Published in final edited form as:
Genes Brain Behav. 2013 August ; 12(6): 666–672. doi:10.1111/gbb.12060.

Delta JunD overexpression in the nucleus accumbens prevents sexual reward in female Syrian hamsters

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Abstract

Motivated behaviors, including sexual experience, activate the mesolimbic dopamine system and produce long-lasting molecular and structural changes in the nucleus accumbens. The transcription factor ΔFosB is hypothesized to partly mediate this experience-dependent plasticity. Previous research in our laboratory has demonstrated that overexpressing ΔFosB in the nucleus accumbens of female Syrian hamsters augments the ability of sexual experience to cause the formation of a conditioned place preference. It is unknown, however, whether ΔFosB-mediated transcription in the nucleus accumbens is required for the behavioral consequences of sexual reward. We therefore used an adeno-associated virus to overexpress ΔJunD, a dominant negative binding partner of ΔFosB that decreases ΔFosB-mediated transcription by competitively heterodimerizing with ΔFosB before binding at promotor regions on target genes, in the nucleus accumbens. We found that overexpression of ΔJunD prevented the formation of a conditioned place preference following repeated sexual experiences. These data, when coupled with our previous findings, suggest that ΔFosB is both necessary and sufficient for behavioral plasticity following sexual experience. Furthermore, these results contribute to an important and growing body of literature demonstrating the necessity of endogenous ΔFosB expression in the nucleus accumbens for adaptive responding to naturally rewarding stimuli.

Keywords

Delta FosB; Sex; Conditioned Place Preference; Adeno-Associated Virus; Plasticity

INTRODUCTION

Plasticity in the striatum, like many areas of the brain, is critical for adaptive responses to environmental stimuli at the molecular, cellular, and behavioral levels. Motivated behaviors, such as sex (Meisel & Mullins, 2006, Pitchers *et al.*, 2010b, Wallace *et al.*, 2008), aggression (Couppis & Kennedy, 2008, Staffend & Meisel, 2012), and wheel running (Greenwood *et al.*, 2011, Vargas-Perez *et al.*, 2003, Werme *et al.*, 2002), as well as chronic exposure to drugs of abuse (Chen *et al.*, 2010, Robinson & Kolb, 2004, Russo *et al.*, 2010), result in activation of the mesolimbic dopamine system and long-lasting changes in the nucleus accumbens (NAc). Structural changes, particularly the formation of dendritic spines, are important components of this experience-based plasticity, which persists long after the

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motivated behavior or drug administration has ceased (Meisel & Mullins, 2006, Pitchers *et al.*, 2010b, Robinson & Kolb, 2004).

Although the intervening molecular events that cause changes in dendritic morphology have not been fully described, the transcription factor ΔFosB appears to be at the heart of the molecular pathway that mediates the enduring structural and behavioral changes that occur following experience with motivated behaviors and/or drugs of abuse (Nestler, 2008). ΔFosB is an alternative splice product of the immediate early gene *fosB* and shares homology with other Fos family transcription factors, which heterodimerize with Jun family proteins to activate AP-1-mediated gene transcription (Robison & Nestler, 2011). Unlike other Fos family proteins that are highly unstable and return to basal levels within hours of stimulation, ΔFosB accumulates following repeated stimulation and remains elevated and stable for extended periods of time (Chen *et al.*, 1997, Nestler, 2008, Robison & Nestler, 2011). This stability is a hypothesized mechanism by which changes in gene expression and dendritic morphology can persist in the absence of further stimulation.

