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Stereotypic mice are aggressed by their cage-mates, and tend to be poor demonstrators in social learning tasks

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1 **Stereotypic mice are aggressed by their cagemates, and tend to be poor**

2 **demonstrators in social learning tasks**

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25   **Abstract**
26   Stereotypic behaviours (SBs) are linked with behavioural inflexibility and resemble

27   symptoms of autism, suggesting that stereotypic animals could have autistic-like social

28   impairments. SBs are also common in caged mice. We therefore hypothesised

29   relationships between stereotypic and social behaviours, predicting that highly stereotypic

30   mice would give/receive more agonism and be less effective in social learning tasks.

31   Experiment One used C57BL/6 and DBA/2 mice in non-enriched or enriched housing (15

32   cages each); Experiment Two, more cages (6 non-enriched, 44 enriched) plus a third

33   strain (BALB/c). Across both experiments, enrichment reduced SB and agonism

34   (aggression, plus 'displacements' where one mouse supplants another at a resource).

35   These effects appeared related: housing effects on agonism became negligible when SB

36   was statistically controlled for; and, at least in enriched cages, SB covaried with receiving

37   aggression. In Experiment Three, 20 DBAs varying in SB from Experiment Two acted as

38   demonstrators in a 'social transmission of food preferences' task. They were fed a novel

39   flavour (shatavari powder), then each mingled with a familiar but flavour-naïve C57

40   observer. Observers were subsequently offered two novel flavours: shatavari or marjoram.

41   Those spontaneously choosing more shatavari (n = 10) tended to have had less

42   stereotypic demonstrators than the other 10 observer mice. Overall, highly stereotypic

43   mice thus received more agonism -- an effect with obvious welfare implications that can

44   be reduced with enrichment -- and seemed potentially less effective at inducing flavour

45   preferences in conspecifics. Such effects are consistent with social impairment,

46   suggesting that reducing SB may perhaps enhance interactions between conspecifics.

47   **Keywords:** *aggression, animal welfare, environmental enrichment, mice, stereotypic*
48   *behaviour, social learning*
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Introduction

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51

52 Most laboratory rodents are housed in small, barren environments. These conditions are

53 associated with poor welfare, as well as impaired learning and memory (Mason &

54 Latham 2004; Simpson & Kelly 2011), and reduced levels of normal activity that are

55 replaced by spending more time standing still, doing nothing (Tilly *et al* 2010) and/or

56 performing stereotypic behaviours, SBs (*eg* Sørensen 1987, Mason & Latham, 2004,

57 Tilly *et al* 2010): repetitive activities arising from frustration, needs to cope, or central

58 nervous system dysfunction (*eg* Campbell *et al* 2013). Whether environments are

59 enriched or impoverished can also affect laboratory rodents' interactions with cagemates.

60 For example, male bank voles *(Clethrionomys glareolus)* housed in enriched rather than

61 barren environments were less aggressive (Sørensen 1987); social play that had been

62 reduced by prenatal stress in rats *(Rattus norvegicus),* was restored to normal levels

63 through enrichment (Morley-Fletcher *et al* 2003); and in laboratory mice *(Mus musculus)*,

64 enriched caging can also improve maternal care (Whitaker *et al* 2009). Enrichment can

65 also affect aggression: in male mice, adding a shelter increased levels of aggression (Van

66 Loo *et al* 2002), perhaps by increasing resource competition, but providing nesting

67 material significantly decreased it, and more recently, providing diverse enrichments has

68 been shown to reduce aggression in mice of both sexes (Turner *et al* under revision).

69

70 Our aims were therefore to study how providing enrichments affects interactions between

71 mice within their cages, and to investigate how any changes relate to alterations in SB.

72 This is because SBs in captive animals share similarities with the repetitive behaviours

73 seen in certain socially disabling human disorders, especially schizophrenia and autism

2

74 (Lam *et al* 2008; Dallaire *et al* 2011): both have been linked to changes in basal ganglia

75 function (*eg* Tanimura *et al* 2008; 2011) that help cause behavioural inflexibility and

76 perseveration (the repetition of responses when no longer appropriate) (*eg* Lopez *et al*

77 2005; Campbell *et al* 2013). Thus to give examples from caged rodents, high levels of SB

78 correlate with elevated perseveration in extinction learning, reversal learning and other

79 tasks in bank voles, African striped mice *(Rhabdomys),* deer mice *(Peromyscus*

80 *maniculatus)* and one strain of laboratory mouse, C57BL/6 (Garner and Mason, 2002;

81 Tanimura *et al* 2008; Jones *et al* 2011, Garner *et al* 2011), and rearing conditions that

82 reduce SB may also reduce perseveration (Tanimura *et al* 2008 and Jones *et al* 2011).

83 Such effects seem likely to be relevant for social interactions, since in autistic humans,

84 perseveration and SB predict reduced social competence. Autistic humans thus often

85 show social impairments if prone to repetitive behaviour (McEvoy *et al* 1993; Lam *et al*

86 2008); furthermore, in autistic children, tendencies to perseverate correlate with poorer

87 social skills (McEvoy *et al* 1993) and reduced social interaction (Memari *et al* 2013). Do

88 barren-housed stereotypic animals also show such social impairments? This topic has

89 been little studied. However, consistent with this hypothesis, 'knockout' mice lacking a

90 dopamine transporter gene show both elevated SB and aggressive, unstable social

91 hierarchies (Rodriguiz *et al* 2005); while in mate choice tasks, non-stereotypic enriched

92 male mink gained more copulations with females than stereotypic non-enriched males

93 (Diez-Leon *et al* 2013).

94

95 The first part of this study used behavioural observations to assess in-cage social and

96 stereotypic behaviour in female C57BL/6 (C57), DBA/2 (DBA) and BALB/c mice,

97 housed in both enriched (EE) and non-enriched (NE) cages. All animals were housed in

98   mixed strain trios, enabling the generality of effects across these widely-used strains to be

99   easily assessed, without increasing the animal numbers used (Walker *et al* 2013). We

100   predicted that mice in non-enriched housing would display more SB and more aggression.

101   We also hypothesized that if SB reflects autistic-like impairments that compromise social

102   functioning, then stereotypic individuals should receive more aggression, and fewer

103   affiliative behaviours such as resting/sleeping together.

