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Research report

The dopamine D2/D3 receptor agonist quinpirole increases checking-like behaviour in an operant observing response task with uncertain reinforcement: A novel possible model of OCD

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HIGHLIGHTS

- We validate a novel operant model of compulsive checking, relevant to OCD.
- The dopamine D2/3 receptor agonist quinpirole selectively increases checking.
- Uncertainty increases checking differently following quinpirole treatment.
- Dopamine D2/3 receptors modulate excessive, non-functional checking.
- OCD checking may respond to treatment augmentation with D2/3 receptor antagonists.

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ABSTRACT

Excessive checking is a common, debilitating symptom of obsessive-compulsive disorder (OCD). In an established rodent model of OCD checking behaviour, quinpirole (dopamine D2/3-receptor agonist) increased checking in open-field tests, indicating dopaminergic modulation of checking-like behaviours.

We designed a novel operant paradigm for rats (observing response task (ORT)) to further examine cognitive processes underpinning checking behaviour and clarify how and why checking develops. We investigated i) how quinpirole increases checking, ii) dependence of these effects on D2/3 receptor function (following treatment with D2/3 receptor antagonist sulpiride) and iii) effects of reward uncertainty.

In the ORT, rats pressed an 'observing' lever for information about the location of an 'active' lever that provided food reinforcement. High- and low-checkers (defined from baseline observing) received quinpirole (0.5 mg/kg, 10 treatments) or vehicle. Parametric task manipulations assessed observing/checking under increasing task demands relating to reinforcement uncertainty (variable response requirement and active-lever location switching). Treatment with sulpiride further probed the pharmacological basis of long-term behavioural changes.

Quinpirole selectively increased checking, both functional observing lever presses (OLPs) and non-functional extra OLPs (EOLPs). The increase in OLPs and EOLPs was long-lasting, without further quinpirole administration. Quinpirole did not affect the immediate ability to use information from checking. Vehicle and quinpirole-treated rats (VEH and QNP respectively) were selectively sensitive to different forms of uncertainty. Sulpiride reduced non-functional EOLPs in QNP rats but had no effect on functional OLPs. These data have implications for treatment of compulsive checking in OCD, particularly

Abbreviations: %ActiveCS, percentage of active lever presses when the active-lever light was illuminated; %ActiveCSoff, percentage of active lever presses when the active lever light was not illuminated; EOLPs, extra observing lever presses (non-functional, no consequence); EOLPinCS, rate of EOLPs relative to observing light illumination period; FT, fixed time schedule; FR, fixed ratio schedule; OCD, obsessive-compulsive disorder; OLPs, observing lever presses (functional, turns on light above active lever); ORT, observing response task; QNP, quinpirole-treatment group; SRI, serotonin reuptake inhibitor; VEH, vehicle-treatment group; VT, variable time schedule; VR, variable ratio schedule.

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for serotonin-reuptake-inhibitor treatment-refractory cases, where supplementation with dopamine receptor antagonists may be beneficial.

1. Introduction

Excessive, or compulsive, checking is a common symptom of obsessive compulsive disorder (OCD; lifetime prevalence of 1–3%) [1,2]. Self-reported checking behaviour was recently shown to be the best, and indeed only significant, predictor of OCD diagnosis compared with other OCD symptom domains [3]. Checking routines may be both time-consuming and highly debilitating, and are well-documented to cause distress, but it is still far from clear how these repetitive and ritualised checking routines develop and escalate from once-functional actions.

Compulsive checking may arise in response to obsessions that focus on threat and danger, although, recently, the opposite has been proposed – that over-expression of checking behaviour might drive the development of obsession, perhaps to rationalise or justify excessive, compulsive action [4]. Thus, compulsive checking may initially arise, without the need for pre-existing obsessional thought, from any one of a number of neurobehavioural mechanisms, such as behavioural inflexibility, inability to terminate security-related behavioural patterns or as a form of information-seeking [5–7]. One hypothesis suggests that checking might provide information to decrease uncertainty, which may subsequently reduce anxiety in unpredictable circumstances. This link between checking and information-gathering is supported by evidence that OCD patients who are predominantly compulsive checkers are also more intolerant of uncertainty compared with other subtypes of OCD, when assessed by questionnaire testing [7]. Rodent models make an important contribution to our understanding of how such compulsive behaviours develop: although these models cannot define the relationship between compulsion and obsession *per se*, they are invaluable to the study of the cognitive, neuropharmacological and/or neuroanatomical factors that drive the development of compulsive behaviour.

Functional neuroimaging studies have linked symptoms of OCD with altered activation within the cortico-basal-ganglia circuitry, in particular within orbitofrontal/anterior cingulate/dorsolateral prefrontal-striato-thalamic circuits [8,9]. However, despite considerable overlap in altered brain activation across symptom dimensions of OCD, there are subtle, but consistent, differences in activation patterns that may impact on treatment strategy and outcome. For example, compulsive checkers often show marked increases in striatal activation during cognitive challenge, compared with healthy controls or compulsive washers [10,11], supporting the hypothesis that the neural basis of washing and checking symptoms is not identical. Although OCD symptom severity can be significantly reduced with serotonin reuptake inhibitors (SRIs), SRI pharmacotherapy is successful in only 40–60% of OCD patients [12], most likely because of the heterogeneous nature of the disorder. Recent advances in OCD treatment have augmented traditionally-prescribed SRIs with atypical antipsychotics or dopamine D2-receptor antagonists in SRI-treatment-refractory cases, suggesting that both serotonin and dopamine are critical to effective control of obsessive-compulsive symptoms [13–15]. Therefore, it is highly likely that different symptom subtypes of OCD, potentially driven by dysfunction within different components of fronto-striatal circuitry and/or neurotransmitter action [10], may be responsive to different pharmacotherapy strategies. As a consequence, a better understanding of the development and maintenance of behaviours associated with different OCD symptom subtypes is essential to successful treatment of the disorder as a whole.

A well-established rodent model of checking behaviour showed that striatal dopamine function is important for the control of checking: following chronic treatment with dopamine D2/3-receptor agonist quinpirole, rats increased open-field locomotor behaviour in a manner that displayed many features of human compulsive checking [16,17]. Rats returned to selected object locations more often, but without the fixed patterns typical of conventional stereotypical behaviour, or the generalised activity increase typical of hyper-locomotion. The quinpirole-induced checking model was sensitive to manipulations within the frontal-basal-ganglia circuitry and to both serotonergic and dopaminergic challenges, highlighting the potential validity of the quinpirole-induced checking model for OCD [18–22].

Using the open-field checking model alone, it is not straightforward to examine the cognitive processes that define the development and escalation of checking behaviour. In the current study, we designed a novel operant task, the observing response task, based on earlier observing tasks (e.g. [23]), in order to expand the investigation of cognitive processes that underpin checking-like behaviour in rats, and to complement open-field checking. Rats can press an observing lever to gain information about which of two additional levers is 'active' (information in the form of light illumination above the active lever; see Fig. 1) and will result in food reward if pressed [24]. Using parametric manipulations, it is possible to use the observing response task to address a large range of specific questions pertaining to the development of checking – questions that are not possible to address using more ethological models alone. Operant tasks have contributed significantly towards understanding of other facets of OCD-like compulsive behaviour, for example, the role of feedback during escalation of compulsive responding in the 'signal attenuation' model (see [25,26] for review).

We validated and evaluated the novel observing response task as a potential model of checking behaviour, by testing the hypothesis that chronic quinpirole treatment would selectively increase observing/checking behaviour, as it does in the open-field checking model. We examined factors that affected the development and escalation of observing in the face of increased task demands, in terms of uncertainty/unpredictability of reinforcement, to extend our current knowledge about the link between compulsive checking and uncertainty [7,27–29]. We predicted that uncertainty would increase checking behaviour, preferentially-so following quinpirole treatment, because of the link between dopamine function and uncertainty [30–32]. We examined possible links between anxiety and checking behaviours by comparing observing responses with marble burying and elevated plus maze [33,34]. Finally, we investigated the role of dopamine D2/3 receptors in checking behaviour by assessing if quinpirole-induced changes in observing behaviour could be reduced by OCD-relevant treatment with the D2/3 receptor antagonist sulpiride [14,15].

2. Materials and methods

2.1. Subjects

Subjects were 24 male Lister-hooded rats (Charles River, UK), housed in groups of four (cages supplied with cardboard tube enrichment). Experiments were conducted during the dark phase of a reversed 12 h light-dark cycle (lights off at 07:30). Rats weighed 259 ± 3 g initially (7–8 weeks of age), 284 ± 4 g at

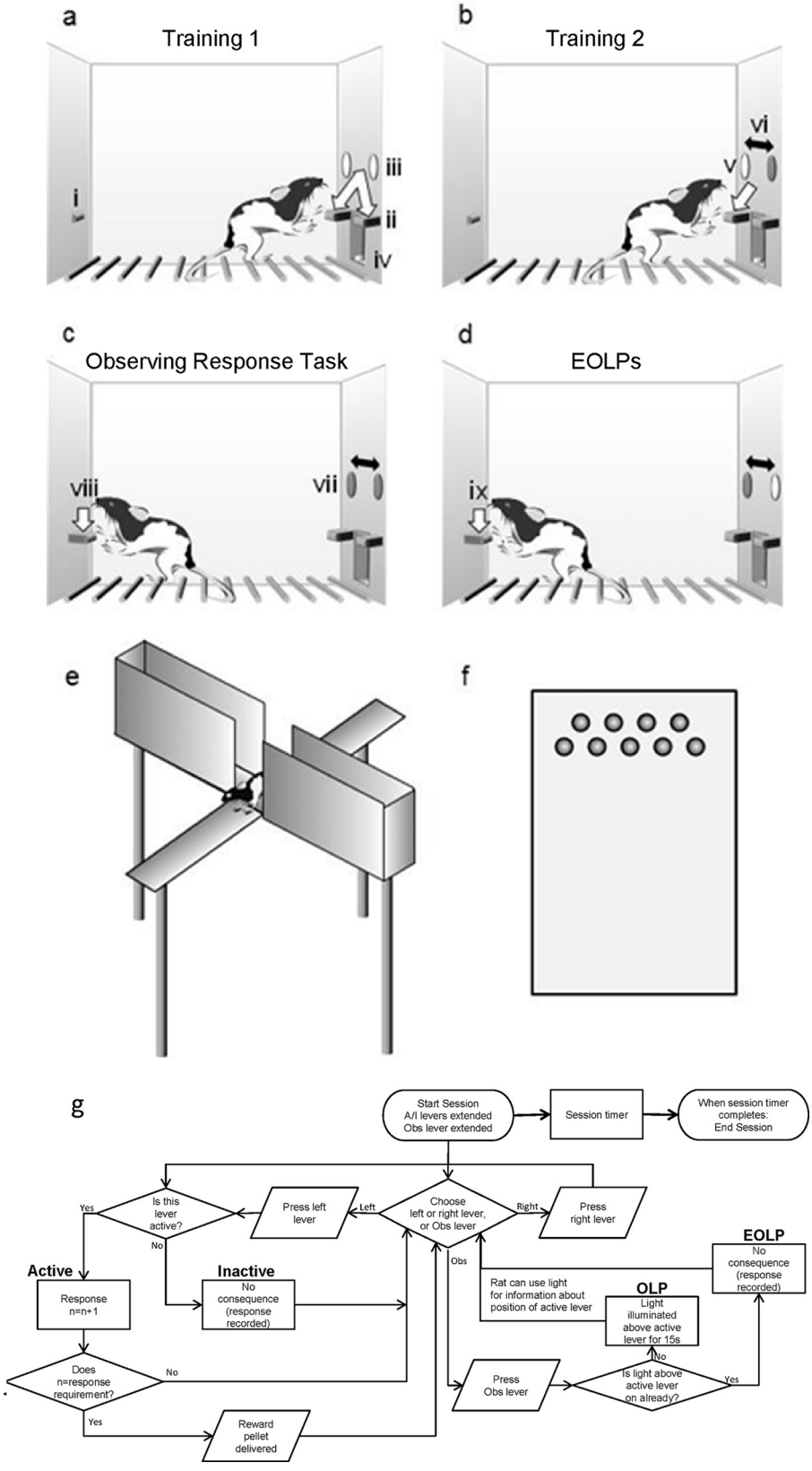

Fig. 1. (a–d) The observing response task. (a) Training 1. The observing lever (i) on the back panel of the box was retracted. Rats were trained to press two levers (ii) on the front panel of the chamber. A light was illuminated above each lever to indicate 'active' status (iii). Completion of lever-presses requirement gave a food pellet in a central food well (iv). (b) Training 2: lever discrimination. One front-panel lever was active and the light above was illuminated (v); the other lever was inactive and the light above was unlit. The active-lever and illuminated-light location switched on a pre-determined schedule (vi). (c) Observing response task. Both levers were extended but neither light was lit above (vii). The observing lever was extended, and a single press on the observing lever (viii) illuminated the light above the active lever for 15 s. (d) Extra observing lever presses (EOLPs) (ix); when the active-lever light was already illuminated had no further consequence, but was recorded. (e) Elevated plus maze. (f) Diagram of marble-burying cage showing the position of the marble array. 20 mm marbles were arranged 6 cm apart along the short wall of the cage. (g) Flowchart of task components in the observing response task, showing active/inactive lever press, observing lever press (OLP) and extra observing lever press (EOLP).

quinpirole treatment and 542 ± 8 g at the end of the study. Weights were maintained at approximately 95% free-feeding weight (rat growth curves; Harlan, UK). Rats received 15–20 g of food daily (task reinforcer pellets plus laboratory chow given 1–2 h following the daily test session) restricting weight gain to approximately 5 g per week. All experiments were conducted in accordance with the United Kingdom Animals (Scientific Procedures) Act, 1986.

2.2. Apparatus

Rats were trained in six operant-conditioning chambers (Med Associates, Vermont, USA). The chamber configuration is shown in Fig. 1. Each chamber had two retractable levers, with a light above each, to the left and right of a central food well (Fig. 1a). Illumination of the light above a lever signaled that the lever was currently active (Fig. 1b). A third lever, the observing lever, was located in the centre panel of the back wall at the same height above the chamber floor as the active/inactive levers. If the observing lever was extended, a lever press turned on the light above the active lever if it was previously unlit (Fig. 1c). A house-light in the chamber roof was illuminated throughout the session. A pellet dispenser delivered 45 mg Noyes formula P pellets (Sandown Scientific, Middlesex, UK) into the food well. Chamber operation and on-line data collection were controlled with the Observing Response Task program (written by A.C. Mar) for the Whisker server platform [35]. Rats were tested 5 days per week unless otherwise stated.

2.3. Observing response task training

The observing response task was based on previous observing tasks. Fig. 1 briefly outlines training stages and the final task, with a flowchart of the task process shown in Fig. 1g. Training parameters are described fully below. Rats received two training sessions per day for 15 training days (session 1–30) before quinpirole treatment, and one session per day during, and subsequent to, quinpirole treatment. Sessions were not divided into discrete trials and there were no correct or incorrect responses (other than some responses having a contribution towards pellet delivery or light illumination, and other responses having no consequence). There was no measure of response omission on this task.

2.3.1. Training 1: lever acquisition (Fig. 1a)

Rats were trained to lever-press for food pellets. Both front-panel levers were presented and active (i.e., resulted in pellet delivery). The light above each lever was illuminated for the whole session. The observing lever remained retracted. Rats were reinforced with a food pellet on an FR1 (session 1–2) or FR3 (session 3) schedule for completing the required presses on one or the other lever. Each session was terminated after 20 min or 200 rewards, whichever was sooner.