We have used female sexual behavior in Syrian hamsters as a model of experience-based plasticity in the brain (Hedges *et al.*, 2010, Meisel & Mullins, 2006). This is a valuable model because repeated sexual experience reliably produces changes analogous to chronic exposure to drugs of abuse (Bradley *et al.*, 2005a, Bradley & Meisel, 2001, Kohlert & Meisel, 1999, Meisel & Joppa, 1994, Meisel *et al.*, 1996, Meisel & Mullins, 2006). Furthermore, previous work in our laboratory has demonstrated that overexpression of ΔFosB in NAc using adeno-associated viral (AAV) vectors mimics the ability of sexual experience to cause the formation of a conditioned place preference (Hedges *et al.*, 2009), suggesting that ΔFosB accumulation in NAc is sufficient to create the behavioral plasticity associated with sexual experience. It remains unknown, however, whether ΔFosB is also necessary for these behavioral consequences of sexual experience. The current study therefore used AAV vectors to overexpress ΔJunD, a dominant negative inhibitor of ΔFosB, in the NAc. We report here that overexpression of ΔJunD prevents the formation of a conditioned place preference following repeated sexual experiences. When coupled with our previous results, these results suggest that ΔFosB in NAc is both necessary and sufficient for behavioral plasticity following sexual experience.

MATERIALS AND METHODS

Subjects—Adult female and male Syrian hamsters (*Mesocricetus auratus*) were purchased from Charles River Laboratories (Wilmington, MA, USA) at approximately 60 days of age. Females were used as experimental subjects, whereas males were used as sex stimulus animals during conditioned place preference tests. Females were housed individually and males were housed in pairs in polycarbonate cages (females: 50.8 × 40.6 × 20.3 cm; males: 43.2 × 22.9 × 20.3 cm). All animals were maintained on a reversed 14 hr light/10 hour dark photoperiod (lights off between 1:00 and 11:00 PM) and all behavioral testing occurred during the dark phase. The animal room was maintained at a controlled temperature of 22°C and food and water were available *ad libitum*. All animal procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23; revised 1996) and approved by the University of Minnesota Institutional Animal Care and Use Committee.

Viral Vectors—AAV is characterized by its ability to efficiently transfect neurons and 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 used an AAV2 (serotype 2) from Stratagene with a titer of over $10^8/\mu l$ expressing green fluorescent protein (AAV-GFP) as well as an AAV vector that contained

constructs for both GFP and ΔJunD (AAV-GFP-ΔJunD). ΔJunD decreases ΔFosB-mediated transcription by competitively heterodimerizing with ΔFosB before binding the AP-1 region within gene promoters. 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 six 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 for the production and use of these vectors are provided in earlier publications (Winstanley *et al.*, 2007, Zachariou *et al.*, 2006).

Surgical Procedures

Ovariectomy—Female hamsters were bilaterally ovariectomized under sodium pentobarbital anesthesia (Nembutal; 8.5 mg per 100 g body weight, i.p.). Subjects' ovaries were removed through bilateral flank incisions via cauterization of the uterine horn and blood vessels. Chromic gut absorbable suture (Henry Schein, Melville, NY, USA) and wound clips were used to close the smooth muscle and skin incisions, respectively.

Viral Vector Delivery—Immediately following ovariectomy, females were given supplemental anesthesia if necessary, and then underwent stereotaxic delivery of either AAV-GFP or AAV-GFP-ΔJunD. Anesthetized subjects were secured in the stereotaxic apparatus such that their skull was level in the anterior-posterior (A-P) and medial-lateral (M-L) planes. Following a midline scalp incision, the skin and temporal muscles were retracted to expose the skull and a hand-operated drill was used to expose dura. All A-P and M-L measurements were taken in mm relative to bregma and all dorsal-ventral (D-V) measurements were taken in mm relative to dura. Bilateral stereotaxic injections were made by lowering a microinjection syringe (model number, Hamilton, Reno, NV, USA) under stereotaxic control (Microinjection Unit, Model 5002, David Kopf Instruments, Tujunga, CA, USA) into bilateral sites targeting NAc and injecting 0.7 μl of AAV. To minimize the flow of AAV up the needle tract, the syringe was left in place for 10 min after each injection. Viral vectors were injected into NAc at least 2 weeks prior to behavior testing to allow ΔJunD overexpression to develop.

