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# ΔFosB overexpression in the nucleus accumbens enhances sexual reward in female Syrian hamsters

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## Abstract

Repeated activation of the mesolimbic dopamine system results in persistent behavioral alterations accompanied by a pattern of neural plasticity in the nucleus accumbens (NAc). As the accumulation of the transcription factor ΔFosB may be an important component of this plasticity, the question addressed in our research is whether ΔFosB is regulated by sexual experience in females. We have shown that female Syrian hamsters given sexual experience exhibit several behavioral alterations including increased sexual efficiency with naïve male hamsters, sexual reward, and enhanced responsiveness to psychomotor stimulants (e.g., amphetamine). We recently demonstrated that sexual experience increased the levels of ΔFosB in the NAc of female Syrian hamsters. The focus of this study was to explore the functional consequences of this induction by determining if the constitutive overexpression of ΔFosB by adeno-associated viral (AAV) vectors in the NAc could mimic the behavioral effects of sexual experience. Animals with AAV-mediated overexpression of ΔFosB in the NAc showed evidence of sexual reward in a conditioned place preference paradigm under conditions in which control animals receiving an injection of AAV-green fluorescent protein (GFP) into the NAc did not. Sexual behavior tests further showed that males paired with the AAV-ΔFosB females had increased copulatory efficiency as measured by the proportion of mounts that included intromission compared to males mated with the AAV-GFP females. These results support a role for ΔFosB in mediating natural motivated behaviors, in this case female sexual behavior, and provide new insight into the possible endogenous actions of ΔFosB.

### Keywords

female sexual behavior; conditioned place preference; adeno-associated virus

## Introduction

Experience with drugs of abuse, motivated behaviors, wheel running behavior or instrumental learning results in the activation of the mesolimbic dopamine system and persistent alterations in the nucleus accumbens (NAc) (Becker *et al.*, 2001, Di Chiara *et al.*, 1998, Harris *et al.*, 2007, Kumar *et al.*, 2005, Meisel & Mullins, 2006, Nestler, 2008, Olausson *et al.*, 2006, Perrotti *et al.*, 2008, Pierce & Kumaresan, 2006, Wolf *et al.*, 2004). Structural changes, particularly the formation of dendritic spines, are an important component of this experience based plasticity (Allen *et al.*, 2006, Lee *et al.*, 2006, Li *et al.*, 2003, Meisel & Mullins, 2006, Norrholm *et al.*, 2003, Robinson & Kolb, 2004), which

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remains long after either the behavioral experience or drug administration has ceased (McClung & Nestler, 2008, Meisel & Mullins, 2006, Wolf *et al.*, 2004).

The transcription factor ΔFosB has molecular properties that make it a good candidate to mediate the enduring structural and behavioral modifications consequent to behavioral or drug experiences (Chen *et al.*, 1997, Chen *et al.*, 1995, Colby *et al.*, 2003, Doucet *et al.*, 1996, Hope *et al.*, 1994, Kelz *et al.*, 1999, McClung & Nestler, 2003, McClung *et al.*, 2004, McDaid *et al.*, 2006, Nakabeppu & Nathans, 1991, Nestler, 2008, Nye *et al.*, 1995, Olausson *et al.*, 2006, Perrotti *et al.*, 2008, Wallace *et al.*, 2008, Werme *et al.*, 2002, Zachariou *et al.*, 2006). ΔFosB is an alternative splice product of the immediate early gene *fos*B (Mumberg *et al.*, 1991, Nakabeppu & Nathans, 1991) and, unlike the full length FosB protein, the truncated ΔFosB has unusual stability resulting in accumulation of the protein following repeated stimulation (Chen *et al.*, 1997, Chen *et al.*, 1995, Hope *et al.*, 1994, Kelz *et al.*, 1999, Perrotti *et al.*, 2008, Zachariou *et al.*, 2006). Although the mechanism by which the *fos*B gene is alternatively spliced remains unknown, the truncation of the protein along with phosphorylation protects the protein from rapid proteasomal degradation producing a greater level of transcriptional activity compared with more transiently-lived FosB family members (Carle *et al.*, 2007, Ulery & Nestler, 2007, Ulery *et al.*, 2006). The postulate is that accumulation of ΔFosB protein produces patterns of gene expression that may underlie the effects of experience on long-term behavioral and cellular plasticity (McClung & Nestler, 2008).

