-phase firing (Figure 8B), albeit at a very low rate. It is possible that with different synaptic parameters or connection topology (for example, STN input that preferentially makes contact with a particular group of GP neurons), the synchronized activity of one group could cause a second group to become synchronized in anti-phase.

### 4. DISCUSSION

#### 4.1. A NEW, BIOLOGICALLY DETAILED, MODEL HELPS US TO STUDY GP DYNAMICS

We have presented what we believe to be a novel model of GP neurons that features much of the biological realism of previous detailed multi-compartmental models but considerably reduced complexity (both computationally and in terms of model construction). This makes our model well-suited to detailed modeling of the dynamics of networks of GP neurons and their connections with other nuclei. We have also introduced a possible computational mechanism for simulating the downregulation of HCN channels and shown that this improves how closely our results fit with biological evidence.

Our results demonstrate a mechanism whereby local inhibitory connections allow two anti-phase oscillatory sub-populations of GP neurons to emerge in response to rhythmic excitatory input from the STN. The two subpopulations appear due to a complex self-organization process and despite the homogeneity of the overall population. This effect is only seen when both the STN input and inhibitory GP-GP coupling are sufficiently strong, and there is good experimental evidence that both STN input to the GP and intra-GP coupling increase in rodent models of Parkinson’s disease. We therefore claim that our model shows a plausible mechanism for those experimental results which show a prominent increase in the number of TA and TI neurons that occurs in the rodent GP after dopamine lesioning (Magill et al., 2001). In our model, HCN channel downregulation makes oscillatory entrainment of the in-phase group of neurons more prominent but is not essential for the two groups to appear. This may explain the result of Chan et al. (2011) whereby artificial up-regulation of HCN channels via viral transfection restored the cells’ ability to autonomously pacemake but did not give any significant improvement to Parkinsonian motor impairment. The fact that we did not see an increase in the number of neurons that were unable to autonomously pacemake following simulations of the Parkinsonian network may indicate that we didn’t set the threshold for HCN downregulation low enough.
4.2. RELATIONSHIP TO PREVIOUS STUDIES ON COUPLED OSCILLATORS

Networks of coupled oscillators are found in many areas of science and the dynamics of such networks have therefore been widely studied from a theoretical standpoint. Our model can be thought of as a network of inhibitory coupled oscillators that receive random, sparse excitatory input with a particular global frequency. Although many theoretical studies of similar systems use reduced models, they may still provide insights into the different dynamical behaviors that our model is likely to exhibit.

Most previous theoretical studies do not include common external input to the coupled oscillators, although some may consider the effects of input that is constant in time. Chow (1998) describes the analysis of a neuronal network that consists of a number of oscillators with heterogeneous spiking frequencies that are all-to-all coupled by inhibitory connections. Such networks are capable of producing a range of dynamics, including almost-synchronous phase-locking, harmonic locking, and suppression. The stability of these states strongly depends on the details of the neurons’ response to synaptic input. This network is similar to our model in the case where STN input is made constant in time, although inhibitory coupling in the GP network is random and relatively sparse rather than all-to-all. Since SWA oscillations are much slower than the GP neurons’ intrinsic pacemaking, we can consider the GP network during the active and inactive phases separately, with constant STN input within each phase. Using the terminology of Chow (1998), during the active phase of SWA (high STN input) the TI neurons are in the suppressed state while in the inactive phase the TA neurons are suppressed. We have not observed synchrony between neurons during active or inactive phases, although it’s possible that the system would converge to these states after a long period of time with constant input.

As the frequency of the cortical modulation is increased to be closer to the GP neurons’ pacemaker frequencies (e.g., into the β band) it no longer makes sense to consider the scenario of constant STN input. In this scenario, theoretical results from oscillator models may be useful for suggesting conditions that support the emergence of in-phase and anti-phase groups. Golomb et al. (1992) describes a network of phase oscillators that all receive common global input that is a function of the phases of the oscillators. This is not explicitly the case in our model, but nevertheless their findings regarding the stability of solutions with clustered phase distributions may be relevant. In particular they show that the fewer clusters a state has, the more stable it is (larger basin of attraction). This could explain why the GP network under β modulation organises into just two anti-phase clusters. Kilpatrick and Ermentrout (2011) study a more biologically realistic model for the emergence of gamma rhythms in a network containing a large population of excitatory neurons with a smaller subpopulation of inhibitory interneurons. Interestingly, they show that the number of clusters that emerge in their model depends on the level of spike frequency adaptation in the excitatory neurons, which arises due to a calcium current. Our model contains a calcium channel that activates during fast firing and causes some degree of spike frequency adaptation, which raises the possibility that using different conductances for this channel may result in patterns with more than two clusters. It has also been shown that networks of neurons that have heterogeneous synaptic interconnectivity may display clustered dynamics if the connectivity structure satisfies certain conditions (Li et al., 2003)—although this has only been shown for excitatory synapses and so it is not clear whether the same would apply to the GP network.

4.3. STIMULATION OF THE STN MAY REDUCE OSCILLATIONS IN THE HYPERDIRECT PATHWAY

The hypothesis that basal ganglia activity is entrained to cortical rhythms via the hyper-direct pathway in Parkinson’s disease offers some explanation of the possible mechanism(s) underlying the clinical effectiveness of STN deep-brain stimulation (DBS), in which an implanted electrode provides constant electrical stimulation of approximately 120 Hz to the STN. The precise effects of this stimulation on neuronal activity in the basal ganglia are not fully understood and are likely to be many and varied (Kringelbach et al., 2010). The computational model of the basal ganglia of Kumar et al. (2011) included the effects of DBS through either a reduction of strength of cortex-STN synapses or inhibitory input onto STN and in both cases DBS was found to reduce oscillatory firing. In this model oscillations appear because the STN and GPe act as a pacemaker circuit due to the excitatory
connections between STN neurons. It is clear that if DBS were added to our model in a similar manner then oscillations in the GP would be much reduced, as they are dependent on reasonably strong input from the STN. Another possible mechanism of DBS that has good experimental support is that it antidromically activates the fibers that project from the cortex to the STN (Li et al., 2007). The effect of this antidromic activation is a reduction of oscillatory activity in the cortical regions that project to the STN. Since our model only includes STN input to the GP, clearly a reduction of oscillatory STN activity would reduce GP oscillations as well. Wilson et al. (2011) found that in a relatively abstract model of the GP consisting of uncoupled phase oscillators synchronized to a common input, chaotic dynamics served to desynchronize the population at frequencies and intensities similar to those that are clinically effective for DBS. The same may also be true of our model when the GP neurons are entrained to β-frequency STN input but further investigation is required. Our model could also help to test and improve the effectiveness of new forms of DBS, such as that proposed by Popovych and Tass (2012) which involves using multiple electrodes to desynchronize groups of neurons that have become entrained to particular rhythms.

4.4. THE EMERGENCE OF TI/TA GROUPS DEPENDS ON STRONG INHIBITORY COUPLING

One possible weakness of our model is that it relies on intra-GP inhibition being much denser than is currently supported by experimental evidence. It has been estimated that the probability of a given GP neuron synapsing onto any other, randomly-chosen, GP neuron is less than 1% (Sadek et al., 2007) but in most of our simulations this value is 20%. Our preliminary experiments with larger networks have suggested that the level of intra-GP coupling can be reduced while preserving the division into TI and TA groups by increasing the size of the network. Further work will involve investigating even larger (more computationally expensive) networks to see how much further the size of the network can be increased while maintaining the same division.

If further increases in network size cannot generate realistic activity with physiological levels of GP-GP coupling, there are several other possible reasons why the connectivity may be greater than has so far been measured experimentally. It has been suggested that the basal ganglia are organized into a series of partially overlapping “channels” (Alexander and Crutcher, 1990), where neurons preferentially synapse onto other neurons in the same channel. We have previously shown modeling evidence that increased coupling between channels may allow the STN-GP circuit to generate oscillations (Merrison-Hort et al., 2013), but in the present study we suggest that our small population of GP neurons could represent part of a single channel. Under this assumption, the proportion of coupled neurons might be much higher than would be seen from picking pairs of neurons from across the whole GP at random. It is also possible that the projection from GP to STN, which is not included in our model, may contribute to the effect of lateral inhibition since the tri-synaptic GP-STN-GP pathway is a route by which GP neurons inhibit other GP neurons, and there is experimental evidence to suggest the strength of this pathway may be increased under Parkinsonian conditions (Johnson and Napier, 1997). However, it is hard to say whether or not this explanation is plausible without more detailed information about the topology of the STN-GP and GP-STN projections.

Similarly, the increase in GP-GP synaptic conductance that occurs under Parkinsonian conditions in our model may be larger than in reality. Although we have used data from paired-pulse experiments to choose the conductance of GP-GP synapses in the healthy case, it is not clear how much this increases by following loss of dopaminergic input. Miguélez et al. (2012) used an optogenetic technique to stimulate a number of GP neurons whilst recording IPSCs and found an increase of approximately 67% after dopamine lesioning. This is considerably smaller than the increase we use under Parkinsonian conditions, where the GP-GP conductance is doubled. However, the increase seen by Miguélez et al. (2012) may be lower than the in conductance at a single GP-GP synapse, since their method simultaneously activates many pre-synaptic GP neurons and the summation of the resulting IPSCs may not be linear.

4.5. FUTURE WORK: STRIATAL INPUT, RECIPROCAL CONNECTIONS AND GP HETEROGENEITY MAY IMPROVE OUR RESULTS

The aim of this study was primarily to investigate whether the hyperdirect pathway alone could account for one characteristic of the Parkinsonian GP and we have therefore only included STN-GP and GP-GP synaptic connectivity. However, the main source of synaptic input to the GP is the striatum, and it is clear that adding simulated inhibitory striatal synaptic input could improve our results. Galati et al. (2009) demonstrated that the delivery of a GABA_A antagonist into the GP also causes cortical entrainment of the neurons there and that this effect is dependent on a functioning STN. They suggest that this demonstrates that inhibitory striatal input is also involved in oscillatory entrainment. This result is more difficult to explain in our model, since it is unlikely that decreasing the level of GP-GP inhibition would cause oscillations to appear in the (otherwise) healthy case. However, if the effect of GABA antagonism is to remove tonic background inhibition (probably from the striatum) then we could include this in our model as a depolarizing current injection to all GP neurons. This would move their membrane potentials closer to the spike threshold and make them more sensitive to the (weak) rhythmic STN input that is present in the healthy case. Furthermore, Tseng et al. (2001) showed that the activity of striatal projection neurons is modulated by cortical SWA and increases after OHDIA lesion. Including the effects of this in our model would probably increase the number of TI neurons and may allow us to reduce the amount of intra-GP inhibition to a more realistic level.

Another possible pathway that could be added to our model is the projection from the GP back to the STN. Computational models of networks that include this connection have shown that the STN and GP can work together to act as a pacemaker circuit (Gillies et al., 2002; Terman et al., 2002; Holgado et al., 2010). Terman et al. (2002) describes the results of simulating a spiking model of the interconnected STN-GPe network in which the tonic activity of both populations can become bursty with a regular bursting rhythm. In fact, the neurons in this model can self-organise into different sized clusters, which allows for the
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possibility of two anti-phase groups under some conditions. We
expect that our model could support similar regimes if the GPSTN connection was added, provided that we also introduced a
more realistic STN neuron model.
Although our model has demonstrated that discrete groups
of neurons can emerge from a population of GP neurons with
homogeneous (unimodally distributed) membrane properties,
there is now some evidence that the neurons in the TA and TI
groups are distinct in some ways, including the nature of local
inhibitory connectivity, the basal ganglia nuclei that they project
to, and in their chemistry (Mallet et al., 2012). Similarly, several
studies (Nambu and Llinás, 1997; Cooper and Stanford, 2000;
Bugaysen et al., 2010) have attempted to categorize GP neurons
based on their electrophysiological properties, and their results
seem to suggest that several distinct groups may exist (although
the boundaries remain fuzzy). It would be very interesting to
incorporate these results into our model, perhaps by making the
parameter noise for the NaP or HCN channels bi- or tri-modal
and by giving one group of neurons a higher degree of local connectivity than another. We expect that this would promote the
emergence of the TI, TA and NM groups and would probably
reduce the degree of GP-GP connectivity that is required in order
to obtain results that are similar to experiments.

