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Electrophysiological and Structural Alterations in Striatum Associated with Behavioral Sensitization to (±)3,4-Methylenedioxymethamphetamine (Ecstasy) in Rats: Role of Drug Context

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Abstract

We examined whether repeated exposure to the increasingly abused amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA; ecstasy) results in long-lasting neurobehavioral changes, and further, the ability of contextual cues to modulate these changes. We focused on dorsal striatum, a brain region implicated in the formation of persistent drug-related habits. Rats were transported to a novel recording chamber and treated with once-daily injections (sc) of (±)-MDMA (5.0 mg/kg) or saline for 5 days, followed by a challenge injection 14 days later either in the same (Experiment 1) or different context (Experiment 2). Chronically implanted micro-wire bundles were used to record from populations of striatal neurons on days 1, 5, and challenge. Twenty-four h after the last injection, brains were removed and processed using a modified Golgi method to assess changes in neuronal morphology. A sensitized locomotor response was observed following MDMA challenge in 11 of 12 rats in Experiment 1 (same context), whereas only 58% of rats (7 of 12) displayed sensitization in Experiment 2 (different context). Furthermore, several alterations in striatal electrophysiology were apparent on challenge day, but only in rats that displayed sensitization. Conversely, structural changes in striatal medium spiny neurons, such as increases in spine density, were observed in MDMA-treated rats regardless of whether they displayed behavioral sensitization. Thus, it appears that reorganization of synaptic connectivity in dorsal striatum may contribute to long-lasting drug-induced behavioral alterations, but that these behavioral alterations are subject to modification depending on individual differences and the context surrounding drug administration.

Keywords

DENDRITIC SPINES; ELECTROPHYSIOLOGY; MDMA; MORPHOLOGY; STRIATUM; SENSITIZATION

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The widely used amphetamine (AMPH) derivative 3,4-methylenedioxymethamphetamine (MDMA; ecstasy) increases synaptic levels of dopamine (DA) and serotonin (5-HT) in striatum and prefrontal cortex (Gudelsky and Yamamoto, 2008), causing acute changes in the functioning of neurons in these regions. For example, we reported that acute MDMA (5.0 mg/kg) administration caused DA- and 5-HT-dependent changes in the activity of dorsal striatal neurons in freely moving rats (Ball et al., 2003; Ball and Rebec, 2005). Moreover, we found that repeated administration of MDMA resulted in relatively long-lasting changes in both the electrophysiology of dorsal striatal neurons (Ball et al., 2006) and the morphology of neurons within ventral striatum (nucleus accumbens; NAc) and medial prefrontal cortex (Ball et al., 2009). Such long-term abnormalities within the so-called 'motive-circuit' are thought to underlie equally long-lasting changes in behavior that increase vulnerability to addiction (Everitt and Robbins, 2005; Moghaddam and Homayoun, 2008).

One of the most enduring behavioral changes induced by drugs of abuse is behavioral sensitization or an augmentation of the psychomotor effects of a drug following repeated administration (Segal and Mandell, 1974; Stewart and Badiani, 1993). Behavioral sensitization is considered a useful animal model for the study of addiction (Kalivas and Stewart, 1991; Robinson and Berridge, 1993) because many of the neural adaptations underlying sensitization overlap with those driving addiction-like behaviors (Tzschentke and Schmidt, 2003; Shaham and Hope, 2005). As with other drug-related behaviors (Caprioli et al., 2007), however, behavioral sensitization is strongly modulated by the context surrounding drug administration (Anagnostaras and Robinson, 1996; Robinson et al., 1998). For example, the expression of behavioral sensitization and associated changes in dendritic spine density and glutamate dynamics in NAc core are observed in rats given cocaine injections in a novel context, but not in animals treated with the identical regimen in their home cages (Bell and Kalivas, 1996; Bell et al., 2000; Li et al., 2004). In freely moving rats we found that changes in the electrophysiology of dorsal striatum were evident only in animals that received MDMA injections in a novel test cage and subsequently displayed behavioral sensitization to a challenge injection of MDMA (Ball et al., 2006).

Given the growing evidence that MDMA use may lead to compulsive drug-seeking behavior in vulnerable individuals (Topp et al., 1999; Soar et al., 2006; Ball et al., 2007), it is important to clarify the factors related to individual differences in the response to MDMA. In the present study, we found that whereas the vast majority of animals developed sensitization when given repeated MDMA injections in a novel environment, a change to a different context following the development of sensitization brought about striking individual differences in the expression of sensitization. Moreover, we found several differences in the electrophysiological characteristics of striatal neurons between animals that did and did not display behavioral sensitization. Finally, in tissue from the same rats used in the behavioral and electrophysiological studies, we observed large MDMA-induced changes in the morphology of striatal medium spiny neurons.

2. Experimental Procedures

2.1. Animals

Adult male, Sprague-Dawley rats weighing 350–450 g and bred from source animals supplied by Harlan Industries (Indianapolis, IN) were used for all experiments. Rats were housed individually under standard laboratory conditions (12-h light cycle from 7:30 AM to 7:30 PM) with *ad libitum* access to food and water. All procedures were conducted between

9:00 AM and 5:00 PM, were in compliance with the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (Academies, 2003), and were approved by the Indiana University Institutional Animal Care and Use Committee.

2.2. Surgery

Recording electrodes were chronically implanted in dorsal striatum as described previously (Ball et al., 2003). Briefly, animals were anesthetized with ketamine HCl (90 mg/kg) and xylazine HCl (10 mg/kg), and placed in a stereotaxic frame. One hole was drilled over dorsal striatum, and the underlying dura was carefully retracted. Five additional holes were drilled for stainless steel support screws. The electrode bundle was implanted unilaterally into dorsal striatum (1 mm anterior, 2.50 mm lateral, and 5.00 mm ventral to bregma), and attached to the skull with support screws and dental acrylic. Half of the rats received either left or right hemisphere implants. Each electrode bundle consisted of eight microwires (Formvar-coated stainless steel, 25 μ m diameter) and one ground wire (stainless steel, 50 μ m diameter), soldered to a 10-pin connector.

2.3. Treatment regimens

($\pm$)-MDMA was provided by the National Institute on Drug Abuse (NIDA, Research Triangle Park, NC). The dose of 5.0 mg/kg (weight of salt; dissolved in 0.9% saline) was used for all injections. We have used this dose in previous studies (Ball et al., 2003; Ball and Rebec, 2005; Ball et al., 2006) because it is a relatively low dose, yet induces robust behavioral activation.

Animals were given at least 6 days to recover from surgery before experiments began. The treatment regimens for Experiments 1 and 2 are described below, and were chosen based on previous work (Ball et al., 2006).