We have used female sexual behavior in Syrian hamsters as a model of experience-based plasticity in the brain (Bradley *et al.*, 2005a, Bradley *et al.*, 2005b, Bradley & Meisel, 2001, Bradley *et al.*, 2004, Kohlert & Meisel, 1999, Kohlert *et al.*, 1997, Meisel *et al.*, 1993, Meisel & Joppa, 1994, Meisel *et al.*, 1996, Meisel & Mullins, 2006). An advantage to working with sexual behavior is the ability to control the level of an animal's experiences by having either completely sexually naïve animals, or by differentially exposing animals to varying levels of sexual experience. We have previously shown that repeated sexual experience results in sensitization of the mesolimbic dopamine system, analogous to that of drugs of abuse (Bradley *et al.*, 2005b, Bradley & Meisel, 2001, Brenhouse & Stellar, 2006, Cadoni & Di Chiara, 1999, Hope *et al.*, 1992, Kelz *et al.*, 1999, Kohlert & Meisel, 1999, Pierce & Kalivas, 1995, Pierce & Kalivas, 1997a, Pierce & Kalivas, 1997b, Robinson & Kolb, 1999a). For example, like the effects of drugs, repeated sexual experience increases dendritic spines in medium spiny neurons of the NAc (Lee *et al.*, 2006, Li *et al.*, 2003, Meisel & Mullins, 2006, Norrholm *et al.*, 2003, Robinson *et al.*, 2001, Robinson & Kolb, 1997, Robinson & Kolb, 1999a, Robinson & Kolb, 1999b, Robinson & Kolb, 2004). Further, we have found that ΔFosB/FosB staining is persistently elevated in the NAc following repeated sexual experience (Meisel & Mullins, 2006).

Given that sexual experience can produce long-lasting expression of FosB family members, the purpose of this study was to manipulate ΔFosB expression to mimic the behavioral consequences of repeated sexual experience. Following viral-mediated overexpression of ΔFosB in the NAc, female Syrian hamsters were tested for enhanced conditioned place preference and also increased copulatory efficiency with naïve male hamsters, two endpoints that have previously been shown to be affected by repeated sexual experience (Bradley *et al.*, 2005b, Meisel & Joppa, 1994, Meisel *et al.*, 1996, Meisel & Mullins, 2006). We report here that by persistently overexpressing ΔFosB in the NAc of female hamsters receiving minimal sexual experience, we are able to produce behavioral changes similar to those females with more extensive sexual experiences.

## Materials and Methods

### Experimental Subjects

Male and female Syrian hamsters were delivered at approximately 60 days of age from Charles River Breeding Laboratories, Inc. (Wilmington, MA). Females were housed individually in plastic cages (50.8 cm long × 40.6 cm wide × 20.3 cm high), while the male stimulus animals were group-housed in identical cages in numbers of three or four. The animal room was maintained at a controlled temperature of 22 °C with a 14:10 hr light-dark schedule (lights off between 1:30 and 11:30 p.m.). Food and water were available to the animals *ad libitum*.

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

### Surgery

Female hamsters were bilaterally ovariectomized under sodium pentobarbital anesthesia (Nembutal; 8.5 mg per 100 gm body weight, i.p.), given supplemental anesthetic and then underwent bilateral stereotaxic surgery for the delivery of viral vectors. During stereotaxic surgery, the head was shaven and the skin and muscle retracted. A small hole was drilled in the skull and a 5 μL Hamilton syringe was lowered to the level of the NAc from a 2° lateral angle to ensure clearance of the lateral cerebral ventricles. The syringe was kept in place for 5 min prior to injections and then either adeno-associated virus (AAV)-GFP or AAV-ΔFosB (0.7 μL) was delivered into the NAc over 7 min, with the syringe then kept in place for an additional 5 min. This procedure was repeated for the contralateral side of the brain.

