Original Article

Oxytocin attenuates phencyclidine hyperactivity and increases social interaction and nucleus accumbens dopamine release in rats

Running Title: Oxytocin enhances social behavior and accumbens dopamine

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Abbreviations: ANOVA (analysis of variance); aCSF (artificial cerebrospinal fluid); BBB (blood brain barrier); L-368,899 hydrochloride ((2S)-2-Amino-N-[(1S,2S,4R)-7,7-dimethyl-1-[[[4-(2-methylphenyl)-1-piperazinyl]sulfonyl]methyl]bicyclo[2.2.1]hept-2-yl]-4-(methylsulfonyl)butanamide); NAc (nucleus accumbens); PCP (phencyclidine); PFC (prefrontal cortex); SR49059 ((2S)-1-[[(2R,3S)-5-Chloro-3-(2-chlorophenyl)-1-[(3,4-dimethoxyphenyl)sulfonyl]-2,3-dihydro-3-hydroxy-1H-indol-2-yl]carbonyl]-2-pyrrolidinecarboxamide); ventral tegmental area (VTA); 5-HT (5-hydroxytryptamine, serotonin).

ABSTRACT

The pituitary neuropeptide oxytocin promotes social behavior, and is a potential adjunct therapy for social deficits in schizophrenia and autism. Oxytocin may mediate pro-social effects by modulating monoamine release in limbic and cortical areas, which was investigated herein using *in vivo* microdialysis, after establishing a dose that did not produce accompanying sedative or thermoregulatory effects that could concomitantly influence behavior. The effects of oxytocin (0.03-0.3mg/kg s.c.) on locomotor activity, core body temperature and social behavior (social interaction and ultrasonic vocalisations) were examined in adult male Lister-hooded rats, using selective antagonists to determine the role of oxytocin and vasopressin V_{1a} receptors. Dopamine and serotonin (5-HT) efflux in the prefrontal cortex (PFC) and nucleus accumbens (NAc) of conscious rats were assessed using microdialysis. 0.3mg/kg oxytocin modestly reduced activity and caused hypothermia but only the latter was attenuated by the V_{1a} receptor antagonist, SR49059 (1mg/kg i.p.). Oxytocin at 0.1mg/kg, which did not alter activity and had little effect on temperature, significantly attenuated phencyclidine (PCP)-induced hyperactivity and increased social interaction between unfamiliar rats without altering the number or pattern of ultrasonic vocalisations. In the same rats, oxytocin (0.1 mg/kg) selectively elevated dopamine overflow in the NAc, but not PFC, without influencing 5-HT efflux. Systemic oxytocin administration attenuated PCP-induced hyperactivity and increased pro-social behavior without decreasing core body temperature and selectively enhanced NAc dopamine release, consistent with activation of mesocorticolimbic circuits regulating associative/reward behavior being involved. This highlights the therapeutic potential of oxytocin to treat social behavioral deficits seen in psychiatric disorders such as schizophrenia.

INTRODUCTION

The cross-species conserved neuropeptide oxytocin is synthesised in the paraventricular and supraoptic nuclei of the hypothalamus (Neumann *et al*, 1993) and acts via central and peripheral oxytocin and vasopressin V_{1a} and V_{1b} receptors, as well as renal V_2 receptors (Borthwick, 2010; Smith *et al*, 2017). In addition to established roles in lactation and parturition oxytocin is a key regulator of social and affective behavior (Grinevich *et al*, 2016; Meyer-Lindenberg *et al*, 2011). Accumulating evidence implicates its dysregulation in several neuropsychiatric disorders (Romano *et al*, 2015), and oxytocin has received considerable recent interest as a potential adjunct therapy for the social deficits in schizophrenia (Bradley and Woolley, 2017; Kirsch, 2015).

Pro-social effects of oxytocin occur across species on multiple facets of interaction, enabling mechanistic studies in rodents. Acute intracerebral administration increases rat maternal behavior (Pedersen and Prange, 1979) and contributes to partner preference in monogamous prairie voles (Insel and Hulihan, 1995), while chronic administration suppresses aggression (Calcagnoli *et al*, 2014) and attenuates stress-induced behavior, cardiac and autonomic changes in rodents (Grippo *et al*, 2009). The oxytocin-induced decrease in 40kHz 'distress' calls by maternally separated rat pups (Insel and Winslow, 1991) was reversed by an oxytocin antagonist, suggesting involvement of this receptor. However, there are no reports of the effect of oxytocin on rat 50kHz ultrasonic vocalisations (USVs) accompanying positive emotional states specific to reward-associated situations which may have translational relevance to communicative deficits associated with negative symptoms in man (Simola, 2015; Wohr *et al*, 2008; Wohr and Schwarting, 2013; Wright *et al*, 2010).

The NMDA receptor antagonist phencyclidine (PCP) can produce 'schizophrenia-like' psychotic behavior and cognitive impairments in normal healthy individuals, and reversal of acute PCP-induced hyperactivity in rodents (associated with enhanced prefrontal cortex (PFC) and nucleus accumbens (NAc) dopamine and 5-HT release) has been widely used to assess potential antipsychotic drug activity (Hackler *et al*, 2010; Jentsch and Roth, 1999; Li *et al*, 2010; Millan *et al*, 1999). The effect of oxytocin on PCP-induced hyperactivity, as performed herein, has not been reported before.

The formation and maintenance of social bonds in mammals is thought to involve oxytocin-dopamine interactions in the mesocorticolimbic system (Fernandez *et al*, 2018; Skuse and

Gallagher, 2009) and reinforcement of social reward requires oxytocinergic enhancement of dorsal raphe serotonergic input to the NAc (Dolen *et al*, 2013). Infusions of oxytocin into the ventral tegmental area (VTA) of Long Evans rats selected for high grooming behavior, increased NAc dopamine release during bouts of pup grooming which was attenuated by VTA-infusion of an oxytocin receptor antagonist (Shahrokh *et al*, 2010). The localisation of oxytocin receptors to brain regions associated with reward, including the VTA, NAc and prelimbic cortex, suggest that bond formation and social attachments may have rewarding properties (Insel, 1997; Love, 2014; Smith *et al*, 2017). In addition, partner preference in female prairie voles is differentially influenced by dopamine receptor subtype activation; D_1 receptors inhibit while D_2 receptors facilitate partner preference (Aragona *et al*, 2006; Gingrich *et al*, 2000; Liu and Wang, 2003). Furthermore, micro-infusion of oxytocin increased NAc dopamine overflow (Young *et al*, 2014) consistent with the proposed involvement of dopamine/oxytocin interaction on social behavior, although the downstream mechanisms involved are unclear. Therefore, we also determined the impact of oxytocin on PFC and NAc dopamine and serotonin (5-hydroxytryptamine 5-HT) release by microdialysis to evaluate their potential role in mediating the pro-social effects observed.

The blood brain barrier (BBB) penetration of oxytocin is extremely low. A fundamental unresolved controversy limiting any translational therapeutic potential (Meyer-Lindenberg *et al*, 2011) is whether changes in CNS function can be achieved via systemic administration of the neuropeptide (Leng and Ludwig, 2016) without concomitant adverse peripheral effects, and the mechanisms underlying such CNS effects are yet to be elucidated. The variable effects of oxytocin in preclinical paradigms evaluating positive, negative and cognitive symptoms of schizophrenia (Feifel *et al*, 2016) may also be due to confounding activation of other receptors such as the V_{1a}. The doses required for 'desired' and side-effects of oxytocin are species and gender specific, perhaps reflecting species variation in receptor distribution and sexual dimorphism in innervation (Smith *et al*, 2017; Uhl-Bronner *et al*, 2005), and may also vary by rat strain. As there are no published effects of oxytocin in Lister-hooded rats, initial dose-response studies established a dose that did not induce hypothermia or hypolocomotion and used oxytocin and V_{1a} receptor antagonists to elucidated the receptor(s) responsible for these unwanted peripheral effects before determining the effect of oxytocin on PCP-induced hyperactivity, social interaction, USVs and monoamine release by microdialysis.

MATERIALS AND METHODS

Animals

Fifty six male Lister-hooded rats (150-200 g; Charles River UK) were housed in groups of four in ventilated cages or, following surgery for microdialysis, in individual cages. Rats were maintained in a controlled environment (21 ± 2 ^{o}C and 55 ± 10 % humidity) on a 12 h light-dark cycle (lights on 06:00h) with food and water *ad libitum*. All experiments were performed in the light phase, and procedures conducted in accordance with the Animals (Scientific Procedures) Act, 1986, the ARRIVE guidelines (Kilkenny *et al*, 2010) and approval of the University of Nottingham Local Ethical Committee. Group sizes were selected on the basis of our previous publications using these behavioral (Watson *et al*, 2016) and microdialysis techniques (Shortall *et al*, 2016).

