

# Sexual Behavior Induction of c-Fos in the Nucleus Accumbens and Amphetamine-Stimulated Locomotor Activity Are Sensitized by Previous Sexual Experience in Female Syrian Hamsters

Katherine C. Bradley<sup>1</sup> and Robert L. Meisel<sup>2</sup>

<sup>1</sup>Graduate Neuroscience Program, and <sup>2</sup>Department of Psychological Sciences, Purdue University, West Lafayette, Indiana 47907-1364

Dopamine transmission in the nucleus accumbens can be activated by drugs, stress, or motivated behaviors, and repeated exposure to these stimuli can sensitize this dopamine response. The objectives of this study were to determine whether female sexual behavior activates nucleus accumbens neurons and whether past sexual experience cross-sensitizes neuronal responses in the nucleus accumbens to amphetamine. Using immunocytochemical labeling, c-Fos expression in different subregions (shell vs core at the rostral, middle, and caudal levels) of the nucleus accumbens was examined in female hamsters that had varying amounts of sexual experience. Female hamsters, given either 6 weeks of sexual experience or remaining sexually naive, were tested for sexual behavior by exposure to adult male hamsters. Previous sexual experience increased c-Fos labeling in the rostral and caudal levels but not in the middle levels of the nucleus accumbens. Testing for

sexual behavior increased labeling in the core, but not the shell, of the nucleus accumbens. To validate that female sexual behavior can sensitize neurons in the mesolimbic dopamine pathway, the locomotor responses of sexually experienced and sexually naive females to an amphetamine injection were then compared. Amphetamine increased general locomotor activity in all females. However, sexually experienced animals responded sooner to amphetamine than did sexually naive animals. These data indicate that female sexual behavior can activate neurons in the nucleus accumbens and that sexual experience can cross-sensitize neuronal responses to amphetamine. In addition, these results provide additional evidence for functional differences between the shell and core of the nucleus accumbens and across its anteroposterior axis.

*Key words:* female sexual behavior; nucleus accumbens; shell; core; c-Fos; sensitization; cross-sensitization; amphetamine

Dopamine neurons originating in the midbrain ventral tegmental area and projecting to various forebrain nuclei, including the nucleus accumbens, are part of the mesolimbic dopamine system. It has been suggested that this dopamine system is important for the regulation of appetitive behaviors (Mitchell and Gratton, 1994; Salamone, 1994, 1996; Ikemoto and Panksepp, 1999), as well as self-administration of drugs of abuse (Pierre and Vezina, 1998; Koob, 1999; Lorrain et al., 1999; McKinzie et al., 1999; Peoples et al., 1999; Bradberry et al., 2000). Systemic administration of a variety of drugs of abuse (e.g., cocaine, amphetamine, and heroin) activates dopamine pathways (Pontieri et al., 1995; Nisell et al., 1997; Pierce and Kalivas, 1997a; Tanda et al., 1997; Tanda and Di Chiara, 1998; Barrot et al., 1999; Cadoni and Di Chiara, 1999), and repeated exposure to these pharmacological agents can sensitize these dopamine-responsive neurons (Robinson et al., 1988; Kalivas et al., 1992; Kalivas and Duffy, 1993; Pierce and Kalivas, 1995; Kuczenski et al., 1997; Nisell et al., 1997; Birrell and Balfour, 1998; Heidbreder and Feldon, 1998; Cadoni and Di Chiara, 1999; Cadoni et al., 2000). Research has provided evidence that the nucleus accumbens also responds to certain properties associated with mating. Extracellular dopa-

mine levels in the nucleus accumbens increase during sexual interactions in female rats (Mermelstein and Becker, 1995; Pfau et al., 1995) and hamsters (Meisel et al., 1993; Kohlert et al., 1997; Kohlert and Meisel, 1999). Similar to repeated drug administration, multiple sexual behavior tests also augment the increases in nucleus accumbens dopamine levels, suggesting that sexual experience can sensitize neurons in the dopamine pathway (Kohlert and Meisel, 1999).

The nucleus accumbens is composed of many anatomically distinct subregions, the most familiar of which are the shell and the core. Anatomical connections of the shell and core diverge, suggesting that these two subregions regulate different functions (Crawley et al., 1985a,b; Heimer et al., 1991; Zahm and Brog, 1992; Brog et al., 1993; Kalivas and Duffy, 1995; Maldonado-Irizarry et al., 1995; Pierce and Kalivas, 1995; Pontieri et al., 1995; Broening et al., 1997; Heimer et al., 1997; Kelley et al., 1997; Stratford and Kelley, 1997; Heidbreder and Feldon, 1998; Lanca et al., 1998; Bassareo and Di Chiara, 1999; Di Chiara et al., 1999b; Groenewegen et al., 1999; Kelley, 1999; McKinzie et al., 1999; Zahm, 1999; Brown and Molliver, 2000). Because the nucleus accumbens is a heterogeneous nucleus, it is not clear whether responses to female sexual behavior are localized to specific subregions of the nucleus accumbens or spread throughout the entire nucleus. Techniques previously used to answer this question (e.g., microdialysis) are not spatially sensitive enough to explore the functional heterogeneity of the accumbens. In contrast, immunocytochemical processing for the c-Fos protein provides a method for examining discrete cellular activation among subregions of the nucleus accumbens. Thus, the first purpose of

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Correspondence should be addressed to Dr. Robert L. Meisel, Department of Psychological Sciences, Purdue University, West Lafayette, IN 47907-1364. E-mail: meisel@psych.purdue.edu.

