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ROLE OF DOPAMINE OF NUCLEUS ACCUMBENS IN BEHAVIORAL SENSITIZATION TO METHYLPHENIDATE

Sheshali J. Wanchoo

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By

Sheshali J. Wanchoo, BS.

APPROVED:

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Dr. Alan C. Swann, Supervisory Professor

_____
Dr. Andrew Bean

_____
Dr. Anne B. Sereno

_____
Dr. Michael Beauchamp

_____
Dr. Thomas Goka

APPROVED:

_____
Dean, The University of Texas
Graduate School of Biomedical Sciences at Houston

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SENSITIZATION TO METHYLPHENIDATE

A

THESIS

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by

Sheshali J Wanchoo, BS.

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Dedications

I dedicate this thesis to my mother, who has always encouraged me to gain knowledge and work hard; and to my father, who through his stories has boosted my morale and taught me perseverance. I also dedicate it to my brother for being both my biggest fan and my worst critic.

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Abstract

Behavioral sensitization is defined as the subsequent augmentation of the locomotor response to a drug following repeated administrations of the drug. It is believed to occur due to alterations in the motive circuit in the brain by stressors, central nervous system stimulants, and similar stimuli. The motive circuit (or mesocorticolimbic system) consists of several interconnected nuclei that determine the behavioral response to significant biological stimuli. A final target of the mesocorticolimbic system is the nucleus accumbens (NAc), which is a key structure linking motivation and action. In particular, the dopaminergic innervations of the Nac are considered to be essential in regulating motivated states of behavior such as goal-directed actions, stimulus-reward associations and reinforcement by addictive substances. Therefore, the objective of this study was to investigate the role of dopaminergic afferents of the NAc in the behavioral sensitization elicited by chronic treatment with methylphenidate (MPD), a psychostimulant that is widely used to treat attention deficit hyperactivity disorder. The dopaminergic afferents can be selectively destroyed using catecholamine neurotoxin 6-hydroxydopamine (6-OHDA). In order to determine whether destruction of dopaminergic afferents of the NAc prevents sensitization, I compared locomotor activity in rats that had received infusions of 6-hydroxydopamine (6-OHDA) into the NAc with that of control and sham-operated animals. All groups of rats received six days of single daily MPD injections after measuring their pre and post surgery locomotor baseline. Following the consecutive MPD injections, there was a washout period of 4 days, where no injections were given. Then, a rechallenge injection of MPD was given. Behavioral responses after

repeated MPD were compared to those after acute MPD to assess behavioral sensitization. Expression of sensitization to MPD was not prevented by 6-OHDA infusion into the NAc. Moreover, two distinct responses were seen to the acute injection of MPD: one group of rats had essentially no response to acute MPD, while the other had an augmented ('sensitized'-like) acute response. Among rats with 6-OHDA infusions, the animals with diminished acute response to MPD had intact behavioral sensitization to repeated MPD, while the animals with increased acute response to MPD did not exhibit further sensitization to it. This suggests that the acute and chronic effects of MPD have distinct underlying neural circuitries.

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Abbreviations

6-OHDA   6-hydroxydopamine

DA   Dopamine

DBH   Dopamine-beta-hydroxylase

ED   Experimental day

HA   Horizontal activity

HPLC   High performance liquid chromatography

MPD   Methylphenidate

NAc   Nucleus Accumbens

NE   Norepinephrine

NOS   Number of stereotypy

PFC   Prefrontal cortex

TD   Total distance

VPm   ventrol medial part of Ventral Pallidum

VP   Ventral Pallidum

VTA   Ventral Tegmental Area

1. <u>INTRODUCTION</u>

Behavioral sensitization is the progressive increase in the motor response to a drug following repeated exposure to it (Dafny and Yang, 2006; Pierce and Kalivas, 1997; Wolf, 1998). It is associated with increased rewarding properties of the drug (Mead et al., 2004), and is related to mechanisms of addiction (Pierce and Kalivas, 1997; Wolf, 1998) and long term adaptations to stress (Sorg and Kalivas, 1993). It is generally believed that behavioral sensitization occurs due to alterations in the motive circuit or mesocorticolimbic system, which consists of dopaminergic connections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) (Dafny and Yang, 2006; Ikegami and Duvauchelle, 2004; Pierce and Kalivas, 1997; Sorg and Kalivas, 1993; Wolf, 1998). The final target of the mesocorticolimbic system, the NAc, is considered a key structure linking motivation and action (Mogenson et al., 1980). Particularly, the dopaminergic innervations of Nac are considered to be essential in regulating motivated states of behavior such as goal-directed actions, stimulus-reward associations and reinforcement by addictive substances (Meredith et. al., 1995). The NAc appears to be involved in the expression of behavioral sensitization (Pierce and Kalivas, 1997; Wolf, 1998). However, the role of NAc, particularly dopamine (DA) in the NAc, in behavioral sensitization to psychostimulants is not known. Methylphenidate (MPD) is an example of a psychostimulant that can cause behavioral sensitization (Gaytan et al., 1996, 1997, 2000; Yang et al., 2003, 2006, 2007). MPD is a widely used treatment for attention deficit disorder, with reports of abuse of MPD by human subjects (Kollins et al., 2001), consistent with a potential link between neural circuits involved in addiction

and behavioral sensitization (Wolf, 1998). Thus, the role of NAc DA in behavioral sensitization to MPD may be relevant to changes caused in the brains of those addicted to MPD or other stimulants, the abuse of which has been reported to be increasing (Wilens et al., 2008).

Research related to behavioral sensitization has widespread implications. Psychostimulants cross-sensitize with each other (Brandon et al., 2001; Itzhak et al., 2003; Achat-Mendes et. al., 2003; Rosine et al., 2009; Valvassori et al., 2007; Yang et al., 2003), with stressors (Nikulina et al., 2004; Prasad et al., 1995), and with other classes of drugs, such as opiates and nicotine (Celik et al., 2006; Cunningham et al., 1997; Santos et al., 2009; Smith et al., 2009). These neural alterations are persistent (Laruelle, 2000): prenatal stress predisposed to behavioral sensitization to stimulants in adulthood (Henry et al., 1995). Therefore, stress, psychostimulants and other classes of drugs seem to activate a common circuitry in the brain. However, not all psychostimulants activate the circuitry in a similar fashion. While loss of DA transmission in PFC eliminated behavioral sensitization to amphetamine (Bjijou et al., 2002), Beyer and Steketee (1999) reported that a decrease in PFC DA transmission caused a sensitized-like response to an acute injection of cocaine. Additionally, research related to behavioral sensitization may also be relevant to psychiatric illnesses. Similarities between neural circuitry underlying behavioral sensitization and the dopaminergic dysfunction related to schizophrenia have been noted: both sensitization and dopaminergic aberrations in schizophrenia have been linked to increased transmission within subcortical dopamine systems (Laruelle, 2000). This

increased transmission has been correlated positively with manifestation of positive

psychotic symptoms like delusions, auditory hallucinations, and thought disorders.

However, Laruelle (2000) states that such a correlation is an oversimplification but,

nevertheless, concludes that schizophrenia can be termed as 'an endogenous

sensitization process'. Thus, understanding the role of specific nuclei within the

circuitry involved in sensitization may also help understand brain alterations in

psychiatric disorders and drug addiction.

     The neural circuitry underlying sensitization, sometimes referred to as the

motive circuit, comprises of several interconnected nuclei and is responsible for

appropriate behavioral responses to biologically relevant stimuli (Kalivas, 2000;

Kalivas and Duffy, 1993; Pierce and Kalivas, 1997; Wolf, 1998). The motive circuit

has been conceptualized as a gateway to decide the threshold of response and

intensity of response to environmental and pharmacological stimuli (Pierce and

Kalivas, 1997), and consists of interconnections that translate information from

limbic nuclei to motor systems (Mogenson et al., 1980). In behavioral sensitization,

the strength of these connections is altered so as to produce augmented behavioral

responses to certain stimuli (Pierce and Kalivas, 1997). The strengthening of

dopaminergic connections between VTA and NAc is believed to increase locomotor

activity in behaviorally sensitized animals (Clarke et al., 1988; Kelly and Iversen,

1976; Oades et al., 1986; Pierce and Kalivas, 1997). The alteration of DA

transmission in NAc may change the locomotor response to subsequent

pscyhostimulant administration, since the NAc is believed to screen the amount

and/or nature of information from the cortex onto lower motor circuitry (Groves,

1983). It has been suggested that drugs that modulate DA transmission within the

NAc (for example, psychostimulants) may modify locomotor activity by altering the

pattern of information that is passed onto the ventral pallidum, afferents from which

ultimately activate locomotion (Koob and Swerdlow, 1988). Thus, the sensitized

locomotor response to MPD may also be due to increased dopaminergic transmission

in the NAc, since MPD acts as indirect dopamine agonist.

Increased DA in the NAc after psychostimulant administration (Akimoto et

al., 1990; Kalivas and Duffy, 1990; Kalivas and Duffy, 1993; Pierce and Kalivas,

1995) has many effects. This increased DA in NAc has been linked to increased

locomotion (Oades et al., 1986), increase in self-administration (Roberts et al., 1980;

Maldonado et al., 1993; Wise et al., 1995) and behavioral sensitization to

psychostimulants (Pierce and Kalivas, 1997; Parson and Justice, 1993; Robinson et

al., 1988; Wolf et al., 1993). DA depletion in NAc suppresses amphetamine-induced

increase in locomotion (Kelly et al. 1975; Koob et al. 1981) and reduces self-

administration of psychostimulants (Caine and Koob, 1994); application of DA

antagonists to the NAc prevented the expression of sensitization to the

psychostimulant 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy") (Ramos

et. al., 2004). It is important to note that drugs that share psychostimulant and

rewarding properties but do not cause addiction (for example, caffeine) fail to

increase DA transmission in the NAc (Acquas, et al., 2002). Thus, increased

dopaminergic transmission in NAc seems to be linked to addictive properties. Since

similar neural circuitry is involved in addiction and behavioral sensitization (Wolf, 1998), it may be deduced that increased dopaminergic transmission in the NAc may be specifically associated with sensitization.

Behavioral sensitization has two phases: induction and expression. The induction phase refers to the transient changes present immediately after repetitive administration of psychostimulants that cause the increased behavioral response; expression of behavioral sensitization is defined as the permanent and / or long-lasting neural changes that maintain the augmented behavioral response to a repeat stimulus despite cessation of continuous psychostimulant administration (Pierce and Kalivas, 1997). In general, the NAc is considered to be involved in the expression phase of behavioral sensitization (Kalivas and Stewart, 1991; Nestler, 1992; Pierce and Kalivas, 1997; White et al., 1995).   These phases of behavioral sensitization are apparently due to changes at different cerebral loci: while the transient changes are believed to be located at the ventral tegmental area (VTA), the NAc is linked to the long-lasting neural changes associated with repeated psychostimulant administration of cocaine and amphetamine (Pierce and Kalivas, 1997; Wolf, 1998). As MPD is pharmacologically similar to amphetamine and cocaine, it can be conjectured that the NAc is involved in the expression of sensitization to MPD.

However, NAc is a functionally heterogeneous brain region consisting of a dorsolateral core and a ventro-medial shell (DiChiara, 2002; DiChiara et. al., 2004; Fuchs et. al., 2008). The roles of the core and the shell of NAc, reported in literature,

differ. For example, it has been reported that while the core is believed to be involved in motor functions, the shell is believed to be more involved in emotion (Alheid et al., 1988, Heimer et al., 1991). Others have also reported the role of the shell in Pavlovian learning and the use of short-term memory in goal-directed behavior, while the expression of motivation is linked to DA transmission in the core (DiChiara, 2002). With regards to psychostimulant administration, the NAc core and shell also differ in the changes in DA transmission. Some report that the effect of psychostimulants on the spatial distribution in NAc is dependent on the dose of the psychostimulant. For example, amphetamine causes a preferential increase in DA transmission in the shell at lower doses, but similar increases across the shell and core at higher doses (DiChiara, 2002). Similar trends were observed for subcutaneous injections of morphine, amphetamine and intraperitoneal injections of cocaine (DiChiara, 2002). There are also contradictory reports about the changes in the nucleus accumbens core and shell associated with psychostimulant sensitization. Some report that the increase in DA transmission in sensitized animals occur in the NAc core (Cadoni et al., 2000), while the NAc shell is involved in the initial action of the same drugs (DiChiara, 2002). Yet, others report that increase in DA transmission occurs in the NAc shell is associated with behavioral sensitization (Pierce and Kalivas, 1995). The above mentioned results indicate that the role of sub-regions of NAc in psychostimulant sensitization is unclear.

The NAc shell and core are connected with the prefrontal cortex (PFC), also involved in behavioral sensitization to MPD (Wanchoo et al., 2009, 2010). Both

glutamate cells of the PFC (Wanchoo et. al., 2009) and dopaminergic afferents of the PFC (Wanchoo et al., 2010) were essential for behavioral sensitization to MPD. Also, activation of glutamatergic afferents from the PFC to the nucleus accumbens core is believed to be essential for reinstatement of drug-seeking behavior of psychostimulants (McFarland et al., 2003) while the dopaminergic afferents of PFC are believed to modulate DA levels in NAc core as well as shell in response to psychostimulants and stress (King et. al., 1997). Moreover, reciprocal connections exist between the shell and core of NAc (van Dongen et al, 2005) such that one takes up the functions of the other following selective destruction of either (Schoenfeld and Hamilton, 1977). Thus, this study did not specifically aim to differentiate between the subregions of NAc with respect to their role in sensitization to MPD.

The NAc has been reported to be structurally altered by repeated administration of MPD: chronic exposure of MPD has been shown to increase the density of dendritic spines on DA receptors in NAc and increase the expression of ΔFosB (Kim et. al., 2009), the transcription of which is linked to addictive properties of psychostimulants (Nestler, 2008). In a previous study (Podet et al., submitted for publication) electrolytic lesion of the nucleus accumbens eliminated the expression of behavioral sensitization to MPD, and caused a significantly greater increase in locomotor activity upon the acute injection as compared to the increase following acute injection in the control group.   Since electrolytic lesions are non-specific, it is possible that the results reported by Podet et al (submitted for publication) may be due to destruction of dopaminergic afferents of NAc. The dopaminergic afferents to

the NAc can be destroyed by administering 6-hydroxydopamine (6-OHDA). 6-OHDA is a selective neurotoxin that selectively eliminates catecholaminergic pathways. Therefore, the hypothesis of the current study was that 6-OHDA lesions of the NAc would reduce baseline locomotor activity, and prevent behavioral sensitization to MPD, without eliminating the acute response to MPD. A standard sensitization protocol was used and locomotor activity was recorded with an automated infrared beam-crossing system.

