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NEUROPLASTICITY IN THE MESOLIMBIC SYSTEM INDUCED BY NATURAL REWARD AND SUBSEQUENT REWARD ABSTINENCE

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Abstract

BACKGROUND—Natural reward and drugs of abuse converge on the mesolimbic system, where drugs of abuse induce neuronal alterations. Here, we tested plasticity in this system following natural reward and the subsequent impact on drug responses.

METHODS—Effects of sexual experience in male rats on behavioral sensitization and conditioned place preference associated with d-amphetamine, and Golgi-impregnated dendrites and spines of nucleus accumbens cells were determined. Moreover, the impact of abstinence from sexual behavior in experienced males on these parameters was tested.

RESULTS—First, repeated sexual behavior induced a sensitized locomotor response to d-amphetamine compared to sexually naïve controls observed 1, 7, and 28 days following last mating session. Second, sexually experienced animals formed a conditioned place preference for lower doses of d-amphetamine than sexually naïve males, indicative of enhanced reward value of d-amphetamine. Finally, Golgi-Cox analysis demonstrated increased numbers of dendrites and spines in the nucleus accumbens core and shell with sexual experience. The latter two alterations were dependent on a period of abstinence of 7–10 days.

CONCLUSIONS—Sexual experience induces functional and morphological alterations in the mesolimbic system similar to repeated exposure to psychostimulants. Moreover, abstinence from sexual behavior following repeated mating was essential for increased reward for drugs and dendritic arbors of NAc neurons, suggesting that the loss of sexual reward may also contribute to neuroplasticity of the mesolimbic system. These results suggest that some alterations in the mesolimbic system are common for natural and drug reward, and may play a role in general reinforcement.

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Keywords

dopamine; nucleus accumbens; psychostimulant; sexual behavior; substance abuse; dendritic spine

INTRODUCTION

The mesolimbic dopamine (DA) system, consisting of dopaminergic neurons in the ventral tegmental area (VTA) with projections to the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC), plays a critical role in the motivating and rewarding aspects of behavior including aggression (1), feeding (2–7), drinking (8), mating (9–11) and social bonding (12–13). Drugs of abuse converge upon the mesolimbic DA system (14–15). Moreover, repeated drug administration can induce neuronal alterations in these pathways, that in turn play a putative role in increasing the susceptibility to drug relapse, or in the transition from drug use to drug addiction (16–18). The behavioral effects of repeated drug administration include a sensitized locomotor response to psychostimulants and opiates (19–21), an enhanced conditioned drug reward (22–24), and increased operant responses for cues associated with prior drug intake (25). Furthermore, repeated drug administration results in long-lasting changes in dendritic morphology and spine density throughout the mesolimbic circuit (16, 26–31), and induces gene expression changes (32–35). Finally, repeated drug administration alters synaptic strength at excitatory and inhibitory synapses on midbrain dopamine neurons (36–41), and neurons in the NAc (42–44). It is currently unclear if similar alterations in the mesolimbic system occur with repeated exposure to natural rewards. Determining whether such alterations overlap with or are unique to drugs of abuse may lead to a better understanding of the cellular mechanisms underlying the differences between normal reward reinforcement versus compulsive seeking of a particular reward.

Supporting the hypothesis that stimuli other than drugs can cause neuronal alterations in the mesolimbic system are findings that stressful stimuli activate dopamine systems (45–47), and cause psychomotor stimulant sensitization (21,48–50) and relapse in self-administration models (51–54). However, few studies have investigated whether natural rewarding behaviors can also produce functional changes in the DA system (6,55–56). Therefore, the hypothesis was tested that male sexual experience causes neuronal alterations within the mesolimbic DA system via analysis of effects of sexual experience on locomotor sensitization, conditioned place preference, and dendrite morphology of NAc neurons. Furthermore, we hypothesized that an abstinence period from sexual behavior (sexual reward) is critical for the onset of these changes, based on recent observations that abstinence from drugs play a key role in the development of neural plasticity associated with repeated drug exposure (40,57–59).

METHODS

Animals

Adult male Sprague Dawley rats (210–250 grams) were obtained from Harlan Laboratories (Indianapolis, IN, USA) or Charles River Laboratories (Senneville, QC, Canada) and housed in Plexiglas cages with tunnel tubes. Males were housed in same sex pairs throughout the experiments (experiments 2–5), except for experiment 1 in which males were single housed at the onset of the study. The temperature-regulated colony room was maintained on 12/12 hr light dark cycle with food and water available *ad libitum* except during behavioral testing. Stimulus females (210–220 grams) for mating behavior sessions were bilaterally ovariectomized and received a subcutaneous implant containing 5% estradiol benzoate and 95% cholesterol. Sexual receptivity was induced by administration of 500μg progesterone in 0.1 ml sesame oil approximately 4 hours before testing. All procedures were approved by the

Animal Care and Use Committees of the University of Cincinnati and the University of Western Ontario, and conformed to NIH and CCAC guidelines involving vertebrate animals in research.