Behavioral Testing

Sexual Experience—Ovariectomized hamsters were hormone-primed for weekly sexual experiences via subcutaneous injections of estradiol benzoate (10 μg in 0.1 ml of cottonseed oil) at approximately 48 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 paired with a sexually-experienced male hamster for a 10 minute session. Measures of female copulatory behavior (lordosis latency and total lordosis duration) were scored live during each sexual experience. Each male and female was only paired once.

Conditioned Place Preference—Our conditioned place preference (CPP) apparatus consists of two lateral chambers (60 × 45 × 38 cm) connected by a clear central chamber (37 × 22 × 38 cm). The lateral chambers are differentiated by the color of the walls and the type of bedding on the chamber floor: one lateral chamber is gray and contains aspen bedding (Harlan Laboratories, IN, USA) while the other lateral chamber is white and contains corncob bedding (Harlan Laboratories, IN, USA). Female hamsters underwent three different types of behavioral tests in the CPP apparatus: a pre-test, 5 conditioning tests, and a post-test. During the pre-test, hormone-primed female hamsters were placed in the clear central chamber and allowed to freely explore the entire apparatus for 10 min in order to establish an initial preference for one of the lateral compartments. During conditioning tests, hormone-primed females were given sexual experience (see above) in their non-preferred

chamber for 10 min. One hour after sexual conditioning in the non-preferred chamber, females were placed into the initially preferred chamber alone for 10 min. The order in which the conditioning stimuli (i.e., sexually-experienced male versus no stimulus) are presented does not impact the formation or strength of a subsequent conditioned place preference (Meisel & Joppa, 1994). Consequently, we chose a single order of presentation in which subjects were always given sexual experience in the non-preferred chamber before being placed alone in the preferred chamber during conditioning tests. After 5 consecutive weeks of conditioning tests, hormone-primed females were given a post-test during which they were again allowed to freely explore the entire apparatus to determine if their initial preference had changed. Previous research has demonstrated that five consecutive sexual experiences are sufficient to detect significant changes in place preference (Hedges *et al.*, 2009, Meisel & Joppa, 1994, Meisel *et al.*, 1996). For both AAV-GFP and AAV-GFP-ΔJunD groups, some animals did not receive sexual experience during the conditioning tests but were hormone-primed and placed alone in each chamber for 10 min. As such, there were four experimental groups in this experiment: A positive control group that received bilateral AAV-GFP injections and were given sexual experience during conditioning ($n = 7$); a negative control group that received bilateral AAV-GFP injections but were not given sexual experience during conditioning ($n = 7$); a second negative control group that received bilateral AAV-GFP-ΔJunD injections but were not given sexual experience during conditioning ($n = 6$); and an experimental group that received bilateral AAV-GFP-ΔJunD injections and were given sexual experience during conditioning ($n = 8$). All tests in the CPP apparatus were conducted and scored by an observer blind to the experimental group of the subjects.

Histology and Injection Verification

Following the last behavioral test, subjects were injected with an overdose of Sleepaway (0.2 ml i.p., Fort Dodge Laboratories, Fort Dodge, IA, USA), injected intracardially with 0.2 ml of heparin sulfate (1000 IU/ml, Sagent Pharmaceuticals, Schaumburg, IL, USA) and intracardially perfused with 25 mM 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 1 hr in 4% paraformaldehyde then placed in a 10% sucrose solution in PBS overnight. Serial coronal sections (40-μm) of frozen brain tissue were sectioned on a microtome and every third section was processed for immunohistochemical localization of either GFP or JunD. Free-floating sections were rinsed in PBS with 0.1% bovine serum albumin (wash buffer) and then incubated in primary antibody (rabbit anti-GFP: 1:1,000, AB3080, Millipore, Billerca, MA, USA; rabbit anti-JunD: 1:5000, sc-74, Santa Cruz Biotechnology, Dallas, TX, USA) in wash buffer with 0.3% Triton-X at room temperature for 24 hr. After rinsing in wash buffer, sections were incubated in a biotinylated secondary antibody (1:200, Vector Laboratories, Burlingame, CA, USA) for 45 min at room temperature, rinsed in wash buffer, and then incubated in an avidin-biotin complex (Vectastain ABC Kit, Vector Laboratories) for 45 min at room temperature. Sections were then rinsed in wash buffer and reacted in a 3, 3′-diaminobenzidine (DAB) solution with 0.003% hydrogen peroxide. After 5 min, sections were rinsed in wash buffer to stop the chromagen reaction. Immunostained sections were mounted onto glass slides, coverslipped, and examined under a light microscope for the location and rostral-caudal spread of AAV injection as compared with published hamster neuroanatomical plates (Morin & Wood, 2001).