Drugs

Oxytocin acetate was purchased from Bachem (Saint Helens, UK), the oxytocin antagonist L-368,899 hydrochloride ((2S)-2-Amino-N-[(1S,2S,4R)-7,7-dimethyl-1-[[[4-(2-methylphenyl)-1-piperazinyl]sulfonyl]methyl]bicyclo[2.2.1]hept-2-yl]-4-(methylsulfonyl)butanamide) from Tocris (Bristol, UK), phencyclidine hydrochloride (PCP) and the V_{1a} receptor antagonist SR49059 ((2S)-1-[[(2R,3S)-5-Chloro-3-(2-chlorophenyl)-1-[(3,4-dimethoxyphenyl)sulfonyl]-2,3-dihydro-3-hydroxy-1H-indol-2-yl]carbonyl]-2-pyrrolidinecarboxamide) relcovaptan, from Sigma Aldrich (Poole, UK). All compounds were dissolved in 0.154M saline (vehicle also containing 5% DMSO for the antagonists) and administered at volume of 1 ml/kg s.c. (oxytocin) or 2 ml/kg i.p. (all other compounds). Doses are expressed as the salt. In all cases the experimenter was unaware of the treatment received both during administration and observation.

Dose-response and antagonist studies with oxytocin on core body temperature and locomotor activity

To establish a suitable dose of oxytocin which would not suppress locomotor activity (LMA) or produce hypothermia during microdialysis studies, rats (n=12) were tested using a within-subjects design on four occasions at weekly intervals following injection of vehicle and each dose of oxytocin (0.03, 0.1 or 0.3 mg/kg s.c.) in a pseudo-random order to serve as their own

control. This range was selected from previous reports showing that oxytocin doses above 0.3mg/kg s.c. or i.p. suppress spontaneous locomotion in other rat strains (Angioni *et al*, 2016; Hicks *et al*, 2014; Klenerova *et al*, 2009) so we included lower doses to identify those devoid of this unwanted effect. To establish the relative contribution of oxytocin and vasopressin V_{1a} receptors to hypothermia produced by the highest dose, a further twelve rats received vehicle or oxytocin (0.3mg/kg s.c.) in the presence and absence of the non-peptide selective V_{1a} receptor antagonist SR49059 (1 mg/kg i.p.) or the selective oxytocin antagonist L-368,899 (2 mg/kg i.p.), on six occasions at weekly intervals (within-subjects design). Although original peptide antagonists for these receptors showed poor stability and selectivity the development of non-peptide antagonists greatly improved pharmacokinetic properties (Manning *et al*, 2012). The current non-peptide antagonists (SR49059 and L-368,889) were selected because they possess the best overall profile of commercially available oxytocin and V_{1a} antagonists; having high affinity, relative selectivity, good BBB penetration and plasma half-life (Manning *et al*, 2012) and are devoid of partial agonist activity. Doses of these brain penetrant antagonists were selected from previous studies showing <15min onset and 2-4h duration in rodents. SR49059 prevented oxytocin-induced pro-social behavior (Ramos *et al*, 2013) and hypothermia (Hicks *et al*, 2014), while L-368,899 (which has brain penetration demonstrated by PET studies (Smith *et al*, 2013)), prevented anxiolytic effects of oxytocin in the open field (Klenerova et al., 2009), reduced conditioned disgust behavior during social interaction (Boulet *et al*, 2016) and attenuated sexual motivation in male rats (Blitzer et al, 2017). The cross-over repeat within-subject design for dose response and antagonist studies greatly reduced the number of rats required and the inter-individual variation of measurements made in line with the 3R's principle.

Temperature microchips

Temperature microchips (Bio-Thermo idENTICHIP; AnimalCare Ltd; York, UK) were implanted s.c. 48h before behavioral testing under brief manual restraint, to enable subsequent temperature recording via a digital chip reader with minimal disturbance to the animal, at 15min intervals during assessment of LMA.

Locomotor Activity

LMA was recorded as described previously (Watson *et al*, 2016). Briefly, rats were placed into individual Perspex boxes (39 x 23.5 x 24.5 cm with removable wire lids) surrounded by a dual-level photobeam activity system (San Diego instruments, CA, USA). A single

ambulation count was recorded for every two consecutive adjacent lower beam breaks and cumulative beam breaks were recorded in 5 min epochs for 2 h. Oxytocin or vehicle were administered following 30 min arena habituation and in the antagonist study antagonists were administered 15 min prior to oxytocin.

Effect of oxytocin on PCP-induced hyperactivity, social interaction and PFC and NAc dopamine and 5-HT efflux

Oxytocin at 0.03 and 0.1mg/kg were selected for further investigation, as these doses did not produce confounding effects on ambulation and body temperature in dose-response studies described above. A separate group of rats (n=32, Figure S1) was used to examine the effect of these two doses on PCP-induced hyperactivity, and on the basis of these findings 0.1mg/kg oxytocin was administered 7 days later, prior to assessment of social interaction and ultrasonic vocalisations (USVs). The following week rats underwent stereotaxic surgery to implant microdialysis probes into the PFC and NAc and after 7 days recovery the effects of oxytocin on dopamine efflux from these brain regions was assessed. One week was left between each of the three protocols (Figure S1) to ensure complete drug wash-out and minimise any carry over effects from the previous procedure.

Locomotor activity

LMA was assessed on a single occasion as described above. Animals received oxytocin or vehicle after 30 min arena habituation, and vehicle or PCP (5.6 mg/kg i.p.; (Hackler *et al*, 2010) an established dose to examine 'antipsychotic-like' activity) 30 min later, resulting in 4 treatment combinations: vehicle + vehicle, PCP + vehicle, PCP + 0.03mg/kg oxytocin, PCP + 0.1mg/kg (n=8/group; between-subjects design).

Social Interaction

Two rats from different litters matched for weight (<30 g difference) and treatment were sprayed on the nape with blue or pink hair dye 45 min prior to the trial to facilitate video tracking. Both rats received 0.1 mg/kg oxytocin or vehicle 45 min before placement into an unfamiliar circular arena (75cm diameter) under low light conditions (40LUX) for 10 min (Bull *et al*, 2003; Watson *et al*, 2016). Ethovision (Noldus) was used to record interactive behaviors, including body sniffing, ano-genital sniffing, following, crawling over-and-under, lying side-by-side, pinning and boxing/biting and to derive total social interaction. As the behavior of each rat depends on that of the partner, an average time was derived for each pair

(n=8 per treatment) rather than each rat. Body temperature was recorded by telemetry immediately before and after social interaction.

Ultrasonic Vocalisations (USVs)

Both 22 kHz (alarm) and 50 kHz (prosocial) USVs were recorded during social interaction as previously described (Watson *et al*, 2016). An electret microphone (Emkay, Avisoft Bioacoustics, Germany) connected to an ultrasound detection unit (Ultrasound Gate, customised model 112, Avisoft Bioacoustics, Berlin) was secured above the arena. The resulting signal was digitalised and saved as .wav files. Temporal and frequency characteristics of 50 kHz calls were extracted using Avisoft analysis software (SAS-Lab Pro, v 4.38, Avisoft Bioacoustic, Berlin) and categorised offline into flat, step or trill subtype, according to pattern analysis (Brenes *et al*, 2016; Simola, 2015). Flat calls had peak frequency changes ≤5 kHz, although the difference between start and end frequencies could be >5 kHz if it had an upward or downward direction. Step calls had at least one short element >5 kHz higher or lower than the fundamental call. Trill calls had at least one frequency modulation with a longer element than step calls and/or a common zig-zag pattern often appearing as inverted-U shapes.

Surgery and microdialysis

One rat from each social interaction pair (n=16 in total) was anaesthetised with isoflurane in O_2 and N_2O, placed in a stereotaxic frame and CMA 12 guide cannulae (Linton Instrumentation; Diss, UK) implanted into the PFC and NAc (AP +3.2, L -0.7, V -2.3 and AP +1.7, L +1.6, V -3.8 respectively from Bregma; (Paxinos and Watson, 1986)). Guide cannulae, two copper-wire tethers and stainless steel screws were secured in place with dental acrylic. Carprofen (1mg/kg s.c.) was administered pre-surgery and lignocaine applied topically around sutures post-surgery.

One week later, rats were briefly re-anaesthetised to insert microdialysis probes (0.5mm x 4.0mm membrane length with molecular cut-off 20,000 Da; CMA 12, Linton Instrumentation) into the guide cannulae. Probes were perfused continuously with aCSF (125mM NaCl, 1.25mM KCl, 0.5mM $MgCl_2$, 13.5mM $NaHCO_3$, 0.2mM NaH_2PO_4, 0.90mM Na_2HPO_4, 0.30mM Na_2SO_4 and 1.2mM $CaCl_2$) at 1 µl/min using dual-syringe pumps (Harvard Apparatus; Cambridge, UK). Rats were placed into an arena (50cm diameter, 45cm height, containing sawdust bedding), with the microdialysis tubing attached to a dual channel

swivel (Linton Instrumentation) and swivel mounted tether (Linton Instrumentation) allowing free movement (Shortall *et al*, 2016). The following day, three baseline samples were collected (in vials containing 5 µL 0.1M perchloric acid (Fisher Scientific, UK) and 0.03% sodium metabisulphite (Sigma Aldrich, UK) and kept on dry ice until storage at -80ºC) at 20min intervals before and for 2h after injection of either oxytocin 0.1mg/kg s.c. or saline (1mg/ml). At the end of the study, rats were euthanised with sodium pentobarbital (Euthatal) i.p. and brains dissected and stored in 4% PFA at 4ºC for histological verification of probe placement.

