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3 Chronic paroxetine treatment prevents disruption of methamphetamine-4 sensitive circadian oscillator in a transgenic mouse model of Huntington's 5 disease

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19 **Abstract**

20 Circadian abnormalities seen in Huntington's disease (HD) patients are recapitulated in several
21 HD transgenic mouse models. In mice, alongside the master clock located in the suprachiasmatic
22 nucleus (SCN), two other oscillators coordinate circadian behaviour. These are the food-
23 entrainable oscillator (FEO) and the methamphetamine-sensitive circadian oscillator (MASCO).
24 SCN- and MASCO- (but not FEO-) driven rhythms are progressively disrupted in the R6/2
25 mouse model of HD. MASCO-driven rhythms are induced by chronic treatment with low dose of
26 methamphetamine and characterised by an increase in period length to greater than 24 hours.
27 Interestingly, the rhythms mediated by MASCO deteriorate earlier than those mediated by the
28 SCN in R6/2 mice. Here, we used a pharmacological strategy to investigate the mechanisms
29 underlying MASCO-driven rhythms in WT mice. In contrast to methamphetamine, chronic
30 cocaine was ineffective in generating a MASCO-like component of activity although it markedly
31 increased locomotion. Furthermore, neither blocking dopamine (DA) receptors (with the DA
32 antagonist haloperidol) nor blocking neurotransmission by inhibiting the activity of vesicular
33 monoamine transporter (with reserpine) prevented the expression of the MASCO-driven
34 rhythms, although both treatments downregulated locomotor activity. Interestingly, chronic
35 treatment with paroxetine, a serotonin specific reuptake inhibitor commonly used as
36 antidepressant in HD, was able to restore the expression of MASCO-driven rhythms in R6/2
37 mice. Thus, MASCO-driven rhythms appear to be mediated by both serotoninergic and
38 dopaminergic systems. This supports the idea that abnormalities in MASCO output may
39 contribute to both the HD circadian and psychiatric phenotype.

40 **Keywords:** cognition, sleep, depression

41 **Chemical compounds studied in this article:**

42 (-)-Cocaine hydrochloride (PubChem CID: 656832); (+)-methamphetamine hydrochloride
43 (PubChem CID: 66124); haloperidol (PubChem CID: 3559); reserpine (PubChem CID: 5770);
44 paroxetine hydrochloride hemihydrate (PubChem CID: 62878).

45

46 **Highlights (maximum 85 characters, including spaces, per bullet point)**

47 • Chronic treatment with cocaine does not induce MASCO-like rhythms in WT mice

48 • Non-selective blockade of dopaminergic receptors does not prevent expression of MASCO
49 in WT mice.

50 • Treatment with the serotonin-specific reuptake inhibitor paroxetine rescues the expression of
51 the MASCO-driven rhythms in R6/2 mice.

52 • Serotonin is involved in the expression of MASCO-driven rhythms.

53 • Deficit in central serotonin may contribute to circadian rhythm dysfunction in HD mice.

54

55

1. Introduction

Circadian abnormalities have been described in Huntington's disease (HD) patients, as well as in rodent and sheep models of HD (Arnulf et al., 2008; Fisher et al., 2013; Loh et al., 2013; Morton, 2013; Morton et al., 2005; Piano et al., 2015). Circadian rhythms are controlled by the suprachiasmatic nucleus (SCN) that is located in the anterior hypothalamus. Under specific circumstances, two other circadian oscillators are able to generate robust behavioural rhythm independent of the SCN in rodents. These are the food-entrainable oscillator (FEO; Mistlberger, 1994) and the methamphetamine (MAP)-sensitive circadian oscillator (MASCO; Honma et al., 1986; Honma and Honma, 1986). The FEO in the transgenic R6/2 mouse model of HD appears to operate normally. Both the R6/2 and the full-length knock-in Q175 mice models of HD, however, show a progressive disruption in the SCN-driven rest-activity rhythms that is also seen in HD patients (Fisher et al., 2016, 2013; Loh et al., 2013; Morton et al., 2005; Ouk et al., 2016). MASCO is severely disrupted in both of these lines of mice (Cuesta et al., 2012; Ouk et al., 2016), with MASCO disruption occurring long before the SCN-mediated disintegration of circadian rhythms was observed. In the R6/2 line, the expression of MASCO-driven rhythms is disrupted in > 95% of the mice by 7.5 weeks of age (Cuesta et al., 2012), many weeks before motor symptoms are seen.

MASCO is induced by chronic MAP treatment at low doses and restores rhythmicity in mice rendered arrhythmic via SCN lesion (Honma et al., 1986, 1987). In SCN-intact WT mice placed in constant darkness (DD), rest-activity rhythms driven by MAP treatment are characterised by a period length that is greater than 24 hrs, and can increase up to the circabidian (48 hrs period) range. Under the influence of MAP, a distinct second component of general

78 activity progressively dissociate from the SCN-driven rhythms, leading to the emergence of
79 MASCO-driven rhythms (Honma et al., 1986; Honma and Honma, 1986; for other references see
80 Cuesta et al., 2012). The mechanisms underlying the expression of the MASCO-driven rhythms
81 are currently unknown.

82 The deficit of MASCO-driven rhythms in HD mice raises many questions, not only about
83 the function of the MASCO, but also about the mechanisms underlying the MASCO. We found
84 previously that chronic treatment with L-DOPA delayed the disruption of MASCO-driven
85 rhythms in R6/2 mice (Cuesta et al., 2012), suggesting an early abnormality of the dopamine
86 (DA) and/or noradrenaline (NA) systems in HD mice. A key role of DA in the expression of
87 MASCO-driven rhythms is consistent with both the pharmacological action of MAP and the
88 involvement of striatal dopaminergic rhythms that might act as ultradian oscillators able to
89 generate rhythms greater than 24 hrs when dopaminergic tone is elevated (Blum et al., 2014).
90 Consistent with this, deficits in both dopaminergic and noradrenergic systems are well described
91 in both patients and HD mice. HD patients, at both early and late stage of the disease, exhibit a
92 disruption in DA signaling, with reduced striatal DA levels and DA receptors (Antonini et al.,
93 1996; Ginovart et al., 1997; Sedvall et al., 1994; Weeks et al., 1996). In R6/2 mice, loss of D1
94 receptors mRNA is evident as early as 4 weeks of age (Cha et al., 1998), with the DA system
95 being functionally impaired and DA release severely compromised by 6 weeks of age (Hickey et
96 al., 2002; Johnson et al., 2006). Noradrenergic receptors β1 are reported to be dramatically
97 reduced in the basal ganglia of HD patients at late stage of disease (Waeber et al., 1991). In R6/2
98 mice, NA neurotransmission is already decreased by 4 weeks of age in the hippocampus
99 (Reynolds et al., 1999). Interestingly, it has been shown that MAP also affected the serotonergic

100 system in neurotransmitter release (Ago et al., 2008) which is disrupted in both HD patients
101 (Steward et al., 1993) and R6/2 mice (Mochel et al., 2011; Yohrling et al., 2003). Thus, it seems
102 possible that the major disruption of MASCO-driven rhythms in R6/2 mice is caused by early
103 abnormalities in one or both of these biogenic amine systems.

