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MK-801 Blocks the Induction of Behavioral Sensitization and C-Fos Expression to Amphetamine

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Abstract

Background: After repeated administration of psycho stimulants such as amphetamine, less of the drug is needed to achieve the initial psychoactive effects. Glutamate has been implicated in the neural plasticity resulting from repeated amphetamine. Therefore, the current work set out to determine if blockade of glutamate acting at the N-methyl-D-aspartate (NMDA) receptor would interfere with the neurobehavioral development of amphetamine sensitization.

Methods: Long-Evans rats (N=24) received a 4-day treatment of either (1) isotonic saline (0.9%), (2) 3.0 mg/kg/ml of amphetamineor (3) dizocilpine hydrogen maleate (MK-801, 0.01 mg/kg/ml) 30 min prior to amphetamine (3.0 mg/kg/ml) every other day. Forty-eight, 72 and 96 h, respectively, after the last amphetamine treatment, animals were given Environmental, Low Dose (amphetamine, 0.75 mg/kg/ml) and High Dose challenges (amphetamine 1.5 mg/kg/ml).

Results: Analyses of behavioral data indicated the 4-day AMPH regimen induced sensitization that was significantly attenuated by pretreatment with MK-801. Postmortem analyses revealed a significant decrease in c-fos expression in the secondary motor cortex, cingulate cortex and nucleus accumbens core in amphetamine-treated rats, whereas an increase in c-fos was observed in the nucleus accumbens (shell and core) and cingulate cortexin animals receiving AMPH+MK-801 treatment.

Conclusions: These results suggest that NMDA receptors are critical for aspects of amphetamine sensitization and distinct forebrain structures may be involved at different stages of this phenomenon.

INTRODUCTION

Repeated administration of amphetamine (AMPH) leads to behavioral sensitization that is characterized by enhanced locomotor activity to a low dose AMPH challenge [1]. The fact that sensitization persists, even after a long period of withdrawal, suggests that there are relatively permanent neuronal changes that take place with repeated administration [2]. Glutamate N-methyl-D-aspartate receptors (NMDARs) have been implicated in this plasticity [3], with evidence that the classic non-competitive NMDAR antagonist, dizocilpine hydrogen maleate (MK-801), produces sensitization. MK-801 and CPP (RS-CPP [(*RS*)-3-(2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid)] an NMDAR antagonist with GluN2 subunits) also blocks the development and expression of AMPH sensitization [2,3], AMPH cross-sensitization [4] and conditioning [5]. These findings and that of others over the past several decades [6] suggest that NMDARs are involved in the initiation of the long-term neuronal changes that lead to and are a consequence of sensitization [3,6].

Multiple brain areas underlie the sensitization that occurs with AMPH, including the ventral tegmental area (VTA) to nucleus accumbens (NAc) and striatal dopaminergic system and the glutamate-containing cells in the medial prefrontal cortex (mPFC) and striatum [7]. The NAc and the striatum are associated with the psychomotor activating and rewarding effects of AMPH and neuronal changes including induction of the immediate early gene (IEG), c-fos, and activation of extracellular kinases following repeated AMPH [8-10]. The mPFC is implicated in associative learning and IEGs in this region are increasingly activated in response to MK-801 and to repeated AMPH [11-14]. Changes in dendritic length and spine density are also apparent in these forebrain structures following repeated AMPH, even after a withdrawal period of 38 days [15]. The dorsal striatum is a terminus for midbrain dopamine neurons and, is postulated to be essential for the acquisition of compulsive drug-seeking behavior [16].

An increase in glutamate levels has long been associated with

neural plasticity [1,17] and thus, a likely target for disrupting sensitization. Pre- and post-synaptic changes in glutamate transmission that occur along the reward pathway, including the VTA, play a key role in neuroplasticity [18]. At doses of 0.01 - 0.3 mg/kg of MK-801, a number of labs have shown elevated motor activity [19] in rats and increased c-Fos expression as well as reduced sensitization to AMPH [11,20].

We designed the current study to evaluate whether pretreatment with a low dose of MK-801 that does not induce sensitization could interfere with the development of behavioral sensitization to AMPH and associated c-fos expression changes along regions of the mesocorticolimbic pathway.

METHODS

Animals

Male and female Long Evans rats were purchased (Charles River Breeding Laboratories, Wilmington, MA) and mated (n=40). Adult male offspring (n=24) with initial weights of 244-254g (average postnatal day 55) were used for this study. Rats were housed in pairs within their treatment group in clear polystyrene cages with food and water provided ad libitum. The environment had a constant temperature (22-24°C) and a 12-h light/dark cycle with lights on at 0700 h. Initial weights and test day weights were recorded to ensure that rats were receiving the correct treatment doses throughout the experiment. Protocols were in strict adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and have been approved by the IACUC of the University of Massachusetts Boston.