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How do the pioneer networks in the axial core of the vertebrate nervous system first develop? Fundamental to understanding any full-scale neuronal network is knowledge of the constituent neurons, their properties, synaptic interconnections, and normal activity. Our novel strategy uses basic developmental rules to generate model networks that retain individual neuron- and synapse resolution and are capable of reproducing correct, whole animal responses. We apply our developmental strategy to young Xenopus tadpoles, whose brainstem and spinal cord share a core vertebrate plan, but at a tractable complexity. Following detailed anatomical and physiological measurements to complete a descriptive library of each type of spinal neuron, we build models of their axon growth controlled by simple chemical gradients and physical barriers. By adding dendrites and allowing probabilistic formation of synaptic connections, we reconstruct network connectivity among up to 2000 neurons. When the resulting “network” is populated by model neurons and synapses, with properties based on physiology, it can respond to sensory stimulation by mimicking tadpole swimming behavior. This functioning model represents the most complete reconstruction of a vertebrate neuronal network that can reproduce the complex, rhythmic behavior of a whole animal. The findings validate our novel developmental strategy for generating realistic networks with individual neuron- and synapse-level resolution. We use it to demonstrate how early functional neuronal connectivity and behavior may in life result from simple developmental “rules,” which lay out a scaffold for the vertebrate CNS without specific neuron-to-neuron recognition.

Key words: axon guidance; growth cone; locomotion; rhythms; Xenopus

Introduction

In vertebrates, molecular mechanisms, like gradients of morphogens, organize dorsoventral (DV) rows of neurons along the developing nervous system (Helms and Johnson, 2003; Goulding and Pfaff, 2005; Lewis, 2006). The neurons then grow axons, and knowledge of the mechanisms allowing axons to navigate is constantly increasing (Dickson, 2002; Chilton, 2006; Zou and Lyuskayutova, 2007). However, it is still unclear how neurons build networks by making synaptic connections (Sperry, 1963; Zupanc, 1986). Our aim is to “grow” realistic model networks to test whether early network assembly could be controlled by a basic set of rules. Critically, to validate model networks we need to show they have the same responses to input as living networks. This raises a big problem for exploring networks in higher brain regions because precise inputs and outputs are not known. In newly hatched larval fish and amphibians, pioneer networks allow them to respond predictably to touch by swimming (McLean and Fetcher, 2009; Roberts et al., 2010). The precise input and output are therefore defined and can be used to validate model networks.

We use hatching Xenopus tadpoles to explore the first formation of a working network (Fig. 1A–C; Roberts et al., 2010). Paired whole-cell recordings have provided detailed evidence on the different spinal neurons and synaptic interactions in the network generating swimming in response to touch stimuli. This evidence provides the foundation for using a developmental model to generate a full-scale functioning locomotor network. The recordings and previous anatomical network modeling suggested a lack of specificity in synaptic connections (Li et al., 2007). Connection probability appeared to reflect the dorsoventral distributions of axons and dendrites (Borisyuk et al., 2008, 2011): dorsal sensory axons contact dorsal sensory pathway neuron dendrites, but not ventral motoneuron dendrites. Our hypothesis is that early axon growth is controlled by simple responses to ba-
sic features like physical barriers and chemical gradient cues (Sperry, 1963; Shimozono et al., 2013); axons synapse probabilistically with any dendrites they contact without specific neuron recognition; this is sufficient for networks to form that are able to function without further refinement.

If the factors controlling network assembly in the early vertebrae brain are simple, a developmental model using basic rules should be sufficient to generate the full scale synaptic connectivity of the functional network controlling swimming in Xenopus tadpoles. We therefore use existing (for review, see Roberts et al., 2010) and new whole-cell recordings to define neuron activity and obtain anatomical data on the seven neuron types involved. We then build an axon growth model to reconstruct the swimming network of ~1400 neurons in the tadpole hindbrain and rostral spinal cord. Crucially, we know exactly what sensory activity induces tadpole swimming as well as the resulting motor output. This means we can test the operation of networks generated by our developmental model by asking if they produce swimming activity in response to sensory stimuli. We conclude that simple mechanisms may lay out the first functioning neuronal networks in the vertebrate nervous system.

Materials and Methods

Animals, behavior, physiology and anatomy

Procedures for obtaining hatchling Xenopus laevis (Daudin) tadpoles of either sex from a captive breeding colony comply with UK Home Office regulations. All experiments and analysis were approved by the local ethical committee and were performed on tadpoles at developmental stage 37/38 (Nieuwkoop and Faber, 1956) at 18–22°C. Videos of 40 tadpoles were recorded at 300 fps with a Casio Exilim ExF1 camera. Methods for extracellular skin stimulation, motor nerve recording, whole-cell electrophysiology, and dye filling with neurobiotin have been described recently (Buhl et al., 2012). Stable whole-cell recordings were made in 74 animals: 77 descending interneurons (dINs; including six pairs simultaneously on opposite sides), 22 dorsolateral commissural interneurons (dlcs; including 11 simultaneously with a dIN), and 3 dorsolateral ascending interneurons (dias). Following experiments, neuron anatomy was revealed using DAB as chromogen and observed using a 100X oil-immersion lens (for review, see Li et al., 2001). The recorded dlc, dl, and dIN neurons were identified by comparison to published data on their responses to skin stimulation, activity during swimming, and anatomy after dye-filling (Li et al., 2003, 2004a, 2006). Chemicals were from Sigma.

To measure the three-dimensional morphology of neurons, neurobiotin-filled neurons were defined for each neuron type relative to the ventral midline (“axes” and their intersection provided datum lines and a CNS framework in which to map neuron positions and features. The type of structures and their 3D coordinates were saved to a text file by Gridstore software. The saved 3D x, y, z coordinate pairs were then corrected for z-axis distortion by light refraction in glass (1.35 x), shrinkage (1.28 x), and straightening of curvature of the ventral midline “axis.” The x, y, z coordinate pairs were converted to .swc format with a single coordinate at the midpoint of the pair plus a radius. Transformations were performed using custom Python and MatLab tools (available on request). Swc files list x, y, z midpoints and their radii along with connectivity and type, and allow 3D visualization of neurons based on a series of connected cylindrical segments using Neuromantic (Myatt et al., 2012). This also allowed the export of images (Fig. 2D–F). For use in the growth model (see below), .swc coordinate data were converted to 2D as if viewed laterally, and the dorsoventral projection patterns of dendrites and axons were defined for each neuron type relative to the ventral midline axis (Fig. 2F, G). The new .swc neuron mapping data (79 neurons; including 8 dlcs fills from recordings made during this study) were combined with new measurements made from existing drawings to trace the individual neurobiotin or HRP-filled neurons (205 neurons). These new measures extended and improved the accuracy and detail of our anatomical library. We measured soma and axon branching positions, axon initial outgrowth angle and tortuosities, dorsolateral dendrite extents, and complete primary and secondary axon trajectories and lengths. Calculations and descriptive statistics were performed in Microsoft Excel and Minitab. All means are given with their SD.

To define CNS anatomy and the axon growth environment, tadpoles were anesthetized in 0.1% MS222, fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, and obtained anatomical data on the seven neuron types involved. The type of structures and their 3D coordinates were saved to a text file. The coordinates were extended and improved the accuracy and detail of our anatomical library. We measured soma and axon branching positions, axon initial outgrowth angle and tortuosities, dorsolateral dendrite extents, and complete primary and secondary axon trajectories and lengths. Calculations and descriptive statistics were performed in Microsoft Excel and Minitab. All means are given with their SD.
Table 1. Channel rate functions for model dIN neurons

<table>
<thead>
<tr>
<th>Channel</th>
<th>Rate function</th>
<th>A (ms⁻¹)</th>
<th>B (ms⁻¹ mV⁻²)</th>
<th>C</th>
<th>D (mV)</th>
<th>E (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>α_n</td>
<td>4.05</td>
<td>0</td>
<td>1.0</td>
<td>-15.32</td>
<td>-13.57</td>
</tr>
<tr>
<td></td>
<td>β_n(V&lt;−25mV)</td>
<td>1.24</td>
<td>0.093</td>
<td>-1.0</td>
<td>10.63</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>β_n(V&gt;−25mV)</td>
<td>1.28</td>
<td>0</td>
<td>1.0</td>
<td>5.39</td>
<td>12.11</td>
</tr>
<tr>
<td>K fast</td>
<td>α_k</td>
<td>5.06</td>
<td>0.0666</td>
<td>5.12</td>
<td>-18.396</td>
<td>-25.42</td>
</tr>
<tr>
<td></td>
<td>β_k</td>
<td>0.505</td>
<td>0</td>
<td>0</td>
<td>28.7</td>
<td>34.6</td>
</tr>
<tr>
<td>K slow</td>
<td>α_k</td>
<td>0.462</td>
<td>8.204 × 10⁻³</td>
<td>4.59</td>
<td>-4.21</td>
<td>-11.97</td>
</tr>
<tr>
<td></td>
<td>β_k</td>
<td>0.0924</td>
<td>-1.335 × 10⁻³</td>
<td>1.615</td>
<td>2.1 × 10⁵</td>
<td>3.33 × 10⁵</td>
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<tr>
<td>Na</td>
<td>α_n</td>
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<td>1.0</td>
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<td>1.0</td>
<td>9.01</td>
<td>9.69</td>
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<tr>
<td></td>
<td>α_n</td>
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<td>0</td>
<td>0</td>
<td>38.88</td>
<td>26.0</td>
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<tr>
<td></td>
<td>β_n</td>
<td>4.08</td>
<td>1.0</td>
<td>1.0</td>
<td>-5.09</td>
<td>-10.21</td>
</tr>
</tbody>
</table>

The time constants and steady-state values of each channel’s gates are dependent on membrane potential, according to functions α(V) and β(V) (see Eq. 4 and 5). These functions are sigmoidal and their shape is determined by parameters A, B, C, D, and E.

Axon growth and synapse formation model

On the basis of our measurements, we open the CNS along its dorsal midline like a book to produce a two-dimensional growth environment representing the outer part of the tadpole CNS where the axons grow (Borisuyk et al., 2011). The axon growth model is described by the following system of three difference equations:

\[
\begin{align*}
x_{n+1} &= x_n + \Delta \cos(\theta_n) \\
y_{n+1} &= y_n + \Delta \sin(\theta_n) \\
\theta_{n+1} &= \theta_n - g_b h_i(x_n) \sin(\theta_n) + g_c h_i(y_n) \cos(\theta_n) \\
&\quad - g_b h_l(y_n) \cos(\theta_n) + \xi_n
\end{align*}
\]

where \((x_n, y_n)\) are the variables defining the coordinates of the axon tip (growth cone) in a two-dimensional field (with a longitudinal \(x\)-axis and the \(y\)-axis representing the DV axis on both sides of the CNS) and where the variable \(\theta_n\) is the growth angle. Parameters are as follows: \(\Delta\) is the growth step (1 μm); \(\xi_n\) is a random variable in the range \([-\alpha, \alpha]\); \(g_c, g_b, g_l\) are sensitivities to rostral, ventral, and dorsal guidance cues. Guidance cues \(h_i, h_l\) are given by their “diffusive” rostral, ventral, and dorsal gradients:

\[
\begin{align*}
h_i(x) &= \exp(-\beta_i x) \\
h_l(y) &= \exp(-\beta_l(y - 100))
\end{align*}
\]

where \(\beta_i, \beta_l, \beta_b\) are slopes. All parameters are universal for all neuron types except for the three gradient sensitivities and random angle fluctuations: \(g_c, g_b, g_l, \alpha\). Values of these four parameters were selected for each axon type using a stochastic optimization algorithm ("pattern-search" routine from the Global Optimization Toolbox of MATLAB) to minimize a cost function. This cost function was given by the weighted sum of two components: (1) the sum of squared differences of the averaged tortuosities (arc–chord ratios) calculated for the generated and measured axons, and (2) the dissimilarity ("distance") between the dorsoventral distributions of measured and modeled axon projections (histograms). The distance between histograms of measured and modeled axons is the normalized sum of squared differences between counts in corresponding bins of the histogram, and this quadratic form is identical to that used in the \(\chi^2\) statistic for testing the homogeneity of two distributions.