2.3.1. Experiment 1—Animals ($n = 12$) were transported from their home cages and received once daily injections (sc) of MDMA (5.0 mg/kg) in an experimental recording chamber for five consecutive days. Two weeks following the fifth injection, animals received a challenge injection (5.0 mg/kg) in the recording chamber. Experiments were conducted on Days 1, 5, and Challenge (see *Electrophysiology* section below). On days 2, 3, and 4, rats were injected and placed in the recording chamber where they remained for 30 min before being returned to their home cages. The context of the recording chamber remained constant throughout the treatment regimen. A separate group of rats ($n = 6$) underwent the identical treatment and recording protocol, except that all injections were saline vehicle.

2.3.2. Experiment 2—The protocol for Experiment 2 was identical to Experiment 1 above, except that the context of the recording chamber changed on the challenge day. Thus, there were two distinct contexts: Context 1 = black walls and smooth floor; Context 2 = white walls and textured floor. Half the rats ($n = 6$) experienced Context 1 on Days 1 through 5 and Context 2 on the Challenge day. The remaining rats ($n = 6$) experienced the reverse order. A separate group of rats ($n = 6$) underwent the identical treatment and recording protocol, except that all injections were saline vehicle.

2.4. Electrophysiology

For all experimental sessions, a flexible, shielded wire harness was plugged into the connector on the rat's head. Rats were then placed into a sound-attenuating, open-field recording chamber (1.2 m²), and the harness was connected to a commutator, allowing the rat unrestricted movement. A white light bulb located at the top of one wall illuminated the chamber, and a video camera mounted above the rat recorded all experiments for subsequent

behavioral analysis. Extracellular spike activity was routed through multiple-channel preamplifiers with $500 \times$ gain and 154 Hz to 8.8 kHz band-pass filters (Plexon, Dallas, TX). The signals were digitized at 40 kHz and acquired by the Multichannel Acquisition Processor system (Plexon). This system and its companion software Sort Client (Plexon) provide direct computer control over amplification, filtering, signal detection, spike-waveform discrimination, and storage. To be classified as single-unit activity, waveforms displayed a signal-to-noise ratio of at least 2.5:1, and exhibited a consistent shape and amplitude. Analysis of autocorrelations also was used to support single-unit activity classification by ensuring the presence of the absolute refractory period. For each unit, data from the entire recording session (which includes pre- and post-MDMA activity) was used for autocorrelation analysis. This ensures that any extraneous noise due to increased movement is not counted as spike activity. Any recordings not meeting the above criteria were excluded from subsequent analyses.

At the beginning of each experimental session, rats were placed into the recording chamber and unit activity was discriminated as described above. Recording sessions were 120 min in length and divided into three successive periods as in our previous studies (Ball et al., 2003; Ball and Rebec, 2005; Ball et al., 2006). Period 1 (10 min) served as pre-baseline, during which no treatment was administered, but single-unit activity was recorded to ensure a stable signal. Period 2 (20 min) served as baseline, and began after the administration of saline vehicle (1ml/kg, sc). Period 3 (90 min) began after an injection of MDMA (5.0 mg/kg, sc).

2.5. Data analysis

Electrophysiological data were analyzed by means of Neuroexplorer version 3.204 (Nex Technologies, Littleton, MA). All statistical analyses used GraphPad Prism version 4.03 (GraphPad Software, San Diego, CA). A mean baseline firing rate was obtained for each unit during the last 10 min before drug injection. Changes in firing rate for individual units were calculated as a percentage of baseline for each 5-min bin beginning 5 min after MDMA administration. The 5-min period immediately following MDMA administration was omitted from analysis to avoid injection-related effects. During quiet resting behavior, the majority of striatal units exhibit fairly regular, low levels of spontaneous activity (Haracz et al., 1998; Ball et al., 2003). However, during phasic behavioral activation (e.g., locomotion), it is not uncommon to observe changes in firing rate of $> 100\%$ (Rosa-Kenig et al., 1993; Ball et al., 2003). Therefore, changes in firing rate of $> 50\%$ were considered a departure from baseline (Haracz et al., 1993; Ball et al., 2003), and units showing increases or decreases of this magnitude for at least three consecutive bins (15 min) were classified as excited or inhibited, respectively. If changes in activity did not reach 50% in either direction, a unit was classified as unresponsive. When analyzing electrophysiological data across treatment days, a Mann Whitney or Kruskal-Wallis test (followed by Dunn's multiple comparison test) was used when firing rates were not normally distributed. Cross-correlograms were constructed to assess the presence of pair-wise spike correlations over the 90-min post-drug period for MDMA-treated animals. All cross-correlograms were calculated for delays of ± 500 ms (bin = 10 ms). All pair-wise spikes with significant signs of deviations from independent firing patterns based on a 95% confidence limit were labeled as correlated. Differences in the frequency of correlated pair-wise spikes across days were evaluated with a Pearson Chi-Square test.

A trained observer, blind to the treatment conditions, viewed videotapes of experimental sessions and scored locomotion using event-recording software (BEST Collection, Educational Consulting Inc., Las Vegas, NV), as described previously (Ball et al., 2003; Ball and Rebec, 2005). To define baseline locomotion, the amount of time spent locomoting was quantified for the last 10 min of Period 2. To assess the level of MDMA-induced

locomotion, three, 10-min samples of locomotor activity were collected during early, middle, and late portions of Period 3 (corresponding to 5–15, 35–45, and 75–85 min post-challenge, respectively). Because statistical analysis revealed no treatment $\times$ time interaction, data from the three phases were collapsed for each day and analyzed by means of one-way or two-way ANOVAs and Bonferroni post-hoc tests.

2.6. Histology

Twenty-four h following the completion of the electrophysiological experiments rats were deeply anesthetized with urethane and marking lesions were made by applying a 7-s, 40 μ A current across those wires from which units were recorded. Rats were then perfused transcardially with 0.9% saline. Brains were removed and a mid-sagittal cut was made. The hemisphere in which electrode bundles were implanted was stored in formalin solution for at least 1 week. After storage in formalin, brains were stored in a 30% sucrose/10% formalin solution for at least 1 week, then frozen, cut (60 μ m sections), mounted on slides, and stained with cresylecht violet to verify recording sites.