Drug preparation

AMPH was dissolved in isotonic saline (SAL) in three concentrations for intraperitoneal (IP) injections: 3.0 mg/kg/ml for the 4-day treatment, 0.75 mg/kg/ml for the low dose challenge and 1.5 mg/kg/ml for the high dose challenge. AMPH challenge doses are in the dose range used by other researchers to assess AMPH sensitization [21]. MK-801 was prepared in 0.9%SAL in one concentration (0.01 mg/kg/ml). AMPH and MK-801 doses and the timing of the injection protocol were worked out in the lab prior to this study. SAL (0.9%) was prepared fresh and used for all control injections (volume of 1 ml/kg) and dilutions.

Behavioral assays

The rats were tested in locomotor activity (LMA) chambers automated to a PC (Med Associates, St. Alban, VT). LMA chambers were43.2cm^3 with clear Plexiglas walls and an opaque Formica floor. The horizontal measures of the animal were recorded via photo beams emitting from metal strips around the center and bottom of each chamber side. Brightly colored paper decorated the walls to help orient the rat to its location. During any testing period, a total of four rats were each placed in a separate LMA chamber. The distance traveled (horizontal) data were collected by an IBM-compatible desktop using Med Associations Inc., Activity Monitor Software v. 4.0.After the animals completed their 30-min habituation period in the LMA chambers, the recording was paused, and each rat was taken out individually and received its treatment and returned to the LMA chambers for 45 minutes of post-injection observation.

During the 4-day treatment, rats were initially put in the LMA for a 30-min habituation period and removed at time point 30 for injections. However, the rats in the MK-801 + AMPH group received MK-801 prior to the habituation period and received AMPH at time point 30 and were returned to the LMA post-injection for 45 min. Forty-eight h after the last AMPH treatment, rats were given an Environmental challenge: all procedures were held constant as stated above; except the rats received a mock injection (syringes did not have needles). Two days later, rats were administered a Low Dose AMPH challenge (0.75 mg/kg/ml) and 48 hours after that all animals received a High Dose AMPH challenge (1.5 mg/kg/ml).

Immunocytochemistry

Upon completion of the High Dose AMPH challenge day, representative rats (n=4) were deeply anesthetized with sodium pentobarbital and transcardially-perfused with 0.9% phosphate buffered saline followed by 4% paraformaldehyde. The brains were removed and stored in the 4% paraformaldehyde solution and later cryoprotected in a20% sucrose-4 % paraformaldehyde solution. The brains were microsectioned coronally at 30 µm and the tissue slices were stored in cryoprotectant until time of immunohistochemistry (IHC). The IHC protocol was adapted from the two-day G. E. Hoffman Protocol [22]. On day one, the tissue was flushed in 0.05 M NaPBS and then incubated in the primary antibody (Ab-5) (4-17) rabbit polyclonal AB (Calbiochem, San Diego, CA 1:8000) in NaPBS and 0.4% Triton-X-100 for one hour at room temperature. The tissue was then incubated for 48 hours at 4°C. On the second day, the tissue was flushed in 0.05 NaPBS followed by a one-hour incubation period in avidin-biotin complex (concentration ratio 1:500 for A and B) at room temperature. The tissue underwent another series of flushes in NaPBS and sodium acetate followed by incubation period in 3-3'-diaminobenzidine (DAB) solution (concentration ratio 1:50), three additional flushes in sodium acetate and three final flushes in NaPBS to complete the staining process.

The stained tissue was then mounted onto 0.5% gelatin subbed glass slides using a free-floating procedure and dehydrated using reverse osmosis. A SPOT Flex monochrome camera mounted on alight microscope was used to image the regions of interest in each of the tissue samples. Cells with c-fos immunoreactivity (Fos-IR) were counted with the aid of Image J software (NIH) at 20 ×magnifications within a 250 µm^2 area in the following regions: NAc (core and shell), primary motor cortex (M1) and secondary motor cortex / medial prefrontal cortex (M2/mPFC) (according to and cingulate cortex (Cg1). Experimenters who did the counts were blinded to group identification and showed high inter-rater reliability (Crohen's kappa=0.90).

Data analysis

Statistical analysis of the behavioral data was performed using Prism Graph Pad for PC (v5.0, Graph Pad Software, Inc., La Jolla, CA) and SPSS (PC version 12.0) was used for analyses of the immunohistochemistry findings. For all analyses, the significance level was set at $p \leq 0.05$.Data are represented graphically as bar graphs of group means ± SEM. For full time-course for locomotor activity, researchers performed a two-way repeated measures analysis of variance (ANOVA; Day× Treatment factors) followed

by Bonferroni multiple comparisons post-hoc tests. We also ran distinct ANOVAs on habituation and post-injection time-course data for the 4-day regimen using factors Day and Treatment. For Challenge Days, we ran separate ANOVAs for Factors: Time and Treatment. One-way ANOVAs were performed for expression of Fos-IR in the studied brain areas followed by post hoc analyses using Tukey's HSD for between-group differences.

