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Nucleus accumbens neuronal activity in freely behaving rats is modulated following acute and chronic methylphenidate administration

Samuel L Chong, Catherine M Claussen, and Nachum Dafny*

Department of Neurobiology and Anatomy, The University of Texas-Medical School at Houston, Houston, TX, 77030

Abstract

Methylphenidate (MPD) is a psychostimulant that enhances dopaminergic neurotransmission in the central nervous system by using mechanisms similar to cocaine and amphetamine. The mode of action of brain circuitry responsible for an animal's neuronal response to MPD is not fully understood. The nucleus accumbens (NAc) has been implicated in regulating the rewarding effects of psychostimulants. The present study used permanently implanted microelectrodes to investigate the acute and chronic effects of MPD on the firing rates of NAc neuronal units in freely behaving rats. On experimental day 1 (ED1), following a saline injection (control), a 30 minute baseline neuronal recording was obtained immediately followed by a 2.5 mg/kg i.p. MPD injection and subsequent 60 min neuronal recording. Daily 2.5 mg/kg MPD injections were given on ED2 through ED6 followed by 3 washout days (ED7 to 9). On ED10, neuronal recordings were resumed from the same animal after a saline and MPD (rechallenge) injection exactly as obtained on ED1. Sixty-seven NAc neuronal units exhibited similar wave shape, form and amplitude on ED1 and ED10 and their firing rates were used for analysis. MPD administration on ED1 elicited firing rate increases and decreases in 54% of NAc units when compared to their baselines. Six consecutive MPD administrations altered the neuronal baseline firing rates of 85% of NAc units. MPD rechallenge on ED10 elicited significant changes in 63% of NAc units. These alterations in firing rates are hypothesized to be through mechanisms that include D1 and D2-like DA receptor induced cellular adaptation and homeostatic adaptations/deregulation caused by acute and chronic MPD administration.

Keywords

Methylphenidate; Nucleus accumbens; Electrophysiology; Behavior sensitization/tolerance

1. Introduction

Methylphenidate (MPD) is a psychostimulant readily prescribed for attention-deficit/hyperactivity disorder (Accardo and Blondis, 2001; Greenhill et al., 2006). It is estimated

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*Corresponding author at: Department of Neurobiology and Anatomy, The University of Texas, Medical School at Houston, P.O. Box 20708, Houston, TX 77225 United States. Tel.: þ1 713 500 5616; fax: þ1 713 500 0621. nachum.dafny@uth.tmc.edu (N. Dafny).

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that MPD (Ritalin) is used in the United States for the treatment of 5-15% of children ages 5 to 18 (Rowland et al., 2001). Moreover, there has been a rapid increase in the illicit use and distribution of prescription drugs, such as MPD, on high school and university campuses. Students are buying MPD in order to get higher grades and provide an edge over their fellow students (Bidwell et al., 2011; Greely et al., 2008; Kollins et al., 2006). Given the widespread use of MPD, it is surprising that little is known about the neural mechanisms underlying the actions of this drug, particularly in those individuals without ADHD who are taking MPD for recreation and cognitive enhancement for educational gain.

MPD has a chemical structure closely related to the structures of amphetamine, methamphetamine and cocaine, drugs that have been shown to have a high probability for abuse (Teo et al., 2003; Volkow et al., 2001). High reward predisposition over natural positive reinforcement is a proposed mechanism for drug abuse (Deadwyler et al., 2004). The high reward, positive reinforcing properties of stimulants are linked to their interactions in the mesolimbic dopamine (DA) system, which includes the nucleus accumbens (NAc) and ventral tegmental area (VTA) (Deadwyler et al., 2004; Koob and Bloom, 1988; Swanson, 1982). Specifically, the NAc receives dense dopaminergic projections from the VTA which interacts with pre frontal cortex (PFC) glutamatergic transmission and mediates the rewarding actions of psychostimulants (Kalivas, 2009; Kalivas et al., 2009; Koob and Bloom, 1988; Swanson, 1982). The NAc also receives excitatory glutamatergic inputs from the thalamus, hippocampus, and amygdala (Kita and Kitai, 1990; McDonald, 1991; Phillipson and Griffiths, 1985; Sesack et al., 1989). These glutamatergic inputs form synapses onto the densely populated spines of medium spiny neurons (MSNs) in the NAc. In summation, the NAc is implicated as a key component of the neuronal circuitry underlying psychostimulant action and the constructs of motivation, reward and locomotion (Berridge et al., 2006; Pessiglione et al., 2006).

Neuropharmacological studies show that the effects of MPD are very similar to those of cocaine (Volkow et al., 1995; Volkow et al., 1999). Psychostimulants exert their effect by binding to the dopamine transport (DAT) and thus prevent the reuptake of DA into the presynaptic terminal (Volkow et al., 1995; Volkow et al., 1999). Studies have shown that increased DA levels in the NAc cause behavioral sensitization, an experimental marker that indicates the potential of the drug to elicit dependence (Podet et al., 2010; Yang et al., 2007a, 2010). The increased sensitivity to psychostimulants following chronic exposure, such as behavioral sensitization, is suggested to model the phenomena of drug dependence and craving that develops in humans who abuse these drugs (Robinson and Berridge, 1993). Additionally, the NAc has been implicated specifically in the induction of behavioral sensitization following chronic psychostimulant administration by studies that have directly injected psychostimulants into the NAc (Bell and Kalivas, 1996).

Previous neurophysiological studies that have targeted the mesocorticolimbic system with psychostimulants have been done in-vitro (Harvey and Lacey, 1996) or conducted in-vivo under anesthesia (Prieto-Gomez et al., 2004; Prieto-Gomez et al., 2005). Additionally, other studies have investigated the electrophysiological effects due to MPD on sensory evoked responses (Yang et al., 2006b, c, d, 2007a). This investigation aims to study the effects of acute and chronic MPD on single NAc units by recording from non-anesthetized, freely behaving animals previously implanted with permanent semi-microelectrodes. During such recordings, NAc neuronal activity can be examined before and after MPD injection for an extended period of time without the modulation of the CNS from anesthesia.

2. Methods

2.1 Subjects

Twelve adult male Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) weighing 150-175 grams upon arrival were housed for 5 to 7 days individually in Plexiglas cages inside a sound-attenuated animal facility room for adaptation. The home cage subsequently was used as the test cage during entire experiment. The room was maintained on a 12-h light/dark cycle (lights on 06:00 to 18:00), at an ambient temperature of $21 \pm 2^\circ\text{C}$ and at a humidity of 58-62%. Rats were supplied food pellets and water ad libitum for the entire duration of the study. 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. Based on previous experiments from our laboratory, repeated daily saline injections have been shown not to alter behavioral locomotor activity, sensory evoked neuronal potentials nor electrophysiological neuronal firing rates (Yang et al., 2006b, c, d, 2007a); therefore, the neuronal recording after saline injection was used as a control to study the drug effects. i.e., each animal was used as its own control.

2.2 Surgery

On the day of surgery, rats were weighed and anesthetized with 50 mg/kg pentobarbital. The top of the rat's head was shaved to expose skin and coated with a thin layer of 2% Lidocaine Hydrochloride Jelly (Akorn, Inc.). The animal was then placed in a stereotaxic instrument. An incision was made and muscle and connective tissue were retracted to expose the skull. A hole frontal to the olfactory bulbs two bilateral .6mm diameter holes were drilled over the NAc in accordance to coordinates derived from Paxinos and Watson (1986) rat brain atlas (1.70mm anterior from bregma, 1.2mm lateral and to implant a reference and four recording electrodes respectively. Prior to electrode placement, 4 (2mm in length, .8mm in diameter) anchor screws were put in vacant areas of the skull to anchor down the dental acrylic skull cap.

Two twisted Nickel-Chromium, Diamel coated (fully insulated except at tips), 60 micron diameter wire electrodes were secured each to a 1cm copper connector pin (A-M systems, INC.) and made prior to surgery. The twisted electrodes were inserted into the drilled hole at an initial depth of 6.8mm. Unit activity was monitored during electrode placement of electrodes by using a Grass emitter Hi Z Probe connected to a Grass P511 series pre-amplifier. Electrodes where fixed to the skull only when spike activity exhibited at least a 3:1 signal to noise ratio. If the activity did not match our spike to noise ratio criteria, the electrode was moved down in approximant increments of 10 microns until they displayed a proper signal to noise ratio neuronal activity. Once a sufficient signal was obtained, the electrode was fixed in the skull with Webglue, cyanoacrylate surgical adhesive (Webster Veterinary). The secondary twisted electrode was implanted using identical procedures in the other hemisphere (Dafny, 1980, 1982, 1983; Dafny et al., 1979; Dafny et al., 1983; Dafny and Gilman, 1973; Dafny et al., 1980; Dafny and Terkel, 1990; Yang et al., 2007a, b). The electrode connector pins were inserted into Amphenol plugs which were positioned on the skull and secured to the skull with dental acrylic cement. Rats were allowed to recover from the surgical procedure for 4 to 7 days. During this recovery period, every day for 2 hours, rats were placed in the experimental cage and connected to the wireless (telemetric) head stage transmitter (Triangle BioSystems, Inc; Durham, NC, USA) for daily acclimation.