104 Here we conducted a pharmacological study using mice to examine some of the
105 mechanisms that might underlie the deficits in the expression of MASCO-driven rhythms in
106 R6/2 mice. We tested four drugs that modulate dopaminergic/noradrenergic or serotoninergic
107 neurotransmission. First, we tested whether a chronic treatment with another dopaminergic
108 stimulant (cocaine) than MAP could induce the expression of MASCO-driven rhythms in WT
109 mice. We then investigated whether blocking the neurotransmission of DA/NA (using the non-
110 selective dopaminergic receptor antagonist haloperidol or the monoamine depleting agent
111 reserpine) was sufficient to suppress the MASCO-driven rhythms in WT mice. Finally, we
112 investigated whether chronic treatment with paroxetine (that inhibits the neuronal reuptake of
113 serotonin (5-HT) by blocking the 5-HT transporter; SERT) had an ameliorating effect on the
114 abnormal MASCO-driven rhythms in R6/2 mice.

115

116 **2. Material and methods**

117 **2.1. Animals and housing conditions**

118 All of the experimental procedures were conducted in accordance with the guidelines of the UK
119 Animals (Scientific Procedures) Act 1986 and with the approval of the University of Cambridge
120 Animal Welfare and Ethical Review Board.

121 R6/2 and WT littermate mice were obtained from a colony established in the University of
122 Cambridge (CBA x C57BL/6J background) following the breeding strategy described by
123 Mangiarini et al. (1996). Genotyping and determination of the CAG repeat lengths were
124 performed by Laragen (Los Angeles, CA, USA) as previously described (Morton et al., 2009).
125 Mice were group-housed and placed under a 12 h light (~300 lux): 12 h dark cycle before the
126 start of the experiment and had *ad libitum* access to food and water. Experiments were conducted
127 in DD. For analysis of individual behavioural rhythms during the drug treatments, mice were
128 then singly housed in a ventilated sound-proof and light-tight cabinet (Scantainer, Scanbur,
129 Denmark). Humidity and temperature were maintained at mean (± SEM) of 55% ± 10 and 22°C
130 ± 1, respectively.

131 We routinely use both male and female mice in our studies. While there are some sex
132 differences (Skillings and Morton, 2016; Wood et al., 2010), using both sexes strengthens the
133 validity of any major differences that are seen due to the HD genotype. A total of 44 WT mice
134 (31 male and 13 female) and 36 R6/2 mice (18 male and 18 female) were distributed between
135 four pharmacological experiments (for details of numbers and treatments see **Table 1**). We used
136 an R6/2 mouse line with a CAG repeat of 250. This is a well-described R6/2 line with
137 reproducible phenotype that exhibits many changes that recapitulate those seen in humans (brain
138 atrophy, aggregates of mutant huntingtin and behavioral abnormalities (Lione et al., 1999;
139 Morton et al., 2000). The behavioral phenotype and end stage brain pathology of the R6/2 250
140 and R6/2 110 (the two line available from Jackson Laboratories) are very similar (Morton et al.,
141 2009). We use the 250 CAG repeat mouse rather than the R6/2 110 line because the development

142 of its phenotype takes a slower trajectory, and thus gives a wider experimental window of
143 opportunity. The R6/2 mice used in this study had a mean ± SEM CAG repeat length of 257 ± 1.

144

145 **2.2. Drugs**

146 (-)-Cocaine hydrochloride (C5776), (+)-methamphetamine hydrochloride (M8750),
147 haloperidol (H1512), reserpine (R0875) and paroxetine hydrochloride hemihydrate (P9623) have
148 been purchased from Sigma Aldrich (UK). Cocaine was dissolved in 0.9% saline and
149 administered to the mice with mini-osmotic minipumps (Alzet, model 1004). Haloperidol was
150 dissolved in either polyethylene glycol vehicle (molecular weight 400 daltons) vehicle (40%
151 PEG 400 in 0.9% saline or 5% ethanol in 0.9% saline). Reserpine was dissolved in 0.9% saline
152 and 0.5% acetic acid. Paroxetine was dissolved in 0.9% saline at 60°C with a sonicator and left
153 to cool to ambient temperature before administration.

154

155 **2.3. Drug treatment**

156 Mice were placed under DD at least a week before the start of the drug treatments.
157 In the cocaine experiment, mini-osmotic pumps containing either 0.9% saline or cocaine
158 hydrochloride dissolved in 0.9% saline were implanted subcutaneously into 12-week-old drug-
159 naïve WT mice under general anaesthesia (0.5-2% isoflurane). These pumps allowed chronic
160 drug infusion for 4 weeks with a dose rate of 30 mg/kg/day. The dose of cocaine tested was
161 chosen based on the literature (Jiang et al., 2013; Li et al., 2005) and pilot experiments (data not
162 shown).

163 For haloperidol, reserpine and paroxetine experiments, the mice were given intraperitoneal
164 injections of drugs in a volume of 100 µl per 10 g of body weight, at random times of day
165 between 7am and 7pm to avoid any entrainment to the injections. The doses of the drugs tested
166 were chosen based on the literature (de Jong et al., 2005; Duan et al., 2004; Fukushiro et al.,
167 2008; Maxwell et al., 2004; O'Leary et al., 2007; Roscoe et al., 2005; Skalisz et al., 2002).

168 In the haloperidol experiment, drug-naive WT mice first received *ad libitum* water (7-7.5
169 weeks) to drink for the single housing habituation period in the circadian cabinet. After this, mice
170 were split into four groups. WT mice were given to drink either water alone or 0.005% MAP
171 diluted in water (from 8 to 16 weeks of age). In addition, each mouse received either a saline
172 (control) or haloperidol injections daily (from 10 to 14 weeks of age) with doses gradually
173 increased from 0.1 mg/kg to 0.3 mg/kg and 0.5 mg/kg. Mice were distributed between 4
174 combinations of injection and drink: saline/water (n = 5; 2 male and 3 female), saline/MAP (n =
175 5; 3 male and 2 female), haloperidol/water (n = 11; 6 male and 5 female) and haloperidol/MAP
176 (n = 7; 4 male and 3 female).

177 In the reserpine experiment, we used the WT mice that had previously received
178 haloperidol. After a wash-out period of 3 weeks during which haloperidol was withdrawn but
179 mice continued to be given MAP, mice previously treated with haloperidol were treated with
180 reserpine (1 mg/kg) associated with either water or MAP from 17 to 22.5 weeks of age.
181 Reserpine or saline was administered intraperitoneally daily for the first 5 days, then every 3
182 days thereafter.

