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1 **Dopamine microinjection into the rat subthalamic nucleus produces a**

2 **conditioned place preference that is modulated by oxytocin**

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Abstract

The subthalamic nucleus (STh) is increasingly recognised as an important region involved in the motivation for drug reward. It is not yet known if dopamine, the neurotransmitter primarily responsible for reward signaling, produces a rewarding effect when microinjected into the STh. Further, there is evidence to suggest that the neuropeptide oxytocin acts within the STh to reduce drug reward. However, a direct interaction between dopamine and oxytocin in the STh has yet to be explored. The current study aimed to determine (i) whether dopamine microinjected into the STh would result in a significant place preference following a single-trial conditioning session, (ii) whether co-administered oxytocin would block the formation of a conditioned place preference (CPP) to dopamine administration, and (iii) whether the selective oxytocin antagonist desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT, when co-administered with oxytocin and dopamine, would reverse the effects of oxytocin and result in a CPP for dopamine administration. Results showed that male Sprague Dawley rats i) formed a preference for the context paired with dopamine (100 nmol/side) administration into the STh ii) that the co-administration of oxytocin (0.6 nmol/side) with dopamine prevented the formation of a CPP, and that a microinjection of the oxytocin antagonist (3 nmol/side) in addition to dopamine and oxytocin resulted in the formation of a CPP. This suggests that dopamine administration into the STh produces rewarding effects that can be reduced by activation of local oxytocin receptors.

Keywords: dopamine, oxytocin, conditioned place preference, subthalamic nucleus, reward

38

39 ## Introduction

40 The subthalamic nucleus (STh) has traditionally been considered a key brain
41 region modulating basal ganglia motor circuitry (Baunez and Lardeux, 2011; Janssen
42 et al., 2012; Tofighy et al., 2003). More recently, this region has been recognized for
43 its involvement with motivational aspects of natural and drug reward (Baunez and
44 Lardeux, 2011). Both bilateral lesions and deep brain stimulation of the STh reduce
45 the reinforcing effects of ethanol and cocaine and increase the motivation for food as
46 measured through the conditioned place preference (CPP) and self-administration
47 paradigms (Baunez et al., 2005; Lardeux and Baunez, 2008; Rouand et al., 2010).
48 The role of dopamine neurotransmission in reward has long been implicated
49 and demonstrated by the self-administration of D_1- and D_2- like receptor agonists
50 (Self and Stein, 1992; Sinnott et al., 1999; Woolverton et al., 1984), the formation of a
51 CPP to systemically administered D_1-like receptor agonists (Abrahams et al., 1998)
52 and the prevention of forming a place preference for amphetamine through pre-
53 treatments of D_1- and D_2- like receptor antagonists (Bardo et al., 1999). In addition,
54 dopaminergic neurons encode reward-related information (Di Chiara, 1995). As STh
55 neurons are also involved in coding reward magnitude and reward-related predictions
56 (Darbaky et al., 2005; Lardeux et al., 2009), and are innervated by midbrain dopamine
57 neurons, it seems likely that STh neuronal activity is modulated by dopamine
58 signaling (Di Chiara, 1995; Lardeux et al., 2009). However a direct effect of
59 dopamine microinjection into the STh on the modulation of reward behaviour has yet
60 to be shown.

61 The neuropeptide oxytocin has been suggested as a potential novel
62 pharmacotherapy for drug dependence. Oxytocin administration modulates the

63 rewarding effects and abuse potential of various illicit drugs, one of which being
64 methamphetamine (METH)(Baracz et al., 2012; Carson et al., 2010a; Carson et al.,
65 2010b; Cui et al., 2001; Kovacs et al., 1985a; Kovacs et al., 1985b; Kovacs et al.,
66 1990; Qi et al., 2009; Qi et al., 2008; Sarnyai et al., 1991). More recently, we have
67 shown that systemically administered oxytocin reduced METH-induced Fos
68 expression in the STh (Carson et al., 2010b) and oxytocin microinjected into this
69 region reduced the formation of a conditioned place preference (CPP) for METH
70 (Baracz et al., 2012). These studies highlight the involvement of oxytocin in reducing
71 drug reward and the involvement of the STh in this process.

72 The ability of oxytocin to attenuate drug-related reward is thought to be
73 through the modulation of dopamine neurotransmission (McGregor and Bowen, 2012;
74 McGregor et al., 2008; Qi et al., 2008; Qi et al., 2009; Yang et al., 2010). Oxytocin
75 and dopamine interact to regulate a number of socio-affiliative behaviours in addition
76 to drug reward. Such behaviours include pair bonding (Liu and Wang, 2003),
77 maternal behaviour (Shahrokh et al., 2010), social memory (Ferguson et al., 2000),
78 and sexual behaviour (Baskerville et al., 2009; Succu et al., 2007). This interaction
79 between oxytocin and dopamine can either be facilitatory or inhibitory, depending on
80 the behaviour (Baskerville and Douglas, 2010) and the brain regions involved
81 (Kovacs et al., 1990).