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this experiment was to determine whether cellular activation after female sexual behavior is localized to specific subregions of the nucleus accumbens.

An interesting property of these dopamine pathways is cross-sensitization. In other words, the dopamine neurons previously sensitized to one drug will exhibit sensitized responses to another drug given for the first time (Cunningham and Kelley, 1992; Pierce and Kalivas, 1997a; Birrell and Balfour, 1998; Taylor and Horger, 1999). In addition to cross-sensitization among drugs, several studies have reported cross-sensitization between repeated exposures to pharmacological agents and natural motivated behaviors (Mitchell and Stewart, 1990a,b; Tidey and Miczek, 1997; Fiorino and Phillips, 1999). Therefore, we examined whether sexually experienced and sexually naive animals would respond differently to a novel stimulus known to activate dopamine pathways (i.e., cross-sensitization), such as amphetamine. If female sexual behavior sensitizes dopamine pathways, then sexually experienced females should display an augmented behavioral response to a single injection of amphetamine.

## MATERIALS AND METHODS

### General methods

**Animals.** Male and female Syrian hamsters were delivered from Charles River Laboratories (Kingston, NY) at ~60 d of age. The females were housed individually, and male stimulus animals were housed in groups of three or four in plastic cages (50.8 × 40.6 × 20.3 cm). The animal colony room was maintained at a constant temperature (22°C) with the lights off between 1:30 and 11:30 P.M. (14/10 hr light/dark cycle). Food and water were available *ad libitum*.

The procedures used in this experiment are in accordance with National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals* and have been approved by the Purdue Animal Care and Use Committee.

**Sexual experience.** Approximately 1 week after the females arrived at the laboratory, they were bilaterally ovariectomized under sodium pentobarbital (Nembutal) anesthesia (8.5 mg per 100 gm body weight, i.p.). After ovariectomy, the females were initially divided into two groups. One group of females received 6 weeks of sexual experience with a stimulus male; the second group remained sexually naive. All of the females were hormonally primed once a week for the 6 week period. At both 48 and 24 hr before the sexual experience, the females were injected subcutaneously with 10 µg of estradiol benzoate in 0.1 ml of cottonseed oil. On the day of the experience test, the females received 500 µg of progesterone in 0.1 ml of cottonseed oil (subcutaneous injection). Females not receiving sexual experience were injected with the hormone regimen and remained in their home cages in the colony room. At 4–5 hr after the administration of progesterone, an adult male hamster that had received sexual experience through use in other sexual behavior studies was placed in the home cage of the experimental female. The order of the cages containing the males was rotated each week to minimize the likelihood that an individual male and female were paired more than once during the 6 weeks of sexual experience.

**Immunocytochemistry.** Female hamsters, killed with an overdose of sodium pentobarbital, were intracardially perfused with a 25 mM PBS solution, pH 7.5, for 2 min (flow rate, 25 ml/min), followed by 4% paraformaldehyde in PBS for 20 min. Brains were post-fixed for 2 hr in the paraformaldehyde and stored in 10% sucrose PBS overnight at 4°C.

Serial coronal 40 µm frozen sections were taken through the entire nucleus accumbens. After three 10 min rinses in PBS, sections were incubated in either primary antibody to c-Fos (1:6000 in PBS with 0.3% Triton X-100; Santa Cruz Biotechnology, Santa Cruz, CA) or in primary antibody to calbindin-D (28 kDa) (1:6000 in PBS with 0.3% Triton X-100; Chemicon International, Temecula, CA) at 4°C for 48 hr. Both the c-Fos and calbindin-D sections were then incubated for 45 min at room temperature in biotinylated anti-rabbit IgG secondary antibody (1:200 in PBS; Elite Vectastain ABC kit; Vector Laboratories, Burlingame, CA), followed by an incubation with an avidin–biotin horseradish peroxidase complex (1:50 in PBS; Elite Vectastain ABC kit) for 45 min at room temperature, with three 10 min rinses in PBS preceding each incubation. After two rinses in PBS and a 10 min rinse in 0.1 M Tris

buffer, pH 7.6, the c-Fos and calbindin-D sections were incubated for 5 and 10 min, respectively, in 0.08% diaminobenzidine (DAB) (Aldrich, Milwaukee, WI) in Tris buffer containing 0.003% hydrogen peroxide and 0.015% nickel chloride. All sections were rinsed again in Tris buffer and deionized water and then mounted onto chrom–alum-coated slides. The slides were dried, dehydrated, cleared, and coverslipped using Permount (Fisher Scientific, Pittsburgh, PA).

**Microscopic analysis.** The neural tissue stained for calbindin-D, which delineates the shell and core of the nucleus accumbens (Jongen-Relo et al., 1994a; Johnson and Wood, 1999) was used to identify one section each at the rostral, middle, and caudal level of the dorsal nucleus accumbens. Sections from the nucleus accumbens at the rostral, middle, and caudal levels stained for calbindin are shown in Figure 1A–C. It has been reported that there are less distinct differences between the core and shell in calbindin-D immunoreactivity in the Syrian hamster compared with the rat, but staining for this peptide is still able to demarcate the subregions of the nucleus accumbens (Johnson and Wood, 1999). A box encompassing a sampling region of 0.1 mm<sup>2</sup> (0.2 × 0.5 mm) was placed over the dorsal shell and core of the nucleus accumbens for each section. An image of each section was printed onto transparency film and the images were then superimposed onto the corresponding c-Fos sections for each animal, ensuring that the box was placed in the same position for all the animals. Figure 1, D and E, illustrates one caudal section from an animal that received 6 weeks of sexual experience and was tested for sexual behavior. The box was placed in the core of the caudal nucleus accumbens in Figure 1D and in the shell of the caudal nucleus accumbens in Figure 1E. A box with the same dimensions was placed in the same tissue section in the medial cingulate cortex and over the medial and lateral dorsal caudate nucleus at each of the three levels sampled for the nucleus accumbens. Because we hypothesized that there could be rostral–caudal variations in the effects of mating on c-Fos, only one section per level was analyzed to increase the anatomical precision of our sampling. The number of c-Fos-immunoreactive cells in each selected area was counted with the aid of a video camera connected to a computerized image analysis system (BioQuant MegM; R & M Biometrics, Nashville, TN).