104

105   The second part of this study aimed to further assess the social normality of stereotypic

106   mice by investigating the effect that SB has on social learning. Social learning involves

107   the transfer of information between individuals (*eg* Kavaliers *et al* 2001), and has been

108   demonstrated in many rodent species, including rats, mice and gerbils (Galef & Wigmore

109   1983; Valsecchi *et al* 1996; Kavaliers *et al* 2001). One commonly used paradigm to

110   assess social learning is the transmission of food preferences, which involves a

111   demonstrator transmitting information about a novel flavour to an observer, as revealed

112   by that observer then preferring diets with this flavour even though they are novel. There

113   has been no research into the role of SB on social learning, despite evidence that the

114   perceived quality of the demonstrator in such tests can be influential. Thus in mice, pups

115   are less effective demonstrators than adults (Choleris *et al* 1997); in gerbils, unfamiliar,

116   unrelated demonstrators are not effective at conferring flavour preferences, while familiar,

117   related demonstrators are (Valsecchi *et al* 1996); in African striped mice, fathers are less

118   effective than mothers at transferring food preferences to their offspring (Rymer *et al*

119   2008); and in deer mice, subordinate demonstrators are less effective than dominant

120   demonstrators (Kavaliers *et al* 2005; see Clipperton *et al* 2008 for potentially similar

121   effects in lab mice). We therefore predicted that if stereotypic animals are perceived as

122 abnormal, subordinate, or receive less attention from observers in the transmission phase

123 of the task, then they would prove poorer demonstrators.

124

125 **Methods**

126 *Ethics Statement*

127 All procedures and husbandry techniques were approved by the University of Guelph

128 Animal Care Committee, and comply with the Canadian Council on Animal Care

129 guidelines (covered by AUPs #1398 and #2430).

130

131 *Experiment One*

132 *Animals and housing*

133 The subjects were part of another ongoing experiment (a long-term study of enrichment

134 effects on activity levels) and were observed opportunistically for this study in order to

135 reduce the total number of animals used (NC3Rs 2015). Animals were adult female

136 C57BL/6 (henceforth 'C57') mice (n = 60) and female DBA/2 ('DBA') mice (n = 30),

137 purchased from Charles River Laboratories (Quebec) at four weeks of age. Mice were

138 housed in groups of three, with two C57 mice and one DBA mouse per cage (see Walker

139 *et al* 2013 for a validation of this mixed strain housing), in either non-enriched 'NE'

140 housing (15 cages) or enriched 'E' housing (15 cages). NE cages were standard

141 laboratory cages, measuring $12H \times 27L \times 16W$ cm. NE mice were provided

142 with Shepherd Enviro-dri$^{©}$ nesting material and a UDEL polysulfone plastic mouse house

143 shelter, but no other enrichments. E cages were each $12H \times 43L \times 21W$ cm in size, and

144 as well as a clear plastic shelter and nesting material (as used in the NE cages), they

145 contained enrichments: a horizontal plastic running wheel, a pinecone, a sock 'hammock'

146   measuring roughly 11 $L \times 7$ W cm, 2 paper cups, a piece of PVC pipe roughly 6.5 L cm,

147   a sponge, and two cotton balls. All mice were maintained at 21°C and were fed a

148   standard laboratory rodent diet (Harlan® Teklad Global Diet [14% protein]). Food and

149   water were given *ad libitum*. Mice were kept on a 12:12 reversed dark/light cycle, with

150   the dark cycle beginning at 1000h.

151

152   *Observations*

153   Mice were six months old at the time of behavioural observations. They were observed *in*

154   *situ* in their home cages, using red room lights and headlamps, via live scan sampling.

155   The positions of E and NE cages were randomised across racks and shelves. To assess

156   home cage activity time budgets, scans were taken every 20 minutes, for a period of four

157   hours per session (based on Walker *et al* 2013, and validated using split-half analyses

158   regressing data from odd and even days [Martin & Bateson 1993]). Sampling periods

159   were from 1130h to 1530h, and from 1730h to 2130h. Eight sampling periods were

160   conducted, for a total of 94 scans (one scanning period only had ten scans). Scans were

161   split between two experimenters (LH and KR), whose inter-observer reliability after

162   training was >95%.

163

164   Social interactions were assessed by LH using a focal sampling regime, since they were

165   relatively rare occurrences. Each cage was observed for five minutes, with all bouts of

166   social behaviour in the cage recorded; the next cage was then moved on to. Observations

167   commenced at 1130h and again at 1700h. This was repeated 10 times per cage (thus 50

168   minutes of focal observation over six days, a regime again confirmed as valid using split-

169   half analyses regressing data from odd and even days). An ethogram of behaviours

170 recorded during scan and focal sampling is provided in Table 1. Note that we chose to

171 assess and analyse being 'still but awake' separately, because being inactive despite being

172 awake seems to increase in mice housed in barren rather than enriched cages (Tilly *et al*

173 2010).

174 <center>*** Table 1 about here ***</center>

175

176 ### *Experiment Two*

177 *Animals and Housing*

178 Again, subjects were part of another on-going experiment (this time a long-term study of

179 enrichment effects on life expectancy), being observed opportunistically for this study to

180 maximise their usefulness and minimize the total number of animals used. This second

181 cohort comprised 150 adult female mice purchased from Charles River Laboratories

182 (Quebec) at 3 to 6 weeks of age, and housed in groups of three in mixed-strain housing.

183 This time three strains were used, BALB/c, DBA/2 and C57BL/6, one mouse of each per

184 cage (thus 50 cages). 50 cages were used as this was the maximum number of cages that

185 could be observed in a single 40-60 minute session of focal observations (one per cage).

186 They were part of an on-going longevity study, and so were housed in one of seven

187 housing types (again randomised across racks and shelves). Six cages were standard

188 laboratory cages, as used in Experiment 1. The other 44 cages were larger, $20H \times 43L$

189 $\times 21W$ cm. Half of these were enriched with 'comforts' (enrichments designed to enhance

190 comfortable rest: a sock 'hammock', Nestlets, a tissue to construct into nests, and a paper

191 cup to shelter in), while half were not. Cross-factored with the presence or absence of

192 comforts, approximately one third of the cages contained a working horizontal plastic

193 running wheel, approximately one third contained a working metal wheel, and

<center>7</center>

194 approximately a third contained a locked wheel (for specifics see Table 2). The same

195 room was used as in Experiment One, mice being provided with the same rodent chow

196 and water *ad libitum*. Mice were kept on a 12:12 reversed dark/light cycle, with the dark

197 cycle beginning at 1000h.

