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Selective bilateral lesion to caudate nucleus modulates the acute and chronic methylphenidate effects

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Abstract

The psychostimulant methylphenidate (MPD) is currently the most prescribed drug therapy for attention deficit hyperactivity disorder (ADHD) and is used by students as a cognitive enhancer. The caudate nucleus (CN) is a structure within the motive circuit where MPD exerts its effects, it is known to contain high levels of dopaminergic cells and directly influence motor activity. The objective of this study was to understand the role of CN in response to acute and chronic administration of MPD using an open field assay.

Specific and non-specific bilateral ablations were created in the CN using electrolytic lesion and injection of the neurotoxin 6-Hydroxydopamine (6-OHDA). Four groups of rats were used: intact control (n=4), sham operated (n=4), CN electrolytic lesion group (n=8) and CN 6-OHDA injected group (n=8). On experimental day one (ED1) all rats received a saline injection and baseline locomotive activity was recorded. On ED2 and ED3 CN sham, electrolytic lesion and/or 6-OHDA injected groups were made followed by four to five days recovery (ED3–7), followed by six daily 2.5 mg/kg MPD injections (ED9–14), three days of washout (ED15–17) and an MPD re-challenge of drug proceeding the washout days (ED18). Locomotor activity was obtained at ED1, 8, 9, 18 using an open field assay.

The results show that the CN electrical lesion group responded to the acute and chronic MPD administration similar to the intact control and sham operated group, while the CN 6-OHDA injected lesion group that selectively depleted the CN dopaminergic neurophil prevented the acute and chronic effect of MPD administration. One possible interpretation why nonspecific electrical lesioning of the CN failed to prevent acute and chronic effects of MPD administration is due to destruction of both direct and the indirect CN pathways which act as an inhibitory/excitatory balance, making the net outcome no change for the electrical lesion group. The selective dopaminergic lesioning prevented the acute and the chronic effects of MPD administration suggesting dopaminergic pathways in CN play a significant role in the acute and chronic effect of MPD.

Keywords

Caudate; Ritalin; 6-OHDA Lesion; Behavior; Electrolytic Lesion

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1. Introduction

Attention deficit-hyperactivity disorder (ADHD) is a neuropsychiatric syndrome recognized in early childhood with persistent symptoms of inattention, hyperactivity and impulsivity (DSM-IV). Recent figures estimate that 7 to 10% of school aged children have been diagnosed with ADHD and 32% of these children are undergoing drug therapy (Froehlich et al., 2007). Amphetamine (AMP) was the preferred treatment for ADHD until the 1930's when it was found to elicit adverse effects such as dependence (Tilson & Rech 1973). AMP as a way to treat ADHD was then replaced with another psychostimulant, Methylphenidate (MPD; Ritalin) (Greenhill et al., 2006). The chemical structure of MPD is close to AMP, and its pharmacological effects closely resemble those of cocaine (Kuczenski & Segal 1997; Teo et al., 2003; Volkow et al., 1995). Similar to cocaine, MPD binds to dopamine transporters (DAT) preventing the Dopamine (DA) reuptake from the synaptic cleft, resulting in increased extracellular DA levels in the mesocorticolimbic system (Ferris et al., 1972; Kuczenski & Segal 1997).

Repetitive exposure to MPD can elicit dose dependent behavioral sensitization or tolerance (Yang et al., 2006, 2007, 2010, 2011). Previous reports state that behavioral sensitization following chronic drug treatment is due to an increase in DA in the mesocorticolimbic DA system (Beyer and Steketee, 1999; Kalivas and Stewart, 1991; Kalivas et al., 1993; Stewart and Badiani, 1993). Behavioral tolerance and/or sensitization are experimental markers for the probability of a drug to elicit dependence (Robinson and Berridge, 1993). Behavioral sensitization is defined as the progressive augmentation of activity as a result of repeated administration of psychostimulants (Chao and Nestler, 2004; Gaytan et al., 1997; Kalivas and Stewart, 1991; Robinson and Berridge, 1993; Yang et al., 2003, 2011).