2. MATERIALS AND METHODS

2.1. Animals

Thirty-seven male Sprague Dawley rats were purchased from Harlan, Madison, Wisconsin, USA. The animals were housed two per cage in a temperature and humidity controlled animal room for at least 3 -5 days before the start of the experiment to acclimatize them. The temperature and humidity of the room were maintained at 71 ± 2ºF and $57 \pm 5\%$. The animals were kept on 12:12 light / dark cycle with the lights on at 06:00 to maintain a stable circadian rhythm, since behavioral sensitization to MPD is time-dependent (Gaytan et al., 2000). The animals had access to food and water throughout the day, except for the time spent in recording chambers (2 hrs). The animals weighed at least 180 g (range 180 – 220 g), i.e., they had reached adulthood at the commencement of the experiment. Adult male Sprague Dawley rats were used since female rats have reproductive cyclicity, which might affect the results of the experiment: for example, estrogen, the levels of which fluctuate throughout the menstrual cycle, augments the acute behavioral and neurochemical responses to psychostimulants (Dafny and Yang, 2006; Kelly et al., 1999; Van Haaren and Meyer, 1991). Upon completion of the experiment, the rats were anesthetized with pentobarbital and perfused intracardially with 10% formaldehyde, and their brains were stored in 10% formaldehyde for at least 48 hours for fixation.

2.2. Experimental protocol

The animals were acclimatized to the recording chamber for 15 – 20 minutes before each injection and / or recording session. On experimental day (ED) 1, animals were injected with 0.8ml of 0.9% saline after 15 minutes of acclimatization to the recording chamber (Table 1), and the locomotor activity baseline was recorded for two hours post injection in an open field assay using Accuscan Analyzer. Locomotor activity was recorded for two hours post injection since the effect of MPD lasts about 50 – 80 minutes depending upon the dose of MPD (Gaytan et al., 1997; Yang et al., 2003, 2007). On ED 2, the animals were divided into the following groups – control, sham-operated, and 6-hydroxydopamine lesion in NAc group. Two sham groups were used – one group in which saline was injected in NAc (surgical shams) while the other group consisted of animals that underwent anesthesia and skin incision without drilling holes in the skull (non-surgical shams). Animals with higher locomotor baseline (> 5000 cm HA) were chosen for the NAc lesion group since 6-OHDA lesions of NAc have been reported to cause a decrease in locomotor activity (Oades et al., 1986; Austin and Kalivas, 1991; Andèn and Jackson, 1975; Costall et al., 1976; Cools, 1977; Makanjoula and Ashcroft, 1982; Selling and Clarke, 2003, 2006). Similarly, animals in the surgical sham group had a higher locomotor baseline comparable to the baseline of the lesion group. The higher locomotor baseline was chosen for the surgical shams to determine whether administering saline in NAc caused changes in locomotor activity. Sham and lesion surgeries were performed on ED 2, with a recuperation period of five days from ED 3 through ED 7. The post-surgery baseline locomotor activity was recorded on ED 8 following saline injection

to determine whether surgery changed the baseline locomotor activity. All groups were then administered a single dose of 2.5 mg/kg MPD daily at around 06:45 am for six days (ED's 9 through 14) (Table 1) and their locomotor activity was recorded. After six days of MPD administration, there was a washout period of four days (ED 15 to 18), where no injections were given. Finally, a rechallenge injection of 2.5 mg/kg MPD was administered to all three groups on ED 19 (Table 1) and locomotor activity was recorded.

Table 1: Experimental protocol

Group	Day 1	Day 2	Days 3-7	Day 8	Days 9 – 14	Days 15-18	Day 19
Control	Saline		No treatment	Saline	2.5mg/kg MPD	Washout	2.5mg/kg MPD
Non-Surgical shams	Saline	Surgery	No treatment	Saline	2.5mg/kg MPD	Washout	2.5mg/kg MPD
Surgical shams	Saline	Surgery – saline in NAc	No treatment	Saline	2.5mg/kg MPD	Washout	2.5mg/kg MPD
Lesion	Saline	Surgery – 6-OHDA in NAc	No treatment	Saline	2.5mg/kg MPD	Washout	2.5mg/kg MPD

2.2. 1. Surgeries

Sham surgeries: On ED 2, rats in the two sham groups, surgical shams and non-surgical shams, were anaesthetized with 40 mg/ kg pentobarbital. When the rat was under anesthesia, an incision was made on the skull to expose the bregma. For surgical shams, holes were drilled in the skull 1.7 mm anterior from the bregma and 1.6 mm lateral to the midline. The position of the co-ordinates was based on Paxinos and Watson (1986). Then, a 27 G needle was inserted to a depth of 6.8 mm, and 2.5 µl of saline was injected over 25 s. After saline was injected, the needle was kept in place for additional six minutes to allow the liquid at the tip of the needle to diffuse (Jaskiw et. al., 1990; Li and Wolf, 1997; Li. et. al., 1999). The procedure was repeated on the other side. The skin was stapled back together, and the rats were allowed to recuperate for five days (ED 3 through ED 7). For the nonsurgical sham group, a similar procedure was carried out under anesthesia, except that holes were not drilled in the skull. The purpose of two sham groups was to determine whether injecting liquid in NAc caused volume damage or altered motor activity.

Bilateral 6-OHDA administration to NAc: Since desipramine, a noradrenergic blocker, may cause its own effects (Ainsworth et al., 1998), desipramine, was not given. Noradrenergic blockers are generally given to ensure that 6-OHDA destroys only the dopaminergic afferents (Beyer and Steketee, 1999; Bjijou et al., 2002). 40 mg/ kg of pentobarbital was administered. Once the animal was under anesthesia, the bregma was exposed by making an incision and two holes were drilled above the NAc at 1.7 mm anterior from the bregma and 1.6 mm lateral from the midline. A 27G needle was inserted into the holes and lowered to depths of 6.8 mm (Paxinos and Watson, 1986) and 2.5 µl of 6-OHDA solution (Taylor and Robins, 1986) was

injected. The 6-OHDA solution constituted of 3 mg 6-OHDA and 2 mg of ascorbic acid per 1 ml of 0.9% isotonic saline. 3% ascorbic acid was added to prevent the rapid oxidation of 6-OHDA (Wanchoo et. al., 2010). The needle was left in position for six minutes to allow diffusion of the drug. 6-OHDA solution was injected on the other side similarly, and the wound closed with staples. The animals rested for five days (ED 3 through ED 7).

2.2. 2. <u>Drugs</u>

A dose of 2.5 mg/kg MPD was selected because previous work showed that this dose was optimal for eliciting behavioral sensitization (Gaytan et al., 1997, 2000; Yang et al., 2003, 2006, 2007). 2.5 mg / ml of MPD was prepared by dissolving methylphenidate hydrochloride in 0.9% isotonic saline. For injections, the animals were weighed and 2.5 mg / kg of MPD was administered. Saline was added to all injection such that the volume of all injections was 0.8 cc. The drug (on ED 9 through 14, and ED 19) or saline (on ED 1 and ED 8) was administered intraperitoneally at around 06:45 am. The time of administration of the drug was chosen because MPD elicits optimal behavioral sensitization when given at the beginning of the light cycle (Gaytan et al., 2000).

2.2.3. <u>Locomotor recordings</u>

Locomotor activity was recorded using computerized animal activity monitoring (CAAM; Accuscan Instrument, Inc., Columbus, OH) in open field cages (40.5 cm×40.5 cm×31.5 cm). The monitoring device consisted of two levels of infrared beams of frequency 100 Hz at 6 cm and 12.5 cm from the base of the cage. There was

one animal per cage. Beam crossings, representing motor activity, were counted in

ten-minute units by Accuscan Analyzer and downloaded into OASIS. Further

analysis of beam interruptions was done in OASIS by sorting and quantifying the data

into different locomotor indices. If two or more consecutive beams were interrupted

at least 1 s apart, then the activity monitoring system interpreted it as a motor

movement. If the same beam was interrupted, it was interpreted as stereotypic

activity. If there was difference of > 1 s between the interruptions of the same beam,

it was counted as a different episode of stereotypic movement (Gaytan et al., 1996).

Horizontal activity (HA) was defined as the overall locomotor activity. Total distance

(TD) was the sum of the total forward ambulation in centimeters, and number of

stereotypic movements (NOS) provided the measure of the total number of repetitive

episodes (Askenasy et al., 2007; Gaytan et al., 1996, 1997, 2000; Yang et al., 2003,

2006, 2007).

2.3. <u>Histology</u>

The animals were anaesthetized with sodium pentobarbital and perfused intracardially

with 10% formaldehyde. The brains were extracted and kept in 10% formaldehyde

for at least 48 hours for fixation. The brains were then sliced into 40 µm sections

using a Microm HM 505E cryostat (Microm GmbH, Germany). The slides were dried

for at least 24 hours, and stained with Cresyl Violet (Riickert et. al., 1997) between

24 to 72 hours after being sliced. The purpose of the staining was to assess the

accuracy of lesions.

2.4. <u>Data Analysis</u>

Locomotor activity was recorded by the CAAM and Accuscan Analyzer as 10 minute activity units for different indices. The sum of the total activity in 2 hours was calculated for HA, TD and NOS (Askenasy et. al., 2007; Gaytan et. al., 1996, 1997, 2000; Yang et. al., 2003, 2006, 2007). The locomotor activity for each group of animals was compared across days to note the effects of treatment. The difference in locomotor activity between days in the lesion group was compared to the differences in locomotor activity between corresponding days in the control group. Statistical analyses used motor activity as dependent variable and day and group as independent variables. To analyze the effect of surgery on locomotor activity, locomotor activity on ED 8 (post-surgery baseline) was compared to pre-surgery baseline (ED 1). Similarly, activity following acute administration of MPD (ED 9) was compared to post-surgery baseline (ED 8) to observe the acute effect of MPD. Acute injection of MPD caused a biphasic response in the lesion group. Based upon their response to the acute injection of MPD, animals in the lesion group were divided into two groups: 1) high acute responders, who had significantly greater locomotor activity upon the acute injection of MPD or 2) acute non-responders, who exhibited little or no change in baseline locomotor activity after the 1st injection of MPD. Data was analyzed for both all the animals in the lesion group combined, and for the two response groups separated: high acute responders and acute non-responders. Activity following the 6th consecutive injection of MPD (ED 14) was compared to post-surgery baseline (ED 8) and acute administration of MPD (ED 9) to determine the effects of repetitive

administration of MPD. Finally, activity following the rechallenge MPD injection (ED 19) was compared to that after acute administration of MPD (ED 9) to determine the expression of sensitization. Analysis of variance (ANOVA) with post hoc analysis using the Fisher Least Significant Difference (LSD) test if ANOVA was significant (Yang et. al., 2003, 2007) was used, and differences were considered statistically significance if two-tailed $p \leq 0.05$.

Alternatively, the locomotor response of the two lesion groups (high acute responders and acute non-responders) was compared to the locomotor response observed in the control group for different days since both groups had similar post-surgery baselines.

3. RESULTS:

Effects of administration of 6-OHDA in the NAc were evaluated by comparing locomotor activity of the 6-OHDA lesioned group in the following locomotor indices: horizontal activity (HA), total distance (TD) and number of stereotypic movements (NOS). The histograms in the figures are group means of the total activity recorded two hours post injection. The error bars show standard errors. The figures labeled 'A' display data for all the groups – control, nonsurgical sham, surgical shams, and lesion groups (all lesion animals grouped together). The figures labeled 'B' indicate the activity of the two lesion group responses separated and compared to the control group. ANOVA was used to compare significance of differences and significance was set at $p \leq 0.05$.

Effect of 6-OHDA administration in NAc on locomotor baseline:

To observe the effect of time (control group) and surgery (surgical shams, non-surgical shams and lesion group) on the locomotor activity, pre- and post-surgery baseline locomotor activity of all groups was compared (Fig. 1). Activities did not differ for control animals in HA ($F_{(1, 7)} = 0.009$, $p = 0.925$), TD ($F_{(1, 7)} = 0.001$, $p = 0.97$) and NOS ($F_{(1, 7)} = 0.012$, $p = 0.91$). Similarly, neither the nonsurgical sham group HA ($F_{(1, 7)} = 0.009$, $p = 0.93$), TD ($F_{(1, 7)} = 0.12$, $p = 0.73$) and NOS ($F_{(1, 7)} = 0.18$, $p = 0.69$) nor surgical shams HA ($F_{(1, 3)} = 0.80$, $p = 0.40$), TD ($F_{(1, 3)} = 0.35$, $p = 0.57$) or NOS ($F_{(1, 3)} = 1.74$, $p = 0.23$) had significantly different baseline activities after surgery in any of the three indices. This is in agreement with previous reports that motor indices are stable over time (Bjork et al., 1998; Gaytan et al.,

1998). However, the 6-OHDA in NAc lesion group had significantly decreased locomotor activity after surgery: HA ($F_{(1, 16)} = 8.52$, $p = 0.006$), TD ($F_{(1, 16)} = 9.05$, $p = 0.005$) or NOS ($F_{(1, 16)} = 7.74$, $p = 0.009$) on ED 8 compared to ED 1. Thus, reduced locomotor activity was seen in the lesion group, while the control and both the sham groups did not differ in their baseline locomotor activity.

FIGURE LEGEND: Figure 1 compares the pre-and post surgery baseline of all the groups. It was seen that the locomotor baseline of rats did not change much with time in horizontal activity (HA; 1st graph), total distance (TD; 2nd graph) and number of stereotypy (NOS; 3rd graph). Similarly, surgery in itself did not cause significant change in locomotor baseline (non-surgical and surgical shams) in all three indices. However, 6-OHDA administration in NAc caused a significant reduction in locomotor baseline (lesion group) in all three indices. Δ indicates significance difference ($p \leq 0.05$) as compared to pre-surgery baseline.