Drug Treatment

D-Amphetamine (AMPH) sulfate (Sigma, St. Louis, MO) was dissolved in sterile 0.9% saline (SAL). Animals received AMPH doses ranging 0.5–5.0 mg/kg body weight, calculated based upon the free base, in a volume of 1mL/kg body weight. Control animals received SAL. All injections were given subcutaneously during the first half of the light phase (2–6 hours after lights on), immediately prior to placement into the behavioral apparatus.

Locomotor Activity Testing

Locomotor activity was measured using custom-designed locomotor activity chambers (LACs), modeled on chambers designed by Segal and Kuczenski (60). Locomotor activity was measured using a 16×16 photobeam array (San Diego Instruments, San Diego, CA) and expressed as crossovers per minute(s). A crossover was recorded each time the animal entered any of the "active zones" of the chamber, depicted as shaded areas in Figure 1A (61).

Sexual behavior testing

In all experiments, sexually naïve male rats were randomly divided into groups that either gained sexual experience or remained naïve. For experience, all mating tests were conducted during the first half of the dark phase (3–8 hours after lights off) under dim red light. Animals that remained sexually naïve were handled and housed in the same rooms as sexually experienced males, hence exposed to similar levels of disturbance, environment novelty and distant female odors as experienced animals. For all experiments, groups of sexually experienced males were matched for sexual experience (based on numbers of ejaculations, and latencies to ejaculation and intromission during last mating session).

Experiment 1—Experiments 1 and 2 utilized different paradigms to test the effects of intermittent mating and environment. In experiment 1, animals in the sexually experienced groups received 5 intermittent mating sessions spaced 3–4 days apart, during which they mated in their home cages with a receptive females for 3 copulatory series (including ejaculation) or 60 minutes, whichever came first. Animals that completed more than five cumulative copulatory series were considered sexually experienced. Sexually naïve animals did not receive female partners. One week following the last mating session, sexually experienced and naïve animals were subdivided into groups receiving AMPH (0.5 mg/kg) or SAL for a total of four groups (Naïve Amphetamine: NA; Experienced Amphetamine: EA; Naïve Saline: NS; and Experienced Saline: ES; n = 6 each).

Experiment 2—This experiment differed from experiment 1 in three ways: 1. Animals mated to one ejaculation during consecutive days; 2. Animals mated in the same cage as in which they received AMPH (in the LACs); 3. Locomotor activity following AMPH was analyzed at three different times following sexual experience. The sexually experienced animals received 7 consecutive daily mating sessions in the LACs and locomotor activity was recorded during the 15 minutes between placement in LACs and introduction of female. The sexually naïve animals were placed in the LACs for seven consecutive sessions without mating. The day following the final mating session (Day 8 of the experiment), animals were placed in the LACs immediately following injection of AMPH (0.5 mg/kg) or SAL (Naïve Amphetamine: NA; Experienced Amphetamine: EA; Naïve Saline: NS; and Experienced Saline: ES; n = 8–9 each) and locomotor activity was recorded. The animals were tested in the LACs again one week following the final mating session (Day 14). Animals that received AMPH on Day 8 received SAL on Day 14, and animals that received SAL on Day 8 received AMPH on Day 14. Half of

the naïve and experienced animals were sacrificed one day later for RNA extraction (data not included in this report). One month following the final mating session (Day 35), the remaining half of the animals (Naïve, n=8; Experienced, n=9) received AMPH and locomotor activity was recorded.

Data Analysis

Locomotor Activity—Data were collected in 3-minute bins for 90 minutes following AMPH or SAL injection. Results are shown as the mean ± SEM for each group and analyzed using two-way ANOVA (experiment 1, experiment 2 days 8–14: factors: sexual experience, drug treatment), or one-way ANOVA (experiment 2 day 35 and activity before mating sessions; factor: sexual experience). *Post-hoc* comparisons were made using Fisher LSD tests with significance set at p-value < 0.05.

Conditioned Place Preference (CPP) Testing

Apparatus—CPP was performed in a three-compartment apparatus (Med Associates Inc., St. Albans, VT, USA) which consisted of two larger, outer chambers (28 × 22 × 21cm) distinguishable by visual and tactile cues, separated by a small central compartment (13 × 12 × 21cm). The apparatus was equipped with photo-beams for automated analysis of tracking and measuring locomotor activity.

Conditioning and Testing—CPP conditioning and testing was conducted during the first half of the light period. A pretest was conducted to determine each animal's initial preference. No significant differences were detected between the times spent in either chamber. On the following day, the male rats were either confined to the AMPH-paired chamber or to the SAL-paired chamber for 30 minutes. Rats received the opposite treatment the following day in a counterbalanced manner. A posttest that was procedurally identical to the pretest was conducted on the final day.