2.3 Data Acquisition

On the experimental day (ED), the rat was placed with his home cage in a Faraday testing box to reduce noise during signal transmission. The wireless Triangle Bio Systems (Durham, NC, USA) head stage was connected to the electrode pins of the skull cap. The

Triangle BioSystem head stage sent neuronal activity signals through a receiver to a Cambridge Electronic Design (CED) analog –to-digital converter (Micro1401-3; Cambridge, England) which then collected and stored the recorded data on a PC. Spike 2.7 software (CED) was used off line to sort for identical spike amplitude and waveforms by examining single unit spike activity exhibiting similar wave form patterns before and after MPD administration for ED1 and ED10 (see table 1) to produce a sequential frequency histogram used to calculate the firing rate in spikes per second (See section 2.4.1 on spike sorting for more details).

2.4 Data analysis

2.4.1 Spike sorting—The Spike 2 version 7 software (Cambridge Electronics Design-CED) was used for spike sorting. The data was captured by the program and processed using low and high pass filters (0.3-3 kHz). There are two window levels, one for positive-going spikes and one for negative-going spikes. Spikes with peak amplitude that were triggered by the window were used to create the templates. 1000 waveform data points were used to define a spike. The spikes were extracted when the input signal enters the amplitude window (previously determined). Spikes with peak amplitude outside these limits were rejected. The algorithm that we used to capture a spike allows the extraction of templates that provide high-dimensional reference points that can be used to perform accurate spike sorting, in both experimental days (ED1 and ED10) despite the influence of noise, spurious threshold crossing and waveform overlap. All temporally displaced templates are compared with the spike event to find the best fitting template that yields the minimum residue variance. Secondly, a template matching procedure is then performed; when the distance between the template and waveform exceeds some threshold (80%) the waveforms are rejected. That means that the spike sorting accuracy in the reconstructed data is about 95%. All these parameters of spike sorting for each electrode were sorted and used for the activity recorded in experimental day 1 (ED1). On ED10 the templates from ED1 were then loaded onto ED10 data to be reclassified. This ensured that the spike amplitude and pattern from ED1 is the same spike on ED10 (See figure 1).

2.4.2 Statistical analysis—The sorted neuronal activity obtained from the fixed template matching system was converted into their firing rates (spikes per second) for the baseline control recording and for the activity following MPD administration. This was accomplished by calculating the average firing rate of the neuronal unit based on a 15 second interval bins. These firing rates were exported into a spread sheet format displaying the rat's number, experimental day, MPD dose and channel (to distinguish hemisphere). Firing rates were evaluated for normality assumptions to determine parametric or non-parametric methods to evaluate differences before and after MPD treatments. The firing rates were determined to not hold normality assumptions, so we assessed differences in firing rates using percent change from baseline control. By using the mean firing rate of the first 30 min of recording and comparing it to the mean firing rate of the subsequent recording post MPD exposure; the changes in firing rates of neuronal units can be evaluated for change. Changes in each unit activity induced by the treatment was considered statistically significant if the mean firing rate after drug treatment differs by at least 2 standard error (S.E.) and exhibits at least a 20% change from the mean observed in the control period (Carelli and Ijames, 2001; Dafny and Terkel, 1990; Ghitza et al., 2004; Reyes-Vazquez et al., 1994; Yang et al., 2000, 2001, 2006b). Those units displaying between $\pm 20\%$ change were classified as “no change” (Ghitza et al., 2004; Stowe et al., 2005).

2.5 Experimental protocol

Experimentation began 4 to 7 days post-surgery and lasted for 10 days. All the recordings and the injections were done in the animal home cage. On experimental day 1 (ED1) prior to

the start of the recording session, animals were again allowed to acclimate to the recording system as before for 20-30 minutes. During this time, the recording systems were organized in order to properly save files and calibrate neuronal activity thresholds. Immediately post i.p. saline (0.8 cc of 0.9%) injection, a 30 minute baseline of neuronal activity was recorded. This was followed by a 2.5 mg/kg i.p. MPD injection and recordings were resumed immediately after injection for another 60 minutes. From ED2 to ED6 rats were injected once daily with 2.5 mg/kg MPD i.p. at the same time as they would have been during recording session days. The injections were done in their home cages (test cages) and on ED7 to ED9, washout days, no injections were given. On ED10, identical experimental protocol as ED1 was followed, with neuronal baseline and neuronal post MPD injection was collected (See table 1). This experimental protocol is the result of previous MPD dose response studies done in our laboratory (Dafny and Yang, 2006; Gaytan et al., 1996, 1997b; Gaytan et al., 2000; Yang et al.; Yang et al., 2006a,c).

2.6 Drug

Methylphenidate hydrochloride (MPD) was obtained from Mallinckrodt Inc. (St. Louis, MO, USA). Based on previous time and dose response experiments (.1 to 40 mg/kg i.p. MPD), the dose of 2.5 mg/kg MPD administered intraperitoneal (i.p.) in the morning elicited behavioral sensitization (Dafny and Yang, 2006; Gaytan et al., 1997b; Gaytan et al., 1998a, b; Gaytan et al., 2000; Yang et al.; Yang et al., 2003; Yang et al., 2006a, 2007a). Moreover, the 2.5 mg/kg MPD dosage yields clinically relevant peak plasma levels and falls within therapeutic range used in treating ADHD (Berridge et al., 2006; Devilbiss and Berridge, 2006; Gatley et al., 1999). Therefore for our study, this dose was chosen to investigate the acute and chronic effect of MPD on NAc unit activity recorded from freely behaving animals previously implanted with permanent electrodes. The MPD was dissolved in 0.9% saline (NaCl) solution and the dose was calculated as free base. All injections were given i.p. in the morning and equalized to 0.8cc's with saline so that all injections volumes were the same for all animals.

2.7 Histological verification of electrode placement

After completion of the last recording session, rats were deeply anesthetized with sodium pentobarbital. The rat's brain was transcardially perfused with 10% formalin solution containing 5% potassium ferrocyanide. A 2 mA DC current was passed through the electrode connector pin for 40 seconds to produce a small lesion. The brain was then excised and stored in 10% formalin for subsequent histological processing. Placements of the electrodes were verified in 60 micron thick coronal sections that were stained with cresyl violet. Coordinate position of the electrode tips were established by matching equivalent locations of the lesion and the prussian blue spot by using Paxinos and Watson Rat Brain Atlas (1986) (See figure 2).

3. Results

Eighty seven (87) NAc neuronal units from 17 animals exhibiting identical spike waveform and amplitude on experimental day 1 (ED1) and on ED10 were recorded. These recordings were obtained from 58 recording electrodes confirmed histologically to be from NAc (Fig. 2).

3.1 Effect of saline (control recording at ED1 and ED10)

Twenty NAc units were recorded following saline treatment only on ED1 and ED10. None of these units responded to saline by changing their firing rate at ED1, nor was their baseline at ED10 changed compared to ED1 baseline. Furthermore, saline injection at ED10 failed to

alter their activity compared to ED10 baseline activity. Figure 3 is a representative frequency firing rate of this group of NAc units.

3.2 Acute effect of MPD – Recording at ED1

Table 2:A summarizes the effects of acute MPD administration on NAc neuronal firing rate activity as compared to their baseline control from ED1. MPD (2.5 mg/kg i.p.) administration to MPD naïve animals elicits significant firing rate changes (increase or decrease) to 54% (36/67) of NAc units (Table 2:A). Of the responding units, 72% (26/36) exhibited increased firing rate activity while 28% (10/36) of the units exhibited decreased neuronal firing rates following MPD administration when compared to their ED1 baseline activity (Table 2:A).

3.2 Chronic effect of MPD – Recording at ED10

Sixty-seven (67) NAc units exhibiting identical spike pattern and amplitude on ED1 and ED10 were used to analyze the chronic effect of MPD on NAc neurons (see methods). These recordings were obtained at ED1 and also after six consecutive daily injections of 2.5mg/kg MPD (ED1 to ED6) and three washout days (ED7 to 9) on ED10 (Table 1). On ED10 neuronal recordings were resumed after a saline (control) and MPD injection similar to the recordings obtained on ED1. The first analysis compares the saline baseline activity on ED10 to the saline baseline activity on ED1.

3.3 Comparing baseline activity of units on ED10 to ED1

Table 2B summarizes the comparison of ED10 NAc neuronal baseline activity to that activity recorded from ED1. Six days of MPD administration and three washout days resulted in a significant increases or decreases in the baseline firing rates of 85% (57/67) of NAc units when ED10 baseline was compared to ED1. From the 57 NAc units that exhibited significant changes in ED10 vs ED1 baseline firing rate activity, 56% (32/57) increased their baseline firing rate, while 44% decreased (Table 2:B).

Of the 32 units with an increased neuronal baseline firing rate at ED10 (Table 2B), 53% (17/32) came from units that had previously shown on ED1 an increase in firing rate after acute 2.5mg/kg i.p MPD administration (Table 3:Aa). The remaining 15 units that exhibited an ED10 increase in their baseline firing rates came from units that were non-responsive to MPD on ED1 (Table 3:Cm).

