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DELTAFOSB IS INCREASED IN THE NUCLEUS ACCUMBENS BY AMPHETAMINE BUT NOT SOCIAL HOUSING OR ISOLATION IN THE PRAIRIE VOLE

C. M. HOSTETLER* AND K. L. BALES

Department of Psychology, University of California, Davis, CA 95616, USA

Abstract—The nucleus accumbens is a key region that mediates aspects of immediate and long-term adaptations to various stimuli. For example, both repeated amphetamine and pair-bonding increase dopamine D1 receptor binding in the nucleus accumbens of the monogamous prairie vole (*Microtus ochrogaster*). This upregulation has significant and stimulus-dependent behavioral consequences. A promising candidate for these and other adaptations is the transcription factor ΔfosB. ΔfosB is a highly stable protein that persists in the brain over long periods of time, leading to increasing and accumulating levels with repeated or continuous exposure to specific stimuli. Within the nucleus accumbens, ΔfosB is specifically increased in medium spiny neurons containing D1 receptors. To explore whether ΔfosB is altered by drug and social experience in prairie voles, we performed three separate experiments. In the first experiment, animals were treated with repeated injections of amphetamine and then brain tissue was analyzed for ΔfosB expression. As expected, 4 days of amphetamine treatment increased ΔfosB in the nucleus accumbens, consistent with previous findings in other laboratory species. In the second experiment, animals were housed for 10 days with one of three social partners: a familiar same-sex sibling, an unfamiliar same-sex partner, or an unfamiliar opposite-sex partner. Here, we predicted that 10 days of housing with an opposite-sex partner would act as a "social reward," leading to upregulation of ΔfosB expression in the nucleus accumbens. In a third experiment, we also investigated whether 10 days of social isolation would result in altered ΔfosB activity. We hypothesized that isolation would lead to decreased levels of nucleus accumbens ΔfosB, as seen in other studies. However, neither opposite-sex cohabitation nor social isolation affected ΔfosB expression in the nucleus accumbens. These findings suggest that social stimuli, in contrast to drugs of abuse, are not mediators of ΔfosB in this region in prairie voles. **© 2012 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: deltaFosB, nucleus accumbens, prairie vole, amphetamine, pair bonding, isolation stress.

The nucleus accumbens (NAc) is a key region mediating both immediate and long-term aspects of drugs of abuse (Russo et al., 2009; Carlezon and Thomas, 2009) and pair-bonding behavior (Gingrich et al., 2000; Liu and Wang, 2003; Aragona et al., 2003, 2006; Aragona and Wang, 2007; Liu et al., 2011). Recent work in prairie voles has shown that both repeated amphetamine and pair-bonding increase dopamine D1 receptor (D1R) binding in the Nac (Aragona et al., 2006; Liu et al., 2010; Young et al., 2011). This upregulation has significant and stimulus-dependent behavioral consequences. Prairie voles treated with repeated amphetamine fail to form partner preferences, and this is mediated via increased NAc D1R (Liu et al., 2010; Young et al., 2011). In contrast, upregulated NAc D1R in pair-bonded voles mediate blunted reward response to amphetamine (Liu et al., 2011). However, the molecular process under which these two very different stimuli may lead to a similar outcome of increased NAc D1R has not been explored.

A promising candidate for these adaptations is the transcription factor ΔfosB. This protein is a highly stable and truncated isoform of the fosB protein that may persist in the brain over long periods, on the order of weeks to months (Nestler, 2008). The long life of ΔfosB results in increasing, accumulating levels of the transcription factor with repeated or continuous exposure to specific stimuli. Drugs of abuse are potent upregulators of ΔfosB (Nestler, 2008; Perrotti et al., 2008), including amphetamine in rats and mice (Nye et al., 1995; Shen et al., 2008; Renthal et al., 2008; Conversi et al., 2008, 2011). This protein is also implicated in natural rewards such as food-reinforcement (Olausson et al., 2006; Christiansen et al., 2011), wheel running (Werme et al., 2002; Greenwood et al., 2011), and sex (Meisel and Mullins, 2006; Wallace et al., 2008; Hedges et al., 2009; Pitchers et al., 2010). Although the regional activation of ΔfosB is stimulus specific, the NAc is a consistent target of ΔfosB response. Within the NAc, ΔfosB is specifically increased in medium spiny neurons containing D1R (MSN-D1; Lee et al., 2006; Kim et al., 2009). Therefore, ΔfosB is an ideal candidate for exploring NAc neuroplasticity in prairie voles. Given that pair bond formation is dependent upon dopaminergic reward pathways in the NAc (Wang et al., 1999; Gingrich et al., 2000; Liu and Wang, 2003; Aragona et al., 2003), it is possible that pair bonding may function as a natural reward, leading to an upregulation of ΔfosB in this region.