### Viral Vectors

AAV is characterized by its ability to efficiently transfect neurons as well as to maintain specific transgene expression for long periods of time (Chamberlin *et al.*, 1998). AAV vectors exist in different serotypes based on the characterization of their capsid protein coat. This experiment utilized an AAV2 (serotype 2) from Stratagene with a titer of over $10^8$/μl expressing green fluorescent protein (AAV-GFP) as well as an AAV vector that had constructs for both ΔFosB and GFP (AAV-ΔFosB-GFP). The viral vectors were injected into the NAc at least 3 weeks prior to behavioral testing to allow for ΔFosB overexpression to develop. These AAV vectors mediate transgene expression in rats and mice that becomes maximal within 10 days of injection and then persists at this level for at least 6 months (Winstanley et al., 2007, Zachariou et al., 2006). Importantly, the vectors infect neurons only and produce no toxicity greater than vehicle infusions alone. Details of the production and use of these vectors are provided in earlier publications (Winstanley et al., 2007, Zachariou et al., 2006).

### Sexual Experience

All ovariectomized female hamsters were primed for sexual experience once a week by giving two daily subcutaneous injections of estradiol benzoate (10 μg in 0.1 ml of cottonseed oil) approximately 48 hr and 24 hr prior to the sexual behavior test followed by a subcutaneous injection of progesterone (500 μg in 0.1 ml of cottonseed oil) 4–6 hr prior to the sexual behavior test. Females that received sexual experience were presented with a sexually experienced male hamster for a 10 min session 4–6 hr after the progesterone injection. Each male and female was only paired once during the duration of the sexual experience tests.

### Conditioned Place Preference

A biased conditioned place preference paradigm was utilized in this experiment (Tzschentke, 1998). Our conditioned place preference apparatus (Meisel & Joppa, 1994, Meisel *et al.*, 1996) consists of one white and one gray compartment (60 × 45 × 38 cm) connected by a clear central compartment (37 × 22 × 38). The main compartments were further differentiated by aspen bedding (Harlan Laboratories, IN) in the gray compartment and corncob bedding (Harlan Laboratories, IN) in the white compartment. The ovariectomized female hamsters were hormonally primed prior to the pre-test, sexual conditioning sessions, and the post-test. During the pretest the animal was placed in the clear central chamber and was free to roam the different compartments for 10 min to establish an initial preference for each compartment. As all animals showed an initial preference for the white chamber, conditioning was performed in the gray chamber. The hormone priming was repeated during the 2 (Groups 2–5) or 5 weeks (Group 1) of conditioning. During conditioning, females were given sexual experience with a male in the gray compartment for 10 min, with female copulatory parameters measured (lordosis latency and total lordosis duration). One hr following the sexual experience test, the female was placed alone in the white chamber for 10 min. A control group of females that did not receive sexual experience were hormonally primed but placed alone in each chamber for 10 min. Following the 2 or 5 weeks of conditioning, animals were given a post-test in which they again were free to roam the chambers for 10 min. Regardless of group, all post-tests were done seven weeks post-stereotaxic surgery, and therefore all animals were sacrificed with the same level of viral expression. There were 5 groups of animals in this experiment: A positive control group of animals received bilateral AAV-GFP and given 5 weekly sexual behavior pairings with a male (Group 1, n=8). Two negative control groups were not given any sexual conditioning for 2 weeks, and received either AAV-ΔFosB (Group 2, n=5) or AAV-GFP (Group 3, n=4). Lastly, there were animals that received 2 weeks of sexual behavior pairings with a male with a bilateral injection of either AAV-ΔFosB (Group 4, n=7) or AAV-GFP (Group 5, n=7).

### Naïve Male Experiment

Previous research has shown that sexually experienced female hamsters can improve the copulatory efficiency of interactions with their sexually naïve male partners (Bradley *et al.*, 2005b). This test was given approximately one week following the conditioned place preference post-test to the two groups of animals that received 2 weeks of sexual conditioning (Groups 4 and 5). Females were hormonally primed for sexual experience as described. During the 10 min test, a sexually naïve male hamster was introduced to the female's home cage and the test session was videotaped for later analysis. The number of mounts and intromissions (including ejaculations) by the male as well as the proportion of total mounts that included intromission (hit rate) were determined from the videotape.