The optimization procedure results in parameter estimates that ensure the value of the cost function is suitably small: in the majority of cases, the value after optimization is in the range (2–16). To judge the quality of optimization, we applied two sample \(t\) tests to show that differences between mean tortuosities for real and modeled axons were not significant (\(p > 0.05\) in all cases). Although the standard statistical tests are not applicable for estimating the similarity between modeled and experimental histograms of dorsoventral axon distributions (data are not independent, being close successive points on the same axon), in the majority of cases, the values of the \(\chi^2\)-based component of the cost-function lay below the appropriate critical value for the \(\chi^2\) statistic (16.92 for 9 df at a significance level of \(p = 0.05\)).

Modeled dendrites were specified by their dorsal and ventral extents, by adding two-dimensional Gaussian noise (equal SDs of 15 and correlation of 0.8) to pairs of measured values. Dorsoventral projections of modeled dendrites were binned (10 μm bins) to produce a histogram. These binned distributions matched those of measured dendrites for each neuron type (\(\chi^2\) test: \(p > 0.05\)). Methods have been described previously for distributing populations of somata for each different neuron type along the CNS, and for allowing the stochastic formation of en-passant axo–dendrite synaptic connections to generate a network (Li et al., 2007; Borisuyk et al., 2011). The axon growth and synapse formation model was implemented using custom-written MatLab code.

Conductance-based neuronal network model

To assess each network, we mapped it onto a physiological, conductance-based, neuronal network model to test whether the connections generated by the growth model could produce swimming-like motoneuron activity in response to brief “sensory” stimuli.

Active channels in model dIN neurons. Hindbrain and spinal cord dINs are central to the Xenopus tadpole swim Central Pattern Generator (Li et al., 2010; Li, 2011). To construct model dINs with appropriate physiological firing properties, we used NEURON (controlled by a Python script; Carnevale and Hines, 2006) to build networks with 30 multicompartiment dINs electrically coupled by gap junctions on their axons. Membrane potential (\(V\)) evolves according to Equation 3. Channel kinetics were based on voltage-clamp data for the currents (Dale, 1995; Winlove and Roberts, 2012). The sodium (\(i_{Na}\)) and potassium currents (\(i_{K}\)) were modeled using a Hodgkin–Huxley–type formulation and the calcium current (\(i_{Ca}\)) was modeled using the Goldman–Hodgkin–Katz formulation. The \(i_{Na}\) current is due to passive membrane leak conductance and \(i_{Ca}\) is an externally applied current that is only used for testing the response of neurons to current injections. Each channel is gated by one or more gating variables. Equation 4 describes the dynamics of an arbitrary gating variable \(X\), where the functions \(X_n(V)\) and \(\tau_\alpha(V)\) give its steady-state value and characteristic time constant, respectively (Eq. 5). These functions are dependent on functions \(\alpha(V)\) and \(\beta(V)\), which control the opening and closing of gates and are written in the form of Equation 6. The values for parameters \(A, B, C, D, E\) and \(F\) are given in Table 1. [Note that the \(\beta\)-rate equation for calcium takes one of two sets of parameters depending on the membrane potential (Dale, 1995).]

cadocyanate buffer, pH 7.4, at 4°C for 90 min, and transferred to buffer to make direct measurements of whole-mount CNS dimensions before dehydration (n = 4). Three specimens were postfixed in 2% osmium tetroxide, dehydrated, embedded in Epon 812, sectioned at 2 μm, and stained with 1% methylene blue. Photographs of sections (Fig. 2A) were used to measure CNS features using NIH ImageJ software.
Parameter sweeps over the channel densities (of Ik, Na, K, Ca) were used to match the responses of the electrically coupled, intracompartmental model dINs to those of experimentally recorded dINs. The responses matched as follows: single action potential to depolarization current; rebound firing after hyperpolarization during depolarization; rhythmic firing during NMDAR activation (Soffe et al., 2009; Li et al., 2010). Since variability is introduced into model parameters (see below), 10 neurons were evaluated at each point.

**Physiological simulation of the full network.** For efficient computational simulations of the entire network with ~1400 neurons, the intracompartmental dIN model was simplified to a single compartment. The forms of the equations remained the same and values for density parameters were converted into whole-cell values by assuming a surface area of 1000 μm². (This is a round number lying within the range of summed soma and dendrite surface areas measured for filled neurons: 754–1594 μm². Inhibitory glycinergic synapses were given a reversal potential of -80 mV.) Following a presynaptic spike, the synaptic open probability was incremented by n/N, where n is the number of open channels and N is the number of closed channels.

Results

The foundations for this study lie in extensive previous work, first, on the neurons controlling tadpole swimming (Roberts et al., 2010; Li, 2011) and, second, on modeling of the operation of the swimming network (Sautois et al., 2007) and its development (Li et al., 2007; Borisyuk et al., 2008, 2011). Our previous axon growth model was not based closely on biology but provided a mathematically simple way to generate the longitudinal component of axon trajectories and reproduce the synaptic contact probabilities found in experiments (Li et al., 2007). We then used this axon growth model to generate model tadpole networks which we analyzed anatomically but not physiologically (Borisyuk et al., 2008, 2011). The present modeling is based on more detailed anatomical analysis of a larger set of filled neurons, new insights into the channel properties of spinal neurons (Winlove and Roberts, 2012), and the properties and roles of excitatory reticulospinal dIN neurons (Moult et al., 2013). It also uses a new, biologically realistic, gradient field-dependent model of axon growth.

**Recordings from tadpole neurons define their properties and connections**

Motor nerve recordings in immobilized tadpoles (Fig. 1D–F) show that a skin stimulus can initiate motor nerve activity (43.6 ± 11.5 ms after the stimulus: 155 trials in 31 tadpoles) matching real swimming behavior in frequency (10–25 Hz) and progressing from head to tail (Kahn et al., 1982).

Whole-cell recordings have been used to trace the chain of events from a skin stimulus to swimming. Overall, the accumulated recordings from ~1500 pairs of dye-filled neurons define the properties and synaptic connections of seven neuron types forming a network that allows the tadpole to swim when touched (Fig. 1G; Soffe et al., 2009; Roberts et al., 2010; Li, 2011; Buhl et al., 2012). Crucial components of the swim network are the excitatory pacemaker dINs, which drive swimming activity. We knew that these were excited when a few sensory RB neurons were excited to spike 4.5–8 ms after local trunk skin stimulation (Soffe, 2007). Synaptic conductances had values based on paired recordings (Sautois et al., 2007) with the following exceptions: sensory RB neuron AMPAR excitation was made stronger (0.6–8.0 nS) onto sensory pathway neurons to compensate for their reduced input resistance in our simplified model; the AMPA conductance of dIN synapses onto inhibitory ascending interneurons (aINs) was reduced (0.6–0.1 nS) so dIN firing during swimming was restricted to a short period following sensory stimulation in agreement with experimental data (Li et al., 2004b); the NMDA conductance of dIN-to-dIN synapses was reduced (0.3–0.15 nS) so the level of steady depolarization in dINs during swimming matched experimental measurements more closely (Li et al., 2006).

Our neuron network model was implemented with custom-written C code. All numerical simulations were performed using the Runge–Kutta–Fehlberg method from the GNU Scientific Library (version 1.15) with an adaptive step size (absolute and relative tolerance 10⁻³). The maximum step size was 0.1 ms and spikes were detected after every step by membrane potential zero crossing. Gaussian noise with a SD equal to a percentage of the mean value was applied to the capacitance and leak/ channel conductances of all neurons (at 2% of the mean), as well as the conductances of every individual synapse (at 5% of the mean). This noise represents the nonhomogeneity of soma sizes and synapse strengths, respectively. Higher noise levels were tested and found to make no major qualitative difference to the results of simulations.

The code for all our anatomical and physiological modeling is available on request from R.Borisyuk@plymouth.ac.uk

<table>
<thead>
<tr>
<th>Parameter</th>
<th>dINs</th>
<th>Other Neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leak conductance (nS)</td>
<td>1.4</td>
<td>2.47</td>
</tr>
<tr>
<td>Leak reversal potential (mV)</td>
<td>-52</td>
<td>-61</td>
</tr>
<tr>
<td>Sodium conductance (nS)</td>
<td>240.5</td>
<td>110</td>
</tr>
<tr>
<td>Sodium reversal potential (mV)</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Fast potassium conductance (nS)</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Fast potassium reversal potential (mV)</td>
<td>-81.5</td>
<td>-80</td>
</tr>
<tr>
<td>Slow potassium conductance (nS)</td>
<td>9.6</td>
<td>1</td>
</tr>
<tr>
<td>Slow potassium reversal potential (mV)</td>
<td>-81.5</td>
<td>-80</td>
</tr>
<tr>
<td>Calcium permeability (cm²/s)</td>
<td>0.014</td>
<td>N/A</td>
</tr>
</tbody>
</table>

N/A Not applicable.
1991), but the detailed timeline for activation of the dINs by trunk stimulation was unclear. We therefore made single or paired whole-cell recordings to define the delays in sensory pathway and dIN neuron responses following trunk skin stimulation above the threshold for evoking swimming monitored by motor nerve recordings (74 tadpoles, with 17 neuron pairs). Sensory pathway neurons on the stimulated side (dlc and dla; Fig. 1C) fire shortly after the sensory RB neurons (7.6 ± 1.8 ms after skin stimulation; 16 neurons). The dINs on each side of the body then receive excitation (13.6 ± 8.1 ms, 58 neurons). This excitation leads to dIN firing on one side or the other after 36.5 ± 23.1 ms (48 neurons), and when this occurs, rhythmic swimming starts (Fig. 1E,F). In this way we have defined the input and output of the whole swim network as well as the precise patterns of neuron activity during swimming (Roberts et al., 2010).

Anatomy of seven brainstem and spinal cord neuron types in the swim network

Detailed anatomical analysis is required to allow us to model the growth of each neuron type, the crux of our developmental strategy, and the formation of the swim network in the young tadpole. The spinal cord has a simple tube-like architecture with a ventral “floor plate” formed of cuboidal cells and a dorsal “roof plate” formed by sensory RB neuron somata (Fig. 2A,B). As in all vertebrates, sensory neurons and functions are located dorsally, while motoneurons and motor functions are more ventral. At least seven types of spinal and hindbrain neuron are active during swimming (Li et al., 2001; Roberts et al., 2010). Using 276 single neuron dye fills from previous and 8 from current whole-cell recordings, details of their soma positions, dendrite dorsal and ventral extents, complete axonal trajectories, and branch points were defined by measuring a sample of each neuron type (Fig. 2C–H; Table 3). Axons were considered to be filled to their ends if they had a well labeled end-bulb. The characteristics of each neuron type were analyzed to define common patterns and generate a library of quantitative anatomical measurements. Most neuron somata lie medial to the marginal zone (MZ) where longitudinal axons make en-passant synapses with mainly radial dendrites. Most axons grow ventrally into the MZ before turning to grow longitudinally on the same side or growing though the floor plate to reach the opposite side before turning longitudinally (Fig. 2B).

The present data on the DV distribution of axons and dendrites extends our previous measurements (Li et al., 2007) in both number and details of measures. These distributions determine where synapses can form and the broad differences between different neuron types are illustrated here by their median DV positions (in micrometers relative to the ventral edge of the MZ; Figs. 2H, 3B,C). Sensory neuron axons lie in the dorsal tract, which is separated from the MZ by a barrier formed by a superficial row of dorsolateral neuron somata belonging to sensory pathway dlla and dlc neurons (cf. adult dorsal column and dorsal horn; dl in Fig. 2A,B; Clarke and Roberts, 1984). Sensory pathway neurons have dendrites distributed in the dorsal MZ and the dorsal tract (median DV positions: dlla, 109.9; dlc, 106.7) where they can contact sensory axons (Fig. 3G; Li et al., 2003, 2004a). The axons of all but the sensory neurons lie in the MZ (Fig. 2A,B), and their DV distributions peak in a mid-DV position (median DV positions: alN, 45.9; dlla, 45.4; dIN, 35.3; dlc, 32.6); in commissural interneurons (cINs) and motoneurons the peak is more ventral (median DV positions: cIN, 23.5; mm (motoneuron), 13.3; Fig. 3B). The dendrites of the nonsensory pathway neurons have distributions extending through the MZ rather like the axons, but with alN and motoneuron dendrites having a more ventral bias (median DV positions: dIN, 44.8; cIN, 42.7; mm, 34.8; aIN 31.0; Fig. 3C).