Psychostimulants exert their effects predominantly on the motive circuit (Manev and Uz 2009; Yang et al., 2007). The motor activating effects of psychostimulants partially depend on an increase in DA transmission in the Caudate Nucleus (CN) (Rebec, 2006). The CN is a subcortical structure belonging to the extrapyramidal motor system which is known to play a role in locomotive regulation and belongs to the motive circuit (Ferris et al., 1972; Rebec, 2006; Yang et al., 2006). The CN receives DA projections from the associative-cognitive areas including but not limited to the prefrontal cortex (PFC) (Cognat et al., 2010). Behavioral sensitization is expressed in the PFC following chronic psychostimulant administration (Lee, 2008; Pierce and Kalivas, 1997; Wanchoo et al., 2010; Wolf, 1998; Yang et al., 2006, 2007). Moreover, it was reported that simply impairing the dopaminergic system in the CN can disrupt sensitive motor performance (Amalric & Koob, 1987). Therefore, it was postulated that the dopaminergic system in the CN might be involved at least in part in the induction or the expression of behavioral sensitization following repetitive MPD administration.

Given the CN involvement in locomotion regulation (Ferris et al., 1972; Rebec, 2006), it is imperative to investigate the role of the CN in the initiation of increased locomotor activity following acute and chronic MPD administration. Limited research exists to understand the CN influence on psychostimulant induced behavioral sensitization or behavioral sensitization to MPD in particular. Previous studies have investigated other motive circuit nuclei involvement in the acute and chronic effect of MPD by creating non-specific and specific destruction in the structure. Bilateral ablation of the PFC and nucleus accumbens (NAc) by electrolytic lesion has been reported to prevent the expression of behavioral sensitization produced by repetitive psychostimulant administration (Lee, 2008; Podet et al., 2010; Tang, 2009). Similarly, bilateral injection of 6-hydroxydopamine (6-OHDA) into the PFC and NAc that specifically eliminated the dopaminergic system in these areas has shown to prevent the behavioral sensitization produced by chronic administration of MPD (Wanchoo et al., 2009). Another experiment investigating the effect of amphetamine

induced taste aversion before and following 6-OHDA injection reported that 6-OHDA treated animals exhibit attenuation to the aversion preference assay (Roberts and Fibiger 1975). By comparing various methods of CN lesions it is possible to expand the knowledge of the role of the CN in response to acute and chronic effects of MPD. The aim of this study was to investigate the role of the CN in response to the acute and chronic effect of MPD in four groups of animals; intact control, CN sham operated, CN bilateral electrolytic lesion and CN bilateral 6-OHDA injected groups, using an open field assay.

2. Methods

2.1 Animals

Twenty-four adult male Sprague-Dawley (SD) rats (Harlan, Indianapolis, IN, USA) were housed individually in home cages. Previous studies have shown differing responses to drug administration when animals are placed in a novel environment for recording purposes, therefore we used the rat's home cage as their test cage throughout experiment (Liao, RM & Lin, HL 2007; Mattson et al., 2007) Rats were allowed to acclimate for 4 to 5 days before beginning experiment. Animals were maintained on a 12 h light/dark schedule (light on at 06:00) with access to food and water ad libitum, the temperature was kept at 21 ± 2 °C with a relative humidity of 37–42%. The animals weighed between 210 and 240 grams on experimental day 1 (ED1). All experiments were approved by our Animal Welfare Committee and carried out in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals. In line with a principle issue of research and testing consideration, our experiment used a reduced number of control (n=4) and sham (n=4) animals. Citing previous studies done in our laboratory that used exact experimental protocol for control and sham operated animals as precedent (Lee et al., 2008; Podet et al., 2010; Wanchoo et al., 2010; Yang et al., 2007, 2008) thus enabling us to use a reduced number of animals without compromising statistical significance.