RESULTS

Locomotor activity

Data were graphed as mean distance traveled ± SEM for full time-course (0-75 min), habituation (0-30 min) and post-injection time period (35-75 min) over the 4-day treatment. In addition, average time-course data were depicted for habituation and post-injection periods. Separate bar graphs and analyses were made for each of the challenge days including average responses for the full time-course. Figure 1 depicts the experimental timeline for the pretreatment, challenges and post-mortem analysis.

Analysis of distance traveled: 4-day treatment habituation time-course: ANOVA revealed that across the 4-day treatment regimen, groups varied for the distance traveled during the 30-min habituation period. A significant main effect of Day and an interaction effect of Day × Treatment were found [F (3,45) =4.970, $p< 0.01$] and [F(6,45) =3.947, $p< 0.01$], respectively. Post-hoc tests indicated that SAL locomotion was lower than AMPH on Day 3 ($p< 0.05$) and higher than MK-801+AMPH on Day 3 and MK-801+AMPH differed from AMPH ($p< 0.05$) (Figure 2A).

Analysis of distance traveled: 4-day treatment post-injection time-course: Activity levels post-injection were augmented across the 4-day treatment. ANOVA showed significant main effects of Day [F(3,72)=172, $p< 0.0001$] and Treatment [F(2,72)=77.36, $p< 0.0001$] and a Day × Treatment interaction [F(6,72)=44.79, $p< 0.0001$]. Pair-wise comparisons indicated the following differences: SAL and AMPH Days 1 through 4 ($p< 0.00001$); SAL and MK-801+AMPH Day 1 ($p< 0.001$) and Days 2 through 4 ($p< 0.00001$); AMPH and MK-801+AMPH Day 3 ($p< 0.0001$) (Figure 2B).

Analysis of distance traveled: 4-day treatment full time-course: Full time course data were analyzed using a mixed model ANOVA for factors: Day and Treatment. Results show there were main effects of Day [F (3,112)=3.050, $p< 0.05$] and Treatment [F(2,112)=63.14, $p<0.0001$]. Post-hoc tests determined significant differences between SAL and AMPH on Day 1 ($p< 0.05$), Days 2, 3 and 4 ($p< 0.0001$). Differences were found between SAL and MK-801+AMPH on Days 2 and 3 ($p< 0.01$) and on Day 4 ($p< 0.0001$). There was also a near significant difference between AMPH and MK-801+AMPH (p=0.0686) on Day 3 (Figure 2C).

Analysis of distance traveled: challenge days: Separate repeated measures ANOVA were run for each challenge day including the following factors: Time and Treatment. For the Environmental Challenge, we obtained main effects of Time [F(14,280)= 45.69, $p< 0.0001$] and Treatment [F (2,280)= 5.392, $p< 0.05$] plus a Time × Treatment [F(28,280)= 2.063, $p< 0.01$] interaction. Pair-wise comparisons indicated SAL and AMPH pretreatment groups differed at time points 40 ($p< 0.001$) and 45 ($p< 0.0001$) and AMPH and MK801+AMPH differed at time point 45 ($p< 0.01$) (Figure 3A).

For the Low Dose AMPH Challenge, the analysis revealed main effects of Time [F (14,280)=19.91, $p< 0.0001$], Treatment [F(2,280)= 5.533, $p< 0.05$ as well as an interaction effect of Time × Treatment [F(28,280)= 2.570, $p< 0.0001$]. Post hoc tests indicated that SAL and AMPH differed at time-points 45-55 and 65 ($p< 0.01$) and 60 ($p< 0.001$) (Figure 3B).

Finally, for the High Dose AMPH Challenge, significant main effects of Time [F (14,280)= 48.81, $p< 0.0001$] and Treatment [F(2,280)= 5.416, $p< 0.05$] as well as a Time ×Treatment interaction [F(28,280)= 5.544, $p< 0.0001$] were found. Post-hoc results showed the following between group differences: SAL v. AMPH at time-points 40, 45 and 70 ($p< 0.001$), time-points 50 and 55 ($p< 0.0001$) and time-point 60 ($p< 0.01$); SAL v. MK-801+AMPH at time-points 45 and 55 ($p< 0.05$) and AMPH v. MK-801+AMPH at time points 45 and 55 ($p< 0.05$) (Figure 3C).