To explore whether ΔfosB is altered by drug and social experience in prairie voles, we performed two separate experiments. Specifically, we focused on the NAc, and analyzed the core and shell components separately. The NAc core and shell are anatomically and functionally distinct regions, with the NAc core playing a role in voluntary

*Correspondence to: C. M. Hostetler, Department of Behavioral Neuroscience, Oregon Health and Science University, 3181 SW Sam Jackson Park Road L470, Portland, OR 97239-3098, USA. Fax: +1-503-494-6877.
E-mail address: caroline.hostetler@gmail.com (C. M. Hostetler).
Abbreviations: DS, dorsal striatum; D1R, dopamine D1 receptor; MSN-D1, D1R-containing medium spiny neurons; NAc, nucleus accumbens.

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motor behavior and the NAc shell mediating limbic processes (Deutch and Cameron, 1992; Di Chiara, 2002; Kelley, 2004). Although both regions are implicated in reward-related behaviors, their distinct behavioral roles warrant individual analysis. The dorsal striatum (DS) was used as a comparison region, as neither repeated amphetamine nor pair bonding alter D1R in this region in prairie voles (Aragona et al., 2006; Liu et al., 2010; Young et al., 2011). In the first experiment, animals were treated with repeated injections of amphetamine, and then brain tissue was analyzed for ΔfosB expression. We expected to see an increase in ΔfosB expression in the NAc of drug-treated subjects, consistent with the literature in other species. In the second experiment, animals were housed with one of three social partners: a familiar same-sex sibling, an unfamiliar same-sex partner, or an unfamiliar opposite-sex partner. Here, we predicted that social contact with an opposite-sex partner would act as a "social reward," leading to an upregulation of ΔfosB expression in the NAc.

In the third experiment, we also investigated whether social isolation would result in altered ΔfosB activity. Social isolation in prairie voles results in a number of depressive-like characteristics, including anhedonia, reduced immune function, and alterations in both endocrine and neuroanatomical variables (Klein et al., 1997; Stowe et al., 2005; Grippo et al., 2007, 2009; Carter et al., 2008; Pan et al., 2009; Bosch et al., 2009; Pournajafi-Nazarloo et al., 2009, 2011). Although the NAc has not been studied in isolated voles, research in rats and mice indicates that isolation leads to a number of alterations in the NAc, particularly in dopaminergic measures (Jones et al., 1992; Hall et al., 1998; McCormick et al., 2002; Fone and Porkess, 2008; Shao et al., 2009; Wallace et al., 2009; Wang et al., 2011; Han et al., 2011; Fabricius et al., 2011). ΔfosB is reduced in the NAc of both isolated mice and clinically depressed humans (Vialou et al., 2010a,b). We hypothesized that isolation would lead to decreased levels of NAc ΔfosB. Additionally, animals were isolated from either a same-sex or opposite-sex partner, to investigate whether each behavioral and ΔfosB responses differed based on the sex of the social partner.

EXPERIMENTAL PROCEDURES

Subjects

The prairie voles (*Microtus ochrogaster*) used in this study were from an outbred stock originally captured in Illinois and reared at the University of California, Davis. Animals were weaned at 21 days of age and housed in same sex pairs in standard "shoebox" mouse cages (27 cm long×16 cm wide×13 cm high) until testing as adults. Colony rooms were maintained under controlled temperature, humidity, and light cycles (14L:10D). Food (Purina high-fiber rabbit diet) and water were available *ad libitum*. All procedures were approved and annually reviewed by the Institutional Animal Care and Use Committee of the University of California, Davis. Different subjects were used for each experiment. All subjects and stimulus animals were tested as adults (60–120 days of age).

Experiment I: The effects of amphetamine exposure on ΔfosB expression

Subjects were adult male and female prairie voles housed in same-sex cages. Subjects were given either amphetamine (5 mg/kg) or saline vehicle i.p. injections once daily for 4 days. This dose results in conditioned place preference in prairie voles (Liu et al., 2010) and is comparable to that shown to increase striatal ΔfosB over 4 days of repeated administration in previous studies (Nye et al., 1995; Conversi et al., 2008; Nestler, personal communication). Sample size was six males for each drug treatment and five females for each drug treatment. Forty-eight hours after the final injection, subjects were euthanized via cervical dislocation under deep isoflurane gas anesthesia and brains removed for histological analysis. At this time, all acute stimulus-induced full-length fosB is degraded, and immunoreactive cells are specifically positive for ΔfosB (Perrotti et al., 2008).

Tissue fixation. Following sacrifice, brains were removed and kept in 4% paraformaldehyde with acrolein at 4 °C for approximately 24 h. Following overnight fixation, tissue was stored at 4 °C in sucrose (25% in dH_2O) with sodium azide until sectioning. Brains were sectioned on a freezing microtome at 40 μm thickness and stored in cryoprotectant at −20 °C until the time of assay.