82 The exact mechanisms by which oxytocin and dopamine interact to reduce
83 drug reward are not well understood. In the STh in particular, very limited research
84 into the function of dopamine and oxytocin has been conducted. Dopamine terminals,
85 as well as D_1 and D_2 receptors have been located within this region (Boyson et al.,
86 1986; Hassani et al., 1997; Johnson et al., 1994), although, dopamine has not been
87 independently investigated for its rewarding effects in the STh. Oxytocin containing

88 cells of the supraoptic nucleus (SON) and paraventricular hypothalamic nucleus
89 (PVN) are known to release oxytocin by volume transmission, affecting diverse
90 midbrain and forebrain areas (McGregor, 2008). In addition, the PVN provides
91 classical synaptic transmission to forebrain areas (McGregor, 2008), however it is not
92 known if these cell project to the STh. It is known that oxytocin receptor mRNA is
93 expressed in STh neurons (Vaccari et al., 1998), yet the lack of visualization of
94 oxytocin receptors in this area limits our knowledge of the existence of these
95 receptors and how these are situated on STh neurons to interact with dopamine
96 neurotransmission.
97 The purpose of the present study was to investigate the possible interaction
98 between dopamine and oxytocin in mediating reward behaviour in the STh using a
99 single-trial CPP paradigm. Firstly, we examined whether dopamine microinjected into
100 the STh would result in a significant place preference. Secondly, we looked at
101 whether co-administration of oxytocin with dopamine would block the formation of a
102 CPP and thirdly we examined the effect of antagonizing the effect of oxytocin at the
103 oxytocin receptor on the formation of CPP produced by dopamine administration.
104
105 **Materials and methods**
106 **Animals**
107 Ninety male Sprague Dawley rats (weighing 200-250 g) were obtained from
108 the Animal Research Centre (Perth, WA, Australia). Rats were housed in pairs (cage
109 size: 40 x 27 x 16 cm) with the exception of a two-day postoperative period of
110 individual housing. Food and water were available *ad libitum* in the home cages and
111 not during experimental procedures. Lighting was kept on a 12-hour light/dark cycle
112 (lights on 06:00), with all experiments conducted during the light cycle. Housing
113 room temperature was maintained at 21°C (±1°C). Prior to the start of

114 experimentation, rats were acclimatized to the facility for seven days and were
115 handled daily for a further seven days. All experimental procedures were conducted in
116 accordance with the Australian Code of Practice for the Care and Use of Animals for
117 Scientific Purposes (7th edition, 2004) and were approved by the Macquarie
118 University Animal Ethics Committee.
119
120 **Drugs**
121 Dopamine hydrochloride (DA) was obtained from Sigma Aldrich (Castle Hill,
122 NSW, Australia). Oxytocin (OXY) was synthesized by AusPep Ltd (Parkville, VIC,
123 Australia). The selective oxytocin receptor antagonist desGly-NH_2,d$(CH_2)_5$[D-
124 Tyr^2,Thr^4]OVT was a gift from Dr. Maurice Manning (Department of Biochemistry
125 and Cancer Biology, The University of Toledo, USA). All drugs were dissolved in
126 saline (0.9%) for injection purposes with the OXY and cocktail solutions freshly
127 prepared for each conditioning day. Vehicle (VEH) administration was a 0.9% saline
128 solution.
129
130 **Apparatus**
131 The CPP apparatus used were as described previously (Baracz et al., 2012).
132 The three chambers consisted of three compartments separated by two removable
133 guillotine doors. The two side compartments were distinguished by distinct odour
134 cues, these being drops of essential oils (Sunspirit aromatherapy oils) in small caps
135 placed on a tray under the opposite far corner of each compartment. Frankincense oil
136 was placed under one compartment and rosewood oil under the other compartment.
137 Infrared cameras were positioned above each compartment and were used to film