The brain hemisphere contralateral to the side in which electrode bundles were implanted was processed using a modified Golgi method (Glaser and Van der Loos, 1981). We have used this modification of the Golgi-Cox method extensively to make reliable and quantitative assessments of alterations in dendritic arborization in a variety of cortical and subcortical areas due to a variety of manipulations (Harmon and Wellman, 2003; Churchill et al., 2004; Cook and Wellman, 2004; Works et al., 2004; Izquierdo et al., 2006; Wellman et al., 2007; Ball et al., 2009). The modification is relatively impervious to the types of fixation artifacts that can result in incomplete staining of distal dendritic processes and therefore inconsistencies in numbers of neurons stained across animals using other techniques such as rapid Golgi (Williams et al., 1978; Buell, 1982; Coleman and Flood, 1987). Brains were immersed in Golgi-Cox solution (a 1:1 solution of 5% potassium dichromate and 5% mercuric chloride diluted 4:10 with 5% potassium chromate) for 10 days [based on previous results (Ball et al., 2009), which showed complete staining for the majority of striatal cells]. Brains were then dehydrated and embedded in celloidin. Coronal sections were cut at 160 μ m on a sliding microtome (Leica Histoslide 2000, Wetzlar, Germany). Free-floating sections were then alkalinized, developed, fixed, dehydrated, cleared, mounted, and coverslipped [see (Glaser and Van der Loos, 1981)].

2.7. Dendritic reconstructions and spine counts

Medium spiny projection neurons from dorsal striatum were drawn. These neurons are readily identifiable in Golgi-stained material based on their characteristic morphology (12–20 μ m diameter cell body, dendrites that taper and become heavily laden with spines; see Fig. 1), which is distinct from the minority of other cell types in striatum (Wilson and Groves, 1980). Neurons selected for reconstruction were completely stained, located in the middle third of the section, did not have truncated branches, and were unobscured by neighboring neurons and glia, with dendrites that were easily discriminable by focusing through the depth of the tissue. Numbers of neurons meeting these criteria did not vary significantly across animals or groups. For each animal, seven neurons were drawn; for branch length, this number yielded a mean ($\pm$ S.E.M.) within-animal error of 8.66 ($\pm$ 0.70) % and 8.21 ($\pm$ 0.61) % for Experiments 1 and 2, respectively. All neurons were drawn at 600X magnification and morphology of dendritic arbors quantified in three dimensions using a computer-based neuron tracing system (Neurolucida, MicroBrightfield, Williston, VT), with the experimenter blind to condition. Several aspects of dendritic morphology were examined. To assess overall changes in dendritic morphology, total length and number of dendritic branches were compared between groups with an independent measures *t*-test and one-way ANOVA for Experiments 1 and 2, respectively. Differences in the amount and

location of dendritic material were assessed by counting the number of intersections of dendrites with an overlay of concentric rings centered on the soma (Sholl, 1956). These data were compared with a two-way, repeated measures ANOVA (treatment $\times$ distance from soma) followed by Bonferroni post-tests.

Spine density also was determined at various branch orders. For each animal, spines were counted from eight neurons; this number yielded a mean ($\pm$ S.E.M.) within-animal error of 8.41 ($\pm$ 0.84)% and 8.17 ($\pm$ 0.61)% for fourth order spine density in Experiments 1 and 2, respectively. For each neuron, one dendritic tree containing at least one first-, second-, and third-order dendrite was chosen (first-order dendrites were defined as branches that emerge directly from the soma). Previous results (Ball et al., 2009) indicated that 96% of medium spiny neuron dendrites were fourth order or less; therefore, only first through fourth order branches were included for analysis. One to two branches at each order [mean ($\pm$ S.E.M.) length = 20.85 ($\pm$ 0.62) μ m and 20.54 ($\pm$ 0.50) μ m for Experiments 1 and 2, respectively] were drawn and spines counted at 1000X magnification. Spine counts at each branch order began at the branch node. Branches chosen for analysis had $\sim$ 20 μ m segments that were unobscured by overlapping branches in the same plane of focus. Spines were identified based on the standard morphological criteria for “mushroom” and “thin” spines (Peters and Kaiserman-Abramof, 1970). Because spine density varies with thickness of the dendrite [and therefore branch order; (Wilson and Groves, 1980)], the lengths of dendritic sections at each order were recorded, and spine densities (spines/10 μ m) for each branch order were then calculated. Comparisons between groups were made by means of two-way, repeated measures ANOVAs (treatment $\times$ branch order) followed by Bonferroni post-tests. At the same time spines were counted, multiple-headed spines, defined as spines with two or more heads connected to a common shaft (Comery et al., 1996), also were counted. An independent measures *t*-test and one-way ANOVA (followed by Bonferroni post-tests) compared these data for Experiments 1 and 2, respectively.

3. Results

3.1. Electrophysiology: Experiment 1

3.1.1. Baseline—A total of 138 and 371 single units were recorded from animals in the saline and MDMA groups, respectively. Fig. 2 shows electrode-bundle placements in dorsal striatum (one animal in the MDMA group was excluded from analysis; see below). Baseline (i.e., Period 2) behavior in both the saline and MDMA groups consisted primarily of quiet rest with brief periods of locomotion or grooming. During this period, neurons exhibited firing rates (<7 spikes/s) and action potential waveforms (<2 ms; biphasic) typical of striatal units in conscious rats (Haracz et al., 1993; Haracz et al., 1998; Ball et al., 2003; Ball and Rebec, 2005; Ball et al., 2006). There were no significant differences in baseline locomotion across days in either saline or MDMA animals [$F(2, 10) = 0.74$, $p = 0.50$ and $F(2, 20) = 3.19$, $p = 0.06$ for saline and MDMA groups, respectively]; however, a two-way ANOVA indicated a significant main effect of treatment, $F(1, 15) = 10.93$, $p < 0.01$. The Bonferroni post-hoc test indicated that MDMA-treated animals displayed significantly more locomotion on Challenge day compared to saline-treated rats, likely indicating conditioned locomotor activity (see Fig. 3A). Overall neuronal firing rates decreased significantly by Challenge day in the saline group ($H = 7.12$, $p < 0.05$; see Fig. 3B). In the MDMA group, units were analyzed separately based on their response to MDMA. This analysis revealed that the baseline firing rates of units that subsequently showed an excitatory response to MDMA (hereafter referred to as “excited” neurons) were significantly higher ($H = 11.80$, $p < 0.05$) compared to earlier in the treatment regimen (see Fig. 3C). Baseline firing rates of units that subsequently displayed an inhibitory response to MDMA (hereafter referred to as

“inhibited” neurons) did not differ significantly across treatment days ($H = 2.01$, $p = 0.37$; see Fig. 3D).