Modeling axon growth for each type of neuron

New anatomical data and their analysis provided the essential underpinning of an axon growth model. The first requirement was a two-dimensional growth environment. This consisted of a rectangular field. It was based on measures from sections of the hindbrain and rostral spinal cord (Fig. 2A) and was made by opening the CNS like a book along its dorsal midline for 2000 μm from the midbrain (Figs. 2B,H, and 3; Li et al., 2007; Borisyuk et al., 2011). Regions >2000 μm caudal to the midbrain were not considered because the narrowing of the spinal cord becomes significant and we have insufficient data on caudal neuron anatomy and physiology. Barriers to axon growth are formed by the edges of the whole CNS, the floor plate ventrally, a dorsolateral row of neuronal somata separating the marginal zone from the sensory dorsal tract (dl in Fig. 2A,B), and the roof plate of sensory RB somata dorsally. The floor plate is the only barrier routinely crossed by commissural axons. In addition to the barriers, the model growth environment was given three axon growth cues: a rostral-caudal polarity cue, and dorsal and ventral cues representing diffusive chemical gradients (Sanes et al., 2006), each with the same exponential form and slope (Fig. 3A). These DV gradients originate at the dorsal edge of the cord and within the floor plate, 5 μm from the ventral midline. The barriers, rostrocaudal (RC) polarity, DV gradients, and overall dimensions define the growth environment.

The axon growth model uses surprisingly few and simple rules to match the trajectories of the real axons of each neuron type. In essence, they are as follows. (1) Axons were assigned an initial axon position (same as soma position), an outgrowth angle, a branch distance and a branch angle, and a final length, all based on generalization from measurements (Table 3; see Materials and Methods). (2) Axons then extend in 1 μm steps, turn after each step in response to the three guidance cues (the angle depending on the modeled sensitivities of the growth cone to each gradient) with a small random element, and some branch to grow a second axon once the first has grown. An optimization process was used to find parameters for guidance cue sensitivities and the degree of random turning for axons of each neuron type giving the best match to real axon trajectories. We found that axons of each neuron type also needed a sequence of different parameter sets at successive stages of their growth. All axons have an initial orientation to longitudinal growth stage, and a main growth stage; commissural axons also have a change in their response to gradients after growing through the floor plate (Moon and Gomez, 2005). After the parameter optimization for each stage, the model generated sets of realistic axonal trajectories and distributions for each neuron type, either to the same or the opposite side of the CNS (Fig. 3B,D).

Modeling the formation of synaptic connections and the swim network

The axon growth model was then used to produce a map of connections from axons onto dendrites in the whole swim network. Defined populations of each neuron type (~30–200) were distributed along each side of the 2D growth environment based on available evidence, for example from transcription factor immunocytochemistry (Li et al., 2001, 2004b). The axon growth model then generated the axons of the ~1400 neurons in our 2000-μm-long model based on the simple gradients and barriers. Since understanding of spinal neuron dendrite growth is lacking
Cline and Haas, 2008; Katsuki et al., 2011), we assigned a single straight “dendrite” to each neuron at its soma position with dorso-ventral limits generalized from measurements (Figs. 2B, 3A, C, 4B; Table 3; Li et al., 2007). Where an axon crossed a dendrite, a synapse was made with a probability of 0.46 in the marginal zone and 0.63 for sensory connections in the dorsal tract based on evidence from paired whole-cell recordings (Li et al., 2007). Each run of this process generated a unique network: a map and list of all the synapses (range: 81,822–91,045; mean number 86,655 ± 1412 SD) between all the neurons in the model network (Fig. 4A; Table 4).

Each network generated by the growth model (we will use this term as a shorthand for: axon growth and connection probability model) reflects the particular distribution of neurons, axons, and dendrites (Fig. 4). Thus, as in life, sensory RB axons in the dorsal tracts synapse with sensory pathway dlc and dla neurons, the only neurons with dendrites lying in this tract (Fig. 4C). These sensory pathway neurons in turn synapse onto the swim neurons like...
dINs. Since their long ascending axons accumulate rostrally, they make more synapses here and fewer caudally (Fig. 4C, dla and dlc; Wolf et al., 2009). The excitatory dINs (~120 per side), which drive swimming, make synapses with each other, with inhibitory cIN and aIN neurons, and with motoneurons. The reciprocal inhibitory cIN neurons make synapses with all except sensory RB neurons on the opposite side (for summary see Fig. 1C, Table 4).

### Table 3. Neuron anatomy parameters for axon growth model

<table>
<thead>
<tr>
<th>Neurons</th>
<th>RB</th>
<th>dlc</th>
<th>dla</th>
<th>aIN</th>
<th>cIN</th>
<th>HdIN</th>
<th>RdIN</th>
<th>CdIN</th>
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<tbody>
<tr>
<td>Soma</td>
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<tr>
<td>Number per side</td>
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<td>999 (298)</td>
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<td>6.9 (9.3)</td>
<td>26.4 (11.2)</td>
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<td>21.2 (18.2)</td>
<td>31.1 (17.3)</td>
<td>13.3 (3.5)</td>
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Measured values are expressed as means (SD). Values in italics were fixed in the model as follows: dorsoventral positions of somata of sensory RB neurons, and sensory pathway dla and dlc neurons so they lay in the strict longitudinal columns that they form in vivo; some initial and/or branch angles because there is negligible variation in vivo; axon branch positions of RB and HdIN (hindbrain descending interneuron) because all occur at the soma; soma numbers because all are based on current best estimates (Borisyuk et al., 2011). Prim and Sec are primary and secondary axons. Branch dist is the distance of an axon branch from the soma. Excitatory dINs were subdivided into three populations (Hindbrain, Rostral, and Caudal) because some features like initial axon outgrowth angle and possession of a secondary ascending axon change with longitudinal position.

### Figure 3

The 2D CNS growth environment with modeled axons and match to real axons. A, 2D plan with examples of real measured axons for each neuron type. Circles show positions of somata and vertical bars show simplified dendrites for 4 neurons (*). The form of the exponential DV gradients is shown at right (dorsal gradient is green, ventral, blue). B, C, Histograms comparing DV distributions of real (measured, open bars) and model (solid bars) axons and dendrites (10 µm bins show proportions) for aINs, cINs, dINs, and dlas. Note that sensory pathway dla dendrites extend into dorsal tract. D, Examples of modeled axon trajectories of dINs and dlas (cf. Fig. 3H).
Can modeled networks produce realistic, whole animal behavior?
Crucially, we can validate and test the adequacy of each “grown” network because we know the real network response to skin stimulation is sustained, coordinated swimming. The generated swim networks were therefore mapped onto a physiological model to ask if a brief stimulus to some of the sensory neurons innervating the skin triggers sustained swimming. Neurons in the physiological model were single-compartment and, for simplicity, most of them had the same, typical, Hodgkin–Huxley-type membrane channels as motoneurons in our previous study (Dale, 1995; Sauvotis et al., 2007). The model dIN neurons alone were given special properties based on recent experimental evidence from whole-cell paired and voltage-clamp recordings and modeling using a population of electrically coupled multicompartment dINs (Roberts et al., 2010; Li, 2011; Winlove and Roberts, 2012). The majority of voltage-gated current is carried through one type of sodium channel (Na), fast and slow potassium channels (K_f, K_s), and a high-voltage-activated calcium channel (Ca). After tests in the multicompartment model, the channel properties and weak
electrical coupling were mapped onto single-compartment dINs in a network model. Injecting current into a single dIN showed that the new model neurons responded with single spikes but could show postinhibitory rebound firing as seen in experiments (Fig. 5A, B; Li et al., 2006). However, we could also show that the electrically coupled population of dINs would show rhythmical pacemaker-like firing in the swimming frequency range during the slow activation of an NMDA conductance (Fig. 5C; Li et al., 2010). In the swimming networks we build, rhythmic firing is based on feedback glutamate excitation activating NMDA and AMPA receptors. It depends on voltage-gated Na⁺, Ca²⁺, and fast and slow K⁺ channels and postinhibitory rebound firing. We used three types of conductance-based synapses: glutamatergic AMPA and NMDA, and glycinegic. Synaptic time course and strengths were based on evidence from paired recordings (Dale, 1995; Sautois et al., 2007) with only minor adjustments to connections strengths which are defined in Materials and Methods.

When a network was mapped onto a physiological model incorporating the special dIN properties, a single spike in two sensory RB neurons (modeling touch to the skin) initiated the alternating motoneuron firing we define as “swimming” (Fig. 6A–C). Stimulation made sensory neurons (yellow/black) fire and recruited sensory pathway neurons (pink and red). These amplified the sensory excitation and distributed it to both sides. Swimming started first reliably on the nonstimulated side, provided that sensory pathway neuron synapses were asymmetric and dlc neurons made stronger synapses on that side (Zhao et al., 1998). Alternating swimming activity occurred reliably in most networks tested (99 of 100 tests), often preceded by several cycles of synchronous left-right firing at double the swimming frequency (82 of 99 tests; range 1–7 cycles, the mode is 1 cycle). The swimming frequency (17.8 ± 0.55 Hz) was within the normal range (10–25 Hz) and left/right motoneuron activity was strictly alternating (phase 0.5 ± 0.01, n = 99) as in the immobilized tadpole (Kahn et al., 1982). If sensory pathway dla neurons projecting to the stimulated side made stronger synapses, motoneuron activity started on the stimulated side (Fig. 6D; 12 of 12), but there was little effect on average swimming frequency (18.2 ± 0.4 Hz). Without such asymmetry the network still swam reliably (11 of 12 tests) with an average frequency 18.1 ± 0.5 Hz, but swimming was preceded by more synchronous motoneuron activity on both sides of the body after stimulation (11 of 12 tests; range 2–7 cycles, the mode is 7 cycles). Head-to-tail progression of motoneuron activity was not clear in the model. However, it was also not found in the rostral 2 mm in vivo and only becomes clear over longer regions of the spinal cord (Tunstall and Roberts, 1991). The importance of the pacemaker properties of dINs was emphasized by the observation that if dINs were given the same properties as other neurons, a reflex occurred, but rhythmic swimming failed (0 of 12; Fig. 6E). In vivo, the initiation pathway is probably more complex because the delay to the first motoneuron spike is longer (43.6 ± 11.5 ms vs 19.6 ± 0.5 ms in the model) and can be on either side (50:50 in 155 trials in 31 tadpoles). Synchrony following stimulation is rare in vivo. However, these results show that complex model networks built by generalizing from small biological datasets and assembled following remarkably simple rules can produce reliable coordinated motor activity like swimming. The patterns of activity shown by each type of neuron are also similar to those seen in whole-cell recordings (Figs. 1E,F, 6B).

Experiments to find the essential features of model network assembly

The physiological model was then used to test the effect of easing anatomical constraints on network self-assembly. Swimming remained reliable but became faster (23.2 Hz ± 0.6, n = 12) when synapse formation probability was increased to 1 from 0.46 and 0.63. Swimming was still reliable when synapse probability was reduced by 12% (12 of 12). After a 25% decrease, most networks swam (9 of 12), whereas the rest showed rhythmic activity on one side of the body only. Scaling the probability down by 25% again (yielding a 44% reduction from the original value) eliminated swimming in all networks (0 of 12). We then tested whether the details of the axon or dendrite distributions of the neurons active during swimming (dIN, cIN, aIN, mn) in the marginal zone were significant for swimming. Swimming was still reliable when these neurons were all given the same axon DV distributions as sensory pathway dlas (at 16.2 ± 0.5 Hz; 12 of 12), or the dendrite DV distributions of inhibitory cINs (at 17.9 ± 1.0 Hz; 12 of 12; Fig. 7A). The next test was to make all CPG neuron dendrites span the whole dorsoventral extent of the marginal zone so they could be contacted by all axons except those of sensory neurons. This test effectively discards all the details of the axon trajectories and dendritic arborizations of each neuron type. Remarkably, these networks produced reliable swimming provided that synapse formation probability for CPG neurons was reduced (to 0.25) so that total synapse number was conserved (swimming at 13.9 ± 0.3 Hz; 12 of 12; Fig. 7B, C).

If the detailed DV positions of axons and dendrites are not critical to swimming network function, we can extend our model to more caudal regions of the spinal cord where anatomical data are scant. We therefore extended the model from 2000 to 3500 μm so it had 1980 neurons. Stimulation produced swimming as previously shown (at 17.5 ± 0.4 Hz; 12 of 12; Fig. 7D,E) but a head-to-tail delay of 4.4 ms mm⁻¹ was now clear between the earliest motoneuron spikes (at ~1000 μm) and the latest (at ~2500 μm). This is within the range of 2–5 ms mm⁻¹ found experimentally (Tunstall and Roberts, 1991).