2.3.2. Training 2: lever discrimination (Fig. 1b)

Rats were trained to discriminate active from inactive levers. Both front-panel levers were presented, one active and one inactive. The observing lever remained retracted. The light above the active lever was lit and the light above the inactive lever was unlit. The position of the active lever/light switched on an FT60s schedule. The sequence always began with left lever active, which promoted more rapid learning; the rats could begin each session with predictably-rewarded active lever location [training data (sessions 14–16) were analysed for evidence of side bias in lever pressing towards the left lever as a result of this training, but there was no bias towards left lever pressing (total lever presses, side $F(1,23) = 0.04$, n.s.)]. Left and right levers were active for equal duration per session. An active lever press delivered food pellets on a pre-determined schedule of reinforcement (see below). Inactive lever pressing gave no consequence. If a rat switched from active to inactive lever, the active lever responses within a partially-completed ratio were not reset to zero. However, schedule requirement was restarted following a switch in location of active lever. Sessions ended after 20 min or 200 reward pellets. Rats were reinforced under the following schedules: FR3 increasing to FR10 (session 4-10), VR5-15 increasing to VR10-20 (session 11-20).

2.3.3. The observing response task (session 23–30) (Fig. 1c,d)

Rats were trained to make observing responses that "produce discriminative stimuli associated with the conditions of availability of primary reinforcement, but do not alter the availability of primary reinforcement" ([36] cited in [23]). At the beginning of the session, both front-panel levers were presented, but there was no light illuminated above either lever. The observing lever was extended. One observing lever press (Fig. 1c) illuminated the light above the currently-active lever for a pre-determined period (detailed below). If the active lever switched location during the observing period, the light position switched correspondingly. While the active-lever light was illuminated, any further observing lever presses had no consequence, but were recorded as extra observing lever presses (Fig. 1d: EOLPs). EOLPs did not extend the period of light illumination.

The active lever switched sides under an FT90s schedule [increased from FT60s in order to accommodate higher ratios of responding]. Rats were reinforced for active lever presses on a VR10–20 schedule. The session ended after 21 min or 200 reward pellets, whichever was sooner. [Increased to 21 min so left and right levers were active for equal total time within each session.] Session 23–28: Training with observing light duration, 30 s. Session 29–30: Baseline test parameters. Observing light duration, 15 s. S29 was the pre-treatment baseline session for assignment to treatment groups. S30 was the pre-treatment baseline session for statistical analysis.

All task parameter sets are described as follows: active/inactive lever switch schedule, active lever response schedule, OLP response schedule (observing light duration), i.e., for the final training session, S30, the schedule was FT90s, VR10-20, OLP FR1 (15s).–20, OLP FR1 (15s).

2.3.4. Behavioural measures

The main measures on the observing response task are detailed below. In order to standardise measures for potential future comparison and translation, data are presented, where possible, as rates (per minute) (session-wide count /session duration).

2.3.4.1. Active lever presses. Responses on the active lever gave access to food pellets. Active lever presses can be categorised as active-(CS) or active-(CS off) dependent on whether the presses were completed when the light above the active lever was illuminated or not. Here we present a total of all active lever presses.

2.3.4.2. Inactive lever presses. Responses on the inactive lever had no consequence. Inactive lever presses can be categorised as inactive-(CS) or inactive-(CS off) dependent on whether the presses were completed when the light above the active lever was illuminated or not. Here we present a total of all inactive lever presses.

2.3.4.3. Observing lever presses (OLPs). Presses on the observing lever that turned on the active-lever light.

2.3.4.4. Extra observing lever presses (EOLPs). Non-functional observing lever presses, completed during the period when the

active-lever light was illuminated, and that had no further consequence. These responses were perseverative, in the sense of being superfluous or non-functional, and could occur throughout the active-lever light period.

2.3.4.5. EOLPinCS. EOLPs were possible only for part of the session in which the active-lever light was illuminated. It follows that if the active-lever light was illuminated for longer (because more OLPs were made) an increase in session-wide EOLPs might be observed, despite no change in EOLP rate. Therefore, we also analysed EOLP rate for the portion of the session in which the active-lever light was illuminated. Extra observing lever presses are presented as activity per minute of CS-illumination, calculated as (session-wide count/number of 15s observing periods) × 4. On rare occasions, a CS-illumination period occurred within 15 s of the end of the session. These periods were included in the calculation, resulting in possible underestimate of EOLPinCS.

2.3.4.6. Rewards. Total reward pellets per session.

2.3.4.7. %ActiveCS. %Active lever presses during the periods when the light above the active lever was lit, calculated as [100 x active/(active + inactive)]. %ActiveCS measured accuracy of responding on the active versus inactive lever during periods when the light gave information about which lever was currently active. We tested the hypothesis that rats were able to use the information from pressing the observing lever (i.e., turning on the light above the active lever) to locate, and therefore press, the active lever for food reward.

2.3.4.8. %ActiveCS off. Calculated as in %ActiveCS above, but for the periods of the session when the light above the active lever was unlit.

2.4. Experiment 1: effects of quinpirole on checking-like behaviour

2.4.1. Experiment 1A: effects of daily quinpirole: (S31–40; Q1–10)

We aimed to test the hypothesis that a pharmacological model of checking behaviour in rats, defined as ten treatments with a specified dose of quinpirole (commonly 0.5 mg/kg [16–18]), would selectively increase checking-like behaviour in the observing response task. Rats were treated with either quinpirole (0.5 mg/kg in saline, administered in 1 ml/kg, i.p.) or vehicle on 10 consecutive days. Drug administration and pre-treatment holding occurred in rooms that were not normally associated with behavioural testing. Following drug administration, rats were placed into separate holding cages, with *ad libitum* access to water for the duration of the post-injection, pre-test period. Quinpirole-induced behavioural sensitization is most effective if rats are exposed to the test context and behavioural requirements during quinpirole treatment [37]. Therefore, it was important that rats had overcome any immediate hypolocomotion or behavioural suppression associated with acute quinpirole treatment [dopamine agonists often produce hypolocomotion or sedation (e.g. [38,39]]. On days 1–3, treatment was given 60 min before testing to allow rats to overcome any immediate post-treatment behavioural suppression induced by acute quinpirole [a brief probe test showed that animals failed to produce any response on task with shorter pre-treatment]. On days 4–10, pre-treatment time was reduced to 20 min as behavioural suppression had diminished across days 1–3 of treatment. Following the pre-treatment period, rats were tested with one 21 min session per day, with parameters set at FT90s, VR10–20, OLP FR1 (15s).

2.4.2. Experiment 1B: post-quinpirole treatment, early effects (S41–50; post-quinpirole PQ1–10), late effects (see baseline PQ49–58)

Rats were tested for 10 consecutive days/sessions in the absence of quinpirole treatment but with the same test parameters: FT90s, VR10–20, OLP FR1 (15s).

2.5. Experiment 2: effects of changing reward uncertainty on checking-like behaviour

2.5.1. Experiment 2A: single-day extinction (PQ11–14): unpredicted reinforcer omission

We tested the hypothesis that removal of reinforcer pellets, when reward was expected, during a single extinction session, would increase observing behaviour. Rats completed one baseline session [FT90s, VR10–20, OLP FR1 (15s)], one extinction-of-reward session and two recovery baseline sessions. During extinction, the session was identical to baseline, but the food reinforcer was delivered outside the test chamber (so all food-delivery cues were identical except for food availability in the magazine). Following extinction, rats received two baseline sessions, with parameters identical to the pre-extinction baseline.

2.5.2. Experiment 2B: increasing response requirement on the active lever (PQ15–38) - uncertain response requirement

We tested the hypothesis that increasing response requirement and variability would increase task uncertainty and consequently increase observing behaviour.

Response requirement, and variability, on the active lever was increased across sessions, using the schedule parameters FT90s, VR10–*x*, OLP FR1 (15s), where *x* (the VRmax) was 20, 30, 40, 50, 60, 70, 80, 90, 100. Rats received two sessions for each value of *x*, with the exception of performance-stabilization during *x* = 60 and *x* = 70, where the rats were tested for 5 sessions at each ratio. Data are presented for the final session at each ratio.

2.5.3. Baseline (PQ39–58)

Task parameters were returned to FT90s, VR10–20, OLP FR1 (15s) until performance stabilised [10 sessions]. A further ten sessions of baseline performance were recorded (PQ49–58), for comparison with PQ1–10, to assess long-term effects of quinpirole.

2.5.4. Experiment 2C: lever switching: uncertain active lever location (PQ59–74)

We tested the hypothesis that increased uncertainty about active lever location would increase observing responses. Uncertainty was increased by switching active/inactive lever location less predictably – from FT90s to VT20–120s schedule (i.e., VT20–120s, VR10–20, OLP FR1 (15s)). Performance was stabilised over 11 sessions and data are presented from the subsequent 5 sessions (16 sessions in total).

2.5.5. Experiment 2D: combined uncertainty of location and reinforcer (PQ75–84)

Rats were further challenged with combined lever-switch uncertainty and higher VRmax with 5 intermediate sessions at VT20-120s, VR10–50 (data not shown), OLP FR1 (15s) and 5 sessions at VT20–120s, VR10–70, OLP FR1 (15s). We compared the 5 day mean of the VT20–120s schedules at VR10–70 with the data from FT90s VR10–70 (from Fig. 6).

2.6. Experiment 3: elevated plus maze and marble burying - the possible role of anxiety in quinpirole-induced checking

Rats were tested on a new baseline schedule VT20–120s, VR10–20, OLP FR1 (15s) between PQ85 and PQ107, in order to

stabilise a higher baseline of observing before the sulpiride experiment (section 2.7). This VT schedule was maintained throughout further manipulations unless otherwise stated. Rats received one elevated plus maze test and two marble burying tests during the period between PQ85 and PQ107.

2.6.1. Elevated plus maze

The elevated plus maze test is considered sensitive to the anxiety state of the animal [33]. We tested the hypothesis that quinpirole might have anxiogenic/anxiolytic properties that could affect observing lever presses in the observing response task. Therefore, QNP and VEH groups might respond differently on the elevated plus maze. Rats received one session of elevated plus maze between sessions PQ93 and 94, in the absence of quinpirole treatment.

Rats were tested with one 5 min session under normal room illumination. Rats were tested on a standard plus maze apparatus, raised 80 cm above the floor, comprising four arms in the shape of a cross (arm dimensions (45 × 10 cm). The arms were joined at the centre by a 10 × 10 cm platform. Two of the arms, opposite each other, had no walls (open arms) and the other two arms (closed arms) had 23 cm high walls. Rats were acclimatised to the test room for 5 min before test. Each rat was placed on the centre of the maze, at the junction between open and closed arms, facing one of the open arms. Performance was recorded by a ceiling-mounted camera above the centre of the maze. A rat was considered to have entered an arm of the maze when all four feet were within the arm. Recorded parameters were: entries into the closed arms, entries into the open arms, total entries percentage of time in open or closed arms. A reduced percentage time in open arm is considered to reflect fear-induced inhibition and may relate to increased 'anxiety' levels experienced by the rat.

2.6.2. Marble burying

The marble-burying test was originally characterised as a model of anxiety and impulsive behaviour in mice [34] but subsequently has been redefined as a potential model of compulsive responding with relevance to OCD, although the relationship between checking and marble burying is unclear (see [25] for review). We used a version of the marble-burying task proposed by Schneider and Popik [40] that is suitable for rats. We tested the hypothesis that marble burying in rats might be linked with either anxiety or checking measures.

Test cages were novel but with the same dimensions as home cage (35 × 53 × 18 cm), containing fresh bedding to a depth of 50 mm. Nine 20 mm marbles were placed on the surface of the bedding at one end of the cage with 60 mm between marble centres (Fig. 1f).

Testing was conducted for 10 min under red light in a test room that was different from the observing response task or elevated plus maze test rooms, and different from the drug administration/pretest housing or home colony rooms. At the end of the session, the rat was removed from the cage and a count was made of the number of marbles more than two-thirds buried. The array was photographed and the buried marble counts were later verified by an observer who was blind to the treatment groupings.

Rats received two marble-burying baseline sessions. Rats were also tested immediately following observing response task testing during sulpiride (60 mg/kg) and vehicle test days.

2.7. Experiment 4: effects of sulpiride on checking-like behaviour in post-quinpirole and post-vehicle rats (PQ108–118)

We tested the hypothesis that quinpirole-induced behaviour was mediated via dopamine D2/3 receptors and that the dopamine D2/3-receptor antagonist sulpiride would reduce quinpirole-induced behavioural differences. All the rats that had previously been treated with quinpirole or vehicle received 0, 20 and 60 mg/kg sulpiride. Rats were allocated to two groups matched for OLPs (VEH rats matched with VEH rats and QNP matched with QNP), to receive either vehicle-then-sulpiride (60 mg/kg) or sulpiride (60 mg/kg)-then-vehicle. Dosing across the full dose range was not fully counterbalanced to allow possible removal of the 20 mg/kg dose from analysis, should drug effects have carried over to intermediate baseline days.

Set 1: week 1 test schedule: baseline, 60 mg/kg sulpiride or vehicle, day off, baseline, 60 mg/kg sulpiride or vehicle, 2 days off.
Set 2: week 2 test schedule: as week 1.
Set 2 continued: week 3 test schedule: baseline, all 20 mg/kg sulpiride, day off, baseline.

Sulpiride doses were selected on the basis of ability to induce behavioural changes in other studies (e.g. [41]). Rats were tested under the VT20–120s, VR10–20, OLP FR1 (15s) schedule throughout.

In addition to regular measures, the effects of sulpiride on OLPs and EOLPs were assessed relative to OLPs and EOLPs baseline levels, respectively. This analysis was performed to determine if any effects of sulpiride were dependent on baseline levels of behaviour. Baseline measures were taken from the session immediately before sulpiride treatment.

Data for the effect of sulpiride on OLPs were plotted as change in OLPs as a result of sulpiride treatment (OLPs with sulpiride – OLPs with vehicle, for each dose of sulpiride) on the *y* axis, against baseline OLPs on the *x* axis. Data for the effect of sulpiride on EOLPs are plotted as change in EOLPs as a result of sulpiride treatment (EOLPs with sulpiride – EOLPs with vehicle, for each dose of sulpiride) on the *y* axis, against baseline EOLPs on the *x* axis.

As baseline levels of EOLPs were lower for the VEH group compared with the QNP group, it was not practical to median-split each of the QNP/VEH groups into high- and low-EOLP baseline groups. Such data groupings would not be directly comparable. Instead, the data are presented as correlations between baseline EOLPs and change in EOLPs following sulpiride treatment.

2.8. Population variability in observing

We investigated natural population variability in observing-lever responses and its impact on subsequent behavioural and pharmacological challenges. During assignment to QNP/VEH groups on S29, rats were also assigned to high-checker (high OLPs) and low-checker (low OLPs), based on a median split of OLP performance (corresponding to possible bimodality of distribution, with low-checkers producing fewer than 6 OLPs per session). Each of the VEH and QNP groups was assigned 6 high-checker and 6 low-checker rats (matched for OLP between VEH and QNP groups).

2.9. Statistical analysis

Results are expressed as responses per 21 min session or in rates (for comparability with future studies in human patients). Behavioural data were subjected to analysis of variance using a general linear model with significance at $\alpha = 0.05$, using full-factorial models. Homogeneity of variance was verified using Levene's test. For repeated-measures analyses, Mauchly's test of sphericity was applied and the degrees of freedom corrected to more conservative values using the Huynh–Feldt epsilon for any terms involving factors in which the sphericity assumption was violated. Corrected degrees of freedom are shown to the nearest integer. Following repeated-measures analyses, simple pre-planned one-way ANOVA or paired *t*-tests were used to investigate within-subjects and between-subjects factors, with α adjusted where appropriate [42].