3.1.2. Effects of MDMA—A sensitized locomotor response to MDMA was observed following MDMA challenge in 11 of 12 rats [$F(2, 10) = 4.23$, $p < 0.01$; see Fig. 4A]. The one animal in the MDMA group that did not display sensitization to MDMA was excluded from all analyses. Sensitization was defined as a $\geq 20\%$ increase in MDMA-induced locomotor activity on Challenge Day compared to Day 1. This is a standard criterion for drug sensitization studies (Bell and Kalivas, 1996; Pierce et al., 1996; Churchill et al., 1999). Locomotion ranged from 0.00 to 176.29, 30.45 to 224.67, and 23.34 to 292.60 s/30 min for Days 1, 5, and Challenge, respectively.

MDMA administration induced both striatal excitations and inhibitions, but excitations predominated across all treatment days (28, 51, and 46% excited and 6, 8, and 15% inhibited for Days 1, 5, and Challenge, respectively). As Fig. 4B illustrates, the magnitudes of neuronal excitation paralleled locomotor activity across treatment days. On Challenge day, there was a significant increase in the maximum firing rate of excited neurons compared to Day 1, $H = 11.60$, $p < 0.01$. When MDMA-induced increases in firing rate were calculated as a percentage of baseline firing rate, changes were similar across treatment days, and there were no significant differences in the maximum percentage increase in baseline firing rate across days, $H = 3.58$, $p = 0.17$. By comparing the mean firing rate of all excited units at early, middle, and late phases of the drug response with mean locomotor activation across treatment days, we found that there was a significant positive correlation between the magnitude of excitation and locomotion ($r = .84$, $p < 0.01$; see Fig. 4C). Mean ($\pm$ S.E.M.) magnitudes of excitation were 3.12 (± 0.71), 4.53 (± 0.61), and 5.13 (± 0.57) spikes/s for Days 1, 5, and Challenge, respectively. Fig. 4D shows the firing rates of inhibited neurons, which were similar across treatment days. There was no significant correlation between the firing rates of inhibited neurons and locomotion across treatment days (data not shown).

To determine the proportion of pair-wise spikes showing correlated activity (at ± 500 ms delay) we conducted a cross-correlation analysis; a large number of pair-wise comparisons was possible [mean ($\pm$ SEM) number of pair-wise comparisons/day = 729.33 (± 38.95)]. This analysis revealed that the proportion of pair-wise spikes showing correlated activity was significantly increased following the challenge injection compared to Day 1 [$\chi^2(1, n = 1382) = 7.12$, $p < .01$; see Fig. 4E]. The proportions of units showing correlated activity were 26%, 30%, and 32% for Days 1, 5, and Challenge, respectively. Examples of cross-correlograms showing correlated and non-correlated activity are shown in Fig. 4F.

3.2. Anatomy: Experiment 1

Six of the 12 MDMA-treated rats from Experiment 1 were randomly chosen for anatomical analysis (excluding the animal that did not display sensitization). Complete impregnation of numerous striatal medium spiny neurons was apparent. The dendritic arbors of all neurons selected were completely contained within a single section.

3.2.1. Effects of MDMA on striatal dendritic length, branching, and distribution

—To assess overall changes in dendritic length and branching, total length and number of dendritic branches were compared between groups. There were no significant group differences for either of these measures [$t(10) = 0.69$, $p = 0.51$ and $t(10) = 1.14$, $p = 0.28$ for length and number, respectively; see Fig. 5A, B]. To determine if MDMA treatment was associated with any long-term changes in the distribution of dendritic material, a Sholl analysis (Sholl, 1956) was performed and revealed no significant differences between treatment groups [$F(1, 10) = 0.38$, $p = 0.55$ and $F(5, 50) = 0.21$, $p = 0.96$ for main effect of

treatment and interaction, respectively in regard to number of intersections and $F(1, 10) = 0.47$, $p = 0.51$ and $F(5, 50) = 0.10$, $p = 0.99$ for main effect of treatment and interaction, respectively in regard to length; see Fig. 5C].

3.2.2. Effects of MDMA on striatal spine density—As expected, spine density varied significantly across branch orders for both treatment groups [main effect of branch order, $F(3,10) = 398.40$, $p < 0.0001$]. Spine density also varied significantly with treatment [main effect of treatment, $F(1,10) = 23.16$, $p < 0.001$]. MDMA treatment increased spine density across all branch orders. These differences were quite large 56%, 114%, 70%, and 20% increases for first-, second-, third-, and fourth-order branches, respectively (see Fig. 6). There also was a significant interaction [$F(3,10) = 10.10$, $p < 0.0001$] in that the effect of MDMA treatment on spine density was statistically significant for the more distal branches (second-, third-, and fourth-order) but not for first-order branches. MDMA treatment also resulted in a large increase in the overall number of multiple-headed spines [$t(10) = 2.88$, $p < 0.05$; see Fig. 6]. Compared to controls, MDMA-treated rats displayed increases in the density of multiple-headed spines of 71%. Fig. 6 shows photomicrographs of dendritic segments from striatal medium spiny neurons in saline- and MDMA-treated rats.

3.3. Electrophysiology: Experiment 2

3.3.1. Baseline—A total of 102 and 218 single units were recorded from animals in the saline and MDMA groups, respectively. Rats in the MDMA group were divided into two categories based on their behavioral response to MDMA challenge. Thus, animals that displayed sensitization (SENS group) and did not display sensitization (NOSENS) on Challenge Day were analyzed separately. Fig. 7 shows electrode-bundle placements in dorsal striatum for saline, SENS, and NOSENS animals. Baseline behavior for all groups consisted primarily of quiet rest with brief periods of locomotion or grooming. During this period, neurons exhibited typical firing rates of <7 spikes/s and biphasic, <2 ms action potential waveforms. There were no significant differences in baseline locomotion across days in any group ($F(2, 5) = 0.22$, $p = 0.81$, $F(2, 6) = 1.89$, $p = 0.19$, and $F(2, 4) = 0.22$, $p = 0.80$ for saline, SENS and NOSENS groups, respectively; see Fig 8A). The overall neuronal firing rate did not change significantly across treatment days in the saline group ($H = 0.17$, $p = 0.92$; see Fig. 8A). Also, there was no change in the baseline firing rate of either excited or inhibited neurons across treatment days in NOSENS animals ($H = 0.21$, $p = 0.90$ and $U = 7.00$, $p = 1.00$ for excited and inhibited neurons, respectively; see Fig. 8B). In SENS animals, however, the baseline firing rates of both excited and inhibited neurons were significantly lower ($H = 7.99$, $p < 0.05$ and $H = 6.22$, $p < 0.05$ for excited and inhibited neurons, respectively) compared to earlier in the treatment regimen (see Fig. 8C).