Statistical analysis

Female copulatory parameters during sexual conditioning tests were compared between AAV conditions (AAV-GFP-ΔJunD vs. AAV-GFP) using one-way repeated measures ANOVAs. Our operational definition for sexual reward was a significant increase in time

spent in the conditioned chamber on the post-test compared to the pre-test. To test our hypothesis that ΔFosB in the nucleus accumbens is necessary for sexual reward, conditioned place preference data from each treatment group were analyzed individually with a repeated measures *t*-test comparing the amount of time spent in the initially non-preferred chamber between the pre-test and the post-test. Bonferroni corrections were applied to minimize the likelihood of Type I error.

RESULTS

Immunohistochemical Characterization of AAV Injections

Brain tissue processed for immunohistochemical localization of either GFP or JunD was examined under a light microscope to determine the placement and extent of AAV injections. Injection placement was determined by tracing residual needle tracks. Bilateral AAV injections were consistently located in the dorsal NAc core (Figure 1A). Of the 29 animals analyzed, 12 animals had injection sites in the mid-caudal NAc core (Bregma + 2.1 mm), 9 animals had had injection sites located slightly more caudally (Bregma + 1.8 mm), and 8 animals had injection sites located slightly more rostrally (Bregma + 2.4 mm).

The extent of AAV infection was determined by examining JunD (Figure 1B) or GFP (Figure 1C) expression. All animals injected with either AAV-GFP (n = 15) or AAV-GFP-ΔJunD (n = 14) had significant transgene expression around the injection site that extended within the NAc core in both the rostral-caudal and dorsal-ventral planes. In most animals (n = 21), expression also extended into the NAc shell, albeit often to a lesser extent. In 6 animals, expression extended into the most rostral aspects of BNST, but labeling here was sparse. In one animal, the injection site was visible within the dorsal NAc core, but infection did not spread significantly outside of the region of the needle tip; as such, this animal was removed from the analysis. Although the exact shape of the spread of infection varied among individual animals, this variability was consistent between experimental groups.

Conditioned Place Preference

During the pre-test, 21 females demonstrated an initial preference for the white chamber and 8 females initially preferred the gray chamber. For those animals who received sexual experience, there was no difference in the average lordosis latency (Figure 2A) or lordosis duration (Figure 2B) between females injected with AAV-GFP and females injected with AAV-GFP-ΔJunD on any test day (all $p > 0.05$), suggesting that the overexpression of GFP or ΔJunD alone does not affect the receptive behavior of females.

For each group, the amount of time spent in the initially non-preferred chamber during the pre-test was compared to the time spent in the same chamber during the post-test (Figure 3). If sexual conditioning was successful, then the amount of time spent in the initially non-preferred chamber should increase significantly following sexual experience. Indeed, the positive control group that received AAV-GFP injections and was given sexual experience spent significantly more time in the initially non-preferred chamber during the post-test compared to during the pre-test ($t(6)= 8.21$, $P < 0.05$). In contrast, the amount of time spent in the initially non-preferred chamber did not differ between the pre- and post-tests for AAV-GFP-injected animals who did not receive sexual experience ($t(6)= 0.58$, $P > 0.05$). Similarly, the amount of time spent in the initially non-preferred chamber did not differ between the pre- and post-tests for AAV-GFP-ΔJunD-injected animals who did not receive sexual experience ($t(5)= 0.92$, $P > 0.05$). Interestingly, females in the AAV-GFP-ΔJunD group who received sexual experience also did not demonstrate a significant increase in the amount of time spent in the initially non-preferred chamber during the post-test ($t(7)= 1.01$,

$P > 0.05$), suggesting that the overexpression of ΔJunD in NAc can prevent the formation of a conditioned place preference as a result of sexual experience.