Experiment 3—Animals in the sexually experienced groups received 5 consecutive daily mating sessions in test cages. Day 1 was assigned to the first mating day. Control males remained sexually naïve, but were placed into a clean test cage for 1 hour each day for 5 consecutive days. Animals were divided into groups receiving different doses of AMPH (mg/kg; s.c.) (Naïve: N0.5, N1.0, N2.5 or N5.0, n = 7–8 each; Experience: E0, E0.5, E1.0, E2.5 or E5.0, n = 6–9 each). Pretest occurred on day 14, conditioning trials on days 15 and 16, and posttest on day 17. This schedule allowed for 10 days of abstinence from sexual behavior before conditioning.

Experiment 4—Sexually experienced males gained sexual experience through 5 consecutive days of mating identical to Experiment 3. The key difference with experiment 4 was that CPP testing occurred while animals were gaining sexual experience, thus there was no period of abstinence from sexual behavior. Instead, conditioning trials began following the first 3 mating sessions. Animals were divided into groups receiving different doses of AMPH (mg/kg; s.c.) (Naïve: N0.5, N1.0, N2.5 or N5.0, n = 6–8 each; Experience: E0, E0.5, E1.0, E2.5 or E5.0, n = 7–11 each).

Data Analysis—CPP scores were calculated for each an imal as the time spent (sec) in the paired chamber during the posttest minus the pretest. Group means were calculated and compared to the SAL-treated group (E0) using unpaired t-tests. For all experiments significance was set at a p-value < 0.05.

Golgi Experiment

Experiment 5—Males in the sexually experienced groups were placed in a test cage with a receptive female and allowed to mate until one ejaculation or 60 minutes, whichever occurred first, during 7 consecutive days. Control males remained sexually naïve, but were taken from their home cage and placed into the clean test cage for 30 minutes each day for seven consecutive days. Groups of experienced or naïve animals were sacrificed either one day (N1; n = 5; E1; n = 7) or 7 days (N7, E7; n = 5 each) following the last mating session or exposure to the test cage. Sexually experienced groups did not differ in experience.

Tissue processing—One day or one week following the last mating session or exposure to test cage, animals were given an overdose of sodium pentobarbital (i.p.) and were perfused with 500 mL of saline. The brains were processed for Golgi-Cox staining using a method adapted from Pugh and Rossi (62). For further details see Supplement 1.

Data analysis—Camera Lucida drawings were made of 5–7 neurons in the caudal NAc core and shell subregions in each animal. Cells were selected for which the entire or the majority of the dendritic branches were visible and easy to distinguish from neighboring cells. Dendritic branches were quantified by centrifugal order (63) and averages per animal were calculated. Dendritic spines were quantified on a 40 μm length of two second order dendrites per cell (4–7 cells per animal). Group means were compared using a two-way ANOVA (factors: sex experience and abstinence period) and Fisher LSD tests for *post hoc* analysis.

RESULTS

Experiment 1

The goal of Experiment 1 was to determine if sexual experience affects the locomotor response to AMPH in male rats. Locomotor activity during a 90-minute period was measured in sexually experienced and naïve rats following treatment with 0.5 mg/kg AMPH or SAL. Results from Experiment 1 are illustrated in Figure 1. Both sex experience ($F_{1,22}$=15.88; p=0.0006) and drug treatment ($F_{1,22}$=45.00; p<0.0001) had significant effects on locomotor activity and a two-way interaction between sexual experience and drug treatment was observed ($F_{1,1,22}$=14.27; p=0.0010). Specifically, both naïve and experienced animals showed a significantly increased locomotor response to AMPH compared to the appropriate SAL controls. Moreover, sexually experienced rats displayed an increased locomotor response to AMPH compared to naïve animals. Sexually experienced and naïve rats did not differ in their responses to SAL.

Analysis of the locomotor responses to AMPH in smaller time intervals of 30 minutes and 3 minutes are illustrated in Figure 1, panels C-F. Sexually experienced males displayed an increased locomotor response to AMPH compared to naïve rats throughout the 90-minute test period. Moreover, sexually experienced rats showed an increased locomotor response to AMPH compared to their SAL controls throughout the 90-minute test period, while naïve animals only displayed a significantly higher locomotor response during the last 30-minute interval (Figure 1; p-values are listed in figure legend).

Experiment 2

The goal of experiment 2 was to test if sexual experience results in locomotor sensitization in animals that mated during consecutive days, and in the same environment as in which they are exposed to AMPH. Exposure to the sex-paired environment caused increased locomotor activity during the 15 minutes prior to each mating session (Figure S1 in Supplement 1), illustrating the learned association between sexual behavior and environment. In addition, experiment 2 investigated the temporal pattern of locomotor sensitization to AMPH in sexually experienced male rats. The locomotor response to AMPH or SAL was measured one day (Day

8), one week (Day 14) and one month (Day 35) following the last mating session. As in experiment 1, sexually experienced rats displayed a greater locomotor response to AMPH compared to naïve animals. Moreover, this effect was evident on all three testing days. Figure 2 illustrates locomotor activity during the last 60 minutes of the tests during which the most robust differences were observed, and data for first 30 minutes are shown in Figure S2 (Supplement 1). Naïve and experienced animals did not differ in their response to SAL on any of the testing days, and rats that received AMPH displayed increased locomotor activity when compared to their SAL controls (Figure 2; p-values are listed in figure legend).