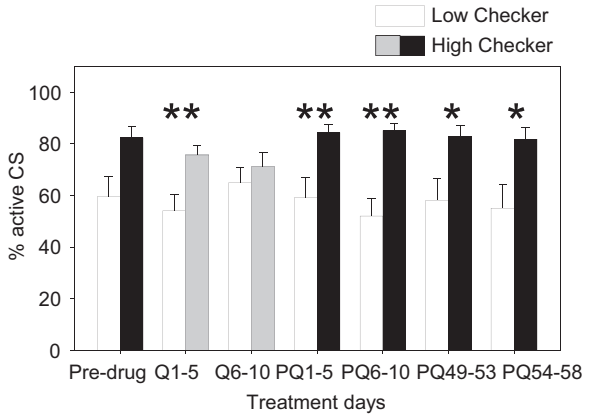

Fig. 2. During active-lever light illumination, percentage of active lever presses (%ActiveCS) for high- and low-checkers. Black bars denote pre- and post-treatment phases, when rats were tested in the absence of quinpirole. Grey bars denote the 10 day quinpirole-administration period. Data are shown for pre-treatment, during 10 days of quinpirole administration (Q1–10), and for both early (PQ1–10) and late (PQ49–58) post-quinpirole periods (in the absence of further quinpirole administration). Error bars represent + 1 s.e.m. Asterisks denote differences between high and low Checker groups $^{*}p < 0.05$, $^{**}p < 0.01$.

P-values greater than 0.1 are reported as non-significant (n.s.). All figures show group means with error bars ±1 s.e.m. QV (quinpirole/vehicle), and HLCheck (checkers) were between-subjects factors. Parametric manipulations across days/phases (e.g. Pre-Q, Pre-PQ, Phase, VR) and SulpDose were within-subjects factors. For marble-burying analyses, repeated measures could not be analysed non-parametrically. Each variable was measured for two sessions and analysed with repeated measures ANOVA. For test-retest reliability, non-parametric Spearman's rank-ordered correlation was most appropriate to assess relative changes to behavioural ranking over time. No rats were systematically excluded from analysis.

3. Results

3.1. Observing response task baseline measures

3.1.1. Baseline matching of performance

Rats were allocated to quinpirole-treatment (QNP) and vehicle-treatment (VEH) groups (matched for OLPs on S29). The task schedule was FT90s, VR10–20, OLP FR1 (15s). Baseline performance was analysed on S30. As expected, there were no pre-treatment baseline differences between QNP and VEH groups on any measure (shown in Figs. 3 and 4; Table 1, PreQ column).

3.1.2. High- and low-checkers: baseline differences in observing behaviour

On training S29, rats were also assigned to high- and low-checker groups based on a median split of OLPs. On S30, high- and low-checker rats retained their difference in baseline levels of OLPs (High-checker mean 0.83 ± 0.12 OLPs/min [17.6 ± 2.6 per session] Low-checker mean 0.20 ± 0.03 OLPs/min [4.3 ± 0.7]; HLCheck, $F(1,22) = 23.51$, $p < 0.0001$). High-checkers illuminated the active-lever light for approximately 21% of the session (264 ± 39 s), whereas low-checkers illuminated the active-lever light for approximately 5% of the session (65 ± 11 s). Fig. 2 shows that high-checkers directed responses towards the active rather than inactive lever during light illumination: during pre-drug baseline, 82.3 ± 4.5% of high-checker responses were active lever, whereas only 59.5 ± 10.3% of low-checker responses were active lever during the illuminated period (HLCheck, $F(1,22) = 4.12$, $p \leq 0.055$). In summary, high-checkers made more observing lever responses than low-checkers and were also more accurate at discriminating the active from inactive lever when the light was illuminated.

High- and low-checkers did not differ on any other measure (HLCheck, all df(1,22); EOLPs $F = 2.10$, EOLPinCS $F = 2.49$, Active $F = 0.19$, Inactive $F = 0.01$, Rewards $F = 0.25$; all n.s.). There were no differences between prospective QNP and VEH groups within the high-checker group or the low-checker group (Table 1).

3.1.3. Analysis of baseline observing lever presses (S30)

3.1.3.1. Do high levels of observing responses result from a generalised increase in lever pressing?. High levels of observing responses (either OLPs or EOLPs) might occur because of generally higher lever-press responding across all levers. However, there was no strong correlation between active lever presses and either OLPs or EOLPs ($n = 24$, OLPs vs. Active $r = 0.34$, n.s.; EOLPs vs. Active $r = 0.12$, n.s.) and, furthermore, there was a negative correlation between OLPs and inactive lever presses ($n = 24$, $r = -0.46$, $p < 0.05$). Therefore, generalised differences in lever pressing were unlikely to have accounted for differences in observing responding. Furthermore, the negative correlation between OLPs and inactive lever presses suggests that one function of OLPs might be to reduce inactive lever responses.

3.1.3.2. Do high levels of observing lever presses result in increased EOLPs?. It is possible that EOLPs and OLPs were not independent measures and that higher EOLPs were a direct consequence of rats making more OLPs. For example, EOLPs may have resulted from rats failing to learn that a single OLP was sufficient to turn on the active-lever light, or because of a failure to terminate the motor action of lever pressing on the observing lever once the light was illuminated. If EOLPs were dependent on OLPs in such a way, we would expect to see a degree of scaling of EOLPs with OLPs. There was no such correlation between baseline OLPs and EOLPs ($n = 24$, $r = 0.26$, n.s.) and, therefore, no clear evidence that the EOLP differences between subjects were directly dependent on between-subject differences in OLPs, under baseline test conditions.

3.1.3.3. Do increased observing lever presses increase rewards earned?. Checking could improve task performance, predicting that rats that made more observing lever presses would be more successful on task, in terms of rewards earned. Overall, there was no strong correlation between OLPs and rewards earned ($n = 24$, $r = 0.35$, $p < 0.1$). However, within the high-checker group, OLPs correlated significantly with rewards earned ($n = 12$, $r = 0.53$, $p < 0.05$), also with active lever presses ($r = 0.52$, $p < 0.05$) and %ActiveCS ($r = 0.51$, $p < 0.05$), but showed a very strong negative correlation with inactive lever presses ($r = -0.78$, $p < 0.001$). Thus, for high-checker rats, there was a strong relationship between observing lever presses and task success. In contrast, there were no correlations between the observing lever presses of low-checker rats and any of these measures ($n = 12$, all $-0.11 < r < 0.11$, n.s.). It is possible that low-checkers adopted a strategy for task performance that was unrelated to checking, using internal cues alone, perhaps by estimating lever press count or duration of lever-press requirement (at least under baseline conditions). It is also possible that low-checkers failed to learn the task discrimination and were unable to gain information from the active-lever light.

3.2. Experiment 1: effects of quinpirole on checking-like behaviour in the observing response task

Figs. 3 and 4 show the effects of daily quinpirole (mean of 5 session blocks, Pre-drug, Q1–5, Q6–10), for the whole group (Fig. 3) and for high/low-checkers (Fig. 4), respectively. The task schedule was FT90s, VR10–20, OLP FR1 (15s). Table 1 shows statistical

Table 1
Statistical comparison of quinpirole vs. vehicle-treated groups before drug treatment (PreQ), during 10 consecutive days of quinpirole/vehicle treatment (Q1–5, Q6–10), during the 10 days immediately following quinpirole/vehicle treatment in the absence of further quinpirole (PQ1–6, PQ6–10) and at a later time-point, again in the absence of quinpirole/vehicle treatment (PQ49–53, PQ54–58). Table shows ANOVA *F* statistic for each comparison (all $p < 0.05$ shown in bold). Degrees of freedom are given above each set. Asterisks denote $^{*}p < 0.05$ and $^{**}p < 0.01$.# denotes $0.05 < p < 0.1$. The final column indicates the qualitative difference between groups.

Whole group	PreQ	Q1–5	Q6–10	PQ1–5	PQ6–10	PQ49–53	PQ54–58	
(d.f.)	*F*(1,22)	*F*(1,22)	*F*(1,22)	*F*(1,22)	*F*(1,22)	*F*(1,22)	*F*(1,22)	
OLP	0.01	**4.87***	**5.29***	3.94#	**4.48***	1.96	1.14	Q > V
EOLP	1.84	2.79	3.80	**6.57***	**15.63****	**6.00***	**5.89***	Q > V
Active	0.12	**39.70****	**27.81****	2.67	1.64	0.08	0.61	V > Q
Inactive	0.37	**46.96****	**7.33***	0.08	0.01	0.03	0.02	V > Q
Rewards	0.10	**38.72****	**30.87****	2.45	1.57	0.07	0.07	V > Q
%ActiveCS	0.13	0.01	0.01	0.67	0.03	0.63	2.11	
%ActiveCSoff	0.90	**39.35****	**32.35****	3.27#	2.04	0.03	0.16	V > Q
EOLPinCS	0.01	2.74	0.53	1.31	**10.11****	**8.51****	**6.49***	Q > V
Low checkers	*F*(1,11)	*F*(1,11)	*F*(1,11)	*F*(1,11)	*F*(1,11)	*F*(1,11)	*F*(1,11)	*F*(1,11)
OLP	3.50	**8.26***	**6.97***	**4.87***	**6.13***	0.21	0.07	Q > V
EOLP	0.77	1.84	3.73#	1.52	**9.32***	1.09	1.71	Q > V
Active	0.01	**10.05****	**8.86***	1.21	0.61	0.08	0.24	V > Q
Inactive	0.05	**26.19****	**5.83***	0.37	0.41	0.03	0.02	V > Q
Rewards	0.01	**10.06****	**9.65***	1.22	0.60	0.07	0.23	V > Q
%ActiveCS	0.05	0.23	0.71	1.26	0.85	0.01	0.9	
%ActiveCSoff	0.02	**7.84***	**7.72***	0.23	0.06	0.01	0.07	V > Q
EOLPinCS	0.05	2.63	0.26	0.86	**9.05***	2.86	4.79#	Q > V
High checkers	*F*(1,11)	*F*(1,11)	*F*(1,11)	*F*(1,11)	*F*(1,11)	*F*(1,11)	*F*(1,11)	
OLP	0.16	1.54	3.65#	3.93#	**5.79***	**7.95***	**7.00***	Q > V
EOLP	1.66	1.75	2.56	**9.93****	**16.45****	**6.67***	**4.92***	Q > V
Active	0.24	**69.60****	**37.9****	1.27	0.94	0.01	0.4	V > Q
Inactive	1.38	**18.18****	2.34	0.17	0.31	0.32	0.36	V > Q
Rewards	0.22	**55.31****	**42.59****	1.03	0.88	0.01	0.4	V > Q
%ActiveCS	0.19	1.24	1.66	0.04	3.83#	4.72#	3.13	
%ActiveCSoff	1.45	**98.14****	**60.35****	**4.94***	3.37#	0.24	0.27	V > Q
EOLPinCS	0.65	0.21	1.34	1.29	2.53	**6.10***	2.66	Q > V

comparison of QNP and VEH groups at each of the experimental stages and time-points.

3.2.1. Experiment 1A: effects of daily quinpirole

Daily quinpirole administration significantly increased OLPs compared with pre-treatment performance (Fig. 3a. Pre-Q x QV, Q1–5, $F(1,20) = 19.56$, $p < 0.0001$; Q6–10, $F(1,20) = 25.89$, $p < 0.0001$; also Table 1). There was no significant difference between high and low-checker groups in this respect (Pre-Q × QV × HLCheck, Q1–5, $F(1,20) = 0.025$, n.s.; Q6–10, $F(1,20) = 0.01$, n.s.).

Quinpirole also increased EOLPs compared with pre-treatment performance (Figs. 3b, 4c and d. Pre-Q × QV, Q1–5, $F(1,20) = 5.14$, $p < 0.05$; Q6–10, $F(1,20) = 6.70$, $p < 0.05$; but see Table 1 – QNP vs. VEH during treatment not independently significantly different). There was no significant difference between high and low-checker groups in this respect (Pre-Q × QV × HLCheck, Q1–5, $F(1,20) = 0.03$, n.s.; Q6–10, $F(1,20) = 0.01$, n.s.).

The quinpirole-induced increase in OLPs/EOLPs was selective and not the result of a generalised increase in responding on all levers. Thus, in contrast to observing responses, quinpirole reduced active and inactive lever presses leading to a reduction in earned rewards (Fig. 3c–e. Pre-Q × QV, df(1,20): Active. Q1–5, $F = 18.67$, $p < 0.0001$; Q6–10, $F = 14.64$, $p \leq 0.001$; Inactive. Q1–5, $F = 38.79$, $p < 0.0001$; Q6–10, $F = 4.88$, $p < 0.05$; Rewards. Q1–5, $F = 18.95$, $p < 0.0001$; Q6–10, $F = 15.96$, $p < 0.001$).

Quinpirole did not change the ability/tendency for rats to use the active-lever light for information about the location of the active lever. There was no significant effect of quinpirole on %ActiveCS (Fig. 3f. Pre-Q × QV, Q1–5, $F(1,20) = 0.26$, n.s.; Q6–10, $F(1,20) = 0.10$, n.s.). The difference between high- and low-checkers remained for part of the quinpirole treatment period (Comparing Pre-Q × QV × HLCheck, Q1–5, $F(1,20) = 3.53$, $p \leq 0.075$; Q6–10, $F(1,20) = 4.90$, $p < 0.05$. Comparing quinpirole/vehicle treatment phase only: HLCheck, Q1–5, $F(1,20) = 6.21$, $p < 0.05$; Q6–10, $F(1,20) = 3.38$, $p \leq 0.08$). It is unlikely that the increased OLPs of quinpirole-treated rats were because quinpirole had decreased the ability to use the light for information about the location of the active lever.

3.2.2. Experiment 1B: post-quinpirole treatment

3.2.2.1. Early post-quinpirole effects (PQ1–10). Figs. 3 and 4 also show ten sessions after quinpirole treatment had ended (sessions PQ1–10 in 2 × 5 day blocks; task schedule FT90s, VR10–20, OLP FR1 (15s), Pre-PQ denotes pre-treatment vs. post-quinpirole treatment). Statistical analyses of QNP versus VEH groups are shown in Table 1.

This study is the first to show long-lasting effects of quinpirole on checking-like behaviour, for many weeks after the final quinpirole treatment. QNP rats made more observing responses, both as OLPs and EOLPs, than VEH rats during the ten days after quinpirole treatment had ended (Fig. 3a,b, Table 1), both within high- and low-checker groups (Table 1). Long-term effects of quinpirole were selective to observing responses. There were no differences between QNP and VEH groups on active and inactive lever presses, nor differences on rewards earned. (Fig. 3c–e, Table 1).

High-checkers maintained their ability to differentiate active and inactive levers when the light was illuminated, showing high %ActiveCS compared with low-checkers during the 10 day post-drug period (Fig. 2. HLCheck, PQ1–5, $F(1,20) = 7.22$, $p < 0.05$; PQ6–10, $F(1,20) = 11.01$, $p < 0.01$), with no difference between QNP and VEH groups (Fig. 3f. Table 1).