2.2 Apparatus

Locomotive activity was recorded using an open field computerized animal activity system (Opto-M3, Columbus Instruments, Columbus, OH). The animals were housed in clear acrylic cages which fit into the recording apparatus thus allowing us to record the animal's behavior in their home cages. The Columbus system infrared beam sensors run 40 cm in length, 20 cm in width, and are set 5 cm above the floor of the cage. Movement across any of the infrared beams resulted in a beam break and was subsequently recorded for 60 minutes after saline or MPD (2.5 mg/kg i.p.) injection and were compiled and downloaded to a PC in 5 minute bin. Two locomotor indices were evaluated; total distance traveled (TD), and horizontal ambulatory (HA) activity.

2.3 Procedure

Rats were given 4 to 5 days to acclimate in the home/test cage before the initial experiment began. On experimental day one (ED 1) animals were then divided into four random groups; intact control group (n=4), sham operated (n=4) group, electrolytic lesion (n=8) and 6-OHDA injected (n=8) groups. A saline injection and baseline activity was recorded for 60 minutes. All the electrolytic and chemical injection surgeries were performed on ED 2 and ED 3. The rats were given 5 days of recovery from ED 3-ED 7. On ED 8 the rats received a saline injection and locomotive activity was recorded immediately for 60 min same as at ED1. On ED 9 through ED 14, the rats received daily injections of 2.5 mg/kg MPD in their home cage with behavioral recordings proceeding for 60 minutes post injection. On ED 15-ED 17 animals underwent washout days, i.e. no injections were given. On ED 18 the rats received a rechallenge injection of 2.5 mg/kg MPD with locomotive recordings resumed post injection for 60 min (Table 1).

2.4 Surgeries

On ED 2 or ED 3 the randomly selected sham (n=4) operated, bilateral electrolytic lesion (n=8) and 6-OHDA bilateral injection (n=8) rats underwent surgery. The rats were all anesthetized i.p. with 50 mg/kg sodium pentobarbital, and then placed into stereotaxic apparatus. An incision was made down the middle of the scalp and cranial muscles and connective tissues were separated to expose the skull.

A. Bilateral electrical lesion group—Two holes were drilled bilaterally above the CN (0.5 mm anterior to bregma, 2.0 mm and 3.0mm lateral to midline on each side) using coordinates derived from the Paxinos and Watson rat atlas (1986). To produce electrolytic lesions a bipolar electrode was made from twisted 80 μ m in diameter stainless steel wires (electrodes) insulated except at the tips. The electrode lowered in CN at a depth of 4.8 mm below the skull and a DC current of 2 mA was applied for 120 seconds. A similar procedure was performed on the other holes. After completion of the surgery, the incision was closed using wound staples.

B. Bilateral 6-OHDA lesion group—Two bilateral holes were created above the CN as described above however, instead of using bipolar electrodes; a 30 gauge stainless steel cannula was used to inject 4 μ l of 6-OHDA solution at a depth of 4.8 mm below the cortex at a rate of 1 μ l/min. The 6-OHDA was dissolved in 2 μ l of 0.9% saline containing 0.2 mg/ml ascorbic acid (Jackson, 1983; Wanchoo et al., 2009). The cannula was left in place for additional three minutes after the completion of each injection to allow diffusion of the 6-OHDA to the surrounding tissue. These steps were repeated in the second hole in the same hemisphere and in the other hemisphere for each additional hole. After surgery the scalp was closed using wound closing staples. The sham rats went under the same procedure however there was no electrode/cannula placement.

2.5 Drugs and Dose

Methylphenidate hydrochloride (MPD) was obtained from Mallinckrodt (St. Louis, MO). Based on previous dose response experiments (0.1 to 40 mg/kg i.p. MPD), it was found that 2.5 mg/kg MPD administered i.p. in the morning elicited behavioral sensitization (Gaytan et al., 1997, 1998, 2000; Yang et al. 2000, 2003, 2006, 2007). Additionally, 2.5 mg/kg MPD yields clinically relevant peak plasma levels and falls within therapeutic range used in treating ADHD (Gatley et al., 1999; Berridge et al., 2006; Devilbiss & Berridge, 2006). MPD was calculated as free-base and dissolved in 0.9% saline to make 2.5 mg/kg. Each animal was weighed prior to injections and each injection was standardized to 0.8 ml with saline. Recordings began immediately after injection at approximately 07:30 (see Table 1).