198

199 *** Table 2 about here ***

200

201 *Observations*

202 All observations were conducted in home cages when mice were seven months old.

203 Activity budgets were again generated using scan sampling. Scans were taken every 40

204 minutes to an hour; scanning times were longer in Experiment Two due to the greater

205 number of cages (120 cages were scored for activity levels as part of the larger longevity

206 study). Sessions commenced twice daily, at 1130h and 1700, with three scans being taken

207 per session (a total of 48 scans/cage). Scans were split between the two experimenters of

208 Experiment One, plus a new, experienced experimenter (MW).

209

210 Social data were again collected by LH using focal sampling, from just the 50 cages used

211 in this study. Each was watched for three minutes (reduced from the five minutes in

212 Experiment One for practical reasons, due to the increased number of cages observed) per

213 focal observation, with observations commencing at 1130h and 1700h per day. Data

214 came from a total of 12 focal periods per cage, over 12 days. All bouts of social

215 behaviours that occurred in each observation period were recorded using the ethogram in

216 Table 1; SB was also recorded during these focal observations.

217

218   *Experiment Three: Social transmission of food preferences*

219   *Animals*

220   The subjects used were 20 pairs of females, one DBA and one C57, now aged 8 months,

221   each pair being selected from one of 20 of the enriched cages used in Experiment Two.

222   Twenty pairs were used because this allowed for a range of SB to be investigated, while

223   still remaining a manageable sample size for the researchers.

224

225   Only E mice were used to ensure that all subjects came from similar housing types (only

226   six NE cages were available), and because only in the E conditions did highly

227   stereotypic mice attract elevated agonism from their cagemates (see Results for

228   Experiment Two). The 20 cages used were selected on the basis of their SB (as

229   quantified in focal observations), as follows. Of the three strains, DBA mice had the most

230   variation in SB, and so these mice were chosen to act as demonstrators in the social

231   learning task. Of the 44 E cages screened in Experiment Two, these 20 cages were chosen

232   because their DBAs displayed the greatest variation in their average levels of SB, from

233   0.44 bouts/minute (two mice) to zero bouts/minute (four mice) C57 mice were selected to

234   be used as observers, since a previous experiment using social transmission of food

235   preferences had been conducted with this strain (Ryan *et al* 2008). As well, all the C57

236   observers in the study had very low amounts of stereotypic behaviour (only one observer

237   mouse showed any stereotypic behaviour during focal observations), so selecting these

238   mice reduced behavioural variation across the observers. All mice continued to be housed

239   as they had been for Experiment Two, including in the same trios of individuals (although

240   the BALB/cs played no active role in Experiment Three). During the social transmission

241   test, each demonstrator was paired with a C57 observer from its home cage: thus

242   demonstrators and observers were familiar.

243

244   *Flavour selection for the social learning test*

245   Non-subject mice in pilot trials were tested to determine suitable flavours for the

246   experiment. Two flavours picked were those that no researchers or technicians had

247   consumed in the past six months (shatavari powder and ashwagandha root powder), while

248   two others had been very little ingested (marjoram and anise seed): important because

249   rodents can pick up flavour preferences from humans (Galef 2001). Flavours were ground

250   up (if needed) and mixed into powdered rodent chow at appropriate concentrations. Mice

251   were each given a choice of two flavours in a specialised test apparatus designed for this

252   purpose: a polyethylene cage (37 x 21 x 19 cm) with two food magazines affixed to one

253   side (Tecniplast SPA, Buguggiate, VA, Italy). The food magazines had removable food

254   trays that hold the flavoured foods; the food trays each had an apron to catch spilled food,

255   so allowing for precise measurements of food intake (Valsecchi *et al* 1989). Each flavour

256   was available for consumption in one of the two compartments, counterbalanced, for a

257   period of four hours. Dishes were weighed and the amount of food eaten was measured at

258   one, two and four hours. Both powdered diets were measured at wet weight. We

259   confirmed whether all mice consumed each flavoured food (defined as eating more than

260   0.1g), and calculated the coefficient of variation across individuals to assess how

261   consistent consumption levels were across mice. The two flavours eaten by all mice with

262   the lowest coefficients of variation were chosen for Experiment Three. These were 2%

263   shatavari powder (Rootalive Inc., Canada) and 2% marjoram (McCormick®, Canada). In

264   these pilot tests, mice consumed more marjoram (mean 0.738+/-0.586g) than shatavari

265   (mean 0.589+/-0.508g); however, because these two flavours were never offered in a

266 pairwise combination, their relative appeal to naïve mice was unknown.

267

268 *Social transmission of food preferences*

269 The protocol described here is adapted from Valsecchi & Galef 1989. Ten cages were

270 tested daily (thus 10 DBA demonstrators and 10 C57 observers), for two days. The

271 demonstrators' variation in SB was balanced across days (*ie* mice spanning similar ranges

272 were tested each day), and the tester was blind to these during the test.

273

274 All cages of subjects were food deprived for 16 hours before trials began, largely over the

275 12 hour light phase (when food consumption is very low: *eg* Clipperton *et al* 2008), thus

276 starting at 1730h, in order to ensure food consumption the morning of the following day.

277 At 0930h each demonstrator mouse was moved to a clean empty cage and fed a powdered

278 diet composed of 2% shatavari powder and powdered rodent chow. Food was presented

279 for two hours to these demonstrators, in jars approximately 7 cm in diameter and 5 cm in

280 depth (with a perforated stainless steel disc placed on the top of the food to prevent

281 digging and spillage). Weights of food given were measured before and after

282 consumption, to ensure that each demonstrator consumed the diet (greater than 0.2g).

283

284 Food was removed from the cage, and each demonstrator was then immediately paired

285 with its corresponding observer (the C57 from its homecage) by placing the observer

286 mouse in the test cage with the demonstrator and the two familiar mice were allowed to

287 interact for one hour. Demonstrators were then moved back to their home cages.

288 Observers were instead each moved to a specialised Tecniplast test cage (as used to

289 screen potential flavours at the start of this study), with each flavour of food available for

290 consumption in one of the two compartments, counterbalanced across cages. The

291 observer mice in these test apparatuses were also provided with a small amount of

292 bedding and water *ad libitum.* Weights of each food were taken before testing and after

293 two hours (after which each observer mouse was then returned to its homecage). The

294 amounts of each diet consumed by each observer mouse were then analyzed, to determine

295 effects of demonstrator levels of SB on observer preferences.

296

297 *Statistical analyses*

298 Statistical analysis was performed using JMP® 11 software, and general linear models

299 (GLMs). Appropriate transformations were performed in order to satisfy the assumptions

300 of parametric models as best as possible; in practice this typically meant that

301 homogeneity of variance was achieved but strict normality of residuals was not;

302 realistically, this is of small concern as these tests are robust to deviations from normality

303 (Rasch & Guiard 2004). All results were considered significant at $P = 0.05$ or lower (and

304 presented as trends if between 0.05 and 0.10). Two-tailed tests were used throughout, to

305 be conservative, even though we made directional predictions. Tukey's tests were used to

306 investigate the drivers of any significant interactions between categorical variables.

