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Sensitized nucleus accumbens dopamine terminal responses to methylphenidate and dopamine transporter releasers after intermittent-access self-administration

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ABSTRACT

Long-access methylphenidate (MPH) self-administration has been shown to produce enhanced amphetamine potency at the dopamine transporter and concomitant changes in reinforcing efficacy, suggesting that MPH abuse may change the dopamine system in a way that promotes future drug abuse. While long-access self-administration paradigms have translational validity for cocaine, it may not be as relevant a model of MPH abuse, as it has been suggested that people often take MPH intermittently. Although previous work outlined the neurochemical and behavioral consequences of long-access MPH self-administration, it was not clear whether intermittent access (6 h session; 5 min access/30 min) would result in similar changes. For cocaine, long-access self-administration resulted in tolerance to cocaine's effects on dopamine and behavior while intermittent-access resulted in sensitization. Here we assessed the neurochemical consequences of intermittent-access MPH self-administration on dopamine terminal function. We found increased maximal rates of uptake, increased stimulated release, and subsensitive D2-like autoreceptors. Consistent with previous work using extended-access MPH paradigms, the potencies of amphetamine and MPH, but not cocaine, were increased, demonstrating that unlike cocaine, MPH effects were not altered by the pattern of intake. Although the potency results suggest that MPH may share properties with releasers, dopamine release was increased following acute application of MPH, similar to cocaine, and in contrast to the release decreasing effects of amphetamine. Taken together, these data demonstrate that MPH exhibits properties of both blockers and releasers, and that the compensatory changes produced by MPH self-administration may increase the abuse liability of amphetamines, independent of the pattern of administration.

1. Introduction

Previous work has shown that methylphenidate (MPH) self-administration in rodents enhances the potency and reinforcing efficacy of amphetamine-related drugs, suggesting that MPH abuse may change the dopamine system in a way that promotes future drug abuse (Calipari et al., 2013a; Calipari et al., 2014a). MPH, the active compound in Ritalin, is commonly used off-label orally as a "study drug" (Teter et al., 2006), and in college students it has also been reported to be used to stay awake longer to party on weekends (Hall et al., 2005; Prudhomme-White et al., 2006; Teter et al., 2003). MPH is also taken intranasally or intravenously (IV) in larger doses, to "get high" (Gautschi and Zellweger, 2006; Levine et al., 1986; Parran and Jasinski, 1991; Sherman et al., 1987; Shaw et al., 2008; Teter et al., 2003); however, currently there is limited information on these patterns or routes of administration in pre-clinical models (Marusich et al., 2010; Calipari et al., 2013a; Calipari et al., 2014a). When taken via the same route of administration, the subjective effects of MPH are indistinguishable from cocaine or amphetamine, two commonly abused and highly addictive drugs (Rosen et al., 1985; Silverman and Ho, 1980). Although the subjective effects of MPH and other stimulants are similar, recent work has shown that the compensatory changes induced by repeated administration of MPH are divergent from other stimulants of the same type, making it difficult to predict the neurochemical consequences of MPH abuse based on literature on compounds such as cocaine, the prototypical dopamine transporter blocker (Calipari et al., 2013a,c; Calipari et al., 2014a,b; Ferris et al., 2011, 2012, 2013a,b). For example, while a five-day history of cocaine self-administration selectively reduced cocaine, but not MPH, potency at the DAT, MPH self-administration

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selectively increased MPH potency (Calipari et al., 2014a). Additionally, although long-access MPH self-administration did not alter blocker potency (cocaine, nomifensine), it enhanced both the potency and reinforcing efficacy of releasers (amphetamine, methamphetamine) (Calipari et al., 2013a), suggesting that these compounds may have an increased abuse liability following repeated MPH exposure.