Analysis of Monoamines

Microdialysis samples were analysed using High Performance Liquid Chromatography with electrochemical detection (HPLC-ED) as described previously (Shortall *et al*, 2016). Defrosted samples were kept on ice before injection (15µl) into a Targa C18 3µM column (100 x 1.0mm; Higgins Analytical) using a Perkin Elmer Series 200 autosampler (Antec Leyden, The Netherlands). Dopamine, 5-HT and their major metabolites; 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) were detected using a mobile phase (20mM potassium dihydrogen phosphate, 20mM sodium acetate, 0.1mM ethylenediaminetetraacetic acid (EDTA), 0.15mM octanesulfonic acid and 10% methanol, pH 3.9) at 0.4ml/min (Dionex P680 pump), and measured against standards with a DECADE II SDC Detector I (Antec; Leyden, The Netherlands) and Clarity software (Data Apex) using a potential of +0.75V. The percentage change from baseline for every microdialysate molecule was calculated for each individual rat. PFC samples were excluded from one rat due to incorrect probe placement and two others because of flow disruption. In one rat NAc dopamine was below the detection limit; n=6/7 per group in the PFC, and n=7/8 in the NAc.

Data analysis

All analyses were perfomed by GraphPad Prism v7 (GraphPad Software Inc.) and SPSS (v24) and data were checked for normality and homogeneity of variance using Shapiro-Wilk's and Levene's tests, respectively. The time course of LMA and body temperature in studies that employed a within-subject design were analysed using two-way repeated measures ANOVA (oxytocin dose response study) or three-way repeated measures ANOVA (antagonist study) with oxytocin, antagonist (where used) and time as factors and total cumulative activity counts used repeated measures ANOVA. Data from PCP and

microdialysis studies, which had a between-subject design, were analysed by two-way ANOVA with treatment as a between subjects factor and time as a repeated measure. ANOVA's were followed by Tukey's or Sidak multiple comparison post hoc tests where appropriate. Data are presented as mean±SEM and $P<0.05$ considered significant.

RESULTS

Effect of oxytocin on locomotor activity and body temperature

During LMA rats habituated to the arena such that ambulation progressively decreased ($F_{(23, 1012)}=86.20$, $P<0.0001$) over 2h. Dose-response studies (Figure 1A) showed acute oxytocin (0.03, 0.1 or 0.3 mg/kg) had no effect, such that ANOVA revealed no significant main effect of treatment (data not shown). There was also no effect of oxytocin on total cumulative ambulatory activity over 90min ($F_{(3, 44)}=1.293$, $P=0.2888$; one-way repeated measures ANOVA, Figure 1B). Although the cumulative activity counts tended to be reduced by the highest dose (0.3 mg/kg) this failed to reach significance in this group.

During the LMA task, rat core body temperature modestly decreased ($F_{(7, 308)}=31.82$, $P<0.0001$), but post-injection the highest dose of oxytocin (0.3 mg/kg) produced significantly greater hypothermia than either saline or the two lower doses of oxytocin ($F_{(3, 44)}=23.73$, $P<0.0001$). There was also a treatment x time interaction ($F_{(21, 308)}=14.29$ $P<0.0001$; ANOVA). Oxytocin at 0.3 mg/kg produced a significantly ($P<0.05$, 0.01 or 0.001; Tukey post-hoc) greater decrease in temperature than vehicle between 15-60 min post-injection, whereas 0.1 mg/kg slightly decreased temperature at the 30 min time point only (Figure 1C).

Role of oxytocin and V_{1a} receptors in oxytocin-induced LMA and hypothermia

Following pretreatment with either a selective oxytocin and V_{1a} receptor antagonist (according to the protocol shown in Figure 2A) three-way ANOVA of the time course of LMA counts confirmed main effect of oxytocin ($F_{(1,11)}=34.776$, $P=0.0001$) and time ($F_{(17,187)}=15.441$, $P=0.0001$) but no significant effect of antagonist and no significant oxytocin x antagonist or oxytocin x antagonist x time interactions. Thus for total cumulative activity counts post-injection, 0.3mg/kg oxytocin produced a modest (and in this group) a significant reduction in the cumulative locomotor activity counts from that seen in vehicle controls ($P<0.01$) which was unaffected by pre-treatment with either L-368,889 or SR49059, neither of which affected activity on their own (Figure 2B). At the same time similar analysis of body temperature (Figure 2C) showed main effects of oxytocin ($F_{(1,11)}=59.162$, $P=0.001$),

antagonist ($F_{(2,22)}$=5.046, *P*=0.016) and time ($F_{(7,77)}$=47.072, *P*=0.0001) and a significant oxytocin x antagonist x time interaction ($F_{(14,154)}$=4.156, *P*=0.0001). Oxytocin produced a marked (approximately 2^{o}C) reduction in body temperature reaching a peak 30 to 60 min post-injection which was significantly lower than that in vehicle treated controls (*P*<0.0001). The hypothermia induced by oxytocin from 30-60 min post-injection was significantly attenuated (*P*<0.05 to *P*<0.001) by the V_{1a} receptor antagonist, SR49059, such that body temperature was not significantly different from vehicle at any time point in the SR49059 + oxytocin group. In contrast, the time course and magnitude of hypothermia produced by oxytocin was comparable in the absence and presence of the oxytocin receptor antagonist L-368,889.

Effect of oxytocin on PCP-induced hyperactivity

A separate group of rats received rats was used to consecutively examine the effect of oxytocin on PCP-induced hyperactivity, social interaction and PFC and NAc dopamine and 5-HT efflux by microdialysis according to the protocol in Figure 3A. The highest dose of oxytocin attenuated the magnitude of the PCP-induced hyperactivity such that in the 90min following injection with oxytocin there was a significant main effect of time ($F_{(17, 476)}$=19.86, *P*<0.0001), treatment ($F_{(3, 28)}$=25.88, *P*<0.0001) and a treatment x time interaction ($F_{(51, 476)}$=3.428, *P*<0.0001; Figure 3B). There was no difference in the magnitude of LMA response between PCP rats that received pre-treatment with saline or the low dose of oxytocin. In contrast 0.1 mg/kg oxytocin produced an approximately 50% reduction, such that LMA was significantly less (*P*<0.05 or *P*<0.01) than both saline and low dose oxytocin groups between 20 and 45min after PCP injection.

As expected the cumulative ambulatory count following injection of PCP was significantly greater than saline, such that there was a significant main effect of treatment ($F_{(3, 28)}$=24.34, *P*<0.0001; one-way ANOVA). Consistent with the time course data, only the highest dose of oxytocin (0.1 mg/kg) significantly attenuated PCP-induced hyperactivity compared to vehicle-PCP treated rats (*P*<0.01, Tukey post-hoc, Figure 3C), which was still significantly greater (P<0.01) than the basal activity seen in saline controls.

The body temperature of rats measured during LMA gradually reduced over time ($F_{(7, 189)}$=20.17, *P*<0.0001; two-way repeated measures ANOVA) by approximately 0.5^{o}C. However, there was no significant effect of oxytocin at either dose on the magnitude ($F_{(3,}$

$_{27)}$=1.325, *P*=0.2868) or time course of the temperature change (treatment x time interaction $F_{(21, 189)}$=0.6389, *P*=0.8860; Figure 3D).

Effect of oxytocin on social interaction

Socially interactive behaviors including ano-genital sniffing, body sniffing, crawling over-and-under, following, pinning, lying side-by-side, as well as aggressive boxing/biting behavior were scored manually post-trial. Both the total time spent undergoing pro-social behavior and engaged in each individual behavioral component was similar to that previously reported by us in group-housed control rats (Watson *et al*, 2016). Comparison of vehicle and oxytocin-treated rats revealed significant main effects of both treatment ($F_{(1, 98)}$=7.001, *P*=0.0095) and behavioral component scored ($F_{(6, 98)}$=123, *P*<0.0001) and a treatment x behavior interaction ($F_{(6, 98)}$=3.241, *P*=0.006). Rats given oxytocin (0.1mg/kg) engaged in significantly more body sniffing (*P*<0.05) and ano-genital sniffing (*P*<0.001; Sidak post-hoc) compared to saline controls (Figure 4A) but there were no differences in any of the other five behavioral components.

Oxytocin also significantly increased the total time spent in social interaction (mean±SEM = 71.6±4.3s), compared to those receiving vehicle (56.9±4.1s; *P*<0.05 unpaired Student's *t*-test; Figure 4B).