### Immunohistochemistry

Immunostaining was performed on all animals to verify both virus injection location and anatomical extent of protein expression. Females were given an overdose of Sleepaway (0.2 ml i.p., Fort Dodge Laboratories, Fort Dodge, IA) and intracardially perfused with 25 mM phosphate buffered saline (PBS) for 2 min (approximately 50 ml) followed by 4% paraformaldehyde in 25 mM PBS for 20 min (approximately 500 ml). The brains were removed and post-fixed for 2 hr in 4% paraformaldehyde then placed in a 10% sucrose solution in PBS overnight at 4°C. Animals that received only bilateral AAV-GFP had serial coronal sections (40 μm) cut from frozen tissue into 25 mM PBS plus 0.1% bovine serum albumin (BSA) (wash buffer) then mounted directly onto slides and coverslipped while still wet with 5% n-propyl galate in glycerin. Animals that received bilateral AAV-ΔFosB had serial coronal sections (40 μm) cut from frozen tissue, and then rinsed 3 times for 10 min in

wash buffer. AAV-ΔFosB animals were only analyzed for ΔFosB expression and therefore were incubated in ΔFosB/FosB primary antibody (1:10000, sc-48 Santa Cruz Biotechnology Inc., Santa Cruz, CA) in wash buffer plus 0.3% Triton-X 100 at room temperature for 24 hr and then moved to 4 °C for 24 hr. This concentration of primary antibody was chosen as it produces only minimal endogenous ΔFosB/FosB staining. Following incubation in primary antibody the sections were rinsed 3 times for 10 min in wash buffer, and then incubated in biotinylated-secondary antibody for 45 min at room temperature (1:200, Vector, Burlingame, CA). The sections were then washed 3 times for 10 min in wash buffer before being incubated in streptavidin Alexa Fluor 594 conjugate (1:500, Molecular Probes, Eugene, OR). Following this incubation, the sections were washed 3 times for 10 min in wash buffer then mounted on slides and coverslipped while still wet with 5% n-propyl galate in glycerin.

### Microscopic Analysis

Slides were analyzed by a Leica DM4000B light microscope with fluorescent capability coupled to a Leica DFC500 digital camera. Digital images of both the right and left injection sites of each section were serially analyzed by fluorescence microscopy to locate the injection placement in the NAc. The sections from each animal were analyzed to find the rostral to caudal spread of viral expression and also the anatomical location of the largest diameter of expression. Further, within these sections the numbers of FosB stained cells were counted in ImageJ from saved digital images. As our goal was simply to obtain approximate cell counts, stereological methods were not used.

## Results

### Time course of viral mediated overexpression of ΔFosB in the NAc of female Syrian hamsters

A separate group of animals were utilized initially to find a time course of viral-mediated ΔFosB overexpression in the female hamster. Analysis of ΔFosB expression at the 3 (n=5), 6 (n=6), and 9 (n=2) week time points revealed that 3 weeks post stereotaxic surgery produced a level of ΔFosB overexpression which was maintained through 6 and 9 weeks post stereotaxic surgery. Viral expression was mostly nuclear, but was also found in the cytoplasm and even the dendrites of some overexpressing cells. Of the thirteen animals that comprised the time course experiment, four animals had rostral NAc core viral injections, one of which spread into the bed nucleus of the stria terminalis (BNST). The remaining nine animals had caudal injection placements, seven in the caudal core, and two in the caudal shell of the NAc. Only one of the caudal shell injections crossed caudally into the BNST, while six of the injections in the caudal core crossed caudally into the BNST. The average largest diameters of viral expression for each time point were found to be 0.9 mm, 1.2 mm, and 1.0 mm for 3, 6, and 9 weeks, respectively. These average diameters were subjected to an analysis of variance and were not found to be significantly different. Therefore, in the following behavioral experiments, behavioral testing began around 3 weeks post stereotaxic surgery, and animals were sacrificed around 9 weeks post stereotaxic surgery to ensure that viral expression was maintained at a consistent level.

### Immunohistological analysis of AAV-ΔFosB and AAV-GFP viral injections

Brain sections from each animal used in the behavioral experiments were serially analyzed in a coronal plane for the anatomical location of viral injection. A total of 12 animals were analyzed for their bilateral ΔFosB expression by cell count, and injection placement, which was determined by tracing the residual needle tracks. Although injection placement was analyzed in a coronal section (Figure 1), protein expression extended in a rostral-caudal ellipse from the injection site, and also spread in a dorsal-ventral ellipse from the injection

site. Of the five animals analyzed from Group 2, 70.5% of the overexperssion cells were in the NAc (median= 16,864 cells, lower quartile= 7,551 cells, upper quartile=20,002 cells, interquartile range=12,451). The seven animals analyzed from Group 4 showed 65.6% viral overexpression in the NAc (median=9,972 cells, lower quartile=5,683 cells, upper quartile=11,213 cells, interquartile range= 5530.). These cell counts represent viral overexpression rather than endogenous staining due to the purposeful dilution of the primary antibody.