2.6 Histology

At the conclusion of the experiment the animals were overdosed with sodium pentobarbital and perfused intracardially with 10% formaldehyde. The brains were removed and soaked in formaldehyde for several days. The brains were then sliced in the coronal plane at 60 μ m thickness and allowed to dry for 24 hours. The slices were then scanned using a high resolution scanner and analyzed for lesion size and location, using the atlas of Paxinos and Watson (1986).

2.7 Data Analysis

Locomotor activity was recorded and summed to 5 min bins for 60min (i.e. 12 bins). A histogram summing the activity under the 60 min temporal graph was created to compare the average sums of the 60 min of activity pre and post-surgical manipulation (ED 8 vs. ED 1) to verify effects of surgery. For acute (ED 9 vs. ED 8) and chronic (ED 18 vs. ED 9)

effect mean ($\pm$ standard deviation) for each bin was sequentially plotted to produce a temporal graph for 60 min after each injection. To determine the significance of changes among the experimental days for each group an ANOVA with repeated measures was used with adjustments for correlation among measurements within each animal. Post ad hoc comparisons were used to estimate changes between days within groups. Three comparisons were made as follows: (1) ED 8 was compared to ED 1 to determine whether the lesion and/or the sham surgeries affected the animals' baseline activity level (see Table 1). (2) ED 9 was compared to ED 8 to evaluate the acute effect of MPD in all of the 4 groups. (3) ED 18 was compared to ED 9 to determine if tolerance or sensitization had been expressed following 6 days of MPD administration and a 3-day washout period followed by a 2.5 mg/kg MPD rechallenge (Table 1). (A p-value <0.05 was considered as statistically significant.

3. Results

3.1. Effects of CN manipulation on baseline activity: comparing ED8 to ED1

Baseline locomotive activity was recorded for all animals prior to surgery on experimental day 1 (ED1) and again several days post-surgery on ED 8 (Table 1). Figure 1 shows the sum of 60 min, horizontal activity (HA), total distance (TD) traveling before and after surgical manipulation of the intact control, sham operated, electrolytic lesion and 6-OHDA injection groups respectively. The data obtained at ED 8 shows that the intact control, sham operated, bilateral CN electrolytic lesion and bilateral 6-OHDA injection to deplete the DA system of the CN had similar baseline locomotive activity when compared to their initial recording obtained at ED1. The CN electrolytic lesion group before the CN lesion (i.e. baseline control ED 1) and after the CN electrolytic lesion exhibited similar baseline level of locomotion (comparing ED 1 to ED 8) however when comparing this group of locomotive activity to the other groups the CN electrolytic lesion group exhibited a lower baseline of activity than the intact control, sham operated and CN 6-OHDA injection groups. Differing baseline of TD, HA and NOS activity between groups of animals has been reported. Animals were randomly assigned groups on ED1 prior to any locomotor recording and each animal was used as its own control to observe the treatment effects (Gaytan et al., 1997, 2000; Yang et al., 2001, 2003, 2007, 2010, 2011).