3.2.2.2. Late post-quinpirole effects (PQ49–58). Around fifty days after quinpirole treatment ended, there was no longer a significant difference between QNP and VEH groups in OLPs for all rats (Fig. 3a, Table 1). Within the high-checker group, OLPs of the QNP rats remained higher than the VEH rats, but OLPs in the low-checker QNP and VEH groups were not significantly different (Table 1). However, there was no QV × HLCheck interaction at either PQ49–53 ($F(1,20) = 2.43$, n.s.) or PQ54–58 ($F(1,20) = 3.09$, $p < 0.09$) so it was

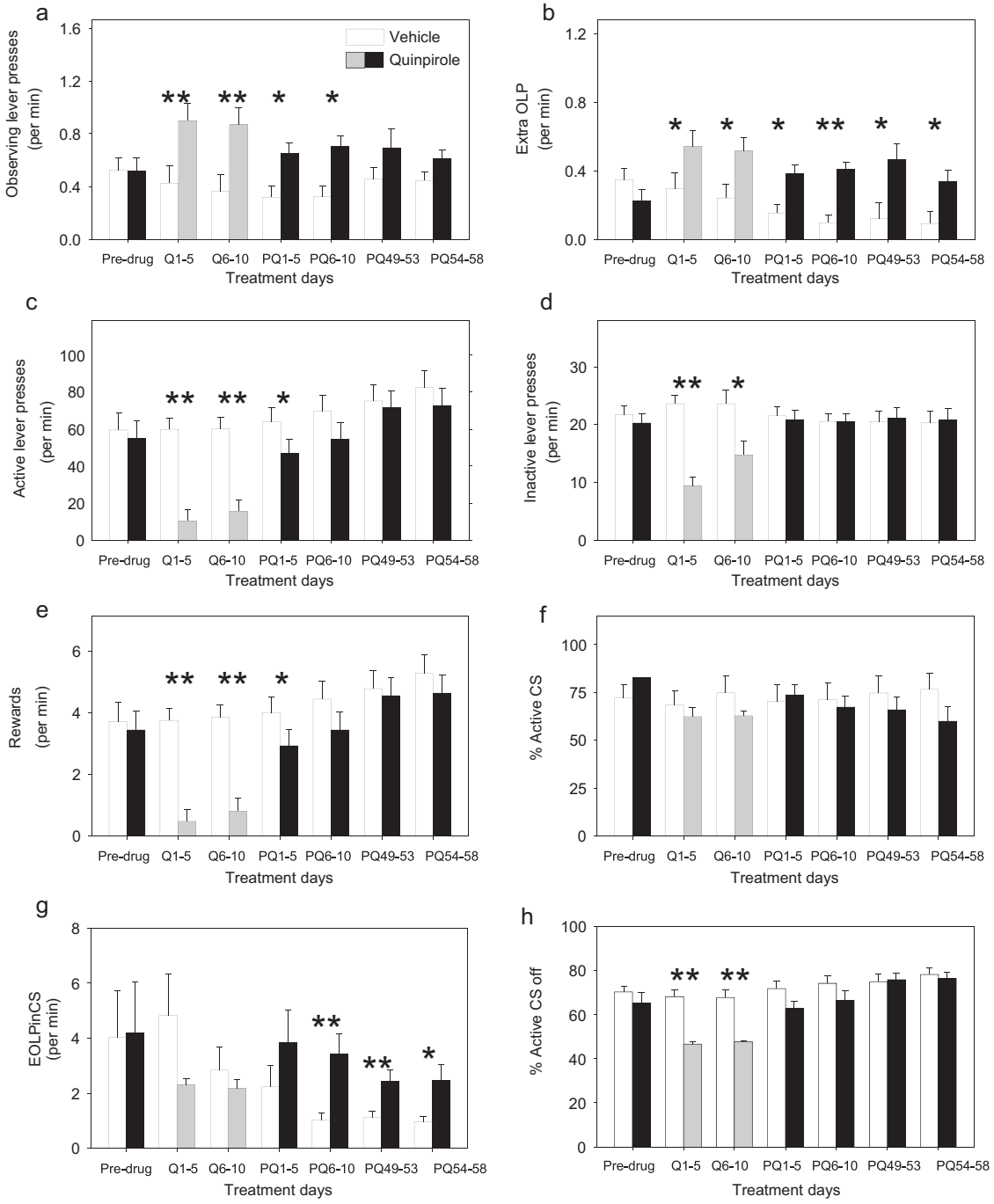

Fig. 3. Performance measures on the observing response task [schedule FT90s, VR10–20, OLP FR1 (15s)], for vehicle (white bars) and quinpirole (filled bars) treated rats. Black bars denote pre- and post-treatment phases, when rats were tested in the absence of quinpirole. Grey bars denote the 10 day quinpirole-administration period. Data are shown for pre-treatment, during 10 days of quinpirole administration (Q1–10), and for both early (PQ1–10) and late (PQ49–58) post-quinpirole periods (in the absence of further quinpirole administration). (a) observing lever presses; OLPs, (b) non-functional extra observing lever presses; EOLPs, (c) active lever presses, (d) inactive lever presses, (e) rewards, (f) percentage of active lever presses when the active-lever light was illuminated %ActiveCS, (g) EOLPs relative to duration of active-lever light illumination EOLPinCS and (h) percentage of active lever presses when the active-lever light was off %ActiveCSoff. Error bars represent + 1 s.e.m. Asterisks denote differences between vehicle and quinpirole treatment groups $^{*}p < 0.05$, $^{**}p < 0.01$.

not possible to conclude that high- and low-checkers differed significantly in their responsiveness to quinpirole in the long term.

In contrast, EOLPs remained high across the whole QNP group compared with the VEH group (Fig. 3b. Table 1). Therefore, in the longer term, the effect of quinpirole on non-functional EOLPs was more robust than the effect of quinpirole on OLPs. This was only independently statistically significant in the high-checker group (Fig. 4c,d. Table 1), but there was no QV × HLCheck interaction at either PQ49–53 ($F(1,20) = 1.54$, n.s.) or PQ54–58 ($F(1,20) = 0.47$, n.s.).

There were no long-term differences between QNP and VEH groups on active and inactive lever presses or rewards (Fig. 3c–e, Table 1). After fifty days in the absence of daily drug/vehicle treatment, long-term effects of quinpirole were still highly selective for the observing responses and not expressed in terms of general lever pressing.

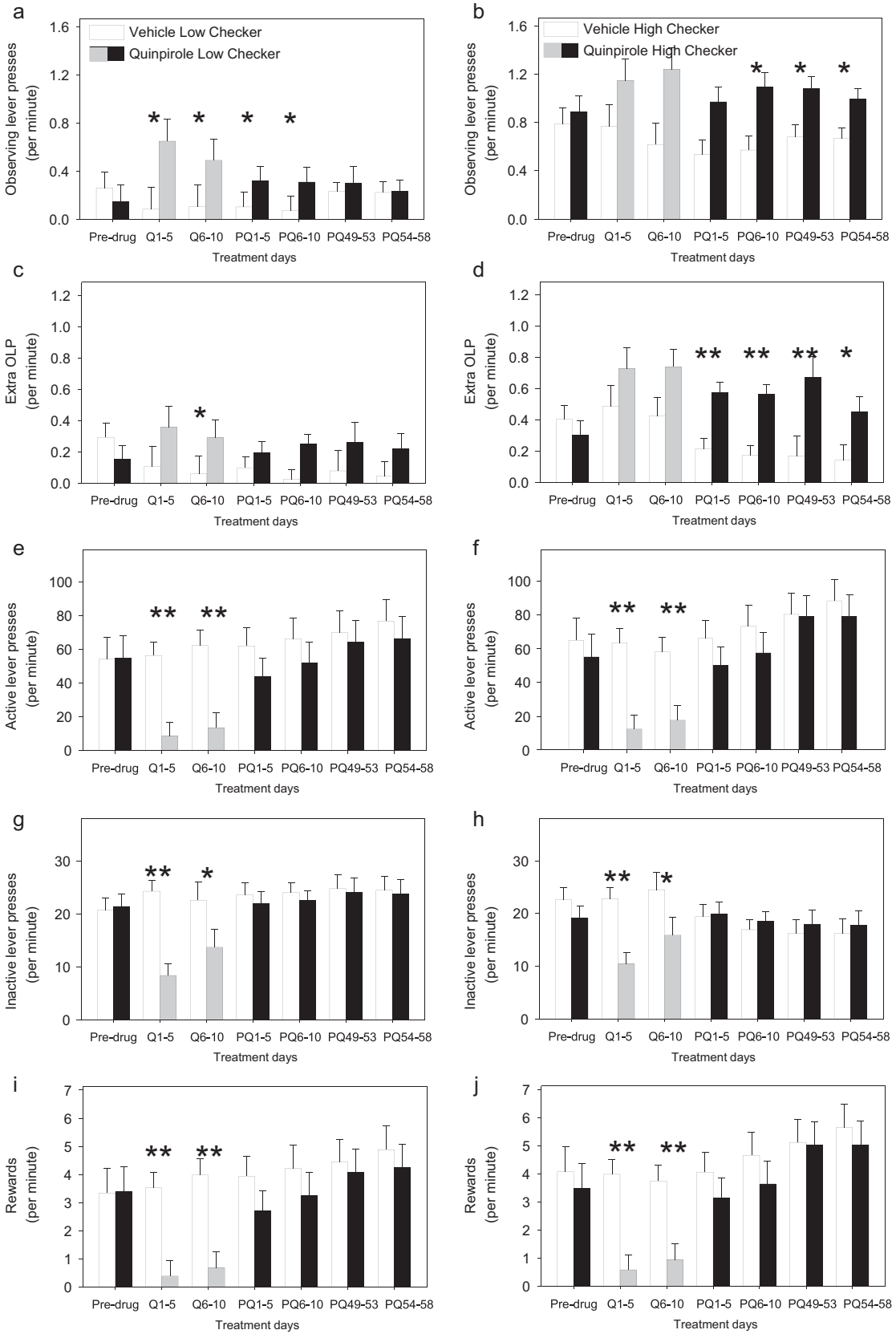

Fig. 4. Performance measures on the observing response task, showing the difference between low (a, c, e, g, i) and high-checkers (b, d, f, h, j), [schedule FT90s, VR10–20, OLP FR1 (15s)], for vehicle (white bars) and quinpirole (filled bars) treated rats. Black bars denote pre- and post-treatment phases, when rats were tested in the absence of quinpirole. Grey bars denote the 10 day quinpirole-administration period. Data are shown for pre-treatment, during 10 days of quinpirole administration (Q1–10), and for both early (PQ1–10) and late (PQ49–58) post-quinpirole periods (in the absence of further quinpirole administration). (a, b) OLPs, (c, d) EOLPs, (e, f) active lever presses, (g, h) inactive lever presses, (i, j) rewards. Error bars represent + 1 s.e.m. Asterisks denote differences between vehicle and quinpirole treatment groups $^{*}p < 0.05$, $p < 0.01$.

Table 2
Test-retest reliability of OLPs and EOLPs measures. Spearman rank-order correlation coefficents r_s. Asterisks denote statistical significance $^*p < 0.05$, $^{**}p < 0.01$ (all $p < 0.05$ shown in bold).

		PQ6–10	PQ54–58
All OLPs	Pre-Q	**0.706****	**0.813****
	PQ6–10		**0.898****
All EOLPs	Pre-Q	0.215	0.146
	PQ6–10		**0.579****
VEH OLPs	Pre-Q	**0.889****	**0.824****
	PQ6–10		**0.963****
VEH EOLPs	Pre-Q	**0.581***	**0.596***
	PQ6–10		**0.686***
QNP OLPs	Pre-Q	**0.847****	**0.831****
	PQ6–10		**0.914****
QNP EOLPs	Pre-Q	0.475	−0.148
	PQ6–10		**0.592***

High-checker rats maintained higher %ActiveCS during post-quinpirole days 59–68 compared to low-checkers (Fig. 3f. HLCheck, PQ49–53, $F(1,20) = 6.51$, $p < 0.05$; PQ54–58, $F(1,20) = 6.11$, $p < 0.05$). This confirms that there were no long-term changes in the ability to use the light for information about the location of the active lever.

3.2.2.3. Test-retest reliability on the observing response task is robust. The test-retest reliability of observing responses was very strong (Table 2 shows Spearman's rank-ordered correlation to investigate the relative rankings of low-to-high observers between Pre-Q, PQ6–10 and PQ54–58 time points). Individual ranking based on OLPs was retained both for pre-post treatment comparisons and for the post-treatment test-retest. As OLPs rank was retained following quinpirole treatment, it is probable that quinpirole increased OLPs in proportion to baseline OLPs response levels.

In contrast, individual ranking based on EOLPs was altered between pre- and post-treatment, although the post-treatment EOLPs rank was robust across post-treatment test-retest. Further analysis showed that the VEH group was robustly ranked during the pre-post analysis and post-treatment test-retest, but the QNP group EOLPs ranking was significantly changed by quinpirole treatment. However, following quinpirole treatment, the QNP group EOLPs ranking remained consistent across post-treatment test-retest. This suggests that the effect of quinpirole on EOLPs was independent of pre-treatment EOLPs, but that any quinpirole-dependent effect was robust over time.

3.2.3. Effects of quinpirole treatment on EOLPs as a function of available observing time (Fig. 3g)

We also analysed EOLPs as a function of the time available with the CS illuminated (EOLPinCS), to determine if any increase in EOLPs was a direct result of having increased the time available to make such responses. During pre-treatment baseline and quinpirole treatment, QNP and VEH groups showed no difference in EOLPinCS (Fig. 3g, Table 1). Thus, despite the increases in both OLPs and EOLPs during the ten days of quinpirole treatment, EOLPinCS remained comparable for VEH and QNP groups, suggesting that quinpirole-induced EOLPs during the quinpirole treatment phase were most likely a direct result of increasing the time available for performance of these perseverative responses. However, during the later post-quinpirole testing, EOLPinCS was higher in the QNP group compared to VEH (Table 1), mainly as a result of the VEH group EOLPinCS reducing over time, whereas the QNP group remained significantly higher. In the longer term, quinpirole-treated rats made significantly more perseverative EOLPs per minute of light illumination than vehicle-treated counterparts. There were no differences between high-and low checkers on this measure (all $F(1,22) < 2.50$, n.s.).

3.2.4. Effects of quinpirole on the function of observing responses (Fig. 3h)

Although quinpirole had no effects on the ability to use the active-lever light to locate the active lever, during the ten days of quinpirole administration, there was a significant effect of quinpirole on the ability to locate the active lever when the active-lever light was turned off (Fig. 3h, Table 1). During the pre-treatment phase, there was no difference between QNP and VEH groups in the relationship between %ActiveCS and %ActiveCSoff, whereas, during the drug-treatment phase, %ActiveCSoff was significantly lower than %ActiveCS for the QNP group (QV × CS/CSoff, %active lever presses, Pre-treatment, $F(1,20) = 0.58$, n.s.; Q1–5 $F(1,20) = 6.12$, $p < 0.05$; Q6–10, $F(1,20) = 4.30$, $p < 0.05$). There was no difference between high and low checkers in this respect (QV × CS/CSoff × HLCheck, %active lever presses, Pre-treatment, $F(1,20) = 1.44$; Q1–5 $F(1,20) = 0.20$; Q6–10, $F(1,20) = 1.91$, all n.s.). Thus, for the quinpirole treatment phase, quinpirole may have disrupted rats' ability to locate the active lever when information about its position was absent.

The negative correlation between OLPs and inactive lever presses before drug treatment suggested that rats might use information from the observing response to reduce inactive lever presses. We investigated the effects of quinpirole on the relationship between OLPs and inactive lever presses within the high-checker group (with the strongest baseline correlation between these two measures). Quinpirole eliminated the negative relationship between OLPs and inactive lever presses, and these effects were long-lasting in the absence of further quinpirole (Table 2). The VEH high-checker group maintained a strong negative relationship between OLPs and inactive lever presses. In contrast, the QNP high-checker group showed a comparable negative correlation between OLPs and inactive lever presses during pre-treatment baseline but not during drug treatment, nor in the long term without quinpirole (see Table 3). There were no significant correlations between these behavioural measures for the low-checker rats ($n = 12$, all $r < 0.67$, n.s.). In high-checker rats, one of the effects of quinpirole may be to dissociate the relationship between OLPs and reduction of inactive lever pressing.

3.3. Summary of findings for experiment 1

- Rats made OLPs to turn on a light above a lever, and they were able to obtain information about which of two levers delivered food reward.
- Non-functional EOLPs, when the light was already illuminated, were not directly correlated with the number of functional OLPs, and were, therefore, considered as a separate response type.
- The population of rats tested showed a range of individual variability in observing behaviour and could be categorised as high- and low-checkers. High-checkers tended to use the information from the light to locate the active lever.
- Within the high-checker group, rats that made more OLPs earned more rewards. This relationship was not found in the low-checker group, nor when the entire cohort was examined. Therefore, checking behaviour was associated with improved task performance, but only for those rats that used the information to locate the active lever.
- There was a strong negative relationship between OLPs and inactive lever responses in untreated rats, suggesting that checking might normally be associated with 'reduction of negative' rather than 'enhancement of positive' responses.
- Quinpirole significantly, and selectively, increased observing responses, both functional and non-functional (OLPs and EOLPs, respectively). This increase in observing responses was long-lasting in the absence of further quinpirole treatment.