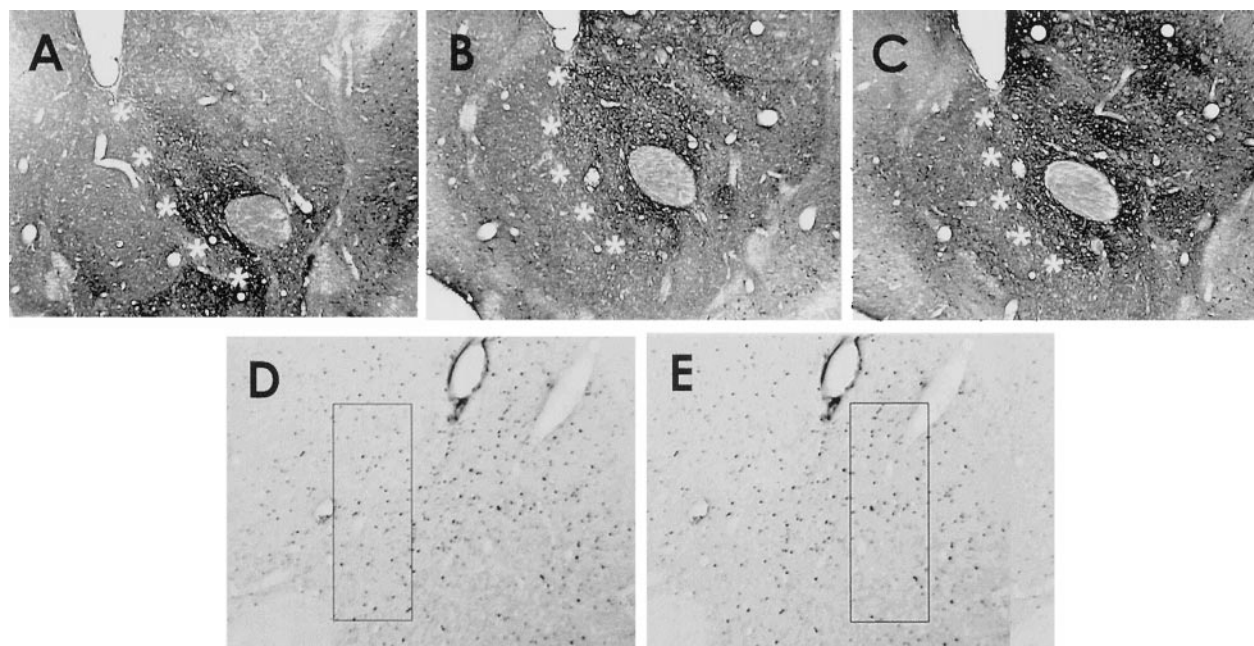
### Experiment 1

The first experiment investigated the effects of sexual experience and testing on c-Fos induction in the nucleus accumbens, dorsal caudate nucleus, and cingulate cortex. The goal of the experiment was twofold. The first goal was to determine whether there were differences in cellular activation in any of the brain regions because of previous sexual experience and/or behavioral testing. If c-Fos expression was altered, it was then determined whether the changes could be localized to specific subregions within the three brain areas analyzed.

Female Syrian hamsters received 6 weeks of sexual experience or remained sexually naive. During the 6 weeks of experience, the cumulative amount of time that the female assumed lordosis (immobility accompanied by a dorsoflexion of the back) was measured for each 10 min test session. No measures of male sexual behavior were recorded. During week 7, the same series of estradiol benzoate and progesterone injections were given. This time, half of the sexually experienced and naive females were tested for sexual behavior by placing an adult male in their home cage. The remaining females were left in their home cages. At 60–90 min after exposure to the male, the females were intracardially perfused, and their brains were processed for c-Fos expression. Those females not tested for sexual behavior were perfused 4 hr after progesterone administration.

**Data analysis.** Because the cell counts did not differ between those females not tested for sexual behavior during week 7, regardless of past sexual experience (see Table 1 for an example), the experimental females were ultimately divided into three treatment groups for analysis. The first group contained the females that received 6 weeks of sexual experience and were tested for sexual behavior (experience/test,  $n = 6$ ). The second group consisted of those females that did not receive any previous experience, but were tested for sexual behavior (no experience/test,  $n = 8$ ). The final group contained all of the female hamsters that were not tested for sexual behavior, regardless of any previous sexual experience (no test,  $n = 13$ ). The two groups not receiving sexual behavior testing were combined to increase statistical power in the analyses. The number of c-Fos-stained cells from the dorsal nucleus accumbens, dorsal caudate nucleus, and cingulate cortex were compared among the three groups.