307

308 In Experiments One and Two, cage was treated as a random effect, and nested within

309 housing type (EE / NE). Strain, housing and their interactions were included as fixed

310 effects in every model. Behavioural variables analysed were stereotypic behaviour, and

311 'still but awake' (both calculated as a percentage of all observations); along with the

312 number of aggressive + displacement acts given or received per minute of observation

313 (pooled under the term 'agonism'), the number of aggressive acts recorded per minute of

314 observation, and time spent nesting together recorded per minute of observation. To

315 avoid problems of non-orthogonality, sequential tests were used when continuous

316 independent variables were included, with the term of interest placed last in the model

317 (Doncaster & Davey 2007, Grafen & Hails 2002).

318

319 Pooled analyses were also conducted, combining data from Experiments One and Two to

320 assess the consistency or otherwise of effects across the two studies, and to run some

321 analyses with greater power. The BALB/c mice were excluded from this pooled dataset,

322 since not present in both studies. In these analyses, cage (again a random effect) was

323 nested within both housing type and experiment, and for categorical variables, all

324 possible two- and three-way interaction terms were included. These analyses aimed to

325 investigate: 1) whether mice in enriched cages were more often out of sight than mice in

326 non-enriched; 2) if so, whether housing type effects on behaviour could still be detected

327 when this problem was controlled for statistically, by incorporating all 'out of sight'

328 observations (active and inactive pooled) into all relevant models; 3) whether housing

329 type effects on aggression or agonism could still be detected if stereotypic behaviour was

330 statistically controlled for, and *vice versa*; and 4) how consistent relationships between

331 SB and social interactions were across the two studies.

332

333 In Experiment Three, the weight of shatavari-flavoured food (the diet eaten by the

334 demonstrators) eaten by each observer was expressed as a proportion of all food eaten,

335 and regressed against how stereotypic each demonstrator was (as a % time budget).

336 Observers were also divided into two groups according to whether the shatavari-

337 flavoured food was qualitatively preferred (*ie* making up more than 50% total weight of

13

338 food consumed) over the control novel food. The stereotypic behaviour levels of the two

339 groups' demonstrators were then compared in a GLM, with 'test day' and its interaction

340 as blocking factors.

341

342 **Results**

343 *Experiment One*

344 Compared to mice in enriched (E) cages, mice in non-enriched (NE) cages performed

345 more SB ($F_{1,34} = 63.35$, $P = <0.0001$), and more 'still but awake' behaviour ($F_{1,32} = 6.85$,

346 $P = 0.01$). They also performed more acts of agonism (aggression + displacement) ($F_{1,32}$

347 $= 5.59$, $P = 0.024$; see Figure 1), although not aggression when considered on its own.

348 For receiving agonism, there was an interaction between strain and housing type

349 (strain*housing: $F_{1,58} = 14.53$, $P = 0.003$), caused by C57 mice in NE cages receiving

350 more aggression than those in enriched cages (Tukey's test, $P = 0.006$). There were no

351 effects on the receipt of aggression when considered on its own. There was no effect of

352 housing type on nesting together.

353

354   *** Figure 1 about here ***

355

356 **Performance of agonistic behaviour in enriched and non-enriched cages.**

357
358 *Experiment Two*

359 No behavioural differences were found between the various large enriched housing types,

360 and so all were pooled as 'enriched cages' for ease of subsequent analysis. Compared to

361 mice in enriched cages, mice in NE cages were more stereotypic ($F_{1,48} = 9.70$, $P =$

14

362 0.0031). For 'still but awake' behaviour there was a strain*housing interaction ($F_{2,96}$ =

363 3.21, P = 0.04), because C57s in NE cages performed more than C57s in enriched cages

364 (Tukey's test, P = 0.010). There were no effects of housing type on all acts of agonism

365 ($F_{1,48}$=1.04, P = 0.31), but for being aggressive, there was a strain*housing interaction

366 ($F_{1,96}$ = 3.30, P = 0.041), because C57 mice in NE cages were more aggressive than those

367 in large enriched cages (Tukey's test, P = 0.035; see Figure 2). There were also no

368 housing type effects on the receipt of agonism ($F_{1,48}$=1.28 $P > 0.10$), but for receiving

369 aggression *per se* there was a trend for NE mice to receive more ($F_{1,48}$=3.21, P = 0.08).

370 Mice in NE cages also spent significantly more time nesting together than those in

371 enriched cages ($F_{1,48}$ = 22.34, $P < 0.0001$).

372   ** *Figure 2 about here* **

373

374 There were no relationships between SB and giving or receiving agonism. However, a

375 significant interaction with strain was found when correlating SB with the performance of

376 aggression (Strain*SB: $F_{1,125.8}$ = 12.16, $P < 0.0001$). Upon further analysis, it was found

377 that C57 mice that performed more aggression had lower levels of SB ($F_{1,46}$ = 16.00, P =

378 0.0002). A significant interaction with housing type was also found regressing SB

379 against receiving aggression (housing*SB: $F_{1,137.8}$ = 17.77, $P < 0.0001$), because in

380 enriched cages, mice that performed more SB also received more aggression ($F_{1,115.3}$ =

381 9.77, P = 0.002). Finally, mice that performed higher levels of SB also spent more time

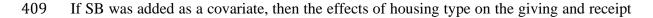382 nesting with cage mates ($F_{1,109}$ = 15.523, $P < 0.0001$).

383

384 **Pooled analyses**

385 Across the two studies pooled, mice in enriched cages were out of sight significantly

386   more often than those in NE cages: ($F_{1,\,74.3} = 24.03$, $P < 0.0001$). All potential housing

387   type effects on behaviour therefore were reinvestigated to check they were not mere side-

388   effects of enriched mice being harder to observe. For SB, there remained a strong overall

389   effect of housing type, enriched mice being less stereotypic ($F_{1,75} = 36.28$, $P < 0.0001$).

390   However, experiment*strain*housing was also significant ($F_{1,\,101} = 6.65$, $P = 0.013$), with

391   Tukey's tests revealing that while enrichment reduced SB in both strains in Experiment

392   One ($P < 0.005$), in Experiment Two it only did so for DBAs ($P < 0.0001$). Being 'still

393   but awake' was consistently reduced by enrichment (with no interactions with strain or

394   experiment) ($F_{1,\,78} = 6.89$, $P = 0.010$).