183 For the paroxetine experiment, drug-naïve R6/2 mice were treated daily between 6 and 12
184 weeks of age with either vehicle or paroxetine at a dose of 20 mg/kg. WT mice have normal

185 levels of 5-HT and express robust MASCO-driven rhythms. Since the purpose of the experiment
186 was to see if paroxetine treatment could restore the MASCO-driven rhythms, and there is a
187 significant risk for 5-HT syndrome in WT mice after SSRI treatment (Haberzettl et al., 2013;
188 Kalueff et al., 2008; AJM unpublished data), we did not treat WT mice with chronic paroxetine.
189 R6/2 mice were examined daily to detect any sign of Straub tail (S-shaped dorsiflexion), a
190 typical sign of 5-HT syndrome. None of the R6/2 mice developed this sign during the study.
191 Concomitantly with paroxetine or vehicle injections, mice were given either water alone or
192 0.005% MAP diluted in water (from 8 to 12 weeks). Mice were distributed into one of 4 groups
193 that received different combination of drug injections and water only or 0.005% MAP diluted in
194 water to drink. Those were: saline/water (n = 6; 3 male, 3 female), saline/MAP (n = 6; 3 male, 3
195 female), paroxetine/water (n = 12; 6 male, 6 female) and paroxetine/MAP (n =12; 6 male and 6
196 female). Note that for the groups treated with paroxetine, the drug treatment was started two
197 weeks before MAP was administered, as while the action of antidepressants on the transporter is
198 rapid and acutely inhibits neurotransmitters reuptake (Hirano et al., 2005), improvements of
199 behavioural disturbances are delayed and observed only after weeks of repeated treatment
200 (Hébert et al., 2001; Montgomery, 1997).
201
202 **2.4. Circadian analysis**
203 General activity was recorded continuously from individually housed mice in the circadian
204 cabinet with infrared movement sensors (Bosch, Germany) placed on top of each cage and linked
205 to a computerised recording system (Clocklab; Actimetrics, Wilmette, IL, USA).

206 Double plotted actograms in 5-min block size were generated with Clocklab software, for
207 analysis of the circadian parameters of the locomotor rhythms. Period lengths were determined
208 by fitting a least-square regression line to the activity onsets of the data periods analysed.
209 Duration of active period was calculated as the difference between the means of the regression
210 lines drawn through the 7 or 14 activity onsets and corresponding offsets. Lomb-Scargle
211 periodograms were used to determine behavioural rhythmicity using a line of significance at
212 0.001. Only rhythmic mice were included in the analysis of period length and duration of active
213 period. General activity during active and rest period was determined using the profile activity
214 function of the Clocklab software.
215

216 **2.5. Statistical analysis**

217 Statistical analyses were performed using Statistica software (version 13.2, StatSoft Inc., Tulsa,
218 USA). Analysis of variance (ANOVA) with repeated measures was performed to investigate
219 differences between groups. When main or interactions effects were identified, a Bonferroni
220 post-hoc test was used to determine significant effects with $P < 0.05$.
221

222 **3. Results**

223 **3.1. Methamphetamine, but not cocaine, induced the expression of MASCO-driven**
224 **rhythms in WT mice**

225 In WT mice placed under DD and given water alone to drink, a typical free-running circadian
226 locomotor activity rhythm was observed with a period length between 23 and 24 hrs (**Figs. 1A**
227 **and D**). When mice were treated with 0.005% MAP in the drinking water, all WT mice

228 expressed two distinct components of the general activity rhythms, as previously described
229 (Cuesta et al., 2012; **Fig. 1B**). These were the SCN component, with a period length of ~24 hrs
230 and the MASCO component, with period lengths > 24 hrs (**Fig 1B, C**).

231 When WT mice were treated with cocaine, we did not observe the emergence of the second
232 component of activity that is typically seen with MAP treatment (**Fig.1E and F**). Nevertheless,
233 the cocaine was pharmacologically effective, since at the beginning of cocaine treatment, there
234 was a pronounced, but transient, increase in general activity that lasted around 48 hrs during
235 which mice were constantly hyperactive. After this hyperactive period, all mice treated with
236 cocaine returned to a ~24 hrs rest-activity rhythm pattern. We did not find any main effect of
237 cocaine treatment on period length (that remained ~24 hrs), active period duration or rest/activity
238 ratio (**Table 2 and supplementary Fig. 1**). There was a main effect (increase with increased
239 time) of treatment duration on activity counts [$F_{(5,70)} = 3.89$; $P < 0.01$but *post-hoc* test did not
240 reveal any significant difference between control mice and cocaine-treated mice at any of the
241 individual weeks analysed.

242

243 **3.2. Haloperidol and reserpine reduce locomotor activity but MASCO-driven rhythms**
244 **can still be invoked in WT mice**

245 We found an interaction effect of haloperidol and dose on activity counts in the active period [F
246 $_{(7,168)} = 2.75$; $P < 0.01$]. Haloperidol significantly reduced the general activity of mice drinking
247 water (**Fig. 2C, Fig. 3A**) compared to the week before the treatment ($P < 0.001$) and compared to
248 washout week ($P < 0.001$). This reduction of general activity was reversible, with the level of
249 general activity returning to normal within ~6 days of stopping the drug treatment (**Fig. 2C;** P <

250 0.001). After ~2 weeks of treatment, haloperidol suppressed activity onset of the SCN
251 component, although activity offset could still be observed (**Fig. 2C and D**). Period length in DD
252 during haloperidol treatment remained stable with < 24hrs (**Fig. 2C and D**), similar to mice
253 treated with vehicle (**Fig. 2A and B**).

254 The general rest-activity patterns of mice in the saline/MAP group (**Fig. 2E and F**) was as
255 expected, with the clear emergence of a MASCO-driven rhythm component within ~2 weeks. In
256 the haloperidol/MAP group, however, while there was a significant reduction in the general
257 activity of the mice (**Figs. 2G and 3B**) compared to the week before haloperidol treatment
258 started and the washout week ($P < 0.01$); the mice still expressed MASCO-like rhythms (**Figs. 2,**
259 **H and 3G**). There was a main effect of haloperidol on the amplitude of both SCN- and MASCO-
260 driven rhythms, which was decreased by the drug but this was not confirmed by *post hoc*
261 analyses (Figure 3F). In 6 out of 7 mice, the period length of the MASCO-driven rhythms ranged
262 between 24.01 and 32.56 hrs. One out of seven of the mice expressed circabidian (48 hrs)
263 MASCO-driven rhythms.