3.3.2. Effects of MDMA—MDMA administration resulted in increased locomotor activity compared to baseline across all treatment days in both SENS and NOSENS animals. As mentioned above, however, only SENS animals expressed behavioral sensitization on Challenge Day. Thus, in the SENS group ($n = 7$), there was significantly more MDMA-induced locomotor activity on Challenge day compared to either Day 1 or Day 5 [$F(2,6)=16.32$, $p<0.001$]. Conversely, in NOSENS animals ($n = 5$), MDMA-induced locomotor activity was significantly *lower* on Challenge Day compared to Day 1 [$F(2,4)=4.61$, $p<0.05$]. We have used this dose and treatment regimen previously (Ball et al., 2006) and found that the sensitization it produces is limited to increases in locomotor activity; thus, the treatment does not cause an increase in stereotypy. In the present study, observations of behavior verified that differences in locomotor activity between SENS and NOSENS animals were not due to differences in stereotypy. Locomotion ranged from 0.35 to 106.97, 53.07 to 138.40, and 71.93 to 217.85 s/30 min for Days 1, 5, and Challenge, respectively for SENS animals, and 83.14 to 358.44, 9.02 to 158.03, and 22.99 to 179.34 s/

30 min for Days 1, 5, and Challenge, respectively for NOSENS animals. Interestingly, a two-way ANOVA revealed an interaction such that the acute locomotor response to MDMA (Day 1) was significantly higher in NOSENS animals compared to SENS animals, whereas on Challenge Day, MDMA-induced locomotion was higher in the SENS group compared to the NOSENS group [$F(2,10) = 15.71, p < 0.0001$; see Fig. 9A].

As in Experiment 1, MDMA administration induced both striatal excitations and inhibitions, but excitations predominated across all treatment days in both SENS (54, 44, and 43% excited and 27, 22, and 23% inhibited for Days 1, 5, and Challenge, respectively) and NOSENS (55, 83, and 64% excited and 13, 3, and 11% inhibited for Days 1, 5, and Challenge, respectively) groups. As Fig. 9D illustrates, however, the magnitudes of neural excitation were lower on Challenge Day compared to Days 1 and 5 in the SENS group. Moreover, there was a significant decrease in the maximum firing rate of excited neurons on Challenge day compared to Day 5, $H = 7.63, p < 0.05$. Also, there was a significant negative correlation between the magnitude of excitation and locomotion across treatment days ($r = -0.78, p < 0.05$; see Fig. 9E). As in Experiment 1, when MDMA-induced increases in firing rate were calculated as a percentage of baseline firing rate, changes were similar across treatment days, and there were no significant differences in the maximum percentage increase in baseline firing rate across days, $H = 0.43, p = 0.81$. Conversely, in the NOSENS group, magnitudes of excitation did not change across treatment days when calculated as absolute firing rate ($H = 1.11, p = 0.57$; Fig. 9B) or percentage of baseline firing rate ($H = 1.81, p = 0.40$). Mean ($\pm$ S.E.M.) magnitudes of excitation were $4.93 (\pm 1.37)$, $4.23 (\pm 0.52)$, and $2.02 (\pm 0.45)$ spikes/s for Days 1, 5, and Challenge, respectively in the SENS group, and $3.92 (\pm 0.70)$, $4.01 (\pm 0.63)$, and $4.50 (\pm 0.61)$ spikes/s for Days 1, 5, and Challenge, respectively in the NOSENS group. In both the SENS and NOSENS groups, magnitudes of inhibition were similar across treatment days (see Fig. 9C, F).

Cross-correlation analysis revealed that the proportion of pair-wise spikes [mean ($\pm$ SEM) number of pair-wise comparisons/day = $154.67 (\pm 15.17)$] showing correlated activity was significantly increased following the challenge injection compared to Day 5 in SENS animals [$\chi^2(1, n = 339) = 5.16, p < .05$; see Fig. 10]. The proportions of units showing correlated activity were 18%, 13%, and 23% for Days 1, 5, and Challenge, respectively in the SENS group. Conversely, in NOSENS animals, the prevalence of correlated pair-wise spikes [mean ($\pm$ SEM) number of pair-wise comparisons/day = $245.67 (\pm 49.46)$] was not significantly different across the course of drug treatment (see Fig. 10). The proportions of units showing correlated activity were 16%, 21%, and 20% for Days 1, 5, and Challenge, respectively in the NOSENS group.

3.4. Anatomy: Experiment 2

All rats from Experiment 2 were included for anatomical analysis. In all groups, complete impregnation of numerous striatal medium spiny neurons was apparent. The dendritic arbors of all neurons selected were completely contained within a single section.

3.4.1. Effects of MDMA on striatal dendritic length, branching, and distribution

—There were no significant group differences in total length or number of dendritic branches ($F(2, 15) = 0.71, p = 0.51$ and $F(2, 15) = 0.25, p = 0.78$ for length and number, respectively; see Fig. 11B). The Sholl analysis revealed no significant differences in dendritic organization between groups [$F(2, 15) = 0.97, p = 0.40$ and $F(10, 75) = 0.96, p = 0.48$ for main effect of treatment and interaction, respectively in regard to number of intersections and $F(2, 15) = 0.71, p = 0.51$ and $F(10, 75) = 1.43, p = 0.19$ for main effect of treatment and interaction, respectively in regard to length; see Fig. 11C].

3.4.2. Effects of MDMA on striatal spine density—Spine density varied significantly across branch orders for all groups [main effect of branch order, $F(3,15) = 439.10$, $p < 0.0001$]. Spine density also varied significantly with treatment [main effect of treatment, $F(2,15) = 8.15$, $p < 0.01$]. MDMA treatment increased spine density across all branch orders, with 100%, 29%, 45%, and 26% increases for first-, second-, third-, and fourth-order branches, respectively in the NOSENS group and 94%, 32%, 55%, and 20% increases, respectively in the SENS group (see Fig. 12). As in Experiment 1, the effect of MDMA treatment on spine density was statistically significant for the more distal branches (third- and fourth-order) but not for first- and second-order branches [treatment $\times$ branch order interaction, $F(6,15) = 3.67$, $p < 0.01$]. MDMA treatment also resulted in a large increase in the overall number of multiple-headed spines [$F(2,15) = 3.68$, $p < 0.05$; see Fig. 12]. Compared to controls, MDMA-treated rats displayed increases in the density of multiple-headed spines of 70% and 49% for NOSENS and SENS animals, respectively; however, the post-hoc test revealed that only the NOSENS group was significantly different from saline ($p < 0.05$). Fig. 12 shows photomicrographs of dendritic segments from striatal medium spiny neurons in saline-treated rats and MDMA-treated rats in the NOSENS and SENS groups.