Table 3
Correlation between OLPs and inactive lever presses for high-checker VEH and QNP groups. $^{*}p < 0.05$, $^{**}p < 0.01$ (all $p < 0.05$ shown in bold).

Response correlated with OLPs	$N = 6$	Pre-Q	Q1–5	Q6–10	PQ1–5	PQ6–10	PQ59–63	PQ64–68
VEH high	Inactive	**−0.88***	**−0.84***	**−0.83***	**−0.83***	**−0.92****	**−0.81***	**−0.99****
QNP high	Inactive	**−0.81***	−0.57	−0.30	−0.18	0.03	0.07	0.07

Quinpirole-treated rats made relatively more EOLPs than OLPs compared with vehicle-treated rats in the long term.

- Quinpirole had no effect on the ability to gain information about active-lever location from observing responses (as measured by %ActiveCS). However, quinpirole reduced the ability of rats to locate the position of the active lever when there was no light information present. Quinpirole also abolished the negative relationship between observing and inactive lever pressing overall. Therefore, quinpirole-treated rats might not be able to retain information about the active-lever location during parts of the session in the absence of an external information source.

3.4. Experiment 2: effects of changing reward uncertainty on checking-like behaviour

3.4.1. Experiment 2A: single-day extinction: unpredicted reward omission (Fig. 5)

When reward was omitted, all rats increased both OLPs and EOLPs (Fig. 5a,b. for baseline vs. extinction, OLPs, Phase $F(1,20) = 24.37$, $p < 0.001$; EOLPs, Phase $F(1,20) = 18.43$, $p < 0.001$). In addition, EOLPinCS increased for all rats (Fig. 5g. Baseline = 1.47 ± 0.26, Extinction 3.07 ± 0.39 EOLPs per minute of light illumination. Phase $F(1,20) = 12.12$, $p < 0.01$). QNP and VEH groups increased observing responses to a similar extent during reward omission (Phase × QV, df(1,20): OLPs, $F = 0.026$, n.s.; EOLPs, $F = 0.267$, n.s.; EOLPinCS $F = 0.59$, n.s.), as did high- and low-checkers (Phase × HLCheck, df(1,20): OLPs, $F = 0.046$, n.s.; EOLPs, $F = 3.91$, $p \leq 0.062$; EOLPinCS $F = 0.03$, n.s.). Most importantly, the extinction session showed that quinpirole-induced high levels of OLPs/EOLPs were not at ceiling levels and could be increased under further challenge.

During extinction, all rats significantly decreased rewards 'earned' and both active and inactive lever presses (Fig. 5c,d,e. Phase, df(1,20): Rewards, $F = 53.82$, $p < 0.001$; Active, $F = 47.79$, $p < 0.001$; Inactive $F = 13.12$, $p < 0.01$). However, low-checker rats changed their response strategy and significantly increased %ActiveCS (Fig. 5f, baseline vs. extinction, %ActiveCS, Phase $F(1,20) = 10.91$, $p < 0.01$; Phase × HLCheck $F(1,20) = 5.19$, $p \leq 0.018$). There was no overall difference between QNP and VEH groups on this measure (Phase × QV $F(1,20) = 0.69$, n.s.; Phase × QV × HLCheck $F(1,20) = 5.19$, $p < 0.05$). However, further analysis showed that the VEH low-checkers significantly increased %ActiveCS whereas QNP low-checkers and both high-checker groups did not (VEH low-checker, $F(1,5) = 9.46$, $p < 0.05$; all others $F(1,5) < 2.58$, n.s.). In contrast, there was a small, but significant, overall decrease in %ActiveCSoff for all rats, independent of quinpirole/vehicle-treatment or high/low checker status (Phase, $F(1,20) = 22.11$, $p < 0.001$; Phase × HLCheck $F(1,20) = 0.22$, n.s.; Phase x QV $F(1,20) = 3.99$, $p \leq 0.06$). This suggests that all rats responded in extinction with a decrease in discrimination between active and inactive levers, when there was no light to signal the position of the active lever, but were effective in discriminating active from inactive levers when the active-lever light was illuminated.

These results show that all rats are responsive to reward omission, regardless of previous quinpirole/vehicle treatment history. The findings also suggest that low-checker rats had learnt the association between the light and position of the active lever during early discrimination training and were able to use the light for information, but had selected an alternative response strategy, such as counting or timing response requirement, under standard baseline conditions.

Extinction-induced changes were transient, and all measures returned to pre-extinction levels within two sessions. OLPs decreased following extinction (OLPs Baseline vs. PostExt1/2, Phase $F(1,20) = 6.17/4.10$, $p < 0.05/p \leq 0.056$). Quinpirole-treated rats made more OLPs than vehicle-treated rats, and high-checkers more than low-checkers (Fig. 5a, PostExt1/2, QV $F(1,20) = 7.20/4.84$, $p < 0.05$; HLCheck $F(1,20) = 18.97/20.48$, $p < 0.001$). Similarly, post-extinction EOLPs returned to near baseline levels (Baseline vs. PostExt1/2, Phase $F(1,20) = 1.12/1.44$, n.s.). Quinpirole-treated rats made more EOLPs than vehicle-treated rats, and high-checkers more than low-checkers (Fig. 5b, PostExt1/2, QV $F(1,20) = 4.86/4.62$, $p < 0.05$; HLCheck $F(1,20) = 3.49/5.36$, $p < 0.05$ for session 2 only). Both active lever presses and rewards returned to pre-extinction levels then increased slightly following extinction. Inactive lever presses returned to baseline (Fig. 6c–e, baseline vs. post-extinction sessions 1/2, df(1,20): active $F = 0.10/\ 9.51$, n.s./$p < 0.01$; rewards $F = 0.21/6.25$, n.s./$p < 0.05$). Inactive $F = 3.35/0.20$, $p \leq 0.082$/n.s.).

3.4.2. Experiment 2B: increasing response requirement: uncertain response requirement (Fig. 6)

OLPs increased overall with response requirement/uncertainty (increased VRmax) but QNP and VEH groups responded differently. Quinpirole-treated rats maintained consistently high OLPs but were unresponsive to VRmax increase, whereas vehicle-treated rats progressively increased OLPs as VRmax increased (Fig. 6a, Table 4). Consequently, at higher values of VRmax, there was no difference in OLPs between QNP and VEH groups (all $F(1,22)$, VR10–20 = 5.11, $p < 0.05$, VR10–30 = 2.52, VR10–40 = 0.29, VR10–50 = 0.74, VR10–60 = 0.30, VR10–70 = 0.23, VR10–80 = 0.09, VR10–90 = 0.22, VR10–100 = 0.01; all n.s.).

This difference between QNP and VEH group OLPs with changing response requirement/uncertainty was seen primarily in the high-checker group. VEH high-checkers increased OLPs with VRmax increase, whereas QNP high-checkers did not (VR × QV × HLCheck, $F(2,160) = 2.21$, $p < 0.05$; High-checker VR × QV, $F(8,80) = 4.01$, $p < 0.001$; VEH VR, $F(6,28) = 8.77$, $p < 0.001$; QNP VR, $F(8,40) = 1.05$, n.s.). Low-checker VEH and QNP groups showed no difference in OLPs (Low-checker VR × QV, $F(5,46) = 0.87$, n.s.).

The apparent inflexibility of OLPs of quinpirole-treated rats was not because these rats had reached ceiling levels of responding. Quinpirole-treated rats had clearly increased OLPs during the previous extinction session. At VR10-100, the QNP group OLPs were significantly lower than during extinction (VR10–100 vs. extinction, Phase × QV, $F(1,20) = 7.01$, $p < 0.05$; Phase, VEH, $F(1,10) = 0.00$, n.s., QNP, $F(1,10) = 14.46$, $p < 0.01$). Thus, all rats could show response flexibility, given changing task demands, but quinpirole-treated rats did not increase OLPs with increasing VRmax.

EOLPs followed a similar pattern to OLPs. Rats increased EOLPs as VRmax increased (Fig. 6b, Table 4). Although there was no overall statistical difference between QNP and VEH groups, EOLPs were similar in form to OLPs: as VRmax increased, EOLPs appeared

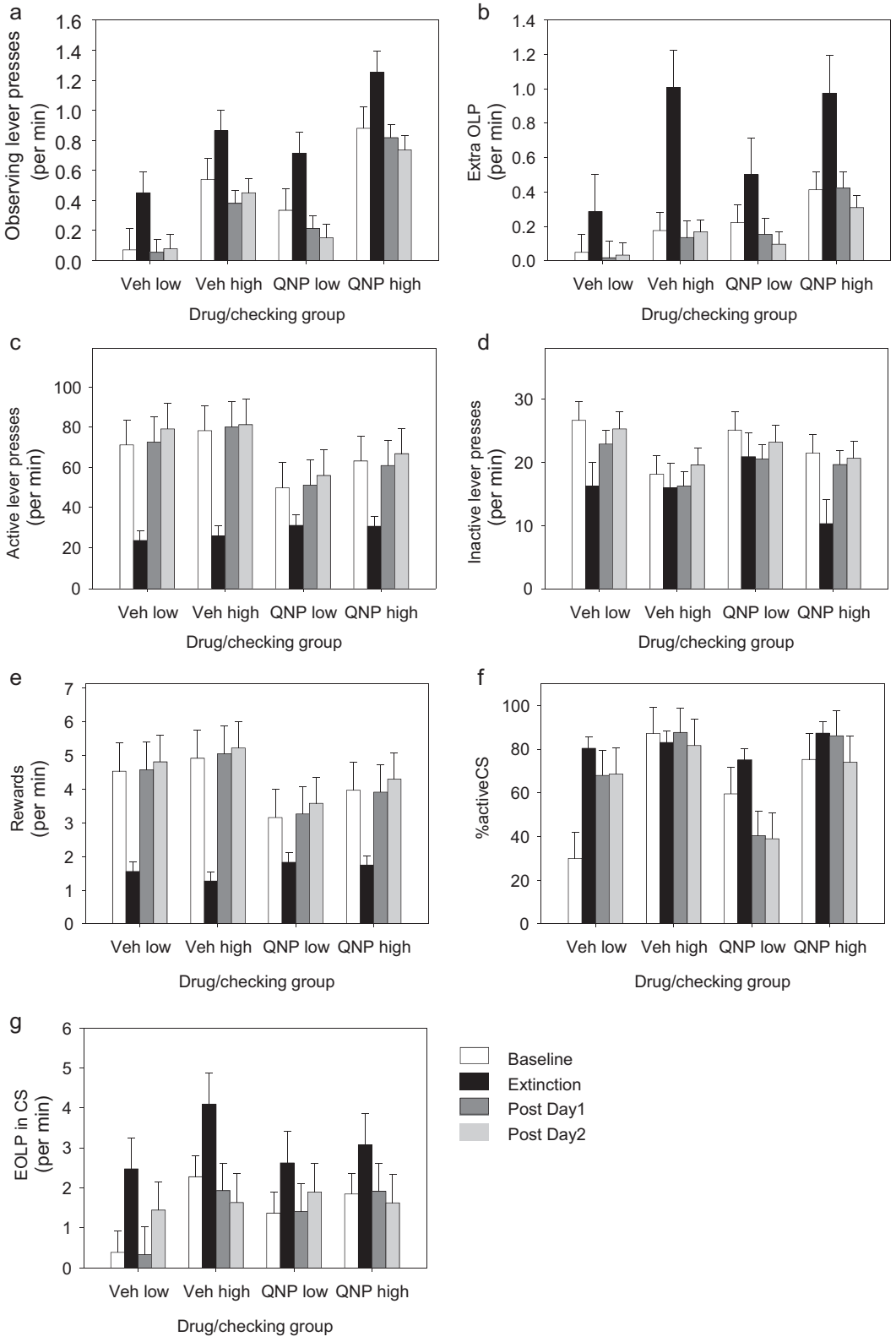

Fig. 5. Single-day extinction. (a) OLPs, (b) EOLPs, (c) active lever presses, (d) inactive lever presses, (e) rewards, (f) %ActiveCS, (g) EOLPinCS for the quinpirole-treated (QNP) and vehicle-treated (VEH) rats. Error bars represent + 1 s.e.m.

high and inflexible in rats treated with quinpirole but increased progressively in vehicle-treated. Comparisons for each response ratio showed that the QNP group made more EOLPs than the VEH group at VR10–20 and VR10–30 but this difference disappeared at higher VRmax (all $F(1,22)$, VR10–20 = 4.31, $p \leq 0.05$, VR10–30 = 7.60, $p < 0.05$, VR10–40 = 1.17, VR10–50 = 3.36 [$p \leq 0.08$], VR10–60 = 0.30, VR10–70 = 0.70, VR10–80 = 1.81, VR10–90 = 0.04, VR10–100 = 0.18 all others n.s.). EOLPs at VR10–100 were lower than extinction EOLPs (VR10–100 vs. extinction, Phase, $F(1,20) = 7.33$, $p < 0.05$; Phase × QV, $F(1,20) = 0.01$, n.s.).

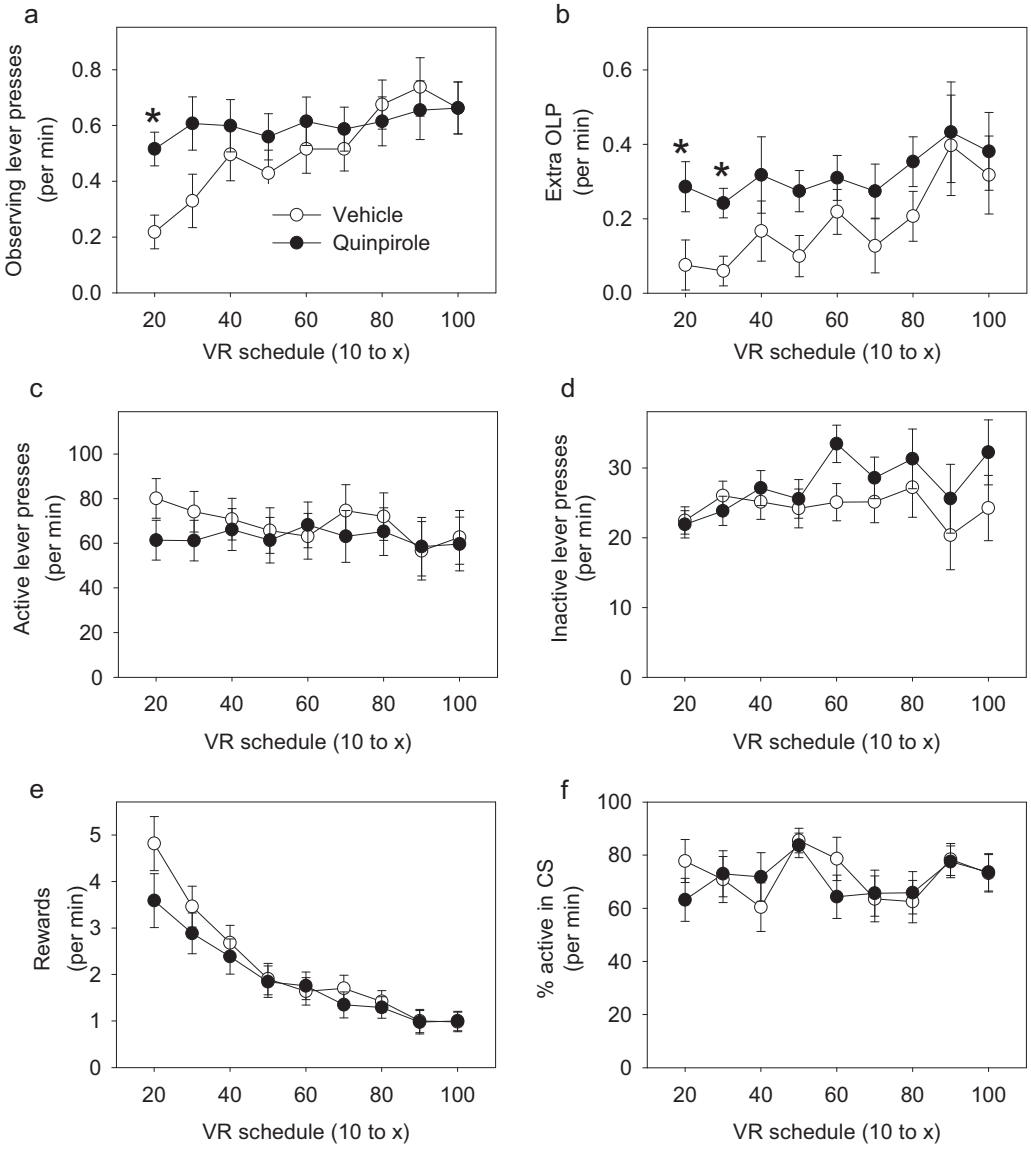

Fig. 6. Effects of increasing response requirement/uncertainty from VR10–20 to VR10–100. (a) OLPs, (b) EOLPs, (c) active lever presses, (d) inactive lever presses, (e) rewards, (f) %ActiveCS. Error bars represent ±1s.e.m. Asterisks denote differences between vehicle and quinpirole treatment groups $^{*}p < 0.05$.