Long-access self-administration of cocaine in rodents results in escalation of intake over sessions, a phenomenon that has been documented in humans and is considered essential to the addiction process (Ahmed et al., 2002, 2003; Ahmed and Koob, 2005; Dackis and O'Brien, 2001). However, while self-administration studies using long-access paradigms have high translational validity for stimulants such as cocaine and amphetamine, which have been reported to be taken in multiple day binges (Dackis and O'Brien, 2001; Koob and Le Moal, 2001), such paradigms may not model MPH abuse as accurately. MPH abuse in humans has been suggested to occur intermittently, in single large doses many hours or days apart (Hall et al., 2005; Prudhomme-White et al., 2006; Teter et al., 2003). Thus, it is important to determine if the same neurochemical changes occur following a more clinically relevant pattern of voluntary MPH intake. Additionally, although previous work has determined the consequences of long-access MPH self-administration on the dopamine system, recent work has shown that the pattern of self-administration (intermittent versus continuous) can play an integral role in determining the neuroadaptations that occur following stimulant exposure. For example, intermittent versus continuous patterns of cocaine or self-administration resulted in opposite adaptations, characterized by the development of sensitization and tolerance to cocaine's effects on the dopamine system, respectively (Calipari et al., 2013b; Calipari et al., 2014c). Hence, we aimed to determine how an intermittent paradigm of MPH administration influences the compensatory changes that occur within the dopamine system as compared to previous work following long-access conditions.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (375–400 g; Harlan Laboratories, Frederick, Maryland) were used for all self-administration experiments. Rats were maintained on a 12:12 h reverse light/dark cycle (3:00 am lights off; 3:00 pm lights on) with food and water *ad libitum*. All animals were maintained according to the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) in Association for Assessment and Accreditation of Laboratory Animal Care accredited facilities. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Wake Forest School of Medicine. All efforts were made to minimize suffering, reduce the number of animals, and to utilize alternatives to *in vivo* techniques.

2.2. Self-administration

Rats were anesthetized and implanted with chronic indwelling jugular catheters and trained for i.v. self-administration as previously described (Calipari et al., 2013b). Following surgery, animals were singly housed, and all self-administration sessions took place in the home cage during the active/dark cycle (9:00 am–3:00 pm). After a 2-day recovery period, animals underwent a training paradigm within which animals were given access on a fixed ratio one (FR1) schedule to a MPH-paired lever, which, upon responding, initiated an intravenous injection of MPH (0.56 mg/kg, infused over 4 s). After each response/infusion, the lever was retracted and a stimulus light was illuminated for a 20 s timeout period, during which the animal had no access to the lever, and thus could not respond for drug. During training, sessions were terminated after a maximum of 20 infusions or 6 h, whichever occurred first. An animal was considered to have acquired upon responding for 20 injections for two consecutive days and a stable pattern infusion intervals was present. Following training, animals were assigned intermittent access after which the dopaminergic alterations that resulted were assessed.

2.3. Controls

Controls were animals housed in the same room, on the same light cycle, with similar handling conditions to the animals that performed self-administration. In previous publications we have confirmed with a number of measures that the surgery, novelty of being present in the self-administration chambers, and/or limited drug self-administration, does not influence the results (Calipari et al., 2013b,c).

2.4. Intermittent access group

Animals were given access to MPH on an intermittent schedule of administration described previously (Calipari et al., 2013b). During each 6 h session animals had access to MPH for 12 five minute trails separated by 25-min timeout periods. Within each five-minute session, there were no timeouts other than during each infusion, and the animal could press the lever on an FR1 schedule to receive a 1-s infusion of MPH (0.140 mg/kg/inf). Upon responding a stimulus light illuminated concurrently with the 1-s infusion of drug.