3.5. Additional Analyses

Although it is possible that the lack of expression of sensitization in NOSENS animals was not due to the context change on challenge day, this possibility is very unlikely. Thus, a chi square analysis indicated a $< 6\%$ chance occurrence of the observed difference in the proportion of sensitized animals in Experiment 2 compared to Experiment 1, $\chi^2(1, n = 24) = 3.56$, $p = .059$. Also, because NOSENS animals could be separated from SENS animals based on their larger locomotor response to acute MDMA administration, we performed an additional analysis in which animals in Experiment 1 were separated into low and high acute response groups based on the median locomotor response to MDMA on Day 1 of injections ($n = 5/\text{group}$), and their locomotor responses to MDMA were compared across treatment days. As shown in Fig. 13, this analysis revealed that both low and high acute responders displayed locomotor sensitization on challenge day when the context did not change. In fact, high acute responders displayed a larger locomotor response on challenge day than did low acute responders. Therefore, it appears that, following the development of sensitization to MDMA, a context change on challenge day (Experiment 2) does, in fact, bring about individual differences in the expression of sensitization that are related to the magnitude of responding to acute MDMA administration.

4. Discussion

A sensitized locomotor response was observed following MDMA challenge in the vast majority of rats (11 of 12) when the context remained constant, whereas only 58% of rats (7 of 12) displayed sensitization when the context was changed on challenge day. Our pattern of results suggests that the decrease in the proportion of animals expressing sensitization in the latter group is directly related to the context change on challenge day. Consistent with the effects of MDMA in NAc (Ball et al., 2009), MDMA treatment was associated with large increases in spine density and the number of multiple-headed spines on striatal medium spiny neurons. Changes in striatal electrophysiology, moreover, were predictive of the expression of behavioral sensitization, extending our previous findings (Ball et al., 2006) implicating dorsal striatum in behavioral sensitization to MDMA.

Exposure to either explicitly drug-unpaired contexts (Stewart and Vezina, 1991) or novel, non-drug contexts (Anagnostaras et al., 2002) has been shown to inhibit the expression of behavioral sensitization to AMPH, a phenomenon called conditioned inhibition (Stewart and

Vezina, 1988, 1991). In the present study, a context change on challenge day unmasked individual differences in rats' expression of MDMA-induced sensitization that were not apparent when the context was held constant. Interestingly, we found that NOSENS animals could be separated from SENS animals based on their larger locomotor response to acute MDMA administration. The reason for this is not clear, but we hypothesize that the larger acute psychomotor response in NOSENS animals facilitated the formation of a stronger association between the unconditioned drug effects and the paired environmental context. Because a larger locomotor response to MDMA in NOSENS animals was not apparent at Day 5 of injections it is possible that a drug-free withdrawal period is necessary for this association to develop fully. In line with the conditioned inhibition hypothesis (Stewart and Vezina, 1988, 1991), a context change on challenge day, therefore, would be expected to exert a greater inhibitory effect on drug-related behaviors in those strongly conditioned subjects. In support of this hypothesis, it was recently shown that the induction of retrograde amnesia for a previous context-specific drug experience reversed the inhibitory effect of a context change such that robust sensitization was expressed in an unpaired environment (Anagnostaras et al., 2002).

We found that only SENS animals displayed a decreased basal firing rate of excited striatal neurons in the non-drug-paired context, whereas sensitized rats in Experiment 1 (drug-paired context) displayed an increased basal firing rate for the same category of neurons. These changes in baseline firing rate were associated with subsequent decreases and increases, respectively, in the magnitude of MDMA-induced excitations. Importantly, NOSENS animals showed no significant changes in striatal activity across treatment days, providing compelling evidence that alterations in synaptic transmission in dorsal striatum contribute specifically to behavioral sensitization, and are not merely the result of drug exposure. The finding that sensitization-related changes in striatal electrophysiology occurred only in cells responsive to MDMA suggests that a distinct population of neurons contributes to drug-induced behavioral adaptations, which is consistent with our previous results using MDMA (Ball et al., 2006), as well as a recent report of changes in medial prefrontal cortical activity following sensitizing regimens of AMPH (Gulley and Stanis, 2009).

Previously, we reported that sensitized rats displayed a decreased basal firing rate in excited neurons in the MDMA-paired context following a short withdrawal period [~ 4 days (Ball et al., 2006)]. The mechanism underlying such a transition from a decreased to an increased basal firing rate in a drug-paired context after extended withdrawal is unknown. Moreover, it is not clear why a change in the signal-to-noise ratio (i.e., a decrease in baseline firing rate, but not firing rate following MDMA administration) correlated with sensitization in our previous study (Ball et al., 2006), whereas a change in *absolute* firing rate, but not signal-to-noise ratio, correlated with sensitization in the present study. These findings emphasize that drug-induced neuroadaptations are highly dynamic and can vary based on the time point during which measurements are obtained. Indeed, the surface expression of GluR1-containing AMPA receptors in NAc was found to be significantly decreased following one day of withdrawal from self-administered cocaine, but significantly increased by withdrawal day 21 (Conrad et al., 2008). Because cocaine, like MDMA, induces relatively large increases in extracellular 5-HT and DA concentrations (Bradberry et al., 1993; Teneud et al., 1996), these time-dependent changes in AMPA receptor expression are a possible explanation for our electrophysiological findings. Such an explanation is tempered, however, by evidence that MDMA's parent drug, AMPH, appears to have very different effects on striatal AMPA receptor expression compared to cocaine (Nelson et al., 2009). Finally, it is not clear why opposite changes in striatal activity would characterize a sensitized response in different contexts, but it is noteworthy that opposite changes in NAc morphology are associated with associative vs. context-independent sensitization [(Singer et

al., 2009); see Discussion below]. Further studies employing techniques that allow for the simultaneous recording of behavior and neuronal activity will be necessary for a more complete understanding of the mechanisms underlying dynamic drug-induced changes in striatal activity and their contribution to behavioral sensitization.