The final test was to make the dendrites of all CPG neurons in the 2000 μm network reach into the dorsal tract so they could be
contacted and excited by sensory RB axons. Sensory stimulation now led to short-latency firing in all types of neurons on the stimulated side, which was never seen in recordings. Swimming always followed (at $19.1 \pm 0.5$ Hz; 12 of 12) but was preceded by more synchrony (mean 3.8 cycles) than in the control case.

**Discussion**

We show that it is possible to use a model of neuronal development to generate the large-scale anatomical pattern of neurons, axons, and synaptic connections forming the core of the vertebrate nervous system. This also leads to the conclusion that the first functional networks in the vertebrate brainstem and spinal cord may develop using surprisingly simple rules. This suggests that complex and large networks can assemble where connections are made without recognition of “correct” target neurons. Axons may only need to distinguish dendrites from glia and axons. They can then synapse with any dendrite they contact (with a certain probability) so long as they grow into an appropriate region and in a broad direction along the nervous system (toward the head or tail; on the same or the opposite side). This important
finding has implications for the longstanding debate on how neuronal connections are made (Sperry, 1963; Zipursky and Sanes, 2010). First, the circuits in developing larval vertebrates are not simply the precursors of more complex networks that only become effective once their connectivity has been refined by additional developmental processes like detailed recognition. They have to function immediately in their own right. As soon as they hatch, fish and frog larvae need to swim to avoid predation, so the networks controlling their first behavior have to work properly. Second, if the formation of spinal circuits relies on simple pro-
cesses, then there is hope for regeneration of spinal circuitry after injury if injected precursor cells only require some simple guidance to navigate the injured environment and integrate into existing circuitry (Bonner et al., 2011; Tuszyński and Steward, 2012).

Simple structural features may be critical for network formation. In the adult spinal cord, sensory axons enter the dorsal horn and dorsal column and are separated from more ventral neurons organizing action. This fundamental segregation of sensory and motor functions is also a feature of the developing frog spinal cord but has received little previous attention. The superficial somata of sensory pathway neurons (in a position corresponding to the adult dorsal horn) separate skin sensory axons in the dorsal tract (Nordlander, 1984, 1987; in a position corresponding to the spinal dorsal column) from the more ventral axons and dendrites of the other neurons. The consequence of this barrier is that sensory axons only make synapses with sensory pathway neurons, because these are the only neurons with dendrites in the dorsal tract. While this segregation of sensory functions is important, our manipulations of model dendrite lengths show that detailed specification of the dorsoventral distribution of axons and dendrite lengths in our models may actually not be necessary for swimming (Fig. 7 B,C). However, the broad pattern of longitudinal neuron distributions, axon projections (ipsilateral or contralateral, ascending and/or descending), and axon lengths are important because they determine the distribution of synapses along the body (Wolf et al., 2009). Our modeling points to these basic features and transmitter phenotypes controlled by transcription factor expression (Lewis, 2006) being necessary in the neuron specification process which can lead to early network formation.

Our study establishes that simple axon guidance mechanisms may be sufficient for the assembly of networks producing the first behavior of developing vertebrates. In fact, the real mechanisms directing axon growth may not be the ones we have used to generate our functional networks (Forbes et al., 2012). In a number of systems there is detailed information on the mechanisms guiding growing axons to their target areas (Dickson, 2002; Chilton, 2006; Zou and Lyuksyutova, 2007; Kastanenka and Landmesser, 2010) and the role of morphogen gradients in this process (Arber, 2012). To allow highly specific synaptic connections, the initial broad mapping of axons is often refined by other mechanisms, sometimes dependent on the timing of neuron spiking activity (Kastanenka and Landmesser, 2010). These processes lead to remarkable examples of precision axon-mapping like retinal ganglion cell projections to the brain (McLaughlin and O’Leary, 2005), motoneurons to specific limb muscles, and muscle stretch receptor afferents to motoneurons (Pecho-Vrieseling et al., 2009). For simplicity, we chose chemical gradients as classic developmental cues (Sperry, 1963) and allowed each neuron to grow its axons independently. This is not what happens in life, where neurons differentiate in a defined dorsoventral and head-to-tail sequence (Lewis, 2006; Chédotal and Richards, 2010). As a result, the axons of more precocious neurons can be followed by fasciculation of those that develop later. The lack of precision in axon trajectories implied by our modeling still leaves many details to be explained, as is apparent from the list of parameters we needed to assign: initial outgrowth angle, orientation to longitudinal growth, distance to axon branching and its angle, axon length (Table 1). With the exception of axon length, control of all these variables occurs physically quite close to the soma. Does this suggest that later control of axon growth is minimal? Is dendrite growth controlled precisely or could it be a simple response to the presence of axons ready to make synapses (Cline and Haas, 2008; Arikath, 2012)? Details of the real processes now need to be investigated experimentally in the light of our results.

Is there a role for subtlety and complexity when the first functioning networks develop in the vertebrate brain? Even in early larval swimming networks there is evidence that homeostatic mechanisms regulate synaptic strengths (Borodinsky et al., 2004), switches occur in the mechanisms controlling axon growth (Moon and Gomez, 2005), the order of neuron differentiation may affect recruitment during swimming (Koyama et al., 2011), and detailed physiological properties of neurons are critical for swimming (Li, 2011). It is highly likely that refinement of connections occurs later in development, as in most systems studied (Sanes et al., 2006), but our results suggest that it is not required for early network function. Specific target recognition may not be necessary until limbs and eyes develop. If simple rules lay out the first scaffold of axons and connections in the vertebrate hindbrain and spinal cord, then the first steps in the construction of networks in other brain regions, even the cerebral cortex (Hill et al., 2012), could be based on similar principles.

What is the significance of this study for understanding of neuronal rhythm generation networks? When the tadpole CNS network was mapped onto a physiological network model where the main excitatory neurons have pacemaker properties, swimming motor output was a remarkably stable response to sensory stimulation. This is what a very young animal may need to survive (at this stage of development, the hatchling tadpole does not appear to have a fast C-start response). The stability almost certainly results from the incorporation of recently described switchable pacemaker properties into models of the dINs which drive swimming (Li et al., 2010). The dINs in the hindbrain may be homologues of reticulospinal neurons in adults. Neurons with related pacemaker properties have been found in many central pattern generators underlying rhythmic activity, where they act in concert with network-based rhythm generation mechanisms (Marder et al., 2005; Huss et al., 2008; Feldman et al., 2013; Moult et al., 2013). In the tadpole, many issues still remain. What is the cellular basis of dIN pacemaker properties? Why does network activity start at shorter latencies in the model networks than in the tadpole? Is there any biological sense in the periods of spontaneous activity on both sides of the body (Fig. 6 C,D) which are also seen in physiological recordings and other models of reciprocally inhibitory networks (Wang and Rinzel, 1992)?

In conclusion, this study has used novel methods to recreate a significant part of the connectivity of the brainstem and spinal cord of a whole vertebrate by generalization from relatively small anatomical datasets defining the features of the main constituent neuron types. Such data, critically including axon trajectories, are far from ideal or complete but are just not available for other animals beyond Caenorhabditis elegans (White et al., 1986), and even here, gaps need to be filled by extrapolation (Haspel and O’Donovan, 2011). Using a simple growth model, we have been able to exploit limited data to generate a biologically realistic baseline network for a significant part of the newly hatched Xenopus tadpole CNS. The details of the distribution of the ~90,000 synapses between ~1400 neurons (Table 3; Fig. 4) are now available for experimental and anatomical testing, and we have already begun to ask what is necessary to ensure proper network function (Fig. 7). In the tadpole we have been able to combine generated networks with detailed physiological data to define the structure and function of a network driving behavior. This process gives insights into the formation as well as the tolerance to
variability and operation principles of such networks. It is only possible because we have detailed information on the neuron types, their anatomy, properties, and synaptic connections. Such details are gradually emerging for the related networks in other vertebrates (Fetcho and McLean, 2010) including mammals (Goulding and Pfaff, 2005; Grillner and Jessell, 2009).

References


Grillner and Pfaff, 2005


Roberts A, Li WC, Sofie SR (2010) How neurons generate behaviour in a
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Many neural circuits are capable of generating multiple stereotyped outputs after different sensory inputs or neuromodulation. We have previously identified the central pattern generator (CPG) for *Xenopus* tadpole swimming that involves antiphase oscillations of activity between the left and right sides. Here we analyze the cellular basis for spontaneous left–right motor synchrony characterized by simultaneous bursting on both sides at twice the swimming frequency. Spontaneous synchrony bouts are rare in most tadpoles, and they instantly emerge from and switch back to swimming, most frequently within the first second after skin stimulation. Analyses show that only neurons that are active during swimming fire action potentials in synchrony, suggesting both output patterns derive from the same neural circuit. The firing of excitatory descending interneurons (dINs) leads that of other types of neurons in synchrony as it does in only neurons that are active during swimming fire action potentials in synchrony, suggesting both output patterns derive from the same instantaneously emerge from and switch back to swimming. Computer modeling supports these findings by showing that the same neural network, in which reciprocal inhibition mediates rebound firing, can generate both swimming and synchrony without circuit reconfiguration. Modeling also shows that lengthening the time window between phasic excitation and inhibition by increasing dIN synaptic/conduction delay can improve the stability of synchrony.

**Key words:** central pattern generator; locomotion; oscillations; spinal cord; swimming; synchrony

### Introduction

Multiple stereotyped behavioral patterns are common in both invertebrates and vertebrates. The underlying neural mechanisms have been better understood in some invertebrate preparations (Kupfermann and Weiss, 2001; Jing and Weiss, 2005; Kristan et al., 2005; Marder et al., 2005; Marder and Bucher, 2007; Briggman and Kristan, 2008). Studies show some neurons can be active in multiple tasks (Weimann et al., 1991; Weimann and Marder, 1994) and circuits can be dynamically reconfigured to generate different outputs (Dickinson et al., 1990; Meyrand et al., 1991, 1994; Popescu and Frost, 2002; Norris et al., 2006; White and Nusbaum, 2011). Much less is known about circuit architecture in vertebrates. It is widely thought that polymorphic neural networks can mediate multiple motor outputs (Getting, 1989; Sofie, 1993; Marder and Calabrese, 1996; Marder et al., 2005; Briggman and Kristan, 2008; Doi and Ramirez, 2008; Rauschen et al., 2009; Klein et al., 2010). However, a number of recent non-mammalian vertebrate studies suggest that circuit reconfiguration is needed to generate different motor responses (Ritter et al., 2001; Kimura et al., 2006; Berkowitz, 2007, 2008; Li et al., 2007; McLean et al., 2007, 2008; Liao and Fetcho, 2008; Frigon, 2009; Satou et al., 2009; Wyatt et al., 2009; Berkowitz et al., 2010).

There are three basic categories of neural activity in central pattern generator (CPG) circuits that control motor outputs: tonic firing, antiphase oscillations, and synchronous oscillations (termed “synchrony” here for simplicity). Movements like walking, running, and axial swimming involve antiphase oscillatory left–right and flexor–extensor muscle activities, known to be coordinated by reciprocal inhibition (Kiehn, 2006). In galloping, hopping, flying, frog- or turtle-style limb-based swimming, left–right synchrony is the operational mode. Whereas neural mechanisms underlying normal left–right synchrony have received little attention, synchrony has been reported in many experimental conditions (Cohen and Harris-Warrick, 1984; Clarke et al., 1991; Cowley and Schmidt, 1995; Kullander et al., 2003). In these studies, genetic mutations or pharmacological blockade led to
functional reorganization of the circuits, and crossed excitation was proposed to couple left–right motor rhythms in synchrony. *Xenopus* tadpoles have been used as a simple vertebrate model for studying neural mechanisms controlling locomotion, and the configuration of the tadpole spinal circuit has been systematically characterized recently using paired whole-cell recordings (Roberts et al., 2010). During tadpole swimming, neuronal activity alternates between the two sides of the spinal cord and hindbrain by way of reciprocal inhibition (Moult et al., 2013). Occasionally, however, the activity on the two sides can become spontaneously locked in synchrony, similar to that reported by Kahn and Roberts (1982) in curarized tadpoles. Synchrony disrupts propulsive swimming and bears no identified behavioral significance to tadpoles. We want to understand how synchrony rhythms are generated by this well studied neural circuit. In contrast to the prevalent evidence that circuit reconfiguration is needed for generating different network outputs, we reveal that the same CPG can generate both swimming and synchrony and that both types of rhythmic activity are coupled by fast reciprocal inhibition.