3.2. MPD acute effect: comparing ED 9 first MPD administration to ED 8 saline baseline

Figure 2 summarizes and compares the HA recorded after the initial 2.5 mg/kg MPD injection at ED 9 to the baseline recording at ED 8, by groups (intact control, sham operated, electrolytic lesion, and 6-OHDA). MPD exposure to the intact control, sham operated, and electrolytic lesion group all showed a significant increase in HA ($p<0.0071$, $F_{3,9}=37.21$), ($p<0.04$, $F_{3,9}=22.13$), and ($p<0.0013$, $F_{3,21}=20.28$), respectively (Fig. 2). TD was also considered significant when comparing ED 9 initial MPD activity to ED 8 baseline activity (data not represented). These results indicate that MPD exposure to intact control, the sham operated and the bilateral electrolytic lesion groups all exhibited similar increases in locomotor activity. However, the 6-OHDA injection group failed to exhibit the acute effect of MPD exposure in all of the locomotor activity indices studied (Fig 2). This failure to exhibit increased activity suggests that 6-OHDA injection of the CN prevents the acute effects of MPD on locomotion.

3.3 MPD Chronic Effect: Comparing ED18 to ED9

The temporal graphs of figure 3 summarizes and compares the HA, TD and NOS recorded following 2.5 mg/kg MPD re-challenge injection at ED 18 to the initial MPD injection at ED 9. ED 18 recordings were obtained after six repetitive days of MPD and three days of washout. After chronic MPD exposure; the intact control, sham operated, and electrolytic lesion group all exhibited significant increases in HA and TD activity ($p<0.006$, $F_{3,9}=37.21$;

Fig 3A HA), ($p < 0.0011$, $F_{3,9} = 21.84$; Fig 3A TD); ($p < 0.0022$, $F_{3,9} = 22.13$; Fig 3B HA), ($p < 0.0008$, $F_{3,9} = 17.76$; 3B TD); ($p < 0.0120$, $F_{3,21} = 20.28$; Fig 3C HA), ($p < 0.0031$, $F_{3,21} = 16.08$; Fig 3C TD), respectively. This significant increase in activity indicates that behavioral sensitization was expressed. Previous studies have shown that chronic exposure to amphetamine results in a statistically significant increase in stereotypic behavior (Gaytan et al., 1996, 1998, 1999). Stereotypic behavior (NOS) is repetitive movements shown to represent motor dysfunction (Gaytan et al., 1996). However, none of these groups expressed a significant change in NOS activity ($p < 0.4258$ $F_{3,9} = 42.24$); ($p < 0.5851$ $F_{3,9} = 7.59$); ($p < 0.2151$ $F_{3,21} = 20.60$), respectively when comparing ED 18 to ED 9. This finding suggests that while amphetamine and methylphenidate share similar chemical structures, their overall modulation of neuronal activity following chronic exposure is different. The 6-OHDA injection group failed to exhibit an increase in activity at ED18 following the MPD rechallenge for all of the locomotor indices compared to the MPD initial injection at ED9. This suggests that the 6-OHDA injection to deplete the DA in the CN prevents the expression of behavioral sensitization.

Discussion

Methylphenidate (MPD) is used as a drug therapy for ADHD patients but more recently MPD has been sought after for cognitive enhancement among students (Greely et al., 2008). There is little and conflicting research on MPDs long term effects and later drug abuse potential; some studies report that MPD treatment can protect youth from later drug dependence (Wilens et al., 2003) whereas others report that MPD use in youth creates susceptibility to later drug use (Piazza et al., 1989; Robinson et al., 1993). MPD primarily acts on the central nervous system (CNS), more specifically on the motive circuit, which consists of the prefrontal cortex (PFC), nucleus accumbens (NAc), Ventral Tegmental Area (VTA), caudate nucleus (CN) and other CNS sites (Kuczenski & Segal 1997; Price & Kalivas 1997). Repetitive exposure to MPD was reported to elicit dose dependent behavioral sensitization or tolerance (Gaytan et al., 1997, 2008; Yang et al., 2000, 2001, 2006, 2007, 2010, 2011). These two behavioral expressions are used as an experimental marker to investigate the liability of the drug (Pierce and Kalivas, 1997). The role of the PFC and NAc in response to acute and chronic MPD administration in eliciting behavioral sensitization has been investigated in other studies using non-specific electrolytic lesioning and injection of selective neurotoxins to deplete the dopaminergic or glutamatergic system by 6-OHDA or ibotenic acid injection respectively, followed by repeated daily MPD administration (Lee et al., 2009; Podet et al., 2010; Wanchoo et al., 2009, 2010). In the reports mentioned above, electrolytic lesion and selective neurotoxin injection of the PFC and NAc alter either the acute and/or chronic MPD effect on locomotion. It was reported that when MPD is given repeatedly for several days the level of DA in the CNS is permanently augmented (Cornish & Kalivas, 2001; Pierce and Kalivas, 1997), this DA augmentation resulted in behavioral sensitization, a known experimental marker to indicate the drug liability. Knowing the role of other CNS sites in response to acute and chronic MPD exposure is imperative to understand the mechanism of MPD action. The aim of this experiment was to create non-specific and specific ablations to the CN to better understand the role of the CN in behavioral sensitization elicited by repetitive MPD exposure.