264 After a 'washout' period of 3 weeks, we treated the mice with reserpine to study the effect
265 of the depletion of catecholamines on the circadian rhythms and the expression of the MASCO-
266 driven rhythms. We found a main effect of reserpine on the activity during the active period of
267 WT mice [$F_{(3,23)} = 7.28$; $P < 0.01$]. This was significantly reduced in reserpine-treated mice
268 drinking water only (**Fig. 3C, Fig. 4C**) during their treatment, compared to the week before the
269 treatment started ($P < 0.001$) or to the mice treated with vehicle (**Fig. 4A**). Period length during
270 reserpine treatment was with a period length of ~24h, similar to that of mice treated with vehicle
271 (**Fig. 4B-D**). In the reserpine/MAP group, while reserpine progressively reduced the general

272 activity of the mice (**Fig. 3D, Fig. 4G**), they still expressed robust MAP-driven rhythms that
273 ranged between 24.27 and 49.51 h (**Fig. 3H, Fig. 4G, H**) as their amplitude was not significantly
274 affected (**Fig. 3H**), with three out of seven of the mice expressing circabidian rhythms. Our
275 results show that lowering DA or blocking DA receptors induced a pronounced downregulation
276 of locomotor activity but did not suppress the induction of the MASCO-driven rhythms.
277

278 **3.3. Paroxetine delays disruption of MASCO-like rhythms in R6/2 mice**

279 Since haloperidol and reserpine could inhibit activity but not suppress the MASCO-driven
280 rhythms in WT mice, we considered the possible involvement of the serotonergic system in the
281 MASCO abnormalities in R6/2 mice. To investigate this, we pre-treated R6/2 mice chronically
282 with paroxetine, a selective 5-HT reuptake inhibitor (SSRI). Paroxetine modulated the general
283 activity patterns in R6/2 mice. In the R6/2 paroxetine/water group, there was a change in general
284 activity that was manifest as a fragmentation of the rest-activity patterns (**Fig. 5C**) compared to
285 R6/2 saline/water group (**Fig. 5A**). Only a single component (SCN-driven) of general activity
286 was observed in these groups (**Fig. 5B and D**). There was no main effect of drug treatment on
287 period length [$F_{(1,75)} = 0.40$; $P > 0.05$; **Table 3 and Fig. 6A and B**] or rest-activity ratio [$F_{(1,80)} =$
288 0.27; $P > 0.05$; **Table 4 and Fig. 7C**]. There was however a main effect of drug treatment on
289 duration of active period in water-drinking mice [$F_{(1,16)} = 6.92$; $P < 0.05$; **Fig 7A**] although *post-*
290 *hoc* tests did not reveal specific timepoints at which the shortening was significant (with
291 maximal shortening at week 4; 13.43 ± 0.21 vs. 14.79 ± 0.44). There was also a main effect of
292 drug treatment on the general activity during active period [$F_{(1,16)} = 14.65$; $P < 0.01$] with
293 activity counts significantly decreased in paroxetine-treated R6/2 mice compared to saline-

294 treated mice at week 3 ($P < 0.05$) and week 6 ($P < 0.01$) of drug treatment (**Table 4 and Fig.**
295 **7E**).

296 R6/2 mice from the saline/MAP group exhibited an increase of total activity in the active
297 period, with a significant main effect of treatment duration on rest-activity rhythms [$F_{(5,75)} =$
298 0.17; $P < 0.001$; **Table 4 and Fig. 7F**]. The mice expressed, however, only the SCN component
299 of the general activity, and did not show the MASCO-like rhythms (**Fig. 5E and F; Fig. 6C)**.
300 This is consistent with our previous study (Cuesta et al., 2012). Analysis of period length of the
301 SCN component revealed no differences between R6/2 mice given water and those given water
302 containing MAP (**Table 3**). Interestingly, seven out of twelve R6/2 mice given MAP and treated
303 concomitantly with paroxetine developed MASCO-like rhythms (See an example in **Fig. 5G and**
304 **H**; **Fig. 6D**), from week 3 to week 4 of MAP treatment when the mice were 10-11 weeks of age.
305 During this time the period length of the MASCO component ranged from 24.04 to 32.06 h. We
306 also found main effects of paroxetine treatment and treatment duration on SCN period length in
307 R6/2 mice drinking methamphetamine [$F_{(1,15)} = 8.50$; $P < 0.05$ and $F_{(5,75)} = 5.10$; $P < 0.001$],
308 although *post hoc* analysis did not reveal any significant differences between paroxetine- and
309 saline-treated mice week per week (**Table 3**). We did not, however, find any effect of paroxetine
310 treatment on the general activity counts (either during rest or active period), rest-activity ratio or
311 duration of active period (**Table 4 and Fig. 7B, D and F**) in mice drinking methamphetamine.

312

313 **4. Discussion**

314 MASCO-driven rhythms are widely believed to be mediated via central dopaminergic
315 mechanisms (Blum et al., 2014; Cuesta et al., 2012). Blocking DA neurotransmission, however,
316 was not sufficient to suppress the MASCO-driven rhythms in WT mice as they were still
317 observed when MAP consumption was combined with chronic treatment of either haloperidol or
318 reserpine. Although 5-HT has not previously been implicated in the mechanisms underlying the
319 MASCO-driven rhythms, we found that in R6/2 mice carrying 250 CAG repeats (that normally
320 do not express them) MASCO-like rhythms could be induced in animals pretreated with
321 paroxetine. Together, our results suggest that abnormalities in the serotoninergic system, in
322 addition to deficits in the dopaminergic/noradrenergic systems, underlie the disruption of the
323 MASCO-driven rhythms in the R6/2 mouse.

324 MAP acts primarily on dopaminergic neurons to increase extracellular DA levels. Thus, an
325 intact dopaminergic system is likely to be necessary for the normal generation of the MASCO-
326 driven rhythms while a deficit associated with abnormal expression of DA may disrupt their
327 manifestation. Consistent with this, L-DOPA was able to partially restore the expression of
328 MASCO-driven rhythms in R6/2 mice carrying 250 CAG repeats (Cuesta et al., 2012).
329 Abnormalities in the dopaminergic system in HD are well-documented in both patients (Andrews
330 et al., 1999; Antonini et al., 1996; Bäckman et al., 1997; Bäckman and Farde, 2001; Bernheimer
331 et al., 1973; Chen et al., 2013; Ginovart et al., 1997; Guo et al., 2012; Kish et al., 1987) and
332 animal models (Chen et al., 2013; Hickey et al., 2002; Squitieri et al., 2015; Tyebji et al., 2015).
333 It seems likely that some of the changes in circadian behaviour in HD mice, and by extrapolation
334 in HD patients, are partially caused by abnormalities in the DA system. For ethical reasons, the

335 existence of the MASCO in humans has not yet been investigated. Therefore, the importance of
336 its possible dysfunction in HD must remain a matter for speculation.

337 There is considerable evidence, both direct and indirect, for a role of DA in both HD and in
338 modulation of circadian rhythms. An important body of evidence has emerged recently showing
339 that the elevation of striatal DA signalling lengthens the circadian period (Blum et al., 2014;
340 Landgraf et al., 2016). It has been shown previously that clock genes are involved in the
341 regulation of striatal DA activity (Hampp et al., 2008; McClung et al., 2005; Roybal et al., 2007)
342 and are down-regulated in HD mice (Morton et al., 2005). Furthermore, dopaminergic receptor
343 agonists influence neuronal clock gene expression (Imbesi et al., 2009). The rhythm of
344 expression of striatal PER2 depends on daily dopaminergic activation of D2 receptors (Hood et
345 al., 2010) and the activation of D2 receptor signalling enhances the circadian regulation by
346 CLOCK and BMAL1 (Yujnovsky et al., 2006).