Changing response requirement/variability had no significant impact on EOLPinCS for either the QNP or VEH groups, although, overall, EOLPinCS tended to increase, rather than decrease, as response requirement increased (Table 4).

Rats earned fewer rewards as VRmax increased (Fig. 6e, Table 4, VR10–20 compared with all other VRs from VR10–30 to VR10–100: all $F(1,11) > 21.30$, all $p < 0.01$). Active lever presses decreased overall as VRmax increased, with no difference for QNP/VEH or high/low-checker comparisons (Fig. 6c, Table 4, VR × HLCheck, $F(6,129) = 1.25$, n.s.). Inactive lever presses were not affected by VRmax, QNP/VEH or high/low-checker comparisons (Fig. 6d, Table 4, VR x HLCheck, $F(7,132) = 1.70$, n.s.).

All rats maintained their ability to distinguish active and inactive levers, under active-lever light illumination, as VRmax increased.

Table 4
Statistical analysis of the effects of increasing response-requirement uncertainty (VR) for quinpirole-treated (QNP) and vehicle-treated (VEH) rats Table shows ANOVA *F* statistic for each comparison (all $p < 0.05$ shown in bold). Degrees of freedom are given alongside each *F* statistic. Asterisks denote $^{*}p \leq 0.05$ and $^{**}p \leq 0.01$. # denotes $0.05 < p < 0.1$. (all $p < 0.05$ shown in bold).

	VR x QV	VR	VR(QNP)	VR(VEH)
OLP	**$F(7,131) = 3.43^{**}$**	**$F(7,131) = 8.41^{**}$**	$F(7,70) = 1.00$	**$F(4,43) = 9.12^{**}$**
EOLP	$F(6,114) = 0.51$	**$F(6,114) = 3.93^{**}$**	$F(5,55) = 0.88$	$F(3,34) = 3.79$
Active	$F(6,129) = 1.71$	**$F(6,129) = 2.28^{*}$**	$F(5,47) = 0.95$	**$F(7,71) = 2.52^{*}$**
Inactive	$F(7,132) = 0.98$	$F(7,132) = 2.04^{\#}$	**$F(6,62) = 2.44^{*}$**	$F(6,63) = 0.73$
Rewards	$F(3,52) = 1.48$	**$F(3,52) = 79.2^{**}$**	**$F(2,19) = 34.92^{**}$**	**$F(3,31) = 45.00^{**}$**
%ActiveCS	$F(8,160) = 0.77$	$F(8,160) = 1.91^{\#}$	$F(4,46) = 1.20$	$F(6,63) = 1.48$
%ActiveCSoff	$F(8,160) = 0.61$	**$F(8,160) = 3.01^{**}$**	$F(8,80) = 1.29$	$F(7,68) = 2.35^{*}$
EOLPinCS	$F(3,54) = 1.15$	$F(3,54) = 2.44^{\#}$	$F(4,44) = 0.59$	$F(2,18) = 2.14$

High-checkers maintained higher response accuracy (%ActiveCS) compared to low-checkers as VRmax increased (Table 4. HLCheck, $F(1,20) = 13.45$, $p < 0.01$; VR × HLCheck $F(8,160) = 1.25$, n.s.), with no differences between QNP/VEH groups (Fig. 6f, Table 4, QV $F(1,20) = 0.04$, n.s.). In contrast, there was a consistent decrease in %ActiveCSoff as VRmax increased, suggesting that rats were less able to discriminate active and inactive levers as VRmax increased, when there was no information available (Table 4). There was no difference between QNP and VEH groups in this respect (Table 4. QV for each VRmax ratio, all $F(1,11) < 0.8$, n.s.). Across all delays, high-checkers were more able to distinguish active and inactive levers when the light was unlit (HLCheck, $F(1,20) = 6.20$, $p < 0.05$).

3.4.3. Experiment 2C: lever switching: uncertain active lever location (Fig. 7)

Rats increased OLPs when the active-lever location was more uncertain, with dependence on QNP/VEH group and high/low-checker status (Phase FT vs. VT, (both VR10–20), $F(1,20) = 9.26$, $p < 0.01$; FT vs. VT × QV × HLCheck, $F(1,20) = 4.16$, $p \leq 0.05$). QNP high-checkers increased OLPs during the VT schedule, whereas VEH high-checkers showed no change (Fig. 7b. FTvsVT, VEH, $F(1,5) = 0.30$, n.s.; QNP, FT vs. VT, $F(1,5) = 8.68$, $p < 0.05$). Neither low-checker group responded differently to increased uncertainty (Fig. 7a. FT vs. VT, VEH, $F(1,5) = 1.65$, n.s.; QNP, $F(1,5) = 1.21$, n.s.).

In contrast to other uncertainty manipulations, there was no effect of lever-switch uncertainty on EOLPs (Fig. 7c,d. FT vs. VT (VR10–20), $F(1,20) = 0.06$, n.s.; FT vs. VT × QV × HLCheck, $F(1,20) = 1.95$, n.s.). Neither QNP/VEH group nor high/low-checker status influenced EOLPs (Fig. 7c,d. FT vs. VT, df(1,5): VEH low-checker, $F = 0.17$; QNP low-checker $F = 1.64$; VEH high-checker $F = 0.01$; QNP high-checker $F = 0.80$; all n.s.). There was no effect on EOLPinCS (FT vs. VT with VR 10–20 for both, $F(1,20) = 0.76$, n.s.; FT vs. VT × QV × HLCheck $F(1,20) = 2.89$, n.s.).

All rats earned consistently fewer rewards when active-lever location was more uncertain (Fig. 7e,f, FT vs. VT(VR10–20), $F(1,20) = 25.58$, $p < 0.001$). This decrease in rewards earned was more pronounced for the high- rather than low-checker rats (FT vs. VT × HLCheck, $F(1,20) = 4.40$, $p < 0.05$), but was unrelated to prior quinpirole or vehicle treatment (FT vs. VT × QV, $F(1,20) = 1.15$, n.s.). There was an overall decrease in active lever presses and an increase in inactive lever presses, suggesting that the rats were less able to differentiate the active from inactive lever (Fig. 7g–j, FT vs. VT, active, $F(1,20) = 19.47$, $p < 0.0001$; Inactive, $F(1,20) = 29.68$, $p < 0.0001$). Under the more variable schedule, all rats were able to maintain active lever pressing while the light above the active lever differentiate between active and inactive levers when the active-lever light was illuminated (%ActiveCS FT vs. VT, $F(1,20) = 0.21$, n.s.) but less so when the active-lever light was unlit (%ActiveCSoff, FT vs. VT, $F(1,20) = 4.31$, $p \leq 0.05$), with the QNP group less sensitive to the change in schedule than the VEH group (%ActiveCSoff, FT vs. VT × QV, $F(1,20) = 12.68$, $p < 0.01$). There was no significant difference between high- and low-checkers in this respect (FT vs. VT, HLCheck, $F(1,20) = 3.88$, $p \leq 0.06$).

3.4.4. Experiment 2D: combined uncertainty of location and response requirement (Fig. 7)

Overall, at higher VRmax, addition of lever-switch uncertainty significantly increased OLPs (Fig. 7a,b, FT vs. VT at VR 10–70, $F(1,20) = 20.39$, $p < 0.001$). This was dependent on QNP/VEH group and high/low-checker status (FT vs. VT × QV × HLCheck ($F(1,20) = 9.93$, $p < 0.01$). QNP high-checker rats significantly increased OLPs with increased lever-switch uncertainty, whereas there was no effect for QNP low-checkers or either VEH group (FT vs. VT, df(1,5): QNP high-checkers $F = 36.61$, $p < 0.01$; QNP low-checkers, $F = 0.22$, n.s; VEH high-checkers, $F = 1.77$, n.s.; VEH low-checkers, $F = 1.48$, n.s.).

EOLPs slightly increased overall (Fig. 7c,d, FT vs. VT with VR 10–70 for both, $F(1,20) = 4.21$, $p \leq 0.054$). Neither QNP/VEH grouping nor high/low-checker status independently affected EOLPs (FT vs. VT, df(1,5): QNP high-checker $F = 3.03$; QNP low-checker $F = 0.87$; VEH high-checker $F = 0.09$; VEH low-checker, $F = 2.61$; all n.s.). No significant changes in EOLPinCS indicated that increased EOLPs were likely a direct result of increased OLPs (all $F < 1.0$, n.s.)

Fewer rewards were obtained when the active-lever location was more uncertain and active lever presses were slightly reduced. However, inactive lever presses were not increased, in contrast to responding with lower VRmax (Fig. 7e–j, FT vs. VT, df(1,20): Rewards, $F = 4.95$, $p < 0.05$; Active, $F = 4.15$, $p \leq 0.055$; Inactive $F = 1.19$, n.s.). Under the more variable schedule, all rats were still able to maintain active lever pressing while the light above the active lever was illuminated (%ActiveCS FT vs. VT, $F(1,20) = 0.86$, n.s.), and the high-checker rats were, again, more accurate than low-checker rats (HLCheck $F(1,20) = 9.12$, $p < 0.01$). At this higher VRmax, there was no significant effect of added uncertainty on the ability to differentiate active and inactive levers when the active-lever light was unlit (%ActiveCSoff, FT vs. VT, $F(1,20) = 2.05$, n.s.; FT vs. VT × QV, $F(1,20) = 0.92$, n.s.).

3.5. Summary of findings for experiment 2

- Each of the basic uncertainty manipulations decreased the ability to discriminate between active and inactive levers when the active-lever light was not illuminated. In contrast the active/inactive lever discrimination was unaffected when the active-lever light was illuminated.
- The effects of quinpirole and vehicle on checking-like behaviour could be dissociated by different manipulations pertaining to task uncertainty.
- Omission of an expected reward increased both functional and non-functional observing responses in all rats, showing that reward omission has the potential to induce high levels of checking behaviour, independent of quinpirole treatment.
- Vehicle-treated rats were sensitive to response requirement uncertainty in the form of increased active-lever VRmax, as predicted by earlier observing response tasks [23] [43]. In contrast, quinpirole-treated rats did not respond as predicted, and showed high, inflexible, levels of observing, despite being responsive to increasing task demands by displaying a decrease in active lever pressing. The selective inflexibility of checking-like responses was not because observing behaviour had already reached ceiling levels.
- In contrast, quinpirole-treated rats were sensitive to increasing uncertainty of active lever position, whereas vehicle-treated rats were less sensitive. Thus, checking induced by different forms of uncertainty may be differentially sensitive to dopamine D2/3 receptor function.

3.6. Experiment 3: elevated plus maze and marble burying: the possible role of anxiety in quinpirole-induced checking

3.6.1. Elevated plus maze (EPM)

There were no differences between QNP/VEH or high/low-checker groups on any measure of performance (time in open arm, closed arm, centre; visits to open arm, closed arm, centre. ANOVA for QV, HChecker QV, LChecker QV, All $F < 1.52$, n.s.). There was no correlation between baseline observing responses and any measure of EPM performance ($n = 24$, all r range between −0.19 and 0.26). It is unlikely that quinpirole induced a higher state of anxiety that could account for increased expression of checking behaviours.

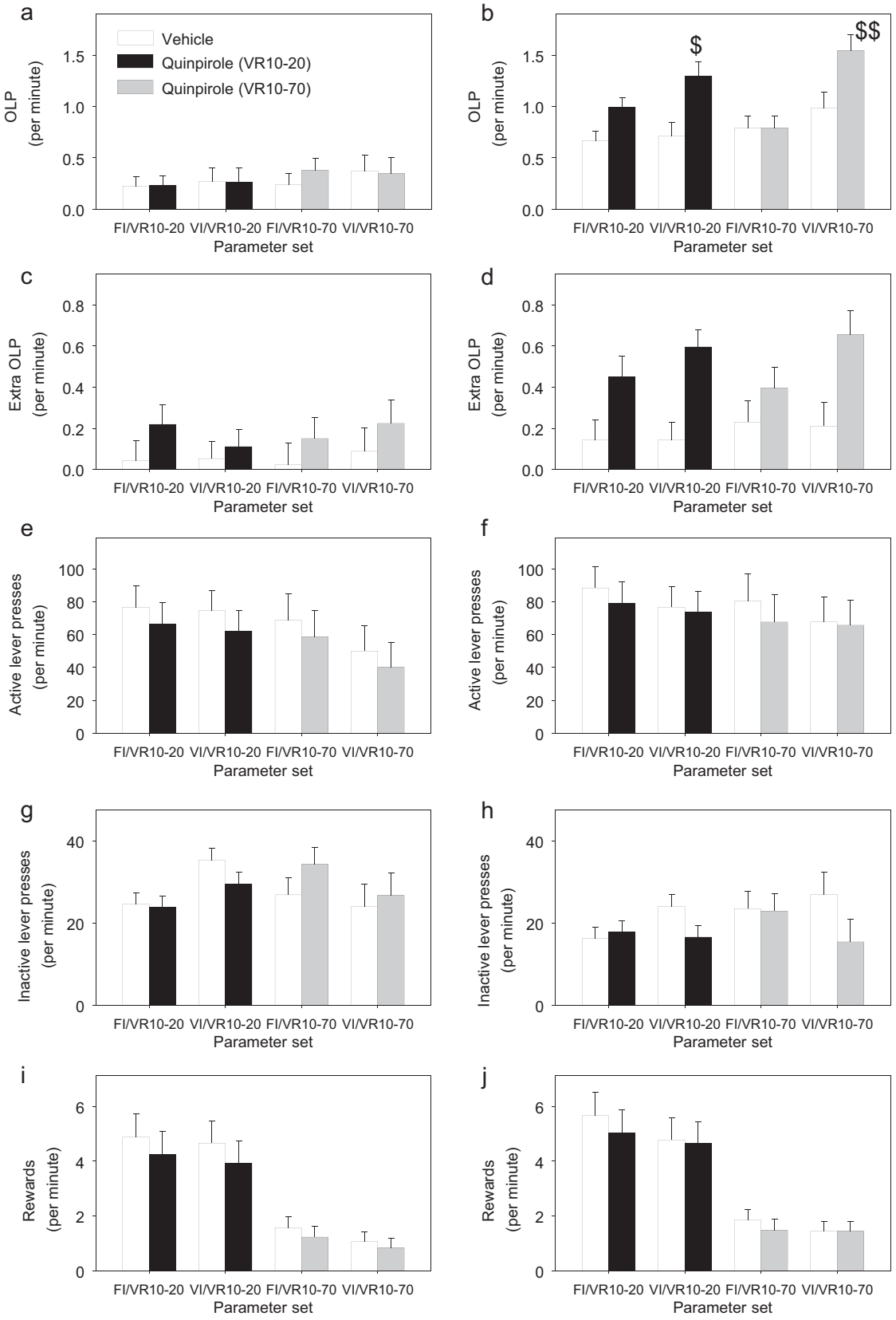

Fig. 7. Increasing uncertainty of active lever location [from FT90s to VT20–120s], for a low response requirement (VR10–20) and a high response requirement (VR10–70). Data are shown for low-checkers (a, c, e, g, i) and high-checkers (b, d, f, h, j). (a,b) OLPs, (c, d) EOLPs, (e, f) active lever presses, (g, h) inactive lever presses, (i, j) rewards, (f) %ActiveCS. Error bars represent + 1 s.e.m. $ denotes difference between FT and VT condition, $$p < 0.05$, $$$p < 0.01$.