Immunohistochemistry. Floating sections were rinsed in 0.01 M KPBS, then incubated for 15 min at room temperature in 3% H_2O_2. Sections were rinsed again with KPBS and then incubated for 1 h at room temperature in a blocking solution of 0.3% Triton-X, 3% normal goat serum in KPBS. Following blocking, tissue was incubated for 48 h at 4 °C in 0.3% Triton-X, 1% normal goat serum, and 1:10,000 fosB primary antibody (*sc*-48; Santa Cruz Biotechnology, Santa Cruz, CA, USA) with blocking reagent in KPBS. After 2 days, the tissue was washed in KPBS, then incubated for 90 min at room temperature in biotin-goat anti-rabbit IgG at 1:200 dilution in blocking solution. Following incubation, the sections were washed in KPBS. Sections were then incubated for 1 h at room temperature in A/B solution. Sections were then rinsed first in KPBS, then in 0.1 M Tris buffer at pH 7.5. Tissue was then incubated in a DAB/nickel/peroxidase substrate solution (Vector Laboratories, Burlingame, CA, USA) for 5 min, until color change was observed. Sections were given final washes in KPBS and mounted within 1 week.

Cell counts. Cell counts were obtained from sections of the NAc and dorsal striatum corresponding to approximately 1.10–1.18 mm rostral from bregma (corresponding to Figs. 21 and 22 of the mouse brain atlas of Franklin and Paxinos, 2008). Photographs were taken at 50× magnification using a Micropublisher 3.3 RTV camera on a Leica DM4000B microscope. We analyzed photographs of both hemispheres from two sections, leading to a total of four counts per subject. Cell counts were collected using the NIH ImageJ software. For each section, the region of interest was manually selected by an experimentally blind observer, according to Franklin and Paxinos (2008), and the total area was recorded. The threshold function was used to identify staining for each section. A fixed threshold value was selected prior to analysis as the threshold that would be sufficient to completely fill strongly stained cells, and was used for all sections within an experiment. Individual cells were counted using the analyze particles function using a size range that captured cells stained with moderate or higher (i.e. strong) intensity.

To allow standardized comparisons between subjects, the density of positive cell counts was calculated for each section as the number of cells per 500 μm^2. Within each subject, the density of positive cells was averaged for each region. Standardized cell counts were then examined with mixed model ANOVA with sex,