Comparison of temperature change from baseline, before and after social interaction showed that core body temperature increased during the trial ($F_{(2, 60)}$=23.3, *P*<0.0001). Oxytocin reduced the extent of hyperthermia ($F_{(1, 30)}$=5.011, *P*=0.0328) such that there was a treatment x time interaction ($F_{(2, 60)}$=2.41, *P*=0.0985). The temperature difference between vehicle and oxytocin-treated rats was close to significance before (*P*=0.0954) but reached significance (*P*=<0.05; Sidak test; Figure 4C) immediately after the social interaction trial.

Effect of oxytocin on USVs

Pro-social 50 kHz USVs emitted from each rat pair during social interaction was quantified according to three defined subtypes; flat, step and trill (Brudzynski, 2015) following Fast Fourier transformation and pattern analysis (Figure 4E). A two-way ANOVA of USVs following a $\log_{10}$ transformation (to normalise data), revealed a significant main effect of the call type ($F_{(2, 42)}$=18.75, *P*<0.0001) emitted (flat being more frequent than step or trill) but this

was unaltered by oxytocin treatment ($F_{(1, 42)}$=0.8109, *P*=0.3730; Figure 4D). No rats emitted any 22 kHz USVs, so no analysis could be performed on these calls.

Effect of oxytocin on PFC and NAc dopamine and 5-HT efflux

Pre-injection basal dopamine and 5-HT levels (average of initial triplicates) were determined in the PFC and NAc and used to calculate the percentage change for each monoamine. Compared to initial basal dopamine levels (0.0728 and 0.176 pmol/ml in the PFC and NAc, respectively) oxytocin (0.1mg/kg) produced a significant ($F_{(1, 12)}$=7.983, *P*=0.0153) persistent (approximately 100%) elevation of overflow in the NAc, but the much more varied trend to increase DA in the PFC did not reach significance ($F_{(1, 11)}$=4.254, *P*=0.0634; Figure 5A). In order to ascertain if the effect on cortical dopamine could be more transient than the NAc analysis was also performed on the data in the first 60 and 80 min post-injection but this was also not significant. There was no significant effect of time nor any treatment x time interaction (p>0.05) in either brain region.

In contrast, basal 5-HT levels (PFC and NAc being 2.212 and 3.677 pmol/ml, respectively) were unaltered by oxytocin injection such that there was no significant main effect of treatment or time nor any treatment x time interaction in either the PFC ($F_{(1, 11)}$=0.06091, *P*=0.8096) or NAc ($F_{(1, 14)}$=0.08213, *P*=0.7786; Figure 5B). Microdialysate levels of the major metabolites of DA and 5-HT (DOPAC, HVA and 5-HIAA) were also unaltered from basal levels by oxytocin or vehicle and remained stable across time post-injection (Figure S2). As extracellular levels of DOPAC, HVA and 5-HIAA reflect intracellular metabolism of their respective neurotransmitter, microdialysate levels of these metabolites would not be expected to mirror nor provide an index of corresponding neurotransmitter release.

Core body temperature slowly and progressively decreased over 2h post-injection ($F_{(8, 104)}$=11.05, *P*<0.0001) and although there was no main effect of treatment ($F_{(1, 13)}$=3.129, *P*=0.1003), there was a significant time x treatment interaction ($F_{(8, 104)}$=2.482, *P*=0.0167; two-way repeated measures ANOVA). Oxytocin only caused significantly greater hypothermia than vehicle at 45min post-injection (*P*<0.05; Sidak post-hoc; Figure 5C).

DISCUSSION

This study demonstrates the ability to dose differentiate the effect of systemic oxytocin injection on reversal of PCP-induced LMA and pro-social behavior from hypothermia and

'sedative-like' hypoactivity in Lister-hooded rats. Importantly oxytocin enhanced social interaction without simultaneously altering 50 kHz pro-social calls, suggesting that this behavior resulted from direct neuronal modulation by oxytocin. Accompanying microdialysis data suggests this may be mediated, at least in part, by selective enhancement of dopamine (and not serotonin) release preferentially in the NAc, and not the PFC, from mesocorticolimbic circuits involved in associative/reward behavior.

Although as little as 0.01% of systemic oxytocin may reach the rat CNS because of poor penetration of the BBB (Meyer-Lindenberg *et al*, 2011; Striepens *et al*, 2013) and the estimated blood half-life is ~5min, the cerebrospinal fluid half-life is considerably longer (at least 20min (Ludwig and Leng, 2006)) and could provide a compartment for more sustained CNS actions (Leng and Ludwig, 2016) similar to those reported herein. Several previous studies show intranasal and intracerebral oxytocin administration enhances rodent social behavior (Calcagnoli *et al*, 2015; Insel and Winslow, 1991; Lukas *et al*, 2011; Witt *et al*, 1992) which may also occur in humans (Gibson *et al*, 2014; Kosfeld *et al*, 2005; Shin *et al*, 2015). As the rat pituitary contains ~1μg oxytocin, even minimal brain penetration of supraphysiological amounts given systemically could alter brain function, since as little as 1ng intracerebroventricular oxytocin produces behavioral effects in rodents (Arletti and Bertolini, 1985). In addition, exogenous oxytocin may activate peripheral afferents or subfornical organ projections to the paraventricular nucleus (Iovino *et al*, 2016) as well as acting directly on magnocellular neurons, all of which may enhance endogenous oxytocin release by positive feedback (Falke, 1989) resulting in diffuse dendritic release (Ludwig and Leng, 2006) and a prolonged effect. Consistent with this proposal fMRI BOLD in rats has suggested systemic oxytocin does not activate the same receptor rich areas as intracerebroventicular administration (Ferris *et al*, 2015) and following subcutaneous injection oxytocin levels double in amygdala and hippocampal microdialysates for 30-60 min (Neumann *et al*, 2013). The current study shows robust changes in behavior and increased NAc dopamine release following subcutaneous oxytocin injection in Lister-hooded rats during a similar time period.

Dose-response studies established that 0.3mg/kg s.c. oxytocin produced hypothermia in Lister-hooded rats (-2^{o}C, peaking at 30-60 min post-injection) and even this high dose caused limited suppression of activity; causing a significant reduction cumulative LMA over 90 min

post-injection in one of the two groups examined. By comparison, lower doses (0.03 or 0.1 mg/kg) of oxytocin produced little hypothermia and no effect on LMA. Thus the current observation that ~0.3mg/kg s.c. oxytocin is at the threshold where attenuation of locomotor activity reaches significance in Lister hooded rats is consistent with findings by Hicks *et al* (2014) and Klenerova *et al* (2009) in Wistar, and Angioni *et al* (2016) in Sprague-Dawley rats, where hypoactivity only occurred with 1 or 2 mg/kg i.p. oxytocin. Similarly, in behavior and microdialysis studies herein, 0.1mg/kg oxytocin had no effect on activity and caused a very small (0.2^{o}C) decrease in body temperature. Of particular note the hypothermia recorded with the highest dose of oxytocin (0.3 mg/kg) in this study was markedly attenuated by the selective V_{1a} receptor antagonist SR49059 but unaffected by the oxytocin antagonist L-368,899 which is brain penetrant (Boccia *et al*, 2007) and attenuates oxytocin-induced hypoactivity in Wistar rats at the dose used in this study (Klenerova *et al*, 2009). Previous biotelemetry studies (Hicks *et al*, 2014) also suggest the thermoregulatory effects of systemic oxytocin primarily involve V_{1a} receptor activation and support the proposal that the pro-social and microdialysis effects reported in this study (with lower doses that cause little hypothermia) may be mediated primarily by oxytocin receptor and not V_{1a} receptor activation. In accord with a previous report in Wistar rats (Klenerova *et al*, 2009) the 'sedative-like' hypoactivity seen with the highest dose of oxytocin was only partially attenuated SR49059 or L-368,889. Therefore this hypoactivity could be mediated by oxytocin acting at distinct receptors, potentially the V_{1b} (located in the pituitary in adults) for which it has modest affinity, or allosteric modulation of dopamine binding at oxytocin-D_2 dopamine receptor heterodimers (Romero-Fernandez *et al*, 2013), if these exist *in vivo*. In contrast, in Sprague-Dawley rats LMA suppression produced by 2 mg/kg i.p. oxytocin was mimicked by microinjection into the substantia nigra and prevented by intracerebroventricular injection of an oxytocin antagonist, and therefore could result from activation of oxytocin receptors on nigrostriatal dopaminergic neurons (Angioni *et al*, 2016).