Figure 1
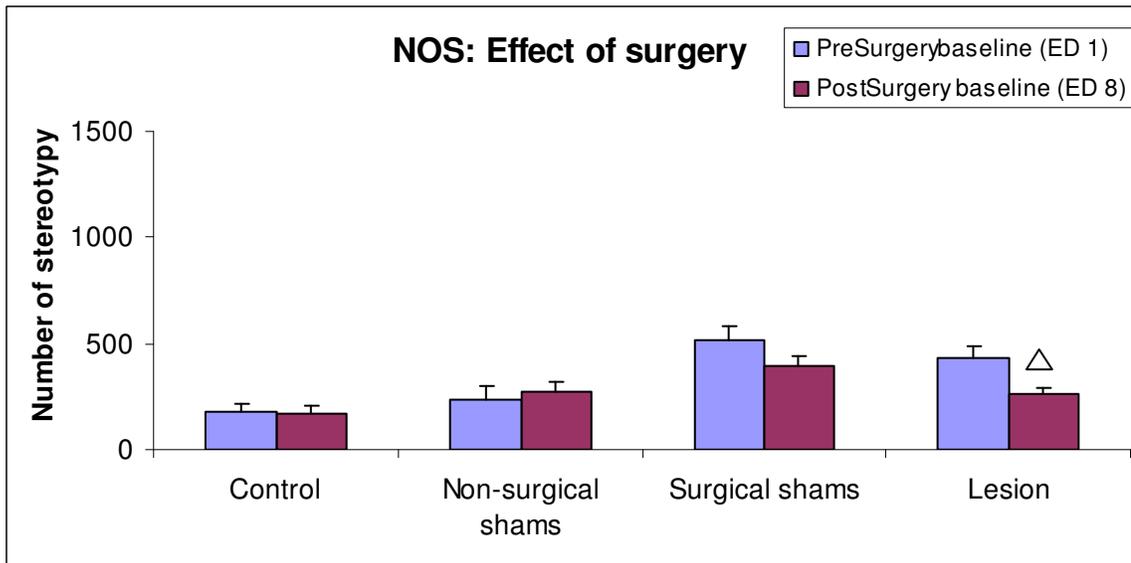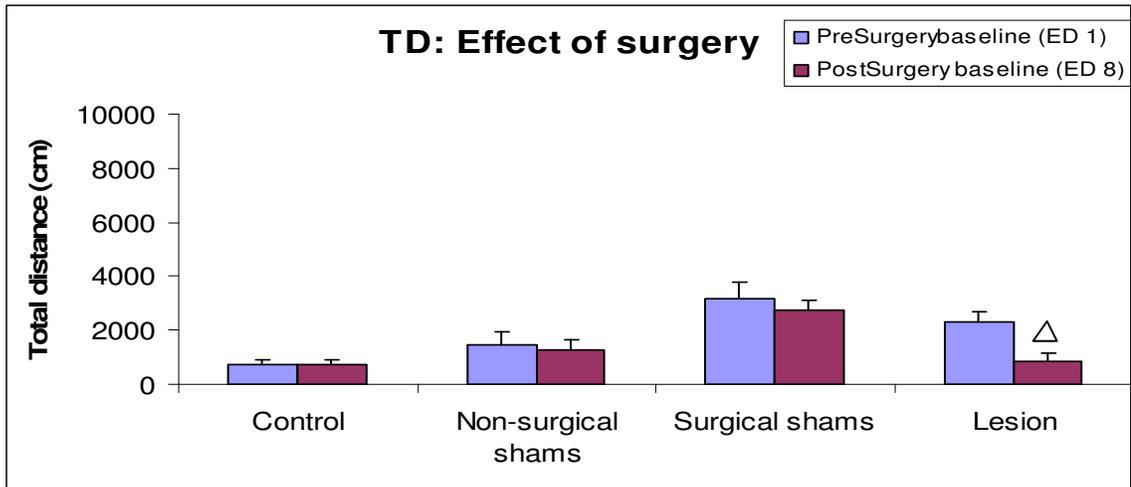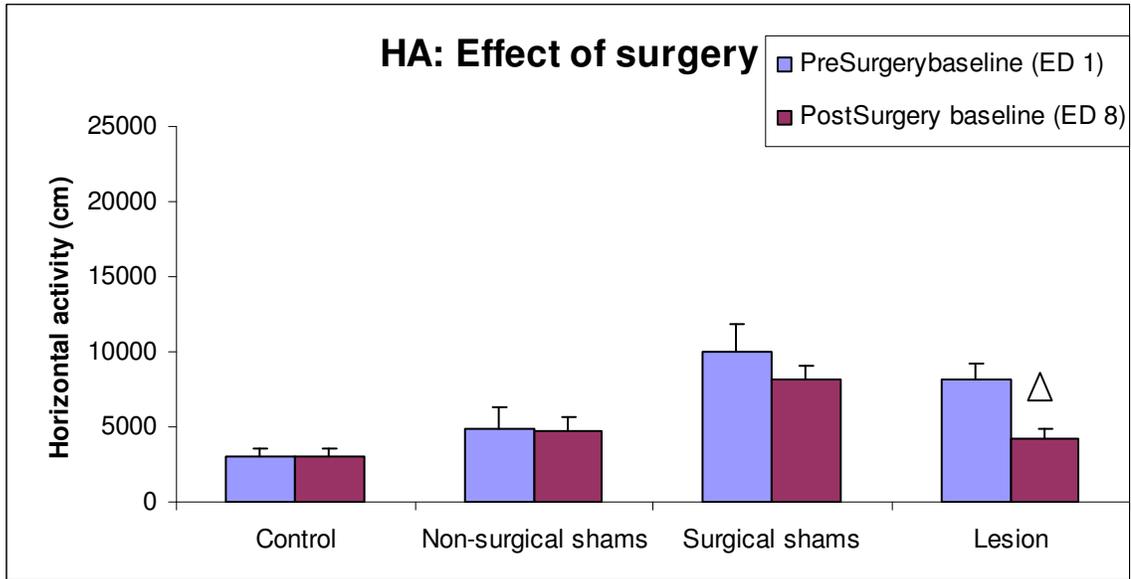
<u>Effect of acute MPD administration:</u> To observe the effect of acute injection of MPD, locomotor activity on ED 9 (after MPD injection) was compared to ED 8 (post-surgery baseline). Acute injection of MPD caused a significant increase in locomotor activity in the control group (Fig 2A) in HA ($F_{(1,7)} = 16.28$, $p = 0.001$), TD ($F_{(1,7)} = 7.16$, $p = 0.02$) or NOS ($F_{(1,7)} = 17.56$, $p = 9 * 10^{-4}$) and in the non-surgical sham group: HA ($F_{(1, 7)} = 4.43$, $p = 0.05$), TD ($F_{(1, 7)} = 4.50$, $p = 0.05$) and NOS ($F_{(1, 7)} = 4.20$, $p = 0.05$) (Fig. 2A). The surgical shams, however, did not have significantly increased locomotor activity following the 1st injection of MPD: HA ($F_{(1, 3)} = 1.37$, $p = 0.28$), TD ($F_{(1, 3)} = 1.45$, $p = 0.27$) and NOS ($F_{(1, 3)} = 1.21$, $p = 0.31$). The lesion group, as a whole, did not have significantly different activity upon the acute injection of MPD either: HA ($F_{(1, 16)} = 2.21$, $p = 0.14$), TD ($F_{(1, 16)} = 2.22$, $p = 0.14$) and NOS ($F_{(1, 16)} = 3.07$, $p = 0.08$) (Fig. 2A).

FIGURE LEGEND: Figure 2A shows the acute response to MPD in control, surgical and non-surgical sham groups. Acute injection of MPD caused a significant increase in all three indices of locomotor activity for control and surgical sham groups. The non-surgical shams showed an increase in locomotor activity, but it did not reach significance probably due to small sample size. The lesion group did not show a significant increase in activity. Δ indicates significance difference ($p \leq 0.05$) as compared to post-surgery baseline.
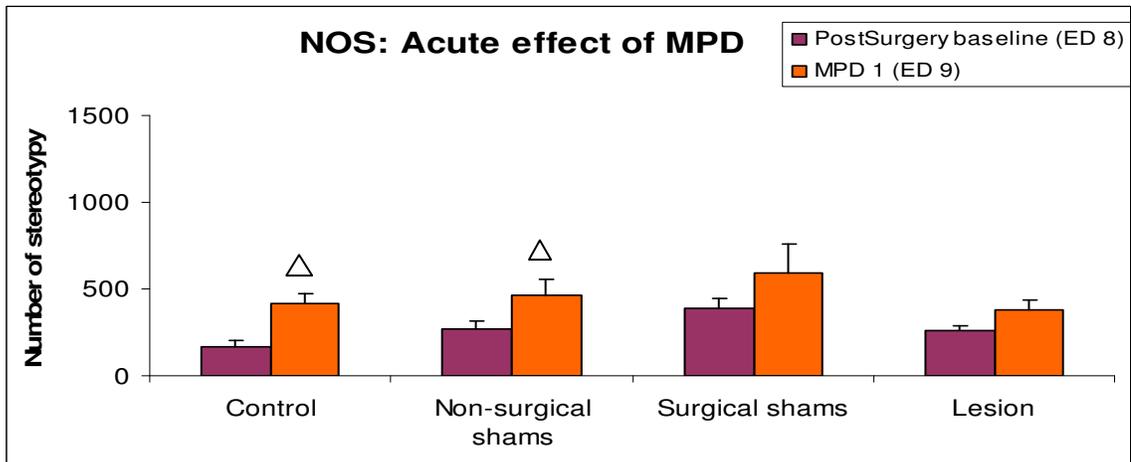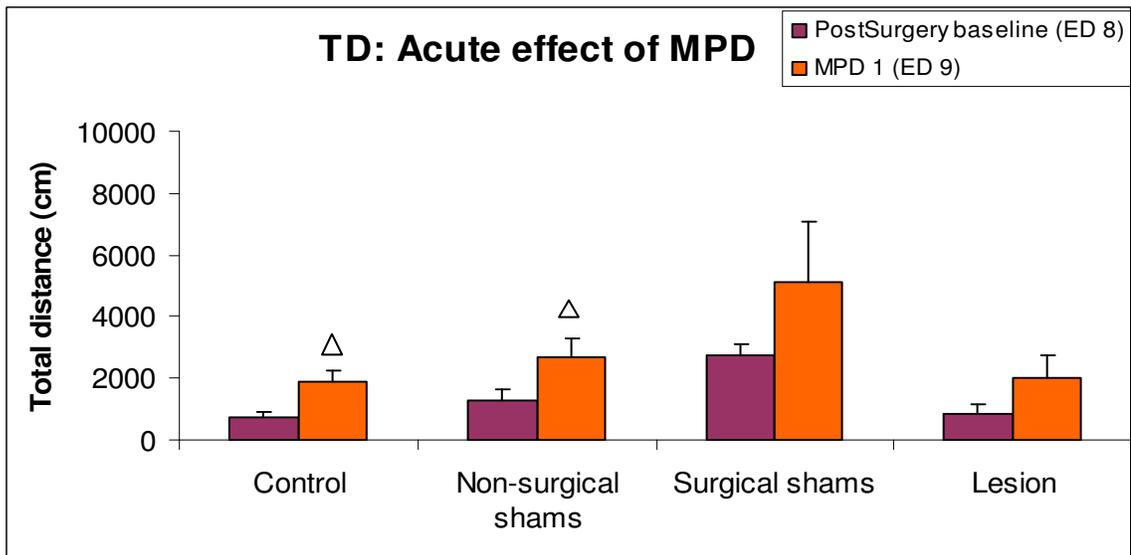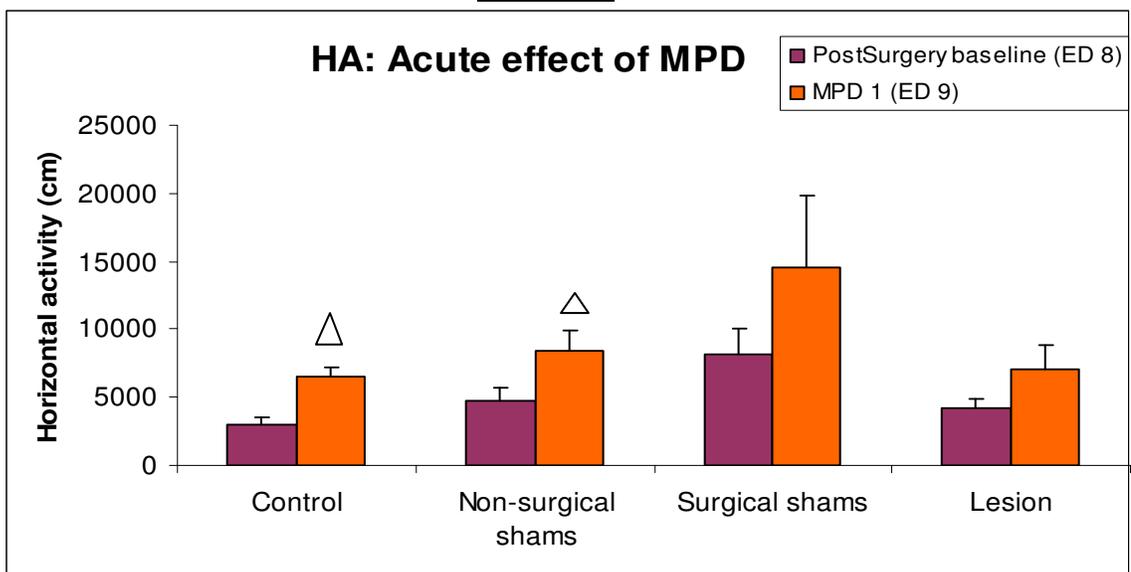
However, among rats with 6-OHDA NAc lesions, there appeared to be two distinct responses to acute MPD administration (Fig. 2B-1 and 2B-2): some animals had significantly increased locomotor activity upon MPD administration, while others had no effect of acute MPD.