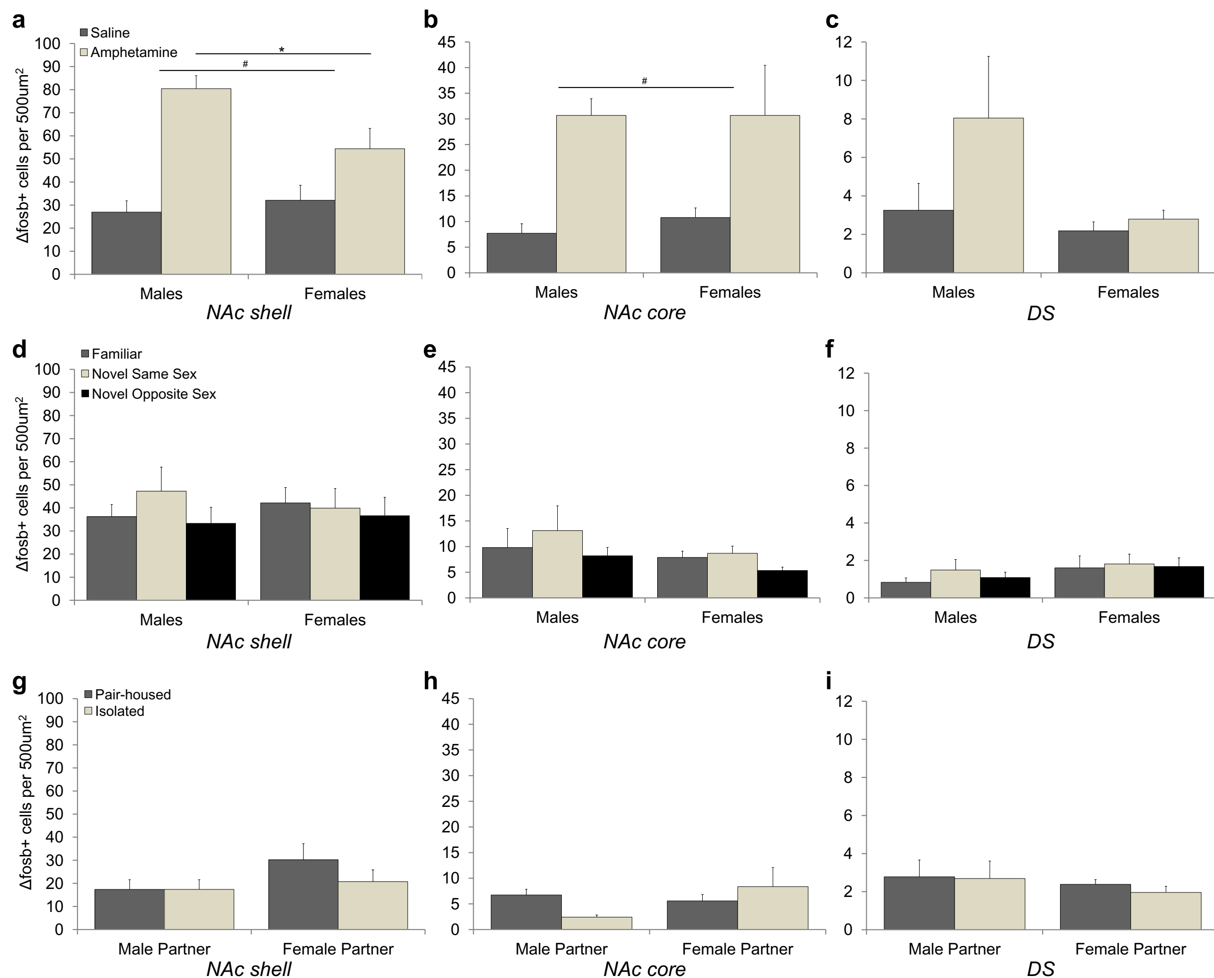

Fig. 1. ΔfosB+ cell counts (means±SE) for each brain region following amphetamine vs. saline treatment (a–c), housing with different social partners (d–f), and isolation (female subjects only; g–i). Bars indicate group differences: # indicates an overall effect of amphetamine treatment ($P<0.001$); * indicates a within-treatment sex difference ($P<0.05$). For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

drug, and sex×drug interaction as fixed factors. Post hoc analyses were performed using least squared means.

Experiment II: The effects of specific social partners on ΔfosB expression

Social pairing. Subjects were gonadally intact adult male and female prairie voles. Animals were housed in a novel cage in one of the following pairings: familiar males ($n=4$ pairs), familiar females ($n=4$ pairs), unfamiliar males ($n=4$ pairs), unfamiliar females ($n=4$ pairs), 1 male and 1 female (both unfamiliar; $n=8$ pairs) for a total of 48 animals. Animals remained in undisturbed pairs for 10 days. This period was selected based on previous studies showing that 10 days is sufficient to detect changes in ΔfosB expression following a number of conditions (Conversi et al., 2008; Perrotti et al., 2004; Vialou et al., 2011), including natural rewards (sucrose: Wallace et al., 2008).

Tissue fixation and immunohistochemistry. As described in Experiment I, the fosB primary antibody binds to both ΔfosB and the fosB protein, which requires the use of additional techniques (such as Western blot or immunohistochemistry subtraction specific for the fosB protein) to verify the specificity of ΔfosB expression. However, due to the prolonged time-period of the experiments (10 days), and the shorter half-life of the fosB protein, changes detected by the pan-fosB antibody in these experiments are inferred as changes in ΔfosB.

Cell counts. Photographs of the DS and NAc were taken at 50× magnification using a Micropublisher 3.3 RTV camera on a Leica DM4000B microscope. Cell counts were made using the NIH ImageJ software as described for Experiment I, with the exception that for some subjects, only two or three sections were analyzed, rather than four, to create an average cell count. Cell counts for each region were examined with mixed model ANOVA with sex, partner (familiar, unfamiliar same-sex, opposite-sex), and sex×partner interaction as fixed factors.

Experiment III: The effects of social isolation on ΔfosB expression

Pair formation and separation. In order to conserve animal numbers, and because the previous experiments failed to find any sex differences in ΔfosB expression, Experiment III was performed in female subjects only. Female subjects were paired either with a novel same-sex or novel male partner in small shoebox cages. In order to prevent confounding effects of sex and parental experience on subjects paired with a male, stimulus

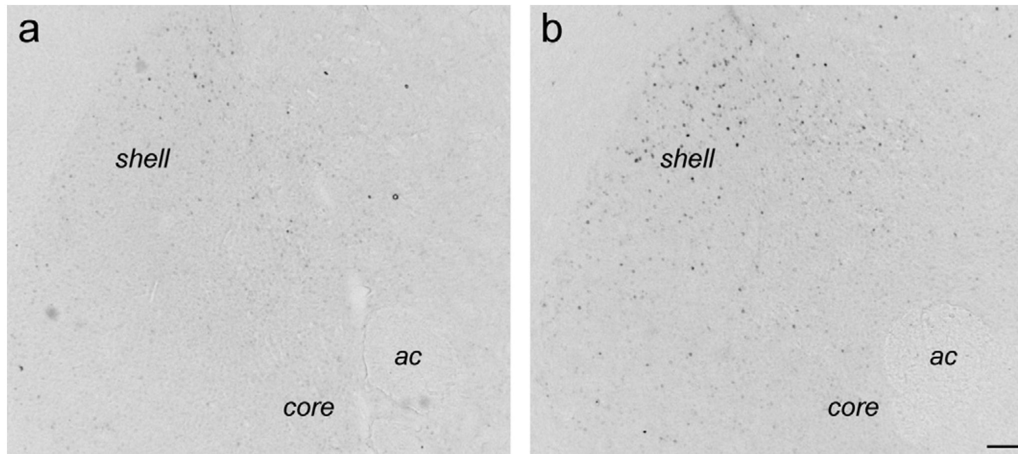

Fig. 2. Representative micrographs at 50× magnification of the nucleus accumbens of saline- (a) and amphetamine-treated (b) subjects (*ac*, anterior commissure). Scale bar indicates 100 μm.