138 activity. Locomotor activity and the time spent in each compartment was recorded by
139 automated video tracking software (Motion Mensura, Cooks Hill, NSW, Australia).
140
141 **Surgery**
142 Rats were anaesthetised with isoflurane gas (3% in oxygen 2L/min) and
143 placed in a stereotaxic apparatus for bilateral implantation of guide cannulae (26
144 gauge; 15 mm) to 1mm above the STh (with nosebar = -3.3 mm, measured from
145 bregma: anterior/posterior, -3.8 mm; lateral, +2.5 mm; dorsal/ventral, -7.0 mm) as
146 previously described (Baracz et al., 2012). Co-ordinates were adapted from the rat
147 brain atlas of Paxinos and Watson (1997). For analgesia, rats were administered with
148 Carpofen (5 mg/kg) subcutaneously (s.c.) at the time of surgery and daily for the
149 following two days. Rats were allowed 5-7 days to recover before experimentation
150 began.
151
152 **Microinjection Procedure**
153 Rats were randomly allocated to one of six treatment groups (n = 15 per
154 group): 1) dopamine (DA; 100 nmol/side), 2) co-administered dopamine and oxytocin
155 (DA+OXY; 0.6nmol/side), 3) the low oxytocin receptor antagonist dose (LOW ANT;
156 1 nmol/side), 4) the high oxytocin receptor antagonist dose (HIGH ANT; 3nmol/side),
157 and co-administered dopamine and oxytocin with the addition of 5) the low oxytocin
158 receptor antagonist dose (COCKTAIL 1) or 6) the high oxytocin receptor antagonist
159 dose (COCKTAIL 2).
160 The dose of dopamine examined in this study was extrapolated from a known
161 pharmacologically effective dose in the nucleus accumbens (Cornish and Kalivas,
162 2000). The oxytocin dose was based on our previous study examining oxytocin in the

163 STh (Baracz et al., 2012). As the oxytocin receptor antagonist has not, to our
164 knowledge, been examined in the STh, the low dose was determined from published
165 studies that microinjected the antagonist into other brain regions (Yang et al., 2011)
166 and a logarithmic scale was used to determine the high dose.

167 Rats received a bilateral infusion of treatment or VEH into the STh at a
168 volume of 200 nl/side. Both microinjectors (33 gauge; 16 mm) were attached by
169 polyethylene tubing to a 1 µl Hamilton syringe with infusions being driven by a
170 microinjection pump (Harvard Apparatus, USA). The microinjectors remained in
171 position 30 s after the completion of the microinjection to ensure the entire dose had
172 infused into the brain region.

173

174 **Conditioned Place Preference (CPP) procedure**

175 The CPP procedure (as previously described in (Baracz et al., 2012)),
176 consisted of a pre-test, conditioning and post-test.

177 **Pre-test:** Rats were placed in the central compartment and were able to freely
178 explore the entire apparatus for 15 min. The odour cues were counterbalanced across
179 side compartments for all treatment groups. Time (s) spent in each compartment was
180 recorded and if, after testing, a large preference was apparent for one compartment,
181 rats were retested up to a maximum of three times until a lesser preference was
182 evident. Thus, rats were retested if their bias was greater than 120 s or if the time
183 spent in the central compartment was more than double the time spent in the two side
184 compartments combined. Typically, most rats reached criterion following the first
185 pre-test session with 25% of the rats requiring testing on the second pre-test and 15%
186 on the third pre-test. These rats were evenly distributed across treatment groups.

187 **Conditioning:** Conditioning sessions began 24 h following the last pre-test
188 day. Two conditioning sessions were conducted (once daily for two consecutive days).
189 On each conditioning day, rats received either a VEH or treatment microinjection
190 with this order counterbalanced such that half the animals received conditioning with
191 one of the treatments and half received VEH on each conditioning day. To prevent
192 any association between the conditioning compartment and the microinjection
193 procedure, and to capture the peak effective period of the treatments administered, the
194 microinjection was administered 5 minutes prior to being placed in a conditioning
195 compartment. After this time, rats were confined to the designated conditioning
196 compartment (VEH or treatment) for 30 min.
197 **Post-test:** Forty-eight h following the last conditioning session for each
198 experimental condition, the post-test was conducted. Rats, in a drug-free state, were
199 placed in the central compartment and were given free access to the CPP apparatus
200 for 15 min. Time (s) spent in each compartment was recorded.
201
202 **Histology**
203 Following the completion of the experiments, rats were deeply anaesthetised with
204 sodium pentobarbitone (135 mg in 1 ml, i.p.) and underwent intracardiac perfusion
205 with 50 ml of 0.9% saline followed by 50 ml of 10% formalin. Brains were extracted,
206 post-fixed in a 10% formalin solution for seven days, and sliced into 60 mm thick
207 coronal sections using a cryostat. Sections were mounted on gel slides. The rat brain
208 atlas of Paxinos and Watson (1997) was used to verify cannulae placement. Only data
209 from rats with correct cannulae placements were analysed.
210
211 **Statistical analysis**