DISCUSSION

The hypothesized role of ΔFosB in mediating the neural and behavioral responses to natural rewards and drugs of abuse is based mainly on studies demonstrating that repeated experience with these stimuli correlates with the accumulation ΔFosB in the NAc (Meisel & Mullins, 2006, Pitchers *et al.*, 2010c, Teegarden & Bale, 2007, Wallace *et al.*, 2008, Werme *et al.*, 2002, Zachariou *et al.*, 2006) or that supraphysiological expression of ΔFosB in the NAc can augment the motivation for or response to these stimuli (Colby *et al.*, 2003, Hedges *et al.*, 2009, Pitchers *et al.*, 2010c, Pitchers *et al.*, 2013, Zachariou *et al.*, 2006). Fewer studies, however, have attempted to experimentally manipulate the endogenous activity of ΔFosB in the brain to directly test the role of ΔFosB in reinforcement and reward. Our study therefore contributes to an important and growing body of literature demonstrating the necessity of endogenous ΔFosB expression in the NAc for adaptive responding to rewarding stimuli (Olausson *et al.*, 2006, Peakman *et al.*, 2003, Pitchers *et al.*, 2010c, Pitchers *et al.*, 2013, Werme *et al.*, 2002).

In the current study, overexpression of ΔJunD was targeted to the NAc core and resulted in minimal spread to the NAc shell or other brain areas outside of the NAc. The ability of core-centered injections of ΔJunD to mitigate the rewarding consequences of sexual experience as measured in a conditioned place preference task is in agreement with previous research in our laboratory demonstrating that sexual experience increases c-Fos (Bradley & Meisel, 2001) and FosB expression (Meisel & Mullins, 2006) specifically within the NAc core. In addition, sexual experience (Meisel & Mullins, 2006) and another naturally rewarding behavior, aggressive experience (Staffend & Meisel, 2012), both increase dendritic spine density exclusively within the NAc core. Furthermore, the neural circuitry mediating the behavioral response to natural (e.g., sexual behavior) or synthetic (e.g., experience with drugs of abuse) rewards likely overlaps substantially, and recent evidence suggests that the NAc core may be particularly important for conditioning to social stimuli. In male rats, the acquisition of CPP for both social interaction and cocaine increases expression of the immediate early gene, Zif268, in the NAc shell and core (El Rawas *et al.*, 2012). In the NAc core, however, CPP for social interaction increased Zif268 expression relative to CPP for cocaine, suggesting that the NAc core may be preferentially involved in the conditioning to naturally rewarding stimuli (El Rawas *et al.*, 2012). It is also possible that separate populations of cells within both the shell and core may respond differentially to naturally rewarding stimuli versus drug stimuli, as neurons in both subregions of the NAc show 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 (Carelli *et al.*, 2000).

As in female hamsters, sexual experience in male rats produces long-lasting neural and behavioral changes that appear to depend on ΔFosB signaling in the NAc. Sexual experience in male rats results in the accumulation of FosB protein in the NAc (Pitchers *et al.*, 2010c). Furthermore, viral overexpression of ΔFosB in the NAc facilitates copulatory efficiency in males, whereas viral overexpression of ΔJunD in the NAc inhibits experience-induced facilitation of male sexual behavior (Pitchers *et al.*, 2010c, Pitchers *et al.*, 2013). This ability of local manipulation of ΔFosB levels in the NAc to affect the overt expression of sexual behavior appears to be unique to males. Whereas overexpression of ΔJunD increases males' latency to mount, intromit, and ejaculate during sexual experience sessions (Pitchers *et al.*, 2013), overexpression of either ΔFosB in a previous study (Hedges *et al.*, 2009) or ΔJunD in the current study affected the rewarding consequences of sexual behavior without affecting

