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Evidence for multiple sites within rat ventral striatum mediating cocaine conditioned place preference and locomotor activation

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Abstract

Considerable evidence suggests psychostimulants can exert rewarding and locomotor stimulating effects via increased dopamine transmission in the ventral striatum. However, the relative contributions of ventral striatal subregions to each of these effects have been little investigated. The present study examined the contribution of different ventral striatal sites to the rewarding and locomotor activating effects of cocaine. Initially, the effects of bilateral 6-hydroxydopamine lesions of the nucleus accumbens core or medial shell on cocaine-induced locomotor stimulation (0.5-1.5 mg/kg i.v. or 5-20 mg/kg i.p.) and conditioned place preference (0.5 mg/kg i.v. or 10 mg/kg i.p.) were examined. A subsequent study investigated the effects of olfactory tubercle *vs.* medial shell lesions on cocaine conditioned place preference and locomotor activity (0.5 mg/kg i.v.). Dopaminergic lesion extent was quantified by radioligand binding to the dopamine transporter. Multiple linear regression was used to identify associations between behavioral effects and residual dopamine innervation in ventral striatal subregions. On this basis, the accumbens core was associated with locomotor stimulant effects of i.v. and i.p. cocaine. In contrast, the medial shell was associated with the rewarding effect of i.v. cocaine, but not of i.p. cocaine. Finally, the olfactory tubercle was identified as an additional site contributing to conditioned place preference produced by i.v. cocaine. Overall, these findings provide additional evidence that the locomotor stimulant and rewarding effects of systemically-administered psychomotor stimulant drugs are segregated within the ventral striatum.

Introduction

The nucleus accumbens (NAcc) plays an important role in the rewarding and locomotor stimulant effects of systemically-administered amphetamine and cocaine (Koob et al., 1998; Wise, 2004). It is anatomically and neurochemically heterogeneous, with a prominent medioventral shell and dorsolateral core (Zahm and Brog, 1992). Recent behavioral studies, largely relying on intracranial microinjections of dopaminergic agonists, have provided evidence for functional compartmentalization within this structure, although certain details are controversial. Thus, the medial shell subregion has been implicated in reward processes (Di Chiara et al., 2004 ; Ikemoto and Wise, 2004), whereas locomotor stimulation has been elicited from core and/or shell injection sites (Boye et al., 2001; Ikemoto, 2002; Sellings and Clarke, 2003 and references therein).

The technique of intracranial drug microinjection, despite its obvious utility, is limited by the fact that local drug concentrations are usually unknown and may not be comparable with those obtained after systemic administration. Using an alternate approach, we recently evaluated the respective roles of accumbens core and shell in amphetamine-induced locomotion and conditioned place preference (CPP) by combining systemic amphetamine challenge with prior 6-hydroxydopamine (6-OHDA) lesions of either structure (Sellings and Clarke, 2003). In this study, DAergic depletion in core and medial shell reduced amphetamine-induced locomotor stimulation and CPP, respectively.

The present study sought to extend these findings to cocaine. Intra-NAcc infusion of cocaine produces both locomotor stimulation and rewarding effects (Ikemoto, 2002; Ikemoto, 2003; Ikemoto and Witkin, 2003; Rodd-Henricks et al., 2003). However, the interpretation of such findings is complicated by possible sympathomimetic and

anesthetic actions within the target tissue (Ikemoto, 2003; Ikemoto and Witkin, 2003). Even after systemic injection, the precise route of administration can be critical. In particular, cocaine is reported to produce DA (dopamine)-dependent or DA-independent rewarding effects, depending on whether it is delivered intravenously or intraperitoneally (Spyraki et al., 1987). In the present study, these two systemic routes of administration were compared.

The less-studied olfactory tubercle (OT) may also play a role in psychomotor stimulant-mediated locomotor activation and reward. This is suggested by studies employing intracranial administration in rats. Thus, direct intra-OT infusions of DA agonists including amphetamine and cocaine produced a marked and prompt locomotor activation (Pijnenburg et al., 1976; Cools, 1986; Ikemoto, 2002), and both these drugs were avidly self-administered at OT sites (Ikemoto, 2003; Ikemoto et al., 2005). Interestingly, intra-OT drug infusions elicited stronger locomotor and reinforcing effects than intra-NAcc infusions (Cools, 1986; Ikemoto, 2003; Ikemoto et al., 2005). Despite these positive findings, we previously tested the impact of profound 6-OHDA lesions of OT on the locomotor stimulant and rewarding (CPP) effects of *systemic* amphetamine challenge, and concluded that DAergic transmission in the OT does not contribute significantly to either behavioral effect (Clarke et al., 1988; Clarke et al., 1990). Hence, at present, it is an open question whether the OT contributes significantly to the locomotor stimulant and rewarding effects of any systemically-administered psychostimulant.

The overall goal of the present study was therefore to localize the ventral striatal actions of *systemically-administered* cocaine. The first experiment investigated whether

the locomotor stimulant effects of i.v. and i.p. cocaine are diminished by DA denervation in the accumbens core or medial shell. The next two experiments determined whether the stimulant and rewarding effects of cocaine could be dissociated by selective 6-OHDA lesions of either structure, as previously seen with amphetamine (Sellings and Clarke, 2003). The final experiment tested for OT involvement in cocaine reward and locomotor activation, again after systemic drug challenge.

Methods

Experimental design. The design of all four experiments is summarized in Table 1.

Subjects. Subjects were male Long–Evans rats (Charles River, St. Constant, Quebec) weighing 250–325 g at time of surgery. Rats were housed individually (Experiment 1) or in groups of three (Experiments 2 - 4) in clear Plexiglas cages in a temperature- and humidity-controlled animal colony, lit from 7 A.M. to 7 P.M. Food and water were available ad libitum except during behavioral testing. All experiments were approved by the McGill Faculty of Medicine Animal Care Committee in accordance with Canadian Council on Animal Care guidelines.

Stereotaxic infusion of 6-OHDA. Surgery was performed 7-10 days prior to the start of behavioral testing. Rats were anesthetized with ketamine HCl (90 mg/kg, i.p.) and xylazine HCl (16 mg/kg, i.p.) prior to placement in a stereotaxic apparatus (Kopf, Tujunga, CA) with the incisor bar set at -3.9 mm. Depending on the experiment (see

Table 1), rats received bilateral infusions of either 6-OHDA or vehicle into either NAcc core, medial shell, or anteromedial olfactory tubercle (amOT). Infusions were made via a 30 gauge stainless steel cannula attached by polyethylene tubing to a 10 µl Hamilton syringe driven by a model 5000 Micro Injection Unit (Kopf) (core or medial shell) or via two separate 10 µl Hamilton syringes driven by a multi-channel syringe pump (amOT; MD-1001, BioAnalytical Systems Inc., West Layette, IN). For greater accuracy, coordinates for all three target subregions were derived from the mean of two coordinate systems. Thus, anterior-posterior coordinates were +10.3 mm from interaural zero and +1.3 mm from bregma for both core and shell, and +10.7 mm from interaural zero and +1.7 mm from bregma for amOT. Lateral coordinates were ±0.6 mm (shell), ±2.4 mm (core) and ±0.8 mm (amOT). Ventral coordinates for shell (three injections) were +2.0, +2.4, and +2.8 mm from interaural zero and -8.0, -7.6, and -7.2 mm from bregma. Ventral coordinates for core were +2.7 mm from interaural zero and -7.3 mm from bregma. For amOT, ventral coordinates were +1.1 mm and –8.9 mm respectively from interaural zero and bregma. All coordinates are based on the atlas of Paxinos and Watson (1997). 6-OHDA or vehicle was infused on each side in a volume of 0.1 µl (core), as three infusions of 0.06 µl (medial shell), or 0.2 µl (amOT) on each side. For core and medial shell, 6-OHDA was infused at a rate of 0.1 µl /min; for amOT, the rate of infusion was 0.1 µl/10 min. The concentration of 6-OHDA used was 80 µg/µl (core) or 48 µg/µl (shell). For amOT, a volume of 0.2 µl of either vehicle or 6-OHDA (40 µg/µl free base) was infused bilaterally over 20 minutes. The different doses of 6-OHDA, infusion volumes and infusion times used at each lesion site were chosen based on pilot studies, and represented the best compromise between efficacy (DA depletion) and anatomical

selectivity. For all three lesion sites, the cannula remained at the final infusion site for 5 min.

Intravenous catheterization. During 6-OHDA lesion surgery, rats were implanted with chronic indwelling silastic catheters (0.51 mm I.D. and 0.94 mm O.D., Fisher Scientific, Montreal, Quebec) in the left jugular vein. Tubing was secured to the vein by surgical silk sutures, led subcutaneously to the skull surface, and was then fitted onto a 22 gauge cannula attached to a plastic connector (Model number C313G-5UP, Plastics One, Roanoke, VA). The cannula/connector was fixed to the animal's skull with small stainless steel screws (Lomir, Notre-Dame-de-L'Ile Perrot, Quebec) and dental cement (Stoelting, Wood Dale, IL). To keep catheters patent, 0.1-0.15 ml heparinized 0.9% saline was administered at the end of surgery, on the first day of behavioral testing, and every 2-3 days thereafter

Locomotor activity testing (Experiment 1). Horizontal locomotor activity was tested in the CPP apparatus (see below for description). Rats were first given one pre-exposure session (20 minutes) in the absence of drug. Each rat then received eight tests on consecutive days with cocaine given i.v. (0, 0.5, 1 or 1.5 mg/kg) or i.p. (0, 5, 10, 20 mg/kg) in a randomized order. Each test session lasted 30 min, starting immediately after injection. Test cages contained one bar and one mesh tile (see below).