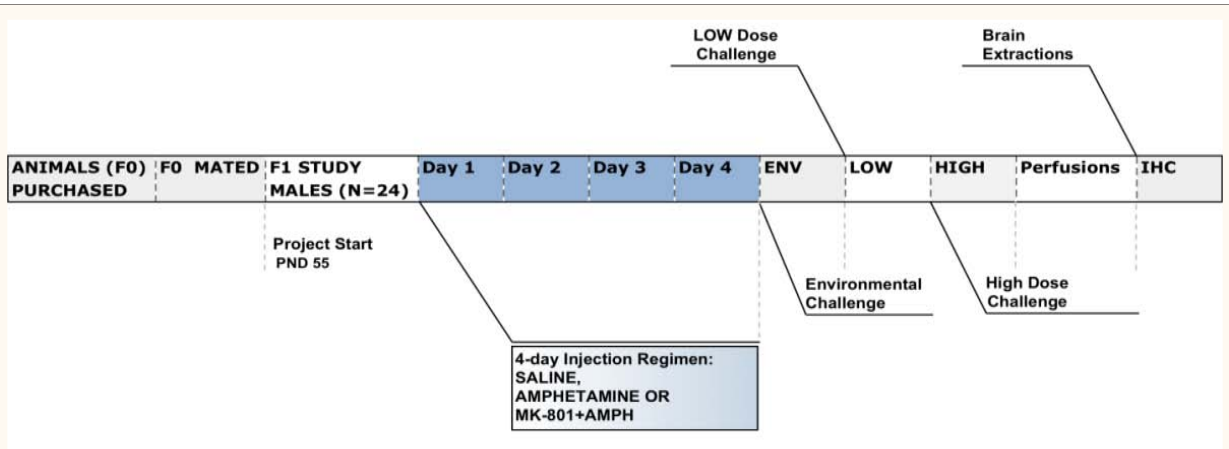

Figure 1 Experimental timeline depicting when treatments and procedures were performed.
Abbreviations: AMPH: Amphetamine (3.0 mg/kg/ml); MK-801+AMPH (30 min pretreatment with MK-801 (NMDA antagonist, 0.01 mg/kg/ml) + AMPH (3.0 mg/kg/ml); SAL: Saline; Env: Environmental Challenge (Test Environment and Mock Drug Injection); Low: Low Dose AMPH challenge (0.75 mg/kg/ml); High: High Dose AMPH challenge (1.5 mg/kg/ml); IHC: Immunohistochemistry

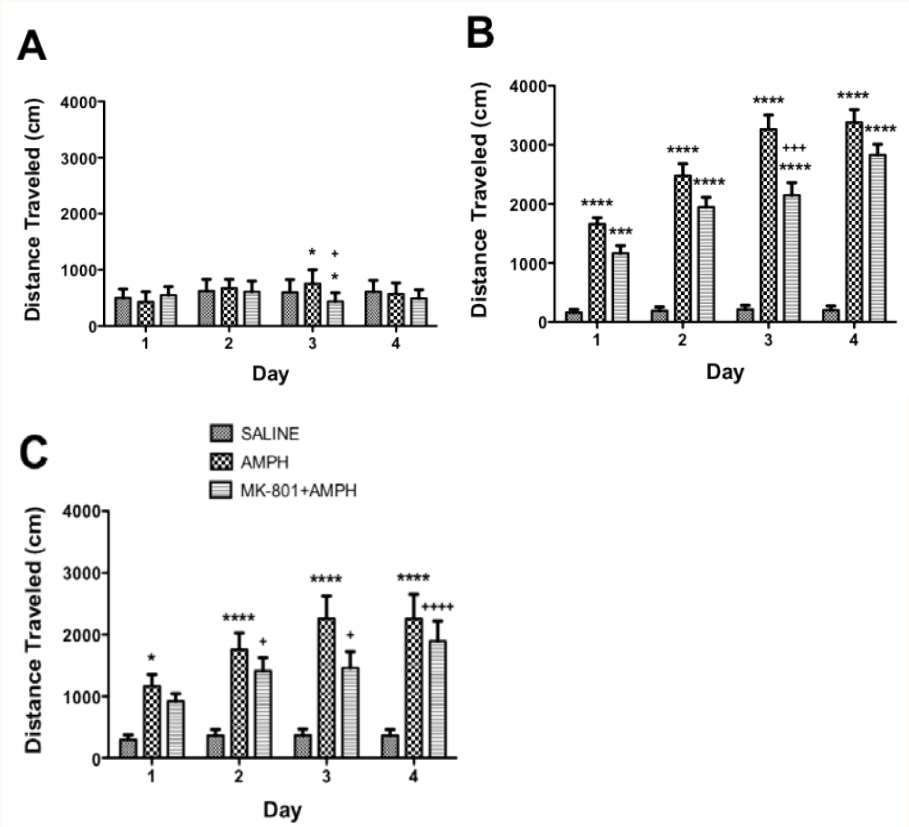

Figure 2 **A:** Locomotor response to intermittent treatment with SAL, AMPH and MK-801+AMPH. Bar graph of mean (± SEM; n=10 rats per group) distance traveled over the 4-day intermittent treatment during (A) the abituation period (0 – 30 min) *p<0.05, AMPH and MK-801+AMPH relative to SAL; +p<0.05 MK-801+AMPH compared to SAL.
B: the post-injection period (35 – 75 minutes) ***p<0.001, SAL and MK-801+AMPH Day 1, ****p<0.00001 Days 2-4; +++p<0.0001, AMPH and MK-801+AMPH Day 3.
C: full 75-min time-course *p<0.05, SAL and AMPH Day 1, ****p<0.0001, Days 2, 3 and 4; +p<0.01 SAL and MK-801+AMPH on Days 2 and 3 and ++++p<0.0001 on Day 4.