**Materials and Methods**

Experimental procedures were similar to those described previously (Li and Moult, 2012), approved by the local Animal Welfare Ethics Committee, and complied with UK Home Office regulations. Pairs of adult *Xenopus* of either sex were given injection of Human Chorionic Gonadotropin to induce mating. Tadpoles at stage 37/38 (Nieuwkoop and Faber, 1956), whose sex cannot be determined at this stage, were briefly anesthetized with 0.1% MS222 (3-aminobenzoic acid ester; Sigma-Aldrich) to allow immobilization with -bungarotoxin (12.5 μM; ToxinCookson) after the dorsal fin was cut open. The animal then was pinned onto a sylgard stage for dissections to expose myotome blocks, spinal cord, and caudal hindbrain. Additional dissections were then made to remove ependymal cells lining the central canal to reveal neuronal somata. Saline contained (in μM) 115 NaCl, 3 KCl, 2 CaCl2, 2.4 NaHCO3, 1 MgCl2, and 10 HEPES, and pH was adjusted to 7.4 with 5 M NaOH. Current-clamp or voltage-clamp whole-cell recordings were made from exposed somata under a Nikon E600FN microscope. Patch pipettes were filled with 0.1% neurobiotin (Vector Laboratories) in the intracellular solution (in μM: 100 K-glucione, 2 MgCl2, 10 EGTA, 10 HEPES, 3 Na3ATP, 0.5 NaGTP, pH adjusted to 7.3 with KOH). Pipette DC resistances ranged from 10 to 20 MΩ. Standard current-clamp or voltage-clamp recordings were made with the Axon-2B or Axon Multi-clamp 700B amplifier (Molecular Devices). Junction potentials (14.7 mV) were not corrected in voltage-clamp recordings. Data were digitized using either CED 1401 plus or Power 1401 mKII, sampled with Signal 5 (Cambridge Electronic Design). Fictive swimming (we use “swimming” in the text for an easier description) was initiated by applying single or repetitive 1 ms current pulses to tadpole skin on the tail or head. Motor nerve (m.n.) recordings from muscle clefts were made normally between the 5th and 10th muscle segments caudal to the otic capsule by using glass nerve (m.n.) recordings from muscle clefts were made normally between the 5th and 10th muscle segments caudal to the otic capsule by using glass nerve (m.n.) recordings from muscle clefts were made normally between the 5th and 10th muscle segments caudal to the otic capsule by using glass nerve (m.n.) recordings from muscle clefts were made normally between the 5th and 10th muscle segments caudal to the otic capsule by using glass nerve (m.n.) recordings from muscle clefts were made normally between the 5th and 10th muscle segments caudal to the otic capsule by using glass nerve (m.n.) recordings from muscle clefts were made normally between the 5th and 10th muscle segments caudal to the otic capsule by using glass nerve (m.n.) recordings from muscle clefts were made normally between the 5th and 10th muscle segments caudal to the otic capsule by using glass nerve.
currents in dINs lack a fast component (W.-C. Li, unpublished observations). All other parameters associated with neurons’ membrane properties were the same as given by Roberts et al. (2014).

The model includes glutamatergic (AMPARs, NMDARs with Mg$^{2+}$-dependent voltage dependency) and glycinergic synapses established in paired whole-cell recordings (Sautois et al., 2007). We introduced synaptic saturation in the present model by increasing synaptic opening $o(t)$ and closing $c(t)$ variables using a varying, rather than constant, increment after a spike. The postspike increment $\Delta o(t)$ is given by the following equation:

$$\Delta o(t) = \frac{1 - c(t) - o(t)}{\sigma}.$$  

Here parameter $s$ is the original increment size (AMPA/NMDA, 1.25; glycine, 3.0), and $\sigma$ is the saturation level (AMPArs and glycine receptors, 1.0; NMDA, 5.0). These values were chosen so that saturation only occurred for step-size control and adjustment was 1.0e$^{-2}$, and spikes are detected by membrane potential zero crossing after every time step (maximum step size, 0.5 ms). Small variability in neuron and synapse strengths was introduced by adding Gaussian noise (SD of 2% of mean values) to cell membrane capacitance and channel conductances and Gaussian noise (SD of 5% of mean values) to the conductance of each synapse.

**Results**

Basic features of synchrony in tadpoles

Left–right synchrony in tadpoles involves simultaneous m.n. bursts on both sides, in contrast to the alternation during swimming (Fig. 2A). We first analyzed the basic properties of synchrony. We arbitrarily define periods with more than five consecutive bursts of synchronous activity (four cycles) as a synchrony bout. Initially, we analyzed spontaneous synchrony bouts in 14 tadpoles where the activity of a left side was active consistently earlier than the other side ($p$ = 0.0008 per cycle). For the same reason, we made a larger change of the strength of dIN to ascending in other neuron types (aINs onto all other neuron types: 0.0435 nS) and motoneurons (MNs) (from 0.593 to 0.54). For the same reason, we made a larger change in the strength of dIN to ascending interneuron (aIN) synapses (from 0.1 to 0.3 nS). Finally, the conductance of the inhibitory synapses made by aINs onto all other neuron types was reduced to 0.0435 nS so that these synapses provided only 10% of the inhibitory strength of dIN synapses.

As in the study by Roberts et al. (2014), all synaptic connections contained a constant delay of 1 ms and a conduction delay that depends on the difference in rostrocaudal position of the presynaptic and postsynaptic neurons according to a conduction speed of 3.5 ms/mm.

The simulation was implemented using custom C code. The simulator uses the fourth-order adaptive Runge-Kutta-Felberg ODE solver from the GNU Scientific Library (version 1.15). The absolute and relative error tolerances for step-size control and adjustment were 1.0e$^{-8}$, and spikes are detected by membrane potential zero crossing after every time step (maximum step size, 0.5 ms). Small variability in neuron and synapse strengths was introduced by adding Gaussian noise (SD of 2% of mean values) to cell membrane capacitance and channel conductances and Gaussian noise (SD of 5% of mean values) to the conductance of each synapse.

Figure 2. Left–right motor synchrony in tadpoles and its basic features. A, A synchrony bout with left and right m.n. activity recorded simultaneously (both fifth/sixth muscle cleft). B, Synchrony after single skin stimulation (arrow). C, Synchrony after repetitive skin stimulation (gray bar) with a simultaneous motoneuron recording. D, Synchrony rhythm frequency and phase (red bars) compared with that of adjacent swimming (black bars). E, Starting time (beginning of red lines) and duration (length of lines) for 37 synchrony bouts after single skin stimulation in 13 tadpoles. F, Cycle-by-cycle phase measurements of two synchrony bouts. Left, Example lacking a phase drift (linear regression coefficient is 0.0004 per cycle; $R^2 = 0.006; p = 0.62$). Right, Example with the clearest phase drift with the progression of synchrony (coefficient is 0.0036 per cycle; $R^2 = 0.41; p < 0.001$). Solid lines are for linear regression. Synchrony bouts are red traces, and swimming activity is black in this and other figures.
lead toward the end of synchrony (Fig. 2F, right). The small phase drifts (0.07 ± 0.02; n = 6, estimated by regression) all ended abruptly with phases jumping to ~0.5 when synchrony switched to swimming. Synchrony frequency (24 ± 0.6 Hz) was reliably twice the frequency of adjacent swimming (12.4 ± 0.4 Hz; 195.5 ± 4.5%; one-sample, two-tailed t test, p = 0.34; Fig. 2D). This feature of abruptly-doubled m.n. frequency fits in with previous descriptions (Clarke et al., 1991). Since swimming frequency only changes gradually after initiation, we used this simple unique feature to identify synchrony when activity was not recorded simultaneously from both sides.

In a bigger sample of 37 tadpoles, we analyzed how often and when synchrony occurred. Within 6 s after single skin stimulation, 37 synchrony bouts were seen in 13 tadpoles (17.5% of 212 trials; Fig. 2B). Within 2.5 s after repetitive skin stimulation (30–40 Hz, 20–50 pulses), 50 bouts of synchrony were seen in 22 tadpoles (26.6% of 188 trials; Fig. 2C). Overall, we observed synchrony in 26 of 37 tadpoles (70.3%) when both types of stimulation were considered. The 37 synchrony bouts evoked by single skin stimulation lasted 0.35 ± 0.03 s (range, 0.09–2.04) or 11 ± 0.8 cycles (range, 4–66). Most of them started within 0.5 s and stopped within 1 s after skin stimulation (32 of 37; Fig. 2E). When the hindbrain was sectioned at the fifth/sixth rhombomere border in another sample of 11 tadpoles, synchrony was seen in 1 of 82 trials after single skin stimulation and in 59 of 521 trials after repetitive skin stimulation of nine tadpoles. This suggests that tadpole spinal cord and caudal hindbrain, which contain the highest density of dINs and are critical for swimming (Li et al., 2006; Soffe et al., 2009), can also generate synchrony.

Neuronal activity during synchrony

To understand how synchrony is generated, we next identified neurons that were active during synchrony. We found that only neurons that were rhythmically active during swimming fired action potentials during synchrony (Table 1). They included all swimming CPG neurons (Roberts et al., 2010): dINs, cINs, aINs, and motoneurons (Fig. 3). We define their firing reliability as the percentage of cycles with action potentials. Their firing reliabilities during synchrony were lower than those during swimming immediately before or after synchrony (Table 1). To see whether there is a change of firing reliability with the progression of synchrony, we compared the reliability in the first five synchrony cycles with that in the last five cycles. In 26 long bouts with an average of 19.4 ± 1.3 synchrony cycles (range, 12–35) from 11 neurons (3 motoneurons, 4 dINs, 2 cINs, and 2 aINs), no difference was found (paired t test, p = 0.74). Neurons that were silent during swimming [dorsolateral commissural interneurons (dINs), dorsolateral ascending interneurons (dlas), excitatory commissural interneurons (ecINs), and repetitive-firing descending interneurons (dlINs)] remained silent during synchrony (Table 1). This lack of recruitment or exclusion of network neurons between the two motor patterns strongly suggests that synchrony is a motor output derived from the swimming circuit.

Analyzes of dIN activity during synchrony

dINs are the only excitatory interneurons in the swimming CPG, and their activity drives swimming (Soffe et al., 2009; Li, 2011). We made 11 paired whole-cell recordings with one dIN and another (non-dIN) CPG neuron at similar locations on the same side. This allowed us to determine directly which neuron fired first in each synchrony or swimming cycle. dIN firing during synchrony preceded the firing of non-dINs (seven cINs, two motoneurons, two unidentified). The latency between dIN and non-dIN firing in synchrony (2.6 ± 0.68 ms; n = 11 bouts) was similar to that in swimming (2.47 ± 0.46 ms; p > 0.05, t test; Fig. 4A–C). Since there was no recruitment of extra types of neurons in synchrony (see above), this suggests that the excitatory dINs were also responsible for driving the firing of other neurons during synchrony, as they are for swimming (Soffe et al., 2009). dIN rebound firing had been proposed as the main mechanism in sustaining swimming (Moutl et al., 2013). To see whether the same rebound mechanism could sustain synchrony, we measured the delay between the start of IPSPs and the time point when the following dIN spike crossed 0 mV. This time (rebound time) should reflect the interaction between different voltage-dependent ionic channels and receptor channels that give rise to dIN rebound firing. No difference was found between rebound time during swimming (36.3 ± 2.6 ms) and that during synchrony (34.3 ± 2.2 ms; n = 7 dINs; two-tailed paired t test, p > 0.05; Fig. 4D,E). These data suggest that the same rebound mechanism that underlies swimming is also responsible for synchrony.