Conditioned place preference and locomotor activity testing (Experiments 2, 3 and 4). The apparatus and general procedure were as previously described (Sellings and Clarke, 2003). Briefly, the procedure consisted of three phases: pre-exposure (one day),

conditioning (six days) and test (one day). All phases were carried out in a one-compartment box (58 cm x 29 cm x 53 cm) with walls made of white plastic-coated particle board. In the pre-exposure phase, Beta-Chip sawdust bedding covered the floor of the cage. In the conditioning phase, two square tactile tiles of either bar or mesh texture were placed in the bottom of the cage, on top of the bedding. During this phase, the video tracking software (EthoVision v 3.0, Noldus Information Technology, Leesburg, VA) measured locomotor activity, expressed as horizontal distance moved (in meters). During the test phase, one bar and one mesh tile were placed on the bottom of the cage. The time spent on bar or mesh texture was measured by EthoVision software. All three phases were carried out under darkroom lighting using a Kodak GBX-2 safelight filter (Vistek, Toronto, Ontario, Canada), to minimize visual cues. Animals do not spontaneously prefer either texture (unpublished observations), and all experiments were as fully counterbalanced as possible with respect to drug-texture pairing and order of drug pairing (drug-saline or saline-drug) within each surgery group. For all experiments, pre-exposure sessions lasted 20 minutes, and the test session 10 minutes. Conditioning trial duration for i.v. COC was 15 minutes; for i.p. COC, 25 minutes.

For i.v. infusion (Experiments 1, 2 and 4), a fluid swivel was fixed above the center of each cage. Each swivel was connected to on one end a 1 ml syringe, and on the other end to a brass connector (Produits MSM, Laval, Quebec) and protective spring (Heiplex, Montreal) via Tygon tubing of 0.51 mm diameter. The cannula fixed to the skull of the rat was attached to the Tygon tubing, and the brass connector fastened to the plastic connector, to secure the tubing to the cannula, hence allowing administration of

drug immediately after placement in the CPP cage. Drug was infused over 25-30 s. Cocaine administered i.p. was injected immediately prior to placement in the CPP cage.

Tissue Preparation. Tissue was prepared for autoradiography and Nissl-staining (cresyl violet) as previously described (Sellings and Clarke, 2003). Briefly, rats were sacrificed 3 to 5 hours following CPP testing, by decapitation under sodium pentobarbital (65 mg/kg, i.p.) anesthesia. Brains were removed, frozen in 2-methylbutane at -50°C for 30 sec, and stored at -40°C.

Coronal sections (20 μm) were taken on a cryostat at several rostrocaudal levels through the ventral striatum. In Experiments 1, 2, and 3, sections were examined at 11.2, 10.7, 10.2, and 9.7 mm anterior to interaural zero; 9.2 and 8.7 mm were also examined in Experiment 4 (Paxinos and Watson 1997). Four adjacent sections were collected for autoradiography and one for Nissl staining with cresyl violet. Sections were thaw mounted onto gelatin-subbed slides, air dried at room temperature for 20–30 min, and stored with desiccant at -40°C.

Quantitative autoradiography. The extent and chemical selectivity of the 6-OHDA lesion was quantified by autoradiographic labeling of the DA transporter (DAT) and the 5-HT transporter (SERT) (Sellings and Clarke, 2003), using a nonsaturating concentration of 3β-(4-iodophenyl)tropan-2-β-carboxylic acid methyl ester ([^{125}I]-RTI-55; 2200 Ci/mmol; NEN-Mandel, Guelph, Ontario).

Sections were thawed at room temperature for 10 min and then placed in a staining dish containing an aqueous buffer solution of 120 mM NaCl, 0.1 M sucrose, 10

mM sodium phosphate buffer, and 10 pM [^{125}I]-RTI-55, with the pH adjusted to 7.4. In the DAT autoradiographic assay, 50 nM citalopram hydrobromide was used to occlude SERT; nonspecific binding was determined by addition of 10 μM 1-(2-[bis(4-flurorphenyl)methoxy]ethyl)-4-(3-phenylpropyl)piperazine dihydrochloride (GBR 12909). For SERT autoradiography, 1 μM 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)piperazine dihydrochloride (GBR 12935) was added to occlude DAT; nonspecific binding was determined by addition of 50 nM citalopram HBr (Sellings and Clarke, 2003). Slides were incubated at room temperature for 2 hr and then washed three times in cold buffer solution (once for 1 min, twice for 20 min) and for 1-2 sec in distilled and deionized water. They were then blow dried and placed in X-ray film cassettes. Kodak BioMax MS film (Amersham Biosciences, Baie d'Urfé, Québec) was exposed to slides for 48 hr (DAT) or 120 hr (SERT) with [^{125}I] autoradiographic standards (Amersham Biosciences). After development of film, DAT and SERT binding were quantified using an MCID M4 imaging system (Imaging Research, St. Catherines, Ontario).

Histological examination. Tissue was stained with cresyl violet to assess nonspecific damage, as previously described (Sellings and Clarke, 2003), and examined under a light microscope (40–200X magnification).

Drugs. Drug sources were as follows: cocaine HCl (gift of National Institute on Drug Abuse, Bethesda, MD); citalopram HBr (gift from H. Lundbeck A/S); dipyrone (Vetoquinol, Quebec, Quebec); ketamine HCl (Vetalar, Vetrepharm, London, Ontario);

xylazine HCl (Anased, Novopharm, Toronto, Ontario); GBR 12909 (NIMH Chemical Synthesis and Drug Supply Program), and GBR 12935•2HCl (Sigma-Aldrich, Oakville, Ontario). Unless otherwise stated, all other chemicals were obtained from Fisher Scientific (Montreal, Quebec).

Cocaine HCl was dissolved in sterile 0.9% saline and injected at 1 ml/kg (i.v. or i.p.). 6-OHDA HBr was dissolved in sterile 0.9% saline containing 0.3 mg/ml sodium metabisulfite (Sigma-Aldrich) as an antioxidant and protected from light. Vehicle solutions, as well as 6-OHDA to be infused into medial shell or amOT, were neutralized to pH 7.3 ± 0.1 with NaOH (to reduce non-specific damage; see Results). Doses of all drugs except 6-OHDA HBr are expressed as the salt. 6-OHDA HBr doses are expressed as free base.

Data analysis. A commercial software program (Systat v10.2, SPSS Inc., Chicago, IL) was used for all data analyses. In all experiments, locomotor responses to cocaine were calculated as the difference of locomotor counts between drug and saline conditioning sessions. In CPP experiments, saline locomotor scores were calculated as the mean activity over all three conditioning sessions with saline, and are expressed as mean ± SEM. After initial data inspection, sham groups were combined within each experiment. Group differences were analyzed by 1-way ANOVA. CPP magnitude was calculated as the difference between time spent on the drug-paired and vehicle-paired sides during the 10-minute test session. Experiments 2 and 4 were each carried out in different batches, due to space constraints in the animal facility; after initial data inspection, the results within each experiment were pooled. The existence of a significant CPP magnitude or

locomotor stimulant effect was determined by one-sample Student's t-test with Bonferroni correction for multiple comparisons. The relationship between behavioral measures *vs.* [^{125}I]-RTI-55 labeling was analyzed by multiple linear regression. A *p* value of less than 0.05 (two-tailed) was considered significant. Group data are expressed as mean ± SEM throughout.

Results

Neurochemical and anatomical selectivity. To assess nonspecific tissue damage, sections were Nissl-stained with cresyl violet. As previously reported (Sellings and Clarke, 2003), only minimal cell loss was evident at the site of infusion for all vehicle groups (Fig. 1A) and for the group infused with 6-OHDA in the core subregion (not shown). Among rats lesioned in medial shell or amOT, most rats (~60%) also showed a minimal degree of cell loss, ~30% of rats possessed a small region of decreased cell density at the infusion site (Fig. 1B), and ~10% of rats showed more pronounced non-selective damage (Fig. 1C). This larger region of non-specific damage did not extend more than 0.3 mm from the site of infusion and was almost always found at only one anterior-posterior level.

Sampling locations for DAT and SERT binding density are indicated in Fig. 2. [^{125}I]-RTI-55 autoradiographs of DAT binding are shown in Fig. 3. Residual DAT binding as a percent of combined sham groups is given in Tables 2 (Experiments 1-3) and 3 (Experiment 4). Radioligand binding to SERT in tissue from lesioned animals was minimally changed by all lesion parameters in all experiments (Tables 2 and 3).

In all Experiments, rats were allowed 7-10 days recovery post-surgery before the start of behavioral testing.