FIGURE LEGEND: Figures 2B-1 and 2B-2 show that some animals in the lesion group responded positively to the psychostimulant (MPD) injection, and others did not exhibit a change in locomotor baseline upon psychostimulant administration. Figure 2B-2 shows the change in locomotor activity of each individual rat in the lesion group after the 1st injection of MPD.
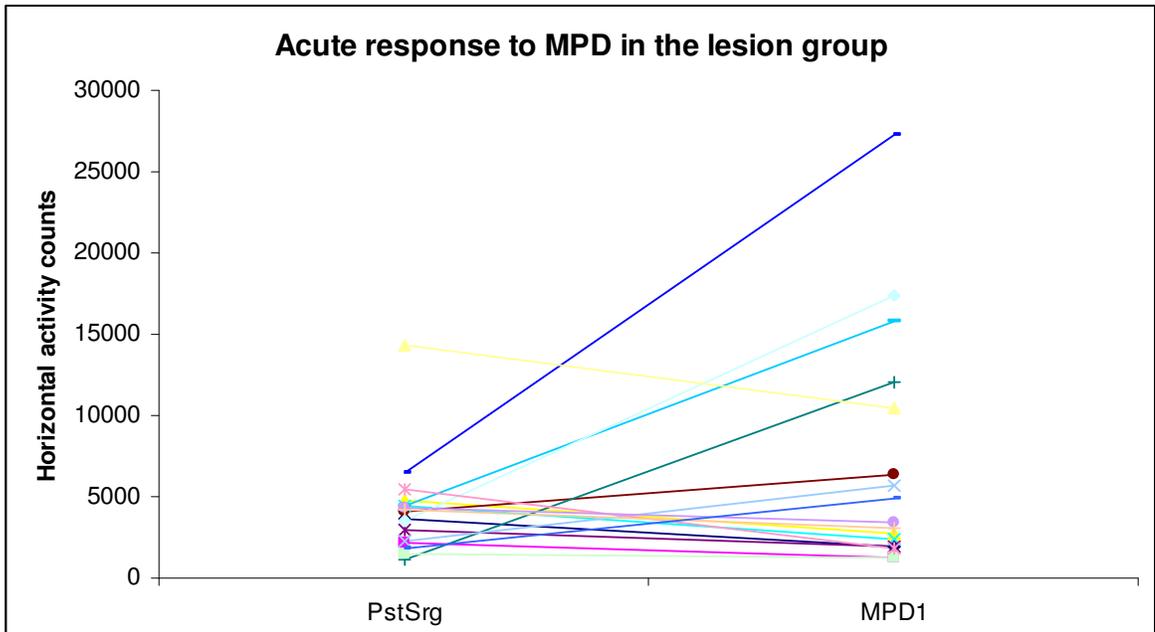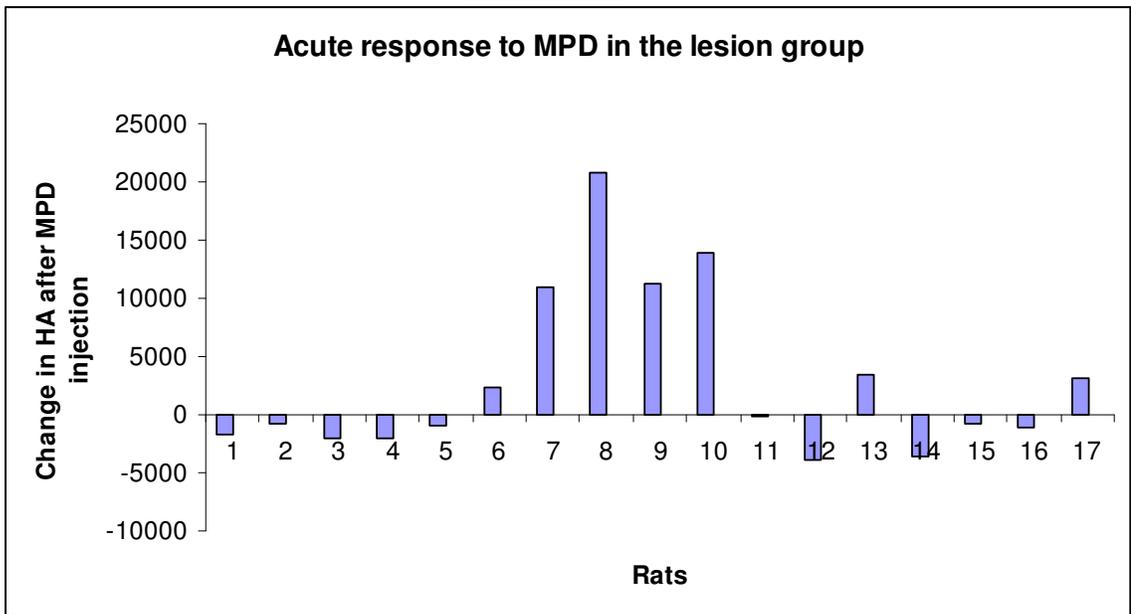
Figure 2B-1

Acute response to MPD in the lesion group

Figure 2B-2

Acute response to MPD in the lesion group

The distinctiveness of the responses persisted throughout the rest of the experiment. Therefore, the animals in the lesion group were divided into two groups – acute non-responders (N= 10), which did not exhibit increased locomotor activity after acute psychostimulant administration (Fig. 2B-2, Fig. 2C), and high acute responders (N= 7), with significantly increased locomotor activity after acute administration of MPD (Fig 2B-2, Fig 2C). Fig 2C compares the activity of the control group to the two lesion groups. In the acute non-responders, acute injection of MPD did not cause any change in locomotor activity in HA (F (1, 9) = 1.91, p = 0.18), TD (F (1, 9) = 0.55, p = 0.46) and NOS (F (1, 9) = 0.92, p = 0.35), while acute injection of MPD caused a significant increase in locomotor activity in high acute responders: HA (F (1, 6) = 8.98, p = 0.01), TD (F (1, 6) = 5.37, p = 0.04) or NOS (F (1, 6) = 21.31, p = 5 * 10^ -4). Thus, acute administration of MPD caused a significant increase in locomotor activity in the control and non-surgical sham groups. Two responses (augmented locomotor activity and baseline locomotor activity) were seen in the lesion group following acute injection of MPD.

FIGURE LEGEND: Figure 2C indicates the acute response to MPD injection of the lesion group, separated out: i.e., compares the response of 1st MPD injection of acute non-responders (the lesion animals that did not have a positive change of locomotor activity in figure 2B-2) and high acute responders (lesion animals with positive change in locomotor activity in figure 2B-2) to the control group.

Figure 2C
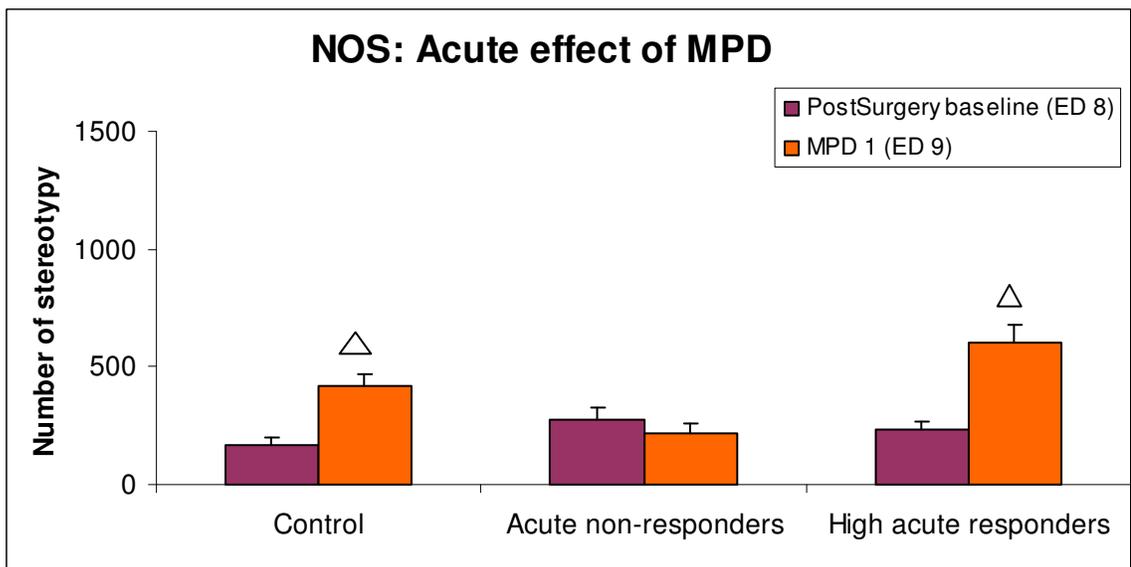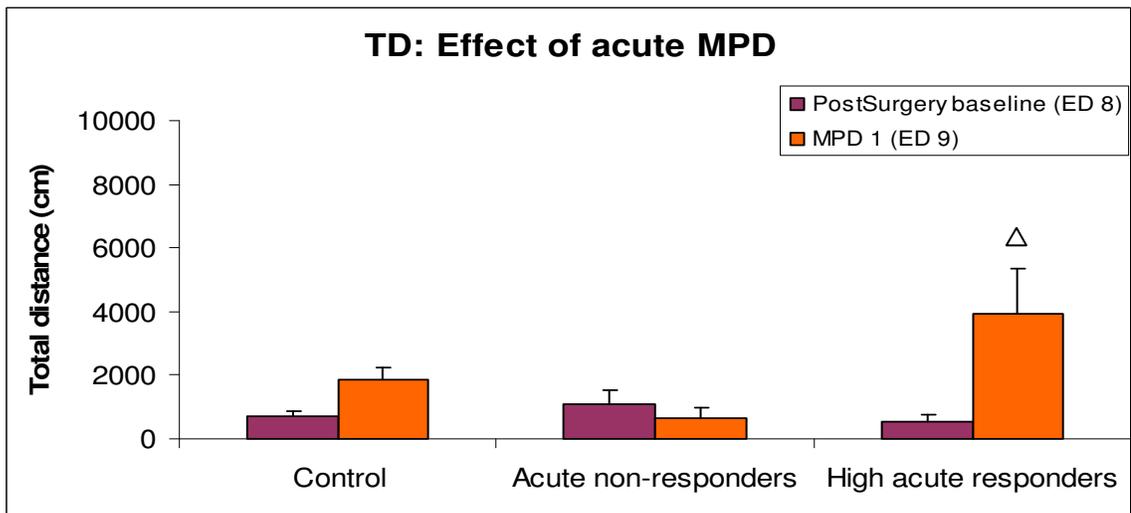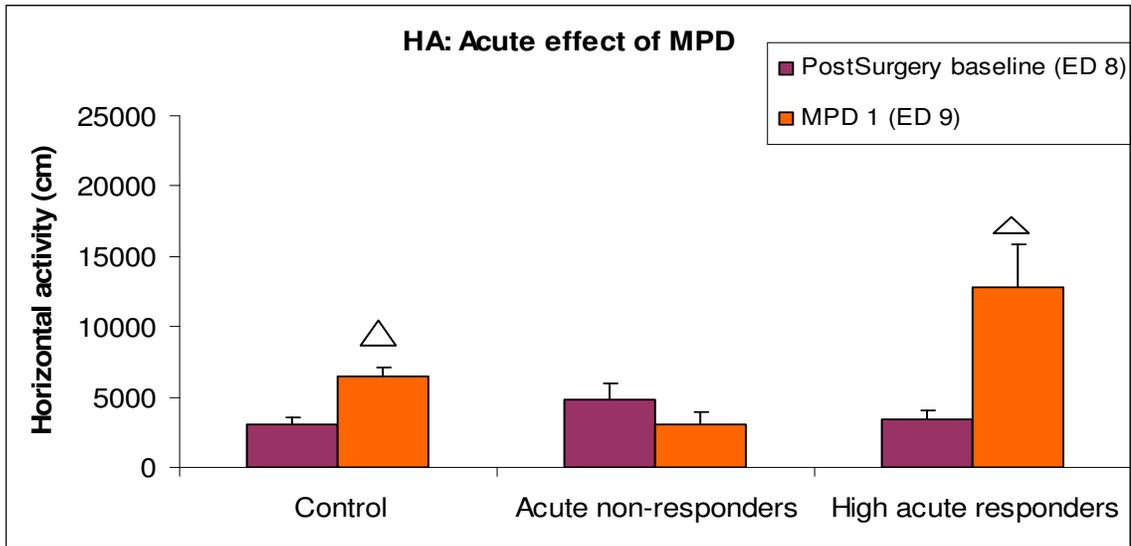
In order to check whether differences in baseline locomotor activity may have contributed to divergent responses to acute injection of MPD, the post surgery baselines of the two groups were compared (Fig. 2D) and no significant differences were found between the groups [HA ($F_{(2, 16)} = 0.85$, $p = 0.37$)]. Thus, the two responses seen in the lesion group after acute MPD injection were not due to differences in locomotor baseline.

FIGURE LEGEND: Since acute response to psychostimulant may differ based on locomotor baseline, figure 2D compares the post-surgery baseline of the acute non-responders and high acute responders. The baselines did not differ significantly and thus differences in baseline locomotor activity did not contribute to the difference in response to acute injection of MPD amongst lesioned animals. Δ indicates significance difference ($p \leq 0.05$) as compared to post-surgery baseline.