Experiment 3

Experiment 3 investigated the effect sexual experience on conditioned AMPH reward. AMPH CPP was tested in sexually naïve and experienced males 10 days following the final mating session. Sexually experienced animals show an enhanced conditioned AMPH reward. Specifically, sexually experienced males formed a strong preference for the AMPH-paired chamber with the lower doses of 0.5 and 1.0 mg/kg but not with the higher doses 2.5 or 5.0 mg/kg. In contrast, sexually naïve males only formed a strong preference for the AMPH-paired chamber with the higher doses, 2.5 and 5.0 mg/kg, and not the lower doses (Figure 3A; p-values are listed in figure legend).

Experiment 4

Experiment 3 demonstrated that sexual experience followed by a period of abstinence resulted in an enhanced conditioned AMPH reward. Experiment 4 investigated whether the effect of sexual experience on conditioned AMPH reward was dependent on this period of abstinence. Results indicated that sexually experienced animals did not show an increased conditioned reward value of AMPH. Sexually experienced and naïve animals showed a strong preference for the AMPH-paired chamber with the higher doses of 2.5 and 5.0 mg/kg. However, neither sexually experienced or naïve males showed an increased CPP score with the lower doses of 0.5 and 1.0 mg/kg dose. The lowest dosage of 0.5 mg/kg even caused an aversion response, but this reached significance only in the sexually experienced animals for the AMPH-paired chamber (Figure 3B; p-values are listed in figure legend).

Experiment 5

The purpose of Experiment 5 was to examine morphological alterations in the mesolimbic system, specifically the NAc, following sexual experience. Morphological alterations were evident one week (Figure 4H, J and L; p-values are listed in the figure legend), but not one day (Figure 4G, I and K), following the last mating session. In particular, significant increases in numbers of dendrites (indicative of increased dendritic branching) were detected in NAc core and shell (Figure 4H and J). In addition, numbers of dendritic spines were significantly increased in both the shell and core regions, one week, but not one day, after sex experience (Figure 4L).

DISCUSSION

This study demonstrates that sexual experience and the post-experience abstinence from sexual behavior induce functional and morphological alterations in the mesolimbic system of male rats. Functional changes were evident in the form of a sensitized locomotor response and an enhanced conditioned reward with AMPH following sexual experience. The sensitized locomotor response was observed as early as 1 day and maintained up to 28 days after last mating session. By contrast, the enhanced conditioned AMPH reward was only evident following an abstinence period from sexual behavior. Morphological alterations in both core and shell subregions of NAc were observed 7 days, but not 1 day, following the last mating session in sexually experienced animals. Together these data demonstrate that sexual

experience induces plasticity in the mesolimbic system and that an abstinence period from mating is critical for the development of some, but not all mesolimbic system changes.

It is well recognized that natural rewarding behaviors and drugs of abuse act within the same neural pathways (64). Indeed, drugs of abuse have been demonstrated to affect the expression of rewarding behaviors (65–67), including male rat sexual behavior (67–70). The alterations in sexual behavior and motivation caused by repeated drug administration are dependent on a withdrawal or abstinence period from drug, as well as the environment in which the drug was presented. The current study showed that exposure to sexual behavior alters responsiveness to drugs of abuse. It was determined that sexually experienced male rats are sensitized to the locomotor effects of AMPH, and that this phenomenon is long-lasting and independent of an abstinence period from mating. Moreover, the sensitized locomotor response was independent of mating schedule or mating environment and was observed following either consecutive or intermittent mating sessions that occurred in the same or different environment as drug exposure. Studies conducted in female hamsters showed that sexually experienced female hamsters display a more rapid onset of AMPH-induced locomotor response compared to sexually naïve controls (71). However, rodents display sexual dimorphic responses to psychostimulants (72–73). Thus, the current studies expand the findings in female hamsters and demonstrate in male rats, the fast onset and the long duration of the enhanced locomotor responses to psychostimulants following sexual behavior.

It is unclear from the current studies which elements of sexual behavior contribute to the AMPH locomotor sensitization and if social interactions are sufficient. Animals in experiment 2 that failed to reach the criteria for sexual experience (displayed mounts and intromissions, but did not copulate to 5 ejaculations during the mating sessions) did not show a sensitized response (Figure S3 in Supplement 1). Therefore, an additional experiment was performed during which males were exposed to a receptive female without physical interaction, or displayed mounts and intromissions, neither of which resulted in sensitized locomotor responses to AMPH (Figure S4 in Supplement 1). Thus, social interactions do not appear to contribute to the effects of sexual experience on AMPH sensitization, but rather copulation including ejaculation appears essential for this form of plasticity.