Table 1: Values are presented as mean (±SEM) number of c-fos-positive cells within each brain region (n=4 per region). ANOVA revealed significance between AMPH and MK-801 + AMPH, **p< 0.0001 in the NAc shell. In the NAc core, ANOVA revealed significance between AMPH and MK-801 + AMPH, *p< 0.05. Separate ANOVAs for the primary motor cortex and secondary motor cortex indicated near significance between AMPH and MK-801 + AMPH where p = 0.096 and p = 0.058, respectively. There was no indicated significance between groups for Cg1.

	Cortical Region				
Treatment Group	**NAcshell**	**NAccore**	**M1**	**M2**	**Cgl**
SAL	179 ± 13.9	180 ± 24.7	267 ± 31.4	307 ± 9.6	86 ± 17.1
AMPH	127 ± 16.6[a]	70 ± 26.1[b]	243 ± 44.3	198 ± 47.3[c]	48 ± 29.9[b,c]
MK-801+AMPH	231 ± 29.2	208 ± 41.0	174 ± 18.1[c]	226 ± 22.2	150 ± 37.0

Abbreviations: NAc: Nucleus Accumbens; M1: Primary Motor Cortex; M2: Prefrontal Cortex, Secondary Motor Cortex (according the criterion outlined in Uylings et al., 2003); Cg1: Cingulate Cortex; SAL: Saline; AMPH: Amphetamine; MK-801+AMPH: Dizocilpine/Amphetamine.
[a]p< 0.01 (compared to corresponding MK-801+AMPH group)
[b]p< 0.05 (compared to corresponding MK+801+AMPH group)
[c]p< 0.05 (compared to corresponding SAL group)

Immunohistochemistry

Histological data are presented as means (± SEM) of the Fos-IR cells in the brain regions listed (Table 1). ANOVA for the NAc shell indicated a main effect of Treatment [F (1, 24) = 218.177, p< 0.0001] (Figure (Figure 4,A-F). Tukey's HSD post-hoc test indicated significant differences between AMPH and MK-801+AMPH (Figure 4G) (p< 0.01) with c-fos expression lower in the AMPH-treated group. For the NAc core, there was also a Treatment effect [F(1, 24) = 71.466, p< 0.0001] and Tukey's HSD post-hoc test revealed there was a near significant difference between AMPH and SAL (p = 0.063) and a significant difference between AMPH and MK-801+AMPH (p< 0.05), again with c-fos expression levels lowest in the AMPH-treated animals. In the primary motor

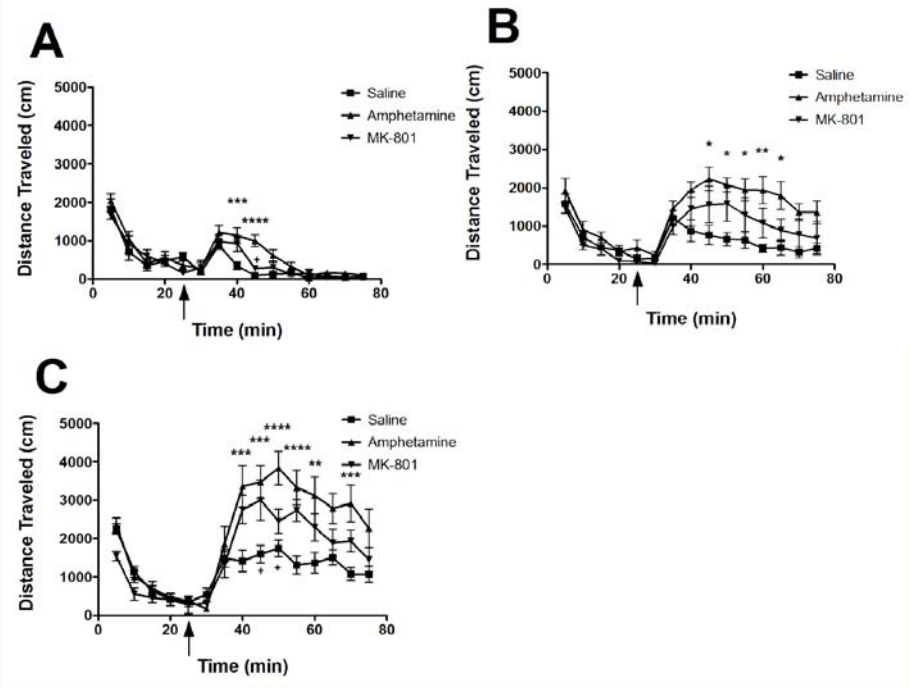

Figure 3 **A:** CIntermittent treatment (4-day) with SAL, AMPH or MK-801+AMPH followed by Environmental and AMPH challenges (Day 6, 8, 10). Line graph of average(± SEM) distance traveled time course for(A) Environmental Challenge (mock injection), ***p<0.001, ****p<0.0001, AMPH relative to SAL; +p<0.01, MK-801+AMPH compared to AMPH.
B: Low Dose Challenge (0.75 mg/kg, AMPH), *p<0.05, **p<0.01 ***p<0.001, AMPH relative to SAL.
C: High Dose Challenge (1.5 mg/kg, AMPH), **p<0.01, ***p<0.001, ****p<0.0001 (SAL relative to AMPH); +p<0.05 (SAL compared to MK-801 + AMPH).