The magnitude of core, but not medial shell, DA denervation predicted locomotor responses to i.p. and i.v. cocaine. The effects of 6-OHDA lesions of core vs. medial shell on cocaine-induced locomotion were tested most extensively in Experiment 1. Locomotor responses to i.p. and i.v. cocaine are shown in Fig. 4A and 4B (absolute values) and Fig. 4C and 4D (saline-subtracted values). Saline test scores did not differ significantly between the three surgery groups (Fig. 4A and 4B). The locomotor stimulant effects of cocaine were blunted only in the core-lesioned group. Multiple linear regression analysis revealed significant positive associations between core DAT binding and the locomotor stimulant response for both administration routes used and at all doses except for 0.5 mg/kg i.v. (range $p < 0.001$ - $p < 0.05$). Significant *negative* associations were observed between medial shell DAT binding and the locomotor stimulant response at several cocaine doses (1 mg/kg i.v., 5 and 10 mg/kg i.p.; $p < 0.05$ – $p < 0.005$).

The effects of core and medial shell 6-OHDA lesions on cocaine-induced locomotion were also tested in two CPP experiments (i.e. Experiments 2 and 3). Locomotor data were obtained from the three drug and saline conditioning sessions. Experiment 2 examined the locomotor stimulant response to cocaine (0.5 mg/kg i.v.). Here, saline locomotor scores did not differ significantly between groups and were as follows: 52 ± 2 m (sham), 54 ± 2 m (core 6-OHDA), and 55 ± 2 m (medial shell 6-OHDA). A significant locomotor stimulant effect was observed in sham-lesioned and medial shell-lesioned animals, but not in the core-lesioned subjects (Fig. 5A). Multiple

linear regression analysis revealed a positive trend between the locomotor response and DAT binding in the core ($p = 0.086$, Fig. 5B) but not medial shell (Fig. 5C).

The locomotor stimulant response to *intraperitoneal* cocaine (10 mg/kg) was also attenuated after core 6-OHDA lesions (Experiment 3, Fig. 6A). No significant group differences were seen for saline locomotor activity. Saline scores were 85 ± 6 (sham), 88 ± 5 (core 6-OHDA), and 87 ± 5 (medial shell 6-OHDA). Multiple linear regression analysis (Fig. 6B and 6C) revealed a positive association between the locomotor response and core DA innervation only ($p < 0.05$).

NAcc medial shell lesions inhibited CPP for i.v. cocaine. In Experiment 2, only the combined sham group and the core-lesioned group exhibited significant CPP (Fig. 5D). Relationships between the CPP magnitude and core *vs.* medial shell DAT binding are shown in Figs. 5E and 5F respectively. The CPP magnitude produced by i.v. cocaine was positively related to medial shell DAT binding ($p < 0.005$, Fig. 5F) with a negative trend in the accumbens core ($p = 0.062$, Fig. 5E).

Conditioned place preference for i.p. cocaine was unaffected by lesions of core or medial shell. In Experiment 3, a significant CPP to i.p. cocaine occurred in the sham-lesioned group, with a similar trend in the two lesion groups (Fig. 6D). No significant relationship was observed between the CPP magnitude and core or medial shell DAT binding (Figs. 6E and 6F).

CPP magnitude for i.v. cocaine was related to OT residual DAT binding. It was recently reported that amOT more robustly supports intracranial self-infusion of cocaine than does medial shell (see Discussion). Therefore, we first re-examined the data from Experiment 2 (i.v. cocaine), to determine if amOT DAT binding may have contributed significantly to the CPP magnitude. However, amOT binding was reduced only slightly in this experiment (by 28% in the core- and 11% in the shell-lesioned group). We therefore addressed the question of amOT involvement by directly comparing the effects of 6-OHDA lesions of the medial shell *vs.* amOT on i.v. cocaine CPP (Experiment 4).

Infusions of 6-OHDA into either amOT or medial shell depleted DAT binding locally, and also tended to produce a smaller and variable depletion in the other structure (Fig. 7). Initial analysis revealed a high degree of co-linearity existing in DAT binding levels between different OT subregions. Accordingly, these values were averaged, and subsequent analyses were carried out using OT rather than amOT values.

Only sham-lesioned animals exhibited significant CPP (Fig. 8A). Multiple linear regression analysis was performed with CPP magnitude as the dependent variable, using residual DAT binding in core, medial shell, ventral shell, ventral caudate putamen and OT as simultaneous predictors. Only OT was retained as a significant predictor ($p < 0.01$, Fig. 8C). Linear regression analysis of CPP magnitude with medial shell as the sole predictor revealed a positive association that bordered on significance ($p = 0.056$). Linear regression analysis of the locomotor stimulant effect revealed that DAT binding in neither medial shell nor OT predicted the degree of locomotor stimulation ($p > 0.5$ for both, data not shown).

Discussion

Novel findings. To our knowledge, the present study is the first to examine the role of ventral striatal subregions in CPP induced by systemically administered cocaine. Cocaine-induced locomotion was related to core DA innervation at several doses of both i.v. and i.p. cocaine. CPP results, in contrast, were more complex. Intravenous cocaine CPP appeared dependent on DA innervation in both OT and medial shell, whereas i.p. cocaine CPP was unaffected by medial shell lesions.

Methodological considerations. The present series of experiments revealed associations between residual DA innervation in various ventral striatal structures and cocaine-induced locomotion or CPP. It is doubtful that these relationships represent segregation between conditioned and unconditioned drug effects rather than between reward and locomotion, as core but not medial shell 6-OHDA lesions abolished amphetamine-induced *conditioned* locomotion (Sellings and Clarke, 2006).

In the present study, quantitative autoradiographic analysis was performed by taking a large number of samples within each structure (e.g. 24 each for medial shell and core). Within each targeted structure, the extent of DAT depletion appeared rather uniform (see Fig. 3 and Sellings and Clarke, 2003), and visual inspection revealed no evidence for smaller sites of preferential depletion. Nevertheless, we cannot rule out the possibility that our behavioral effects resulted from damage to functionally important "hot spots" within the targeted structures.

It is unlikely that non-specific damage caused these lesion effects, since only minimal changes were observed in SERT binding levels, and Nissl staining revealed only

slight non-specific damage in a subset of medial shell and medial OT lesioned animals (Fig. 1). However, 6-OHDA infusion almost certainly depleted noradrenaline as well as DA. Preservation of noradrenergic terminals by using systemic desipramine proved impossible, since in pilot studies the routinely used dose of 25 mg/kg (Kelly and Iversen, 1976) caused significant mortality (>25%). Nevertheless, for several reasons, it is unlikely that the observed lesion effects were due to loss of noradrenergic terminals. First, neither noradrenergic agonists nor antagonists when injected into ventral striatum affected locomotion (Pijnenburg et al., 1975; Pijnenburg et al., 1976). Second, noradrenergic denervation of ventral striatum does not alter locomotor stimulant responses to cocaine and amphetamine (Roberts et al., 1975; Kelly and Iversen, 1976). Third, noradrenergic afferents to NAcc largely avoid the core (Delfs et al., 1998), where lesion effects on locomotor stimulation occurred. Fourth, stimulation of noradrenergic transmission did not produce CPP (Martin-Iverson *et al*, 1985; Subhan *et al*, 2000). Fifth, neither α nor β adrenergic receptor antagonists affected the rewarding effects of i.v. cocaine as reflected by self-administration behavior (Johanson and Fischman 1989). Sixth, the disruptive effects of 6-OHDA lesions on cocaine self-administration appear unrelated to noradrenaline depletion (Roberts et al, 1977; Roberts et al, 1980). Lastly, self-administration of cocaine directly into the amOT was blocked by co-infusion of a D1 or D2 DA receptor antagonist (Ikemoto, 2003). On this basis, it seems reasonable to conclude that our 6-OHDA lesions produced their behavioral effects via local depletion of DA.

The accumbens core and locomotor activation. There is currently no consensus on the role of core *vs.* shell in psychostimulant-induced locomotion (Boye et al., 2001; Ikemoto,

2002 and references therein). In particular, studies employing intra-accumbens microinjection of direct or indirect DAergic agonists have implicated core, shell, or both structures, depending on the drug. For example, amphetamine acted with similar potency at either injection site, whereas cocaine stimulated locomotor activity most strongly after injection into medial OT and medial shell (Ikemoto, 2002). Importantly, locomotor responses from accumbens core injections of cocaine may have been weakened by local anesthesia (Ikemoto and Witkin, 2003).

The present experiments show that the locomotor stimulant effects of systemically administered cocaine are associated with DAergic neurotransmission in core rather than medial shell. This result generalized to several doses of the drug and to both i.p. and i.v. routes of administration. These findings accord with observations using systemic amphetamine (Boye et al., 2001; Sellings and Clarke, 2003 and references therein) and methylphenidate (Sellings et al., submitted). Taken together, they suggest a general mechanism by which systemically administered psychostimulants produce activating effects. Whether core DA transmission directly mediates the locomotor stimulant action of these drugs, or plays an indirect enabling role, remains a question for the future.