Figure 2D

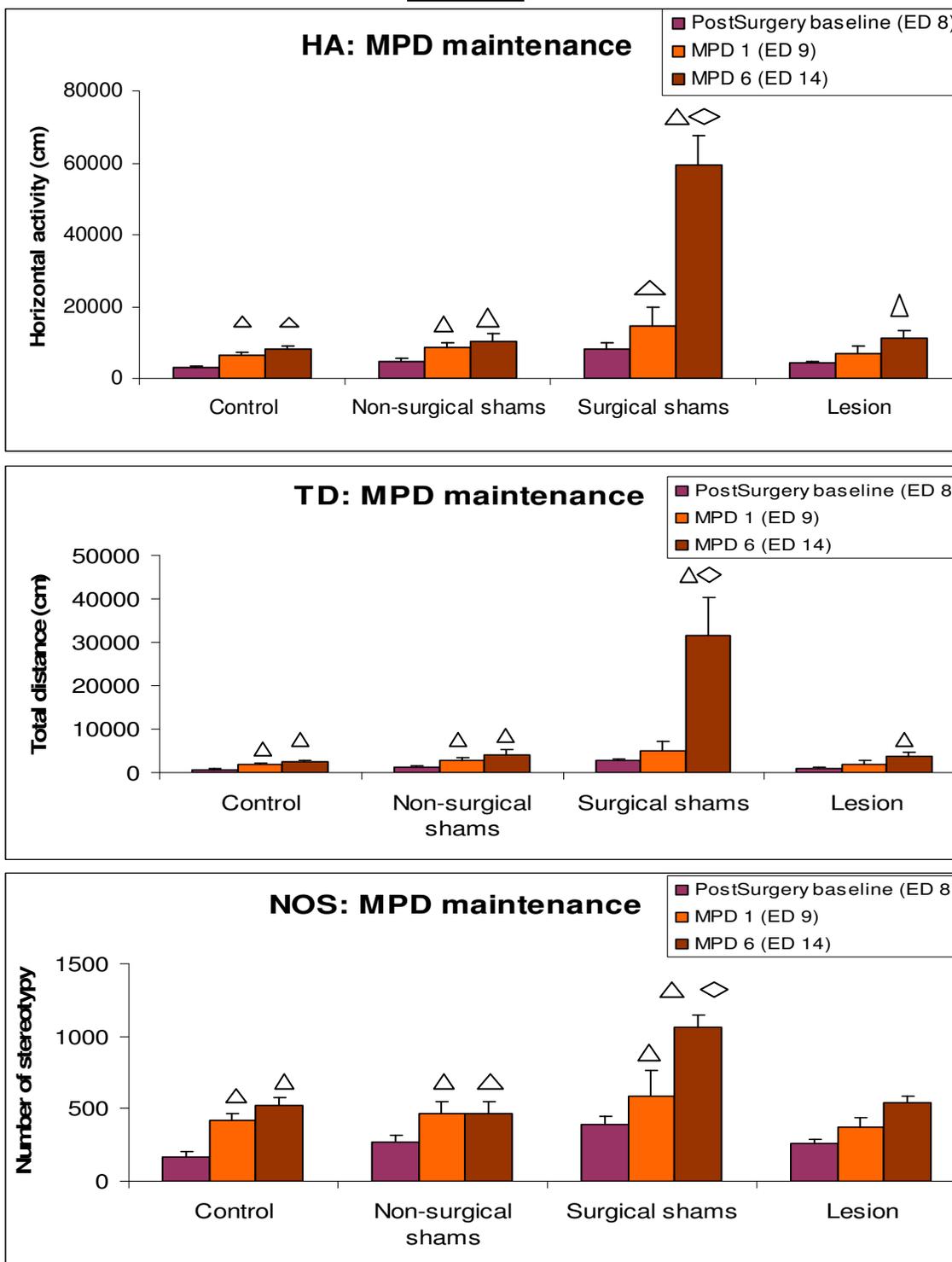**HA: Comparing post surgery baseline of high acute responders with acute non-responders**
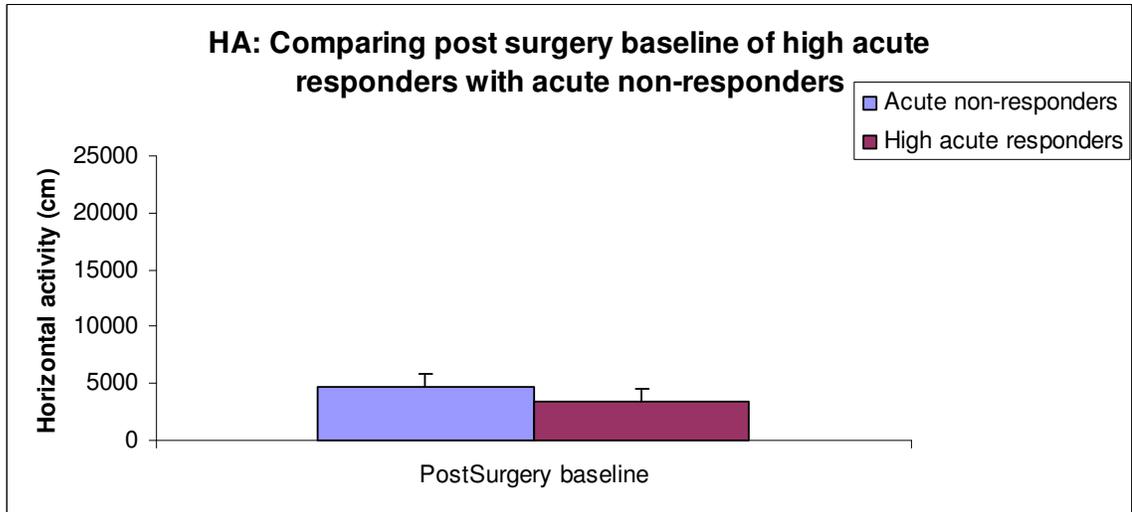
Effect of 6th consecutive injection of MPD:

The increase in locomotor activity after the acute administration of MPD was maintained following the 6th consecutive daily injection of MPD in the control group in [ED 8 compared to ED 14: HA ($F_{(1, 7)}$ = 29.13, p = 9.4 *10^-5), TD ($F_{(1, 7)}$ = 9.46, p = 0.008) or NOS ($F_{(1, 7)}$ = 30.16, p = 7.93 * 10^ -5)] (Fig. 3A). However, the locomotor activity after 6th injection of MPD was not significantly different from the acute effect of MPD for the control group: HA ($F_{(1, 7)}$ = 3.07, p = 0.10), TD ($F_{(1, 7)}$ = 0.61, p = 0.45) and NOS ($F_{(1, 7)}$ = 1.98, p = 0.18). Similar results were seen in the non-surgical sham group, i.e., the increase in locomotor activity was maintained after the 6th injection of MPD [ED 8 compared to ED 14: HA ($F_{(1, 7)}$ = 5.70, p = 0.03), TD ($F_{(1, 7)}$ = 4.62, p = 0.04) and NOS ($F_{(1, 7)}$ = 4.94, p = 0.04)]. However, like controls, the activity on ED 14 was not significantly different from that on ED 9 for non-surgical shams in HA ($F_{(1, 7)}$ = 0.32, p =0.58), TD ($F_{(1, 7)}$ = 1.03, p = 0.33) and NOS ($F_{(1, 7)}$ = 3 * 10^ -4, p = 0.98)]. Contrary to controls and non-surgical shams, the surgical shams had a significantly greater increase in locomotor activity on the 6th consecutive day on MPD injection than on ED 8 [HA ($F_{(1, 3)}$ = 36.94, p = 9 * 10^ -4), TD ($F_{(1, 3)}$ = 11.28, p = 0.01) and NOS ($F_{(1, 3)}$ = 51.72, p = 3.6* 10^ -4)] and ED 9 [HA ($F_{(1, 3)}$ = 20.31, p = 0.004), TD ($F_{(1, 3)}$ = 9.05, p = 0.02) and NOS ($F_{(1, 3)}$ = 6.35, p = 0.04)]. The lesion group, as a whole, had significantly greater activity on the 6th day of MPD injections as compared to post-surgery baseline [HA ($F_{(1, 16)}$ = 13.73, p = 7 * 10^-4), TD ($F_{(1, 16)}$ = 9.67, p = 0.0039) and NOS ($F_{(1, 16)}$ = 25.81, p = 1.57* 10^-5)] but not significantly different

compared to the 1st injection of MPD (ED 9): [HA (F (1, 16) = 2.21, p = 0.15), TD (F (1, 16) = 2.23, p = 0.15) and NOS (F (1, 16) = 3.07, p = 0.089)] (Fig. 3A)

FIGURE LEGEND: Figure 3A compares the locomotor response to the 6th injection of MPD (ED 14, MPD 6) to the locomotor response after 1st injection of MPD (ED 9, MPD 1) and post-surgery – baseline (ED 8). In the control and surgical shams, similar levels of increased activity were seen after the acute and 6th consecutive daily injection of MPD, as compared to the post-surgery baseline. The surgical shams had significantly elevated activity compared to both the acute injection of MPD and the post-surgery baseline. In the lesion group (n = 17), locomotor activity after 6th injection was significantly elevated as compared to the post-surgery baseline. Δ and <> indicate significantly different ($p \leq 0.05$) as compared to post-surgery baseline and acute injection of MPD respectively.

Figure 3A

With the lesion group divided into two based upon their response to the acute injection of MPD, the high acute-responders exhibited a significantly greater increase in locomotor activity following the 6th consecutive daily injection of MPD compared to their post-surgery baseline in HA (F (1, 9) = 4.41, p = 0.05), and NOS (F (1, 9) = 10.53, p = 0.004), even though they did not have increased activity after the acute MPD injection (Fig. 3B). However, TD did not show a significant increase in locomotor activity even after the 6th injection (F (1, 9) = 2.71, p = 0.11). The increase in locomotor activity seen after the 6th injection in acute non-responders was significant compared to activity on ED 9 in all three indices: HA (F (1, 9) = 8.36, p = 0.009), TD (F (1, 9) = 4.31, p = 0.05) and NOS (F (1, 9) =17.41, p = 5 * 10^-4). The high acute responders maintained similar increases in locomotor activity to that on ED 9, after the first injection of MPD [ED 14 compared to ED 8: HA (F (1, 6) = 10.52, p = 0.007), TD (F (1, 6) = 8.01, p = 0.01) or NOS (F (1, 6) = 15.34, p = 0.02)] (Fig. 3B). This increase was not significant compared to activity on ED 9: HA (F (1, 6) = 0.001, p = 0.98), TD (F (1, 6) = 0.13, p = 0.73) and NOS (F (1, 6) = 0.15, p = 0.70).

FIGURE LENGED: Figure 3B compares the MPD response after 6th

injection to the acute response and post-surgery baseline for control group, and acute

non-responders and high acute responders (lesion group separated, based on their

acute response). Locomotor activity after the 6th injection was equally augmented in

all three groups, irrespective of their initial response to MPD. Δ indicates

significantly different (p $\leq$ 0.05) as compared to post-surgery baseline, while <>

indicates significantly increased activity as compared to the 1st injection of MPD.

Figure 3B
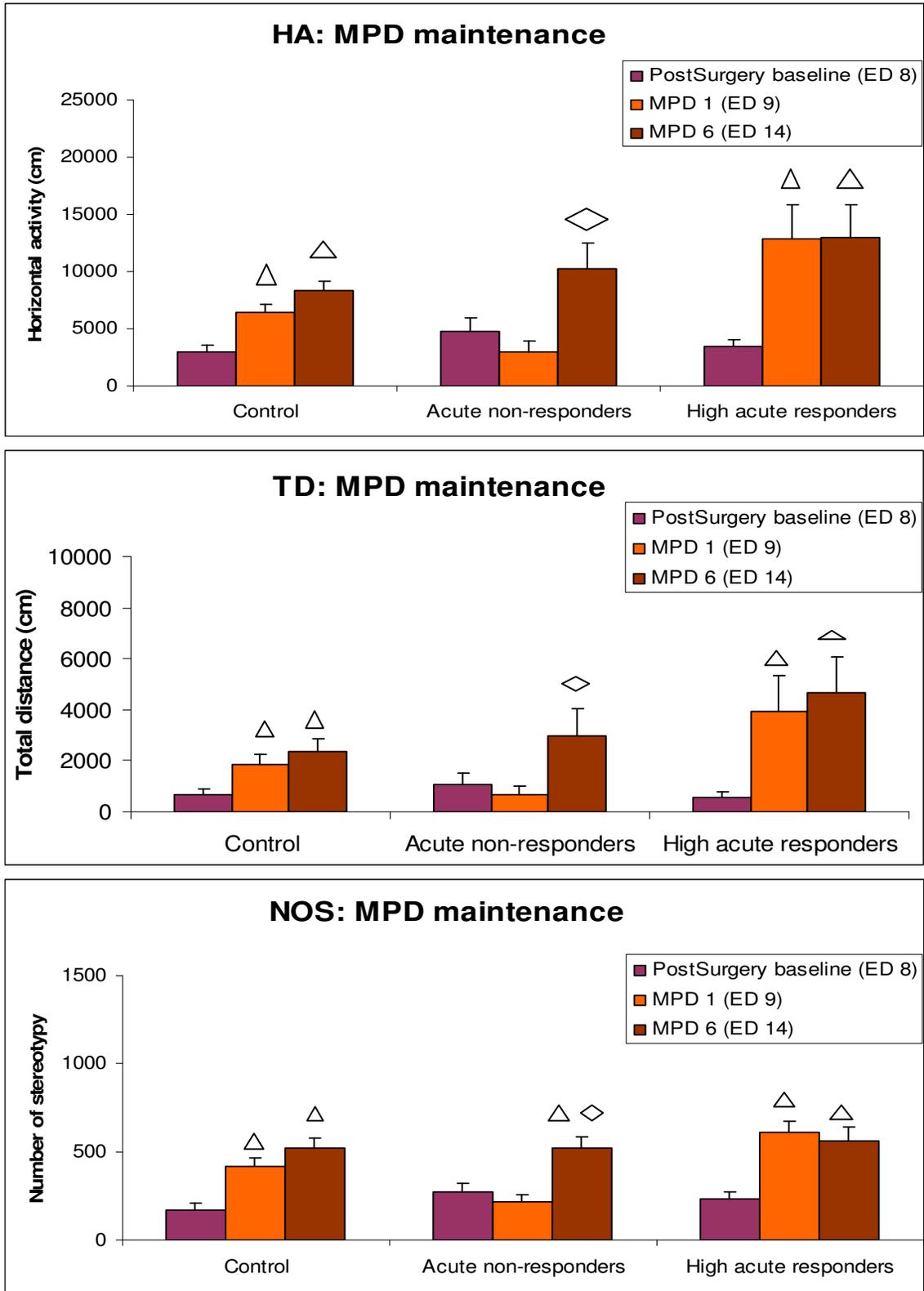
Thus, locomotor activity after the 6th injection of MPD was maintained but not significantly elevated in the controls and non-surgical shams. The surgical shams had significantly greater locomotor activity, even compared to the acute injection of MPD. In the acute non-responders, locomotor activity was significantly elevated compared to the response to the acute injection, while the high acute responders had similar levels of locomotor activity on both the 1st and 6th injection of MPD.