From the 25 NAc units which exhibited a decreased baseline firing rate activity on ED10 when compared to ED1 (Table 2:B), 32% (8/25) came from units that exhibited increased firing rate after MPD injection on ED1 (Table 3:Ac). 68% of these units (17/25) were from the group of units that exhibited significant decreases or no change after administration of acute 2.5 mg/kg i.p. MPD at ED1 (Table 3: Bi& Co).

3.4 Effect of 2.5mg/kg MPD administration on NAc neurons at ED10

The 67 units that exhibited identical spike amplitude and patterns on ED10 and ED1 were rechallenged on ED10 after a baseline recording with 2.5 mg/kg MPD to study of the chronic effect of MPD on NAc neurons similar to that obtained on ED1 (see Table 1). Table 2:C summarizes the NAc neuronal responses to MPD rechallenge at ED10 as compared to their ED10 baseline. After the MPD administration on ED10, 63% (42/67) of NAc units exhibited significant change (Table 2C: 26 and 16 units increase or decrease respectively) when compared to their baseline recording on ED10. 62% (26/42) of the responding units exhibited increased neuronal activity, while 38% of units showed a decreased in their firing rate. The subsequent firing rate increases and decreases after MPD rechallenge suggest the neurophysiological sensitization or tolerance to the drug is expressed.

3.5 Effect of 2.5 mg/kg MPD on ED10 in units that exhibited at ED1 increased activity following MPD administration

39% of the 67 total NAc units had an increased firing rate in response to acute MPD administration (i.e. at ED1) (Table 3:A). 65% (17/26) of these units exhibited an increase in their baseline firing rate activity on ED10 (Table 3:Aa). These 17 units, were rechallenged on ED10 with MPD, 71% (12/17) exhibited decreases in their neuronal firing rate activity (Table 2: A1b) (See Figure 3). The remaining 29% (5/17) failed to respond to MPD at ED10 (Table3:Ab). These observations suggest that these units exhibited tolerance to MPD rechallenge, either by not responding to the drug or by changing their response direction from increase neuronal activity on ED1 to decrease neuronal activity at ED10 to MPD administration.

Eight of the remaining 9 units from the above 26 NAc units (Table 3:Ac), exhibited decreased baseline activity on ED10 compared to ED1 baseline. When these 8 units were rechallenged with 2.5mg/kg MPD on ED10, 88% (7/8) expressed an increase in their firing rates (Table 3:Ad). This type of response to MPD on ED10 suggests that these NAc units exhibited neurophysiological homeostatic adaptation due to the drug.

3.6 Effect of 2.5 mg/kg MPD on ED10 in units that exhibited decreased activity on ED1 following MPD administration

Ten NAc units exhibited a significant decrease in neuronal activity on ED 1 after MPD injection (Table3:B); 60% (6/10) of these NAc units exhibited a decrease in their baseline activity at ED10 due to six daily MPD administrations, i.e. chronic effects of MPD. Following rechallenge with MPD at ED10, three out of the six units (50%) exhibited increases, while the other 50% exhibited non-significant changes in their neuronal firing rates (Table 3:B i&j) (See Fig.5). These results suggest the NAc units underwent neurophysiological homeostatic adaptations and/or tolerance respectively.

The remaining 4 (of the 10) units that exhibited decreased firing rate post acute MPD exposure on ED1, exhibited similar baseline firing rate at ED10 compared to ED1. 75% (3/4) of these units continued to express no changes in activity after 2.5 MPD rechallenge, while 1/4 (25%) increased after MPD rechallenge (Table3:Bk&l).

3.7 Effect of 2.5 mg/kg MPD Rechallenge on ED10 in units that exhibited non-significant changes in activity on ED1 following MPD administration

Thirty-one units (31/67) did not significantly alter their firing rate from baseline post the initial MPD injection on ED1 (Table2:A). However, 84% (26/31) of these NAc units exhibited significant alterations of their ED10 neuronal baseline activity when compared to ED1 baseline activity. This change is suggested to be a result of six daily exposures to MPD. 58% of these units (15/26) that modified their baseline activity on ED10 exhibited increased baseline neuronal activity at ED10, while 42% (11/26) expressed decreases in their neuronal activity on ED10 baselines (Table 3: m&o).

Of the 15 units that exhibited an increase in baseline activity on ED10; 40% (6/15) show further increase (i.e. these NAc units exhibited what can be interpreted as electrophysiological sensitization), 27% (4/15) exhibit decrease (i.e. these NAc units exhibited what can be interpreted as electrophysiological tolerance and/or homeostatic adaptation) and 33% (5/15) failed to express an effect (i.e., tolerance) in neuronal firings rates after 2.5mg/kg MPD rechallenge on ED10 (Table3:C m,o&q).

11 units exhibited a decreased neuronal firing rate in their baseline neuronal activity on ED10; 73% (8/11) of these units responded to MPD rechallenge by increasing their neuronal

activity. These observations suggest that these NAc units underwent neurophysiological homeostatic adaptation. The remaining 3 units (27%) failed to express any effects to MPD rechallenge at ED10 (Table 3: Ao&p). Of the 5 units that exhibited similar baseline activity on ED1 and ED10; 4 of them following rechallenge with 2.5 mg/kg MPD, failed to respond to the drug (Table 3: Cq&r) while one had an increase in firing rate.

4. Discussion

Methylphenidate (MPD) is a mild psychostimulant of the central nervous system (CNS) with structural and neuropharmacological profile similarities to cocaine, amphetamine and methamphetamine (Kuczenski et al., 1995; Teo et al., 2003; Volkow et al., 1995). MPD is the drug of choice to treat ADHD disorder (Accardo and Blondis, 2001; Levin and Kleber, 1995; Solanto, 1998) and has been prescribed to millions of children and adults (Arnsten and Vijayraghavan, 2006). However, with the increase in competitiveness of high school and college, more students are using MPD for cognitive enhancement and recreationally (Bidwell et al., 2011; Greely et al., 2008). Psychostimulant can cause adverse behavioral effects after repetitive (chronic) exposure such as paranoia, schizophrenia, tolerance and behavioral sensitization (Gessa et al., 1995; Liberman, 1997; Pierce and Kalivas, 1997). In rodents behavioral sensitization and/or tolerance has been used as an experimental model to study the risk of other behavioral disorders and to predict a drug's ability to elicit dependency (Robinson and Berridge, 1993) (Robinson and Becker, 1986). Behavioral sensitization, characterized by a progressive increase in locomotion activity, in animals chronically treated with psychostimulants, and has been suggested to be a model of addiction studies (Gaytan et al., 1997b; Kalivas, 1995; Robinson and Becker, 1986; Robinson and Berridge, 1993; Wolf, 1998; Yang et al., 2000; Yang et al., 2006a, c, 2007b, 2010). The addictive properties and consequences of acute and chronic MPD use have been debated; i.e., reports suggest that MPD exposure can protect youth from later drug dependence (Wilens et al., 2003) while others report that MPD use creates susceptibility to later drug use (Piazza et al., 1989; Robinson and Berridge, 1993). Regardless with the high volume of MPD use, controversy still remains over the effects of MPD on the CNS.

Neuronal circuits of the brain are adaptive and alter following changes in the environment (Burrone and Murthy, 2003). Specifically, the mesolimbic dopamine (DA) system has been implicated as the fundamental neuronal circuit that adapts to repetitive psychostimulant exposure to cause behavioral sensitization or tolerance. Behavioral sensitization and tolerance, due to chronic use of psychostimulants, is considered to be the underlying mechanism of the "psychomotor stimulant theory of addiction" (Berridge et al., 1993; Everitt and Wolf, 2002; White et al., 1993; Wise and Bozarth, 1981). Therefore, behavioral sensitization and tolerance in animals chronically treated with psychostimulants has been suggested to be an experimental model to study the liability of a drug (Nestler, 2004; Robinson and Berridge, 1993; Wolf, 1998). The NAc is composed of two parts, the NAc core and NAc shell. This study recorded the neuronal events from NAc core (see fig. 1). Most of the NAc core is comprised of medium spiny neurons (MSN). We assume that most of our recordings were from the MSN. The NAc is involved in the locomotor and reinforcing effect of psychostimulants (Deadwyler et al., 2004; Podet et al., 2010). Previous behavior studies have shown that the NAc is essential in the mesolimbic dopamine system for reward and behavioral sensitization (Deadwyler et al., 2004). This assumption was supported by Podet et al. (2010) in using experimental models that went through the ablation of the NAc. In addition an electrophysiological investigation of the effects of cocaine and amphetamine on the NAc, reported that psychostimulants alter the synaptic transmission of NAc neurons (Nicola et al., 1996). The objective of the present study is to investigate the acute and chronic effects of 2.5mg/kg intraperitoneal (i.p.) MPD, a dose reported to elicit behavior sensitization (Gaytan et al., 1997a; Gaytan et al., 1997b; Gaytan et al., 2000; Yang

et al., 2000; Yang et al., 2001; Yang et al.; Yang et al., 2006a, c, 2007a, b; 2011), on NAc neuronal activity in MPD-naïve rats (experimental day 1 [ED1]) and in the same animal after chronic administration of a MPD on ED10. This was accomplished by recording the neuronal activity from the NAc in non-anesthetized, freely behaving rats previously implanted with permanent semi-microelectrodes (Claussen and Dafny, 2011; Dafny, 1983; Dafny and Terkel, 1990; Yang et al., 2006b, d, 2007a).