Of the 24 bilateral injection sites, twelve were in the rostral core of the NAc, six of which had viral expression that caudally spread into the BNST. The remaining twelve injection sites were in the caudal NAc. One of the twelve injections was in the caudal shell and spread caudally into the BNST. The last eleven injection sites were all in the caudal core of the NAc, eight of which spread caudally into the BNST. All injections were centered around the anterior commissure except the one injection in the caudal shell of the NAc which was slightly more medial than the anterior commissure (Figure 2). All animals showed appropriate overexpression of either GFP or ΔFosB and were therefore used in subsequent behavioral analysis. No animals were excluded from the study because of poor anatomical injection placement. Further, because all injections were aimed at the accumbal core and only one injection included the shell no statistical analysis was done on the injection sites.

## AAV vector overexpression of ΔFosB in the NAc of female Syrian hamsters results in enhanced sexual reward

To assess whether the overexpression of ΔFosB in the NAc had an effect on sexual reward we used the conditioned place preference paradigm. In this test, animals underwent either 0, 2, or 5 weeks of sexual conditioning. During sexual conditioning, lordosis latency and duration were recorded for each female hamster. Neither lordosis latency (Group 1: 553 sec ± 7 sec, Group 4: 552 sec ± 7 sec, Group 5: 561 sec ± 7 sec,) nor lordosis duration (Group 1: 485 sec ± 15 sec, Group 4: 522 sec ± 10 sec, Group 5: 522 sec ± 12 sec) during sexual conditioning differed significantly among groups throughout testing regardless of viral injection. Therefore neither the overexpression of GFP nor ΔFosB had any effect on the receptive behavior of the females.

Each group from the conditioned place preference procedure was analyzed individually with a repeated measure t-test between the amount of time spent in the conditioning compartment (gray compartment) during the pre-test and the post-test. The statistical analysis was not extended between groups. Previous research has shown that five conditioning sexual experiences are sufficient to detect significant changes in place preference (Meisel & Joppa, 1994, Meisel *et al.*, 1996). Indeed, the positive control group consisting of female animals overexpressing GFP in the NAc that were given five conditioning sexual experiences spent significantly more time during the post test in the gray chamber paired with the sexual experience compared with the pre-conditioning performance, $t(8) = -3.13$, $P < 0.05$. As anticipated, animals that were not given any conditioning sexual experiences did not change significantly the amount of time in either chamber regardless of viral injection. Females overexpressing GFP that were given 2 conditioning sexual experiences did not demonstrate place conditioning, whereas females that were given two conditioning sexual experiences with overexpression of ΔFosB spent significantly more time in the chamber paired with sexual experience during this post test, $t(7) = -2.48$, $P < 0.05$ (Figure 3).

## AAV vector overexpression of ΔFosB in the NAc of female Syrian hamsters improves their copulatory efficiency with naïve males

One week following the conditioned place preference post-test, females with 2 weeks of sexual conditioning tests (Groups 4 and 5) were subjected to a naïve male sexual behavior

test. In this test, AAV-ΔFosB females with 2 prior sexual experience tests significantly improved their copulatory efficiency more than did AAV-GFP females with 2 prior sexual experiences (Figure 4). The hit rate (the proportion of total mounts that included intromission) of sexually naïve males that were paired with the AAV-ΔFosB females was significantly higher than the hit rate of naïve males paired with AAV-GFP females, $t(14)= 4.089$ $p<0.005$.

## Discussion

Previous experiments that utilized AAV vectors for overexpression of ΔFosB were conducted in either rat or mouse model systems (Wallace *et al.*, 2008, Winstanley *et al.*, 2007, Zachariou *et al.*, 2006). We validated the viral expression patterns in the hamster brain by immunohistochemical staining. This analysis demonstrated effective expression of ΔFosB that appeared as soon as 3 weeks after intracranial injection and remained elevated for 9 weeks in our time course analysis and up to 12 weeks in the behavioral experiments.