cortex (M1), ANOVA indicated a marked Treatment effect [F(1, 24) = 160.141, p< 0.0001], further analyses indicating a near significant difference between the AMPH and the MK-801+AMPH groups (p = 0.059), and a significant difference between MK-801+AMPH and SAL (p< 0.05).It is probable that with a larger n for the IHC analyses the differences between AMPH and SAL for NAc, and AMPH and MK-801 would have reached significance. There was a Treatment effect for M2/mPFC [F (1, 24) = 303.063, p< 0.0001] with post-hoc tests implicating a significant difference between the AMPH and MK-801+AMPH (Figure 4D) groups (p<0.05). For Cg1, ANOVA revealed a Treatment effect [F (1, 24)= 62.525 (p< 0.0001)] due to differences between SAL and AMPH, and AMPH and MK-801+AMPH groups (p< 0.05) (Figure 4A-C) photomicrographs; Table 1).

DISCUSSION

The current study investigated the role of MK-801 in modulating AMPH-induced locomotor sensitization. Our findings show that repeated intermittent administration of 3.0 mg/kg/ml AMPH heightens locomotor activity in rats as has been reported previously [9]. The administration of 0.01 mg/kg/ml MK-801 before AMPH treatment dampens this locomotion sensitization. Further, we observed depressed levels of c-fos expression in the NAc core and shell, and Cg1 in the AMPH sensitized animals compared to rats treated with the MK-801+AMPH cocktail, implicating long-term depression of c-fos in these regions is blocked by NMDAR antagonism. There is evidence indicating that activation in NAc decreases with repeated psycho stimulant drugs, as more limbic and learning structures are recruited, and that excitatory amino acids acting at NMDARs are involved [23].

Analysis of distance traveled: 4-day pretreatment habituation and post-injection

One of the hallmarks of repeated drug administration in the presence of contextual cues (i.e., experimenter handling, transport, environmental cues) is the shifting of the dose-response curve for the behavioral effects [24]. That is, even prior to actual drug administration these contextual cues (movement to a distinct environment) and not discrete cues (light, tone and/or odor) have the ability to alter the drug sensitization profile [24]. We further tested this phenomenon by monitoring the pre- and post-injection distance traveled of animals across a 4-day treatment. While we did note small between- and within-group differences for habituation data, we found markedly greater locomotor changes in the AMPH and MK-801+AMPH groups for the post-injection time points, though this effect was decreased in the NMDA antagonist group.

Analysis of distance traveled: challenge days

Sensitization is thought to occur as a consequence of contextual cues being salient enough to activate associative learning processes, and these processes have also been implicated in the incentive sensitization theory of drug addiction [25]. That is, after a certain number of pairings between the environment and the drug, the environment alone can cause the subject to experience activity levels similar to when the drug was administered [26].