This study makes an effort to record the same unit at ED1 and on ED10 using state of the art software to sort the same spike amplitude and pattern in the two recording days. However, it is impossible to guarantee that the unit sorted at ED1 is the same unit at ED10. In this study we made the assumption that we evaluated the same unit at ED1 and ED10. This study shows the susceptibility of the NAc to neuronal plasticity through the acute and chronic use of MPD. Extracellular neuronal recordings of NAc units show that the majority of NAc units responded by significantly altering their firing rate activity after acute administration of 2.5 mg/kg MPD; 72% by increasing and 28% via decreasing their firing rates. On ED10, after six daily 2.5 mg/kg i.p. MPD injections and three days of washout, 85% of NAc units had altered their neuronal baseline activity and were exhibiting a baseline that was significantly higher or lower than that of ED1. 63% of the NAc units responded to MPD rechallenge at ED10. Of these units, the majority (62%) responded by increasing while 38% decreased their firing rates at ED10 when compared to their ED10 baseline. Rechallenge with MPD at ED10 induced, which was interpreted as, electrophysiological sensitization seen in those cells that did not respond to acute MPD at ED1 but then saw an increase in baseline at ED10 and further increased firing rate after MPD rechallenge. Some of the NAc units responded with the opposite effect, which was interpreted as electrophysiological tolerance. In this group of units, two types of tolerance were observed; some NAc units responded to MPD at ED1 with decreased firing rates while on ED10 they failed to respond to MPD and the second type was from NAc units that did not respond to acute MPD at ED1 however, saw changes in baseline but did not further act in a manner consistent with the change in baseline activity.

How to interpret the observation that acute MPD exposure elicits both excitatory and attenuation of nucleus accumbens neuronal activity

The effects of MPD on NAc neurons can be modulated directly i.e. on the NAc neurons or indirectly affecting on remote areas that ascend into the NAc. MPD is known to bind mainly to dopamine transporter (DAT) thereby increasing extracellular DA levels (Gerasimov et al., 2000b; Kuczenski and Segal, 1997; Kuczenski et al., 1997). DA receptors can be subdivided into two distinct classes, D1 and D2-like receptors that result in excitatory and/or inhibitory effect respectively (Girault and Greengard, 2004). Both D1-like and D2-like DA receptors are found in high concentrations in the NAc and thus either subtype could mediate the effect of MPD on synaptic actions (Girault and Greengard, 2004). One possible interpretation of the excitatory effects of acute MPD is that these units display D1-like DA receptors. The increased extracellular DA levels that result from the blockage of the DAT by MPD activated the D1-like DA receptors of these cells and thereby enhanced neuronal activity. While the recording from neurons that exhibit decreased in firing rate are due from the drug activation of D2-like DA receptors and or the presynaptic DA auto receptors that exerts an inhibitory effect on neuronal activity (Ruskin et al., 2001) that lead to attenuation of the firing rates (Einhorn et al., 1988; Shi et al., 1997).

The second possibility in interpreting the effects of MPD on the NAc is that MPD affects remote structures that ascend into the NAc, and our recordings are the result of the activity involving this neuronal circuit. i.e., it is also possible that all or some of the NAc unit responses are due to activation of remote CNS sites. The NAc receives glutamatergic afferent innervations from the pre-frontal cortex, thalamus, hippocampus, and amygdala

(Kelley et al., 1982; Kita and Kitai, 1990; Sesack et al., 1989; Swanson, 1982) and the activity obtained in the NAc unit are the result of this connection. Generally, intraperitoneal (i.p.) administration of MPD lead to peak plasma levels at much faster and higher level than oral administration. The binding of MPD to DAT increases DA concentration in the synaptic cleft following MPD i.p. exposure; while oral MPD administration activates mainly norepinephrine (NE) in addition to DA (Gerasimov et al., 2000a; Kuczenski and Segal, 2002) since we treat our animals with i.p. MPD it is possible to assume that the data we record is the result of affecting mainly the DA system. In addition it is possible that all the excitatory activity of NAc units after MPD exposure is the result of MPD direct effect and the attenuation effects on neuronal activity are from NAc units which get inhibitory input from remote CNS structures that result in cognitive enhancement through activation of α 2 adrenoceptors (Arnsten and Dudley, 2005).

How to interpret the observation that chronic MPD exposure modulates the baseline activity of Nucleus accumbens neuronal activity

The majority (85%) of the baseline activity recorded from NAc units on ED10 were significantly different compared to the baseline recorded at ED1. This phenomenon can be attributed to the chronic exposure of MPD since ED10 baseline recordings were obtained after six consecutive daily MPD exposures and three days of washout. It is likely to suggest these changes in baseline activity were due to increases or decreases in D1 and/or D2-like DA receptors in the membranes NAc units and/or from D1 and D2-like DA receptors that ascend excitatory or inhibitory input to the NAc which are involved in reward reinforcing effects (Wolf et al., 2004). These molecular increases alter the chronic state of a neuron due to the increase or decrease activation of these DA receptors by endogenous dopamine which in turn have cellular and effects on gene expression (Chao and Nestler, 2004; Wolf et al., 2004). Another possibility is that chronic MPD exposure and withdrawal stimulates NAc units to undergo cellular augmentation. Cellular augmentation is a core component of neuronal responses to psychostimulants and has been linked in the modulation of chronic neuronal responses (Chao and Nestler, 2004; Claussen and Dafny, 2011; Nestler, 2004). Recent studies report that acute and chronic psychostimulant administration induces change in the regulations of several cellular proteins, such as Δ FosB and cAMP response element binding protein (CREB). Specifically, the Fos family of proteins have been implicated in the role of drug addiction (Chao and Nestler, 2004; Nestler, 2004). Upon acute administration of a psychostimulant (such as cocaine), upregulation of the transcription factor Δ FosB occurs. Δ FosB has been shown to mediate excitatory effects and has been implicated as a cellular marker for behavior sensitization (Chao and Nestler, 2004; Nestler, 2004). Conversely, increased cellular production of CREB has been implicated in regulating the resulting attenuation effects of chronic psychostimulant use (Chao and Nestler, 2004; Nestler, 2004). Our results suggest that these cellular modulations and upregulation of these transcription factors after chronic MPD administration may regulate permanent changes in neuronal firing rates.

How to interpret the neuronal activity of NAc units after MPD rechallenge on ED10

In previous experiments using identical experimental protocol and recording from the NAc the sensory evoked potential (Yang et al., 2006c, d, 2007a), it was observed the acute (ED1) dose response to MPD administration elicited dose response attenuation of the average sensory evoked response. At ED10 the baseline amplitude of the average sensory evoked potential showed decreases compared to the baseline recording at ED1. When rechallenge of MPD was administered at ED10 further decreased in the NAc average evoked responses were observed (Yang et al., 2006b, d). This further attenuation of evoked potentials was interpreted as electrophysiological sensitization since the attenuation effect at ED10 was significantly more than those observed at ED1. Based on this interpretation we can assume

that the NAc units that exhibited the opposite activity may reflect electrophysiological properties underlying behavioral tolerance; which indicate that chronic administration of MPD induced neuro adaptation in the NAc neurons that altered the way these neurons will respond to MPD rechallenge at ED10.

63% of NAc units responded to the rechallenge of 2.5mg/kg MPD on ED10. The majority (62%) of responding NAc units responded to MPD rechallenge by increasing their neuronal firing rates (excitation). This excitation response can be attributed to several factors such as sensitization of the NAc D1-like DA receptors after exposure to repetitive MPD administration (Kalivas and Stewart, 1991; Pierce et al., 1995) or upregulation of the transcription factor Δ FosB (Chao and Nestler, 2004; Nestler, 2004). Acute administration of psychostimulants causes the rapid induction of many Fos proteins which overtime, have been shown to mediate cellular excitability synonymous with sensitization (Chao and Nestler, 2004; Nestler, 2004). Additionally, cAMP response element binding protein (CREB) has been shown to mediate a form of tolerance and dependence to chronic psychostimulant use (Chao and Nestler, 2004; Nestler, 2004). These cellular neuronal mechanisms have provided the foundation for the modulation of the mesolimbic dopamine system and subsequent probability of resulting in behavioral changes; previous studies using similar experimental protocol show that 2.5 mg/kg i.p. MPD induces behavioral sensitization (Gaytan et al., 1997a; Gaytan et al., 1996, 1997b; Yang et al., 2000; Yang et al., 2001; Yang et al., 2003; Yang et al.; Yang et al., 2006a, b, c, d, 2007a, b, 2010). Similarly, the observations of this study show that this MPD dose (2.5 mg/kg i.p.) elicits a neuronal electrophysiological response that can be interpreted as electrophysiological sensitization, homeostatic adaptations and/ or tolerance due to the chronic administration of MPD. Studies indicate that both the rewarding properties of psychostimulants and subsequent behavioral sensitization induced by their use have been dependent on synaptic transmission (Kalivas and Stewart, 1991; Koob and Bloom, 1988; Wolf, 1998; Wolf et al., 2004; Wolf and Xue, 1998). Therefore, this suggests that the increased neuronal baseline in this study and subsequent chronic effect negatively correlate to the rewarding properties of MPD, or sensitization of glutamatergic neurons in remote areas of the CNS that ascend to the NAc (Lee et al., 2008; Wanchoo et al.; Wanchoo et al., 2009).