395

396   Enriched mice also still performed fewer agonistic acts ($F_{1,\,87} = 5.85$, $P = 0.018$), and

397   fewer acts of aggression *per se* ($F_{1,78} = 4.27$, $P = 0.042$): housing effects that did not

398   interact with experiment (or strain), *ie* were consistent across populations. The pooled

399   analyses also revealed a three-way effect of strain*housing*experiment effect on agonism

400   received ($F_{1,112} = 8.83$, $p = 0.004$). A Tukey's test showed that this was driven by

401   enrichment only reducing the receipt of agonism in C57s in Experiment One ($P = 0.006$).

402   When looking at the receipt of aggression only, the pattern was the same as described for

403   agonism (strain*housing: $F_{1,104} = 6.83$, $P = 0.01$). Finally, for nesting together, the tests

404   revealed another three-way effect of strain*housing*experiment ($F_{1,104} = 6.124$, $P = $

405   0.015), driven by both strains in Experiment Two doing more co-nesting in NE housing

406   (for C57s: Tukey's $P < 0.0001$; for DBAs, $P = 0.0005$), but no such effects in

407   Experiment One.

408

409   If SB was added as a covariate, then the effects of housing type on the giving and receipt

16

410 of agonistic behaviour were all reduced or even eliminated. Enrichment effects on the

411 performance of aggression thus became non-significant ($F_{1,85} = 0.42$, $P = 0.51$);

412 enrichment effects on the production of agonistic behaviours were reduced to a trend

413 ($F_{1,96} = 3.54$, $P = 0.06$). This suggests that at least in part, the effects of housing type on

414 agonism reflected its effects on SB. In contrast, if performing aggression or all agonism

415 were added as covariates, or if receiving aggression or all agonism were, the effects of

416 housing type on SB remained very similar: enriched mice remained significantly less

417 stereotypic ($P < 0.0001$ in all models), with experiment*strain*housing also remaining

418 significant ($P < 0.05$ in all models), and Tukey's tests again revealing that while

419 enrichment reduced SB in both strains in Experiment One ($P < 0.01$ in all models), in

420 Experiment Two it only did so for DBAs ($P < 0.01$ in all models). This in turn thus

421 suggests that the effects of housing type on SB were *not* dependent on its effects on

422 agonism.

423

424 The last pooled analyses re-investigated the potential relationships between SB and social

425 interactions. No relationships were found between performing SB and being dominant or

426 aggressive (nor were there any significant interactive effects). For receiving all acts of

427 agonism, however, across both studies together there was a near positive trend with SB

428 ($F_{1,176} = 2.69$, $P = 0.102$). Furthermore, for receiving aggression only, there was a

429 significant interaction with housing type ($F_{1,153.} = 16.57$, $P < 0.0001$). Splitting the data

430 by housing type revealed a strong positive relationship between performing SB and

431 receiving aggression within enriched mice, regardless of strain or experiment ($F_{1,121} =$

432 8.79, $P = 0.004$), but not within NE mice ($F_{1,45} = 0.001$, $P = 0.98$). Finally, for co-

433   nesting, there were no consistent patterns, but instead two significant three-way

434   interactions, and a trend effect for a third (SB*housing*strain: $F_{1,122} = 7.58$, $P = 0.007$;

435   SB*strain*experiment: $F_{1,130} = 4.43$, $P = 0.037$; SB*housing*experiment: $F_{1,155} = 2.72$,

436   $P = 0.101$). Splitting data into subsets to try and investigate why, revealed no significant

437   main effects of SB.

438   *** Table 3 about here ***

439

440   **Experiment Three**

441   A regression revealed no significant linear relationship between how stereotypic the

442   demonstrators were and how much shatavari-flavoured diet was selected by their

443   observers ($F_{1,16} = 0.16$, $P = 0.692$). However, the 20 observers divided into two equal

444   sized groups as to whether or not they chose to eat more shatavari-flavoured diet than

445   marjoram-flavoured control. When these groups' demonstrators were compared, the

446   observers who chose to eat more shatavari tended to have had less stereotypic

447   demonstrators than observers who ingested equal amounts of the two flavours or ate more

448   marjoram ($F_{1,16} = 3.58$, $P = 0.077$; see Figure 3). The amount of aggression received by

449   these demonstrators in their home cages, in contrast, appeared unrelated to whether or not

450   their observers favoured the shatavari-flavoured diet ($F_{1,16} = 0.96$, $P = 0.34$).

451   *** Figure 3 about here ***

452   **Discussion**

453

454   In Experiments One and Two, mice raised and housed in standard non-enriched (NE)

455   cages broadly performed more stereotypic behaviour than those in enriched cages, just as

18

456   expected. NE mice also spent significantly more time being 'still but awake'. Being

457   inactive despite being awake has previously been found to increase in barren enclosures

458   in mice (Tilly *et al* 2010), and similar effects have been seen in other species too

459   (reviewed Meagher & Mason 2012). In mink, this behaviour appears to indicate

460   boredom-like states (Meagher & Mason 2012). In mice, its welfare significance is

461   unknown, and it was not the focus on our research; however, we do flag this behaviour as

462   a potentially interesting topic for future study.

463

464   In terms of social interactions within the home cage, in Experiment Two, mice in NE

465   cages spent more time nesting together than those in enriched cages, but this effect was

466   not consistent across both studies. However, across both Experiments One and Two

467   together, as predicted, agonistic social interactions were consistently more frequent in NE

468   housing. As reviewed in the Introduction, this finding joins several previous studies in

469   showing that barren housing can have adverse social effects on laboratory rodents. Such

470   effects occur beyond this taxonomic group too: NE housing can promote agonistic

471   interactions between conspecifics in non-rodent species (*eg* as reviewed Diez-Leon *et al*

472   2013, Diez-Leon & Mason subm.). For example, NE conditions can exacerbate

473   aggressive behaviour in primates (Honess & Marin 2006, Márquez-Arias *et al* 2010) and

474   newly weaned pigs (Schaefer *et al* 1990), while in farmed mink, NE males are less

475   successful with females in a mate choice experiment (Diez-Leon *et al* 2013). It would be

476   interesting to explore such effects further, perhaps using less enriched NE cages to create

477   more contrast, or studying males, as the more aggressive sex. Possible mechanisms for

478   elevated agonism could also be investigated; these include greater levels of frustration in

479   NE mice (since frustration can exacerbate aggression; reviewed Papini 2003); reduced

480   behavioural competition, with NE mice having fewer opportunities than enriched mice to

481   perform behaviours other than aggression (Turner *et al* under review); reduced abilities of

482   NE mice to physically remove themselves from each other, and/or use enrichment objects

483   to hide in order to diffuse social tension; and more abnormal brain development, perhaps

484   increasing agonistic interactions by making mice poorer at learning and/or more prone to

485   repeat activities that are counter-productive.