Cell counts were analyzed using multifactor ANOVAs. Simple main-effect ANOVAs and *post hoc* Newman–Keuls tests were performed



**Figure 1.** Nucleus accumbens tissue sections stained for calbindin-D and c-Fos. *A–C* are sections from the rostral (*A*), middle (*B*), and caudal (*C*) nucleus accumbens stained (midline is left) for calbindin, illustrating the division between the shell and core subregions (asterisks). There are 320  $\mu\text{m}$  between the rostral and middle sections and 240  $\mu\text{m}$  between the middle and caudal sections. The bottom images (*D*, *E*) are examples of c-Fos staining from the caudal core (*D*) and shell (*E*) of the nucleus accumbens (midline is right) of a sexually experienced female killed after a sexual behavior test. The rectangle illustrates the sampling area (0.2  $\times$  0.5 mm).

**Table 1. Comparison of mean  $\pm$  SEM number of c-Fos-immunoreactive cells in the nucleus accumbens shell and core between the no test treatment groups**

Anatomical division	Treatment group	
	Experience/ no test	No experience/ no test
Nucleus accumbens shell		
Rostral	29.50 $\pm$ 5.8	36.14 $\pm$ 5.6
Middle	25.67 $\pm$ 2.7	24.57 $\pm$ 3.8
Caudal	15.50 $\pm$ 3.3	28.86 $\pm$ 5.0
Nucleus accumbens core		
Rostral	19.17 $\pm$ 4.5	23.71 $\pm$ 5.4
Middle	19.67 $\pm$ 2.8	23.0 $\pm$ 4.9
Caudal	18.33 $\pm$ 2.9	19.86 $\pm$ 3.1

The number of c-Fos-stained cells was compared between the two control no test treatment groups (experience/no test and no experience/no test). A three-way ANOVA (treatment times rostral–caudal level times shell–core) failed to indicate a significant three-way interaction and significant main effects of the treatment groups in either the shell or core of the accumbens, or through the rostral–caudal dimensions of the nucleus.

where appropriate. Behavioral data (lordosis durations) were analyzed using a two-tailed *t* test.

## Experiment 2

The second experiment compared the ability of a novel stimulus, amphetamine, to produce behavioral sensitization in sexually experienced and sexually naive female hamsters. c-Fos expression in the nucleus accumbens, dorsal caudate nucleus, and cingulate cortex was analyzed again to determine whether the pattern of cellular activity was similar to the results obtained in Experiment 1.

Female Syrian hamsters were given 6 weeks of sexual experience or remained sexually naive. At week 7, all females were transported to a novel environment (i.e., a 10 gallon glass aquarium in an unfamiliar room) 4 hr after progesterone administration. The females were placed in the 10 gallon glass aquarium for 10 min, after which time half of the

sexually experienced and sexually naive females were administered D-amphetamine sulfate (1 mg per 1 kg body weight in 1.0 ml of 0.9% NaCl; a gift from Dr. David Nichols, Purdue University). The remaining females were injected with 0.9% NaCl (1 mg per 1 kg body weight). The females were then placed back into the 10 gallon aquarium for an additional 60 min. The 70 min sessions were videotaped for analysis of the general locomotor activity of the females. Within 30 min after the general activity test, the females were intracardially perfused, and their brains were processed for c-Fos expression.

**Videotape analysis.** During week 7, the 70 min sessions testing locomotor activity were videotaped. The 10 gallon glass aquariums were divided into three equal areas on the video screen, and the locomotor activity of the females was recorded in terms of the number of area crosses.

**Data analysis.** Past sexual history did not influence the locomotor activity of the female hamsters injected with saline; therefore, the experimental females were divided into three treatment groups for analysis. The first group contained the females that received 6 weeks of sexual experience and were injected with amphetamine (experience/amphetamine,  $n = 8$ ). The second group consisted of those females that were administered amphetamine but did not receive any sexual experience (no experience/amphetamine,  $n = 8$ ). The final group contained all of the female hamsters that were injected with saline, regardless of any previous sexual experience (saline,  $n = 15$ ). The mean locomotor activity of the females was compared among the three treatment groups across the 70 min of testing (in 10 min periods) using two-factor ANOVAs. Simple main-effect ANOVAs and *post hoc* Newman–Keuls tests were performed where appropriate.

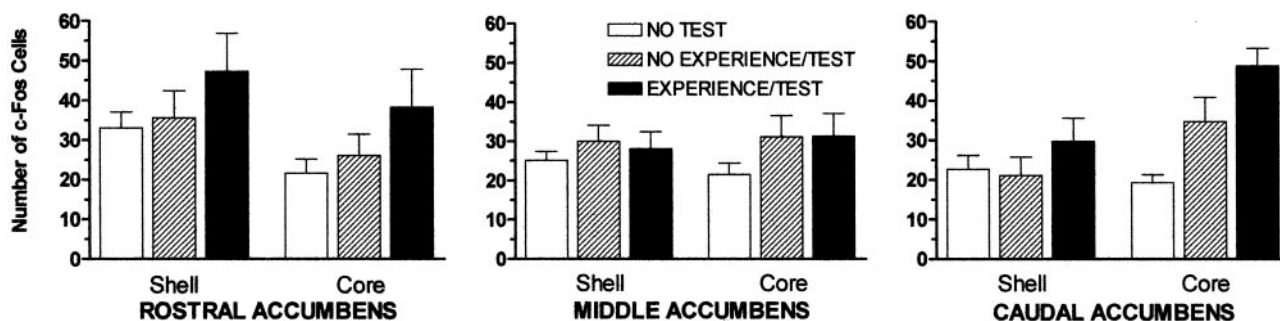
The number of cells stained for c-Fos did not differ between those females injected with saline, regardless of past sexual experience. Therefore, the number of c-Fos-stained cells from the dorsal nucleus accumbens, dorsal caudate nucleus, and cingulate cortex were compared among the same three treatment groups as in the first experiment. Cell counts were analyzed using multifactor ANOVAs. Simple main-effect ANOVAs and *post hoc* Newman–Keuls tests were performed where appropriate.