Another possible interpretation is the observation reported (Kolb et al., 2004) in which chronic psychostimulant exposure induced neuro adaptation that resulting in molecular cellular activity that results in increase of the soma and dendritic spine neurophil that contain DA receptors and MPD now activates more DA receptors either D1 or D2-like DA receptors. Further activation of the D1-like DA receptor may result in electrophysiological sensitization while further activation of D2-like DA receptors result in attenuated effects of MPD administration that can be explained as tolerance. This possibility can be supported by the report that super sensitivity of D1-like DA receptors in the NAc, as a result of repetitive exposure to psychostimulants, leads to the expression of the sensitized response (Wolf et al., 2004; Wolf et al., 1994). Psychostimulant exposure induced changes in the release characteristics of DA (Kalivas and Duffy, 1990a, b; Robinson et al., 1988; Vezina, 1993) and/or alteration in DA-stimulated signal transduction mechanisms (Miserendino and Nestler, 1995; Steketee, 1994; Steketee and Kalivas, 1991; Steketee et al., 1992) are responsible for the alteration of the electrophysiological activity and animal behavioral such as behavioral sensitization and/or tolerance. Similar interpretations can be in NAc remote areas that ascend to the NAc and following rechallenge with MPD exert either excitatory or inhibitory effect on NAc units.

An observation of our acute and chronic results points to a phenomena known as homeostatic adaptation. The majority of NAc units significantly alter their firing rate activity

in a reversed manner than that was previously being observed. i.e., Of the 26 units that increased firing rate activity after acute MPD administration on ED1, 8 expressed a decreased baseline firing rate activity. However, after MPD rechallenge at ED10, 7 out of the 8 units increased firing rate activity, while none further decreased their firing rate activity (Table 3:A c&d). Moreover, some of the MPD responding NAc units at ED1 failed to respond to MPD at ED10. These phenomena can be interpreted as electrophysiological tolerance. Also homeostatic adaptation and dysregulation of homeostasis have been seen in previous cocaine research within the NAc (Huang et al., 2010). Homeostatic adaptations occur as an adjustment mechanism in order to restore or correct neuronal output due to changes in the environment (Turrigiano and Nelson, 2004). Homeostatic adaptation can occur at the cellular and synaptic level and, in our study, present in multiple fashions; if the baseline at ED10 was influenced by the modification in neuronal activity at ED1 after initial MPD exposure and at ED10 after MPD rechallenge exhibited the opposite effect, i.e., acute MPD elicits neuronal increase in activity at ED1 resulting in increased baseline activity at ED10 but decreased activity at ED10 after MPD rechallenge. Secondly, if the baseline on ED10 was the opposite effect of the modulation of acute MPD at ED1 and the unit exhibited the opposite effect of the baseline at ED10 after MPD rechallenge, i.e., acute neuronal increase at ED1 resulting in baseline decrease at ED10, followed by increased neuronal activity after ED10 MPD rechallenge. Moreover, some NAc units did not respond to MPD at ED1 while at ED10 their baselines were significantly different from their baselines at ED1 and they respond to MPD rechallenge at ED10 – they were dormant at ED1 and repetitive MPD administration resulted inactivation of these units.

5. Conclusion

These findings provide a unique insight to the modulation and adaptations into the neuronal process by which the NAc participates in psychostimulant-induced neuronal behavior. The DA receptor action of the D1 and D2-like family of receptors are suggested to modulate the firing rates of NAc neuronal units after acute and chronic MPD administration. This modification of neuronal activity over chronic administration of MPD induced synaptic and cellular neuronal changes of NAc neurons and to those units ascending into the NAc. Additionally, changes in neuronal protein regulation are postulated to have affected the neuronal firing rates after the rechallenge of MPD on ED10. To our knowledge, this study is the first to investigate the acute and chronic effects of MPD on NAc neurons in non-anesthetized, freely behaving rats previously implanted with permanent electrodes. The resulting electrophysiological excitation and inhibition of NAc cells leaves to question the effect of role of MPD in changes of behavior. Like drugs of abuse such as cocaine, our study has shown MPD to cause NAc neuronal units to undergo homeostatic adaptations and dysregulation. Ultimately, it is critical to integrate electrophysiology of the NAc and behavioral sensitization to understand how psychostimulant exert their effects on the brain to result in reward seeding behavior.

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References

- Accardo P, Blondis TA. What's all the fuss about Ritalin? J Pediatr. 2001; 138:6–9. [PubMed: 11148505]

- Arnsten AF, Dudley AG. Methylphenidate improves prefrontal cortical cognitive function through alpha2 adrenoceptor and dopamine D1 receptor actions: Relevance to therapeutic effects in Attention Deficit Hyperactivity Disorder. *Behav Brain Funct.* 2005; 1:2. [PubMed: 15916700]
- Arnsten AF, Vijayraghavan S. Staying in touch with methylphenidate: ADHD and sensory processing. Focus on “methylphenidate enhances noradrenergic transmission and suppresses mid- and long-latency sensory responses in the primary somatosensory cortex of awake rats”. *J Neurophysiol.* 2006; 96:524–525. [PubMed: 16835361]
- Bell K, Kalivas PW. Context-specific cross-sensitization between systemic cocaine and intra-accumbens AMPA infusion in the rat. *Psychopharmacology (Berl).* 1996; 127:377–383. [PubMed: 8923575]
- Berridge CW, Arnsten AF, Foote SL. Noradrenergic modulation of cognitive function: clinical implications of anatomical, electrophysiological and behavioural studies in animal models. *Psychol Med.* 1993; 23:557–564. [PubMed: 8234565]
- Berridge CW, Devilbiss DM, Andrzejewski ME, Arnsten AF, Kelley AE, Schmeichel B, Hamilton C, Spencer RC. Methylphenidate preferentially increases catecholamine neurotransmission within the prefrontal cortex at low doses that enhance cognitive function. *Biol Psychiatry.* 2006; 60:1111–1120. [PubMed: 16806100]
- Bidwell LC, McClernon FJ, Kollins SH. Cognitive enhancers for the treatment of ADHD. *Pharmacol Biochem Behav.* 2011; 99:262–74. [PubMed: 21596055]
- Burrone J, Murthy VN. Synaptic gain control and homeostasis. *Curr Opin Neurobiol.* 2003; 13:560–567. [PubMed: 14630218]
- Carelli RM, Ijames SG. Selective activation of accumbens neurons by cocaine-associated stimuli during a water/cocaine multiple schedule. *Brain Res.* 2001; 907:156–161. [PubMed: 11430899]
- Chao J, Nestler EJ. Molecular neurobiology of drug addiction. *Annu Rev Med.* 2004; 55:113–132. [PubMed: 14746512]
- Claussen CM, Dafny N. Acute and chronic methylphenidate modulates the neuronal activity of the caudate nucleus recorded from freely behaving rats. *Brain Res Bull.* 2011
- Dafny N. Neurophysiological evidence for tolerance and dependence on opiates: simultaneous multiunit recordings from septum, thalamus, and caudate nucleus. *J Neurosci Res.* 1980; 5:339–349. [PubMed: 7191906]
- Dafny N. The hypothalamus exhibits electro physiologic evidence for morphine tolerance and dependence. *Exp Neurol.* 1982; 77:66–77. [PubMed: 7200913]
- Dafny N. Interferon modifies EEG and EEG-like activity recorded from sensory, motor, and limbic system structures in freely behaving rats. *Neurotoxicology.* 1983; 4:235–240. [PubMed: 6195567]
- Dafny N, Brown M, Burks TF, Rigor BM. Unit activity recorded simultaneously from medial thalamus and caudate nucleus in naive and morphine-dependent rats. *Exp Neurol.* 1979; 64:216–224. [PubMed: 570933]
- Dafny N, Burks TF, Bergmann F. Dose effects of morphine on the spontaneous unit activity recorded from the thalamus, hypothalamus, septum, hippocampus, reticular formation, central gray, and caudate nucleus. *J Neurosci Res.* 1983; 9:115–126. [PubMed: 6842623]
- Dafny N, Gilman S. Characterization of spontaneous unit activity in hypothalamus and reticular formation recorded with semi-microelectrodes. *Brain Res.* 1973; 59:243–255. [PubMed: 4747755]
- Dafny N, Rigor BM, Burks TF. Dependence and tolerance: multiunit recording from central gray, mesencephalic reticular formation, and medial thalamus in freely behaving rats. *Exp Neurol.* 1980; 68:217–227. [PubMed: 7363992]
- Dafny N, Terkel J. Hypothalamic neuronal activity associated with onset of pseudo pregnancy in the rat. *Neuroendocrinology.* 1990; 51:459–467. [PubMed: 2111893]
- Dafny N, Yang PB. The role of age, genotype, sex, and route of acute and chronic administration of methylphenidate: a review of its locomotor effects. *Brain Res Bull.* 2006; 68:393–405. [PubMed: 16459193]
- Deadwyler SA, Hayashizaki S, Cheer J, Hampson RE. Reward, memory and substance abuse: functional neuronal circuits in the nucleus accumbens. *Neurosci Biobehav Rev.* 2004; 27:703–711. [PubMed: 15019420]