Differences between i.p. and i.v. cocaine CPP. In the present study, i.v. cocaine produced CPP that appears dependent on DA transmission in both medial shell and OT. In contrast, i.p. cocaine CPP did not appear dependent on accumbens DA transmission. This finding is consistent with reports suggesting that i.v. cocaine produces DA-dependent CPP, and i.p. cocaine DA-independent CPP (Morency and Beninger 1986; Spyraki et al., 1987). Although neuroadaptation may account for the lack of lesion effect

on i.p. cocaine CPP, this appears unlikely considering that similar medial shell lesions reduced CPP both for i.v. cocaine and for amphetamine (Sellings and Clarke 2003). Our results do not rule out other forms of accumbens involvement; indeed glutamatergic and serotonergic manipulations within this structure affect i.p. cocaine CPP (Kaddis et al, 1995; Harris et al, 2001).

Since cocaine produces CPP more potently after i.v. than i.p. administration (Spyraki et al., 1987; O'Dell et al., 1996), care was taken in the present study to select submaximal i.p. and i.v. doses of cocaine approximately matched in terms of CPP magnitude. Hence, it is likely that the differential sensitivity to DA depletion reflected route of administration and not dose.

The neurochemical basis of this differential susceptibility cannot readily be related to changes in extracellular DA. The i.v. dose used (0.5 mg/kg) has been reported to increase dialysate DA levels in the medial shell but not the core (Pontieri et al., 1995), whereas the i.p. dose (10 mg/kg) robustly increased DA levels in both subregions (Cadoni et al., 2000). Another reported difference between i.v. and i.p. cocaine administration is that only the former caused significant increases in glucose metabolism in NAcc and OT (Porrino, 1993); in the latter study, the use of a wide range of doses suggests strongly that route of administration was the critical factor. The basis for route-dependent effects on cerebral glucose utilization, and the possible relation to cocaine reward, remain to be elucidated.

Cocaine CPP: dependence on both medial shell and OT. Although there is a rich literature linking the NAcc to drug reward, possible OT involvement has been largely

unexamined (Clarke et al., 1990; Kornetsky et al., 1991; Ikemoto, 2003; Ikemoto, 2005; Ikemoto and Donahue, 2005). The present results suggest both medial shell and OT play important roles in mediating i.v. cocaine reward.

Self-administration of cocaine directly into the ventral striatum appears strongly site-dependent; responding was vigorous for infusions into amOT, marginal in medial shell, and negligible within accumbens core (Rodd-Henricks et al., 2002; Ikemoto, 2003). In addition, only cocaine infusion at amOT sites produced CPP at the doses tested (Ikemoto, 2003). However, the behavioral effects of focal cocaine infusion into the NAcc (shell or core) may be masked by local anesthesia (Ikemoto and Witkin, 2003). Nevertheless, DA antagonist microinjection experiments suggest that it is medial shell rather than core that mediates the reinforcing effects of self-administered i.v. cocaine (Bari and Pierce, 2005).

In Experiment 2, lesions of the medial shell reduced i.v. cocaine CPP independently of accumbens core; in this experiment, DA denervation in the OT was minimal. When 6-OHDA infusions of medial shell and OT were directly compared (Experiment 4), only OT DA innervation significantly predicted i.v. cocaine CPP. These results may indicate that the OT is a stronger mediator of cocaine reward, as concluded from findings based on intracranial cocaine infusion (Ikemoto, 2003). It is unlikely that these lesion effects represent disruptions of memory or learning, as medial shell lesions did not affect CPP induced by morphine (Sellings and Clarke, 2003) or i.p. cocaine (present study), and extensive 6-OHDA lesions of OT did not disrupt amphetamine CPP (Clarke et al., 1990).

Several factors could determine the relative contributions of OT *vs.* medial shell to psychostimulant CPP. First, the nature of the CPP paradigm used may be a factor. Our CPP procedure is based on tactile cues; other types of stimuli may engage other ventral striatal subregions. Another factor of potential importance is the drug in question. Our results suggest that i.v. cocaine CPP engages OT mechanisms. This does not appear to be the case for i.p. amphetamine CPP (Clarke et al., 1990).

Conclusions. The increase in locomotor activity observed after psychostimulant administration appears related to increased DA transmission in NAcc core. In contrast, CPP appears more complex, likely depending on drug and route of administration. The present study suggests that DA transmission in both medial shell and OT is important for i.v. cocaine CPP. Our findings build on recent evidence suggesting that distinct ventral striatal subregions participate in different aspects of drug reward (Sellings and Clarke, 2003; Ikemoto, 2003; Ikemoto and Donahue, 2005; Pecina and Berridge, 2005). Whether these structures act in concert or independently remains a question for further study (van Dongen et al., 2005).

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Footnotes

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Legends for Figures

Figure 1. Representative photomicrographs of Nissl staining in sham-lesioned (A) and amOT-lesioned (B, C) animals adjacent to the infusion site. In some (30%) of amOT lesioned rats, a small region of reduced cell density was observed compared to sham-lesioned rats (black arrow, Panel B). Larger regions of decreased cell density were seen in a subset (~10%) of lesioned animals (black arrow, Panel C). Scale bar 100 μm. Abbreviations: ac, anterior commissure; Tu, medial olfactory tubercle.

Figure 2. (A) Locations of sampled [^{125}I] RTI-55 binding in core, medial shell, ventral shell, olfactory tubercle and ventral caudate putamen. Each rat was sampled at four anterior-posterior levels. Numbers are distances (in millimetres) anterior to interaural zero. Sampling areas were circles of 0.3 mm diameter. (B) Sampling regions for olfactory tubercle subregions (anteromedial (amOT), anterolateral (alOT) and posterior (pOT)) in Experiment 4, and in post-hoc analyses of Experiment 2. At levels 11.2, 10.7 and 10.2, both amOT and alOT were sampled. At levels 9.7, 9.2 and 8.7, only pOT was sampled. Figures adapted from Paxinos and Watson (1997).

Figure 3. Representative autoradiographic images of [^{125}I] RTI-55 binding to DAT in animals from medial shell-lesioned, anteromedial olfactory tubercle-lesioned and sham-operated groups in Experiment 4. Since binding was similar between groups that received vehicle in medial shell and medial olfactory tubercle, the latter group has been omitted. Numbers designate distance anterior to interaural zero (in millimetres). Radioligand

binding was obtained at a nonsaturating concentration of radioligand. Arrows refer to the medial shell. Arrowheads (pointing upward) refer to the anteromedial olfactory tubercle.

Figure 4. Effect of 6-OHDA lesions of NAcc medial shell or core on locomotor responses to a range of i.p. and i.v. cocaine doses (Experiment 3). Each rat (n = 5-10 per group) was tested with i.v. (0-1.5 mg/kg) and i.p. (0-20 mg/kg) cocaine in a repeated measures design. Absolute locomotor activity at all doses of i.p. and i.v. cocaine are shown in panels A and B respectively. The stimulant effect of cocaine (i.e. cocaine-saline difference score) is illustrated in panels C and D. Locomotor response correlated positively and significantly with DAT binding in core at all doses except 0.5 mg/kg i.v. Shell refers to medial shell.

Figure 5. Effect of bilateral 6-OHDA infusion into either NAcc core or medial shell on locomotor response and CPP to i.v. cocaine (Experiment 2). Rats were allowed 7-10 d recovery after jugular catheter implantation and stereotaxic surgery prior to conditioning with i.v. cocaine (0.5 mg/kg). Locomotor responses (panels A-C) are expressed as the difference between the mean distance moved (m) during conditioning sessions with i.v. cocaine *vs.* saline. CPP magnitude (Panels D-F) is expressed as the difference between time spent on the drug-paired and saline-paired floor textures on test day (in s, 600 s test). DAT labeling in core or medial shell is expressed as a percent of combined sham-lesioned groups. Both sham and shell-lesioned groups exhibit significant locomotor stimulation (Panel A). Locomotor response tended to correlate positively with DAT binding in core (Panel B). Both sham and core-lesioned groups exhibit significant CPP

(Panel D). CPP magnitude correlated positively and significantly with DAT binding in medial shell (Panel F), and tended to correlate negatively with DAT binding in core (Panel E). CV, Core vehicle; CL, core lesioned; SV, medial shell vehicle; SL, medial shell lesion. Shell refers to medial shell.

Figure 6. Effect of bilateral 6-OHDA infusion into either NAcc core or medial shell on locomotor response and CPP to intraperitoneal (i.p.) cocaine (Experiment 3). Rats were conditioned with i.p. cocaine (10 mg/kg). Data are presented as in Figure 3. All groups exhibit significant locomotor stimulation (Panel A), but that of core-lesioned animals was smaller than that of the sham- and shell-lesioned groups ($p < 0.05$). Only sham rats exhibited significant CPP, but core and shell-lesioned animals also tended to exhibit CPP (Panel D). Locomotor response correlated positively and significantly with DAT binding in core (Panel B). No other behavioral responses correlated with DAT labeling in either structure (Panels C, E and F). CV, Core vehicle; CL, core lesioned; SV, medial shell vehicle; SL, medial shell lesion. Shell refers to medial shell.