Synaptic currents in dINs during synchrony

Knowing that dIN activity is likely to drive synchrony, we next looked at the synaptic currents dINs receive during synchrony to understand how they could support dIN firing. dINs receive excitation from other dINs during swimming, with NMDAR components contributing to tonic inward currents and AMPAR and nicotinic receptor components giving rise to the phasic on-cycle EPSCs (Fig. 5A). Additionally, cINs from the opposite side produce midcycle IPSCs, and ipsilateral aINs produce early-cycle IPSCs, which appear between the on-cycle dIN EPSCs and cIN IPSCs (Li and Moutl, 2012; Fig. 5B, arrows). First, membrane potential was clamped around IPSC reversal (approximately −55 mV) to compare EPSCs. EPSCs were smaller in synchrony (−119 ± 13 pA) than those in swimming just before or after synchrony (−150 ± 19 pA; n = 11 bouts in 11 neurons; paired t test, p < 0.01; Fig. 5A). Second, membrane potentials were clamped around 0 mV to reveal IPSCs. During synchrony, aINs and cINs fire synchronously, and both contribute to the IPSCs. The mixed IPSCs during synchrony (262 ± 42 pA) were smaller than the midcycle cIN-mediated IPSCs in swimming (322 ± 51 pA; n = 8 bouts; paired t test, p < 0.05; Fig. 5B). This fits with the observation above that neuronal firing during synchrony is less reliable than during swimming (Table 1). Third, the delay between EPSCs and IPSCs in swimming or synchrony cycles was measured when membrane potential was clamped at

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Table 1. Neuronal firing reliability (percentage of cycles with firing) during synchrony and adjacent swimming

<table>
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<th>Swimming</th>
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The identification of dINs and cINs was based on their synaptic outputs in paired recordings, activity during swimming, and anatomy (Li et al., 2007). Significance levels are as follows: *p < 0.05; **p < 0.01; ***p < 0.001.
approximately \(-20\) mV. This was measured as the time difference between the EPSC trough and the IPSC peak. The delay was \(7.9 \pm 1\) ms during synchrony, which was much shorter than that during swimming (\(41 \pm 2.3\) ms; \(n = 8\) bouts; paired \(t\) test, \(p < 0.01\); Fig. 5C). This delay is important because if inhibition arrives during the rising phase of a dIN action potential, then the spike may be suppressed, which reduces spike reliability (Table 1; Figs. 3D, 5D). Instead, inhibition must arrive during the decaying phase of the dIN action potential to trigger another postinhibitory spike. The fact that during synchrony IPSCs arrive very shortly after dIN spiking may contribute to the instability of synchrony, since a small difference in the timing of inhibition may cause it to arrive before dIN spiking.

Midcycle firing in dINs may initiate synchrony
A critical feature of synchrony is that its frequency is double that of swimming. Abnormal midcycle dIN firing, which doubles the frequency of dIN activity, may initiate synchronous activity because dIN activity drives the network. We looked at the incidences of single, spontaneous midcycle firing (Fig. 6A) in seven paired recordings from a dIN and a non-dIN at similar locations on the same side (non-dINs include one motoneuron and six cINs). These recordings allowed us to determine whether dINs or non-dINs were more likely to fire midcycle spikes. One hundred and ten midcycle firing events were found (in \(>5000\) cycles) without evidence for synchrony in the m.n. recordings. In 81 cases (74%), the dIN fired midcycle action potentials without simultaneous midcycle firing in non-dINs. In 23 cases (21%), both the dINs and non-dINs fired. Only non-dINs fired action potentials in the remaining six cases (5%). The higher probability of dIN midcycle firing supports the proposal that occasional dIN spiking may initiate midcycle activity and synchrony. The phase of midcycle dIN spikes in the swimming cycle was \(0.54 \pm 0.004\) (\(n = 104\)). This was not different from the phase for midcycle...
IPSPs in dINs (median, 0.54; \( n = 308 \) cycles in five tadpoles analyzed; \( p = 0.69 \), independent sample nonparametric median test). However, a dIN spike can appear a few milliseconds earlier than IPSPs in individual cases (Fig. 6B), allowing it to drive postsynaptic firing. Therefore, this type of abrupt doubling of dIN firing frequency during swimming in the form of midcycle spikes may be key to synchrony initiation.

As we have shown above, most synchrony bouts occur within 1 s of skin stimulation. Synaptic currents during this initial period differ from the following periods in that there are bigger tonic inward currents and more reliable aIN IPSCs in dINs (Li and Moult, 2012). Higher depolarization can promote firing at midcycle position. We injected positive DC (60–200% threshold current for evoking firing at rest) into 15 dINs to mimic higher background excitation to see whether this could induce midcycle dIN firing during swimming. In three dINs, this led to doublet on-cycle firing in 48 of 49 swimming cycles. In the other 12 dINs, DC injection led to midcycle dIN spikes on many cycles (Fig. 6C; 269 of 796 cycles). In three trials in three different dINs, this DC injection coincided with synchrony during the injection period (Fig. 6F). However, such coincidence was not repeatable. Injecting DC into non-dINs (55–200% threshold current for firing; five motoneurons, five cINs, four aINs, and two unidentified CPGs) mainly induced on-cycle multiple firing (265 of 502 cycles) with occasional midcycle firing (Fig. 6D; 31 cycles). There were higher percentages of cycles with midcycle firing in dINs (\( p < 0.01 \)) and multiple firing in non-dINs (\( p < 0.001 \), independent samples median test; Fig. 6E). These results indicate that dINs, most of which were recorded in the caudal hindbrain and rostral spinal cord, tend to fire midcycle spikes during enhanced excitation. In some cases, the midcycle firing of dINs may directly lead to synchrony.

The midcycle dIN firing evoked by DC current injections during swimming tended to appear in the latter half-cycle (Fig. 6C, filled triangles), potentially reducing its ability to evoke synchrony. This asymmetrical firing pattern is presumably caused by different synaptic events in a swimming cycle, first strong reliable on-cycle dIN excitation and then weak unreliable early-cycle aIN inhibition and strong reliable midcycle cIN inhibition (Li and Moult, 2012; Fig. 5B). To have better control over the timing of evoked midcycle dIN firing, we used the dynamic-clamp technique to time the injection of artificial excitatory synaptic currents into the recorded dINs. The synaptic currents were triggered by the dIN action potentials with an artificial delay of approximately half of the swimming cycle period (20–25 ms). We failed to induce any synchrony bouts in this way (\( n = 12 \) dINs, 259 trials, 3–50 cycles per trial with current injections, data not shown).

Effects of blocking inhibition on synchrony generation

Blocking glycinergic inhibition can induce synchrony in neonatal rats (Cowley and Schmidt, 1995) and lamprey (Cohen and Harris-Warrick, 1984). In tadpoles, bath-applied 2.5 \( \mu \)M strychnine and 20 \( \mu \)M SR95531 (GABA antagonist) led to an initial silent period during which initiation of swimming by single skin stimulation was very difficult. After \( \sim 23 \) min, the rhythms recovered to some extent after multiple skin stimulation, but with little synchrony (Moult et al., 2013). Application of lower concentra-
tions of strychnine (0.07–0.25 μM) increased swimming frequency, but the incidence of synchrony did not increase (Li and Moult, 2012). Fortuitously, two batches of tadpoles showed an unusual tendency for synchronous activity (Fig. 7A; data were not included for preparing Fig. 2E). In these animals, 21.1 ± 4.6% of rhythmic activity (including both synchrony and swimming) was synchrony (n = 63 swimming episodes). Microperfusing 0.5 μM strychnine in the hindbrain area decreased synchrony to 1.8 ± 0.6% (n = 66 episodes, 7 tadpoles; p < 0.01, paired t test). This recovered to 23.3, 17.8, and 5.3% in wash in three tadpoles (>20 min; Fig. 7A). Injecting positive DC in two of the seven dINs, which could induce midcycle dIN firing in control (cycles with midcycle firing are 33 of 57 and 23 of 115, respectively) as shown above, evoked little dIN midcycle firing in strychnine (0 of 20 and 1 of 84; Fig. 7B). These data imply that inhibition may play an important role in the generation of synchrony.

Synchrony initiation and stability in a computational model of swimming

The excitation from single midcycle spiking in one dIN in whole-cell recordings above was very unlikely to be sufficient to drive substantial midcycle cIN firing and start synchrony. Therefore, we injected brief midcycle currents into multiple dINs simultaneously in a computational tadpole swimming network to see whether synchrony could be initiated.

We performed multiple simulations of the neuronal network model to investigate the dynamical characteristics of synchrony and swimming. Importantly, there was only reciprocal inhibitory connection between the left and right sides (Fig. 1). The network displays stable and realistic swimming activity that is initiated by stimulation of sensory neurons. We injected brief step currents (5 ms, 150 pA) into dINs on the right side during swimming once to make them fire midcycle spikes. Though injections into as few as 10 dINs (still too many to be able to manipulate experimentally here) could evoke brief synchrony bouts, the most stable synchrony was evoked when all dINs were injected with currents. The synchrony bouts evoked by current injections into all dINs lasted for an average of 16.1 cycles (median, 11; range, 6–47; n = 12 trials; Fig. 8A). The respective swimming and synchrony frequencies were very similar across trials and remained stable. Autocorrelation of motoneuron activities on one side across 12 trials gave an average swimming frequency of 20.4 Hz and a synchrony frequency of 43.7 Hz. Cross-correlation of motoneuron activity on both sides gave a phase value of 0.5 for swimming and 1 for synchrony. Both types of measurements matched the exper-

Figure 5. Synaptic currents and potentials in dINs during synchrony and swimming. A. Rhythmic on-cycle EPSCs (membrane potential clamped at approximately −55 mV). The right bar chart summarizes averaged measurements in 11 neurons. B. Rhythmic IPSCs (membrane potential clamped at −0 mV). The right bar chart is for averaged midcycle IPSC measurements in eight neurons. Arrows point at early-cycle IPSCs from aINs. C. Delay from on-cycle EPSP peak (triangles) to midcycle IPSP peak (filled triangles). The right bar chart shows the delay is much shortened during synchrony compared with that in swimming. Significance values as follows: * p < 0.05; ** p < 0.01; *** p < 0.001. D. dIN spiking is inhibited by IPSPs on some cycles during a synchrony bout (asterisks). dINs are recorded in voltage-clamp mode (A–C) and in current-clamp mode (D). Synch, Synchrony.
During synchrony, neuronal firing reliability was lower than periods of swimming (paired t test, \( p < 0.001 \) in each case; \( n = 12 \) synchrony bouts; 193 synchrony cycles and equivalent swimming periods compared; Fig. 8B). AMPA receptor conductance was smaller during synchrony (4.39 ± 0.71 nS) than during swimming (5.12 ± 0.79 nS; \( p < 0.01 \), paired t test; \( n = 10 \) dINs). Similarly, combined inhibitory conductance during synchrony (3.87 ± 0.86 nS) was smaller than cIN conductance during swimming (5.28 ± 1.17 nS; \( p < 0.01 \), paired t test; \( n = 10 \) dINs; Fig. 8C). Reducing inhibition strength by up to 60% failed to convert normal swimming to synchrony (12 trials). Further depression of inhibition made it difficult (three successes in 12 trials at 70%) or impossible (above 70% reduction) to start swimming, similar to what was seen experimentally (Moult et al., 2013).

As shown above, the delay between EPSCs and IPSCs is much shorter during synchrony, and from this we hypothesize that the short delay (Fig. 5C) reduces neuronal firing reliability and is responsible for the unstable nature of synchrony. To test this, we first advanced dIN activity on one side to further shorten the gap. We injected some brief depolarizing currents into all dINs (3 ms, 100 pA) on the left side to advance neuronal activity by 2.27 ± 0.15 ms during the fifth synchrony cycle (12 trials). This reliably shortened synchrony bouts (related-samples Wilcoxon signed rank test, \( p < 0.01 \); Fig. 9A) with 10 of 12 bouts ending in the following cycle or with one cycle delay. We next artificially lengthened the EPSC–IPSC gap by increasing the synaptic/conduction delay at dIN to cIN synapses to see whether we could improve synchrony stability. Synaptic delay is about 1 ms in the tadpole swimming circuit, and dIN spike conduction velocity is 0.36 mm/ms (360 \( \mu \)m will increase conduction delay by 1 ms; Soffe et al., 2009). Increasing dIN–cIN transmission/conduction delay is comparable with adding relay interneurons between dINs.