At 0.1 mg/kg oxytocin markedly attenuated PCP-induced hyperactivity, whilst the low dose (0.03 mg/kg) had no effect. No previous study has examined the effect of oxytocin on NMDA antagonist-induced hyperactivity, but notably oxytocin (2 mg/kg i.p.) decreased methamphetamine-induced hyperactivity in rats (Carson *et al*, 2010a) accompanied by attenuation of induced Fos protein expression in the subthalamic nucleus and NAc core (Carson *et al*, 2010b), suggesting these regions may be involved. Furthermore, oxytocin also attenuated cocaine-induced hyperactivity and the concomitant reduction in post-mortem NAc

dopamine content in mice (Kovacs *et al*, 1990). Both PCP and methamphetamine significantly increased ambulatory activity, and PFC and NAc microdialysate dopamine and 5-HT levels in rodents following acute injection (Li *et al*, 2010; Millan *et al*, 1999). Furthermore, dopamine release measured by chronoamperometry was enhanced by oxytocin infusion into the NAc and VTA (Love, 2014; Shahrokh *et al*, 2010), suggesting that oxytocin can activate mesolimbic dopaminergic neurons. As both PCP and oxytocin enhance NAc dopamine release it appears paradoxical that this could explain oxytocin attenuation of PCP-induced LMA. However, when given in combination, oxytocin reduces the methamphetamine increase in NAc dopamine turnover in mice (Qi *et al*, 2008). Compared with amphetamine, PCP more markedly elevates NAc microdialysate 5-HT and PCP-induced LMA is attenuated by 5-HT_{2A} receptor antagonists (Millan *et al*, 1999). However, as oxytocin did not alter NAc or PFC 5-HT release in the current study it is unlikely that direct modulation of serotonergic function contributes to the attenuation of PCP-LMA observed. Although a small decrease (0.2-0.4^{o}C) in body temperature was seen 30 to 45min after 0.1mg/kg oxytocin administration in the current dose-response and microdialysis studies, this is unlikely to account for the selective elevation in NAc DA overflow observed since hypothermia (Brannan *et al*, 1992) reduces striatal DA levels in microdialysates. Acute PCP activates PFC glutamatergic efferents to the VTA which also contribute to the motoric enhancement observed (Takahata and Moghaddam, 2003). The locomotor hyperactivity and elevation in microdialysate dopamine efflux in the NAc are both attenuated by injection of the dopamine receptor antagonist flupenthixol into the NAc (Del Arco *et al*, 2008), consistent with corticolimbic pathways contributing to PCP hyperactivity. Furthermore, oxytocin attenuates the medial PFC methamphetamine-induced increase in glutamate in an oxytocin receptor sensitive manner (Qi *et al*, 2012). So far no studies have examined whether a similar interaction occurs with PCP. Oxytocin-induced facilitation of mesolimbic dopaminergic function and accompanying facilitation of NAc dopamine release have been shown to regulate maternal behavior and chemical lesions or microinjection of dopamine antagonists disrupt this (Insel, 1997; Love, 2014; Shahrokh *et al*, 2010). Oxytocin containing parvocellular neurons of the paraventricular nucleus project to the NAc and VTA, while oxytocin neurons of the medial preoptic area innervate the VTA whose dopamine neurons in turn innervate the NAc and the PFC (Love, 2014). Both the VTA and NAc have oxytocin receptors, which would be targets of peripheral administration or brain penetrant drugs (Love, 2014; Smith *et al*, 2017). By using oxytocin receptor Cre mice (Peris *et al*, 2017) it has been shown that glutamatergic neurons in the VTA also express oxytocin receptors and oxytocin

likely also modulates glutamatergic neuronal function, as oxytocin receptor knockout mice show larger deficits in sensorimotor gating following PCP than wildtype mice (Caldwell *et al*, 2009). Thus glutamatergic modulation by oxytocin might also contribute to attenuate PCP-induced LMA.

Activation of oxytocinergic neurons in the PVN and supraoptic hypothalamic nuclei (which express 5-HT_{1A} receptors (Uvnas-Moberg *et al*, 1996)) by input from dorsal raphe 5-HT neurons promotes oxytocin release from their terminals in the NAc serving to enhance social reinforcement (Arakawa, 2017; Emiliano *et al*, 2007; Fernandez *et al*, 2018). In addition, oxytocin heteroreceptors enhances 5-HT release from raphe nerve terminals in the NAc, reinforcing the rewarding property of social interaction in mice (Dolen *et al*, 2013). While NAc DA may signal acute reward reinforcement serotonergic neuronal activation in the NAc may contribute to delayed gratification reward and social attachment (Fernandez *et al*, 2018). Consistent with the current microdialysis observations oxytocin may have little impact on basal 5-HT release but only enhance NAc 5-HT during social reinforcement. Consistent with this proposal the oxytocin antagonist, tocinoic acid, does little to social interaction when given alone but attenuates the facilitation of interaction produced by MDMA or the 5-HT_{1A} agonist 8-OHDPAT (Thompson *et al*, 2007).

In this study, oxytocin (0.1 mg/kg) increased pro-social interaction most notably due to increased ano-genital and body sniffing. Rodents are thought to sniff ano-genital regions of conspecifics as part of social recognition to decode identity, and an increase might reflect altered acceptance of familiarity of strangers and facilitate social reciprocity. Similar enhancement of total pro-social behavior has previously been observed in both mice and rats (Bowen *et al*, 2011; Calcagnoli *et al*, 2014; Huang *et al*, 2014; Lee *et al*, 2005; Ramos *et al*, 2013) following oxytocin administration. The only previous report of peripheral oxytocin (0.5 mg/kg i.p. in Long Evans rats) on social interaction found increased lying side-by-side and reduced ano-genital sniffing, both prevented by the V_{1a} receptor antagonist, SR49059, but not by the oxytocin antagonist, C25 (Ramos *et al*, 2013). Several groups have shown a range of doses of both oxytocin and vasopressin produce similar pro-social behavior during social interaction, also implicating V_{1a} receptors or downstream signalling crosstalk between oxytocin and V_{1a} receptors in these effects in rats and hamsters (Ramos *et al*, 2013; Song *et al*, 2014; Suraev *et al*, 2014). The precise brain regions involved in the pro-social effect is unclear but both peripheral and microinjection of the oxytocin antagonist L-368,899 into the

NAc reduced social preference in adult male mice in a social reward paradigm (Dolen *et al*, 2013). By using viral vectors it has been shown that social interaction enhances activity of PVN oxytocin neurons innervating VTA neurons projecting to the NAc which can be attenuated by an oxytocin receptor antagonist (Hung *et al*, 2017) suggesting oxytocin receptors are also involved. The potential sites of interaction of oxytocin with circuits connecting the NAc via activation of oxytocin and/or V_{1a} receptors and consequential alterations in dopamine, GABA, and glutamate are elegantly discussed in a recent review (Baracz and Cornish, 2016) and further evaluation such as by microinjection with selective antagonists or lesions using the neurotoxin, saporin-oxytocin, would help delineate the brain region/s and receptor/s involved.

In contrast to effects on social behavior, oxytocin did not concomitantly alter the number or pattern of high frequency USVs consistent with a previous report that arginine-vasopressin failed to alter 50 kHz USVs in rats (Lukas and Wohr, 2015). 50 kHz USVs are emitted in positive behavioural or rewarding situations (including social play and sexual behavior), and thought to reflect positive affective state (Brudzynski, 2015), and if played back to rats induce approach behavior (Seffer *et al*, 2015). Furthermore 50 kHz USVs are attenuated by dopamine D_1 and D_2 antagonists and 6-hydroxydopamine lesion of the VTA dopamine neurons (Burgdorf *et al*, 2007). Since USVs were unaltered by oxytocin herein the change in social behavior between rat pairs probably results from activation of mesocorticolimbic circuits (Wohr and Schwarting, 2013) and is not driven by altered communicative calls.

Despite evidence that high doses of oxytocin cause receptor internalisation (Huang *et al*, 2014; Klein *et al*, 2011), repeated low dose oxytocin exposure causes enduring increases in social interaction in rats (Suraev *et al*, 2014), potentially resulting from synaptic plasticity enhancing the serotonergic input to the NAc, reinforcing the rewarding impact of social behavior (Dolen *et al*, 2013). The extent of BBB penetration following intranasal oxytocin administration in man is controversial (Leng and Ludwig, 2016). This study in rats show that peripheral oxytocin administration can enhance social interaction and increase NAc dopamine overflow, without producing accompanying sedative or thermoregulatory effects, warranting further investigation as a potential therapy for antecedent social behavioral deficits such as seen in ASD and schizophrenia.

DISCLOSURE

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FIGURE LEGENDS

Figure 1 Effect of oxytocin (OXY, 0.03, 0.1 and 0.3mg/kg; s.c., administered after arena habituation) or vehicle (VEH, 0.154M saline 1ml/kg) administered according to the protocol shown in A on four occasions at 7 day intervals so that each rat received every dose on B; total locomotor activity (LMA, cumulative ambulatory counts, mean±SEM) and C; change in body temperature (from basal recorded 5 min pre-injection, mean±SEM, ^{o}C) in adult Lister hooded rats (n=12, within-subjects design) recorded in an activity box for 90 min post-injection. C: *P<0.05, ***P<0.001 VEH vs. 0.3 or 0.1mg/kg OXY; $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001 0.3mg/kg OXY vs. 0.1mg/kg OXY; ^{++}P<0.01 0.3mg/kg OXY from 0.03mg/kg OXY; Tukey's post-hoc test.