212 Data are presented as the mean ± the standard error of the mean (SEM). CPP
213 was assessed as the difference in time (s) spent in the treatment-paired compartment
214 from pre to post-test in comparison to the difference in time spent in the VEH-paired
215 compartment from pre to post-test. This was analysed using a two-tailed paired
216 samples t-test (Baracz et al., 2012; Herzig and Schmidt, 2004; Tzschentke, 2004).
217 Therefore a significant difference between the shift in time spent in each side
218 compartment, where more time was spent in the treatment-paired compartment post
219 conditioning, indicated that treatment was rewarding and that a CPP had developed.
220 Locomotor activity during conditioning sessions in each experiment was
221 analysed using a mixed analysis of variance (ANOVA) model, with treatment as the
222 between-subjects factor and conditioning days as the within-subjects factor. This
223 ANOVA was followed by post-hoc pair-wise comparisons among the treatment
224 conditions using Bonferroni corrections.
225 Statistical analysis was undertaken using SPSS 19 Graduate Student Version
226 for Mac. Statistical significance was set at $P < 0.05$ for all statistical tests, and for post
227 hoc tests, statistical significance was set in accordance to the Bonferroni decision rule
228 at $P < 0.0083$.
229
230 **Results**
231
232 **Locomotor activity**
233 As shown in Fig. 1, locomotor activity was not altered by the administration of the
234 treatment or vehicle microinjection across all of the treatment groups during the
235 conditioning sessions. Analysis of locomotor activity on the conditioning days
236 showed no significant difference between treatment and vehicle conditioning days
237 ($F(1,37)$ 1.037, $p = 0.315$), a significant group effect ($F(3,37)$ 2.878, $p = 0.049$) and

238 no significant conditioning day x treatment interaction (F(3,37) 0.171, p = 0.915) .
239 Post-hoc pairwise comparisons revealed that there was no difference in locomotor
240 activity following treatment administration across the groups (DA vs. DA+OXY, p =
241 0.074; DA vs. COCKTAIL 1, p = 0.026; DA vs. COCKTAIL 2, p = 0.799; DA+OXY
242 vs. COCKTAIL 1, p = 0.905; DA+OXY vs. COCKTAIL 2, p = 0.115; COCKTAIL 1
243 vs. COCKTAIL 2, p = 0.050).

244 A paired samples t-test indicated no significant difference in locomotor
245 activity for the rats in the LOW OXY ANTAG and the HIGH OXY ANTAG groups
246 (t(11) -1.921, p = 0.081; t(10) -1.765, p = 0.108, respectively).

247 **Insert figure 1 here**

248 **Conditioned place preference**

249 The DA group exhibited a significant preference for the treatment-paired
250 compartment in relation to the vehicle-paired compartment from pre to post-test (t(10)
251 = 3.201, p = 0.009; Fig. 2). A preference for the treatment-paired compartment was
252 not evident in the DA+OXY group (t(10) = 0.951, p = 0.364).

253 The COCKTAIL 1 group did not exhibit a significant preference for the
254 treatment-paired compartment relative to the vehicle-paired compartment from pre to
255 post-test (t(11) = 0.492, p = 0.632). The COCKTAIL 2 group did display a significant
256 preference for the treatment-paired compartment relative to the vehicle-paired
257 compartment from pre to post-test (t(6) = 2.898, p = 0.027).

258 Both the low and the high oxytocin antagonist groups did not display a
259 significant place preference for the treatment-paired compartment relative to the
260 vehicle-paired compartment from pre to post-test (t(11) = -1.527, p = 0.155; t(10) =
261 0.968, p = 0.356, respectively). The data trends towards a dose-dependent effect of
262 producing a reward association to antagonist treatment, with the low dose unable to

263 shift the bias of the preference test and the high dose tending to encourage more time
264 spent in the treatment-paired compartment (*M* = 38.2, *SE* = 26.5) than in the vehicle-
265 paired compartment (*M* = -11.7, *SE* = 36.2) from pre to post-test.

266 **Insert figure 2 here**

267 **Histological verification**

268 Figure 3 illustrates the correctly located cannulae in the STh. Rats were removed if
269 the cannulae were not bilaterally located within this brain region. Due to the small
270 brain area this resulted in a total of 26 excluded rats, producing sample sizes of DA =
271 11, DA + OXY = 11, COCKTAIL 1 = 12, COCKTAIL 2 = 7, LOW OXY ANTAG =
272 12, HIGH OXY ANTAG = 11). There were 7 animals that received dopamine
273 microinjections dorsal to the STh into the zona incerta. Dopamine administration
274 dorsal to the STh did not result in a CPP ($t(6) = -0.642$, $p = 0.544$; treatment-paired
275 *M* = -19.9, *SE* = 26.9, saline-paired *M* = 12.0, *SE* = 47.1).