males were castrated and fully recovered (at least 7 days post-operation) before pairing. Animals remained in these pairs under regular colony husbandry for 6 weeks. A 6-week period was used to examine whether a longer period of cohabitation than used in Experiment II would lead to differences in ΔfosB expression based on the sex of the social partner. At 6 weeks, half of the subjects in each pairing group were then moved individually to a novel shoebox cage with a cotton nestlet. Subjects remained in their respective pair- or isolation-housing for additional 10 days. This isolation period is based on studies showing that repeated stress over 10 days is sufficient to increase striatal ΔfosB (Conversi et al., 2008; Perrotti et al., 2004; Vialou et al., 2010b). A total of 30 females were used in this study: seven in each male-paired group, and eight in each female-paired group.

Behavioral observations. For all subjects, repeated home-cage video-taped observations were conducted. During the fifth week post-pairing ("Pre-Isolation"), subjects were recorded for both morning and afternoon observations. On the morning of isolation ("Isolation"), all isolated subjects were video-taped for the first hour following separation. One week following isolation ("Post-Isolation"), all subjects (regardless of housing condition) were recorded for morning and afternoon observations. All morning observations were recorded between 830 and 1130 h, and between 1300 h and 1500 h for afternoon sessions. Subjects were only recorded once a day. Videos were later scored using BehaviorTracker 1.5 (www.behaviortracker.com) by an experimentally blind observer. The behaviors scored were duration of each autogrooming, exploratory rearing, allogrooming, side-by-side contact, nest building, and in nest. Allogrooming and side-by-side contact measures were limited to observations of socially housed subjects, and both nest building and time in nest were limited to observations of isolated animals.

Tissue fixation and immunohistochemistry. On the tenth day of isolation, animals were sacrificed and brain tissue was collected. Tissue was post-fixed, stored, and assayed as described for Experiments I and II.

Cell counts. Photographs of the DS and NAc were taken at 50× magnification using a Micropublisher 3.3 RTV camera on a Leica DM4000B microscope. Cell counts were made using the NIH ImageJ software as described for Experiment I, with the exception that for some subjects, only two or three sections were analyzed, rather than four, to create an average cell count. Cell counts for each region were examined with mixed model ANOVA with isolation, sex of partner, and isolation×partner interaction as fixed factors.

Behavioral data analysis. In order to determine whether behaviors within a condition differed based on the time of observation, each pair of behaviors from Pre- and Post-Isolation week observations was analyzed for within-subject effects of time of day (AM and PM) using paired *t* tests. There were no differences based on time of day for any behavior, and an average was calculated for each pre- and post-isolation behavior for subsequent analyses. For the pre-isolation period, each behavior was analyzed via paired *t* tests based on the sex of the social partner. For isolated subjects only, we performed a repeated measured ANOVA using a main within-subjects effect of partner's sex, and housing period as the repeated measure. Post hoc analyses were performed using paired *t* tests.

RESULTS

Experiment I: Repeated amphetamine

Repeated amphetamine exposure increased ΔfosB in the NAc. In the NAc core there was a significant main effect of drug ($F_{1,20}=21.02$, $P=0.0002$), with amphetamine-treated animals having more ΔfosB+ cells than saline-treated controls (Fig. 1a,b). There was also a significant main effect of drug treatment in the NAc shell ($F_{1,21}=36.81$, $P<0.0001$), with amphetamine-treated subjects having more ΔfosB+ cells than saline controls. Representative photomicrographs are presented in Fig. 2. There was also a significant interaction between sex and treatment ($F_{1,18}=5.63$, $P=0.03$). Post hoc tests revealed that within each sex, amphetamine-treated subjects had higher ΔfosB+ activity in the NAc shell than saline controls (males: $P<0.0001$; females: $P=0.03$), and among amphetamine-treated animals males had significantly higher ΔfosB+ activity than females ($P=0.01$). There was no significant sex difference among saline controls. There was no significant main effect of sex in this region ($F_{1,18}=2.51$, $P=0.13$).