347 Methamphetamine and cocaine both increase levels of DA in synapses (Sofuoglu and
348 Sewell, 2009) but chronic treatment with cocaine in WT mice did not induce the expression of a
349 second component of general activity as is seen after chronic MAP treatment. Due to the
350 relatively short half-life of cocaine and its poor solubility, we did not attempt to deliver cocaine
351 via drinking water, as this would have required the mice to drink constantly to maintain high
352 enough cocaine levels in the brain to release DA at similar levels to MAP. Instead, we used an
353 osmotic minipump to deliver cocaine chronically. While we did not measure plasma levels of
354 cocaine, we are confident that the cocaine was released from the minipumps, since we saw a
355 clear increase in activity in response to cocaine at onset of drug treatment. In fact, the animals
356 were hyperactive and moved constantly for the first two days after minipump implantation. This

357 is likely to correspond to the period of sensitisation/intensification of behavioural stereotypy
358 induced by cocaine described in the literature (Johansson et al., 1992). Thereafter, the cocaine-
359 treated mice habituated to the drug and resumed a circadian cycle, albeit with a different period
360 and a more fragmented locomotor activity (**Fig. 1E and F**). It has been shown that the route of
361 cocaine administration influenced the drug effects (King et al., 1992). An attenuation of
362 behavioural response upon chronic administration of cocaine has been described, with
363 continuous infusion of cocaine via Alzet minipumps, but not with daily cocaine intraperitoneal
364 injection, producing tolerance (Reith et al., 1987). This tolerance to cocaine appears to be a
365 robust compensatory mechanism and is manifest for at least 7 days after the cocaine treatment is
366 stopped (King et al., 1992).

367 The mechanisms of action of cocaine and MAP are different (for review, see; Chiu and
368 Schenk, 2012). Cocaine increases DA levels in the presynaptic cleft by blocking the DAT and
369 preventing DA reuptake back in the neuron. By contrast, MAP not only competes with DA at the
370 DAT to prevent its reuptake but is also transported into the presynaptic terminal by DAT. Once
371 in the cytosol, MAP is transported into the vesicles where it induces the release of
372 neurotransmitter. MAP also slows the inactivation of DA and NA by inhibiting monoamine
373 oxidase (Egashira and Yamanaka, 1993). Moreover, a study reporting the effects of MAP and
374 cocaine on inhibitory postsynaptic current mediated by D2 autoreceptors (Branch and Beckstead,
375 2012) suggested that the two drugs exerted differential kinetics in intracellular actions for
376 dopamine transmission. Therefore, the expression of MASCO-driven rhythms may be rendered
377 possible by complex pharmacokinetic and dynamic actions of MAP rather than to a single
378 increase in DA in the synapse cleft. Furthermore, it seems that MAP also influence the circadian

379 circadian via the expression of clock genes. The induction of MASCO-driven rhythms does not
380 require the full repertoire of the clock genes (Mohawk et al., 2009). Nevertheless, since
381 MASCO-driven rhythms were observed in lines of mice with single or double mutations in
382 principal clock genes (Per1/Per2, Cry1/Cry2, Bmal and Clock) even if they were arrhythmic
383 (Honma et al., 2008; Masubuchi et al., 2001; Mohawk et al., 2012), it is possible that clock genes
384 also play a role in the timing of MASCO. Interestingly, it has been reported that MAP treatment
385 specifically induced a shift of the expression of Per 1 and Per 2 in the striatum from a nocturnal
386 to a diurnal rhythm while their expression in the SCN was unaffected (Iijima et al., 2002).
387 Furthermore, although Per gene knockout mice express MASCO-driven rhythms, these are
388 atypical. For example, MASCO-driven rhythms in Per2 knock-out mice were reported to be
389 uncoupled from SCN rhythms (Pendergast et al., 2013). MASCO-driven rhythms in a triple
390 knockout mouse for Per1, 2 and 3 with lesioned SCN were unusually short (around 21h;
391 Pendergast et al., 2012), while double knockout of Per1 and 2 mice exhibited MASCO rhythms
392 that alternated between short (22h) and long period (27h; Pendergast et al., 2012). A role for Per
393 genes in the expression of MASCO-driven rhythms is relevant in our case as both Per1 and Per2
394 expressions are dysregulated in the striatum of R6/2 mice (Morton et al., 2005), with a truncation
395 of the peak expression. This may explain the MASCO-driven rhythms observed in R6/2 mice
396 treated with paroxetine with short periods of e.g. 24.04 h. Therefore, the dysregulated expression
397 of Per genes in R6/2 mice may also contribute to the MASCO dysfunction.

398 We found that blocking the DA/NA neurotransmission with either haloperidol or reserpine
399 in MAP-treated WT mice reduced the level of general activity of the mice (showing that the
400 drugs were efficacious). Neither drug, however, suppressed the MASCO-driven rhythms. This

401 suggests that the expression of MASCO-driven rhythms is not dependent solely on DA/NA.
402 Although circadian rhythms were masked by both the decrease in general activity and
403 fragmentation of the rhythms, both SCN- and MASCO-driven rhythms reappeared quickly
404 (within 1-2 days) once the drug administration stopped. It was not possible to use a higher dose
405 of haloperidol to treat the mice as blocking DA receptors reduced motivation to move, eat and
406 drink (Zis and Fibiger, 1975).

407 It has been shown that chronic MAP treatment also modulated the serotonergic system
408 (Ago et al., 2008; Tanaka et al., 2016). A role for 5-HT in the MASCO-driven rhythms however,
409 has not been suggested previously. It is, however interesting to consider this as an underlying
410 cause of an HD-related abnormality in MASCO, since a decrease in 5-HT and its metabolite 5-
411 hydroxyindoleacetic acid (Mochel et al., 2011), as well as a reduction in the activity of
412 tryptophan hydroxylase, the rate-limiting enzyme in biosynthesis of 5-HT (Yohrling et al., 2002)
413 have been described in both patients and HD mice models. Deficits in serotoninergic
414 transmission have been implicated in depression, a psychiatric disturbance often diagnosed in
415 HD patients (Paulsen et al., 2005) and commonly treated with SSRIs such as paroxetine (Nevels
416 et al., 2016). Depressive-like behaviours have been described in R6/2 mice (Ciamei et al., 2015;
417 Pang et al., 2009). Interestingly, SSRIs have been shown to improve cognitive and affective
418 behavioral disorder in R6/2 and other lines of HD mice (Duan et al., 2008; Peng et al., 2008). For
419 example, fluoxetine improves hippocampal-dependent cognitive and depressive-like behavioural
420 symptoms (Grote et al., 2005) and sertraline improved the deficits seen in the forced swim test in
421 female R6/1 mice (Renoir et al., 2012). Furthermore, SSRIs including fluoxetine, sertraline and

422 paroxetine increase levels of brain-derived neurotrophic factor (BDNF; a protein necessary for
423 neuron survival), and improved neurogenesis deficits in HD mice (Peng et al., 2008).