276 **Insert figure 3 here**

277 **Discussion**

278 The aim of the present study was to investigate: (i) the effect of a dopamine
279 microinjection in the STh on the formation of a CPP using a single-trial conditioning
280 procedure, (ii) the effect of co-administration of oxytocin with dopamine on the
281 acquisition of a CPP, and (iii) the effect of microinjecting a cocktail of the oxytocin
282 antagonist, oxytocin and dopamine on the formation of a CPP. We determined that
283 using a single-trial conditioning session, dopamine microinjection in the STh resulted
284 in the formation of a place preference. We also identified that the co-administration of
285 oxytocin and dopamine in the STh prevented the formation of the CPP to dopamine
286 administration. In addition, we showed a dose-dependent effect of the oxytocin
287 antagonist on the formation of a CPP when co-administered with dopamine and

288 oxytocin. The low dose of the antagonist did not alter the attenuating effects of
289 oxytocin on dopamine treatment, resulting in the prevention of a place preference. In
290 contrast, the high dose antagonist appeared to reduce the effect of oxytocin and
291 permitted the formation of a CPP to dopamine administration. It is also of interest that
292 locomotor activity was not affected by any of the treatment conditions.

293 Dopamine is well described for regulating motor control (Ikemoto, 2010). As
294 D_1 and D_2 receptors have been identified in the STh (Boyson et al., 1986; Johnson et
295 al., 1994) and this nucleus plays a crucial role in movement disorders such as
296 Parkinson's disease (Coyle and Snyder, 1969; Greer and Williams, 1963), it would
297 seem likely that local administration of dopamine into this nucleus would alter
298 locomotor activity in some way. However, we found no difference in locomotor
299 activity following dopamine administration into the STh when compared to vehicle
300 controls.

301 The literature on dopamine activity in the STh is sparse, and largely consists
302 of electrophysiological studies. The local application of dopamine agonists or
303 iontophoretic stimulation of D_2 receptors in the STh has produced inconsistent
304 findings, where either firing patterns did not change (Kreiss et al., 1997), firing rates
305 were inhibited (Hassani and Feger, 1999), or firing rates were increased in intact rats
306 (Mintz et al., 1986). As the association between neuronal activity and motor responses
307 is not as strong as previously thought (Wilson and Bevan, 2011), and none of the
308 aforementioned studies examined behavioural responsivity, it is difficult to discern
309 what changes in locomotor activity, if any, would have occurred. Research involving
310 lesions in regions connected to the STh however, have determined that ablations to
311 the striatum or substantia nigra impact on dopamine neuronal firing in the STh in
312 addition to other basal ganglia regions and the motor system, to alter motor output

313 (Hassani and Feger, 1999; Janssen et al., 2012; Lintas et al., 2012). Together this
314 suggests that the STh is not solely involved in motor co-ordination, but interacts with
315 other regions to produce a behavioural outcome.

316 Dopamine is strongly associated with reward-related learning (Hyman et al.,
317 2006; Koob, 2009; Schultz, 2000) and is primarily involved with the rewarding
318 effects of psychostimulants such as METH (Cruickshank and Dyer, 2009; Elkashef et
319 al., 2008). Again, the effect of dopamine on reward has not been previously examined
320 within the STh. Our study showed that a CPP formed following a microinjection of
321 dopamine into the STh, suggesting that increases in dopamine activity in the STh
322 produces a rewarding effect. However, we have not directly assessed the involvement
323 of dopamine receptors in the CPP to microinjected dopamine. It is important to
324 mention that while dopamine administration to the STh may produce a CPP, we
325 cannot discount any non-selective effects that may have occurred. However, we have
326 demonstrated that the co-administration of oxytocin prevented the formation of a CPP
327 to dopamine microinjected into the STh. Using exactly the same procedures, we have
328 recently determined that an independent microinjection of oxytocin into the STh did
329 not alter baseline preferences (see Baracz et al., 2012). This suggests that oxytocin is
330 not producing an aversive outcome to negate dopamine administration, but rather is
331 inhibiting the behavioural effects elicited by dopamine treatment. A possible
332 dopamine/oxytocin interaction supports previous postulations that oxytocin modulates
333 METH-related reward through reductions in dopamine neurotransmission (McGregor
334 and Bowen, 2012; McGregor et al., 2008; Qi et al., 2008; Yang et al., 2010). It is also
335 known that oxytocin may independently increase reward associations, however this
336 effect appears to be regionally specific (Kovacs et al., 1990), with systemic
337 administration enhancing reward (Baracz et al., 2012), and intracerebroventricular or

338 local microinjection of oxytocin into the STh or nucleus accumbens having no effect
339 in the place preference paradigm (Baracz et al., 2012, Qi et al., 2009).