Figure 2 Determination of the effect of pre-treatment with the selective V_{1a} receptor antagonist, SR49059 (1mg/kg), or the selective oxytocin receptor antagonist, L-368,899 (2mg/kg), according to the experimental protocol shown in A on B; the cumulative locomotor ambulatory counts and C; the change in body temperature (^{o}C) over 90 min following injection of oxytocin (OXY, 0.3mg/kg. s.c., administered 15 min after the antagonist) or vehicle (VEH, 1ml/kg saline). Data are presented as mean±SEM, n=12 per treatment (within-subjects design). In B **P<0.01 from VEH + VEH controls. In C * P<0.05 and *** P<0.001 from VEH + VEH controls, $^{+}$ P<0.05, $^{++}$ P<0.01 and $^{+++}$ P<0.001 0.03mg/kg OXY from

0.3mg/kg OXY and ###*P*<0.001 0.1mg/kg OXY from 0.3mg/kg OXY, following Tukey's post-hoc test.

Figure 3 A; Experimental protocol used to consecutively examine the effect of oxytocin on phencyclidine-induced behavior (this figure), social interaction (Figure 4) and prefrontal cortex and nucleus accumbens dopamine and 5-HT efflux by microdialysis (Figure 5), for full details of the methods see Figure S1. Comparative effect of oxytocin (OXY, 0.03-0.1mg/kg; s.c) and vehicle (VEH, 0.154M saline 1ml/kg) on (mean±SEM) the B; time course of phencyclidine (PCP, 5.6mg/kg; i.p.)-induced locomotor activity (LMA; cumulative counts/5min epoch), C; total ambulatory counts in 1 h (following injection with PCP/VEH), and D; change in body temperature (from basal, 5min prior to injection, ^{o}C) in adult Lister hooded rats recorded in an activity box. In B and C: **P*<0.05, ***P*<0.01, ****P*<0.001 compared to VEH-SAL; $^{+}$*P*<0.05, $^{++}$*P*<0.01 compared to VEH-PCP; $^{\#}$*P*<0.05, $^{\#\#}$*P*<0.01 compared to 0.03mg/kg OXY-PCP; Tukey post-hoc) VEH=saline vehicle, OXY=oxytocin; n=8 per group (between-subjects design) as indicated VEH-VEH; VEH-PCP; OXY 0.03mg/kg-PCP; OXY 0.1mg/kg-PCP.

Figure 4 Effect of oxytocin (OXY, 0.1mg/kg; s.c.) and vehicle (VEH, 0.154M saline 1ml/kg) on social interaction between two male Lister hooded rats from different litters paired by similar weight and the same previous drug treatment, recorded over 10 min. A; individual behaviors (s), B; total time in social interaction (s), C; body temperature change from basal (immediately prior to injection), before (45 min post-injection) and after the interaction trial (55 min post-injection as indicated; ^{o}C) and D; the number of pro-social 50 kHz ultrasonic vocalisations (USVs) emitted by both rats in each pair. All data are presented as mean±SEM. E; Representative spectrographs showing pro-social 50 kHz USVs emitted from rat pairs defined into three subtypes; flat, step and trill, following Fast Fourier transformation and pattern analysis. A; *p=0.05; Student's unpaired *t*-test. C; **P*<0.05 from OXY. D; Following $\log_{10}$ transformation, **P*<0.05 ****P*<0.001 VEH vs. OXY; Sidak post-hoc; VEH=saline vehicle, OXY=0.1mg/kg oxytocin; n=8 pairs/group. NB. A different time scale is used to show ano-genital and body sniffing from other, less frequent, behaviors.

Figure 5 Comparison of the effects of oxytocin (OXY, 0.1mg/kg; s.c.) or vehicle (VEH, 0.154M saline 1ml/kg) on A; dopamine and B; serotonin (5-HT) overflow (mean±SEM, pmol/ml) in the prefrontal cortex (PFC) and nucleus accumbens (NAc) measured by

microdialysis, and concomitant C; core body temperature (mean±SEM; ^{o}C) in freely-moving rats. A; Changes to dopamine from baseline in the PFC (0.0728 pmol/ml) and NAc (0.176 pmol/ml) over the 2h period. B; Changes in 5-HT levels from baseline in the PFC (2.212 pmol/ml) and NAc (3.677 pmol/ml). PFC: Dopamine: n=7 VEH, n=6 OXY, 5-HT: n=7 VEH, n=6 OXY; NAc: Dopamine: n=7 VEH, n=7 OXY, 5-HT: n=8 VEH, n=8 OXY; VEH=saline vehicle, OXY=0.1mg/kg oxytocin; PFC; Prefrontal Cortex, NAc; Nucleus Accumbens. C; *P<0.05 VEH vs OXY; Sidak post hoc. VEH=saline vehicle, OXY=0.1mg/kg oxytocin; n=8 per treatment group; between-subjects design.

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Figure 1

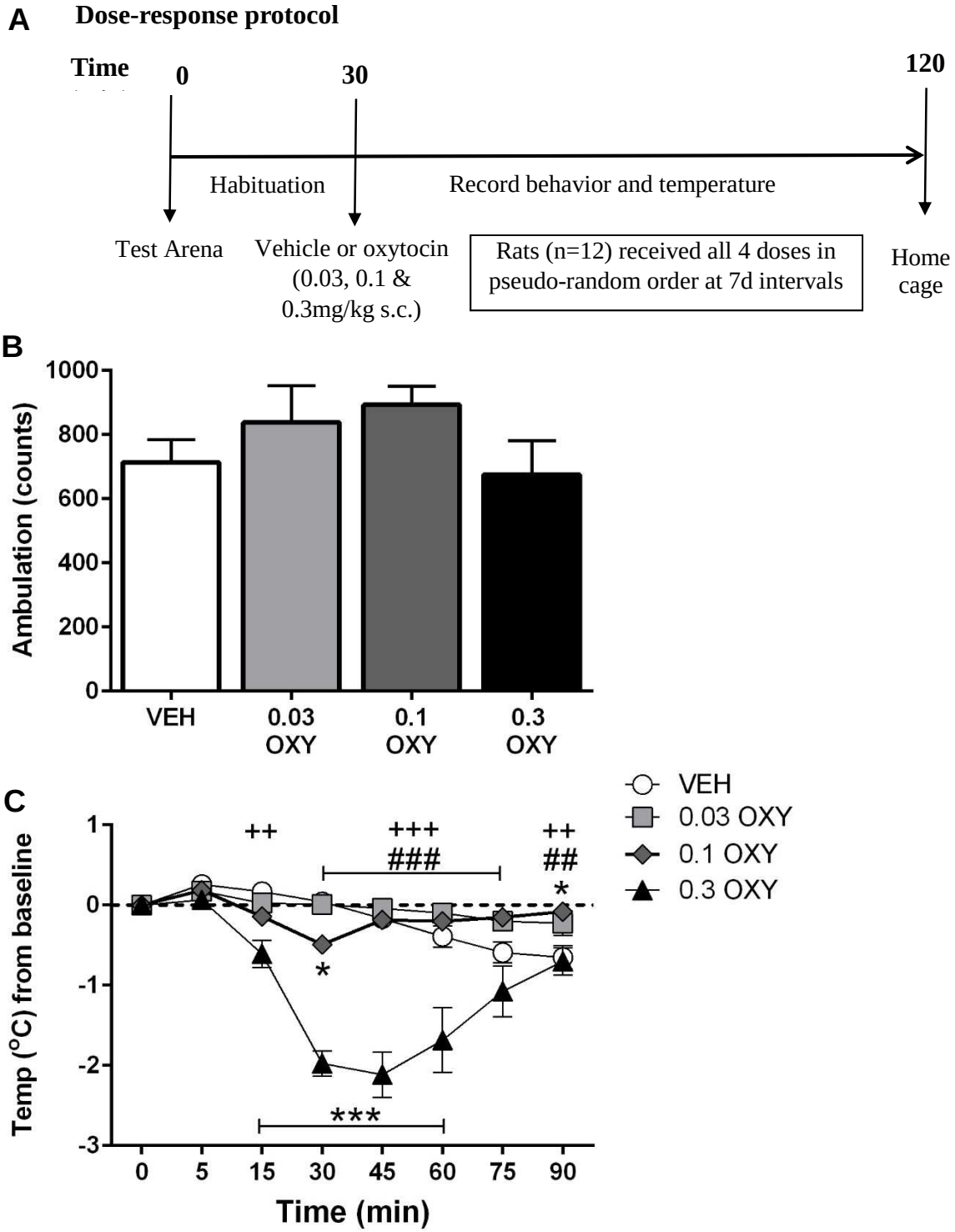
A
Dose-response protocol
Time
0
30
120
Habituation
Record behavior and temperature
Test Arena
Vehicle or oxytocin (0.03, 0.1 & 0.3mg/kg s.c.)
Rats (n=12) received all 4 doses in pseudo-random order at 7d intervals
Home cage
B
Ambulation (counts)
1000
800
600
400
200
0
VEH
0.03 OXY
0.1 OXY
0.3 OXY
C
Temp (°C) from baseline
1
0
-1
-2
-3
++
+++
###
++
##
*
*
***
0
5
15
30
45
60
75
90
Time (min)
VEH
0.03 OXY
0.1 OXY
0.3 OXY