## RESULTS

### Experiment 1

#### Sexual behavior measures

The lordosis durations during the test for sexual behavior on week 7 were compared between the experience/test and the no



**Figure 2.** c-Fos expression in the shell and core of the nucleus accumbens at the rostral, middle, and caudal levels for each of the treatment groups. A three-way ANOVA (treatment times rostral–caudal level times shell–core) was used to examine the effects of sexual experience and behavior on the mean  $\pm$  SEM number of c-Fos cells. No significant main effects of treatment and no three-way interaction among treatment, accumbens level, and shell–core were found.



**Figure 3.** c-Fos expression in the shell and core of the nucleus accumbens, collapsed across the rostral–caudal level. The three-way ANOVA revealed a two-way interaction between treatment and mean  $\pm$  SEM number of c-Fos cells in the shell and core of the nucleus accumbens (treatment times shell–core;  $F_{(2,24)} = 4.243$ ;  $p < 0.026$ ). A one-way ANOVA probing this interaction found significant main effects of the treatment groups only in the nucleus accumbens core ( $F_{(2,24)} = 7.341$ ;  $p < 0.003$ ) and not in the shell of the accumbens ( $F_{(2,24)} = 1.271$ ;  $p > 0.1$ ). Different letters indicate significant differences between groups.

experience/test groups. The average lordosis duration during the 10 min test was  $341 \pm 53$  sec for the experience/test group and  $478 \pm 20$  sec for the no experience/test group. Females in the no experience/test group had assumed lordosis for a significantly longer duration than had the females in the experience/test group ( $t_6 = 5.131$ ;  $p = 0.05$ ). Furthermore, sexual experience did not affect lordosis duration. The analysis showed no significant differences between the average durations for week 1 ( $399 \pm 44$  sec) and week 7 ( $341 \pm 53$  sec) for females in the experience/test group.

#### c-Fos expression in the nucleus accumbens

A three-way ANOVA of treatment times rostral–caudal level times shell–core found no significant main effects of treatment and no three-way interaction among treatment, accumbens level, and shell–core (Fig. 2); however, two important two-way interactions (treatment times shell–core and treatment times rostral–caudal level) were detected.

Probing of the treatment times shell–core interaction revealed significant main effects of the treatment groups only in the core of the nucleus accumbens (Fig. 3). Pairwise multiple comparisons showed that those females tested for sexual behavior during week 7 (experience/test and no experience/test) had significantly more c-Fos-stained cells in the core of the nucleus accumbens than did those females that were not tested (no test) (Newman–Keuls,  $p < 0.01$ ). No effects of testing were observed in the shell of the nucleus accumbens. Furthermore, there were no explicit effects of

sexual experience on the number of cells expressing c-Fos in either the shell or core of the accumbens.

Probing of the treatment times rostral–caudal level interaction found significant main effects of the treatment groups in both the rostral and caudal levels but not in the middle level of the nucleus accumbens (Fig. 4). Newman–Keuls *post hoc* tests indicated that females that had received 6 weeks of sexual experience and were tested for sexual behavior (experience/test) had more c-Fos-positive cells in the rostral nucleus accumbens than females that were tested but that did not receive any previous sexual experience (no experience/test;  $p < 0.05$ ) and those females that were not tested for sexual behavior (no test;  $p < 0.01$ ). The *post hoc* tests revealed similar results for the caudal nucleus accumbens. Females in the experience/test group had a higher number of cells expressing c-Fos in the caudal nucleus accumbens than females in the no experience/test group ( $p < 0.05$ ) and no test group ( $p < 0.01$ ). Therefore, testing for sexual behavior during week 7 had increased the number of c-Fos-stained cells in the rostral and caudal nucleus accumbens only for those females that had received the 6 weeks of experience.

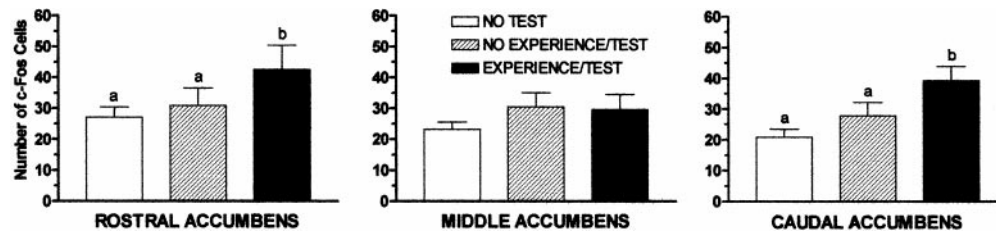
#### c-Fos expression in the caudate nucleus and cingulate cortex

Cell counts from the dorsal caudate nucleus were also analyzed using a three-way ANOVA. The analysis revealed only an interaction between treatment and c-Fos expression in the medial and lateral caudate nucleus ( $F_{(2,24)} = 3.514$ ;  $p < 0.046$ ). However, separate analysis of the medial and lateral caudate nucleus by one-way ANOVA indicated no difference in the number of c-Fos-stained cells between the experience/test, no experience/test, and no test groups (Table 2). In addition, no main effects of sexual experience or behavior on the number of cells expressing c-Fos, or any interactions, were found in the cingulate cortex (data not shown).