340 To gain a greater understanding of a possible interaction between oxytocin and
341 dopamine in modulating reward in the STh, we co-administered the selective oxytocin
342 receptor antagonist desGly-NH_2,d(CH_2)$_5$[D-Tyr^2,Thr^4]OVT. This oxytocin receptor
343 antagonist is 95 times more selective for the oxytocin receptor than for the V1a
344 receptor (Manning et al., 2008). For this reason, it has been used to examine the role
345 of the oxytocin receptor in numerous behaviours such as pain modulation (Yang et al.,
346 2011) and anxiety (Figueira et al., 2008). However, the oxytocin receptor antagonist
347 has not been previously studied in relation to drug reward and abuse.

348 The inclusion of the oxytocin receptor antagonist in our study produced an
349 interesting outcome when co-administered with dopamine and oxytocin. The low
350 oxytocin receptor antagonist dose in COCKTAIL 1 was not sufficient to alter the
351 attenuating effect of oxytocin on the CPP produced by dopamine administration. The
352 higher dose in COCKTAIL 2, however, blocked oxytocin activity, resulting in a
353 preference for dopamine treatment that appeared greater than when dopamine was
354 solely administered. It is possible that a tonic level of endogenous oxytocin is present
355 in the STh, which could have reduced the rewarding effect of independently
356 administered dopamine. A tonic level of oxytocin would also contribute to the
357 ineffectiveness of the low oxytocin receptor antagonist dose on combined dopamine
358 and oxytocin administration. Furthermore, the robust rewarding effect of COCKTAIL
359 2 may be the result of enhancing the effect of administered dopamine, through high
360 dose antagonism of both exogenous and endogenous oxytocin. This possibility is also
361 consistent with the effect of oxytocin antagonist administration alone, as there was a

362 trend towards a significant place preference when the high dose was administered, yet
363 not with low dose antagonist administration.

364 The oxytocin receptor has been identified in numerous brain regions, however,
365 only oxytocin receptor mRNA expression has been reported in the STh (Vaccari et al.,
366 1998), which does not assure that functioning oxytocin receptors are situated within
367 this brain region. If the oxytocin receptor is acting within this region, it may be of low
368 affinity and has not yet been detected by traditional methods such as autoradiography
369 (Freund-Mercier et al., 1988). Indeed, our results demonstrate that within the STh, the
370 high dose of a highly selective oxytocin receptor antagonist blocked oxytocin activity,
371 suggesting that oxytocin receptor activation reduces the CPP to dopamine
372 administration in this region. As the oxytocin receptor has not been visualized on STh
373 neurons, it is unclear if they are located pre- or post-synaptically, to inform how
374 oxytocin modulates this interaction with dopamine administration. In addition to the
375 OT oxytocin receptor, it has been proposed that a further oxytocin receptor subtype
376 exists, and it is not yet known if either subtype is present in the STh (Adan et al.,
377 1995; Chan et al., 2003). Future studies will importantly characterise oxytocin
378 neurotransmission in the STh, and how this may interact with dopamine receptors,
379 including the use of specific receptor ligands across a range of doses.

380 The ability for oxytocin to reduce METH-related reward, METH-induced
381 hyperactivity and relapse to METH-seeking behaviour highlights its potential as a
382 pharmacological treatment for METH abuse. The ability for oxytocin to modulate
383 dopamine reward in the absence of reducing motivational behaviours (Gordon et al.,
384 2011; Gordon et al., 2010; Melis and Argiolas, 2011) further highlights this peptide as
385 a pharmacological treatment for the effective treatment of drug abuse (Izzo et al.,
386 2001; Kovacs et al., 1990; Velazquez-Sanchez et al., 2011). As oxytocin also reduces

387 the behavioural effects of other psychostimulants such as cocaine (Kovacs et al.,
388 1990; Sarnyai et al., 1991), reduces cannabis withdrawal symptoms (Cui et al., 2001)
389 and physical tolerance and dependence on morphine in rodents (Kovacs et al., 1985b),
390 its applicability as a pharmacotherapy extends beyond METH to include other drugs
391 of abuse. Additionally, we (Baracz et al., 2012) and others (Qi et al., 2009) have
392 previously shown that oxytocin, when administered alone via a central route, does not
393 produce a rewarding effect, further emphasizing the potential of oxytocin as an
394 intranasally administered pharmacotherapy. Oxytocin has already been used pre-
395 clinically as an effective intranasal treatment in human populations, largely examining
396 the involvement of oxytocin in stress responses in drug dependent individuals. A
397 number of clinical trials are listed on the National Institute of Health Clinical Trials
398 registry (USA) and the Australian New Zealand Clinical Trials registry.