Figure 2

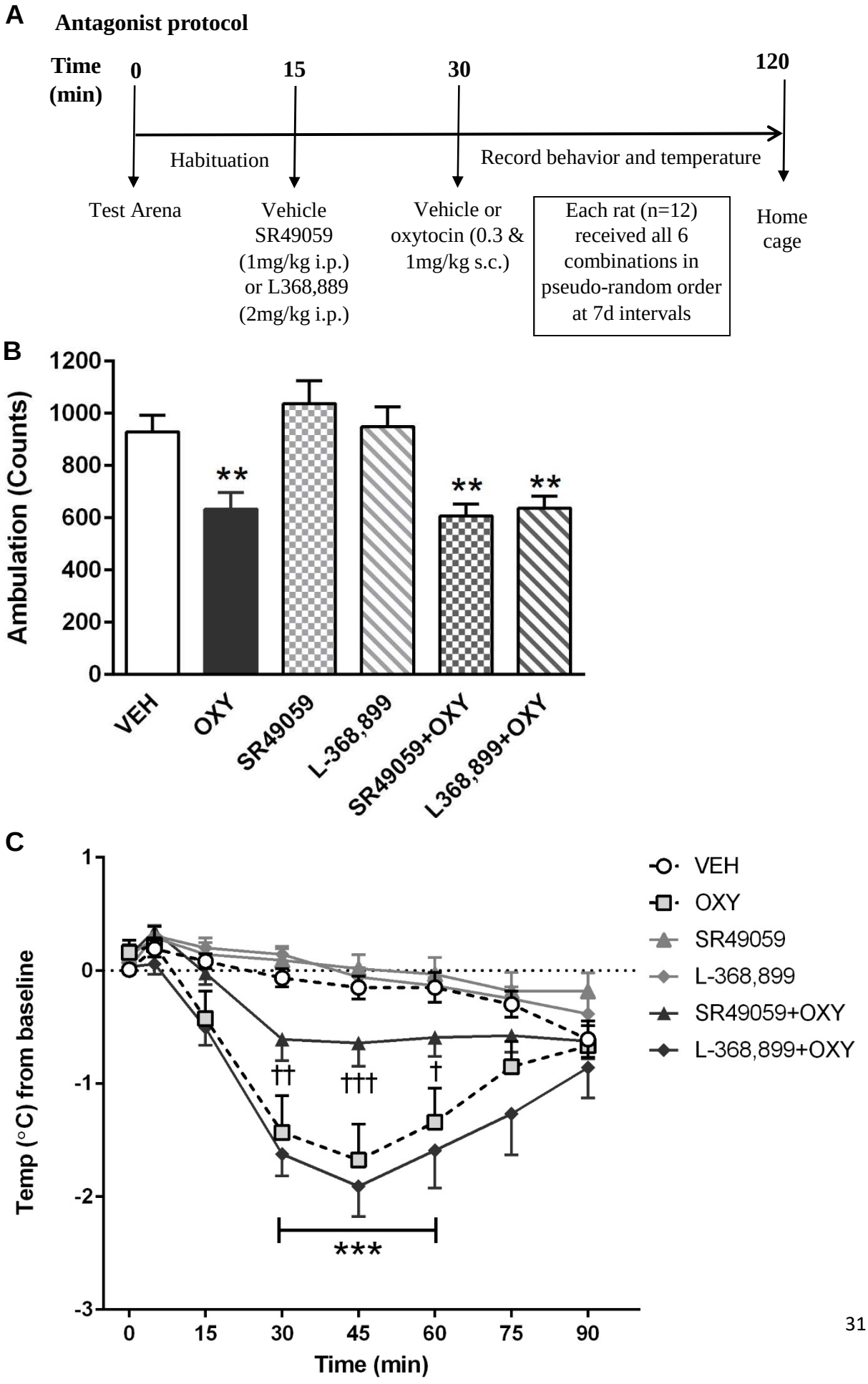
A
Antagonist protocol
Time (min)
0
15
30
120
Habituation
Record behavior and temperature
Test Arena
Vehicle SR49059 (1mg/kg i.p.) or L368,889 (2mg/kg i.p.)
Vehicle or oxytocin (0.3 & 1mg/kg s.c.)
Each rat (n=12) received all 6 combinations in pseudo-random order at 7d intervals
Home cage
B
Ambulation (Counts)
1200
1000
800
600
400
200
0
**
**
**
VEH
OXY
SR49059
L-368,899
SR49059+OXY
L368,899+OXY
C
Temp (°C) from baseline
1
0
-1
-2
-3
0
15
30
45
60
75
90
Time (min)
VEH
OXY
SR49059
L-368,899
SR49059+OXY
L-368,899+OXY
††
†††
†
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Figure 3

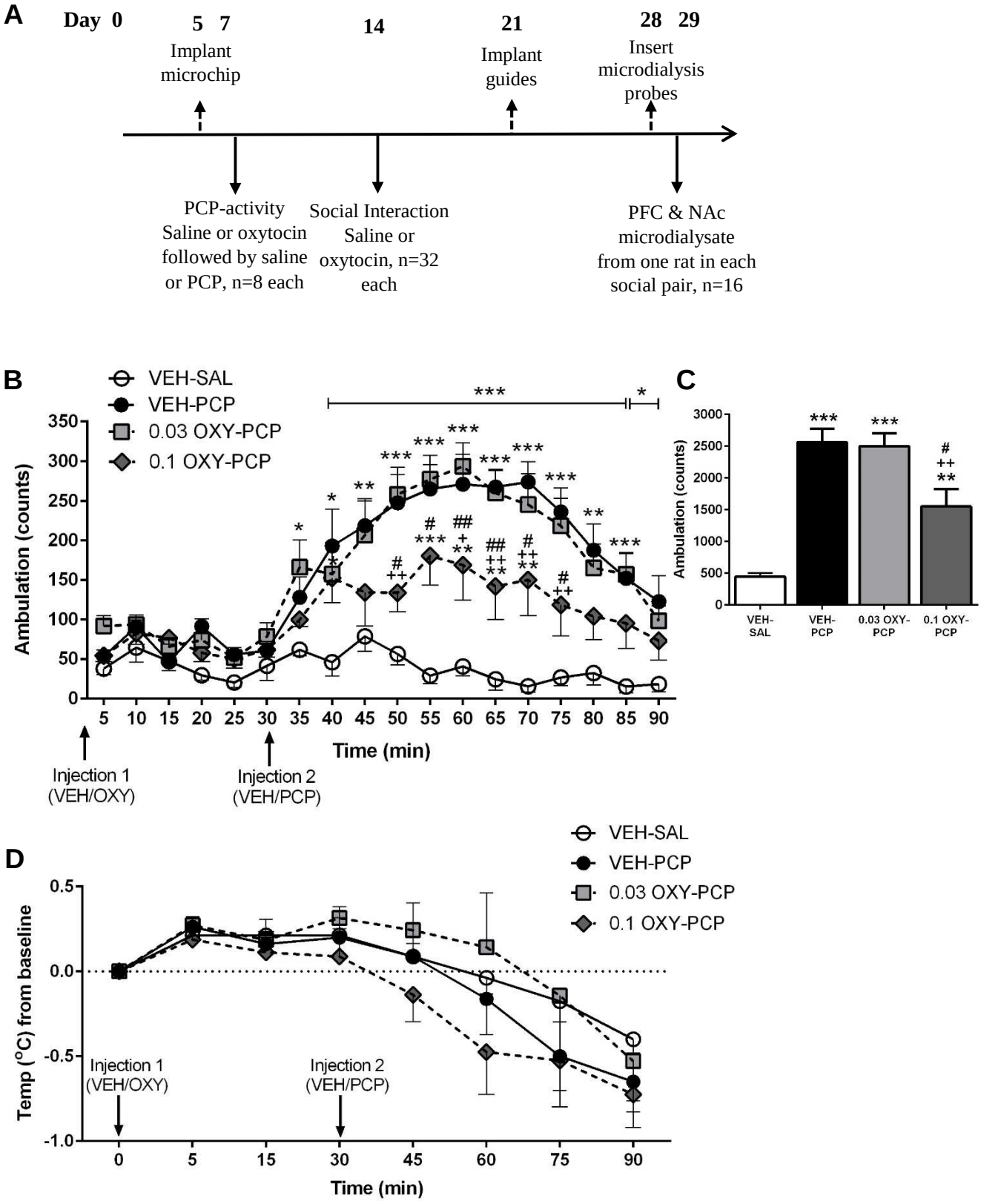

A
Day 0
5 7
Implant microchip
14
21
Implant guides
28 29
Insert microdialysis probes
PCP-activity Saline or oxytocin followed by saline or PCP, n=8 each
Social Interaction Saline or oxytocin, n=32 each
PFC & NAc microdialysate from one rat in each social pair, n=16
B
VEH-SAL
VEH-PCP
0.03 OXY-PCP
0.1 OXY-PCP
Ambulation (counts)
Time (min)
Injection 1 (VEH/OXY)
Injection 2 (VEH/PCP)
C
Ambulation (counts)
VEH-SAL
VEH-PCP
0.03 OXY-PCP
0.1 OXY-PCP
D
Temp (°C) from baseline
Time (min)
Injection 1 (VEH/OXY)
Injection 2 (VEH/PCP)