424 The antidepressant action of paroxetine involves increased availability of 5-HT in the
425 synapse and has been shown to improve 5-HT agonism on 5-HT2A receptors in the cortex of
426 young patients with depression after 6 weeks of treatment (Meyer et al., 2001). R6/2 250 CAG
427 repeat mice develop sleep abnormalities such as increase of REM sleep during the dark period,
428 slowing down of REM sleep theta rhythms and abnormal low gamma oscillations seen in sleep
429 electroencephalography (EEG; Kantor et al., 2013). We have shown recently that chronic, but
430 not acute, treatment of R6/2 250 CAG repeat mice with paroxetine was able to prevent
431 electroencephalographic abnormalities in R6/2 mice if treatment started before symptom onset.
432 This suggests that correcting abnormalities in the 5-HT system stabilized cortical circuits in R6/2
433 mice (Kantor et al., 2017). Here we found that when chronic paroxetine was started at a
434 presymptomatic age, it allowed MASCO-driven rhythms to be induced at a time when they are
435 not normally inducible in R6/2 mice. Together these data suggest that normal cortical function
436 may be necessary for the development of MASCO-driven rhythms.

437 The most parsimonious interpretation of our data is that both 5-HT and DA are important
438 for the expression of MASCO-driven rhythms. In future studies this should be explored further.
439 For example, dopaminergic neurons in the mid striatum have been shown to directly influence
440 the MAP-driven rhythms as silencing them with the DDREADs technique suppressed the
441 rhythms (Blum et al., 2014). It would be very interesting to take a similarly direct approach to
442 determining the role of the serotonergic neurons of the Raphe nucleus in the generation of the
443 MASCO-driven rhythms. The interactions between dopaminergic and serotonergic systems are

444 complex. For example, several lines of evidence show that chronic paroxetine not only acts on
445 serotoninergic neurotransmission by downregulating SERT (Hirano et al., 2005), but also exerts
446 a modulation on the dopaminergic and the noradrenergic receptor systems (Nakayama, 2002;
447 Redrobe et al., 1998). Depressed patients treated with paroxetine exhibited elevated DAT
448 binding (Kugaya et al., 2003). Furthermore, local action of SSRIs in the nucleus accumbens
449 (NAc) mediates a DA efflux from that structure (Parsons and Justice, 1993), resulting in the
450 enhancement of cocaine-evoked hyperactivity (Bubar et al., 2003). Other studies showed that the
451 5-HT_3 antagonist ganisetron inhibited the paroxetine-induced increase in extracellular DA levels,
452 but did not change 5-HT levels in rat medial prefrontal cortex (Nakayama, 2002) or olfactory
453 bulb slices (Zazpe et al., 1994). This suggests that paroxetine could increase DA levels via the
454 stimulation of 5-HT_3 receptors present on dopaminergic neurons. Interestingly, modulatory
455 effects of paroxetine on NA system have also been reported. In patients with major depression
456 treated with paroxetine, NA uptake was inhibited (Gilmor et al., 2002). Moreover, paroxetine
457 inhibits the neuronal uptake of NA, as well as 5-HT (Redrobe et al., 1998). The ability of
458 paroxetine to restore the MAP-driven rhythms in R6/2 mice when the treatment started at
459 presymptomatic age, similar to what we had shown previously with L-DOPA (Cuesta et al.,
460 2012), may be due not only to its ability to increase the levels of 5-HT, but to indirect
461 modulatory effects on DA and NA neurotransmission.

462 Beneficial effects of paroxetine have been observed in a number of animal models of
463 neurodegenerative diseases. For example in a mouse model of Parkinson's disease, treatment
464 with paroxetine prevented degeneration of nigrostriatal dopaminergic neurons, increased striatal
465 DA levels and improved motor function (Chung et al., 2010). Paroxetine delayed neuronal

466 degeneration and motor dysfunction, increased brain-derived neurotropic factor and improved
467 energy metabolism, insulin sensitivity and the survival in the HD-N171-82Q mouse model of
468 HD (Duan et al., 2004) that express the human N-terminal truncated huntingtin with 82
469 polyglutamine repeats driven by a mouse prion protein promoter (Duan et al., 2004). Effects of
470 paroxetine on circadian rhythms have not been described in mouse models of other neurological
471 diseases. This would be worth studying further.

472 In conclusion, we have shown that abnormalities in MASCO in R6/2 mice can be
473 ameliorated with chronic paroxetine treatment. Our findings suggest that, in addition to the
474 dopaminergic/noradrenergic systems dysfunction previously described, an early abnormality in
475 the serotoninergic system may contribute to the impairment of the MASCO observed in the R6/2
476 mouse. Given the propensity for depression in HD patients, deficits in 5-HT may also be
477 involved in the circadian abnormalities and resulting behavioural disturbances observed in HD
478 patients.

479

480 **Conflict of interest statement**

481 The authors declare that they have no conflict of interest.

482

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485

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802

803 **Legend to figures**

804

805 **Figure 1. Methamphetamine but not cocaine induces a second component of general**
806 **activity with period length > 24 hours in WT mice**

807 Double-plotted actograms (A-F) show rest-activity patterns recorded from WT mice in DD
808 between 6 to 14 weeks (A-C) or from 10 to 19 weeks (D-F) of age. The arrows on the right of the
809 actograms show when mice were given water (A-F; dotted line) or 0.005% methamphetamine
810 (MAP) (B and C; solid line) via the drinking bottle. The arrows with solid line on the right of the
811 actograms D-F show infusion of saline (D) or 30mg/kg cocaine (E and F) via osmotic minipump.
812 The dashed red and solid blue lines within actogram B represents the period length of SCN- and
813 MASCO-driven rhythms, respectively. Actogram B is replotted in C with the x-axis changed to
814 the value of the period length of MASCO-driven rhythms (~ 29 h). Actogram E is replotted in F
815 with the x-axis changed to the value of the period length of SCN-mediated rhythms (~ 23.7 h).
816 Asterisks in D, E & F indicate day of osmotic minipump implantation.

817

818 **Figure 2. Haloperidol blocks diurnal activity but not the expression of the MASCO-driven**
819 **rhythms in WT mice.** Double-plotted actograms (A, C, E and G) show rest-activity patterns in
820 WT mice in DD from 8 to 16 weeks. The arrow on the right of the actograms shows the period
821 during which daily injections of saline (A and E) or haloperidol (C and G) were given. The
822 dotted and the dashed lines on the right of the actograms indicate the period during which mice
823 had water only (A and C) or 0.005% MAP (E and G) to drink. Periodograms (B, D, F and H)
824 with dotted line of significance fixed at $P < 0.001$, were determined for the weeks (w) indicated
825 by the black bar at the left of each corresponding actogram (A, C, E and G).