In the DS there was no effect of drug treatment ($F_{1,27}=1.98$, $P=0.18$) or sex ($F_{1,27}=2.31$, $P=0.15$) on ΔfosB+ cell counts.(Fig. 1c)

Experiment II: Social partners

There was no effect of specific social partners on ΔfosB+ activity in any region examined (Fig. 1d-f). There was no

Table 1. Time in seconds (means±SE) engaged in home cage behaviors following 6 wks of cohabitation (female subjects only, * indicates difference due to partner's sex, $P<0.001$)

	Partner	
	Female	Male
Exploratory rearing	743±126	1048±129
Autogrooming	449±52*	205±22
Side by side contact	1089±126	1149±148
Allogrooming	188±29	200±35

significant main effect of either sex ($F_{1,42}$=1.90, P=0.18) or partner ($F_{2,42}$=1.16, P=0.32) on ΔfosB+ activity in the NAc core. There was no significant main effect of either sex ($F_{1,42}$=0.01, P=0.92) or partner ($F_{2,42}$=0.59, P=0.56) on ΔfosB+ activity in the NAc shell. In the DS, there were no significant effects of sex ($F_{1,42}$=2.11, P=0.15) or partner ($F_{2,42}$=0.43, P=0.66) on ΔfosB+ activity. There were no significant interactions between sex and partner in any region (NAc core: $F_{2,42}$=0.11, P=0.89; NAc shell: $F_{2,42}$=0.41, P=0.67; DS:$F_{2,42}$=0.11, P=0.89).

Experiment III: Social isolation

Behavioral observations. Five weeks after initial cohabitation, females that were housed with another female spent significantly more time autogrooming than females housed with a male (t=4.12, df=28, P=0.0003). No other behaviors differed between the two groups (Table 1, $P\geq0.10$ for all behaviors).

Behaviors for isolated subjects across the three observation periods are presented in Table 2. Time spent engaging in exploratory rearing was not affected by partner's sex at any time period ($P\geq0.42$ for all periods). There was a main effect of housing period on exploratory rearing ($F_{2,26}$=8.11, P=0.0018), with pre-isolation rearing being significantly lower than both isolation (P=0.014) and post-isolation (P=0.0006), and no difference between isolation and post-isolation (P=0.21). There was no interaction of housing period with the partner's sex ($F_{2,26}$=0.66, P=0.53).

For autogrooming, there was a significant main effect of partner's sex during the pre-isolation period ($F_{1,14}$=7.05, P=0.02), and a trend during initial isolation ($F_{1,14}$=4.12, P=0.06). In both cases, females housed with a female partner autogroomed more than those housed with a male partner. These differences are not apparent 1 week following isolation ($F_{1,14}$=0.61, P=0.45). There was also a main effect of housing period ($F_{2,26}$=3.63, P=0.04) as well as a significant interaction between housing period and partner's sex ($F_{2,26}$=5.33, P=0.01; Table 2). For all subjects, time spent autogrooming during the initial isolation period was lower than each the pre- and post-isolation periods (P=0.05 and 0.002, respectively). However, when females were analyzed separately based on the sex of their partner, the effects of housing period are driven by females housed with male partners. Whereas females housed with a female partner showed no difference in autogrooming across housing periods ($P\geq0.14$ for all comparisons), females housed with males spent more time autogrooming 1 week following isolation compared to each pre- and isolation observations (P=0.01 and 0.001, respectively). Additionally, there was a trend for autogrooming to be higher during the initial isolation compared to pre-isolation levels (P=0.08).

There were no effects of partner's sex on nest building or time spent in the nest ($P\geq0.23$ for all observations). There was a significant main effect of housing period on time spent in the nest ($F_{1,13}$=6.43, P=0.02), with females spending more time in the nest following a week of isolation than during the initial isolation period. This is likely due to the nest not being constructed during the initial isolation period.

Quantification of ΔfosB expression. Isolation did not affect ΔfosB+ levels in any region examined (Fig. 1g-i). In the NAc core, there was no significant main effect of either partner's sex ($F_{1,26}$=1.25, P=0.27) or isolation ($F_{1,26}$=0.07, P=0.79) on ΔfosB+ activity. There was no significant main effect of either partner's sex ($F_{1,26}$=2.13, P=0.16) or isolation ($F_{1,26}$=0.82, P=0.37) on ΔfosB+ activity in the NAc shell. There was no significant interaction between partner's sex and isolation for the NAc core ($F_{1,26}$=2.51, P=0.13) or shell ($F_{1,26}$=0.20, P=0.41). Similarly, there was no significant main effect of either partner's sex ($F_{1,25}$=0.86, P=0.36) or isolation ($F_{1,25}$=0.20, P=0.66) on ΔfosB+ activity in the DS. There was no significant interaction between the sex of the partner and isolation status ($F_{1,25}$=0.08, P=0.78).

DISCUSSION

This study examined expression of the transcription factor ΔfosB under different drug and social conditions in the prairie vole. We found that 4 days of amphetamine treatment increased ΔfosB in the NAc, but not the DS. This is consistent with previous findings in other laboratory spe-

Table 2. Time in seconds (means±SE) engaged in home cage behaviors for each observation period, in isolated female subjects only

	Female partner (n=8)			Male partner (n=7)		
	Pre-isolation	Isolation	Post-isolation	Pre-isolation	Isolation	Post-isolation
Exploratory rearing	839±179	1650±277	1899±329	927±199	1361±270	1567±203
Autogrooming	425±70	272±51	324±46	211±32	150±27	380±55
Nest building		709±177	53±17		1677±809	55±25
In nest		69±30	620±258		33±26	725±155

cies. None of the social manipulations altered ΔfosB activity in any region examined. In two separate experiments, ΔfosB was not increased following cohabitation with an opposite-sex partner. Additionally, isolation did not affect ΔfosB in any region examined. These findings suggest that social stimuli, in contrast to drugs of abuse, are not mediators of ΔfosB in the striatum.