lordosis latency or duration in female hamsters. As our belief is that nucleus accumbens ΔFosB is a mediator of sexual motivation in both males and females, the apparent difference in the effects of ΔJunD on copulation in males and females may lie more in the fact that copulatory parameters in males includes both motivational and motoric components, whereas the lordosis response in females is primarily a “reflexive” behavior uncoupled from sexual motivation (Rivas & Mir, 1991).

Although the mechanisms underlying sexual experience-induced changes in ΔFosB signaling have not been completely defined, research in our laboratory and others suggests that elevations in NAc dopamine (DA) may drive this plasticity via D1 receptor activation. In female hamsters, sexual experience increases extracellular DA in the NAc (Kohlert & Meisel, 1999) and 6-hydroxydopamine lesions of the NAc block the experience-induced increase in female copulatory efficiency in future tests with males (Bradley *et al.*, 2005b). Our assumption is that these effects are mediated by dopamine D1 neurotransmission, as sexual experience increases D1 receptor-mediated cyclic AMP production in the NAc compared with the response in sexually naïve female hamsters (Bradley *et al.*, 2004). Further, our recent work demonstrates that sexual experience increases dendritic spine density specifically on D1-bearing medium spiny neurons in the NAc core of female hamsters (Staffend and Meisel, in preparation). Complementary evidence from male rats has demonstrated that sexual experience-induced increases in ΔFosB accumulation are dependent on mating-induced D1 receptor activation in the NAc as well. For example, ΔJunD overexpression blocks the sexual experience-induced increase in dendritic spine density in the NAc and pharmacological blockage of D1 receptors during sexual behavior prevents increases in dendritic spine density in males (Pitchers *et al.*, 2013). Our working hypothesis is that sexual experience drives behavioral and structural plasticity through dopamine D1-mediated activation of signaling pathways that induce ΔFosB expression.

The model of experienced-based changes in ΔFosB signaling depending on D1 activation in the NAc is not limited to naturally rewarding experiences such as sexual behavior. Recent research by Grueter *et al.* (2013) has demonstrated that overexpression of ΔFosB in the NAc changes the synaptic properties of D1-expressing neurons in the NAc and increases dendritic spine density specifically on D1-bearing neurons in the NAc. Furthermore, selective overexpression of ΔFosB in D1 but not D2 neurons enhances locomotor sensitization to cocaine as well as CPP for cocaine (Grueter *et al.*, 2013). Given the shared neural substrates for responding to natural rewards and drugs of abuse, and the knowledge that sexual experience can sensitize behavioral responses to drugs of abuse (Bradley & Meisel, 2001, Pitchers *et al.*, 2010a, Pitchers *et al.*, 2013), it is important to understand how experience with natural rewards can impact the vulnerability to drug addiction. By comparing the neural changes that result from naturally rewarding behaviors to those seen after exposure to drugs of abuse, we can separate the neural properties of drug addiction from the endogenous plasticity that underlie activities in everyday life.