826

827 **Figure 3. Haloperidol and reserpine both decrease general activity but do not prevent the**
828 **expression of MASCO-driven rhythms.** Scatter plots show activity count during active period

829 (A-D), amplitude of period lengths (E and F) of the rhythms driven by SCN (open squares) or
830 MASCO (filled squares) and values of period lengths of SCN- and MASCO-driven rhythms (G
831 and H) for individual WT mice. Mice were given to drink water only (A and C) or 0.005% MAP
832 (B and D), and received concomitantly either haloperidol (0.5mg/kg; A, B and E) or reserpine
833 (0.1mg/kg; C and D, F) injections. The bars above the x-axis in E and F indicate the
834 experimental weeks with methamphetamine either alone (white) or concomitantly given with
835 treatment of haloperidol or reserpine (grey). *P < 0.05; **P < 0.01; ***P< 0.001. The section
836 sign (§) in E indicates the experimental weeks for which MASCO-driven rhythms were not
837 observed.
838
839 **Figure 4. Reserpine downregulates activity but does not prevent the expression of the**
840 **MASCO rhythms in WT mice.** Double-plotted actograms (A, C, E and G) show rest-activity
841 patterns in WT mice in DD from 18 to 22 weeks. The arrow on the right of the actograms shows
842 the period during which daily injections of saline (A and E) or reserpine (C and G) were given. The
843 dotted and dashed lines on the right of the actograms indicate the period where mice were given
844 water alone (A and C) or 0.005% MAP (E and G) to drink. Periodograms (B, D, F and H) with
845 line of significance fixed at P < 0.001, were determined for the periods (weeks (w) 1, 5)
846 indicated by the black bars at the left of each corresponding actogram.
847
848 **Figure 5. Chronic paroxetine partially restores the expression of the MASCO-driven**
849 **rhythms in R6/2 mice.**

850 Double-plotted actograms (A, C, E and G) show rest-activity patterns in R6/2 mice in DD from 6
851 to 12 weeks. The arrow on the right of the actograms shows the period of the daily injections of
852 saline (A and E) or paroxetine (C and G). The dotted and dashed lines on the right of the
853 actograms indicate the duration of water alone (A and C) or 0.005% methamphetamine treatment
854 (E and G). Periodograms (B, D, F and H) with dotted line of significance fixed at $P < 0.001$,
855 were determined for the weeks (w) indicated by the black bar at the left of each corresponding
856 actogram.

857

858 **Figure 6. Paroxetine prevents the disruption of the MASCO-driven rhythm expression in**
859 **presymptomatic R6/2 mice.** Scatter plots show period lengths of the rhythms driven by SCN or
860 MASCO for individual WT mice treated with water only (A, B) or 0.005% MAP (C, D),
861 concomitantly with saline (A, C) or paroxetine (20mg/kg; B, D) intraperitoneal injections. The
862 bars above the x-axis indicates the experimental weeks during which treatment was with MAP
863 (black) or water (white).

864

865 **Figure 7. Effect of paroxetine on circadian parameters.** Duration of active period (A, B), rest-
866 activity ratio (C, D), activity during the active phase (E, F) and during the rest phase (G, H) are
867 shown for paroxetine/water mice (A, C, E, G), paroxetine/MAP mice (B, D, F, H) and for the
868 respective controls during the 6 weeks of drug treatment (between 6 and 11 weeks of age).
869 Saline/water group is in white histograms, paroxetine/water in black histograms. Saline/MAP
870 group is shown in grey-hatched and paroxetine/MAP group in black-hatched histograms. Data
871 were averaged across 7 days and are presented as means ± SEM. $*P < 0.05$, $**P < 0.01$.

872

873 Supplementary Figure 1. **The effect of cocaine on circadian parameters.** Period length (A),
874 duration of active period (B), general activity (C) and rest-activity ratio (D) are shown for
875 cocaine-treated mice (black histograms) and in saline-treated mice (white histograms) before
876 drug delivery by osmotic minipump (experimental weeks 1-2), during drug delivery (weeks 3-6)
877 and during drug withdrawal (weeks 6-7). Data were averaged across 7 days and are presented as
878 means ± SEM.

879

880 **Table 1. Numbers of WT and R6/2 mice tested in the pharmacological studies**

881

experiment	genotype	saline + water		saline + MAP		drug + water		drug +MAP	
		males	females	males	females	males	females	males	females
cocaine	WT	10	0	0	0	6	0	0	0
haloperidol	WT	2	3	3	2	6	5	4	3
reserpine	WT	2	3	3	2	6	5	4	3
paroxetine	R6/2	3	3	3	3	6	6	6	6

882

883 **Table 2. Effect of cocaine 30mg/kg on circadian parameters of WT mice**

treatment period	treatment group	period length	duration of active period	general activity	rest/activity ratio
		mean ± SEM (number of mice)			
pre-treatment	saline	23.70 ± 0.04 (10)	13.38 ± 0.66 (10)	2800 ± 262 (10)	0.16 ± 0.03 (10)
	cocaine	23.55 ± 0.08 (6)	13.79 ± 0.90 (6)	2775 ± 265 (6)	0.24 ± 0.05 (6)
week 1	saline	23.76 ± 0.07 (10)	14.10 ± 0.47 (10)	3369 ± 199 (10)	0.19 ± 0.02 (10)
	cocaine	23.66 ± 0.08 (6)	14.70 ± 0.72 (6)	3878 ± 572 (6)	0.30 ± 0.05 (6)
week 2	saline	23.66 ± 0.07 (10)	14.09 ± 0.57 (10)	3757 ± 204 (10)	0.25 ± 0.05 (10)
	cocaine	23.61 ± 0.06 (6)	15.80 ± 0.67 (6)	3592 ± 350 (6)	0.23 ± 0.05 (6)
week 3	saline	23.80 ± 0.13 (10)	15.09 ± 0.51 (10)	3170 ± 182 (10)	0.21 ± 0.05 (10)
	cocaine	23.56 ± 0.15 (6)	16.09 ± 0.82 (6)	3434 ± 268 (6)	0.27 ± 0.09 (6)
week 4	saline	23.83 ± 0.08 (10)	14.94 ± 0.60 (10)	3035 ± 213 (10)	0.22 ± 0.08 (10)
	cocaine	23.65 ± 0.04 (6)	15.20 ± 0.69 (6)	3132 ± 318 (6)	0.18 ± 0.06 (6)
washout	saline	23.95 ± 0.12 (10)	14.95 ± 0.63 (10)	2737 ± 226 (10)	0.32 ± 0.08 (10)
	cocaine	23.63 ± 0.02 (6)	15.25 ± 0.91 (6)	2891 ± 412 (6)	0.36 ± 0.12 (6)