Effect of MPD rechallenge injection:

On rechallenge with 2.5 mg/kg MPD after four days of washout, a sensitized response i.e., a significantly greater increase in locomotor activity than that after the initial injection of MPD, was seen in the control group (Fig. 4A) in HA ($F(1, 7) = 9.37$, $p = 0.008$), and TD ($F(1, 7) = 11.31$, $p = 0.004$), but not NOS ($F(1, 7) = 0.20$, $p = 0.66$). A similar sensitized response was seen in the nonsurgical sham group for HA ($F(1, 7) = 4.84$, $p = 0.04$), and TD ($F = 4.25$, $p = 0.05$), but not NOS ($F = 0.35$, $p = 0.55$). However, the surgical shams did not exhibit a sensitized response for any of the three indices: HA ($F(1, 3) = 2.88$, $p = 0.14$), TD ($F(1, 3) = 3.53$, $p = 0.11$) and NOS ($F(1, 3) = 0.91$, $p = 0.38$). The lesion group, as a whole, did not show a sensitized response, i.e., locomotor on ED 19 (rechallenge injection) was not significantly different than the locomotor activity seen on ED 9 (1st injection) (Fig. 4A): HA ($F(1, 16) = 1.30$, $p = 0.26$), TD ($F(1, 16) = 0.49$, $p = 0.48$) and NOS ($F(1, 16) = 1.76$, $p = 0.19$).

FIGURE LEGEND: Figure 4A compares the activity after the MPD

rechallenge injection, administered after the 6 consecutive injections and four days of

washout, to the locomotor activity after the 1st injection. Control and surgical shams

displayed a sensitized response, i.e., significantly elevated locomotor activity as

compared to the activity after the 1st injection. Non-surgical shams had increased

locomotor activity, but the increase was not significant. The lesion group (n = 17) did

not show a sensitized response.

Figure 4A
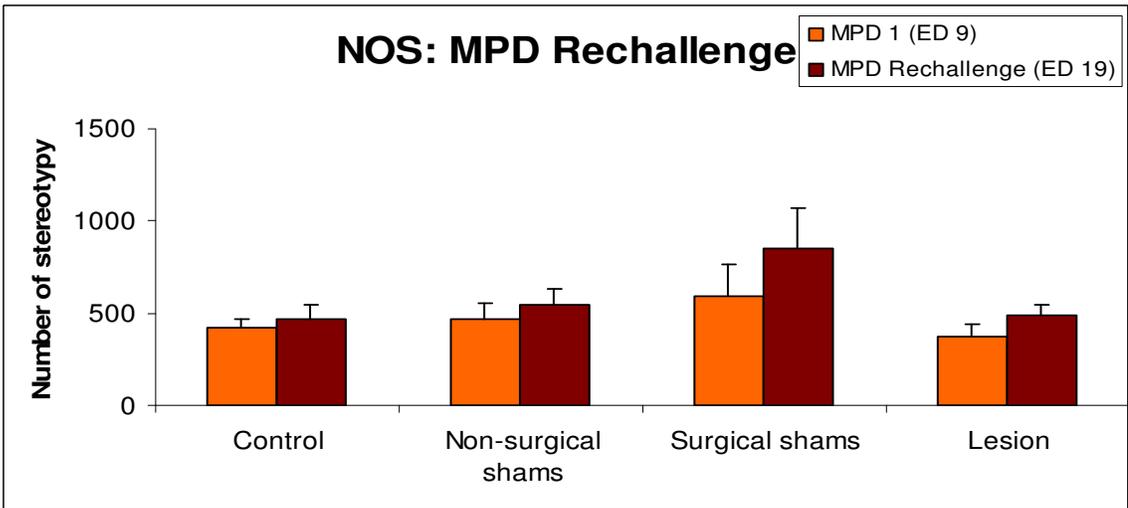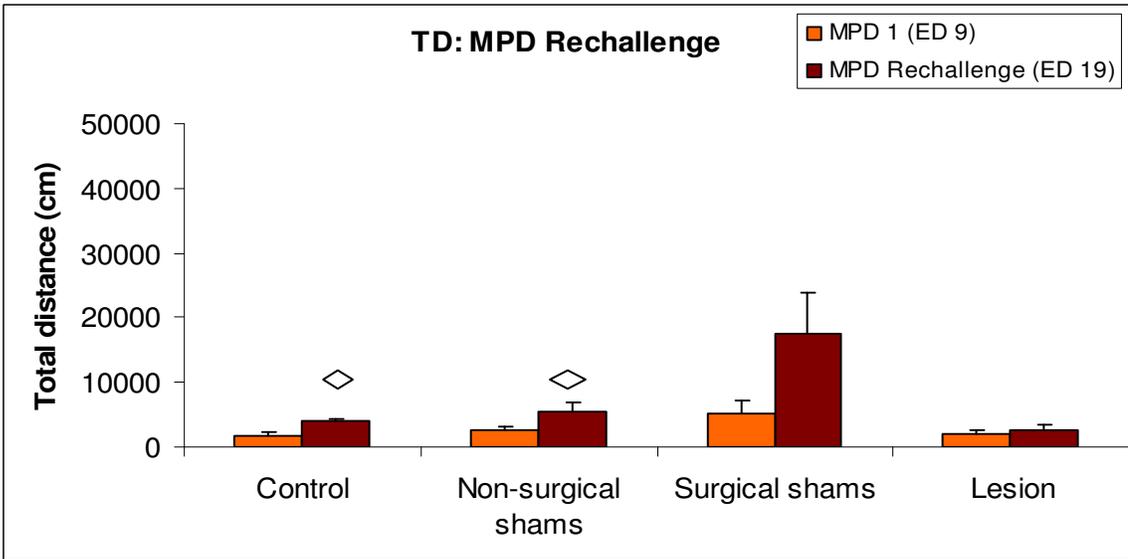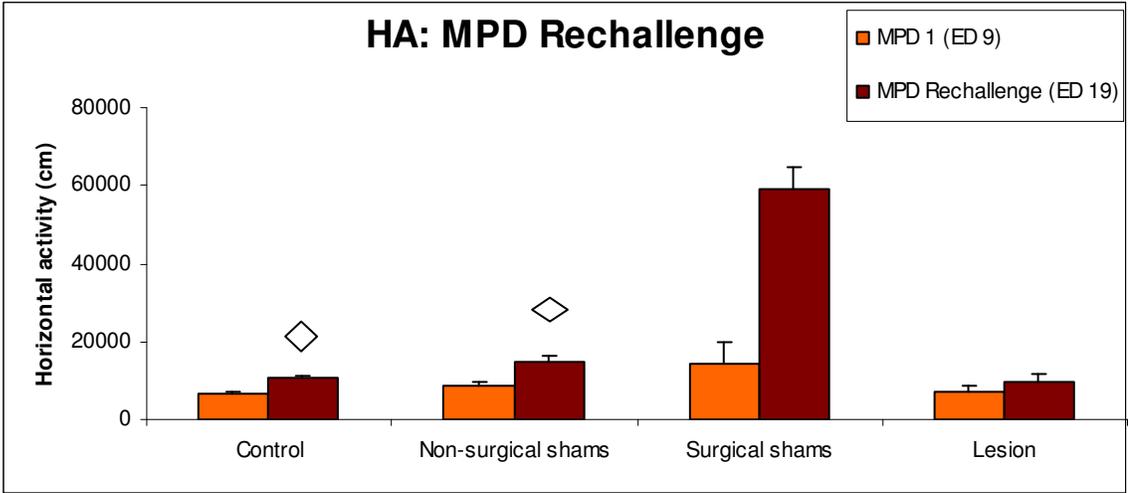
The lesion group, divided by their acute response to MPD, exhibited other patterns. The acute non-responders (Fig. 4B) had an augmented locomotor response upon rechallenge compared to the acute injection, for HA ($F_{(1, 9)} = 7.60$, $p = 0.01$) and NOS ($F_{(1, 9)} = 17.42$ $p = 5 *10^{-4}$) but not TD ($F_{(1, 9)} = 3.12$, $p = 0.09$), while in the high acute responders, the locomotor response to the rechallenge injection was not significantly different from that to the acute injection [HA ($F_{(1, 6)} = 0.06$, $p = 0.81$), TD ($F_{(1, 6)} = 7* 10^{-4}$, $p = 0.98$) and NOS ($F_{(1, 6)} = 0.66$, $p = 0.43$)]. Thus, a significantly increased locomotor response was seen upon the rechallenge injection in the control, non-surgical sham and acute non-responders (lesion) groups. The high acute responders exhibited similar levels of locomotor activity to both the first and rechallenge injection of MPD.

FIGURE LEGEND: Figure 4B compares the locomotor response to the

rechallenge injection to the locomotor response to the 1st injection of MPD. The

control group expressed sensitization, i.e., had significantly elevated activity. The

acute non-responders had significantly elevated activity, comparable to the sensitized

response of the control group, even though the 1st injection of MPD had not elicited a

significant increase in activity. The high acute responders had similar levels of

activity on the acute as well as rechallenge injection; both of which were of similar

magnitude to the sensitized response in the control group. Thus, this suggests that

neural circuitry underlying acute response to MPD is different than that underlying

the expression of behavioral sensitization.

Figure 4B
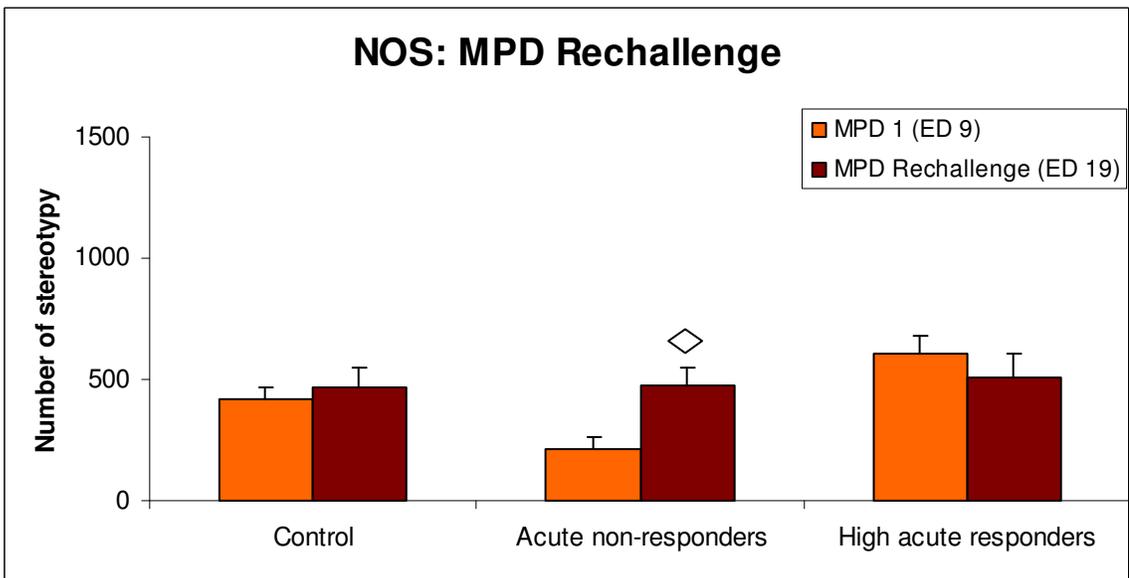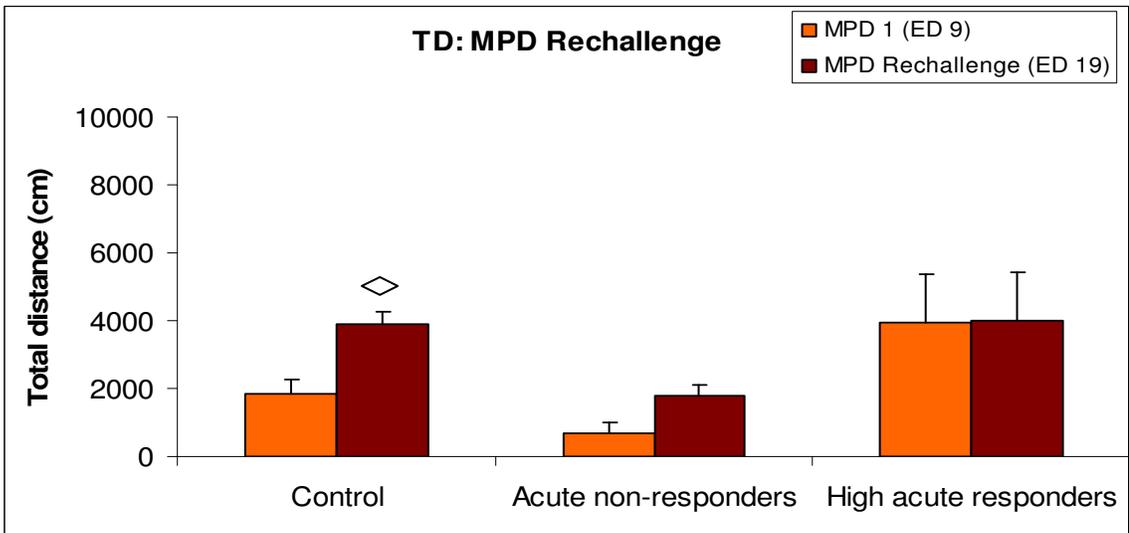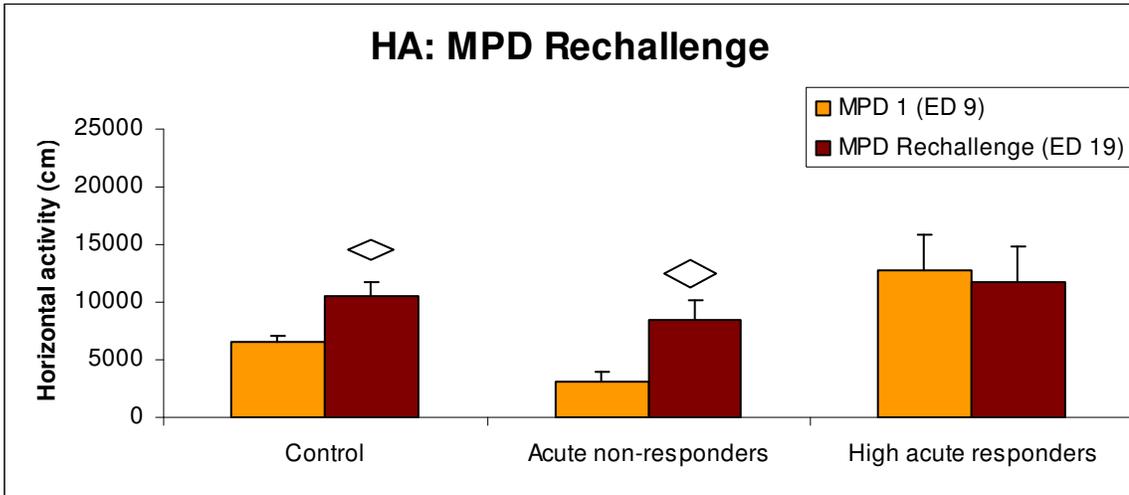
<u>Similar response to rechallenge injection in both lesion groups</u>:

Since both the lesion groups had similar baseline activity to the control group, the locomotor response to the MPD rechallenge injection in the control and the lesion groups was compared using one-way ANOVA with group as the variable .There were no significant differences in response to the rechallenge injection between control and acute non-responders for HA ($F_{(2, 17)} = 0.87$, $p = 0.36$) and NOS ($F_{(2, 17)} = 0.007$, $p = 0.93$)] and between the control and the high acute responders [HA ($F_{(2, 14)} = 0.14$, $p = 0.71$), TD ($F_{(2, 14)} = 0.003$, $p = 0.95$) and NOS ($F_{(2, 14)} = 0.13$, $p = 0.72$)]. TD travelled after the rechallenge injection was significantly greater in the control group as compared to the acute non-responders ($F_{(2, 17)} = 8.23$, $p = 0.01$). Thus, similar levels of activity were seen in the control and both the lesion groups upon the rechallenge injection.

<u>'Sensitized-like' augmented response to acute injection in high acute responders</u>:

No significant difference was found between the acute and rechallenge injection in acute high responders [HA ($F_{(1, 6)} = 0.06$, $p = 0.81$), TD ($F_{(1, 6)} = 7*10^{-4}$, $p = 0.98$) and NOS ($F_{(1, 6)} = 0.66$, $p = 0.43$)]. Moreover, the change in locomotor activity in the acute non-responders was significantly greater than that in the control group in HA ($F_{(2, 14)} = 5.36$, $p = 0.04$), but not in TD ($F_{(2, 14)} = 3.01$, $p = 0.11$) and NOS ($F_{(2, 14)} = 2.13$, $p = 0.16$). Thus, significantly greater increase in locomotor activity, relative to the increase in the control group, was observed upon acute injection of MPD in the high responders group i.e., a 'sensitized-like' response was seen to the acute injection of MPD itself.

4. DISCUSSION:

The aim of the study was to determine whether 6-OHDA lesions of NAc eliminated the expression of behavioral sensitization to MPD. The first specific aim was to verify whether 6-OHDA lesions reduced baseline locomotor activity and it was confirmed that eliminating the dopaminergic afferents to the NAc reduced the locomotor activity of the lesion group. The second specific aim was to determine whether the expression of behavioral sensitization was eliminated in the lesion group, but a sensitized response was seen in the lesioned group that had low acute response to MPD. Therefore, injection of 6-OHDA into the NAc did not prevent the expression of sensitization, as hypothesized. However, other results were found. Notably, half the animals in the lesion group had significantly increased locomotor activity (high acute responders) after acute injection of MPD, while the other half exhibited no significant change from baseline locomotor response (low acute responders) to the acute injection of MPD. One possible explanation for this, discussed below, is that only part of the NAc is involved in the acute response to MPD. Moreover, even though the animals in the lesion group varied in the acute response to MPD, all the animals in the lesion group had an increased response by the time of the final injection, suggesting that the neural circuitry underlying acute effect of MPD is different from the neural circuitry underlying long-term changes caused by MPD. Lastly, it was noted that the animals that exhibited increased locomotor response (high acute responders) had an increased response to the acute injection of MPD.

The different groups of animals, control, surgical, non-surgical and lesion animals had differing baselines. It has been reported that animals with differences in

individual baseline locomotor activity have different susceptibility to sensitization to psychostimulants (Hooks et al., 1991). These authors found that the rats with higher locomotor activity had a greater locomotor response to acute injection of amphetamine and that a direct correlation existed between response to novelty and magnitude of locomotor sensitization. Results similar to Hooks et al. (1991) were reported by Demimere et al., (1989) and Piazza et al., (1989). In our experiment, the non-surgical shams and lesion animals had higher locomotor baselines (pre-surgery baselines), though locomotor activity after 6-OHDA administration (post-surgery baseline) of the lesion group was expected to decline. Thus, according to the results of Hooks et al (1991), a greater locomotor response to acute injection and augmented magnitude of locomotor sensitization was expected from animals with higher baseline activity. Since previous reports from the laboratory had shown sensitization in animals with the lower locomotor baselines (seen in control and surgical sham group here), we did not expect differences in locomotor baseline to cause any change in acute or chronic effect of MPD.

Locomotor activity in rats has been reported to be stable over time (Bjork et al., 1998; Gaytan et al., 1998). Thus, changes in locomotor activity can be attributed to the treatment given. Additionally, it has been demonstrated that handling and injecting saline in rats does not affect locomotor activity for more than 5 minutes, and repeated injections of saline do not have any effect on locomotion (Yang et al., 2007). Similar to these reports, it was observed that locomotor activity on experimental day 1 following saline injection (pre-surgery baseline) was similar to the locomotor activity on experimental day 8 following saline injection (post-surgery baseline) in

control and both the sham groups. In contrast, in the 6-OHDA in NAc lesion group, there was a decrease in baseline activity. While most studies report that DA in the NAc is correlated with increased locomotor activity, consistent with reduction in motor activity after 6-OHDA lesions of the NAc (Pijnenburg and VanRossum, 1973; Oades et al., 1986; Austin and Kalivas, 1991; Anden and Jackson, 1975; Costall et al., 1976; Cools, 1977; Makanjoula and Ashcroft, 1982; Selling and Clarke, 2003, 2006), Kelly and Iversen (1976) reported hyperactivity in 6-OHDA NAc lesioned rats compared to the control rats, while Weissenborn and Winn (1992) reported no changes in spontaneous locomotor activity following 6-OHDA lesions of the NAc. The hyperactivity reported by Kelly and Iversen (1976) has been called 'paradoxical;' and they rationalized that this hyperactivity may represent the failure of the lesioned rats to habituate to the novel environment. Our results agree with reports (Makanjoula and Ashcroft, 1982; Selling and Clarke, 2003, 2006) of a decrease in spontaneous activity following 6-OHDA lesions of the NAc. Since the decrease in locomotor activity following 6-OHDA lesions of NAc was anticipated, animals exhibiting slightly higher baseline activities (> 5000 cm in HA) were chosen for the lesion group so that locomotor activity on ED 8 would be similar across all three groups. Similar to the higher locomotor baseline of the lesion group, surgical shams had higher locomotor activity. This was chosen to determine whether non-specific damage due to administration of saline affected locomotor activity. Administering saline to NAc (surgical shams) did not alter locomotor activity, but administering 6-OHDA to NAc reduced locomotor activity. The decrease in locomotor activity following 6-OHDA administration to NAc was consistent with the fact that DA in the NAc is believed to

have a role in locomotion via its circuitry to ventral pallidum / substantia innominata (Austin and Kalivas, 1991).

However, the accuracy of the lesion is unknown as the lesions could not be verified independently. It can be argued that the lesions caused DA depletion in the NAc since the lesioned animals had significantly reduced locomotor activity, and DA depletion in NAc has been reported to cause a decrease in locomotor activity. But, it is possible that the 6-OHDA may have caused DA depletion in other structures. In close proximity to the nucleus accumbens are the caudate and putamen, which are also believed to be involved in locomotion (Makanjoula and Ashcroft, 1982). Makanjoula and Ashcroft (1982) reported that 6-OHDA lesions of either NAc or caudate putamen reduced spontaneous locomotor activity. They also reported the loss of psychostimulant-induced locomotor activity following 6-OHDA administration to the NAc and reduction in psychostimulant-induced stereotypic activity following 6-OHDA administration to the caudate-putamen. The current results showed loss of the MPD-induced increase in locomotor and stereotypic activity, so it is possible that the lesion may have extended to the caudate-putamen or have lesioned the caudate-putamen. But, the caudate-putamen is generally believed to have a role in stereotyped activity than locomotion (Cho et al., 1999; Horner et al., 2010; Naylor and Olley, 1972). Thus, it seems more likely that the NAc was lesioned as compared to caudate-putamen.

Upon acute injection of MPD, significant increases in locomotor activity were seen in control and non-surgical sham groups for all three indices of activity analyzed, namely HA, TD and NOS. The surgical shams did not have a significant

increase, but this may have been due to the small number of rats in this group (n = 4).

The ability of psychostimulants like MPD to elicit a locomotor response may be attributed to their ability to affect the VTA-accumbens-pallidal circuitry. Psychostimulants cause an increase in dopaminergic transmission from the VTA to the NAc. This dopamine released from VTA neurons in the NAc inhibits the GABA neurons in the NAc, which project to the terminal in ventral pallidum (VP) / susbtantia innominata (SI) (Austin and Kalivas, 1991). It has been reported that injecting picrotoxin, a GABA antagonist, in the ventral pallidum caused an increase in locomotion (Mogenson and Nielson, 1983). Therefore, the inhibition of GABA neurons in the NAc, by the DA released in NAc from VTA, results in increased locomotion. Though exact mechanism(s) for this effect are unknown, it is believed that the connections of ventral pallidum to the mediodorsal thalamic nucleus may be involved. It is so conjectured since the mediodorsal thalamic nucleus projects to the anterior cingulated cortex, which has been termed as the 'likely forerunner of the motor cortex' due to its direct connections to the supplementary motor cortex and the premotor cortex (Heimer et al., 1982).

The VTA-accumbens-pallidal circuitry described above may explain the reduced acute response to MPD after 6OHDA lesion of the NAc. However, electrolytic lesions of NAc did not prevent the acute response to MPD (Podet et al., submitted for publication). Therefore, the hypothesis was that destruction of dopaminergic afferents to the NAc would not affect the acute response to MPD. However, this was not found to be true. In fact, there were two distinct responses in rats with 6-OHDA lesion of the NAc. Fig. 2C shows that while some animals

exhibited no increase in locomotor activity after the acute injection of MPD (acute non-responders), others showed a significantly elevated locomotor activity (high acute responders) following the acute administration of MPD. Each group (acute non-responders and high acute responders) responded differently throughout the length of the experiment. Therefore, the lesion group was further divided into two groups, 'acute non-responders' and 'high acute responders', based on acute response to MPD.

Both no response, and augmented response, to acute injection of psychostimulant in 6-OHDA in NAc lesioned animals have been reported (Kelly et al., 1975; Kelly and Iversen, 1976). While Kelly et al.(1975) reported that the NAc (6-OHDA) lesioned rats did not show the characteristic locomotor response to an acute injection of amphetamine, Kelly and Iversen(1976) reported augmented locomotor activity response to amphetamine 3 days after the 6-OHDA NAc lesion. However, as the time between the 6-OHDA lesion and the acute injection of amphetamine differed between the two experiments (Kelly et al., 1975; Kelly and Iversen, 1976), they rationalize that this difference in response to the acute amphetamine injection in the NAc (6-OHDA) lesioned rats can be attributed to either increased transmitter release from degenerating terminals or to super sensitization of post-synaptic receptors, which may have caused the augmented locomotor response to the release of transmitter. Such reasoning does not apply to the two distinct responses seen in the current experiment since same amount of time was allowed between the administration of 6-OHDA in NAc and the acute injection of MPD in both the acute non-responders and the high acute responders.

It is, however, possible that the difference in acute response to psychostimulant administration may be due to differences in the size or location of the lesion, i.e., different subregions of NAc may have been lesioned, contributing to the difference in acute response seen. The NAc is not a unitary structure (Pontieri et al., 1995; Pierce and Kalivas, 1995); and psychostimulant drugs (DiChiara, 2002; Pontieri et al., 1995) as well as stress (Deutch and Cameron, 1992) activate dopamine transmission selectively in the NAc shell (Kalivas and Duffy, 1995). Acute administration of psychostimulants has also been shown to preferentially increase glucose utilization in the NAc shell compared to the core. Moreover, it has been reported that microinfusions of DA D1 receptor agonists, or amphetamine, into the NAc shell produce a greater increase in locomotor activity than injections into the core (Swanson et al., 1997; Heidbreder and Feldon, 1998). Additionally, it has been reported that it is the NAc shell that selectively innervates the ventromedial part (VPm) of the subcommissural VP (Zahm and Heimer, 1993; Zahm and Heimer, 1990; Heimer et al., 1991), which, in turn, projects to the thalamic mediodorsal nucleus (Zahm, 2006). Thus, it is possible that the acute non-responders, not showing augmentation of locomotor activity upon the acute injection of MPD, may have had lesions that destroyed dopaminergic afferents of both the core and the shell, while the lesion in animals of high acute responders, that had increased locomotor activity following acute injection of MPD, may have had destruction of dopaminergic afferents of the core only.