Amphetamine

Repeated exposure to amphetamine increased ΔfosB in both the core and shell of the NAc (Fig. 1a, b). This is consistent with previous work in rats and mice (Nye et al., 1995; Shen et al., 2008; Renthal et al., 2008; Conversi et al., 2008, 2011), suggesting that prairie voles show a typical ΔfosB response in the NAc to repeated exposure to drugs of abuse. We did not see any effect of amphetamine on ΔfosB expression in the DS (Fig. 1c), which contrasts with work in mice (Conversi et al., 2008, 2011). However, the effects of repeated amphetamine on ΔfosB in the DS are strain specific, as C57BL/6J mice show increased activity in this region, whereas DBA mice show no change (Conversi et al., 2008, 2011). Likewise, a species difference in DS responsiveness to amphetamine is not unexpected.

Repeated psychostimulant exposure increases the density of dendritic spines NAc MSN-D1, and this morphological change is associated with increase in ΔfosB in these cells (Lee et al., 2006; Kim et al., 2009). Although this has not been explicitly studied with amphetamine, the fact that this ΔfosB-associated change in dendritic morphology is seen with methylphenidate and cocaine supports a common mechanism for psychostimulants on MSN-D1 (Lee et al., 2006; Kim et al., 2009). ΔfosB is thought to play role in either the formation or maintenance of dendritic spines in MSN-D1 of the NAc under conditions of repeated psychostimulant exposure (Lee et al., 2006). We propose that the increase in ΔfosB in the NAc of amphetamine-treated prairie voles seen here provides a potential molecular mechanism for increased D1R mRNA and protein levels previously reported in similarly treated animals (Liu et al., 2010; Young et al., 2011). It is interesting to note that the relative increase in ΔfosB following repeated amphetamine is smaller in females than males in both the NAc shell and DS, although this difference is not significant. Increases in striatal D1R are more robust in males than females (Liu et al., 2010; Young et al., 2011). This provides further support for a role of ΔfosB in amphetamine-induced alterations of D1R in the prairie vole.

Social partners

Ten days of social housing with a novel partner of either sex did not affect ΔfosB levels in the NAc or DS (Experiment II, Fig. 1d–h). Eight weeks of cohabitation with a castrated male partner also did not increase NAc ΔfosB in female subjects, when compared with those housed with another female (Experiment III, Fig. 1g–i). A likely explanation for our findings is that pair bonding is not a sufficient reward to increase ΔfosB levels in the NAc. Although there are some studies examining changes in neurophysiology and behavior that follow pair bond maintenance (Bowler et al., 2002; Aragona et al., 2006; Gobrogge et al., 2007, 2009), the majority of research on pair bonding in prairie voles has focused on mechanisms of partner preference formation. Reward clearly plays key role in the formation of partner preferences; however, our current data suggest that pair bond maintenance is not associated with reward. These findings support the idea that formation and maintenance are separate processes in both their behavioral and physiological regulation (Depue and Morrone-Strupinsky, 2005; Bales et al., 2007; Dunbar, 2010) and highlight the need for future research focusing on the neurobiology of partner preference maintenance in prairie voles.

Given that similar increases in NAc D1R are seen with both pair bonding and amphetamine, we expected to see similar patterns of ΔfosB expression in Experiments I and II. However, there are a number of differences in the outcomes of these conditions that contradict a common underlying mechanism. First, the timeline of changes in receptors differs. Increased D1R may be seen after 3 days of amphetamine treatment (Liu et al., 2010; Young et al., 2011), whereas changes following pair bonding do not emerge for 2 weeks following pairing (Aragona et al., 2006; Liu et al., 2011). Second, increases in NAc ΔfosB are associated with an increase in the rewarding properties of drugs of abuse (Colby et al., 2003; Nestler, 2008). However, pair bonding reduces the rewarding properties of amphetamine (Liu et al., 2010). These factors and our findings suggest that ΔfosB is not a likely mediator of alterations in NAc D1R following pair bonding. A limitation of this interpretation is that we did not perform partner preference tests in these subjects, therefore the presence of a pair bond in these subjects was not verified. However, given the established timeline for the formation of a pair bond in prairie voles, it is highly likely that the subjects had already formed a bond (DeVries and Carter, 1999).