In addition to a sensitized behavioral response, sexual experience enhances the conditioned reward value of AMPH, but only following abstinence from sexual reward. Previous work using CPP has shown that repeated exposures to psychostimulants or opiates augment drug-induced rewarding effects in line with the drug-induced locomotor sensitization (22–24). Repeated administration for 5 days of either cocaine (10 mg/kg), d- amphetamine (0.5 mg/kg) or morphine (5 mg/kg) sensitizes the rewarding effects of cocaine when tested 3 days following the cessation of drug pre-treatment. The sensitized effect was displayed by observing a conditioned preference with fewer conditioning trials (from 3 to 2) and with lower drug doses compared to SAL pre-treated control animals. The sensitized conditioned reward caused by repeated cocaine was found 7 days, but not 14 days, after final pretreatment of cocaine (23). A similar study utilizing 5 days of morphine (5.0 mg/kg) shows an augmented conditioned reward response to morphine when conditioning started 3, 10, or 21 days after drug pre-treatment. This augmented response was absent 1 day following morphine pre-treatment (24). Such findings suggest that a period of drug withdrawal of at least 3 days is required for the sensitized or cross-sensitized conditioned reward for both psychostimulants and opiates. Sexual experience, like repeated drug administration, may be instilling similar neuroadaptations in the mesolimbic system responsible for this sensitized drug responsiveness once the reward has been removed. It is currently unclear if reward abstinence is associated with stress and thus acts as a psychological stressor contributing to the observed alterations.

Clearly, there is an interplay between the effects of natural and drug reward. Reward cross-sensitization suggests that the long-lasting effects of both sexual behavior and drugs are mediated by common cellular or molecular mechanisms. Therefore, it is hypothesized that the sex behavior-induced alterations regulate the reinforcing components of sexual behavior and thus may be critical for positive reinforcement of rewarding behaviors in general. However, a subsequent abstinence from sexual reward may induce a state of increased reward seeking, or vulnerability to the effects of addictive substances similar to the effects of abstinence an 'incubation of drug craving' (25,33,74). In general, sexual behavior in male rodents does not causes compulsive seeking for sex, shown using copulation-malaise associative conditioning experiments (75), although the influence of abstinence has not been tested.

Dendritic morphology has been examined in depth in the fields of learning and memory (76–77) and addiction (59,78–79), and is known to be influenced by environmental (80) and hormonal factors (81–82). Since the synaptic inputs are predominantly on dendrites or dendritic spines, they are the most likely target of experience-induced neuroplasticity (26,83). Natural fluctuations or administration of gonadal hormones have been found to cause dendritic changes within several hours (84–87). Also, perturbations to the system, such stress (88) or chronic cocaine (79), cause dendritic alterations detectable within 24 hours. Here, changes to dendritic morphology of medium spiny neurons in both the NAc core and shell were not observed within 24 hours, and instead required a period of abstinence following sexual experience. The structural alterations induced by sexual experience and subsequent abstinence resemble those seen following repeated exposure to psychostimulants (16–17,26,30). By contrast, DA depletion in the NAc results in a decreased number of dendrites and complexity in the shell (18,89). Hence sexual experience-induced changes may be dependent on endogenous DA action in the NAc. However, mating-induced morphological alterations were only evident 7 days following the last mating session and coincide with the enhanced conditioned AMPH reward in sexually experienced animals. These data suggest that these increases in dendritic arborization and spines are not required for the expression of short-term locomotor sensitization to AMPH, yet may play a role in the maintenance and long-term expression of the sensitization. Previous studies of repeated drug administration have also noted a disconnect between long-term sensitization and morphological alterations in the NAc (89–94). It remains unclear what the functional relevance is of the morphological alterations, but it may play a role in the long term changes in function and gene expression.