An electrophysiological correlate of sensitization, regardless of context, was an increase in the proportion of pair-wise spikes showing correlated activity following MDMA challenge. Temporal coordination between the firing of neuron pairs or spike correlations appear to be important for the encoding of information in cell assemblies (Sakurai, 1999; Salinas and Sejnowski, 2001) and are thought to reflect a highly synchronized network state (Schneidman et al., 2006). Abnormalities in spike correlations have been implicated in a variety of brain disorders, including schizophrenia, epilepsy, autism, Alzheimer's disease, Huntington's disease, and Parkinson's disease (Uhlhaas and Singer, 2006; Hammond et al., 2007; Miller et al., 2008; Miller et al., 2010). Although it is known that increased glutamatergic activity in striatum drives both behavioral sensitization (Bell and Kalivas, 1996; Pierce et al., 1996) and drug seeking (Cornish and Kalivas, 2000; Park et al., 2002; Sari et al., 2009) our results suggest that, in addition, increased *correlated* activity in striatum may underlie these behaviors. Such correlated striatal activity may serve to increase the salience of drugs or drug-related cues in recovering addicts, thereby increasing the chance of relapse. It is noteworthy that the increase in spike correlations we observed does not simply reflect behavioral feedback due to increased locomotor activity. Indeed, loss of voluntary movement (akinesia) in Parkinson's disease is associated with *increased* correlated activity in basal ganglia (Brown et al., 2001; Williams et al., 2002). Primate models of Parkinson's disease also have shown that normally low levels of correlated activity in striatum (Lebedev and Nelson, 1999; Raz et al., 2000) are increased following DA depletion (Raz et al., 2000; Raz et al., 2001). Finally, correlated activity in striatum is suppressed during a visuomotor task in behaving monkeys (Courtemanche et al., 2003). Thus, our results suggest that increased coordination of striatal spike activity may be a mechanism driving lasting changes in behavior such as sensitization and, perhaps, addiction.

AMPH-like stimulants, including MDMA, can produce enduring changes in the structure of forebrain neurons (Robinson and Kolb, 2004; Ball et al., 2009). Moreover, these changes are sometimes strikingly subregion specific (Jedynak et al., 2007; Ball et al., 2009). Although the exact way in which these structural changes contribute to drug-induced behavioral plasticity is not known, certain changes are related to the expression of sensitization. For example, Li et al. (2004) reported that a treatment regimen of cocaine administered in a novel test cage induced robust sensitization and also increased spine density in NAc core and shell, but the same regimen of cocaine administered in the home cage failed to induce sensitization and increased spine density only in NAc shell. Extending these findings, it was recently shown that only systemic AMPH administration that produced conditioned locomotion in a drug-paired context resulted in increased spine density in NAc (Singer et al., 2009); conversely, intra-ventral tegmental area injections of AMPH, which produced locomotor sensitization, but not conditioned locomotion, resulted in *decreased* spine density in NAc. This pattern of results suggests that increases in spine density underlie associative drug conditioning, but are not required for purely pharmacological sensitization. Our results, moreover, suggest that altered excitatory transmission as a result of structural changes is subject to context-dependent modification in some animals. Indeed, despite similar increases in striatal spine density, we found very different striatal electrophysiological characteristics between SENS and NOSENS animals. Increases in spine density in striatal regions, therefore, may be necessary, but not sufficient, to drive sensitization under certain conditions. In fact, since increases in spine density appear to reflect associative learning (Leuner et al., 2003; Singer et al., 2009), it is conceivable that animals displaying greater increases in spine density would be *less* likely to display sensitization when the context is

changed due to conditioned inhibitory mechanisms (see Discussion above). It is noteworthy that although we observed similar increases in spine density in SENS and NOSENS animals only NOSENS animals displayed a significant increase in multiple-headed spines. Considering the putative role of multiple-headed spines in synaptic plasticity and learning (Geinisman et al., 1989; Comery et al., 1996; Andersen and Soleng, 1998), this finding is consistent with the hypothesis that strong associative learning between drug and context should inhibit the expression of sensitization when the context is changed.

4.1. Conclusion

We found that a change to a new context following the development of sensitization unmasked individual differences in the expression of MDMA-induced sensitization, and that these differences correlated with specific changes in dorsal striatal functioning. Given that dorsal striatum is a brain region implicated in the formation of persistent drug-related habits (Robbins and Everitt, 1999; Gerdeman et al., 2003), an investigation into how these distinct neurobehavioral responses are related to differences in drug-seeking behaviors will be an important focus of future studies.

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Abbreviations

AMPH	amphetamine
DA	dopamine
MDMA	3,4-methylenedioxymethamphetamine
NAc	nucleus accumbens
SENS	group of animals that expressed sensitization on challenge day in Experiment 2
5-HT	serotonin
NOSENS	group of animals that did not express sensitization on challenge day in Experiment 2

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Figure 1. Digital light micrograph of Golgi-stained medium spiny neuron in the dorsal striatum of a saline-treated rat from Experiment 1. Four photomicrographs were taken at different Z-levels and merged to increase the number of dendritic branches in focus. Scale bar = 50 μ m.

Figure 2. Schematic illustration [modified from (Paxinos and Watson, 1998)] of coronal sections from the rat brain showing approximate location of electrode-bundle tips in dorsal striatum (gray shading). Circles and squares represent placements for saline and MDMA groups, respectively.

Figure 3.

(A) Baseline locomotor activity in saline and MDMA groups. There were no significant differences in baseline locomotion across days in either treatment group; however, MDMA-treated animals displayed significantly more locomotion on Challenge day compared to saline-treated rats. Bars represent mean (+ SEM). * $p < 0.05$. (B) Baseline firing rate of all units in saline group. There was a significant decrease in the overall firing rates on Challenge Day compared to Day 1. Bars represent mean (+ SEM). * $p < 0.05$. (C and D) Baseline firing rates for all units that subsequently showed an excitatory (C) or inhibitory (D) response to MDMA. There was a significant increase in the baseline firing rate of excited neurons on Challenge Day compared to earlier treatment days. Bars represent mean (+ SEM). ** $p < 0.01$ compared to Day 1. # $p < 0.05$ compared to Day 5.

Figure 4.

(A) MDMA-induced locomotion across treatment days. A sensitized locomotor response was observed on Challenge Day compared to both Day 1 and Day 5. Bars represent mean (+ SEM). $**p < 0.001$ compared to Day 1. $\# p < 0.05$ compared to Day 5. (B) Magnitude of excitation for all units classified as excited by MDMA. Curves represent mean (+ SEM) firing rate of excited units for each 5-min bin beginning 5 min after MDMA injection. $*p < 0.01$ when comparing maximum firing rates. (C) There was a significant positive correlation between the firing rate of excited units and locomotor activation across treatment days ($p < 0.01$). Points represent mean firing rate of excited units and mean locomotion for early, middle, and late periods and for each treatment day. (D) Magnitude of inhibition for all units classified as inhibited by MDMA. Curves represent mean (+ SEM) firing rate of inhibited units for each 5-min bin beginning 5 min after MDMA injection. (E) Proportion of all possible single-unit pairs showing correlated activity at ± 500 ms delay. As determined by means of a Pearson Chi-Square test, the proportion of pair-wise spikes showing correlated

activity was significantly increased following the challenge injection compared to earlier injections. $*p < 0.01$ compared to Day 1. (F) Representative cross-correlation histograms depicting either correlated unit pairs (top row) or uncorrelated unit pairs (bottom row). Note the peaks that exceed the 95% confidence intervals in the top two histograms indicating correlated unit activity.