Acknowledgments

The authors wish to thank Calyn Maske for her assistance collecting and scoring behavioral data. Research reported in this publication was supported by DA013680 to RLM and MH51399 to EJN. In addition, LEB was supported by The National Institutes on Drug Abuse under award number T32DA007234. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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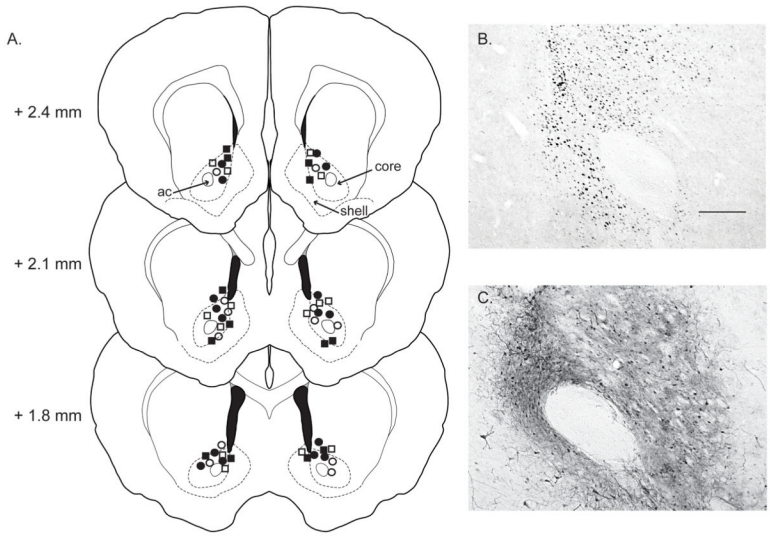

Figure 1. AAV injections

A) AAV injections for the four experimental groups were localized to the dorsal nucleus accumbens core. Circles represent animals injected with AAV-GFP-ΔJunD, whereas squares represent animals injected with AAV-GFP. Filled symbols represent animals that received sexual experience, whereas open symbols represent animals that remained sexually-naïve. Numbers represent distance relative to bregma, ac, anterior commissure. B) Photomicrograph of representative nuclear immunostaining for JunD protein, scale bar = 200 μm. C) Photomicrograph of representative immunostaining for GFP in cell bodies and processes.

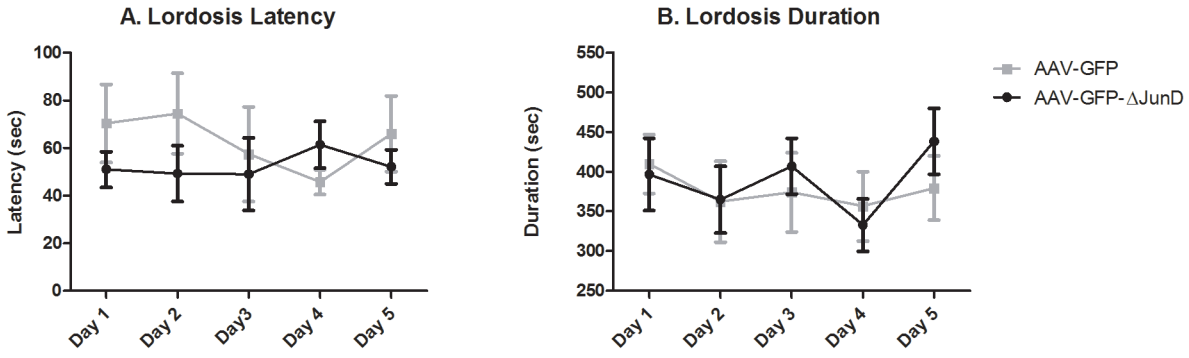

Figure 2. Overexpression of ΔJunD in the NAc does not affect the expression of female sexual behavior

During sexual experience trials, females injected with AAV-GFP-ΔJunD did not differ from females injected with AAV-GFP in A) the latency to express lordosis or B) the total duration of lordosis on any test day. Data expressed as means ± standard errors.