Four groups of animals were used in this experiment, intact control, sham operated, bilateral electrolytic CN lesion and bilateral CN 6-OHDA injection group. The main findings of this study show that the sham operated group, the CN bilateral electrolytic lesion group and the 6-OHDA CN bilateral injection group had no effect on baseline locomotive activity when compared to intact control (i.e. ED8 was compared to ED1). On experimental day 9 (ED9) rats received the first 2.5 mg/kg MPD injection. The intact control, sham operated and electrolytic lesion groups all showed a statistically significant increase in locomotive activity

following the acute MPD administration (ED9) while the pretreated bilateral 6-OHDA CN injected group failed to exhibit an acute effect of the drug as assessed by the open field assay, i.e. no increase in locomotion. Following six days of repeated MPD administration, three days of washout (no injection) and a rechallenge MPD administration at ED18 the intact control, sham operated and electrolytic lesion group all showed a statistically significant increase in locomotive activity following the rechallenge of MPD (behavioral sensitization was expressed when comparing ED18 to ED9). The bilateral CN 6-OHDA injection group failed to exhibit any response to MPD rechallenge i.e., 6-OHDA injection to the CN prevents the acute and chronic effects of MPD on locomotor activity as assessed by the open field assay. These observations are consistent with previous similar experiments which show that electrolytic lesion of the CN, PFC or the NAc did not alter the acute effects of MPD or amphetamine (Lee et al., 2008; McKenzie, 1972; Podet et al., 2010; Tang et al., 2009). McKenzie, 1972 also found that an electrolytic lesion of the CN did not produce attenuation of stereotypic movement. Limited research exists to understand the role of MPD induced activity following 6-OHDA injection to the CN; however, previous reports have studied the role of 6-OHDA injection to the CN followed by amphetamine administration. Kelly and Iversen 1975 reported that 6-OHDA activity in CN lesioned results initially in decreased activity, however after 14 days their locomotor activity increased to a level similar to the sham group except for the stereotypic activity which remained attenuated. A similar decrease in stereotypic movement was reported by Creese and Iversen 1974. The initial decrease in locomotion for the first three days is consistent with the results found in the current study in response to MPD, and also consistent with the observation that the 6-OHDA lesion group exhibited attenuation of stereotypic movement. The prevention of the acute and chronic effects of MPD in the animal group treated with CN bilateral 6-OHDA injection suggests that the CN plays a role in the induction of behavioral sensitization following repetitive MPD exposure. These results give two distinct observations; the CN electrolytic lesion did not affect the response to MPD exposure while selective chemical injection of the CN DA system altered the acute and chronic effect of MPD on locomotion. This opposing response has been observed in a previous similar experiment in which the substantia nigra (SN) was damaged using both electrolytic and 6-OHDA injection resulting in different responses between the groups (Costall et al., 1976). It is well understood that DA plays a significant role in regulation of animal movement via the CN; a way to interpret these differing results in response to electrolytic and 6-OHDA injection is that the dopaminergic system was completely depleted by the chemical diffusion of 6-OHDA whereas the electrolytic lesion was not sufficient enough to destroy all of the DA receptors in order to completely abolish the locomotor response to MPD (Di Chiara 1995; Podet et al., 2010; Robinson & Berridge 1993; Sullivan & Brake 2003). MPD binds with high affinity to DA transporters to prevent the reuptake of DA into the presynaptic neuron resulting in increased extracellular DA levels in the synaptic cleft. (John & Jones, 2007; Volkow et al., 1999) This increase in DA activates the basal ganglia pathway; resulting in locomotor activation. The main CN efferent projections are to the thalamus via the ansa lenticularis and lenticular fasciculus (Carpenter, 1976) and from the thalamus to the cortex. These projections consist of a direct and indirect pathway to the thalamus (Carpenter, 1976). These two pathways exert opposite regulation similar to a push-pull mechanism (Zhang et al., 2004). The direct pathway initiates the execution of voluntary movement while the indirect pathway inhibits competing motor commands from being executed at the same time (Zhang et al., 2004). These direct and indirect pathways are modulated by the activation of CN medium spiny neurons (MSN). The direct pathway innervates the mesocorticolimbic system by way of the nigrostriatal pathway MSN which project to the substantia nigra pars reticulata (SNr) and from the SNr projects to the thalamus via the internal globus pallidus (GPi) (Kreitzer & Malenka, 2008). This pathway contains a high expression of D1-like DA receptors which mediate extracellular signal-kinase (ERK), therefore during acute MPD administration ERK is increasing. Increase in ERK upregulates the activation of the gene expression factor Δ Fos