Figure 4

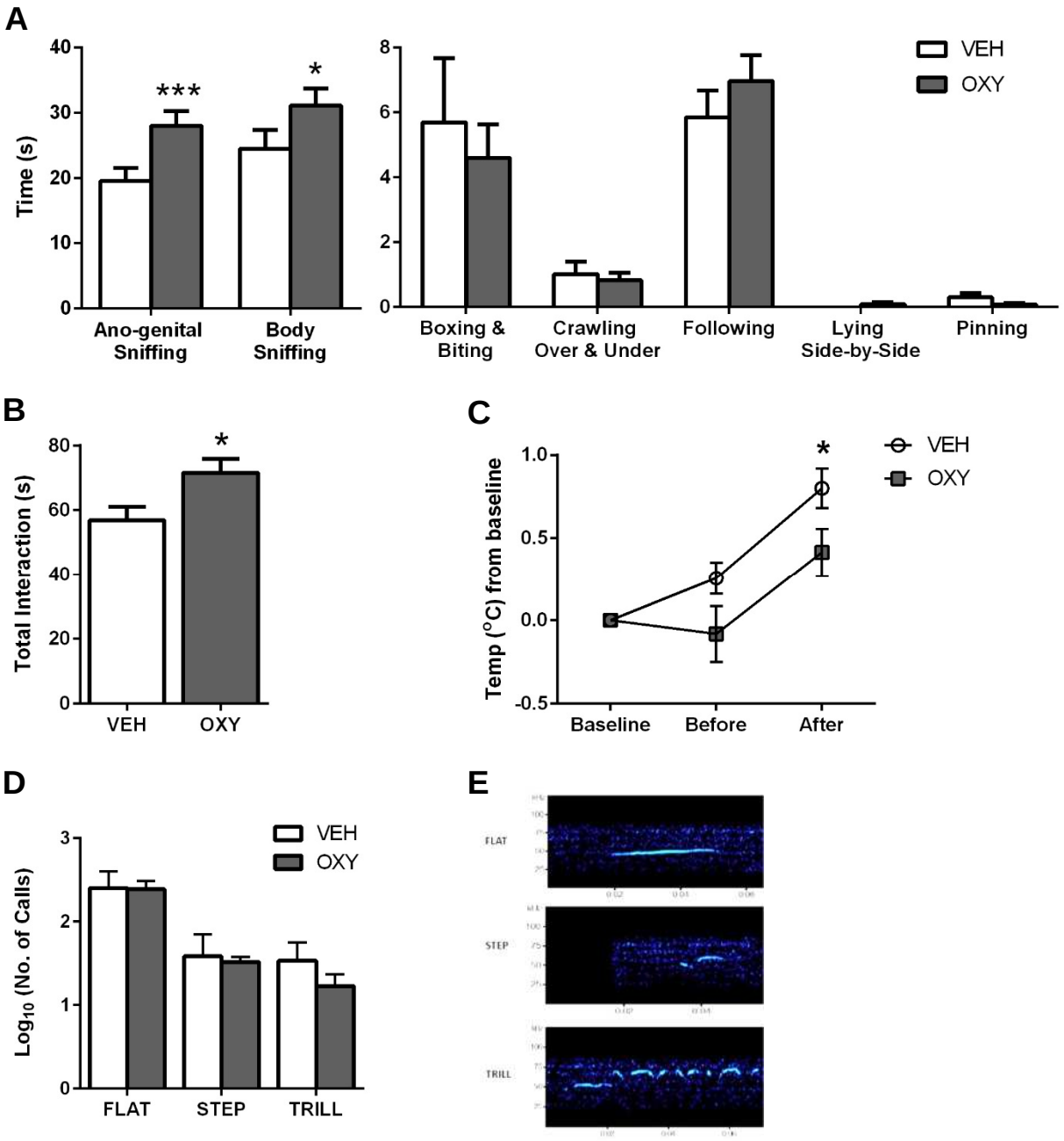
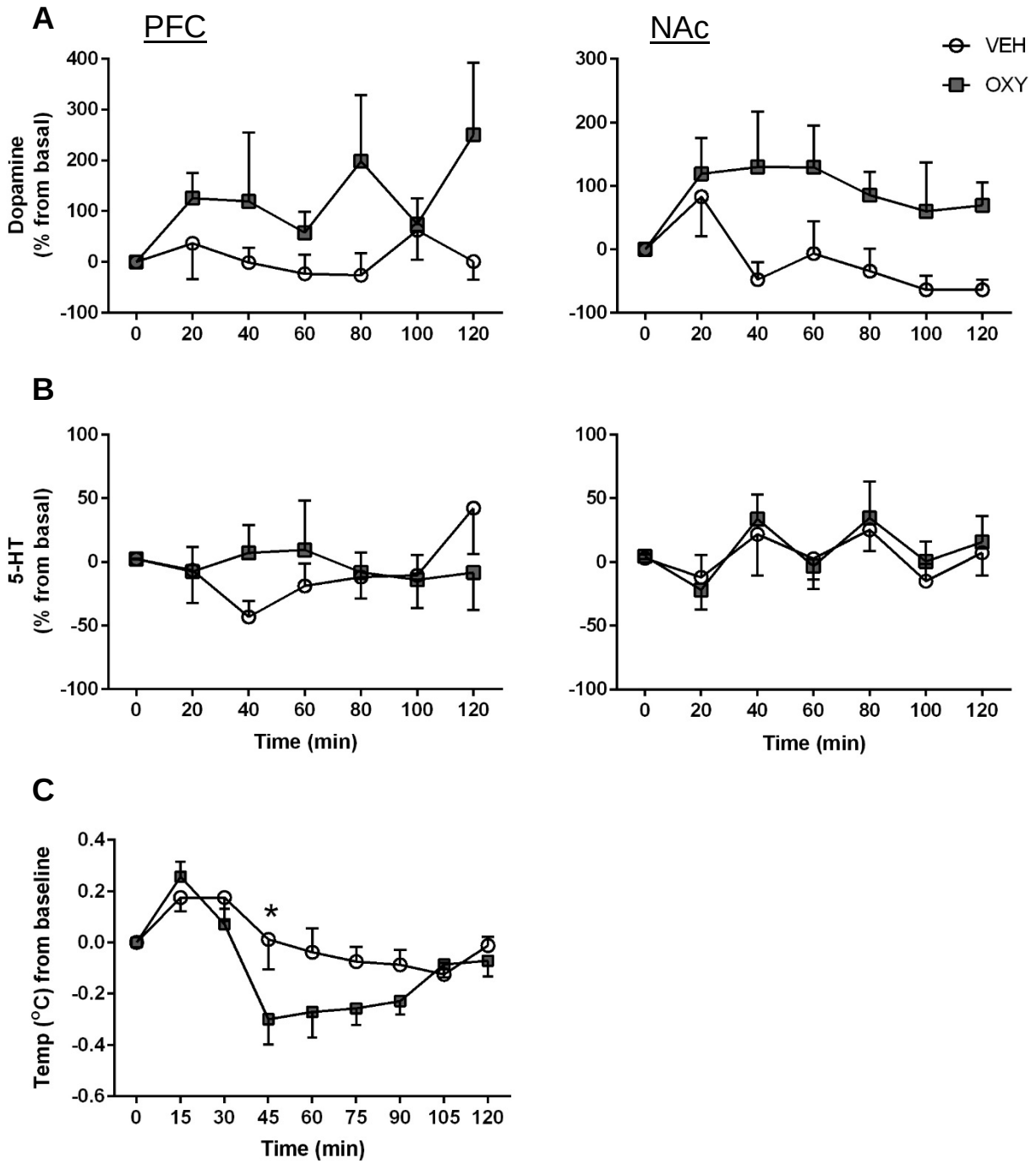

A
Time (s)
***
*
Ano-genital Sniffing
Body Sniffing
Boxing & Biting
Crawling Over & Under
Following
Lying Side-by-Side
Pinning
VEH
OXY
B
Total Interaction (s)
VEH
OXY
C
Temp (°C) from baseline
Baseline
Before
After
VEH
OXY
D
Log10 (No. of Calls)
FLAT
STEP
TRILL
VEH
OXY
E
FLAT
STEP
TRILL

Figure 5


A
PFC
NAc
VEH
OXY
Dopamine (% from basal)
B
5-HT (% from basal)
Time (min)
Time (min)
C
Temp (°C) from baseline
*
Time (min)