## Experiment 2

### Locomotor activity

A two-way ANOVA (treatment times testing period), comparing the mean activity of the females in the experience/amphetamine, no experience/amphetamine, and saline treatment groups across the 70 min of testing revealed an interaction between treatment group and testing period. To examine this interaction, the individual treatment groups were probed separately with one-way ANOVAs. The analyses indicated significant changes in the mean general activity during the 70 min of testing for the two groups of females that were injected with amphetamine (experience/amphetamine and no experience/amphetamine). However, the gen-



**Figure 4.** c-Fos expression through the rostral–caudal dimension of the nucleus accumbens, collapsed across the core and shell. Although a three-way ANOVA indicated that the two-way interaction between treatment groups and the mean  $\pm$  SEM number c-Fos cells through the rostral–caudal levels of the nucleus accumbens only approached significance ( $F_{(4,48)} = 2.365$ ;  $p < 0.066$ ), we probed each level of the nucleus accumbens separately for an effect of treatment on c-Fos staining. A one-way ANOVA revealed significant main effects of the treatment groups in both the rostral level ( $F_{(2,48)} = 5.230$ ;  $p < 0.009$ ) and caudal level ( $F_{(2,48)} = 7.455$ ;  $p < 0.002$ ) but not in the middle level ( $F_{(2,48)} = 1.744$ ;  $p > 0.1$ ) of the nucleus accumbens. Different letters indicate significant differences between groups.

**Table 2.** Mean  $\pm$  SEM number of c-Fos-immunoreactive cells in the medial and lateral dorsal caudate nucleus

Anatomical division	Treatment group		
	Experience/ test	No experience/ test	No test
Medial caudate nucleus			
Rostral	35.67 $\pm$ 7.0	27.63 $\pm$ 5.6	20.77 $\pm$ 2.4
Middle	35.17 $\pm$ 4.9	34.75 $\pm$ 6.9	22.69 $\pm$ 3.2
Caudal	39.67 $\pm$ 7.9	29.88 $\pm$ 7.4	25.31 $\pm$ 4.4
Lateral caudate nucleus			
Rostral	24.17 $\pm$ 5.9	9.75 $\pm$ 3.0	16.15 $\pm$ 2.9
Middle	20.33 $\pm$ 5.7	19.25 $\pm$ 4.2	17.62 $\pm$ 2.6
Caudal	17.50 $\pm$ 4.8	17.00 $\pm$ 7.8	17.46 $\pm$ 4.5

Analysis of the dorsal caudate nucleus found no significant treatment effects across either the rostral–caudal or medial–lateral dimensions of the nucleus.

eral activity of the females that received saline did not change significantly in the 70 min (Fig. 5). Newman–Keuls *post hoc* tests were then used to determine which 10 min testing periods differed. Pairwise multiple comparisons revealed that the general activity of the sexually experienced females given amphetamine significantly increased 10 min after injections ( $p < 0.05$ ). Furthermore, compared with the 10 min before injections, females in the experience/amphetamine treatment group remained significantly more active 20 min ( $p < 0.05$ ) and 30 min ( $p < 0.05$ ) after injections. In contrast, the effects of amphetamine in sexually naive females were not evident until 20 min after injections. At this time point, these females were significantly more active compared with the 10 min before injections ( $p < 0.05$ ). In addition, the activity of the sexually naive females given amphetamine remained significantly increased for 30 min ( $p < 0.05$ ) and 40 min ( $p < 0.01$ ) after injections.

#### c-Fos expression

A three-way ANOVA (treatment times rostral–caudal level times shell–core) was used to examine the effects of sexual experience and amphetamine on c-Fos expression in the nucleus accumbens. No significant main effects of treatment and no three-way interaction among treatment, accumbens level, and shell–core were found. Furthermore, the analysis did not reveal any interactions between the treatment groups and c-Fos expression in the shell and core of the accumbens or between the treatment groups and c-Fos labeling in the rostral, middle, and caudal levels of the nucleus accumbens (data not shown).

Cell counts from the dorsal caudate nucleus were also analyzed

using a three-way ANOVA. Initial analysis revealed no significant effect of previous sexual experience or amphetamine on the number of c-Fos-positive cells. In addition, no effect of previous sexual experience or amphetamine on the number of cells expressing c-Fos was found in the cingulate cortex using a two-way ANOVA (data not shown).

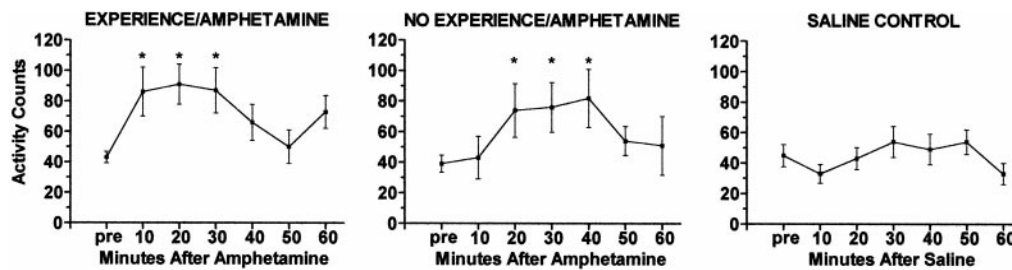
## DISCUSSION

The purpose of this investigation was twofold. We first examined the effects of sexual experience on cellular activity in different subregions of the nucleus accumbens. The second issue, whether previous sexual experience could sensitize the mesolimbic dopamine pathway, was investigated by comparing the behavioral responses of sexually experienced and naive animals to an amphetamine injection. Our findings not only indicate that female sexual behavior can activate neurons in the nucleus accumbens but also that sexual experience can cross-sensitize neuronal responses to amphetamine.