486

487   This last idea led us to investigate how SB and agonistic behaviours inter-relate.

488   Experiment One yielded no information on this, perhaps because of its relatively small

489   sample size, but Experiment Two yielded several interesting results, as did analyses of

490   both datasets pooled. One of our predictions, based on studies of dopamine transporter

491   knockout mice (Rodriguiz *et al* 2004) was that stereotypic mice would show increased

492   levels of aggression. However, our results did not support this. Our second prediction was

493   that highly stereotypic mice would receive high levels of aggression, and this received

494   more support. Receiving aggression positively correlated with SB, although only in

495   enriched cages: stereotypic enriched mice consistently received the most aggression from

496   their cagemates. Why this was only manifest in the enriched cages is unclear, but could

497   perhaps reflect the masking effects of other potentially abnormal behavioural changes in

498   non-enriched mice (*eg* more time spent still but awake).

499

500   At least in the enriched populations, this pattern is thus consistent with the most

501   stereotypic mice being perceived as abnormal by cagemates, or acting socially oddly in

502   competitive situations, thence becoming targets for aggression. Another potential

503   explanation, however, is that mice receiving high levels of aggression then develop more

504   SB in response to increased stress (*cf eg* Akre *et al* 2011). To tease apart these two

505   possibilities, analyses were run to investigate whether housing type effects on agonism

506   were still detectable if variation in SB was statistically controlled for, and conversely,

507   whether housing type effects on SB were still detectable if variation in agonism was

508   statistically controlled for. These analyses revealed that effects of housing type on SB

509   were still evident even when variation in agonism was factored out, but the converse was

510   not true: housing effects on agonism were reduced or even eliminated when variation in

511   SB was factored out. These patterns are consistent with SB being a key driver of the

512   housing effect on agonistic social interactions, just as predicted. Future research should

513   test this hypothesis experimentally, for instance in longitudinal studies to assess which

514   behavioural differences appear first, and/or by moving mice between cages to investigate

515   whether transferring high or low SB individuals to new social groups differentially

516   influences the expression of agonistic behaviour by their cagemates. We also recommend

517   that any such future studies use video rather than direct live observation, to assist the

518   quantification of more nuanced aspects of social interaction (*eg* affiliative versus

519   aggressive allogrooming: Warne 1947, Grant & Macintosh 1963).

520

521   Experiment Three then investigated the social abilities of stereotypic mice in a different

522   way. We tested the hypothesis that stereotypic mice would be less effective

523   demonstrators in a social learning task. This was inspired by previous findings of

524   demonstrator effects in rodent studies (see Introduction). Mixed-strains were used, as this

525   is how mice were housed in the experiment; as far as we know this is the first study to

526   investigate the social transmission of food preferences between different strains of mice.

527   As predicted, low SB demonstrators appeared to possibly be more effective than high SB

528   demonstrators at inducing a qualitative relative preference in observer mice for a novel

529   flavour the demonstrators had recently eaten. However, since this is the first ever

530   indication of such an effect, and it was also merely a trend, we recommend that repeat

531   studies are now conducted to assess whether this result can be replicated. Such studies

532   should use flavours *a priori* shown to be equally preferred by naïve observers (we did not

533   have such data), to allow a clearer, more quantitative assessment of the social

534   transmission of preference. They should also involve videoing the interactions between

535   the demonstrator and observers -- particularly oronasal investigations, since olfactory

536   cues are essential for the social transmission of food preferences (Valsecchi & Galef

537   1989) -- to both identify how these vary in quality or quantity (*cf eg* Choleris *et al* 2011),

538   and assess the impact of any SB performance during this interaction phase. Replicate

539   studies might also benefit from using feeding regimes for the demonstrators that have

540   been shown to induce weaker, more variable flavour preferences in observers, since

541   social effects might be more easily detected using such paradigms (*cf* Galef *et al* 1998).

542

543   Overall, the findings from these three experiments together suggest that non-enriched

544   mice who develop SB do not just have a motor symptom consistent with autism, but

545   possibly also the social and communicative deficits that characterise this condition (*eg*

546   Silverman *et al* 2010, Patterson 2011). Future research should therefore test this

547   hypothesis further*,* both in the ways already suggested, and also by investigating whether

548   low SB mice are preferred as social or sexual partners to high SB mice (*cf* the mink

549   studies of Diez-Leon *et al* 2013).

550

Animal welfare implications and conclusions

This study shows that in addition to reducing levels of stereotypic behaviour, housing female mice in larger, enriched laboratory cages decreases the undesirable social behaviours of aggression and displacement, with obvious implications for their welfare. Furthermore, the two effects seemed related, with high SB mice appearing more prone to being the targets of aggression. Since being aggressed and subordinate is stressful (Lumley *et al* 2000, Bartolomucci *et al* 2005), this suggests that in mice, the welfare of highly stereotypic individuals is of particular concern. It also suggests that these mice may be abnormal in ways that render them socially impaired – an idea tentatively supported by high SB mice tending to be relatively ineffective demonstrators in a social learning task. Whether SBs and their underlying causes truly render mice socially compromised, and if so, how, needs future research, as it could have welfare implications, not only for high SB individuals but also for their cagemates, and not only in mice, but all captive species prone to SB.

575 **References**

576 **Akre A K M, Bakken M, Hovland A L, Palme R and Mason G** 2011. Clustered

577 environmental enrichments induce more aggression and stereotypic behavior than

578 do dispersed enrichments in female mice. *Applied Animal Behaviour Science* 131:

579 145-152.

580 **Bartolomucci A, Palanza P, Sacerdote P, Panerai A E, Sgoifo A, Dantzer R and**

581 **Parmigiani S** 2005. Social factors and individual vulnerability to chronic stress exposure.

582 *Neuroscience & Biobehavioral Reviews 29*: 67-81.