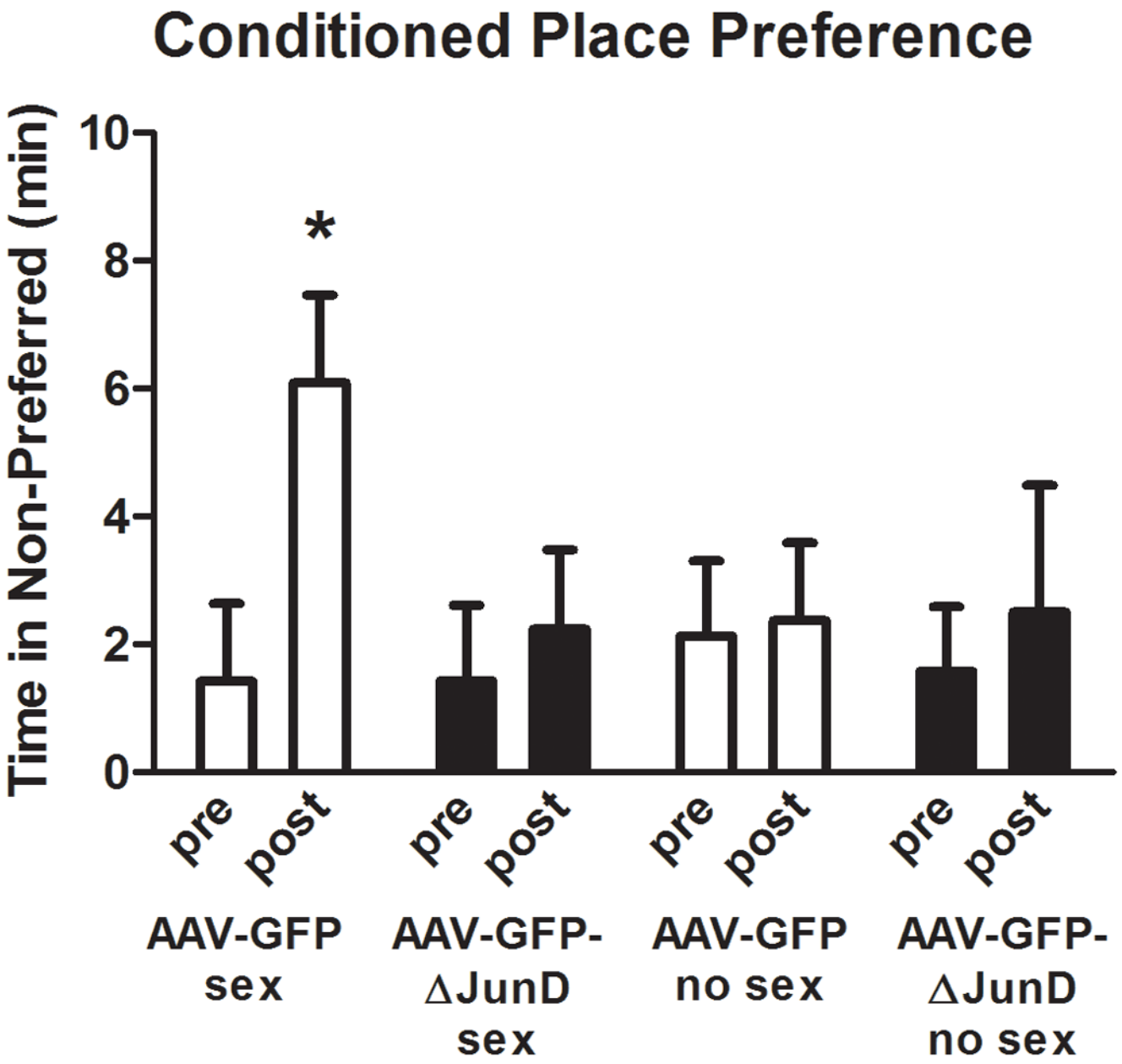

Figure 3. Overexpression of ΔJunD in the NAc prevents the formation of conditioned place preference following sexual experience

During the post-test, females who were injected with AAV-GFP and received sexual experience spent significantly more time in their initially non-preferred chamber (i.e., the chamber in which they received sexual experience) than they did during the pre-test. In contrast, the amount of time spent in the non-preferred chamber did not differ between the pre- and post-test for females injected with AAV-GFP-ΔJunD that received sexual experience. Likewise, females who were injected with either AAV-GFP or AAV-GFP-ΔJunD and did not receive sexual experience did not differ in the amount of time they spent in the non-preferred chamber during the pre- and post-tests. Data expressed as means ± standard errors. *significant difference between pre- and post-test ($p < 0.05$)