### Sexual behavior effects on c-Fos expression in the shell and core of the nucleus accumbens

Sexual behavior testing increased c-Fos expression in the core, but not the shell, of the nucleus accumbens, supporting previous research showing that a single sexual encounter can activate neurons in the nucleus accumbens in female rodents (Meisel et al., 1993; Joppa et al., 1995; Mermelstein and Becker, 1995; Pfau et al., 1995; Kohlert et al., 1997; Kohlert and Meisel, 1999). Literature addressing the functional dichotomy of the nucleus accumbens is composed of numerous reports of differential changes in dopamine transmission within the shell and core of the nucleus accumbens in response to pharmacological and physiological stimuli. Administration of several drugs of abuse results in selective increases in extracellular dopamine levels in the shell of the nucleus accumbens (Pontieri et al., 1995; Nisell et al., 1997; Pierce and Kalivas, 1997a; Tanda et al., 1997; Tanda and Di Chiara, 1998; Barrot et al., 1999; Cadoni and Di Chiara, 1999). In a similar manner, highly palatable foods (Tanda and Di Chiara, 1998; Di Chiara et al., 1999a; Kelley, 1999), mild stress (e.g., foot shock) (Kalivas and Duffy, 1995; Tidey and Miczek, 1997; Bruijnzeel et al., 1999; Wu et al., 1999), and environmental novelty (Rebec et al., 1997; Rebec, 1998) also selectively increase dopamine transmission in the shell of the nucleus accumbens.

Our findings are consistent with the hypothesis that the shell and core are functionally distinct, although we found the core, and not the shell, to be responsive to sexual behavior. It is possible, however, that there were changes in c-Fos immunoreactivity in the shell of the nucleus accumbens, but these changes



**Figure 5.** Effects of amphetamine on the general activity of sexually experienced and sexually naive female hamsters. A two-way ANOVA (treatment times testing period) revealed an interaction between treatment group and testing period ( $F_{(12,150)} = 2.288; p < 0.011$ ) for mean  $\pm$  SEM activity counts. A one-way ANOVA probing the individual treatment groups showed significant changes in the general activity for females in the experience/amphetamine ( $F_{(6,150)} = 3.0468; p < 0.008$ ) and no experience/amphetamine ( $F_{(6,150)} = 3.893; p < 0.001$ ) treatment groups. The activity of the females injected with saline did not change ( $F_{(6,150)} = 1.619; p < 0.1$ ). *Post hoc* tests indicated that the sexually experienced females responded more rapidly to amphetamine, showing an increase in activity within the first 10 min after injection. Sexually naive females did not respond to the amphetamine until 20 min after injection. \* $p < 0.05$  versus the period before testing.

were not detected. The shell is complexly organized into different subregions, a medial, ventral, and lateral shell, with the ventral and dorsal areas of the medial shell possibly being two more distinct subregions (Groenewegen et al., 1999). These subregions of the shell, as well as the medial and lateral parts of the core, receive different combinations of inputs from cortical and subcortical areas (Groenewegen et al., 1999). Furthermore, located within these subregions are functionally distinct ensembles of neurons that are organized into distinct anatomical compartments (Groenewegen et al., 1999). Because the effects of sexual behavior on c-Fos expression in this study were examined in only the dorsomedial shell, it is possible that the number of c-Fos-positive cells actually changed in a different shell subregion.

Despite the observations that many nucleus accumbens functions are localized to the shell region, it is reasonable to hypothesize that different neural circuits within the nucleus accumbens mediate the reinforcing properties of different behaviors. Carelli et al. (2000) have reported recently that nucleus accumbens neurons in rats exhibit similar neuronal activity during operant responding to two natural reinforcers (i.e., food and water) but different firing patterns during responding for a natural reinforcer versus cocaine. They have concluded that separate neural circuits in the nucleus accumbens process information about food and water reinforcement versus cocaine reward (Carelli et al., 2000).

#### Sexual experience effects on c-Fos expression through the rostral-caudal axis of the nucleus accumbens

The literature examining the subnuclear organization through the rostral-caudal nucleus accumbens is small; however, clear functional and anatomical differences have been observed. Our findings are consistent with studies that have reported differential regulation of neurochemical and motor responses across the rostral-caudal axis of the accumbens. Cholecystokinin (CCK) differentially modulates dopamine-induced effects in the rostral and caudal nucleus accumbens (Crawley et al., 1985a,b), potentiating dopamine-induced hyperlocomotion when infused into the caudal accumbens, a region innervated by CCK neurons colocalized with dopamine (Crawley et al., 1985a,b; Lanca et al., 1998). However, CCK is behaviorally inactive when injected into the rostral nucleus accumbens, a region that receives separate CCK and dopamine projections (Crawley et al., 1985a,b; Lanca et al., 1998). It has also been reported that direct infusion of amphetamine into the rostral shell, caudal shell, or core differentially affects behavioral activity and extracellular dopamine and serotonin levels (Heidbreder and Feldon, 1998). Regulation of opioid

peptides, substance P, dopamine D1 receptors (Voorn and Docter, 1992; Jongen-Relo et al., 1994b; Voorn et al., 1994), and acetylcholine release (Jongen-Relo et al., 1995) by dopamine and dopamine receptor agonists also differs between the rostral and caudal parts of the accumbens, with the rostral accumbens being more sensitive to dopamine depletion and administration. Although these functional differences between the rostral and caudal nucleus accumbens have been reported, why these functional differences exist is still not fully understood.