399 In addition to an examination of the applicability of oxytocin as a
400 pharmacotherapy, the involvement of the STh in the effect of oxytocin on METH-
401 related reward, and addiction in general, should be investigated further. This region
402 has recently been considered an input structure of the basal ganglia due to the direct
403 projections it receives from a number of substrates (Baunez and Lardeux, 2011).
404 Additionally, some of the connections are to regions associated with reward,
405 including the prefrontal cortex, ventral pallidum, nucleus accumbens and midbrain
406 dopamine nuclei (Lardeux et al., 2009). It is thought that the STh integrates
407 information received from these regions, and modulates the output of the reward
408 system depending on the salience and nature of the reward (Baunez and Lardeux,
409 2011). This highlights the involvement of the STh in motivational processes, which is
410 typically considered a frontal function, and its critical role in complex output

411 modulation. Thus a potential central role for the STh in reward and addiction needs to
412 be clarified.

413 In conclusion, the present study showed that the local application of dopamine
414 into the STh elicits a place preference that is blocked by co-administered oxytocin,
415 acting at local oxytocin receptors. This suggests that the oxytocin receptor is present
416 in the STh and also supports important roles for dopamine, oxytocin and the STh in
417 the modulation of drug-related reward.

418

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424

425

Figure Captions

Fig 1. Effect of single-trial treatment conditioning on locomotor activity. (DA, n = 11; DA+OXY, n = 11; COCKTAIL 1, n = 12; COCKTAIL 2, n = 7; LOW OXY ANTAG, n = 12; HIGH OXY ANTAG, n = 11). Locomotor activity was recorded during the 30 minute conditioning sessions and is expressed as the mean ± SEM. * $P < .05$ difference in locomotor activity relative to the control group.

Fig 2. Effect of single-trial treatment conditioning on CPP for dopamine (DA, n = 11; DA+OXY, n = 11; COCKTAIL 1, n = 12; COCKTAIL 2, n = 7; LOW OXY ANTAG, n = 12; HIGH OXY ANTAG, n = 11). Place preference was determined as the difference in time (seconds) spent in the treatment and VEH-paired compartments from pre to post-test (mean ± SEM). Time spent in the middle compartment has been omitted, as there was no significant difference between testing days. * $P < 0.05$, ** $P < .005$, treatment-paired compartment vs. VEH-paired compartment.

Fig 3. Anatomical coronal diagrams depicting the microinjection sites in the STh. Sites for each treatment group were equally distributed across the AP axis. The numbers to the left of the image depict the distance in mm from bregma.

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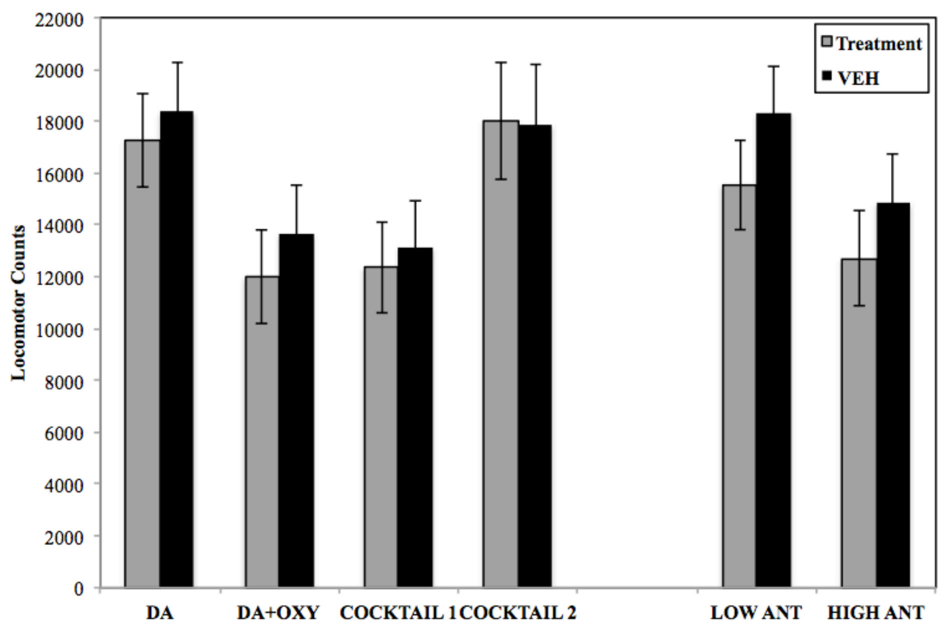

