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

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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

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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,

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