- Devilbiss DM, Berridge CW. Low-dose methylphenidate actions on tonic and phasic locus coeruleus discharge. *J Pharmacol Exp Ther*. 2006; 319:1327–1335. [PubMed: 16980569]
- Einhorn LC, Johansen PA, White FJ. Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: studies in the ventral tegmental area. *J Neurosci*. 1988; 8:100–112. [PubMed: 3339402]
- Everitt BJ, Wolf ME. Psychomotor stimulant addiction: a neural systems perspective. *J Neurosci*. 2002; 22:3312–3320. [PubMed: 11978805]
- Gatley SJ, Volkow ND, Gifford AN, Fowler JS, Dewey SL, Ding YS, Logan J. Dopamine-transporter occupancy after intravenous doses of cocaine and methylphenidate in mice and humans. *Psychopharmacology (Berl)*. 1999; 146:93–100. [PubMed: 10485970]
- Gaytan O, al-Rahim S, Swann A, Dafny N. Sensitization to locomotor effects of methylphenidate in the rat. *Life Sci*. 1997a; 61:PL101–107. [PubMed: 9275016]
- Gaytan O, Ghelani D, Martin S, Swann A, Dafny N. Dose response characteristics of methylphenidate on different indices of rats' locomotor activity at the beginning of the dark cycle. *Brain Res*. 1996; 727:13–21. [PubMed: 8842378]
- Gaytan O, Ghelani D, Martin S, Swann A, Dafny N. Methylphenidate: diurnal effects on locomotor and stereotypic behavior in the rat. *Brain Res*. 1997b; 777:1–12. [PubMed: 9449407]
- Gaytan O, Swann A, Dafny N. Diurnal differences in rat's motor response to amphetamine. *Eur J Pharmacol*. 1998a; 345:119–128. [PubMed: 9600626]
- Gaytan O, Swann A, Dafny N. Time-dependent differences in the rat's motor response to amphetamine. *Pharmacol Biochem Behav*. 1998b; 59:459–467. [PubMed: 9476996]
- Gaytan O, Yang P, Swann A, Dafny N. Diurnal differences in sensitization to methylphenidate. *Brain Res*. 2000; 864:24–39. [PubMed: 10793183]
- Gerasimov MR, Franceschi M, Volkow ND, Gifford A, Gatley SJ, Marsteller D, Molina PE, Dewey SL. Comparison between intraperitoneal and oral methylphenidate administration: A microdialysis and locomotor activity study. *J Pharmacol Exp Ther*. 2000a; 295:51–57. [PubMed: 10991960]
- Gerasimov MR, Franceschi M, Volkow ND, Rice O, Schiffer WK, Dewey SL. Synergistic interactions between nicotine and cocaine or methylphenidate depend on the dose of dopamine transporter inhibitor. *Synapse*. 2000b; 38:432–437. [PubMed: 11044890]
- Gessa GL, Pani L, Serra G, Fratta W. Animal models of mania. *Adv Biochem Psychopharmacol*. 1995; 49:43–66. [PubMed: 7653334]
- Ghitza UE, Fabbriatore AT, Prokopenko VF, West MO. Differences between accumbens core and shell neurons exhibiting phasic firing patterns related to drug-seeking behavior during a discriminative-stimulus task. *J Neurophysiol*. 2004; 92:1608–1614. [PubMed: 15152017]
- Girault JA, Greengard P. The neurobiology of dopamine signaling. *Arch Neurol*. 2004; 61:641–644. [PubMed: 15148138]
- Greely H, Sahakian B, Harris J, Kessler RC, Gazzaniga M, Campbell P, Farah MJ. Towards responsible use of cognitive-enhancing drugs by the healthy. *Nature*. 2008; 456:702–705. [PubMed: 19060880]
- Greenhill L, Kollins S, Abikoff H, McCracken J, Riddle M, Swanson J, McGough J, Wigal S, Wigal T, Vitiello B, Skrobala A, Posner K, Ghuman J, Cunningham C, Davies M, Chuang S, Cooper T. Efficacy and safety of immediate-release methylphenidate treatment for preschoolers with ADHD. *J Am Acad Child Adolesc Psychiatry*. 2006; 45:1284–1293. [PubMed: 17023867]
- Harvey J, Lacey MG. Endogenous and exogenous dopamine depress EPSCs in rat nucleus accumbens in vitro via D1 receptors activation. *J Physiol*. 1996; 492(Pt 1):143–154. [PubMed: 8730590]
- Huang YH, Schlüter OM, Dong Y. Cocaine-induced homeostatic regulation and dysregulation of nucleus accumbens neurons. *Behavioural Brain Research*. 2010; 216:9–18. [PubMed: 20708038]
- Kalivas PW. Interactions between dopamine and excitatory amino acids in behavioral sensitization to psychostimulants. *Drug Alcohol Depend*. 1995; 37:95–100. [PubMed: 7758408]
- Kalivas PW. The glutamate homeostasis hypothesis of addiction. *Nat Rev Neurosci*. 2009; 10:561–572. [PubMed: 19571793]
- Kalivas PW, Duffy P. Effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. *Synapse*. 1990a; 5:48–58. [PubMed: 2300906]

- Kalivas PW, Duffy P. Effect of acute and daily neurotensin and enkephalin treatments on extracellular dopamine in the nucleus accumbens. *J Neurosci.* 1990b; 10:2940–2949. [PubMed: 1697899]
- Kalivas PW, Lalumiere RT, Knackstedt L, Shen H. Glutamate transmission in addiction. *Neuropharmacology.* 2009; 56(Suppl 1):169–173. [PubMed: 18675832]
- Kalivas PW, Stewart J. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Res Brain Res Rev.* 1991; 16:223–244. [PubMed: 1665095]
- Kelley AE, Domesick VB, Nauta WJ. The amygdalostratial projection in the rat--an anatomical study by anterograde and retrograde tracing methods. *Neuroscience.* 1982; 7:615–630. [PubMed: 7070669]
- Kita H, Kitai ST. Amygdaloid projections to the frontal cortex and the striatum in the rat. *J Comp Neurol.* 1990; 298:40–49. [PubMed: 1698828]
- Kolb B, Pellis S, Robinson TE. Plasticity and functions of the orbital frontal cortex. *Brain Cogn.* 2004; 55:104–115. [PubMed: 15134846]
- Kollins S, Greenhill L, Swanson J, Wigal S, Abikoff H, McCracken J, Riddle M, McGough J, Vitiello B, Wigal T, Skrobala A, Posner K, Ghuman J, Davies M, Cunningham C, Bauzo A. Rationale, design, and methods of the Preschool ADHD Treatment Study (PATs). *J Am Acad Child Adolesc Psychiatry.* 2006; 45:1275–1283. [PubMed: 17023869]
- Koob GF, Bloom FE. Cellular and molecular mechanisms of drug dependence. *Science.* 1988; 242:715–723. [PubMed: 2903550]
- Kuczenski R, Segal DS. Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: comparison with amphetamine. *J Neurochem.* 1997; 68:2032–2037. [PubMed: 9109529]
- Kuczenski R, Segal DS. Exposure of adolescent rats to oral methylphenidate: preferential effects on extracellular norepinephrine and absence of sensitization and cross-sensitization to methamphetamine. *J Neurosci.* 2002; 22:7264–7271. [PubMed: 12177221]
- Kuczenski R, Segal DS, Cho AK, Melega W. Hippocampus norepinephrine, caudate dopamine and serotonin, and behavioral responses to the stereoisomers of amphetamine and methamphetamine. *J Neurosci.* 1995; 15:1308–1317. [PubMed: 7869099]
- Kuczenski R, Segal DS, Todd PK. Behavioral sensitization and extracellular dopamine responses to amphetamine after various treatments. *Psychopharmacology (Berl).* 1997; 134:221–229. [PubMed: 9438672]
- Lee MJ, Swann AC, Dafny N. Methylphenidate sensitization is prevented by prefrontal cortex lesion. *Brain Res Bull.* 2008; 76:131–140. [PubMed: 18395622]
- Levin FR, Kleber HD. Attention-deficit hyperactivity disorder and substance abuse: relationships and implications for treatment. *Harv Rev Psychiatry.* 1995; 2:246–258. [PubMed: 9384909]
- Lieberman RP. Schizophrenia practice guideline. *Am J Psychiatry.* 1997; 154:1792–1793. [PubMed: 9396968]
- McDonald AJ. Topographical organization of amygdaloid projections to the caudatoputamen, nucleus accumbens, and related striatal-like areas of the rat brain. *Neuroscience.* 1991; 44:15–33. [PubMed: 1722890]
- Miserendino MJ, Nestler EJ. Behavioral sensitization to cocaine: modulation by the cyclic AMP system in the nucleus accumbens. *Brain Res.* 1995; 674:299–306. [PubMed: 7796110]
- Nestler EJ. Molecular mechanisms of drug addiction. *Neuropharmacology.* 2004; 47(Suppl 1):24–32. [PubMed: 15464123]
- Nicola SM, Kombian SB, Malenka RC. Psycho stimulants depress excitatory synaptic transmission in the nucleus accumbens via presynaptic D1-like dopamine receptors. *J Neurosci.* 1996; 16:1591–1604. [PubMed: 8774428]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates.* 2. Academic Press; Orlando: 1986.
- Pessiglione M, Seymour B, Flandin G, Dolan RJ, Frith CD. Dopamine-dependent prediction errors underpin reward-seeking behaviour in humans. *Nature.* 2006; 442:1042–1045. [PubMed: 16929307]
- Phillipson OT, Griffiths AC. The topographic order of inputs to nucleus accumbens in the rat. *Neuroscience.* 1985; 16:275–296. [PubMed: 4080159]