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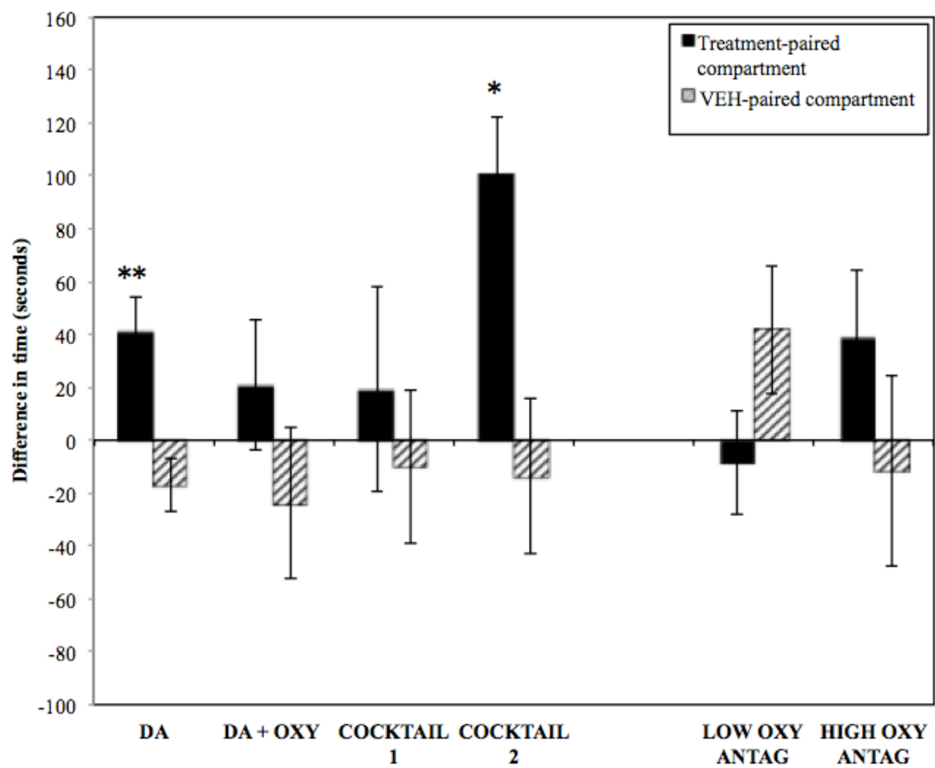

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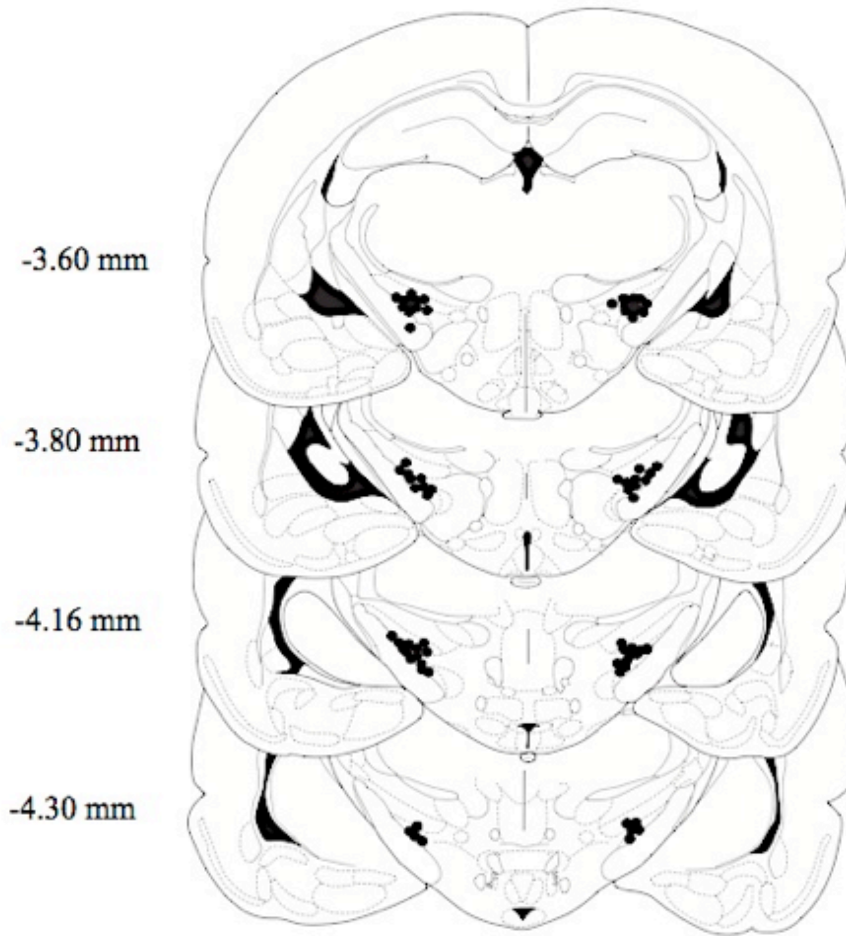
666 Figure 3



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