In our model of sexual experience, repeated copulatory interactions by the male leads to a sensitization of dopamine release in the NAc (Kohlert & Meisel, 1999, Kohlert *et al.*, 1997) which has reinforcing consequences in a conditioned place preference paradigm (Meisel & Joppa, 1994, Meisel *et al.*, 1996). This dopamine sensitization, as well as the ability of female hamsters to regulate successful intromission by the mounting male as a result of repeated sexual encounters, demonstrates an associative response (Bradley *et al.*, 2005b). We have shown that this reinforced sexual behavior can be enhanced by overexpression of ΔFosB in the NAc in the context of subthreshold sexual experience, analogous to the enhancement in instrumental responses to cocaine, morphine, or food consumption following similar overexpression of ΔFosB (Colby *et al.*, 2003, Olausson *et al.*, 2006, Zachariou *et al.*, 2006). This enhancement in sexual interactions with the male following sexual experience was mirrored by the acquisition of a conditioned place preference. It is reasonable to consider ΔFosB as acting as a transcriptional nexus that is mediating both long-term modifications in behavior and the underlying neuronal plasticity consequent to the activation of the downstream targets of ΔFosB.

Given that elevation of ΔFosB produces these effects, the underlying mechanisms should be considered. There are very few identified molecular consequences that result from the accumulation of ΔFosB. Microarray studies of mice overexpressing ΔFosB indicated increases in serine/threonine cyclin dependent kinase-5 (Cdk5), nuclear factor kappa B (NF-κB), GluR2 subunit of the glutamate receptor, and dynorphin (Ang *et al.*, 2001, Bibb, 2003). It is unclear how these molecular events might affect plasticity and dendritic spine formation, although Cdk5 has known role in increasing dendritic spine density (Bibb, 2003, Cheung *et al.*, 2006, Kumar *et al.*, 2005, Norrholm *et al.*, 2003), and GluR2 subunits or NF-κB have been implicated in synaptic (Ang *et al.*, 2001, Nestler, 2001, Peakman *et al.*, 2003). In future studies we plan on concentrating on these and other potential downstream transcriptional targets of ΔFosB to determine how their activity fluctuates with the accumulation of ΔFosB following repeated sexual behavior.

There is a vast literature postulating distinct roles that the shell and core of the NAc play in motivated behaviors (Brenhouse & Stellar, 2006, Cadoni & Di Chiara, 1999, Perrotti *et al.*, 2008, Pierce & Kalivas, 1995). Previous research in our laboratory has consistently identified cellular effects of sexual experience on the core of the accumbens (Bradley *et al.*, 2005a, Bradley *et al.*, 2005b, Bradley & Meisel, 2001, Bradley *et al.*, 2004, Kohlert & Meisel, 1999, Kohlert *et al.*, 1997, Meisel *et al.*, 1993), forming the basis for our targeting of the NAc core in this study. Our analysis of the anatomical extent of ΔFosB overexpression indicated that though the injections were indeed targeted to the caudal core of the NAc,

ΔFosB expression often spread caudally into the rostral BNST. Although the caudal NAc and rostral BNST are certainly anatomically distinct nuclei, they are not necessarily functionally distinct as both regions modulate many of the mechanistic elements key to motivational processes (e.g., Koob et al., 2004). In our microdialysis studies of female hamsters (Kohlert *et al.*, 1997), we noted an inability to distinguish rostral BNST probe placements from those in the caudal NAc in terms of basal dopamine levels, dopamine responses to sexual interactions with males, or patterns of dopaminergic afferent innervation. Rather than viewing the spread of infection into the BNST as methodologically problematic, these results support the idea of a functional continuum between the NAc and BNST.

Although we have shown that overexpression of ΔFosB in female hamsters is *sufficient* to produce a conditioned place preference to sexual responding and to enhance copulatory interactions with males, it remains unknown whether ΔFosB expression is also *necessary* for these behavioral consequences of sexual experience. Recent studies have utilized an AAV-ΔJunD virus, which decreases ΔFosB mediated transcription by competitively heterodimerizing with ΔFosB before binding the AP-1 region on genes (Winstanley *et al.*, 2007). By using the AAV-ΔJunD to knockdown ΔFosB mediated transcription, we hope to determine if ΔFosB is required for the behavioral plasticity we have observed following sexual behavior experience, which will complement the results of the study presented here. If the accumulation of ΔFosB and its subsequent activation of downstream targets are causing both behavioral and cellular plasticity, then the knockdown of ΔFosB should abolish these effects.