583 **Campbell DLM, Dallaire JA, Mason GJ** 2013 Environmentally enriched rearing

584 environments reduce repetitive perseveration in caged mink, but increase spontaneous

585 alteration. *Behavioural Brain Research* 239: 177-187

586 **Choleris E, Guo C, Liu H, Mainardi M and Valsecchi P** 1997 The effect of

587 demonstrator age and number on duration of socially-induced food preferences in house

588 mouse (*Mus domesticus*). *Behavioural Processes* 41: 69-77

589 **Choleris E, Clipperton-Allen AE, Gray DG, Diaz-Gonzalez S and Welsman RG**

590 2011 Differential effects of dopamine receptor D1-type and D2-type antagonists and

591 phase of the estrous cycle on social learning of food preferences, feeding, and social

592 interactions in mice. *Neuropsychopharmacology* 36: 1689-1702

593 **Clipperton A E, Spinato, J M, Chernets C, Pfaff D W and Choleris E** 2008

594 Differential effects of estrogen receptor alpha and beta specific agonists on social

595 learning of food preferences in female mice. *Neuropsychopharmacology* 33: 2362-2375

596 **Clipperton-Allen AE, Almey A, Melichercik A, Allen CP and Choleris E** 2011

597 Effects of an estrogen receptor alpha agonist on agonistic behaviour in intact and

598 gonadectomized male and female mice. *Psychoneuroendocrinology* 36: 981-995

599 **Dallaire JA, Meagher RK, Díez-León M, Garner JP and Mason GJ** 2011 Recurrent

600 perseveration correlates with abnormal repetitive locomotion in mink but is not reduced

601 by environmental enrichment. *Behavioural Brain Research* 224: 213-222

602 **Díez-León M** 2014. Effects of Environmental Enrichment on Stereotypic Behaviour and

603 Reproductive Success in American mink *Neovison vison* (PhD thesis). Guelph:

604 University of Guelph.

605 **Díez-León M, Bowman J, Bursian S, Filion H, Galicia D, Kanefsky J, Napolitano**

606 **A, Palme R, Schulte-Hostedde A, Scribner K and Mason GJ** 2013 Environmentally

607 enriched male mink gain more copulations than stereotypic, barren-reared competitors.

608 *PLoS One* 8: 1-11

609 **Díez-León M and Mason GJ** Submitted, 2014. Effects of environmental enrichment on

610 aspects of maternal behavior, and infant growth and survival, in a model carnivore

611 species, the American mink (*Neovison vison*). *Zoo Biology.*

612 **Doncaster CP and Davey AJH** 2007 *Analysis of variance and covariance. How*

613 *to choose and construct models for the life sciences.* Cambridge: Cambridge

614 University Press

615 **Friske JE and Gammie SC** 2005 Environmental enrichment alters plus maze, but not

616 maternal defense performance in mice. *Physiology and Behaviour* 85: 187-194

617 **Galef B G** 2001. Social influences on food choices of Norway rats and mate choices of

618 Japanese quail. *International Journal of Comparative Psychology 14*: 1-24.

619 **Galef BG and Wigmore SW** 1983 Transfer of information concerning distant food: a

620   laboratory investigation of the 'information-centre' hypothesis. *Animal Behaviour*. 31:

621   748-758

622   **Galef B G, Rudolf B, Whiskin, E E, Choleris E, Mainardi M and Valsecchi P** 1998

623   Familiarity and relatedness: effects on social learning about foods by Norway rats and

624   Mongolian gerbils. *Animal Learning & Behavior* 26: 448-454

625   **Garner JP and Mason GJ** 2002 Evidence for a relationship between cage stereotypies

626   and behavioural disinhibition in laboratory rodents. *Behavioural Brain Research* 136:

627   83-92

628   **Garner JP, Thogerson CM, Dufour BD, Würbel H, Murray JD and Mench JA**

629   2011 Reverse-translational biomarker validation of Abnormal Repetitive Behaviours in

630   mice: An illustration of the 4P's modelling approach. *Behavioural Brain Research* 219:

631   189-196.

632   **Grafen A and Hails R** 2002 *Modern Statistics for the Life Sciences.* New York: Oxford

633   University Press.

634   **Grant EC and Mackintosh JH** 1963 A comparison of the social postures of some

635   common laboratory rodents. *Behaviour* 21: 246-259

636   **Honess PE and Marin CM** 2006 Enrichment and aggression in primates.

637   *Neuroscience and Biobehavioural Reviews* 30: 413-436

638   **Kavaliers M, Choleris E and Colwell DD** 2001 Learning from others to cope with

639   biting flies: Social learning of fear-induced conditioned analgesia and active avoidance.

640   *Behavioural Neuroscience* 115: 661-674

641   **Kavaliers M, Colwell DD and Choleris E** 2005 Kinship, familiarity and social status

642   modulate social learning about "micropredators" (biting flies) in deer mice.

643   *Behavioural Ecology and Sociobiology* 58: 60-71

644   **Jones M A, Mason G J and Pillay N** 2011. Correlates of birth origin effects on

645   the development of stereotypic behaviour in striped mice *Rhabdomys*. *Animal*

646   *Behaviour* 82: 149-159

647   **Lam KSL, Bodfish JW, and Piven J** 2008 Evidence for three subtypes of repetitive

648   behaviour in autism that differ in familiality and association with other symptoms. *The*

649   *Journal of Child Psychology and Psychiatry* 49: 1193-1200

650   **Lopez BR, Lincoln AJ, Ozonoff S and Lai Z** 2005 Examining the relationship

651   between executive function and restricted, repetitive symptoms of autistic disorder.

652   *Journal of Autism and Developmental Disorders* 35: 445-460

653   **Lumley LA, Charles RF, Charles RC, Hebert MA, Morton DM and Meyerhoff JL**

654   2000 Effects of social defeat and of diazepam on behaviour in a resident-intruder test in

655   male DBA/2 mice. *Pharmacology, Biochemistry and Behvaviour* 67: 433-447

656   **Márquez-Arias A, Santillán-Doherty A M, Arenas-Rosas R V, Gasca-Matías M P**

657   **and Muñoz-Delgado J** 2010 Environmental enrichment for captive stumptail

658   macaques (*Macaca arctoides*). *Journal of Medical Primatology 39*: 32-40.

659   **Martin P and Bateson PPG** 1993 *Measuring Behaviour: an Introductory Guide*.