2.5. In vitro voltammetry

Voltammetry experiments were conducted during the dark phase of the light cycle 18 h after commencement of the final self-administration session. Following the completion of the self-administration paradigms animals were deeply anesthetized with isofluorane and rapidly decapitated. The brain was rapidly removed and the tissue was immediately immersed in ice-cold oxygenated artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl (126), KCl (2.5), NaH_2PO_4 (1.2), $CaCl_2$ (2.4), $MgCl_2$ (1.2), $NaHCO_3$ (25), glucose (11), L-ascorbic acid (0.4) and pH was adjusted to 7.4. A vibrating tissue slicer was used to prepare 400 μm thick coronal brain sections containing the NAc. Once sliced, the tissue was transferred to the testing chambers containing bath aCSF (32 °C), which flowed at 1 ml/min. After a 30-min equilibration period, a cylindrical carbon fiber microelectrode (100–200 μM length, 7 μM radius) and a bipolar stimulating electrode were placed into the core of the NAc. The nucleus accumbens (NAc) was selected because of the important role in the reinforcing and rewarding actions of cocaine. Further, our previous research has concentrated on plasticity of dopamine transporters in the core and demonstrated that these alterations occur due to self-administration history of a number of drugs (Calipari et al., 2013a,b,c; Calipari et al., 2014a,b,c,d; Ferris et al., 2011, 2012, 2013a,b). Fast scan cyclic voltammetry was used to characterize baseline dopamine system kinetics, D2-like autoreceptor activity, and the ability of psychostimulants to inhibit dopamine uptake. Endogenous dopamine release was evoked by a single electrical pulse (300 μA, 4 ms, monophasic) applied to the tissue every 5 min. Extracellular dopamine was recorded by applying a triangular waveform (−0.4 to +1.2 to −0.4 V *vs* Ag/AgCl, 400 V/s). Once the extracellular dopamine response was stable for three consecutive stimulations, cocaine (0.3–30 μmol/L), MPH (0.3–30 μmol/L) and (amphetamine 0.1–10 μmol/L) were applied cumulatively to the brain slice to determine the effects of cocaine self-administration on drug-induced uptake inhibition. In order to determine D2-like autoreceptor sensitivity following these self-administration paradigms, we also ran dose–response curves for quinpirole (0.01–1 μmol/L), a D2-like receptor agonist, and reversed the effects with sulpiride (2 μmol/L), a D2-like antagonist.

2.5.1. Data analysis

For all analysis of FSCV data Demon Voltammetry and Analysis software was used (Yorgason et al., 2011). To evaluate the effects of MPH self-administration on dopamine system kinetics, evoked levels of dopamine were modeled using Michaelis–Menten kinetics as described previously (Calipari et al., 2012). Immediately following the completion of each concentration–response curve, recording electrodes were calibrated by recording their response (in electrical current; nA) to a known concentration of dopamine in aCSF (3 μM) using a flow-injection system. This value was then used to convert electrical current to dopamine concentration. For cocaine, MPH, and amphetamine dose–response curves, K_m, a measure of apparent affinity for the dopamine transporter, was used to determine changes in ability of the psychostimulants to inhibit dopamine uptake in the NAc relative to baseline. Because quinpirole is a D2-like agonist it concentration-dependently reduces the peak height of the dopamine signal. As a result we can assess the functional sensitivity of D2-like autoreceptors by determining if there is a shift in the concentration–response curve for quinpirole. All curves for D2-like function are expressed as percent baseline dopamine release.

2.5.2. Calculating K_i values

As described by Jones et al. (1995), inhibition constants (K_i) were determined by plotting the linear concentration–effect profiles and determining the slope of the linear regression. The K_i was calculated by the equation K_m/slope. K_i values are reported in μM and are a measure of the drug concentration that is necessary to produce 50% uptake inhibition.

2.6. Statistics

Graph Pad Prism (version 5, La Jolla, CA, USA) was used to statistically analyze data sets and create graphs. Pre-drug measures of stimulated dopamine release, dopamine uptake, and K_i values were compared using a two-tailed Student's *t*-test. Release data and data obtained after perfusion of cocaine, MPH, amphetamine, or quinpirole were subjected to a two-way analysis of variance (ANOVA) with