3.6.2. Marble burying

There were no differences between QNP/VEH or high/low-checker groups on marble burying (marbles > 2/3 buried: QV ($F(1,22) = 0.13$, n.s.; HLCheck $F(1,22) = 0.03$, n.s). We also assessed marble-burying behaviour within the sulpiride experiment (section 3.8) for 0 and 60 mg/kg sulpiride treatment. There was no effect of acute sulpiride treatment on marble-burying (for 2 presentations of veh/60 mg/kg – Sulpiride vs. Vehicle $F(1,22 = 0.05$, n.s.). These data suggest there is little effect of dopamine D2/3-receptor manipulations on marble burying in rats.

The relationship between original baseline observing responses (S30) and marble burying was assessed for the VEH group. There were no clear correlations between any of the performance measures on the observing response task and marble burying (all $n = 12$, $r < 0.3$, n.s.). There was no correlation between EPM measures and marble burying ($n = 24$, r range $-0.22 < r < 0.20$, all n.s.)

3.7. Summary of findings for experiment 3

- This study found no clear relationship between EPM, marble burying and observing response task measures.
- EPM and marble burying were not affected by past quinpirole treatment, nor were they dependent on high/low-checker status of the rats.

3.8. Experiment 4: effects of sulpiride on checking-like behaviour in post-quinpirole and post-vehicle rats (PQ108–118) (Figs. 8 and 9)

Sulpiride affected neither OLPs nor EOLPs overall (OLPs, Fig. 8a, SulpDose $F(2,40) = 0.62$, n.s.; EOLPs, Fig. 8b, SulpDose $F(2,40) = 0.41$, n.s.). Furthermore, neither OLPs nor EOLPs were affected by prior quinpirole/vehicle treatment history and high/low-checker groupings (OLPs, SulpDose × QV × HLCheck, $F(2,40) = 1.27$, n.s.; SulpDose × QV, $F(2,40) = 0.14$, n.s.; SulpDose × HLCheck, $F(2,40) = 1.59$, n.s. EOLPs, SulpDose × QV × HLCheck, $F(2,40) = 0.68$, n.s.; SulpDose × QV, $F(2,40) = 0.92$, n.s.; SulpDose × HLCheck, $F(2,40) = 0.32$, n.s.). No paired-dose comparisons between 0 mg/kg and either 20 or 60 mg/kg sulpiride were significant (all $F(1,10) < 1.00$, n.s.). There was no effect of sulpiride on EOLPinCS (SulpDose $F(2,40) = 2.02$, n.s.).

However, further analysis showed that the baseline level of EOLPs, immediately before sulpiride treatment, influenced the effect of sulpiride on EOLPs. Across the whole group, there was a correlation between the effect of sulpiride on EOLPs (compared with vehicle) and baseline EOLPs, a relationship that was independently borne out for quinpirole-treated rats but not vehicle-treated rats at both doses (Fig. 9, correlation between baseline EOLPs and change in EOLPs between vehicle and sulpiride treatment. 20 mg/kg, all rats, $n = 24$, $r = -0.76$, $p < 0.001$; QNP $n = 12$, $r = -0.91$, $p < 0.001$; VEH $n = 12$, $r = -0.32$, n.s.; 60 mg/kg, all rats $n = 24$, $r = -0.81$, $p < 0.001$; QNP $n = 12$, $r = -0.90$, $p < 0.001$; VEH $n = 12$, $r = -0.27$, n.s.). There was no effect of sulpiride on functional OLPs (all $-0.21 < r < 0.20$, n.s).

Sulpiride significantly affected other performance measures in a straightforward, dose-dependent manner, but differed for VEH and QNP groups. Rewards and active lever presses were reduced in VEH rats by 60 mg/kg sulpiride with no effect of 20 mg/kg sulpiride. Conversely, rewards and active lever presses were increased in the QNP group by 20 mg/kg sulpiride but there was no effect of 60 mg/kg sulpiride (Fig. 8e, Rewards: SulpDose $F(2,40) = 16.70$, $p < 0.001$; VEH 0 vs. 20 mg/kg $F(1,10) = 0.96$, n.s., 0 vs. 60 mg/kg $F(1,10) = 12.91$, $p < 0.01$; for QNP 0 vs. 20 mg/kg $F(1,10) = 13.87$, $p < 0.01$, 0 vs. 60 mg/kg $F(1,10) = 0.04$, n.s. Fig. 8c, Active lever presses: SulpDose $F(2,40) = 18.68$, $p < 0.001$; for VEH 0 vs. 20 mg/kg $F(1,10) = 0.39$, n.s., 0 vs. 60 mg/kg $F(1,10) = 13.55$, $p < 0.01$; for QNP 0 vs. 20 mg/kg $F(1,10) = 18.63$, $p < 0.01$, 0 vs. 60 mg/kg $F(1,10) = 0.07$, n.s.). For each sulpiride dose (plus vehicle) there was a significant QV x SulpDose interaction for active lever presses (QV × SulpDose 0 vs. 20 mg/kg $F(1,23) = 6.90$, $p \leq 0.015$; 0 vs. 60 mg/kg $F(1,23) = 6.98$, $p \leq 0.015$) and rewards (QV × SulpDose 0 vs. 20 mg/kg $F(1,23) = 7.60$, $p \leq 0.011$; 0 vs. 60 mg/kg $F(1,23) = 7.02$, $p \leq 0.011$). It is possible that the different effects of sulpiride in QNP and VEH groups arose because of baseline differences between the groups. However, this could not be further verified because there was no independent significant difference between the QNP and VEH groups for 0 mg/kg sulpiride (vehicle) for either measure (active lever presses $F(1,22) = 1.41$, n.s.; rewards $F(1,22) = 1.28$, n.s.).

Sulpiride had no significant effect on inactive lever presses (Fig. 8d, SulpDose $F(2,40) = 2.79$, $p \leq 0.073$; post-hoc comparisons, 0 vs. 20 and 0 vs. 60 mg/kg for VEH and QNP groups, all $F(1,10) < 1.46$, n.s.). Sulpiride did not affect %ActiveCS (Fig. 9f, SulpDose $F(2,40) = 1.24$, n.s; 0 vs. 20 and 0 vs. 60 mg/kg for both QNP and VEH groups, all $F(1,10) < 0.59$, n.s.), nor did it affect %ActiveCSoff (SulpDose $F(2,40) = 1.00$, n.s.).

3.9. Summary of findings for experiment 4

- Sulpiride reduced EOLPs if they were previously high and increased EOLPs if they were previously low, suggesting that non-functional observing responses are directly influenced by dopamine D2/3 receptor blockade, albeit in a baseline-dependent manner.
- In contrast, sulpiride had no effect on functional OLPs, suggesting that functional observing is less influenced by dopamine D2/3 receptor blockade.
- Sulpiride affected appetitive instrumental behaviours differently in quinpirole- and vehicle-treated rats, promoting increased active lever pressing in the former, and decreased active lever pressing in the latter. Quinpirole-treated rats were responsive to lower doses of sulpiride. These findings are not inconsistent with a hypothesis that quinpirole-treated rats might have reduced post-synaptic dopamine D2/3 receptor activity.

4. Discussion

This study validated and evaluated the observing response task, a novel, operant test of checking-like behaviour in rats. Checking was described in terms of observing responses, through which rats could gain information about the task. We validated observing responses as being: i) selectively sensitive to repeated quinpirole administration, an established pharmacological model of checking in rodents; ii) selectively responsive to further dopaminergic manipulations when administered sulpiride, a dopamine D2/3 receptor antagonist that has potential relevance as a treatment augmentation for SSRI-refractory OCD and iii) sensitive to uncertainty, a factor that influences human compulsive checking. Each of these validation criteria is pertinent to current and future research with OCD patients. The observing response task has considerable potential for improving our understanding of how and why compulsive checking behaviour develops.

4.1. Validation of observing responses as a model of checking

The chronic-quinpirole model has been well-documented to selectively increase checking in open-field tests [16,17]. In the observing response task, repeated quinpirole administration selectively increased checking behaviour in the form of observing lever responses. Rats 'observed' more after quinpirole treatment compared with vehicle-treated rats. In contrast, lever presses that were more directly associated with goal or reward (the active/inactive levers) were either unaffected or reduced by quinpirole, showing

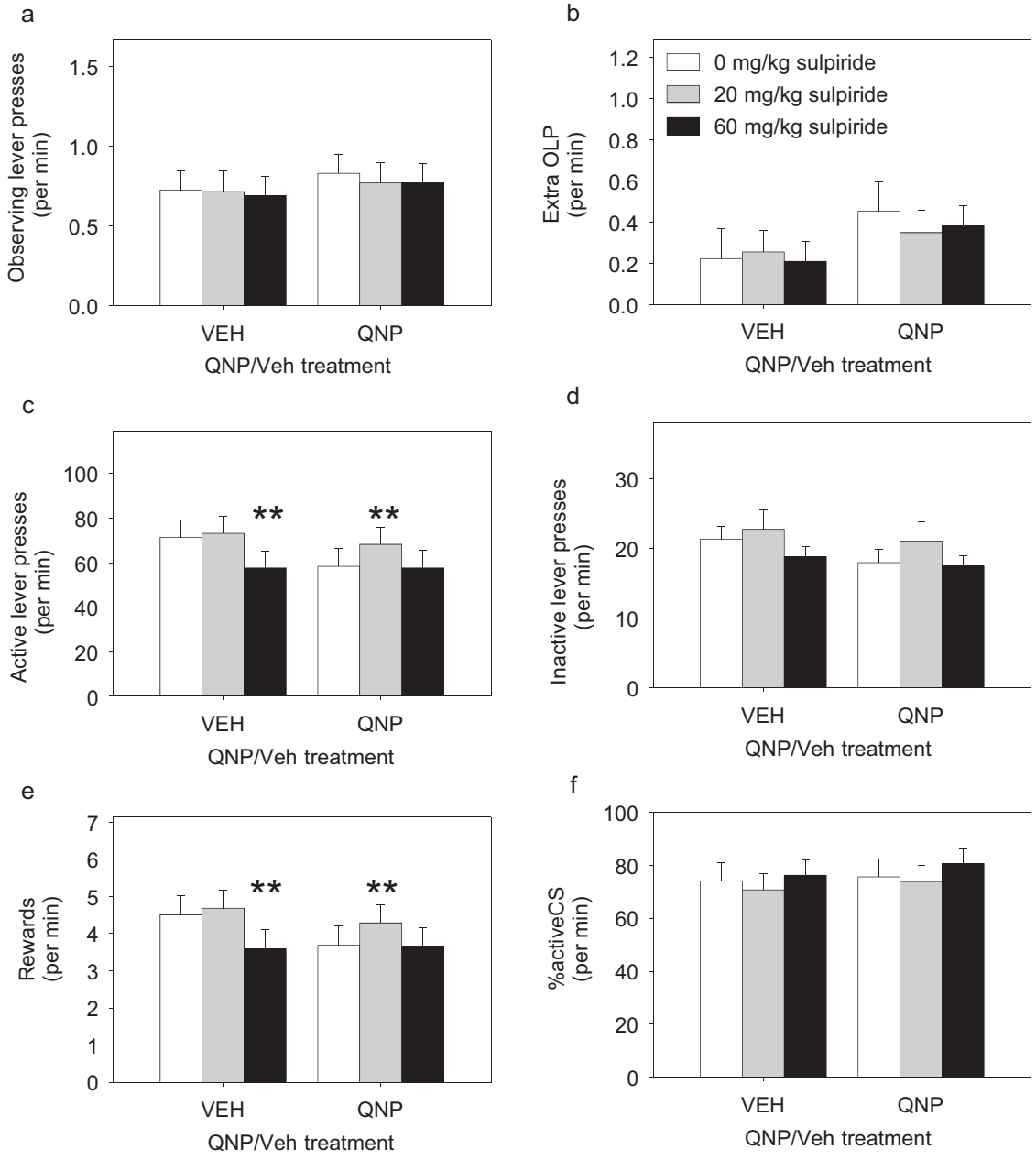

Fig. 8. Effects of sulpiride (vehicle, 20 and 60 mg/kg, i.p) on observing response task measures for the quinpirole-treated (QNP) and vehicle-treated (VEH) rats. (a) OLPs, (b) EOLPs, (c) active lever presses, (d) inactive lever presses, (e) rewards, (f) %ActiveCS. Error bars represent + 1 s.e.m. Asterisks denote difference between sulpiride dose and vehicle $^{**}p < 0.01$.

the selectivity of quinpirole to the checking lever responses. Thus, the observing response task immediately fulfils two of the criteria proposed by Szechtman and colleagues [16,44] to validate animal models of compulsive checking; firstly, there must be a selective increase in checking-like behaviour and, secondly, return time to the site of checking must be reduced. This confirms the observing response task as a potential model of checking that has relevance to OCD studies.

Furthermore, quinpirole-induced checking in the observing response task was long-lasting, in the absence of further quinpirole treatment. This study is the first to show such long-lasting effects of quinpirole on checking behaviour. The quinpirole-induced increase in observing responses persisted for many weeks, in particular for non-functional, extra observing responses. Test-retest reliability was confirmed and, if quinpirole induced high levels of checking, these rats remained high-checkers several weeks later. Einat and Szechtman previously showed long-lasting effects of quinpirole on some aspects of behaviour, including perseveration during extinction in Morris water maze, but found no long term retention of the behavioural rigidity and route stereotypy characteristic of open-field checking, once quinpirole treatment had ceased [45]. Therefore, the observing response task may provide important longer-term information about the development of checking that is not available from other OCD checking models.