660   Cambridge University Press

661   **Mason GJ and Latham NR** 2004 Can't stop, won't stop: is stereotypy a reliable

662   animal welfare indicator? *Animal Welfare* 13: 57-69

663   **McEvoy RE, Rogers SJ and Pennington BF** 1993 Executive function and social

664   communication deficits in young autistic children. *Journal of Child Psychology and*

Psychiatry 34: 563-578

Meagher RK and Mason GJ 2012 Environmental enrichment reduces signs of boredom in caged mink. *PLOS One* 7: 1-10

Memari AH, Ziaee V, Shayestehfar M, Ghanouni P, Mansournia MA and Moshayedi P 2013 Cognitive flexibility impairments in children with autism spectrum disorders: Links to age, gender and child outcomes. *Research in Developmental Disabilities* 34: 3218-3225

Morley-Fletcher S, Rea M, Maccari S and Laviola G 2003 Environmental enrichment during adolescence reverses the effects of prenatal stress on play behaviour and HPA axis reactivity in rats. *European Journal of Neuroscience* 18: 3367-3374

National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) 2015 The 3Rs. https://www.nc3rs.org.uk/the-3rs#What%20are%20the%203Rs? (accessed Feb. 3, 2015)

Papini M R 2003 Comparative psychology of surprising non-reward. *Brain, Behavior and Evolution* 62: 83–95

Patterson P H 2011 Modeling autistic features in animals. *Pediatric Research*, 69: 34R-40R

Rasch D and Guiard V 2004 The robustness of parametric statistical methods. *Psychology Science* 46: 175-208

Rodriguiz R M, Chu R, Caron M G and Wetsel W C 2004 Aberrant responses in social interaction of dopamine transporter knockout mice. *Behavioural Brain Research* 148: 185-198

Ryan BC, Young NB, Moy SS and Crawley JN 2008 Olfactory cues are

688   sufficient to elicit social approach behaviours but not social transmission of food

689   preference in C57BL/6J mice. *Behavioural Brain Research* 193: 235-242

690   **Rymer T, Schradin C and Pillay N** 2008 Social transmission of information about

691   novel food in two populations of the African striped mouse, *Rhabdomys pumilio. Animal*

692   *Behav*iour 76: 1297-1303

693   **Schaefer AL, Salomons MO, Tong AKW, Sather AP and Lepage P** 1990 The effect

694   of environment enrichment on aggression in newly weaned pigs. *Applied Animal*

695   *Behaviour Science* **27: 41-52**

696   **Silverman J L, Yang M., Lord C and Crawley J N** 2010 Behavioural

697   phenotyping assays for mouse models of autism. *Nature Reviews Neuroscience*

698   11: 490-502

699   **Simpson J and Kelly JP** 2011 The impact of environmental enrichment in laboratory

700   rats – Behavioural and neurochemical aspects. *Behavioural Brain Research* 222: 246-

701   264

702   **Sørenson G** 1987 Stereotyped behaviour, hyperaggressiveness and "tyrannic" hierarchy

703   induced in bank voles (*Clethrionomys glareolus*) by a restricted cage milieu. *Progress in*

704   *Neuropsychopharmacology and Biological Psychiatry* 11: 9-21

705   **Tanimura Y, King MA, Williams DK and Lewis MH** 2011 Development of

706   repetitive behaviour in a mouse model: Roles of indirect and striosomal basal ganglia

707   pathways. *International Journal of Developmental Neuroscience* 29: 461-467

708   **Tanimura Y, Yang MC, and Lewis MH** 2008 Procedural learning and cognitive

709   flexibility in a mouse model of restricted, repetitive behaviour. *Behavioural Brain*

710   *Research* 189: 250-256

711 **Tilly SC, Dallaire J and Mason GJ** 2010 Middle-aged mice with enrichment-resistant

712 stereotypic behaviour show reduced motivation for enrichment. *Animal Behaviour* 80:

713 363-373

714 **Turner M** 1999 Repetitive behaviour in autism: A review of psychological research.

715 *Journal of Child Psychology and Psychiatry* 40: 839-849

716 **Turner PV, Wozniak L, Batista L, Ovari J, Clipperton-Allen AE, Hammermueller**

717 **J, Ingrao JC, JN, Bienzle D and Choleris E**, under revision, 2015. Long term provision

718 of environmental improvements alters behaviour but not physiology or neuroanatomy of

719 male and female BALB/c and C57BL/6 mice. *JAALAS.*

720 **Valsecchi P, Choleris E, Moles A, Guo C and Mainardi M** 1996 Kinship and

721 familiarity as factors affecting social transfer of food preferences in adult Mongolian

722 gerbils (*Meriones unguiculatus*). *Journal of Comparative Psychology* 110: 243-251

723 **Valsecchi P and Galef BG** 1989 Social influences on the food preferences of house

724 mice (*Mus musculus). The International Journal of Comparative Psychology* 2: 245-

725 256

726 Valsecchi P, Mainardi M, Sgoifo A and Taticchi A 1989. Maternal influences on food

727 preferences in weanling mice *Mus domesticus. Behavioural Processes* 19: 155-166

728 **Van Loo PLP, Kruitwagen CLJJ, Koolhaas JM, Van de Weerd HA, Van Zutphen**

729 **LFM and Baumans V** 2002 Influence of cage enrichment on aggressive behaviour and

730 physiological parameters in male mice. *Applied Animal Behaviour Science*. 76: 65-81

731 **Van Praag H, Kempermann G and Cage FH** 2000 Neural consequences of

732 environmental enrichment. *Nature Reviews Neuroscience* 1: 191-198

733   **Walker M, Fureix C, Palme R and Mason G** 2013. Co-housing rodents with different

734   coat colours as a simple, non-Invasive means of individual identification: Validating

735   mixed-strain housing for C57BL/6 and DBA/2 mice. *PLOS One* 8(10): e77541.

736   **Warne M C** 1947 A time analysis of certain aspects of the behavior of small groups of

737   caged mice. *Journal of Comparative and Physiological Psychology*, *40*: 371.

738   **Whitaker J, Moy SS, Godfrey V, Nielsen J, Bellinger D and Bradfield J** Effects of

739   cage size and enrichment on reproductive performance and behaviour in C57BL/6Tac

740   mice. *Lab Animal* 38: 24-34

741

742 **Tables and figures**

743

744 Table 1 Behaviours recorded during scan and focal sampling in Experiments One and

745 Two (adapted from Grant & Mackintosh 1963; Tilly *et al* 2010; Clipperton-Allen *et al*

746 2011)

747

748 Table 2. Types of cages and number of cages per housing type for Experiment Two

749

750 Table 3: Overview of how the home cage behaviours of non-enriched (NE) and

751 enriched mice compare across both studies

752

753 Figure 1: The effects of housing type on the performance of agonistic behaviours; there

754 was a significant main effect of housing type and no interaction with strain (see text for

755 details). Data presented are means and SEs of raw data.

756

757 Figure 2: Performance of aggression (bouts/min) in enriched and non-enriched cages in

758 Experiment Two (data shown are means and SEs of raw data); housing type interacted

759 with strain, an effect driven by the elevated aggression of NE C57s (see text for

760 details)

761

762   Figure 3: Level of stereotypic behaviour in the demonstrators of shatavari flavour,

763   compared for observers who ate either more marjoram (n = 10) or more shatavari (n = 10)

764   in a two choice test. Shown are means and SEs of raw data (see text for details)