Figure 6. Extra midcycle dIN firing may initiate synchrony. A, Midcycle dIN firing (filled triangles) before and after a synchrony bout. B, Some examples of midcycle dIN firing showing the relative timing of the firing and cIN inhibition (triangles). Note some spikes are narrowed by inhibition (*). Lack of midcycle burst in the overlapped left m.n. recording implies the absence of synchrony in the circuit. The illustrated cycles are normalized based on dIN spiking. C, Depolarizing DC injection (gray bar) into a dIN during swimming results in reliable midcycle firing (filled triangles). Note the midcycle spikes do not divide the swimming cycle equally. D, DC injections into a motoneuron result in multiple firing (*) in many cycles and some midcycle firing (filled triangles). E, Summary of 12 dINs’ (filled bar) and 16 non-dINs’ (unfilled) firing after DC injections during swimming. Significance values as follows: ** \( p < 0.01 \); *** \( p < 0.001 \). F, Midcycle firing of a right-side dIN coincides with synchrony in the left m.n. recording during the DC injection period (filled triangle marks one “swimming-like” cycle; compare with filled triangles in C). C, E and F are from the same dIN.
and cINs or increasing dIN projection distance (e.g., as is the case when the spinal cord size increases during development). The percentage of simulations with stable synchrony (continuous synchrony from initiation to the end of the 3 s simulation, \( \geq 100 \) cycles) initiated by midcycle dIN current injections increased with longer delays (Fig. 9B, C). When the delay was 2 ms and above, all evoked synchrony bouts were stable. With longer dIN–cIN synaptic/conduction delays and stable synchrony, all CPG neurons fired more reliably during synchrony (\( p < 0.001 \) in each neuron type, one-way ANOVA; Fig. 9D). We also examined whether there was significant drift of phase during the stable synchrony bouts in simulation with a 2 ms dIN–cIN delay. Linear regression revealed some small drift in 6 of the 12 simulations (drifting rate, 0.000074 \( \pm \) 0.000009 per cycle; \( p < 0.05 \)). The modeling, therefore, provides theoretical support that tadpole swimming circuit can generate synchrony and dIN–cIN synaptic/conduction delay can directly determine synchrony stability.

**Discussion**

We propose that left–right motor synchrony in tadpoles is mediated by the swimming CPG. The extra midcycle firing of dINs during swimming can initiate synchrony, and the mismatches of timing in left and right activity can convert synchrony to swimming (Fig. 10A). dIN midcycle firing will drive ipsilateral cINs to fire action potentials and produce extra crossed inhibition on the opposite side. This extra inhibition means dINs on both sides will fire simultaneously on postinhibitory rebound and start synchrony. When the activity on one side comes earlier than the opposite side, the crossed inhibition it produces will arrive earlier, inhibiting the activity on the opposite side.
Then, the synchrony pattern breaks down and swimming resumes.

The production of different rhythms in CPGs has been associated with circuit reconfiguration after different sensory inputs (Marder et al., 2005; Briggman and Kristan, 2008; Berkowitz et al., 2010) or under neuromodulatory influences (Rauschen et al., 2009; Combes et al., 2012). The synchrony shown here does not seem to involve reconfiguration of the swimming CPG. Both swimming and synchrony can occur after the same brief skin stimulation, and they involve the same types of neuron, without recruitment of extra or inhibition of existing types of neuron. The same rebound mechanism that sustains swimming appears to operate in synchrony, since dIN firing precedes that of other neurons in both patterns and the rebound times during both outputs are not different. The phase of cIN inhibition during swimming is 0.54, indicating dIN rebound firing can take place within less than half a cycle. This leaves scope for halving the activity cycle period and may explain the strict relationship between synchrony and swimming frequencies (Fig. 10B). In comparison, the generation of struggling rhythms requires sustained sensory inputs and the recruitment of specialized interneurons, and, furthermore, struggling frequency is not locked to swimming rhythms in any fixed manner (Li et al., 2007).

We have identified midcycle dIN firing as one potential trigger for swimming to switch to synchrony. This extra firing can result from enhanced excitation, e.g., at the beginning of a swimming episode when sensory inputs are still present. In accordance with this, most synchrony bouts occur within 0.5–1 s of single skin stimulation. Although we have recently shown that pacemaker firing is not sufficient to sustain swimming rhythms, higher excitation may support firing at high frequencies in some dINs (Fig. 6A–C). Once synchrony is initiated, the narrow window for neuronal firing (Fig. 5C) determines that the firing of some dINs may be prevented by IPSPs (Table 1; Figs. 3D, 5D) and account for the unreliable firing of CPG neurons during synchrony. It also means that synchrony will stop when the activity on both sides drift out of synchrony slightly and explains why most spontaneous synchrony bouts are short. Indeed, drifts in phase with time have been seen only in some longer synchrony bouts. In modeling, evoking midcycle dIN firing during swimming can initiate synchrony, and advancing activity on one side even for only a couple of milliseconds can convert synchrony back to swimming. Synchrony evoked in the model resembles recorded synchrony in frequency and phase features. Artificially broadening the EPSC–IPSC delay by increasing dIN–cIN synaptic/conduction delay increased CPG firing reliability during synchrony and made synchrony more stable. Therefore, our biological data and modeling both support that the transitions between swimming and synchrony are unlikely to involve circuit reconfiguration.

It was originally proposed that a circuit with two half-centers coupled by reciprocal inhibition could theoretically generate both swimming and synchrony found in the tadpole (Kahn and Roberts, 1982). Later computer modeling, using pairs of neurons with feedback excitation and reciprocal inhibition, suggested that stable synchrony could be evoked by simultaneous sensory activation on both sides or when strong feedback inhibition was added (Roberts and Tunstall, 1990). The previously proposed basic circuit structure has recently been confirmed within the
Figure 10. Hypothesis on synchrony initiation and termination. A, Illustrative traces show alternating dIN rebound firing in swimming (top traces); simultaneous rebound firing during synchrony triggered by a midcycle spike in the right dIN (red arrow; the other 3 red spikes are “extra” rebound firing; middle traces; and the termination of synchrony when left dIN spiking is advanced (red arrow), the subsequent cIN IPSP arrives earlier and inhibits dIN spiking on the right side (dotted trace; bottom). B, Simplified tadpole swimming CPG with aINs and motoneurons omitted to explain neuronal activity within a swimming and synchrony cycle. The triangle synapse is excitatory, and the filled circle is inhibitory. The black arrowed-line indicates activity sequence during swimming. Lines with red arrows indicate activity starts simultaneously in dINs and cycle time is halved during synchrony.

tadpole swimming circuit in a large number of paired whole-cell recordings (Roberts et al., 2010; Li, 2011). This has enabled detailed analyses of neuronal activity and synaptic events during swimming and synchrony here and also allows us to build a computational swimming model of large populations of neurons with biologically realistic properties (Sautois et al., 2007; Borisyuk et al., 2011, 2014). Both our biological and modeling data now provide strong support that reciprocal inhibition, mediated by fast glycinergic synapses, can synchronize network activity. The spontaneous synchrony does not involve different sensory inputs, circuit configuration (Roberts and Tunstall, 1990), or changes in cellular properties like decreased fast potassium currents (Wall and Dale, 1994; Dale, 1995).

The left–right coordination of spinal activity is mediated by crossed inhibition (Kiehn, 2006), but direct commissural excitatory interneurons have been found in some vertebrates. Direct commissural glutamatergic synapses onto motoneurons were reported in mice (Quinlan and Kiehn, 2007). In lamprey, commissural excitatory interneurons have been identified in paired recordings (Buchanan, 1982; Parker and Grillner, 2000), but their role in swimming is primarily unknown (Parker, 2006). Although some interneurons with commissural axons were found to be positive for vesicular glutamate transporters in the larval zebrafish spinal cord (Higashijima et al., 2004), no left–right motor synchrony has been reported in zebrafish. In the tadpole swimming circuit, there is little evidence supporting the existence of crossed excitation from paired recordings except from the sensory interneurons, which are not active during swimming (Dale, 1983; Li et al., 2007). Recording from neurons caudal to hemisections only revealed cIN inhibition during swimming (Soffe and Roberts, 1982). Blocking glycineric inhibition may transform alternating flexor–extensor and left–right motor rhythms into bilateral and also flexor–extensor synchrony at lowered frequencies in rodents (Cowley and Schmidt, 1995; Cazalets et al., 1998; Beato and Nistri, 1999). Similar left–right synchrony after inhibition blockade was seen in lamprey (Cohen and Harris-Warrick, 1984; Alford and Williams, 1989; Hagevik and McClellan, 1994) and mudpuppy (Jovanovic et al., 1999). It is thought that in the absence of strong reciprocal inhibition, the weak crossed excitation becomes dominant and couples the activity on the two sides in synchrony (Cohen and Harris-Warrick, 1984; Quinlan and Kiehn, 2007). It is not known whether strychnine could recruit neurons in the circuit that are inhibited in normal rhythms in these cases. The spontaneous synchrony we have studied here cannot be explained by increased weak crossed excitation since there is no evidence for its presence during swimming or synchrony. Instead, inhibition seems to play a role in sustaining spontaneous synchrony as demonstrated by our experiments on the tadpoles with a high tendency for synchronous activity. Left–right synchrony coupled by crossed excitation has also been reported in some mutant mice. One example comes from mice lacking V2a interneurons (ChX10 DTA), which form ipsilateral excitatory synapses onto commissural interneurons. These mice show galloping gait at high-locomotion speed instead of normal trotting (Crone et al., 2009). This was thought to result from weakened activation of crossed inhibition pathways (Crone et al., 2008, 2009). A second example comes from mutants lacking Ephrin type-A receptor 4, in which ipsilateral excitatory interneurons grow aberrant commissural axons. It was proposed that this led to reciprocal overexcitation of CPG that overcame the coordination mediated by reciprocal inhibition and gave mutants the rabbit-like hopping behavior (Kullander et al., 2001, 2003). Another example involves the deletion of spinal V0 interneurons, which constitute pure commissural interneurons with ascending axons (70% inhibitory, 30% excitatory). This manipulation could increase left–right synchrony during fictive locomotion (Lanuza et al., 2004). In notochordless Xenopus tadpoles, normal alternation of activity between the left and right sides is replaced with synchrony (Clarke et al., 1991). In these tadpoles, many motoneurons have dendrites on both sides of the spinal cord. It is likely that they are excited by dINs on both sides and mediate synchrony.

It is worth pointing out that the aforementioned left–right synchronies were all observed in experimental conditions. Unlike the cases of flying, galloping, hopping, and frog-style swimming, left–right synchrony in axial swimming animals like tadpoles and lamprey is not locomotive. In our modeling, a small increase in dIN-to-cIN synaptic/conduction delay could transform synchrony to a stable regime. Such an increase can take place in the much bigger spinal cord in adult Xenopus. This may provide the mechanism for frog-style swimming, which requires left–right synchrony. Whereas biological evidence from the spinal cord studies above supports that synchronous activity may be coupled by mutual excitation, some computer modeling has suggested that mutual inhibition can also synchronize network activities. Some studies propose that synchrony takes place when the reciprocal inhibition has slow kinetics (Wang and Rinzler, 1992, 1993; Van Vreeswijk et al., 1994; White et al., 1998). This has gained some biological support from studies of pairs of stomatogastric neu-
ron's artificially connected with reciprocal inhibition useful the dynamic-clamp technology (Sharp et al., 1993; Marder, 1998; Elson et al., 2002). A recent modeling study has suggested that fast reciprocal inhibition could also synchronize bursting neurons (Jalil et al., 2010). Similar inhibitory synchronization has also been reported in larger modeled networks. In reticular thalamic (Destexhe et al., 1994), hippocampal, and neocortex (Whittington et al., 1995; Jefferys et al., 1996; Wang and Buzsaki, 1996) computational networks, GABAergic inhibition could synchronize neuronal activity. Biological support for this type of inhibitory synchronization, however, is scarce (Whittington et al., 1995).

Antiphase oscillations and synchrony are arguably the two most common neural rhythms in the CNS. The former is important in locomotion, chewing, and respiration (Kiehn, 2006; Doi and Ramirez, 2008). The latter is common in many cognitive and perceptual functions and shows decrease or enhancement in some neurological or psychiatric disorders (Schnitzler and Gross, 2005; Uhlhaas and Singer, 2006; Brown, 2007; Colgin, 2011; Jirsa et al., 2013). We show that both rhythms are intrinsic to a rebound-based neural circuit that relies on reciprocal inhibition. Additional work needs to thoroughly identify how different factors, including sensory and modulatory inputs and synaptic and neuronal intrinsic properties, stabilize one output preferentially over the other.

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