Figure 7. Relationship of DAT labeling in nucleus accumbens medial shell *vs.* olfactory tubercle in Experiment 4 (n=46 rats). [^{125}I]RTI-55 autoradiography for DAT was used to assess residual DA innervation (see Materials and Methods), and expressed as a percentage of the mean value of the sum of medial shell-vehicle and olfactory tubercle-vehicle groups. Correlational analysis revealed a significant relationship between medial shell and olfactory tubercle binding (r=0.39, $p < 0.01$). OTV, olfactory tubercle vehicle; OTL, olfactory tubercle lesioned; SV, medial shell vehicle; SL, medial shell lesion.

Figure 8. Effect of 6-OHDA lesions of olfactory tubercle and medial shell on i.v. cocaine CPP (Experiment 4). CPP magnitude was calculated as the difference between the time spent on the drug-paired and saline-paired sides. CPP magnitude correlated positively and significantly with DAT binding in olfactory tubercle (Panel C), but not with DAT binding in medial shell (Panel B). OTV, olfactory tubercle vehicle; OTL, olfactory tubercle lesioned; SV, medial shell vehicle; SL, medial shell lesion. Shell refers to medial shell.

Table 1. Experimental parameters for Experiments 1-4. Sham groups represent a combination of rats infused with vehicle in core and shell (Experiments 1-3) or shell and amOT (Experiment 4).

Expt	Lesion site[a]	Dose (mg/kg)	Route	Behavior[b]	n[c]
1	core or shell	0.5-1.5 i.v., 5-20 i.p.	i.v.	LMA	11
2	core or shell	0.5	i.v.	CPP, LMA	10-14
3	core or shell	10	i.p.	CPP, LMA	12-14
4	amOT or shell	0.5	i.v.	CPP	15-16

[a] Shell refers to medial shell.

[b] CPP, conditioned place preference; LMA, locomotor activity.

[c] n is number of rats per surgery group (core, medial shell or anteromedial olfactory tubercle, and the combined sham-operated groups).

Table 2. Residual DAT and SERT binding in rats lesioned in core or medial shell in ventral striatal subregions (Experiments 1, 2 and 3). mSh, medial shell; vSh, ventral shell; OT, olfactory tubercle; vCP, ventral caudate putamen. Values given are mean ± SEM as a percent of combined sham group.

Experiment	1			2			3		
6-OHDA site	sham	core	mSh	sham	core	mSh	sham	core	mSh
DAT									
Core	100±4	40±8	85±5	100±3	25±1	86±5	100±7	20±3	95±5
mSh	100±11	60±9	31±7	100±5	47±3	42±3	100±8	48±4	36±6
vSh	100±5	63±12	76±4	100±7	45±3	92±7	100±6	35±6	98±8
OT	100±4	74±11	80±5	100±5	47±5	76±6	100±12	46±4	80±4
vCP	100±4	66 ± 8	92±4	100±5	45±2	102±7	100±10	50±7	107±6
SERT									
Core	100±9	97±7	91±5	100±5	83±4	93±4	100±5	108±6	97±3
mSh	100±6	105±6	95±7	100±2	93±5	90±4	100±4	97±5	94±3
vSh	100±5	102±6	100±5	100±2	90±4	99±4	100±6	118±8	108±3
OT	100±6	110±6	100±5	100±3	100±5	101±5	100±6	116±5	108±4
vCP	100±9	99±6	98±5	100±4	90±5	107±3	100±3	88±3	93±3

Table 3. DAT and SERT binding in medial shell- or anteromedial olfactory tubercle (amOT)- lesioned rats in imaged ventral striatal subregions (Experiment 4). Values are expressed as mean ± SEM. mSh, medial shell; vSh, ventral shell; amOT, anteromedial olfactory tubercle; alOT, anterolateral olfactory tubercle; pOT, posterior olfactory tubercle; vCP, ventral caudate putamen.

6-OHDA site	sham	medial shell	amOT
DAT			
Core	100±5	87±6	89±5
mSh	100±4	40±5	66±7
vSh	100±5	77±7	75±3
amOT	100±8	57±7	34±8
alOT	100±9	70±6	55±6
pOT	100±10	85±67	51±6
vCP	100±4	100±5	95±4
SERT			
Core	100±14	110±4	97±9
mSh	100±11	100±4	90±8
vSh	100±15	109±6	97±9
amOT	100±13	101±5	92±10
alOT	100±14	109±4	105±12
pOT	100±13	108±6	95±12
vCP	100±15	123±7	110±10