However, the converse has also been reported: that activation of locomotor activity by amphetamine is blunted by 6-OHDA lesions of the core but not the medial

shell (Boye et al., 2001). Therefore, other reasons for the two responses seen may be correct versus incorrect lesioning of the NAc, differences in locomotor baseline between the two groups, and differences in DA depletion between the two lesion groups. It can be argued that the diversity of response to the acute injection following acute injection of MPD may suggest that while one group had accurate lesions of the NAc, the other group may have had inaccurate lesions. However, since both the lesion groups had significantly reduced locomotor activity after surgery (post-surgery baseline), and reduction in locomotor activity has been linked with 6-OHDA lesions of NAc, this suggests that accurate versus inaccurate lesioning of NAc may not be the reason for the diverse responses to acute injection of MPD.

Differences in baseline locomotor activity are also known to contribute to differences in acute response to psychostimulants (Hooks et al., 1991; Mathews et al., 2010) and therefore, the post surgery baseline of the two lesion groups: high acute responders and acute non-responders was compared (Fig. 2D). The post-surgery baselines of the two lesion groups were not significantly different, so the two responses to acute injection of MPD seen in the lesion group are not explained by differences in baseline locomotor activity.

Another rationale for diversity of responses following acute injection of MPD may be due to different amounts of DA depletion in NAc. Differences in DA depletion may have caused differential reduction in the activation of distal excitatory synaptic transmission (Nicola et al., 1996) and thus differences in the locomotor response to acute injection of MPD. Further support for this rationale comes from Bainton et al. (2000), who reported decreased response to acute cocaine in Drosophila

with pharmacologically reduced DA levels. Thus, it may be that the animals in the high acute responders group had less DA depletion, whereas animals in acute non-responders group may have had significant DA depletion in the NAc. As neither high performance liquid chromatography (HPLC) nor tyrosine hydroxylase staining was done, it is possible that differential depletion of DA may have resulted in two responses in the NAc lesioned group to the acute injection of MPD. However, as the same amount of 6-OHDA was administered in all animals in the lesion group, and the different roles of NAc subcompartments are noted (DiChiara, 2002; DiChiara et al., 2004), it is more likely that the high acute responders and acute non-responders differed in the size or location of the lesion.

Following the 6th consecutive injection of MPD, the increase in locomotor activity after the acute injection of MPD was maintained in the control group. Similarly, in the non-surgical sham group, the increase after the 6th consecutive injection of MPD was maintained. Generally, a sensitized response, i.e., significantly elevated locomotor activity after repeated psychostimulant administration as compared to the acute injection of the psychostimulant is obtained. However, this sensitized response is not always reported (Martin-Iversen et al., 1988). A sensitized response was seen in the surgical shams. But the increase seen in surgical sham group was unexpectedly high. The reason for this is unclear and may be related to alterations in the NAc due to saline during surgery. Alternatively, the induction of sensitization observed in the surgical sham group might be exaggerated because they had higher locomotor baseline. Hooks et al (1991) found that the magnitude of sensitization was positively correlated to spontaneous activity. In the NAc 6-OHDA

lesioned animals, the acute non-responders showed a significantly greater increase in locomotor activity as compared to that observed after the acute MPD injection (Fig. 3B). This may suggest that DA in the NAc core and shell may not be essential for induction of behavioral sensitization. However, the acute injection of MPD failed to elicit an increase in locomotor activity in the acute non-responders, so the above interpretation may not be correct. The increase in activity following the 6th consecutive injection of MPD in high acute responders was similar to the increase in activity following the acute injection of MPD. Comparing the locomotor increases following the 6th consecutive injection of MPD in the two lesion groups and the control group, it can be noted that all three groups had similar increase in locomotor activity by the 6th injection of MPD. This suggests that there is dissociation between the neural circuitries underlying acute and chronic effects of MPD.

Finally, the rechallenge injection on ED 19 caused a significant increase in locomotor activity compared to the acute injection of MPD i.e., sensitization was expressed in the control and non-surgical sham group for the indices of HA and TD. The surgical shams showed robust increases in HA, TD and NOS, but these did not reach statistical significance. Given the increase seen in the surgical shams, the difference may not have been statistically significant because of inadequate statistical power, since there were only four rats in this group. In the NAc lesioned animals, the acute non-responders had significantly higher locomotor activity i.e., sensitization was expressed, while the high acute responders had similar levels of activity on both the 1st and the rechallenge injection of MPD. Thus, the response of the high acute responders may be interpreted in two ways: 1) expression of sensitization was

prevented in the high acute responders and 2) a sensitized response was elicited by the 1st MPD injection itself. Since similar levels of locomotor activity were seen in the control animals and the high acute responders and acute non-responders upon the MPD rechallenge injection, it may be suggested that expression of sensitization was not prevented by the lesion, and that a 'sensitized-like' response was observed upon the 1st injection itself in high acute responders.

Previously, Beyer and Steketee (1999) reported that 6-OHDA lesions of the medial PFC caused sensitized-like behavioral and neurochemical responses to an acute injection of cocaine. However, they conjecture that this might be because the DA depletion of mPFC (by cortical 6-OHDA lesions) removes the inhibition on cortical glutamatergic neurons, which have connections to and influence NAc and VTA (Christie et al., 1985; Sesack and Pickel, 1992). Thus, by removing the inhibition, this allows for greater excitation of DA neurons in VTA and NAc. Therefore, they rationalize that decreased DA in mPFC causes an increase in DA of NAc, which correlates with the enhanced response to the acute injection of cocaine. By contrast, we report that 6-OHDA lesions of NAc core, i.e., decrease of DA in the NAc core is correlated with the sensitized response to the acute injection of MPD. This might be due to pharmacological differences between the drugs or it might be that DA depletion in mPFC may not correlate to increased levels of DA in NAc as hypothesized by Beyer and Steketee (1999). Support for the latter explanation comes from the findings of King et al. (1997), who reported that dopamine depletion in the mPFC attenuated the amphetamine-induced increase in extracellular dopamine in the NAc core. Thus, it is possible that the enhanced response to acute injection of cocaine

reported by Beyer and Steketee (1999) may be correlated with decreased levels of DA in mPFC and NAc, since they measured DA levels in only mPFC and not NAc.

This finding, that the sensitized response is correlated with decreased DA in NAc core, however, is not in concordance with other psychostimulants: for example, Cadoni et al., 2000 reported that sensitization to amphetamine and cocaine (low doses) was correlated with sensitization of DA transmission in the NAc core and not to changes in the NAc shell. For higher doses of cocaine, Cadoni et al. (2000) reported a reduction in DA transmission in the shell in addition to strengthened DA transmission in the core. However, contradictory findings have also been reported: Pierce and Kalivas (1995) reported that sensitization to amphetamine is correlated with robust increase in DA transmission in the NAc shell. Thus, the changes, associated with NAc structures and psychostimulant sensitization are conflicting even for a single psychostimulant.

Moreover, if the difference in the responses of high acute responders and acute non-responders are due to differences in size of the lesion, it would appear paradoxical that the dopamine depletion in core and shell caused the response to acute injection of MPD to be eliminated, while the dopamine depletion of the core alone caused an increased response to the acute injection of MPD (Fig. 5). It is possible that the DA in NAc shell is essential for the acute effect of MPD and that the sensitized response is linked to reduced levels of DA in the NAc core in the presence of intact DA transmission in the NAc shell. This may be a possibility since NAc core and shell are known to exhibit differential resposiveness to drugs of abuse (Pontieri et al., 1995) and conventional rewards (Bassareo and Di Chiara, 1999). Further support for

this paradoxical finding comes from Broening et al., 1997 who reported that methamphetamine administration, which is neurotoxic to the dopaminergic neurotransmitter system, destroyed dopaminergic innervation in the nucleus accumbens core, whereas shell inntervation was resistant. They concluded that their results support the hypothesis that there exists differential innervation to core and shell by mesencephalic dopamine neurons. Thus, it is quite possible that the acute injection of MPD specifically activates the innervation to the shell, and that the innervation to the core is altered in sensitization to MPD.

FIGURE LEGEND: Figure 5 shows the heterogeneity of NAc and suggests

that the acute effect of MPD is modulated by DA transmission in NAc shell, and the

'sensitized-like' augmented response may be due to alteration of DA transmission in

the NAc core.

Figure 5

Core (long term effect)

Shell (acute effect)

DA depletion in shell = loss of acute effect of MPD
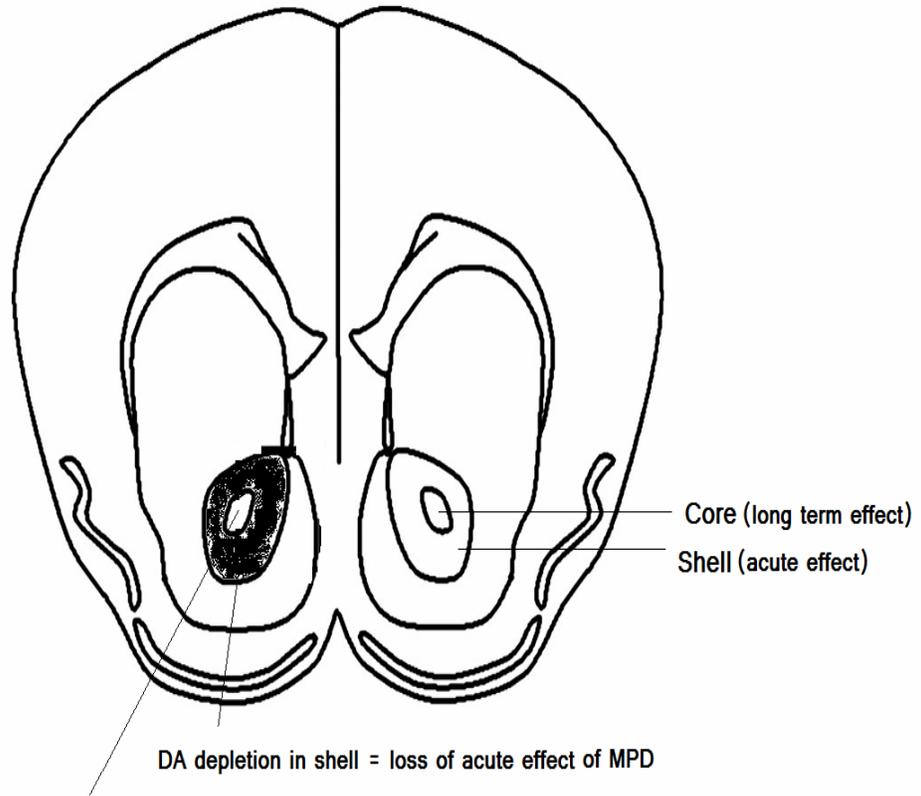
DA depletion of core in presence of intact shell = 'sensitized-like' augmented response to acute injection

The exact roles of NAc core and shell in the acute and chronic effects of MPD reported here are speculations since the DA depletion and the accuracy of the lesions are not known. The accuracy of the lesions could have been better determined with the following methods instead of the cresyl violet staining used:

1)   High Pressure Liquid Chromatography: This is the most common way of measuring the efficacy of 6-OHDA lesions and is used by most studies performing 6-OHDA lesions (Beyer and Steketee, 1999; Bjijou et al., 2002; Carter and Pycock, 1980; Rassnick et al., 1993). In HPLC, the brain tissue from the area of interest is homogenized and then assayed for DA and DOPAC concentrations using electrochemical detection following separation by HPLC. This method would have assessed the effectiveness of dopamine depletion by the lesions, but would not thrown light on the location of the DA depletion. Since NAc is a heterogenous structure and diverse responses were seen upon acute psychostimulant administration (high acute responders and acute non-responders), performing HPLC would not have distinguished whether the difference in response was due to differences in location of DA depletion.

2)   Calbindin immunohistochemistry: This method would have been best to distinguish 'shell' from 'core' in the histological verification of probe location (DiChiara, 1999, 2002). The core of NAc is reported to stain darker with calbindin than the shell (Meredith et al., 1996). However, since calbindin is a calcium binding protein (Timurkaan and Taracki, 2004), calbindin immunohistochemistry would not reveal anything about DA depletion, and thus might have been less useful about highlighting the reason for the differences seen.

3)   Double labeling of tyrosine hydroxylase labeling and dopamine-beta-

hydroxylase (DBH) immunofluorescence: This double labeling would be most

useful since it has helped to assess DA depletion in the core and DA and

norepinephrine (NE) depletion in the shell, as well check the extent of DA

depletion. Tyrosine hydroxylase labels catecholaminergic neurons, whereas DBH is

more specific for noradrenergic and adrenergic neurons (Berod et al., 1982). As

described by Berod et al., 1982, the advantage of combining immunofluorescence

and immunoperoxidase techniques is that they yield very different staining and thus

making it easier to observe.

Future directions to verify these findings may include micro-dialysis studies to

correctly assess DA changes in core and shell associated with behavioral sensitization

to MPD. Also, it might be helpful to perform selective 6-OHDA lesions of only the

core or the shell to elucidate the role of each of the subcompartments of NAc in the

acute and chronic effects of MPD.

In conclusion, there were two patterns of response to MPD after 6OHDA

infusion. Low acute responders had no initial increase in activity, but developed

behavioral sensitization to repeated MPD. High acute responders had an enhanced

motor response to acute MPD, but had no further increase in motor activity. This

suggests that the subregions of NAc may have different roles in locomotor activity

induced by MPD. Further, even though some animals responded to the acute injection

of MPD and others did not; all the animals had similar levels of increased locomotor

activity by the 6th consecutive injection of MPD and the rechallenge injection. Thus,

the neural circuitry underlying the acute effect of MPD appears different from the

neural circuitry underlying behavioral sensitization to MPD.

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Vita

Sheshali Wanchoo earned her Bachelor of Science in Biology Major with a Neurobiology Option from University of Wisconsin – Madison. After undergrads, she moved to Houston to be closer to family and worked as a research technician for a year before she joined her thesis lab. After initially enrolling as a non-degree seeking student, she became a degree seeking student at the Graduate School of Biomedical Sciences (GSBS), University of Texas Health Science Center at Houston in January 2009. While a student of GSBS, she presented her findings at Society for Neuroscience (Chicago, USA; 2009) and the 1st International Congress on Neurobiology and Clinical Psychopharmacology (Thessaloniki, Greece; 2009), and was an enthusiastic volunteer for the GSBS Outreach Program.

sheshali.wanchoo@gmail.com