In summary, the data presented here demonstrate that sexual behavior – a natural rewarding stimulus – can induce long-lasting neuroadaptations in the mesolimbic system. Our findings suggest that behavioral plasticity, particularly a sensitized locomotor response, is an immediate and long-term outcome of sexual experience. Moreover, an abstinence period may allow for neuroadaptations critical for observed morphological changes in the NAc and subsequent enhanced conditioned drug reward. This behavioral and neural plasticity follows a similar, but not identical, profile as seen in drug-sensitized animals. These data are of particular interest since we show that an abstinence from the natural reward induces a vulnerable state to drug administration. Understanding how both natural behaviors and drugs of abuse activate these systems causing neuroadaptations may provide us with a better understanding of reinforcement and reward in general, and provide further insight into the mechanisms of drug addiction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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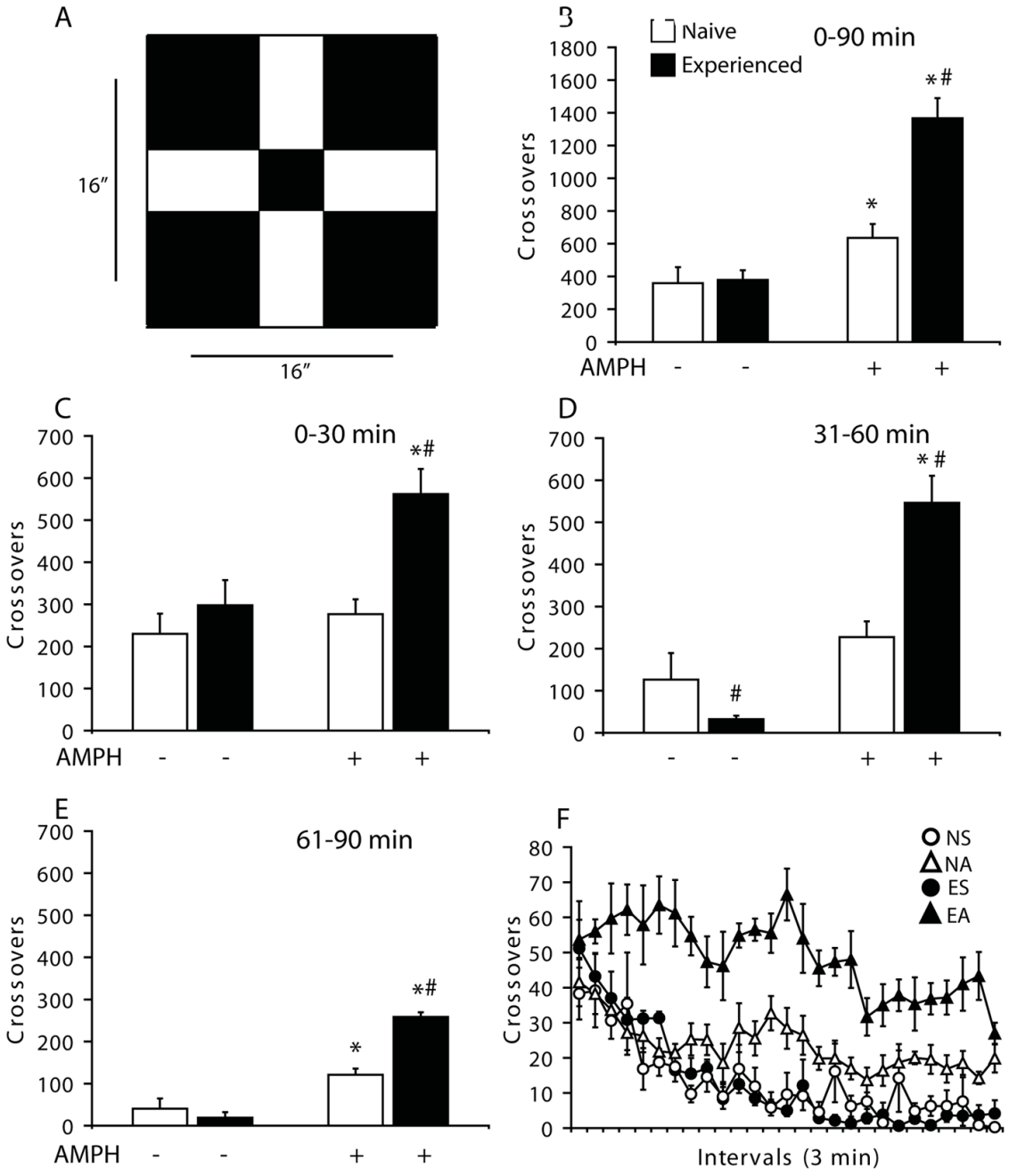

Figure 1.

Locomotor response of sexually experienced and naïve animals to saline or amphetamine administration. A is a schematic diagram of the zone map used to measure locomotor activity. A crossover is recorded each time the animal enters one of the black zones. Mean +/− SEM of total number of crossovers over 90 minutes (B) and in 30-minute intervals (C-E; C, 0–30 minutes. D, 31–60 minutes. E, 61–90 minutes). * indicates a statistically significant difference between AMPH and saline. # indicates a statistically significant difference between experience and naive groups ($p < 0.05$). F displays crossovers per 3-minute interval over 90-minutes (statistical differences are not indicated) (p-values: B, NA vs. NS, $p = 0.0422$; EA vs. ES, $p <$

0.0001, EA vs. NA; $p < 0.0001$; C–E, EA vs. NA; $p < 0.001$, EA vs. ES; $p < 0.001$; E, NA vs. NS; $p = 0.0024$).