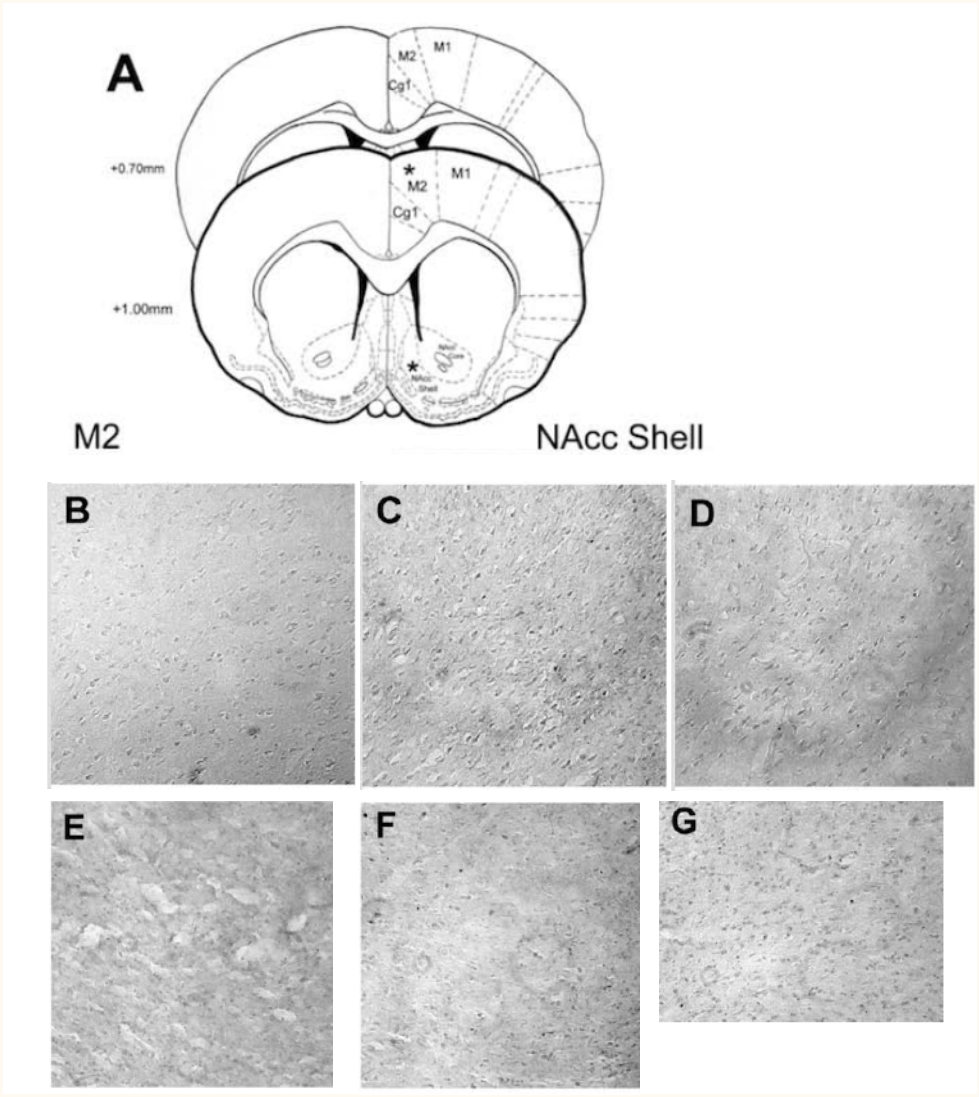

Figure 4 **A:** Schematic representation of parts of reward circuitry (NAc core and shell, M2/mPFC) and primary motor cortex (M1) sketched on sections from stereotaxic plates from Paxinos and Watson (2004) (**A**). The numbers indicate coordinates (mm) rostral to the bregma. The approximate area for Fos-immunoreacted cell counts was 250 μm^2 within the region of interest. The locations of the Fos-immunoreacted areas used for counts are indicated by asterisks.
(B-D): Immunocytochemistry for Fos protein showing bright field Fos-IR at low power (20 X) in M2 (left panel images) and NAc (right panel images) for representative animals from the following pretreatment groups: (**B**) saline (SAL), (**C**) amphetamine (AMPH), (**D**) MK-801+AMPH.
(E-G): (**E**) SAL, (**F**) AMPH (**G**) MK-801+AMPH. Scale bar in **G** is for all histological images and equals 100 µm.

In support of this, we showed elevated motor activity on the Environmental Challenge day, where animals received a mock injection and were placed in the LMA chamber. A combination of the experimenter cues and the drug-paired environment likely influenced the hyperactivity and enhanced Fos-IR within critical brain areas implicated in locomotor sensitization as has been reported previously [26]. The discrete environment in which a drug is administered affects the neural systems involved in psychomotor activation and sensitization [24], and this likely contributes to the enhanced neuronal activation along motor and reward cortices observed currently. This was further supported by our findings that prior AMPH increased motor activity on Low and High Dose challenge days (72 and 96 h after the last AMPH treatment).

Analysis of Immunocytochemistry: NAc, Cingulate and Motor Cortices

Earlier findings demonstrate that behavioral sensitization to AMPH does not induce c-fos expression in ventral striatal regions [27,28] but does elevate Fos-IR in the dorsal striatum [27]. Currently, we looked at c-fos expression in the NAc shell and core and the motor cortices, both located in areas long implicated in sensitization, addiction and locomotor activity [12,14]. C-Fos is thought to be induced in brain areas implicated in motor activation, reward and learning and memory [12,26,29]. It is also known that as an animal becomes familiar with a stimulus, such as an injection of AMPH, the amount of c-fos and other immediate early genes (e.g., Zif-268) expressed decreases [5,29], while still

other research suggests that different brain areas may show heightened activation of c-fos over time [30].

In the present study, we found diminished Fos-IR in discrete brain areas implicated in reward and behavioral sensitization (NAc (shell and core) and M2, and Cg1) in the animals that received prior repeated AMPH injections. By contrast, higher Fos-IR was induced in the NAc (shell and core) and Cg1 in MK-801+AMPH pre-treated rats in comparison to AMPH pre-treated animals. These results are in agreement with previous research showing MK-801 treatment (0.1 mg/kg/ml) resulted in higher Fos-IR in reward-related structures [11,]. It is important to note that previous studies used a larger dose of MK-801 to achieve these effects while in the present work we used a ten-fold smaller dose and obtained similar results.