884

885

886 **Table 3. Effect of chronic methamphetamine and paroxetine 30mg/kg treatments on period**
887 **length and duration of active period in R6/2 mice**

treatment period	**treatment**	**drug**	**period length (SCN)**	**period length (MASCO output)**	**duration of active period**
			mean ± SEM (number of mice)		
week 1	water	saline	23.61 ± 0.11 (6)	none	14.03 ± 0.26 (6)
	water	paroxetine	23.78 ± 0.07 (12)	none	13.06 ± 0.38 (12)
	MAP	saline	23.61 ± 0.09 (6)	none	13.19 ± 0.70 (6)
	MAP	paroxetine	23.96 ± 0.06 (12)	none	13.44 ± 0.24 (12)
week 2	water	saline	23.64 ± 0.03 (6)	none	13.92 ± 0.43 (6)
	water	paroxetine	23.83 ± 0.03 (12)	none	13.09 ± 0.33 (12)
	MAP	saline	23.72 ± 0.08 (6)	none	13.21 ± 0.74 (6)
	MAP	paroxetine	23.74 ± 0.06 (12)	none	13.00 ± 0.15 (12)
week 3	water	saline	23.62 ± 0.09 (6)	none	12.65 ± 0.66 (6)
	water	paroxetine	23.74 ± 0.05 (12)	none	12.55 ± 0.24 (12)
	MAP	saline	23.62 ± 0.10 (6)	none	13.11 ± 0.74 (6)
	MAP	paroxetine	23.80 ± 0.05 (12)	25.61 ± 0.96 (4)	13.34 ± 0.32 (12)
week 4	water	saline	23.50 ± 0.18 (6)	none	14.30 ± 0.20 (6)
	water	paroxetine	23.69 ± 0.07 (12)	none	13.03 ± 0.29 (12)
	MAP	saline	23.53 ± 0.15 (6)	none	14.42 ± 0.62 (6)
	MAP	paroxetine	23.75 ± 0.06 (12)	25.31 ± 0.62 (4)	14.37 ± 0.33 (12)
week 5	water	saline	23.57 ± 0.19 (6)	none	14.79 ± 0.44 (6)
	water	paroxetine	23.65 ± 0.08 (12)	none	13.43 ± 0.21 (12)
	MAP	saline	23.52 ± 0.16 (6)	none	15.19 ± 0.98 (6)
	MAP	paroxetine	23.76 ± 0.08 (12)	25.83 ± 0.62 (5)	15.08 ± 0.41 (12)
week 6	water	saline	23.46 ± 0.16 (6)	none	14.41 ± 0.61 (6)
	water	paroxetine	23.64 ± 0.13 (11)	none	13.54 ± 0.32 (12)
	MAP	saline	23.29 ± 0.16 (6)	none	14.48 ± 1.06 (6)
	MAP	paroxetine	23.67 ± 0.06 (12)	27.74 ± 1.92 (4)	14.44 ± 0.38 (12)

888

889 **Table 4. Effect of chronic methamphetamine and paroxetine 30mg/kg treatments on**
890 **general activity in R6/2 mice**

treatment period	treatment	drug	activity (active period)	activity (rest period)	rest/activity ratio
			mean ± SEM (number of mice)		
week 1	water	saline	1734 ± 329 (6)	543 ± 125 (6)	0.33 ± 0.07 (6)
	water	paroxetine	1012 ± 68 (12)	377 ± 52 (12)	0.39 ± 0.06 (12)
	MAP	saline	1498 ± 231 (6)	366 ± 43 (6)	0.26 ± 0.02 (6)
	MAP	paroxetine	1047 ± 125 (12)	359 ± 43 (12)	0.39 ± 0.06 (12)
week 2	water	saline	2341 ± 501 (10)	369 ± 84 (6)	0.17 ± 0.03 (6)
	water	paroxetine	1165 ± 167 (12)	240 ± 47 (12)	0.22 ± 0.03 (12)
	MAP	saline	1821 ± 261 (6)	252 ± 44 (6)	0.14 ± 0.02 (6)
	MAP	paroxetine	1105 ± 173 (12)	201 ± 32 (12)	0.22 ± 0.04 (12)
week 3	water	saline	2726 ± 470 (6)	306 ± 55 (6)	0.13 ± 0.03 (6)
	water	paroxetine	1388 ± 167 (12)*	211 ± 24 (12)	0.19 ± 0.05 (12)
	MAP	saline	2481 ± 534 (6)	306 ± 46 (6)	0.14 ± 0.04 (6)
	MAP	paroxetine	2265 ± 453 (12)	223 ± 30 (12)	0.13 ± 0.02 (12)
week 4	water	saline	2410 ± 334 (6)	252 ± 47 (6)	0.10 ± 0.02 (5)
	water	paroxetine	1218 ± 165 (12)	158 ± 23 (12)	0.14 ± 0.02 (12)
	MAP	saline	2659 ± 619 (6)	252 ± 51 (6)	0.10 ± 0.01 (6)
	MAP	paroxetine	1882 ± 360 (12)	159 ± 24 (12)	0.10 ± 0.01 (12)
week 5	water	saline	2701 ± 547 (6)	451 ± 194 (6)	0.17 ± 0.08 (6)
	water	paroxetine	1108 ± 193 (12)	137 ± 15 (12)	0.14 ± 0.02 (12)
	MAP	saline	2008 ± 279 (6)	208 ± 54 (6)	0.11 ± 0.01 (6)
	MAP	paroxetine	1383 ± 266 (12)	202 ± 30 (12)	0.17 ± 0.02 (12)
week 6	water	saline	1901 ± 460 (6)	521 ± 226 (10)	0.32 ± 0.13 (6)
	water	paroxetine	922 ± 173 (12)**	194 ± 33 (12)	0.27 ± 0.07 (12)
	MAP	saline	1327 ± 430 (6)	254 ± 48 (6)	0.24 ± 0.05 (6)
	MAP	paroxetine	960 ± 225 (11)	181 ± 39 (11)	0.28 ± 0.08 (11)

891 **$P < 0.01$ compares paroxetine/ water to saline/water group
892 No significant differences were observed between paroxetine/MAP and water/MAP group

893 **ABBREVIATIONS**

894 ANOVA – analysis of variance

895 DA – dopamine

896 DAT – dopamine transporter

897 DD – dark-dark (constant darkness)

898 FEO – food-entrainable oscillator

899 HD – Huntington's disease

900 LD – light-dark

901 MASCO – methamphetamine-sensitive circadian oscillator

902 MAP – methamphetamine

903 NA – noradrenaline

904 NAc – nucleus accumbens

905 SCN – suprachiasmatic nucleus

906 SERT – serotonin transporter

907 SSRI – selective serotonin reuptake inhibitor

908 WT – wild type

909 5-HT – serotonin