Figure 5.

(A) Computer-assisted reconstructions of representative dorsal striatal medium spiny neurons 24 h after the last saline (left) or MDMA challenge (right) injection. Scale bar = 25 μm . (B) Mean (+ SEM) branch length and number for dorsal striatal medium spiny neurons in saline- and MDMA-treated rats. No significant differences between groups were observed for either of these measures. (C) Mean (+ SEM) intersections of dendrites with and length of dendrites within 10 μm concentric spheres (data have been summed into six bins). There were no significant differences in dendritic organization between saline- and MDMA-treated animals.

Figure 6.

(Top, left) Mean ($\pm$ SEM) spine density on first-, second-, third-, and fourth-order branches of medium spiny neurons in dorsal striatum 24 h after the last saline or MDMA challenge injection. MDMA-treated rats displayed increases in spine density across all branch orders (main effect of treatment, $p < 0.001$). There also was a significant interaction ($p < 0.0001$) in that the increases in spine density were larger on second-, third-, and fourth-order branches compared to first-order branches. ** $p < 0.01$. *** $p < 0.001$. (Top, right) Overall density of multiple-headed spines in saline- and MDMA-treated rats. MDMA treatment resulted in a large increase in the overall number of multiple-headed spines. Bars represent mean ($\pm$ SEM). * $p < 0.05$. (Bottom) Photomicrographs of Golgi-stained dendritic segments from dorsal striatal medium spiny neurons in saline- and MDMA-treated rats. Several photomicrographs were taken at different Z-levels and merged to increase the number of spines in focus. Scale bar = 10 μm .

Figure 7. Schematic illustration [modified from (Paxinos and Watson, 1998)] of coronal sections from the rat brain showing approximate location of electrode-bundle tips in dorsal striatum (gray shading). Circles, squares, and diamonds represent placements for saline, NOSENS, and SENS groups, respectively.

Figure 8.

(A, left) Baseline locomotor activity in saline, SENS, and NOSENS groups. There were no significant differences in baseline locomotion across days in any group. Bars represent mean (+ SEM). (A, right) Baseline firing rate of all units in saline group. There were no significant differences in the overall firing rates across treatment days. Bars represent mean (+ SEM). (B and C) Baseline firing rates for all units that subsequently showed an excitatory (left) or inhibitory (right) response to MDMA in NOSENS and SENS groups. There were not a sufficient number of inhibited units for analysis on Day 5 in the NOSENS group. In SENS animals only, there was a significant decrease in the baseline firing rate of both excited and inhibited neurons on Challenge Day compared to earlier treatment days. Bars represent mean (+ SEM). * $p < 0.05$ compared to Day 5.

Figure 9.

(A) MDMA-induced locomotion across treatment days. In SENS animals, a sensitized locomotor response was observed on Challenge Day compared to both Day 1 and Day 5 ($p < 0.001$ and $p < 0.01$, respectively). In NOSENS animals, MDMA-induced locomotion was significantly lower on Challenge Day compared to Day 1 ($p < 0.05$). The acute locomotor response to MDMA (Day 1) was significantly higher in NOSENS animals compared to SENS animals, but on Challenge Day, MDMA-induced locomotion was higher in the SENS group compared to the NOSENS group. $p < 0.01$. (B) Magnitude of excitation for all units classified as excited by MDMA in the NOSENS group. (C) Magnitude of inhibition for all units classified as inhibited by MDMA in the NOSENS group. (D) Magnitude of excitation for all units classified as excited by MDMA in the SENS group. $p < 0.05$ when comparing

maximum firing rates. (E) There was a significant negative correlation between the firing rate of exited units and locomotor activation across treatment days in the SENS group only ($p < 0.05$). (F) Magnitude of inhibition for all units classified as inhibited by MDMA in the SENS group.

Figure 10.

Proportion of all possible single-unit pairs showing correlated activity at ± 500 ms delay. As determined by means of a Pearson Chi-Square test, the proportion of pair-wise spikes showing correlated activity was significantly increased following the challenge injection compared to earlier injections in the SENS group. In the NOSENS group, the prevalence of correlated pair-wise spikes was not significantly different across the course of drug treatment. * $p < 0.05$ compared to Day 5.

Figure 11.

(A) Computer-assisted reconstructions of representative dorsal striatal medium spiny neurons 24 h after the last saline (left) or MDMA challenge injection in NOSENS and SENS groups (middle and right, respectively). Scale bar = 25 μm . (B) Mean (+ SEM) branch length and number for dorsal striatal medium spiny neurons in saline, NOSENS, and SENS groups. No significant differences between groups were observed for either of these measures. (C) Mean (+ SEM) intersections of dendrites with and length of dendrites within 10 μm concentric spheres (data have been summed into six bins). There were no significant differences in dendritic organization between any groups.

Figure 12.

(Top, left) Mean (+ SEM) spine density on first-, second-, third-, and fourth-order branches of medium spiny neurons in dorsal striatum 24 h after the last saline or MDMA challenge injection. MDMA-treated rats in both the NOSENS and SENS groups displayed increases in spine density across all branch orders (main effect of treatment, $p < 0.01$). There also was a significant interaction ($p < 0.01$) in that the increases in spine density were larger on third- and fourth-order branches compared to first- and second-order branches. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ compared to saline. (Top, right) Overall density of multiple-headed spines in saline, NOSENS, and SENS groups. MDMA treatment resulted in a large increase in the overall number of multiple-headed spines in both the NOSENS and SENS groups; however, the post-hoc test revealed that only the NOSENS group was significantly different from saline. All bars represent mean (+ SEM). $*p < 0.05$ compared to saline. (Bottom) Photomicrographs of Golgi-stained dendritic segments from dorsal striatal medium spiny neurons in saline-treated, NOSENS, and SENS rats. Several photomicrographs were taken at different Z-levels and merged to increase the number of spines in focus. Scale bar = 10 μm .

Figure 13.

MDMA-induced locomotion across treatment days in low and high acute responders from Experiment 1. Animals in Experiment 1 were separated into low and high acute response groups based on the median locomotor response to MDMA on Day 1 of injections ($n = 5/\text{group}$). This resulted in a significant difference in the acute locomotor response between low and high responder groups ($*p < 0.01$). Locomotor sensitization was evident in both groups on Challenge Day and on Day 5 in the low acute responder group ($\#p < 0.05$ compared to Day 1).