Figure 1

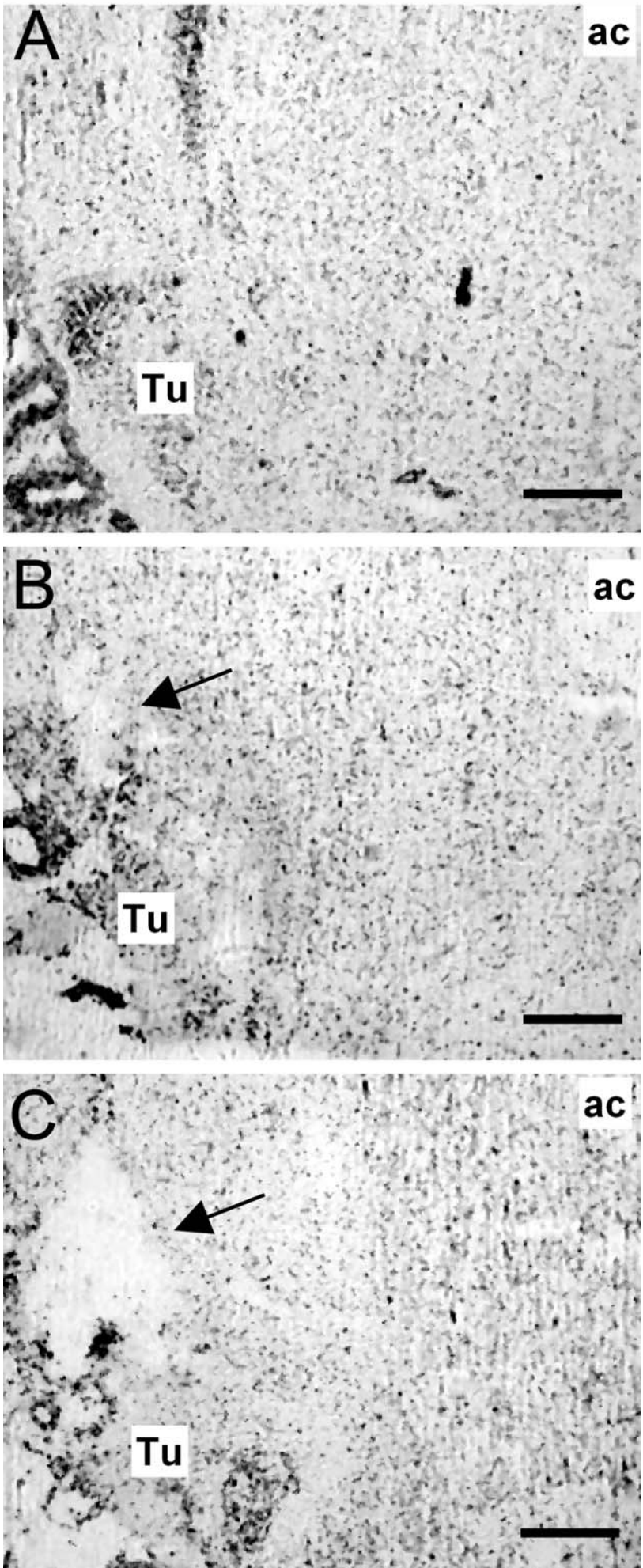


Figure 2

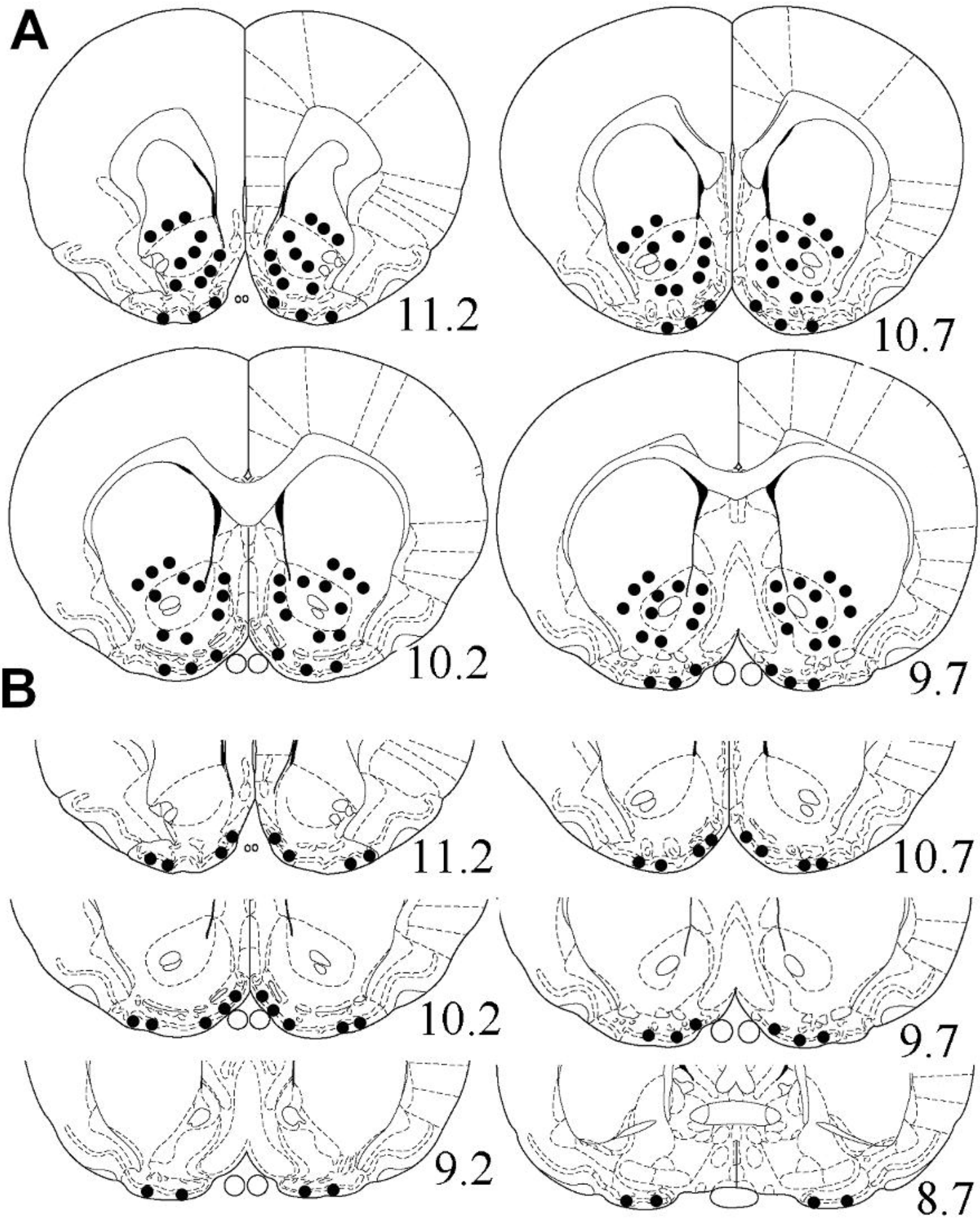


Figure 3

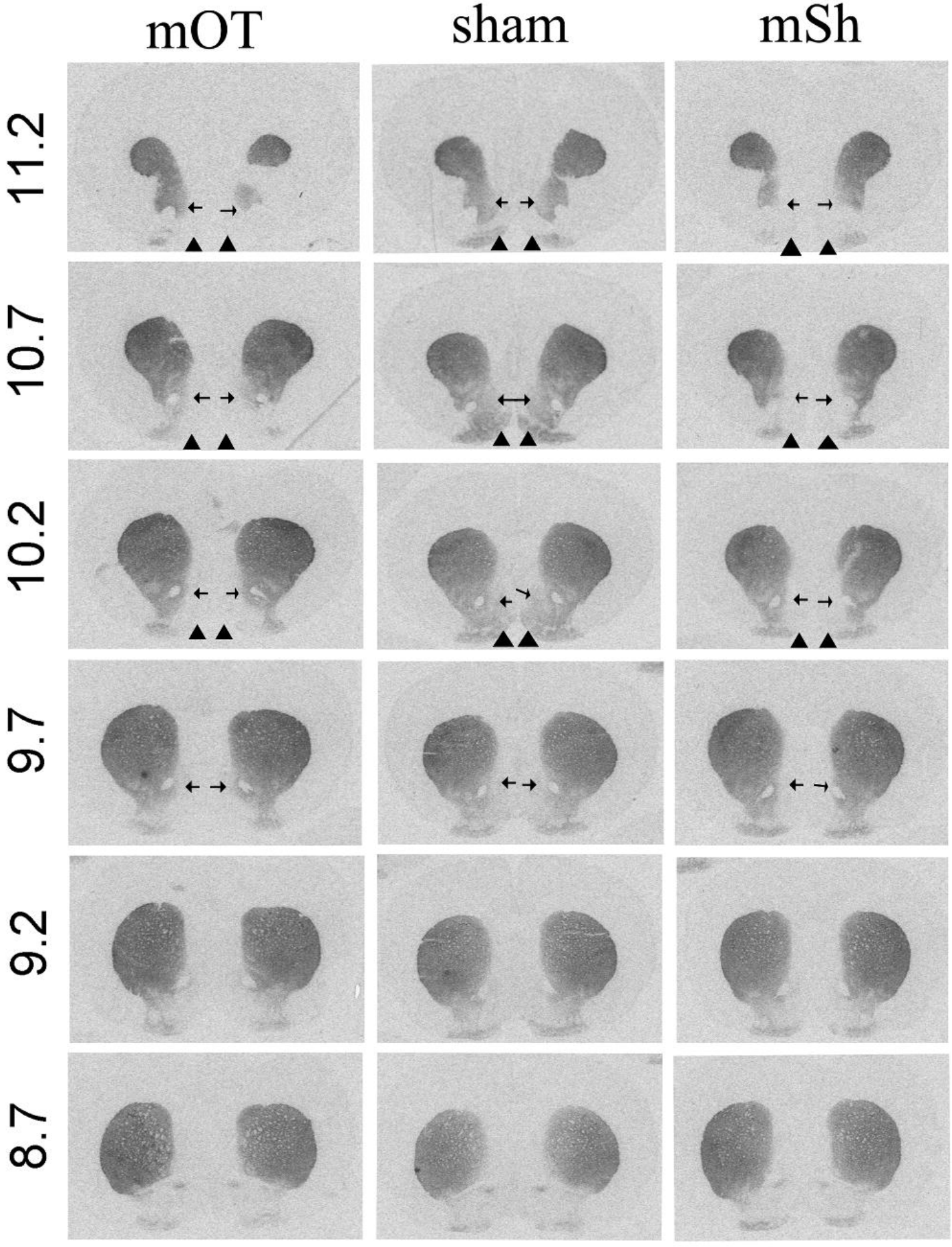


Figure 4

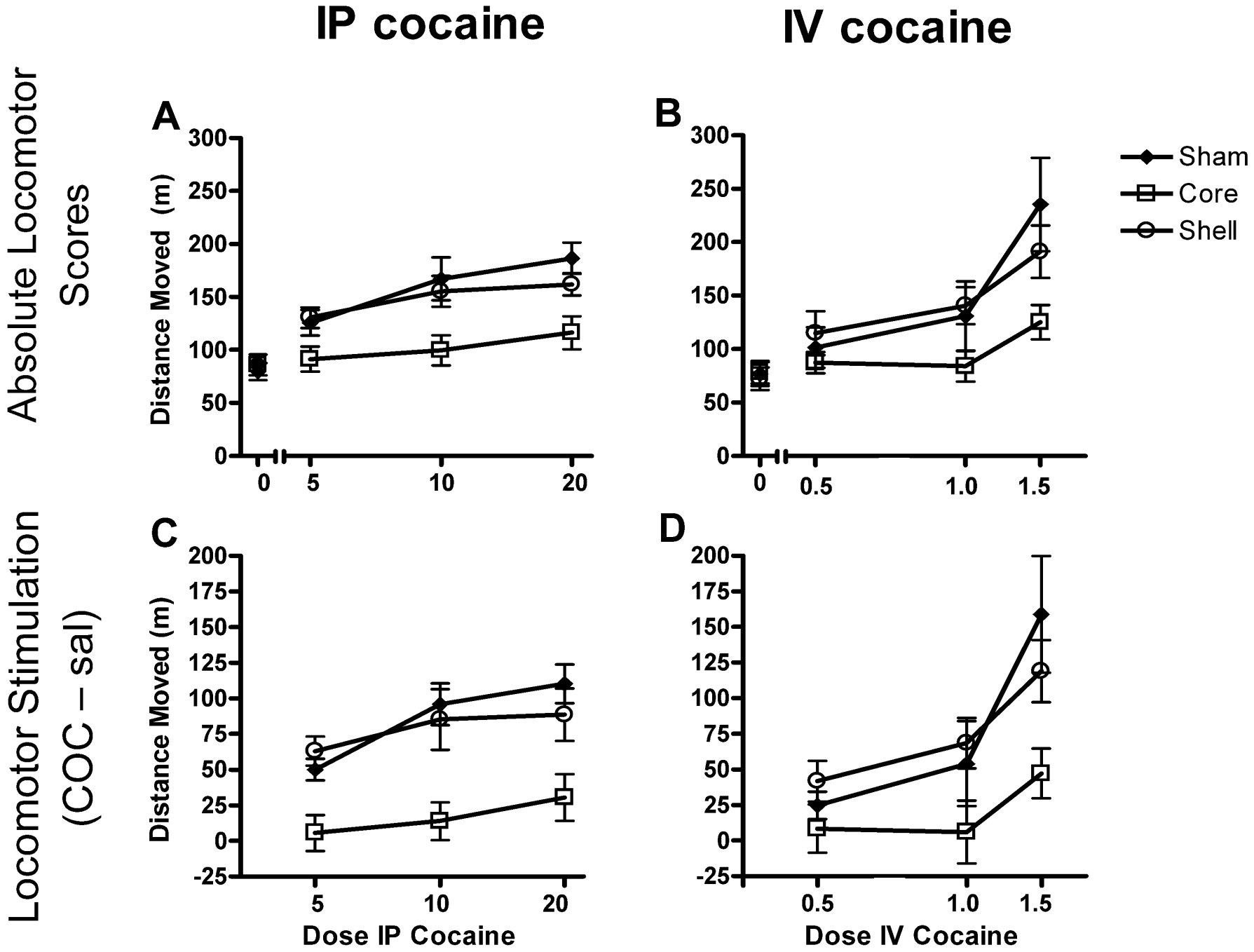

IP cocaine
IV cocaine
Absolute Locomotor Scores
Locomotor Stimulation (COC – sal)
A
B
C
D
Distance Moved (m)
Dose IP Cocaine
Dose IV Cocaine
Sham
Core
Shell
300
250
200
150
100
50
0
175
125
75
25
-25
0
5
10
20
0.5
1.0
1.5