- Piazza PV, Deminiere JM, Le Moal M, Simon H. Factors that predict individual vulnerability to amphetamine self-administration. *Science*. 1989; 245:1511–1513. [PubMed: 2781295]
- Pierce RC, Duffy P, Kalivas PW. Sensitization to cocaine and dopamine auto receptor sub sensitivity in the nucleus accumbens. *Synapse*. 1995; 20:33–36. [PubMed: 7624827]
- Pierce RC, Kalivas PW. A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. *Brain Res Brain Res Rev*. 1997; 25:192–216. [PubMed: 9403138]
- Podet A, Lee MJ, Swann AC, Dafny N. Nucleus accumbens lesions modulate the effects of methylphenidate. *Brain Res Bull*. 2010; 82:293–301. [PubMed: 20470871]
- Prieto-Gomez B, Benitez MT, Vazquez-Alvarez AM, Yang PB, Reyes Vazquez C, Dafny N. Dopaminergic ventral tegmental neurons modulated by methylphenidate. *Life Sci*. 2004; 74:1581–1592. [PubMed: 14738903]
- Prieto-Gomez B, Vazquez-Alvarez AM, Martinez-Pena JL, Reyes-Vazquez C, Yang PB, Dafny N. Methylphenidate and amphetamine modulate differently the NMDA and AMPA glutamatergic transmission of dopaminergic neurons in the ventral tegmental area. *Life Sci*. 2005; 77:635–649. [PubMed: 15921995]
- Reyes-Vazquez C, Prieto-Gomez B, Dafny N. Alpha-interferon suppresses food intake and neuronal activity of the lateral hypothalamus. *Neuropharmacology*. 1994; 33:1545–1552. [PubMed: 7760977]
- Robinson TE, Becker JB. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. *Brain Res*. 1986; 396:157–198. [PubMed: 3527341]
- Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Brain Res Rev*. 1993; 18:247–291. [PubMed: 8401595]
- Robinson TE, Jurson PA, Bennett JA, Bentgen KM. Persistent sensitization of dopamine neurotransmission in ventral striatum (nucleus accumbens) produced by prior experience with (+)-amphetamine: a microdialysis study in freely moving rats. *Brain Res*. 1988; 462:211–222. [PubMed: 2847849]
- Rowland AS, Umbach DM, Catoe KE, Stallone L, Long S, Rabiner D, Naftel AJ, Panke D, Faulk R, Sandler DP. Studying the epidemiology of attention-deficit hyperactivity disorder: screening method and pilot results. *Can J Psychiatry*. 2001; 46:931–940. [PubMed: 11816314]
- Ruskin DN, Bergstrom DA, Shenker A, Freeman LE, Baek D, Walters JR. Drugs used in the treatment of attention-deficit/hyperactivity disorder affect postsynaptic firing rate and oscillation without preferential dopamine auto receptor action. *Biol Psychiatry*. 2001; 49:340–350. [PubMed: 11239905]
- Sesack SR, Deutch AY, Roth RH, Bunney BS. Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. *J Comp Neurol*. 1989; 290:213–242. [PubMed: 2592611]
- Shi WX, Zheng P, Liang XF, Bunney BS. Characterization of dopamine-induced depolarization of prefrontal cortical neurons. *Synapse*. 1997; 26:415–422. [PubMed: 9215600]
- Solanto MV. Neuropsychopharmacological mechanisms of stimulant drug action in attention-deficit hyperactivity disorder: a review and integration. *Behav Brain Res*. 1998; 94:127–152. [PubMed: 9708845]
- Steketee JD. Intra-A10 injection of H7 blocks the development of sensitization to cocaine. *Neuroreport*. 1994; 6:69–72. [PubMed: 7703432]
- Steketee JD, Kalivas PW. Sensitization to psycho stimulants and stress after injection of pertussis toxin into the A10 dopamine region. *J Pharmacol Exp Ther*. 1991; 259:916–924. [PubMed: 1941636]
- Steketee JD, Sorg BA, Kalivas PW. The role of the nucleus accumbens in sensitization to drugs of abuse. *Prog Neuropsychopharmacol Biol Psychiatry*. 1992; 16:237–246. [PubMed: 1579639]
- Stowe ZN, Landry JC, Tang Z, Owens MJ, Kinkead B, Nemeroff CB. The electrophysiological effects of neurotensin on spontaneously active neurons in the nucleus accumbens: an in vivo study. *Synapse*. 2005; 58:165–172. [PubMed: 16108007]

- Swanson LW. The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. *Brain Res Bull.* 1982; 9:321–353. [PubMed: 6816390]
- Teo SK, Stirling DI, Thomas SD, Khetani VD. Neurobehavioral effects of racemic threo-methylphenidate and its D and L enantiomers in rats. *Pharmacol Biochem Behav.* 2003; 74:747–754. [PubMed: 12543241]
- Turrigiano GG, Nelson SB. Homeostatic plasticity in the developing nervous system. *Nat Rev Neurosci.* 2004; 5:97–107. [PubMed: 14735113]
- Vezina P. Amphetamine injected into the ventral tegmental area sensitizes the nucleus accumbens dopaminergic response to systemic amphetamine: an in vivo microdialysis study in the rat. *Brain Res.* 1993; 605:332–337. [PubMed: 8386970]
- Volkow ND, Ding YS, Fowler JS, Wang GJ, Logan J, Gatley JS, Dewey S, Ashby C, Liebermann J, Hitzemann R, et al. Is methylphenidate like cocaine? Studies on their pharmacokinetics and distribution in the human brain. *Arch Gen Psychiatry.* 1995; 52:456–463. [PubMed: 7771915]
- Volkow ND, Wang G, Fowler JS, Logan J, Gerasimov M, Maynard L, Ding Y, Gatley SJ, Gifford A, Franceschi D. Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain. *J Neurosci.* 2001; 21:RC121. [PubMed: 11160455]
- Volkow ND, Wang GJ, Fowler JS, Fischman M, Foltin R, Abumrad NN, Gatley SJ, Logan J, Wong C, Gifford A, Ding YS, Hitzemann R, Pappas N. Methylphenidate and cocaine have a similar in vivo potency to block dopamine transporters in the human brain. *Life Sci.* 1999; 65:PL7–12. [PubMed: 10403500]
- Wanchoo SJ, Lee MJ, Swann AC, Dafny N. Bilateral six-hydroxydopamine administration to PFC prevents the expression of behavioral sensitization to methylphenidate. *Brain Res.* 1312:89–100. [PubMed: 19932692]
- Wanchoo SJ, Swann AC, Dafny N. Descending glutamatergic pathways of PFC are involved in acute and chronic action of methylphenidate. *Brain Res.* 2009; 1301:68–79. [PubMed: 19747456]
- White FJ, Hu XT, Henry DJ. Electrophysiological effects of cocaine in the rat nucleus accumbens: microiontophoretic studies. *J Pharmacol Exp Ther.* 1993; 266:1075–1084. [PubMed: 8355182]
- Wilens TE, Faraone SV, Biederman J, Gunawardene S. Does stimulant therapy of attention-deficit/hyperactivity disorder beget later substance abuse? A meta-analytic review of the literature. *Pediatrics.* 2003; 111:179–185. [PubMed: 12509574]
- Wise RA, Bozarth MA. Brain substrates for reinforcement and drug self-administration. *Prog Neuropsychopharmacol.* 1981; 5:467–474. [PubMed: 7041154]
- Wolf ME. The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. *Prog Neurobiol.* 1998; 54:679–720. [PubMed: 9560846]
- Wolf ME, Sun X, Mangiavacchi S, Chao SZ. Psychomotor stimulants and neuronal plasticity. *Neuropharmacology.* 2004; 47(Suppl 1):61–79. [PubMed: 15464126]
- Wolf ME, Xue CJ. Amphetamine and D1 dopamine receptor agonists produce biphasic effects on glutamate efflux in rat ventral tegmental area: modification by repeated amphetamine administration. *J Neurochem.* 1998; 70:198–209. [PubMed: 9422363]
- Wolf ME, Xue CJ, White FJ, Dahlin SL. MK-801 does not prevent acute stimulatory effects of amphetamine or cocaine on locomotor activity or extracellular dopamine levels in rat nucleus accumbens. *Brain Res.* 1994; 666:223–231. [PubMed: 7882032]
- Yang P, Beasley A, Swann A, Dafny N. Valproate modulates the expression of methylphenidate (ritalin) sensitization. *Brain Res.* 2000; 874:216–220. [PubMed: 10960607]
- Yang P, Singhal N, Modi G, Swann A, Dafny N. Effects of lithium chloride on induction and expression of methylphenidate sensitization. *Eur J Pharmacol.* 2001; 426:65–72. [PubMed: 11525772]
- Yang PB, Amini B, Swann AC, Dafny N. Strain differences in the behavioral responses of male rats to chronically administered methylphenidate. *Brain Res.* 2003; 971:139–152. [PubMed: 12706230]
- Yang PB, Cuellar DO 3rd, Swann AC, Dafny N. Age and genetic strain differences in response to chronic methylphenidate administration. *Behav Brain Res.* 218:206–217. [PubMed: 21111006]
- Yang PB, Swann AC, Dafny N. Acute and chronic methylphenidate dose-response assessment on three adolescent male rat strains. *Brain Res Bull.* 2006a; 71:301–310. [PubMed: 17113960]