#### Sexual experience effects on amphetamine-induced locomotor activity

Results reported here and in an earlier study suggest that previous sexual experience sensitizes neuronal responses to sexual behavior testing, indicating sensitized increases in dopamine release (Kohlert and Meisel, 1999) and cellular activity in the nucleus accumbens (this study). One concern, however, is that experienced females in the previous study may have responded to both sexual behavior testing and environmental cues because sexual experience and testing were conducted in the same room. Environmental cues conditionally associated with motivated behaviors can acquire incentive properties and further increase dopamine levels in the nucleus accumbens (Reid et al., 1996, 1998; Watson and Little, 1999). A second concern is that because measures of male sexual behavior were not recorded, it is not known whether the two groups of females tested for sexual behavior received comparable amounts of vaginocervical stimulation. It has been reported that vaginocervical stimulation is necessary for dopamine release in the nucleus accumbens during mating (Kohlert et al., 1997). Perhaps the sexually experienced females received more vaginocervical stimulation (not measured in this study), thus increasing c-Fos induction. Therefore, to validate that female sexual behavior sensitizes the mesolimbic dopamine pathway, we investigated whether sexually experienced and naive females responded differently to an amphetamine injection, another stimulus known to mediate its effects via dopamine pathways. Furthermore, to ensure that the sensitized responses observed were because of repeated sexual behavior and not because of conditioned association of the environment to sexual behavior, the behavioral responses of the hamsters to amphetamine were tested in a novel environment.

Amphetamine increased general activity in all female hamsters. However, sexually experienced females responded sooner to amphetamine than did sexually naive females. These results validate the hypothesis that repeated sexual behavior can sensi-

**Table 3. Mean  $\pm$  SEM basal number of c-Fos-immunoreactive cells in the nucleus accumbens shell and core for control animals in experiments 1 and 2**

Anatomical division	Control group	
	Experiment 1	Experiment 2
Nucleus accumbens shell		
Rostral	33.08 $\pm$ 4.0	65.67 $\pm$ 4.6
Middle	25.08 $\pm$ 2.3	77.67 $\pm$ 4.8
Caudal	22.69 $\pm$ 3.6	72.33 $\pm$ 5.8
Nucleus accumbens core		
Rostral	21.62 $\pm$ 3.5	53.33 $\pm$ 4.5
Middle	21.46 $\pm$ 2.9	52.93 $\pm$ 3.8
Caudal	19.15 $\pm$ 2.1	61.0 $\pm$ 4.2

Although it is not statistically appropriate to compare the levels of c-Fos expression between the control animals in experiments 1 and 2, it is clear that there are two- to three-fold higher baseline cell counts in experiment 2.

tize neurons in the mesolimbic dopamine pathway and suggest that changes in the pathway produce sensitized behavioral responses both to the natural motivated behavior and to a psychomotor stimulant (cross-sensitization).

These findings are consistent with the hypothesis that there are convergent neural mechanisms mediating responses to drugs and sexual behavior (Robinson and Berridge, 1993; Pierce and Kalivas, 1997b). Several recent studies have observed cross-sensitization between repeated drug exposure and natural motivated behaviors. Social defeat stress reduces the acquisition time for cocaine self-administration in rats (Tidey and Miczek, 1997). An environment paired with repeated morphine injections can facilitate sexual behavior in male rats (Mitchell and Stewart, 1990a,b). Amphetamine pretreatment also facilitates sexual behavior in sexually naive male rats and is correlated with augmented dopamine release in the nucleus accumbens (Fiorino and Phillips, 1999).

c-Fos expression was analyzed in the nucleus accumbens after amphetamine treatment. It was hypothesized that amphetamine would increase c-Fos expression in the nucleus accumbens, and to a greater extent in sexually experienced females. However, no effects of amphetamine on the number of cells expressing c-Fos were found in any of the subregions of the nucleus accumbens. It is evident from Table 3 that the control animals in experiment 2 (saline females) had a higher number of c-Fos-positive cells compared with control animals in experiment 1 (no test females). Badiani et al. (1998) reported that novelty increased *c-fos* mRNA content in the nucleus accumbens, and that this effect of novelty on *c-fos* content was so strong in several brain regions that the administration of amphetamine in a novel environment did not produce an additional incremental response. Thus, it seems possible that in our study the stress of being transported to the novel environment of the testing room activated synthesis of the c-Fos protein, thereby masking changes in c-Fos expression induced by amphetamine and sexual experience.

### Potential significance

These experiments join a growing list of studies (Mitchell and Stewart, 1990b; Fiorino and Phillips, 1999; Miczek et al., 1999) indicating that the experiences of an animal can sensitize the responsiveness of the mesolimbic dopamine pathway both to behaviors that are part of the natural repertoire of an animal and to certain drugs that humans are known to abuse (Wise and Bozarth, 1987). A key issue in research on drug abuse is individ-

ual vulnerability to the effects of drugs (Newcomb, 1992; Robinson and Berridge, 1993), and collectively this research may offer insights into the development of addiction in people.

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