B which has been reported to induce long-term neuroadaptions in the brain (Shi & McGinty, 2010; Zhang et al., 2004). It was reported that chronic administration of psychostimulants results in intracellular increase of Δ Fos B that lead to persistent and long sustaining excitatory neuronal effects. Elevated levels of Δ Fos B are correlated with increased sensitivity to behavioral effects and increased motivation for the drug, i.e. behavioral sensitization (Nestler, 2001, 2004). Ferguson et al., (2010) reported that blockade of the D1-like DA receptors will inhibit the activation of ERK, preventing c-Fos transduction and subsequently inhibiting locomotion. When D1 receptors are inhibited either by blockade or ablation there is an increase in the phosphorylation of cAMP response element binding protein (CREB) which mediates the opposite spectrum of the push-pull mechanism by acting on the indirect pathway to inhibit locomotion. CREB activation in the NAc by psychostimulants has been previously shown to create homeostatic negative feedback adaption, inhibiting sensitivity to future drug administration i.e. upregulation of CREB induces behavioral tolerance (Chao & Nestler, 2004; Nestler, 2004). Kalivas & Stewart (1991) reported that the direct CN pathway is critical for the acute response to psychostimulants, moreover that when the D1-like DA receptors are destroyed in the CN the afferent projections can no longer upregulate DA, resulting in decreased DA concentration thus preventing the acute response of MPD. The indirect pathway MSN contain a high expression of D2-like DA receptors (Kreitzer & Malenka, 2008). The D2-like DA receptor exerts inhibitory effects (Ferguson et al., 2010; Kreitzer & Malenka, 2008; Zhang et al., 2004). Zhang et al., (2004) used D3-like DA receptor (subclass of D2 receptors) on mutant mice to exhibit an increase in ERK activation, thus increasing sensitivity to the drug and moreover the lack of D3 receptors caused further increase in activation of ERK at the D1 receptors. An upset of the balance between the direct and indirect pathways results in the motor dysfunctions that characterize the extrapyramidal motor system. Based on these results it can be postulated that 6-OHDA injection destroys the direct pathway thus causing inhibition of locomotion; however, an electrolytic lesion effects both direct and indirect thus destroying the push pull mechanism causing DA to bypass the CN. Therefore the CN has only modulatory effect on the acute and chronic MPD response.