- Yang PB, Swann AC, Dafny N. Chronic methylphenidate modulates locomotor activity and sensory evoked responses in the VTA and NAc of freely behaving rats. *Neuropharmacology*. 2006b; 51:546–556. [PubMed: 16824558]
- Yang PB, Swann AC, Dafny N. Dose-response characteristics of methylphenidate on locomotor behavior and on sensory evoked potentials recorded from the VTA, NAc, and PFC in freely behaving rats. *Behav Brain Funct*. 2006c; 2:3. [PubMed: 16417623]
- Yang PB, Swann AC, Dafny N. Sensory-evoked potentials recordings from the ventral tegmental area, nucleus accumbens, prefrontal cortex, and caudate nucleus and locomotor activity are modulated in dose-response characteristics by methylphenidate. *Brain Res*. 2006d; 1073-1074:164–174. [PubMed: 16473326]
- Yang PB, Swann AC, Dafny N. Chronic administration of methylphenidate produces neurophysiological and behavioral sensitization. *Brain Res*. 2007a; 1145:66–80. [PubMed: 17335781]
- Yang PB, Swann AC, Dafny N. Methylphenidate treated at the test cage--dose-dependent sensitization or tolerance depend on the behavioral assay used. *Crit Rev Neurobiol*. 2007b; 19:59–77. [PubMed: 19166391]
- Yang PB, Swann AC, Dafny N. Psychostimulants given in adolescence modulate their effects in adulthood using the open field and the wheel-running assays. *Brain Res Bull*. 2010; 82:208–17. [PubMed: 20362034]

- > Acute and Chronic MPD administration modifies NAc neuronal firing rates.
- > Acute administration of MPD elicits changes in firing rate to 54% of recorded NAc neuronal units.
- > 85% of NAc neuronal units exhibited an increased or decreased neuronal baseline after chronic MPD administration.
- > Homeostatic changes and tolerance in NAc neuronal firing rates occur do to chronic administration of MPD.

Figure 1.

shows screen shots of NAc units taken from Spike 2's wave mark window. The dashed lines indicate the positive and negative values used in FTMS window discrimination. Three spikes patterns can be seen using Spike 2; negative deflection only (a), positive deflection only (b) and both positive and negative (c).

a depicts a negative deflected NAc unit being captured by the negative window of the FTMS.

b depicts a positively deflected NAc unit being captured by the positive window of the FTMS.

c depicts a full spike being captured by the FTMS window discriminators.

d depicts a template classification; the Spike 2's FTMS has classified a neuronal spike to one of the two distinct unit templates. The FTMS has mapped out 32 trigger points once the spike has crossed the window discriminator threshold and has classified it to fall at least 80% within template #02 (the "02" is lit blue by the system to indicate a spike to template match) based on amplitude and slope differentials. Real time numbers of spikes superimposed in the template are designated by the first number in the upper second box (see circled), for these templates in this time scale 12 and 5 spikes have been matched to template 1 and 2 respectively. These templates (along with their parameters) are then saved and loaded into the ED10 FTMS for template matching analysis in the same manner as ED1.

Fig 2.

Reconstructs histologically verified electrode tip placements along with screen shot of a lesion with a Prussian blue spot in the NAc. The rat atlas plates represent the NAc in serial coronal sections. The number on the top right corner of each section represents the anterior/posterior distance (mm) from bregma. Black circles are designated to be taken from the right hemisphere, while open circles were found in the left hemisphere. The grey rectangles represent electrode placement of those that missed the NAc. Eighteen of the twenty-four electrodes were securely placed in the NAc and the recordings from these electrodes were evaluated.

Fig 3.

Represents a frequency histogram of NAc neuron before and after saline injection of ED1 and at ED10. The arrow indicates the time of injection (0.8ml saline). The superimposed neuronal spike representation is a screen shot taken from the wave mark window at 10 minutes after the beginning of the recording and 10 minutes after saline injection on ED1 and on ED10. Baseline activity post saline injection at ED1 and ED10 were the same with non-significant minute fluctuation.

Fig 4.

Depicts graphs representing the frequency of a neuronal firing rate in spikes per second over time. The arrow over the 30 minute time point marks the time of injection (2.5 mg/kg MPD i.p.). The neuronal spike representation is a screen shot of the spike template taken from the wave mark window at the 1st (left side of graph) and 90th (right side of graph) min in each recording day. The superimposed spikes within each template are actual density representations of the firing rate of the corresponding min. On Experimental day 1 (ED1) this neuronal unit exhibits a significant increase in firing rate after MPD injection (arrow). On ED10, the same neuronal unit expresses a mean baseline firing rate that is higher than that expressed on ED1. Post MPD rechalleng, the NAc unit's mean firing rate decreases when compared to the firing rate at ED10 baseline.

Fig 5.

Represents the frequency graph of a NAc neuron firing rate in spikes per second over time. The arrow over the 30 minute time points marks the time of injection (2.5 mg/kg i.p.). The superimposed neuronal spike representation is a screen shot taken from the wave mark window at the 1st and 90th min in each recording day. On experimental day 1 (ED1) this neuronal unit decreases its firing rate after the MPD injection. On ED10, the same neuronal unit expresses a mean baseline firing rate that is lower than that expressed on ED1. Post ED10 MPD rechallenge, the NAc neuronal unit's mean firing rate significantly increases above the ED10 baseline firing rate.

Table 1

Experimental protocol

Experimental day	1	2-6	7-9	10
Treatment	Saline	2.5mg/kg	Washout	Saline
2.5mg/kg	2.5mg/kg			

On experimental day 1 (ED1), neuronal recordings were performed after saline injection (baseline) for 30 minutes and continued for 90 minutes following 2.5mg/kg injection of MPD. Rats on ED2-6 were injected once a day in the morning (8 am) with 2.5mg/kg MPD. ED7 through ED9 were washout days (drug abstinence). Neuronal recordings resumed on ED10 after saline (baseline) and 2.5mg/kg MPD. On ED10 rats were treated identical as in ED1.

Table 2

Acute and chronic effects of MPD on 67 NAc units

A. Acute effect of MPD 2.5mg/kg			
ED1 MPD 2.5mg/kg	increase	decrease	no change
N=67	26	10	31
B. Comparing ED10 baseline activity to ED1 baseline activity			
ED10 Baseline	increase	decrease	no change
N=67	32	25	10
C. Effect of MPD rechallenge on ED10			
ED1 MPD 2.5mg/kg	increase	decrease	no change
N=67	26	16	25

A. represents the summary of the effects of MPD on NAc neuronal activity on ED1. *B.* compares the baseline neuronal activity recorded on ED10 compared to ED1. *C.* shows the NAc neuronal units responses to MPD rechallenge at ED10.

Table 3

Tree diagram of the effects of acute and chronic MPD administration on NAc units.

EXPERIMENTAL DAY 1 (ED1)	EXPERIMENTAL DAY 10 (ED10)			
A: Increase in NAc activity on ED1 N: 26	ED 10 Baseline N=26	increase	decrease	no change
		a. 17	c. 8	e. 1
	Response	I D NC	I D NC	I D NC
	ED10 2.5mg/kg MPD	b. 0 12 5	d. 7 0 1	f. 0 0 1
B: Decrease in NAc activity on ED1 N: 10	ED 10 Baseline N=10	increase	decrease	no change
		g. 0	i. 6	k. 4
	Response	I D NC	I D NC	I D NC
	ED10 2.5mg/kg MPD	h. - - -	j. 3 0 3	l. 1 0 3
C: No response in NAc activity on ED1 N: 31	ED 10 Baseline N=31	increase	decrease	no change
		m. 15	o. 11	q. 5
	Response	I D NC	I D NC	I D NC
	ED10 2.5mg/kg MPD	n. 6 4 5	p. 8 0 3	r. 1 0 4

A. represents the summary of the effects of MPD on NAc neuronal units which showed increased activity after MPD on ED1 and how those units responded on ED10. B. summarizes the effects of MPD on NAc neuronal units which showed decreased activity after MPD on ED1 and how those units responded on ED10. C. summarizes the effects of MPD on NAc neuronal units which failed to significantly respond after MPD on ED1 and how those units responded on ED10. I represents increase, D represents decrease and NC represents no change.