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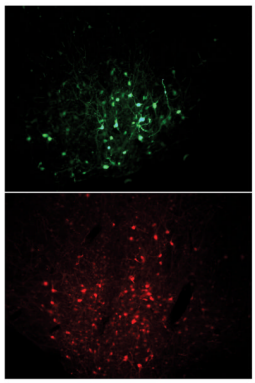

**Figure 1.**
Protein expression levels mediated by AAV-GFP or AAV-ΔFosB 12 wks post-injection. **Top.** GFP overexpression was mostly nuclear but was also found to spread to the cytoplasm and dendrites of cells. **Bottom.** ΔFosB protein expression mimicked the expression pattern of the AAV-GFP overexpression cells.

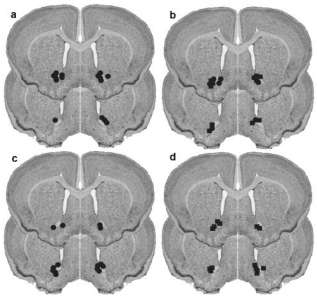


**Figure 2.**

Anatomical localization of viral injection placements of experimental animals. Circles represent AAV-GFP placement and squares represent AAV-ΔFosB placement. **a.** AAV-GFP injection placements for animals with 5 wks sexual conditioning (Group 1). **b.** AAV-GFP and AAV-ΔFosB injection placements for animals with no sexual conditioning (Groups 2 and 3). **c.** AAV-GFP injection placements for animals with 2 wks sexual conditioning (Group 5). **d.** AAV-ΔFosB injection placements for animals with 2 wks sexual conditioning (Group 4).

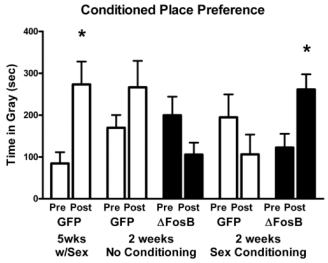


**Figure 3.**

Conditioned place preference following viral injection. This graph shows the mean (±S.E.M) number of seconds during the pre-conditioning test (Pre) and the postconditioning test (Post) that each group of hamsters spent in the gray compartment. AAV-GFP animals received either 0 weeks (no conditioning), 2 weeks, or 5 weeks of conditioning with a male. AAV-ΔFosB animals received either 0 weeks (no conditioning) or 2 weeks of conditioning with a male.

* $p<0.05$ vs. Pre-Test

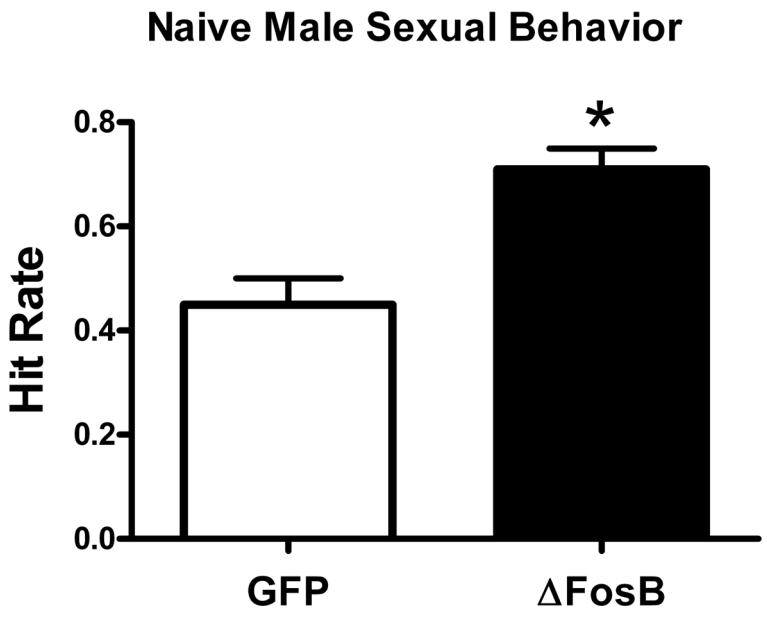


**Figure 4.**

Copulatory efficiency of naïve male hamster partners. This graph shows the mean (± S.E.M) hit rate (the proportion of total mounts that included intromission) of the naïve male hamsters that were paired either with AAV-GFP females or AAV-ΔFosB females. The males paired with AAV-ΔFosB females had a significant increase in the hit rate compared to males paired with AAV-GFP females.

* $p<0.05$ vs. GFP