In addition to checking behaviour, the observing response task measured how rats used the information available from checking. Rats employed different strategies of observing behaviour, used information gained from checking to different extents and checking behaviour did not simply correlate with improved task success. Thus, the high-checking rats showed a graded success on task,

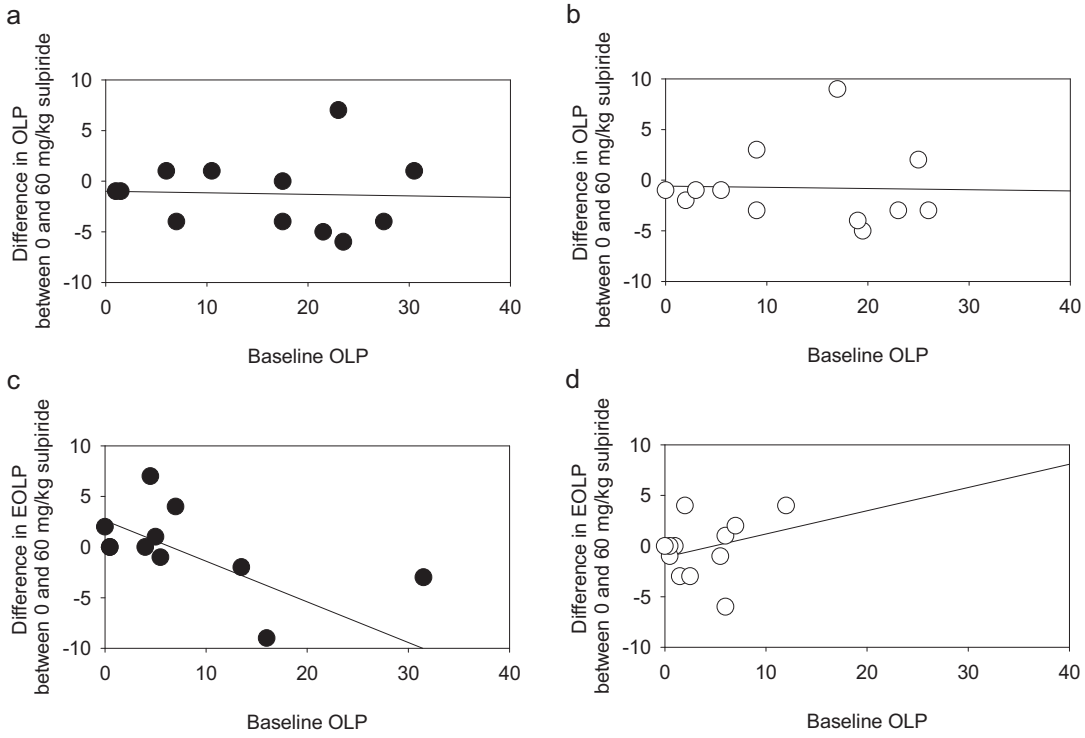

Fig. 9. Baseline-dependent effects of sulpiride on observing and extra observing lever presses. (a,b) Difference in observing lever responses during sulpiride (60 mg/kg) treatment compared with vehicle, plotted against baseline OLPs (during the no-drug baseline sessions in the sulpiride test phase of the experiment) for (a) quinpirole and (b) vehicle treated rats. (c,d) Difference in extra observing lever responses during sulpiride (60 mg/kg) treatment compared with vehicle, plotted against baseline EOLPs for (c) quinpirole and (d) vehicle treated rats. There was a significant negative correlation between EOLPs response to sulpiride and baseline EOLPs for the quinpirole-treated rats.

in terms of pellets earned, that was dependent on the extent of checking. For low-checking rats, task success was independent of checking and may have relied on other strategies. In general, higher checking was found during phases of the task where, when no information was present, the ability to discriminate between active and inactive levers were low. Higher checking by the quinpirole-treated rats was not linked with better task performance than vehicle-treated rats. However, it is plausible that more frequent checking by quinpirole-treated rats was necessary to maintain task success at a level that vehicle-treated rats were able to attain without checking. This may have resulted from a quinpirole-induced shift towards dependence on external stimuli and away from reliance on internal cues to signal that lever-response requirement had been completed. This is consistent with studies of OCD patients who may suffer from impaired sensitivity to internal negative feedback from a security motivation system that diminishes their ability to terminate behavioural sequences appropriately [5]. We predict that quinpirole-treated rats would, therefore, exhibit lower task success if they were tested under conditions that prevented normal checking behaviour.

4.2. Differentiation of functional and non-functional checking

In normal human behaviour, checking is functional, for example, to gain information about whether or not a door is closed properly, but the same actions may become excessive and dysfunctional in OCD patients. In this context, the observing response task differentiates between functional observing responses (OLPs that provide information) and non-functional, perseverative responses (EOLPs with no consequence). Quinpirole increased both forms of observing response, implicating dopamine D2/3 receptor function in the control of both functional and superfluous checking.

Rats learned to use OLPs for information about active-lever location, and to subsequently reduce pressing on the inactive lever. Many aspects of our study suggest that the functional, information-giving, OLPs have a different neural basis from non-functional EOLP responses on the same lever. There was no clear relationship between baseline levels of functional and non-functional observing, suggesting that EOLPs were not a straightforward failure to terminate the lever-press response following a single, functional OLP (we might predict that a simple stereotypical continuation of observing lever pressing of one or two EOLPs each time would scale with OLPs; this was not the case). Additionally, although quinpirole administration initially increased both OLPs and EOLPs to a similar extent, in the longer term, quinpirole-related effects were biased towards the superfluous, non-functional EOLPs. Furthermore, OLPs and EOLPs were differentially sensitive to uncertainty challenges, with, for example, functional OLPs being selectively sensitive to uncertainty about the location of the active lever. Finally, observing responses could be further dissociated by administration of a dopamine D2/3-receptor antagonist sulpiride. OLPs were unresponsive to sulpiride. In contrast, sulpiride changed EOLPs in a baseline-dependent fashion, decreasing EOLPs for rats with high baseline EOLP responding but increased EOLPs in rats with low baseline EOLP responding, particularly in rats that had previously received quinpirole.

It is not yet fully clear if rats were able to distinguish functional from non-functional responses. This problem is common to all rodent tasks in which superfluous responses are unpunished and it is possible that EOLPs resulted from rats' failure to learn the observing lever schedule of FR1. Nevertheless, this study has shown the first evidence that functional and non-functional checking behaviour may be differentially modulated by dopamine D2/3 receptors. Given the potential resemblance of EOLPs to the superfluous, non-functional checking behaviour in OCD, one

could tentatively suggest that SRI-treatment augmentation with dopamine D2/3 receptor antagonists might be most beneficial for patients with excessive checking, but may promote checking behaviour in patients with previously low checking symptoms.

4.3. The observing response task and uncertainty

Patients with OCD checking symptoms are often highly intolerant of uncertainty. Although intolerance of uncertainty is not a phenotype unique to OCD, being also shown strongly by patients with generalised anxiety disorder, the concept of uncertainty, as defined by 'intolerance of uncertainty' questionnaires, encapsulates many important cognitive phenomena commonly expressed by OCD checkers [46]. Dopamine function is strongly implicated in responding to uncertainty [30–32]. These cognitive components of uncertainty may impact differently on the development of compulsive checking behaviour and influence how dopaminergic dysfunction contributes to symptom development.

We predicted that under conditions of uncertainty, quinpirole-treated rats would increase observing responses to a greater extent than vehicle-treated rats. Three different manipulations of task uncertainty – reward omission, response-requirement uncertainty and active-lever location uncertainty – each resulted in increased observing behaviour, possibly as a result of reduced ability to discriminate active from inactive levers, when no external signals guided lever choice, and under conditions of increased uncertainty. However, quinpirole-treated rats were not uniformly more sensitive to these manipulations than vehicle-treated rats. Therefore, the simple relationship between uncertainty-induced task difficulty and checking cannot explain the main effects of quinpirole on checking behaviour.

Quinpirole increased OLPs (but not EOLPs) when the active-lever location was made more uncertain; high-checking quinpirole-treated rats were selectively more sensitive to this increased uncertainty. This finding is consistent with OCD-checkers' self-report of increased sensitivity to uncertainty, suggesting that high-checking quinpirole-treated rats might be the best group to model OCD-like behaviour under uncertain task conditions. Only functional OLPs were sensitive to uncertain active lever switching, so uncertainty in this form may serve only to reset functional checking to a higher level, rather than promote non-functional checking. However, this high level of functional checking may be sufficient to render an individual more susceptible to additional factors that might increase non-functional checking to levels comparable to those of OCD patients.

In contrast, quinpirole-treated rats were unresponsive to increased variability of response requirement on the active lever, both in terms of functional OLPs and non-functional EOLPs. Instead, vehicle-treated rats responded as we had predicted, checking more as the response requirement for each reward pellet (VRmax) increased. Quinpirole-treated rats had not reached ceiling levels of observing responses because both OLPs and EOLPs increased further during extinction. It is possible that quinpirole-treated rats exhibited behavioural inflexibility, which is often associated with disruption of frontostriatal dopamine (reviewed in [47]), although the flexibility of observing response output in other phases of the study suggests that such a simple account of response-insensitivity is inadequate. Certainly, there was no evidence that quinpirole-treatment rendered rats either universally hyper- or hypo-flexible to the other changes in task demands. Nevertheless, it is possible that quinpirole-treated rats were particularly inflexible and unresponsive to the specific contingency changes expressed during this single phase of the task. Chronic administration of quinpirole to rats during adolescence significantly impairs responsivity to contingency degradation [48], and acute quinpirole can change responsiveness to variability of response requirement [49], both of which might be relevant to this phase of the observing response task, where both the contingency and variability of response requirement was changed. A third possibility is that the already-high level of observing by quinpirole-treated rats provided the rats with sufficient observing opportunity to meet the increasing demands of the higher VRmax phases of the task. These latter hypotheses could be examined by increasing VRmax further, to test if quinpirole-treated rats remained inflexible at demands that increased vehicle-treated observing above the existing quinpirole-treated levels. However, when the schedule became suitably demanding, the rats stopped responding. Nevertheless, an interesting concept for further study is that excessive checking may be triggered from a once-appropriate high level of checking behaviour that does not subside once the need for high-level checking diminishes.

4.4. Evidence for alternative interpretations of checking and experimental limitations

It is important to consider that quinpirole might increase checking-like behaviour through processes unrelated to information gathering. For example, dopamine D2/3 receptor agonists are commonly found to induce superfluous, or perseverative responding in a number of rodent models of compulsive response control. Quinpirole increased lever pressing for water, both on an FR3 schedule and during contra-freeloading, when rats were given a choice between operant or free access to water [50]. Quinpirole also impaired reversal learning, increasing perseverative responses on the previously-rewarded response [51]. Joel et al. [52] showed that quinpirole treatment increased compulsive lever-press responding in an OCD-relevant signal-attenuation task. Within the observing response task, quinpirole did not produce indiscriminate perseveration on all levers; increased responding was selective to the observing lever, implying that quinpirole had not induce a simple response perseveration on all levers. Quinpirole treatment did induce a change in balance of active/inactive responses when there was no light information present, consistent with increased perseveration on a previously-rewarded, goal-directed response (i.e., perseveration on the previously-active lever after it had switched position). However, this behaviour was absent when active-lever light information was available. A more plausible explanation for the quinpirole-induced change in active/inactive selection is that rats were less able to retain information from the active-lever light during periods when no information was present. In support of this hypothesis, there is limited evidence from previous studies that quinpirole disrupts spatial working memory [53]. It is possible that quinpirole reduces memory of, or memory confidence for, the spatial location of the active lever, once the observing light has extinguished and this might be one of the mechanisms driving increased observing in quinpirole-treated rats. Through this mechanism, increased spatial uncertainty (but not response-requirement variability on a spatially more predictable lever), combined with reduced internal feedback signals from incorrect responding and reduced memory for active lever location could explain the heightened observing in quinpirole-treated rats.

Quinpirole-induced selectivity to increase observing responses may have been artefacts of a number of other behavioural effects of quinpirole. For example, quinpirole may have made the rats hyperactive (thus increasing the encounter rate with the observing lever), may have induced baseline-dependent changes in lever-press rates (dopaminergic manipulations increasing low rates of responding and decreasing high rates of responding [54,55]), or may have induced an anxiogenic state in the rats. Chronic quinpirole induces hyperactivity that is strongly dependent on the environment or context of administration [37,45]. However, there is no evidence that such locomotor hyperactivity is maintained in the longer

term, in the absence of quinpirole administration [45,48]. Thus, quinpirole-induced hyperactivity could explain increased observing during drug administration, but could not account for the high observing later in the study, nor could it easily account for the pattern of observing during the uncertainty manipulations. It is also possible that quinpirole-induced observing was an artefact of dopaminergic elevation of low-rate and suppression of high-rate responding across the task manipulations, but the lack of consistent inverse relationship between observing and active lever pressing makes this account unlikely. Finally, neither elevated plus maze behaviour nor marble burying behaviour indicated quinpirole treated rats to be more prone to exhibit anxiety-dependent responses, in agreement with an earlier study that found no effect of quinpirole on marble burying [56]. It is unlikely that behavioural changes in the observing response task were the result of an altered anxiety state.

Thus, the clear validity and scientific potential of the observing response task to measure OCD-relevant checking behaviour is established. However, despite evidence of strong, long-term effects on checking behaviour, the current study did not produce truly compulsive behaviour, i.e., behaviour that is maintained despite adverse consequences. Although quinpirole-induced behavioural changes reduced reward acquisition to a limited extent, future studies should show the potential of the observing response task to generate compulsive and debilitating checking behaviour. Nevertheless, the current study has established the observing response task as a strong base to investigate how increased checking behaviour might eventually transition from elevated, non-functional behaviour to the debilitating and compulsive levels characteristic of OCD.

4.5. Dopamine function and checking

We have made progress towards understanding the neurobehavioural processes underlying the development of compulsive checking and that these are, in part, influenced by quinpirole and dopamine function. However, it is not clear how dopamine function is affected by prolonged quinpirole treatment, especially in the long term. Sullivan et al. studied dopamine (DA) and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC), and found decreased dopamine (DA) and increased DOPAC/DA (a marker of dopamine turnover) in the dorsal striatum, but not nucleus accumbens, prefrontal cortex or amygdala, of adult rats, following a 10 day quinpirole administration that was directly comparable to the current study [57]. In contrast, acute quinpirole increased DA in nucleus accumbens and prefrontal cortex, and decreased DOPAC/DA in nucleus accumbens and amygdala, but had no effects in the dorsal striatum [57]. Adolescent rats with extended quinpirole (25 treatments between days 21 and 70) showed decreased dopamine in prefrontal cortical regions and nucleus accumbens, as well as dorsal striatum, with no effect on DOPAC/DA, implying reduced DA synthesis but not metabolism in these rats [48]. In mice, six days of continuous quinpirole infusion down-regulated striatal D2 receptors and increased mu-opioid, but not delta-opioid, receptors [58]. Furthermore, in each of these studies, brain tissue was analysed immediately, or within a day of the final quinpirole dose, so longer-term changes in dopamine function are not known. Despite the lack of longer-term data, decreased dopamine and increase dopamine turnover, coupled with down-regulation of dopamine D2 receptors, in the dorsal striatum, may underlie at least some of the changes in checking behaviour on the observing response task.

Functional and non-functional observing responses were differentially sensitive to dopamine D2/3 receptor manipulations. Whereas quinpirole increased both OLPs and EOLPs, only EOLPs were responsive to subsequent D2/3 receptor antagonism with sulpiride. This supports a hypothesis that modulation of EOLPs is directly, albeit baseline-dependently, via action of dopamine at D2/3 receptors. Quinpirole-treated rats showed the greatest reduction in EOLPs following sulpiride treatment. However, vehicle-treated rats did not show high natural levels of EOLPs, so it is possible that the effect of sulpiride on quinpirole-treated rats was not a unique property of an altered neuropharmacological state induced by quinpirole *per se*, but rather that quinpirole treatment shifted these rats to the high end of normal variation in D2/3-dependent checking behaviour. Nevertheless, such a dependence of perseverative, non-functional checking on dopamine D2/3-receptor mechanisms is potentially important for OCD patients who are SRI-refractory, for whom treatment augmented with dopamine D2-receptor blocking compounds is common. The lack of sensitivity of OLPs to direct D2/3 antagonism suggests that quinpirole-dependent functional observing response increases may result from more complex mechanisms that require further study.

5. Conclusions

The cognitive basis of components of checking behaviour can be addressed in rats using the observing response task. This task has the future potential for direct translation to human study, thus enabling cognitive features of checking behaviour to be studied in both patient and rodent populations. We have examined some critical features of checking-like behaviour, and validated its relevance to OCD research. We have also confirmed the potential of the observing response task task for studying both normal checking behaviour and vulnerability factors that could escalate checking from functional to non-functional and eventually to compulsive levels, relating this to dopaminergic function. We showed that a dopamine D2/3 receptor agonist can produce long-term changes in checking behaviour and suggested that this may have resulted from an altered internal feedback mechanisms and a consequent reliance on external feedback signals. Based on the groundwork of the current study, future work will expand on our current findings, for example, to examine development of highly-debilitating levels of checking that is more compulsive in nature. This will enable us to probe further into the neural circuitry and neurochemical changes underpinning compulsive checking behaviour.

Author contributions

The observing response task concept was developed by D.M.E., A.C.M, G.P.U., S.M-Z. and T.W.R. with equal contribution. The task was programmed by A.C.M.

For this study

D.M.E. designed research; D.M.E., C.d'A., C.Noschang., C.Noble., J.O.D., M-L.D. conducted experiments; D.M.E. and C. Noschang analysed and interpreted data; D.M.E. and T.W.R. wrote the paper. D.E.T. assisted with pharmacology.

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