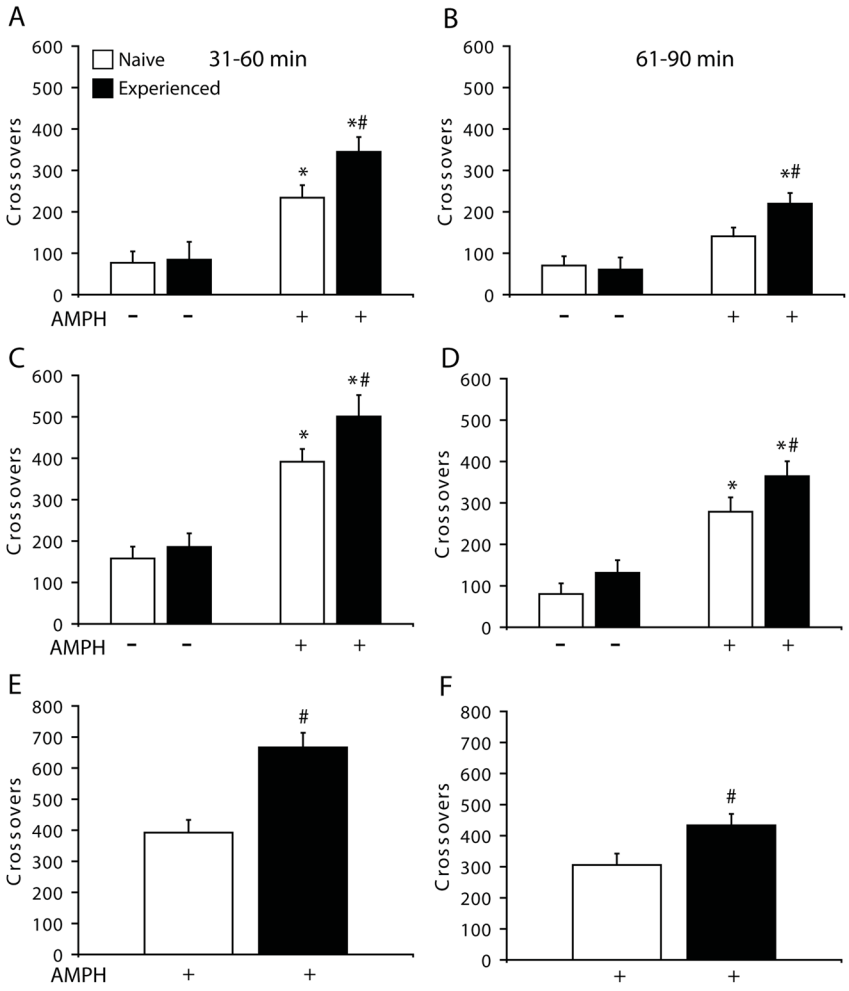

Figure 2.

Locomotor response of sexually experienced and naïve animals to saline or amphetamine administered one day (Day 8; A,B), one week (Day 14; C,D) or one month (Day 35; E,F) following the last mating session. Mean +/− SEM of total number of crossovers over the last 60 minutes divided into 30-minute intervals (31–60 minutes; A,C,E. 61–90 minutes; B,D,E). * indicates a statistically significant difference between AMPH and saline (NA vs NS: A; 0.0009, B; 0.0190, C; < 0.0001, D; 0.0002. EA vs ES: A; < 0.0001, B; 0.0002, C; < 0.0001, D; < 0.0001). # indicates a statistically significant difference between experience and naïve groups (p-values: EA vs NA: A; 0.0154, B; 0.0170, C; = 0.05, D; = 0.05, E; 0.0014, F; 0.0400).

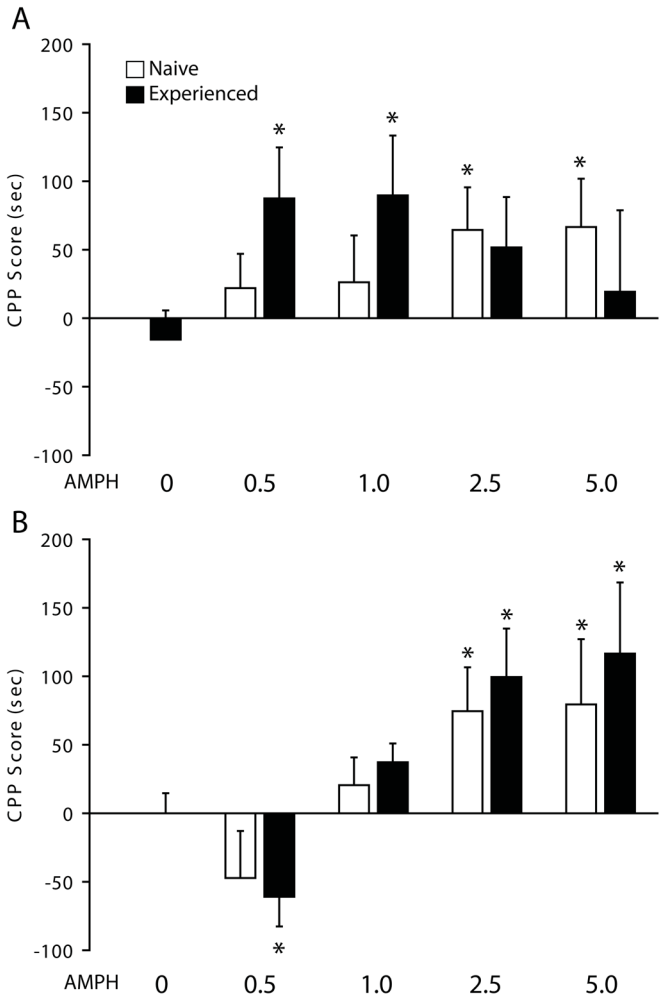

Figure 3.