Figure 5

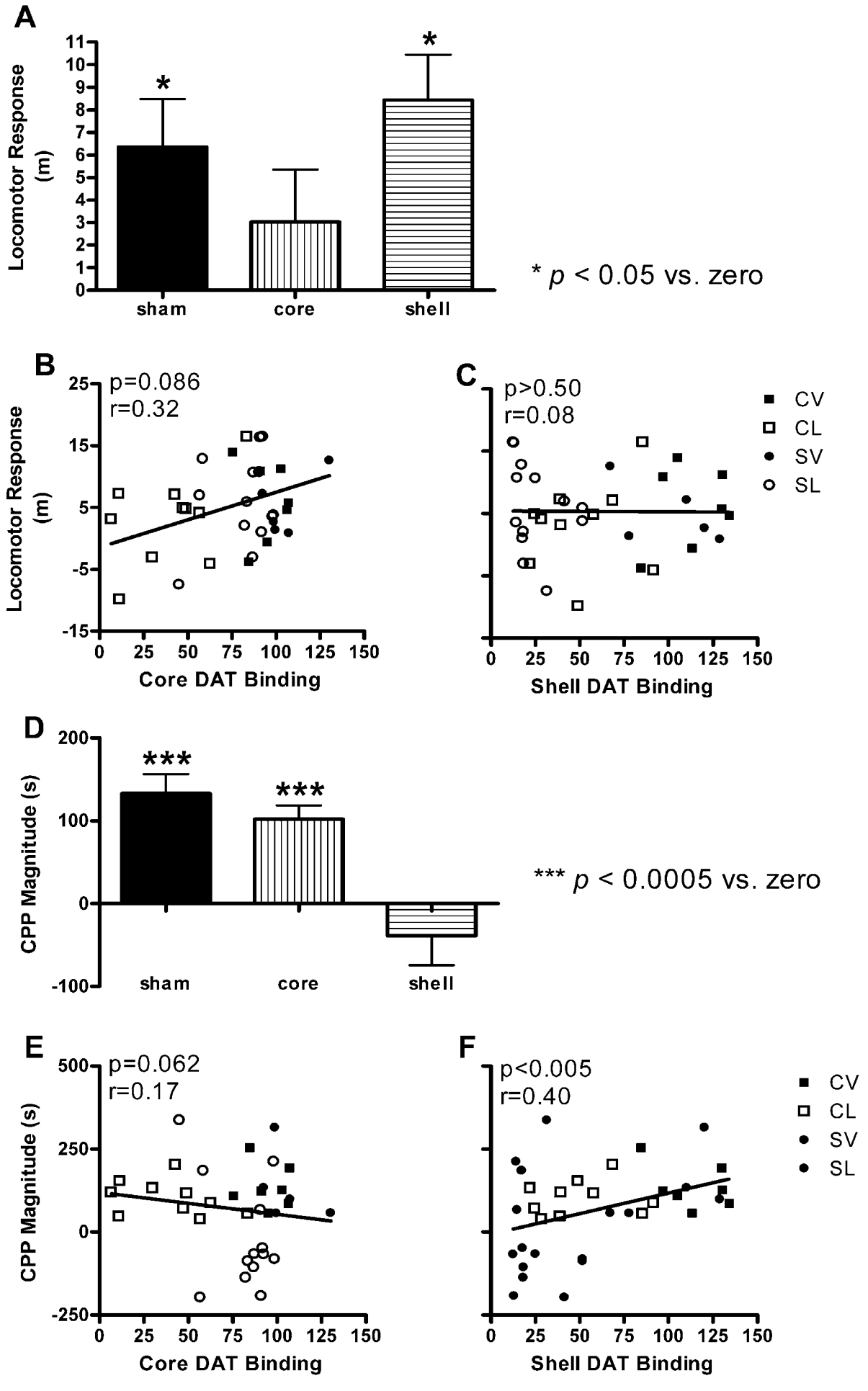

A
Locomotor Response (m)
sham
core
shell
* p < 0.05 vs. zero
B
p=0.086
r=0.32
Locomotor Response (m)
Core DAT Binding
C
p>0.50
r=0.08
CV
CL
SV
SL
Shell DAT Binding
D
CPP Magnitude (s)
***
***
sham
core
shell
*** p < 0.0005 vs. zero
E
p=0.062
r=0.17
CPP Magnitude (s)
Core DAT Binding
F
p<0.005
r=0.40
CV
CL
SV
SL
Shell DAT Binding

Figure 6

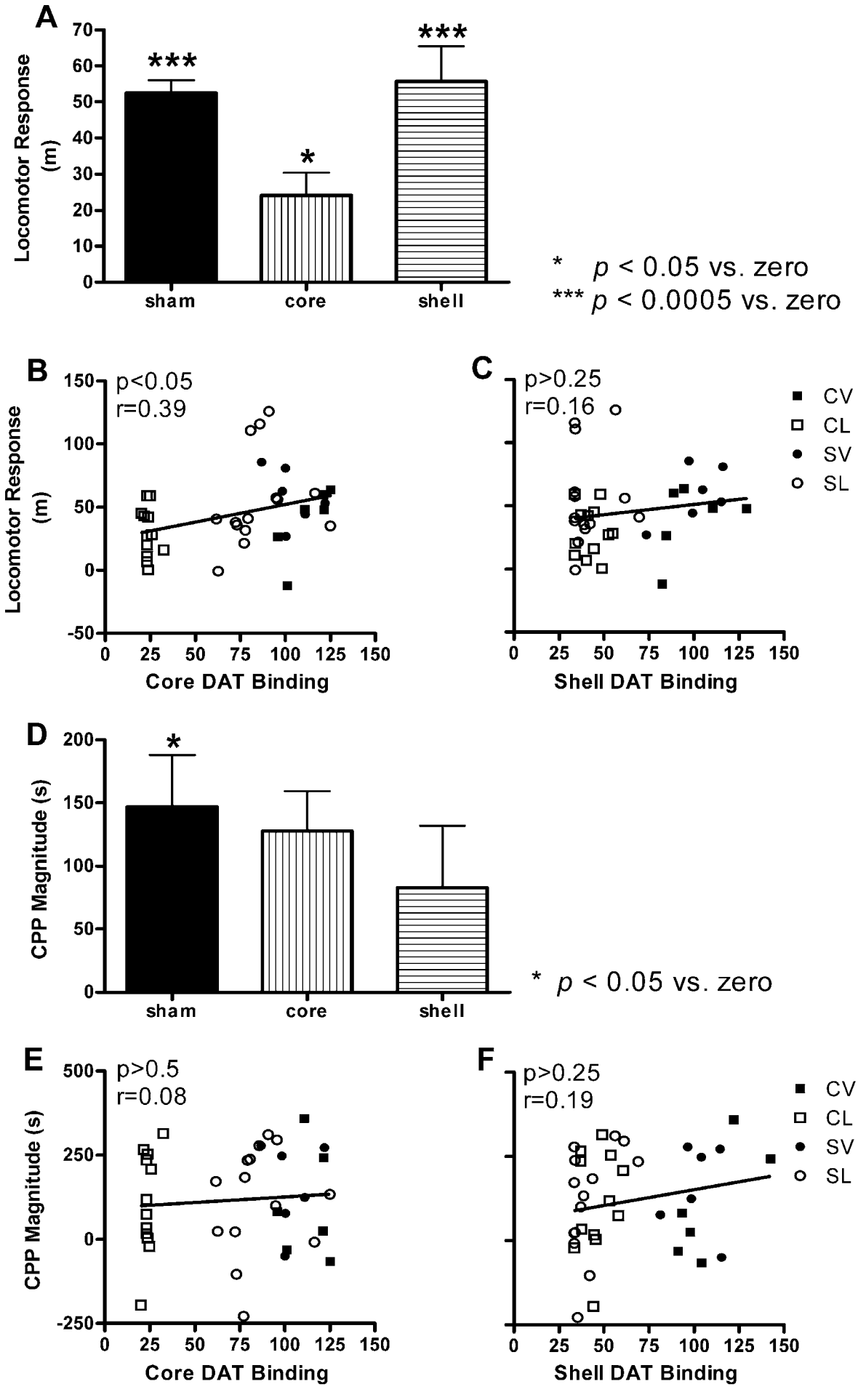

A
Locomotor Response (m)
70
60
50
40
30
20
10
0
***
*
***
sham
core
shell
* p < 0.05 vs. zero
*** p < 0.0005 vs. zero
B
p<0.05
r=0.39
Locomotor Response (m)
150
100
50
0
-50
0 25 50 75 100 125 150
Core DAT Binding
C
p>0.25
r=0.16
CV
CL
SV
SL
0 25 50 75 100 125 150
Shell DAT Binding
D
CPP Magnitude (s)
200
150
100
50
0
*
sham
core
shell
* p < 0.05 vs. zero
E
p>0.5
r=0.08
CPP Magnitude (s)
500
250
0
-250
0 25 50 75 100 125 150
Core DAT Binding
F
p>0.25
r=0.19
CV
CL
SV
SL
0 25 50 75 100 125 150
Shell DAT Binding