Our data also suggest potential functional differences in immediate early gene activation following an AMPH sensitization regimen; particularly along the medial prefrontal cortex since c-fos-IR in the prelimbic (Cg1) region was reduced by AMPH treatment and this diminution was impeded with MK-801 pretreatment. However, this was not the case in the M2/anterior cingulate region [31]. In earlier work by [32], the authors reported that ibotenic lesions along sub regions of the mPFC blocked the development of cocaine but not AMPH sensitization, while other work has demonstrated that whole mPFC lesions did effectively attenuate the development of AMPH sensitization [33]. Given that there is evidence for structurally and functionally distinct regions of the mPFC, more work is warranted to understand the apparent differences in NMDA stimulation and AMPH sensitization observed in the current work.

Role of NMDARs in AMPH sensitization

Interestingly, NMDARs tend to be localized to pyramidal cells within the mPFC [34,35]. Stimulation of these receptors increases GABA release resulting in a diminution in dopamine overflow while antagonism of NMDARs results in an increase [36]. This indirect influence on dopamine efflux in the neocortex is thought to underlie some aspects of cocaine and amphetamine sensitization [37]. We utilized a systemic protocol in the current work and thus, it is likely that administration of the antagonist MK-801 blocked NMDARs throughout the brain. Since we observed elevated Fos-IR in the NAc (shell and core) and Cg1 regions following the MK-801+AMPH regimen compared to AMPH alone, this may reflect the indirect effect of NMDA antagonism on increased neuronal activity in this region. The fact that this NMDA antagonism alone can induce sensitization, and yet it did not have a synergistic effect with AMPH locomotor sensitization may be due to activation at hippocampal receptor sites that could have dampened the associative learning mechanisms critical for the augmented response. While not demonstrated presently, this critical role of hippocampal glutamate activity has been established by others showing methamphetamine and cocaine sensitization resulted in a decline in hippocampal glutamate function while simultaneously causing deficits in contextual learning [38,39]. The finding that prior administration with MK-801 and more recently, CPP (NMDAR antagonist with GluN2 subunits) successfully blocked the development of behavioral sensitization to AMPH has been reported earlier for locomotor sensitization [3,20], and in conditioning paradigms [5]. However, we used a lower dose to establish this MK-801 effect (0.01 mg/kg) compared to the 0.25 mg/kg MK-801 dose used previously, and we administered the pretreatment every other day for only four days (at a dose of 3.0mg/kg, IP), relative to the daily 1 mg/kg AMPH injections of [20]. Still other studies use a range of 2.0 mg/kg – 5.0 mg/kg of AMPH and longer treatment regimens to establish sensitization [9,40].

CONCLUSIONS

To summarize, we report here that intermittent treatment with 3.0 mg/kg dose of AMPH for four days (over eight days) augmented the locomotor responses across treatment days, as well as on Environmental, Low and High Dose Challenge days beginning 48 h after last AMPH treatment. Furthermore, we found MK-801 (0.01 mg/kg) prior to AMPH administration during the treatment successfully attenuated the sensitized response on Day 3 post-injection and during the High Dose Challenge. We used low doses of AMPH and MK-801 – and were able to successfully tease apart the combined sensitization effects observed in other work using higher doses of MK-801 and AMPH [41]. Therefore, the current findings implicate NMDARs in the development of AMPH sensitization that is independent of glutamate sensitization. c-fos expression was attenuated in the motor cortex group exposed to both MK-801 and AMPH, while, as expected, the reduced c-fos expression in the NAc (shelland core) and Cg1, resulting from AMPH treatment, was reversed by MK-801 pretreatment. The Fos-IR profile supports the diminution in motor response observed in the AMPH group that received prior MK-801 treatment given the lower Fos-IR in M1. The decrease in c-fos expression in the NAc (shell and core) parallels reports implicating a depression in excitatory efflux/activity within NAc shell after repeated psycho stimulants [42] as well as research that intra-accumbens AMPH does not induce sensitization [43]. Moreover, since co-administration of an NMDA antagonist reversed this depression, it is likely that blocking glutamate activation at its NMDARs might occur in other limbic areas involved in the induction of amphetamine sensitization [44-46]. Since association cues and conditioning play important roles in all aspects of addiction, future research should further explore the timing of NMDA antagonism necessary for attenuating sensitization and attempt to localize the site(s) of action of the effect [46-48].

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CONTRIBUTORS

Author S. Tiffany Donaldson designed the study along with Dr. Alexia Pollack and wrote the protocol. Authors Wagner, Calhoun, Plotkin, and Trawczynski managed the literature searches and summaries of previous related work. Authors Trawczynski and Mathias undertook the statistical analysis, and author Wagner wrote the first draft of the manuscript and did all the imaging. Author Plotkin worked on revisions and updating references. All authors contributed to and have approved the final manuscript.

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