Conditioned place preference of sexually experienced and naive animals in response to amphetamine either 10 days following (A) or during (B) mating sessions. Mean +/− SEM of CPP score, defined as the time spent in the AMPH-paired chamber in the post-test minus the pre-test (seconds). * indicates a statistically significant difference from males receiving saline (E0) ($p < 0.05$). (p-values: A, E0.5 p = 0.014; E1.0 p = 0.017; N2.5 p = 0.016, N5.0 p = 0.022; B, E0.5 p = 0.014, E2.5 p = 0.005, E5.0 p = 0.001; N2.5 p = 0.007, N5.0 p = 0.037)

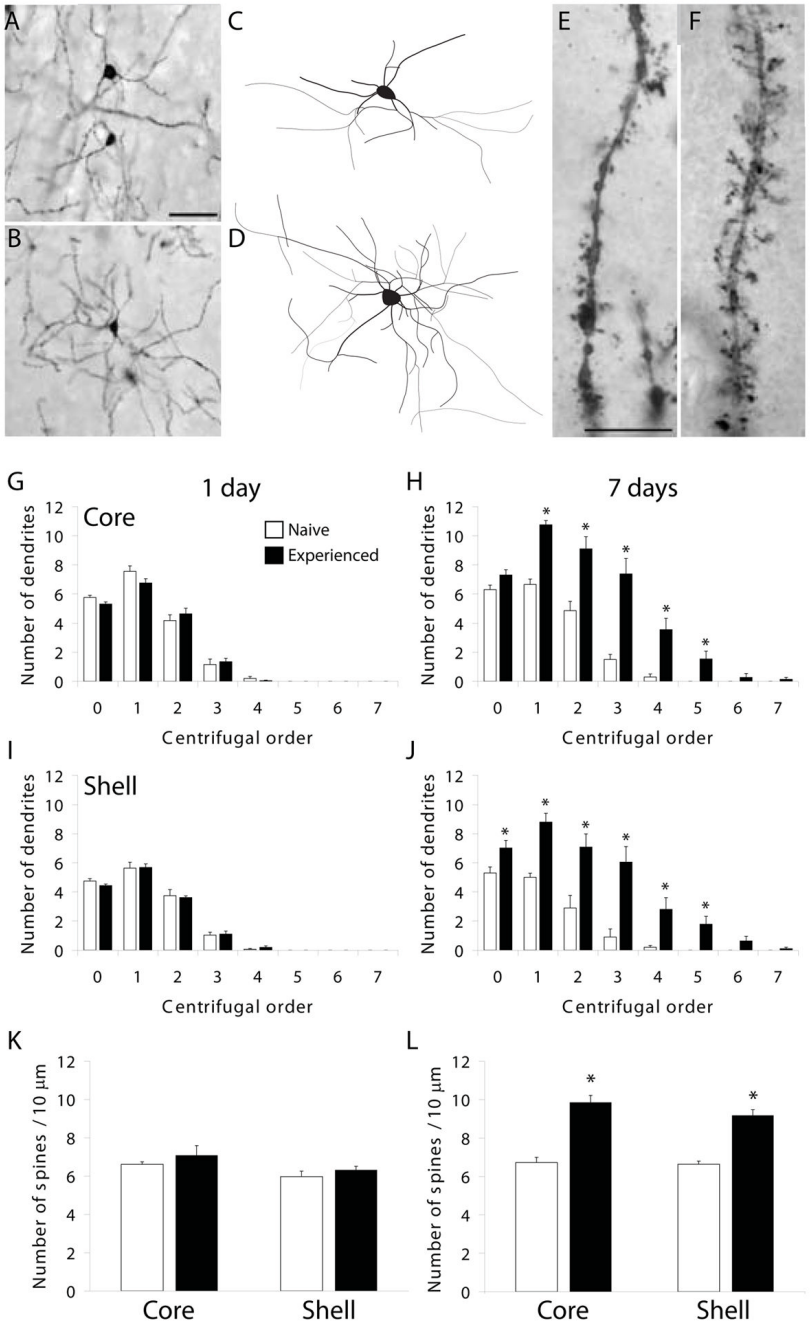

Figure 4.

Dendritic morphology in the NAc of sexually experienced and naïve animals. Sexual experience caused an increase in numbers of dendrites and dendritic spines, illustrated by images (A,B) and camera lucida drawings (C,D) of representative NAc shell neurons as well as spine density on second order dendrites (E,F) in NAc shell of naïve (A,C,E) or experienced (B,D,F) animals, 7 days following last treatment. Scale bar indicates 50 (A,B) or 10 (E,F) μm. Quantitative analysis shows significant increases of numbers of dendrites per centrifugal order (G–J) and numbers of spines (K,L) in NAc core (G,H) and shell (I,J) in sexually experienced males (black bars) compared to naïve males (white bars), but only 7 days following last mating (H,J,I). * indicates a statistically significant difference from naïve males (p-values: G: 0: 0.013

1: < 0.001; 2: <0.001; 3: <0.0001; 4: < 0.001; 5: < 0.001; I: 0: 0.002; 1: < 0.0001; 2: < 0.001; 3: < 0.001; 4: < 0.001; 5: < 0.001; L: core: 0.001; shell: < 0.001).