Figure 7

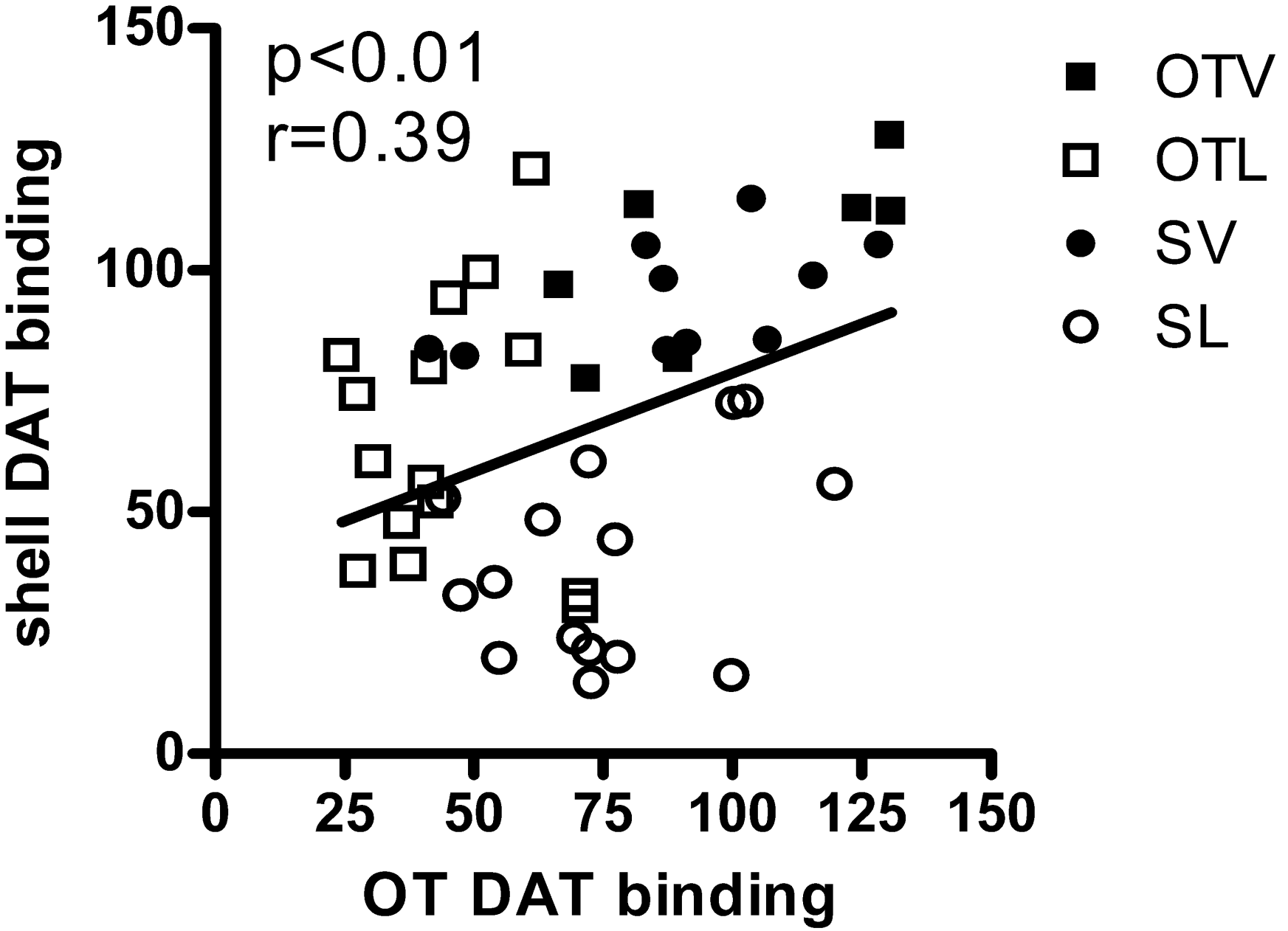
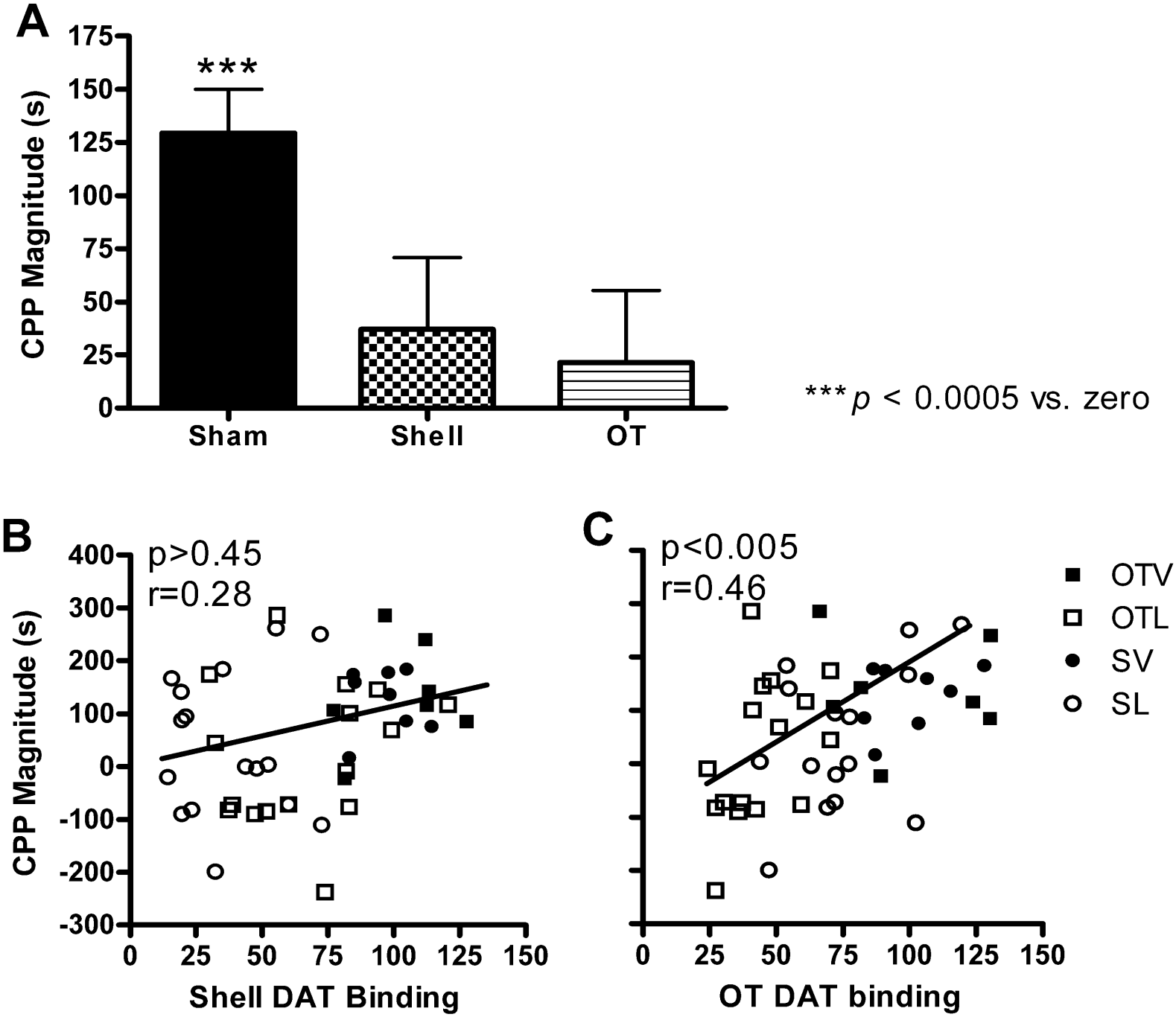

p<0.01
r=0.39
shell DAT binding
OT DAT binding
0
50
100
150
0
25
50
75
100
125
150
OTV
OTL
SV
SL

Figure 8


A
175
150
125
100
75
50
25
0
CPP Magnitude (s)
***
Sham
Shell
OT
***p < 0.0005 vs. zero
B
p>0.45
r=0.28
400
300
200
100
0
-100
-200
-300
CPP Magnitude (s)
0
25
50
75
100
125
150
Shell DAT Binding
C
p<0.005
r=0.46
OTV
OTL
SV
SL
0
25
50
75
100
125
150
OT DAT binding