Previous findings in other rodent species have shown that sexual experience increases ΔfosB in the NAc (Meisel and Mullins, 2006; Wallace et al., 2008; Pitchers et al., 2010). Although we did not verify or quantify mating bouts in the opposite-sex pairs studied here, previous work has shown that prairie vole pairs mate an average 120 times over the first 4 days following pairing (Witt et al., 1988). These mating frequencies are comparable or even higher than those shown to increase ΔfosB levels in other rodent species (Meisel and Mullins, 2006; Wallace et al., 2008; Pitchers et al., 2010). This suggests the intriguing possibility that ΔfosB is not affected by sexual experience in prairie voles, highlighting a potential species difference that warrants further investigation.

Social isolation

Home cage observations of subjects before, during, and following isolation highlight interesting behavioral differences in female subjects housed with a male vs. another female. During the initial social housing period, females that were housed with another female spent more time autogrooming than those housed with males. This may indicate differences in anxiety based on the sex of a social

partner. This is further supported by findings in isolated subjects. Patterns of autogrooming over time were dependent on the sex of the partner. Females that were separated from a male partner showed an increase in autogrooming after 1 week of isolation, whereas isolation from a female partner did not affect autogrooming behavior. Bosch and colleagues (2009) also found that passive stress-coping behavior in the forced swim test differs between male prairie voles isolated from a same sex vs. opposite sex sibling. Therefore, the sex of the social partner may influence the anxiety state of an individual, with persistent effects beyond the social interaction period. Duration of exploratory rearing was increased during and following isolation. This increased activity was independent of the sex of the social partner, and suggests that arousal levels may be increased in isolated animals.

Our initial hypothesis that social isolation would lead to down-regulation of ΔfosB in the NAc was not supported, regardless of whether subjects were isolated from a same-sex or opposite-sex partner. There were no effects of isolation on ΔfosB levels (Fig. 1g–i). This contrasts with recent research in mice and humans showing that ΔfosB is reduced in isolated mice and depressed patients (Vialou et al., 2010a,b). The 10-day isolation period used in this study is markedly shorter than the 8-week separation used in mice (Vialou et al., 2010b), but was chosen based on previous studies showing that repeated stress over 10 days increases striatal ΔfosB (Conversi et al., 2008; Perrotti et al., 2004; Vialou et al., 2010b). It is possible that although some behavioral and physiological adaptations to isolation are shown within 1 to 2 weeks (Stowe et al., 2005; Ruscio et al., 2007; Grippo et al., 2011), a longer period of isolation is required to induce changes in ΔfosB. Certain depressive-like behaviors in prairie voles do not emerge until at least 4 weeks of isolation (Grippo et al., 2007). Exploring the effects of a longer separation period, comparable to that used in mice, may yield similar decreases in ΔfosB and this remains a pertinent direction for future research. An additional explanation may be that lack of ΔfosB response to isolation in prairie voles reflects a species difference.

A potential confound for the isolation experiment was the use of castrated males as social partners. The hormonal status of the partner should not affect the formation of a pair bond (DeVries et al., 1997) and mating is not required to form a partner preference (Winslow et al., 1993; Cho et al., 1999). In contrast, it is possible that maintenance of a pair bond is dependent on gonadal status and/or successful reproduction (Curtis, 2010). A related limitation of the present study is that we did not measure partner preference in these subjects; therefore, we cannot definitively conclude that pair bond maintenance is not associated with changes in ΔfosB expression. However, the majority of behavioral and physiological consequences of isolation in prairie voles are independent of the sex of the partner, and we would still expect to see a general decrease of ΔfosB in all isolated subjects. Additionally, the current study only examined the effects of isolation on female subjects. While we did not find sex differences in ΔfosB response to either amphetamine or with different social partners, it is possible that a sex difference in response to isolation may exist in this species. We conclude that ΔfosB does not appear to mediate the physiological or behavioral changes observed following isolation, at least with respect to the parameters explored in these studies. These findings suggest that the ΔfosB response may be highly specific to certain types of stressors and that this response may be species-specific.

It should be noted that the relative time courses for examining ΔfosB expression in these experiments are not parallel. Thus an intriguing possible explanation for our findings is that the time course for ΔfosB response to stimuli in prairie voles may peak at 4 days. This remains to be investigated in future studies.

CONCLUSIONS

This study investigated a potential molecular mechanism underlying neural and behavioral plasticity underlying amphetamine exposure, pair bonding, and isolation in the prairie vole. We focused on the transcription factor ΔfosB, which has been implicated as a mediator of neural plasticity following each reward and stress. Repeated treatment with amphetamine increased ΔfosB expression in the NAc, consistent with previous work in other species. However, we found that neither cohabitation with an opposite-sex partner nor social isolation affect ΔfosB expression in the NAc, a region implicated in both prairie vole behavior and ΔfosB activity. Our findings suggest that the maintenance of the pair bond is not characterized by reward. This provides further evidence for a separate neurobiology of each formation and maintenance. Further research into the mechanisms underlying prairie vole attachments are maintained over time will provide crucial insight into the neurobiology of affiliation.

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