In conclusion, the role of the CN using non-selective electrolytic lesion and selective 6-OHDA neurotoxin injection created differing results in response to acute and chronic MPD administration, similar to previous study using electrolytic and 6-OHDA lesioning to destroy the SN (Costall et al., 1976). In current study neither lesion nor injection had an effect on baseline locomotor activity; however, after MPD administration the electrolytic group expressed both an acute and chronic response to MPD whereas the 6-OHDA injection group failed to exhibit an acute or chronic response to MPD. These results suggest that DA passageways are critical to the induction and expression of behavioral sensitization; however, the CN DA pathway is only involved in the modulation of locomotion following MPD exposure. Further studies would be necessary to investigate the neural circuitry to understand why the electrolytic lesion and 6-OHDA lesion altered locomotor activity differently.

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Highlights

- In this study we investigate the role of the CN in response to acute and chronic MPD administration
- We ablate the CN using electrolytic and 6-OHDA injection
- Our results indicate that electrolytic lesion to the CN did not alter MPD response however 6-OHDA lesion did
- This indicates that the CN plays only a modulatory role in the response to MPD administration

Figure 1. summarizes and compares the Horizontal activity (HA), Total Distance Traveled (TD) baseline activity on experimental day one (ED1) prior to surgery compared to the same group of animals post-surgery baseline locomotive activity on ED8 for intact control (C), sham operated (S), CN electrolytic lesion (E) and CN 6-OHDA injected group (6-OH). The consistent activity levels within each groups show that the surgical procedures did not have an effect on baseline activity.

Figure 2.

summarizes and compares the horizontal activity in all four groups after first day of MPD 2.5 mg/kg injection on ED9 with post surgery baseline activity of ED8. The temporal graph shows the sequential locomotive activity of locomotive activity for one hour. The intact control, sham operated and electrolytic lesion group all showed significant ($p < 0.05$) increase in locomotive activity on ED9 (first day MPD) when compared to ED8 (baseline, saline injection). The 6-OHDA injection group failed to exhibit a significant increase in locomotive activity compared to the other groups.

A. Control **B. Sham**

C. Electrolytic Lesion

D. 6-OHDA Lesion

Figure 3.

compares and summarizes all the locomotive activity indices: HA, TD, and NOS after the re-challenge injection of MPD on ED18 (after six consecutive days of MPD injection and three days in washout i.e. no injection) to the initial MPD injection on ED9. The intact control, sham operated and electrolytic groups all showed a significant ($p < 0.05$) increase in locomotive activity after MPD administration for HA and TD, indicating that behavioral sensitization is expressed (Fig 3. A,B,C,D). The 6-OHDA injection group failed to exhibit change in locomotive activity following rechallenge of 2.5 mg/kg MPD on ED18 of HA or TD (Fig 3D), i.e. CN 6-OHDA injection alters the chronic effect of MPD. None of the groups exhibited significant NOS movement.

A.

B.

Figure 4. represents histological reconstruction of CN lesions. Group A represents electrolytic lesions by size and group B indicates the tip of cannula tract in CN during 6-OHDA injection.

Table 1

Drug administration schedule.

Group	Experimental Day						
	Day 1 *	Day 2	Day 3-7	Day 8 *	Days 9-14	Days 15-17	Day 18 *
Control	Saline			Saline	2.5 mg/kg MPD i.p. injection	Washout	2.5 mg/kg MPD i.p. injection
Sham	Saline	Surgery	Recovery	Saline	2.5 mg/kg MPD i.p. injection	Washout	2.5 mg/kg MPD i.p. injection
Electrical lesion	Saline	Surgery	Recovery	Saline	2.5 mg/kg MPD i.p. injection	Washout	2.5 mg/kg MPD i.p. injection
6-OHDA lesion	Saline	Surgery	Recovery	Saline	2.5 mg/kg MPD i.p. injection	Washout	2.5 mg/kg MPD i.p. injection

This table shows the experimental protocol for the four rat groups. Displayed are the days drugs were administered and the washout period in which no injections were made. All injections were i.p. = intra peritoneal, given at 7:30 am with all volumes consistent (0.8ml).

* Indicates recording day.