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DeltaFosB in the nucleus accumbens is critical for reinforcing effects of sexual reward

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Abstract

Sexual behavior in male rats is rewarding and reinforcing. However, little is known about the specific cellular and molecular mechanisms mediating sexual reward or the reinforcing effects of reward on subsequent expression of sexual behavior. The current study tests the hypothesis that Δ FosB, the stably expressed truncated form of FosB, plays a critical role in the reinforcement of sexual behavior and experience-induced facilitation of sexual motivation and performance. Sexual experience was shown to cause Δ FosB accumulation in several limbic brain regions including the nucleus accumbens (NAc), medial prefrontal cortex, ventral tegmental area and caudate putamen, but not the medial preoptic nucleus. Next, the induction of c-Fos, a downstream (repressed) target of Δ FosB, was measured in sexually experienced and naïve animals. The number of mating-induced c-Fos-IR cells was significantly decreased in sexually experienced animals compared to sexually naïve controls. Finally, Δ FosB levels and its activity in the NAc were manipulated using viral-mediated gene transfer to study its potential role in mediating sexual experience and experience-induced facilitation of sexual performance. Animals with Δ FosB over-expression displayed enhanced facilitation of sexual performance with sexual experience relative to controls. In contrast, the expression of Δ JunD, a dominant-negative binding partner of Δ FosB, attenuated sexual experience-induced facilitation of sexual performance, and stunted long-term maintenance of facilitation compared to GFP and Δ FosB over-expressing groups. Together, these findings support a critical role for Δ FosB expression in the NAc for the reinforcing effects of sexual behavior and sexual experience-induced facilitation of sexual performance.

Keywords

sexual behavior; sensitization; recombinant adeno-associated viral vector; anterior cingulate area; neuroplasticity; learning

INTRODUCTION

Sexual behavior is highly rewarding and reinforcing for male rodents (Coolen et al. 2004; Pfau et al. 2001). Moreover, sexual experience alters subsequent sexual behavior and

reward (Tenk *et al.* 2009). With repeated mating experience, sex behavior is facilitated or “reinforced”, evidenced by decreased latencies to initiate mating and facilitation of sexual performance (Balfour *et al.* 2004; Pfaus *et al.* 2001). However, the underlying cellular and molecular mechanisms of sexual reward and reinforcement are poorly understood. Sexual behavior and conditioned cues that predict mating have been shown to transiently induce expression of immediate-early gene *c-fos* in the mesolimbic system of male rats (Balfour *et al.* 2004; Pfaus *et al.* 2001). Moreover, it was recently demonstrated that sexual experience induces long-lasting neuroplasticity in the male rat mesolimbic system (Frohman *et al.* 2009; Pitchers *et al.* 2010). In addition, in male rats, sexual experience has been shown to induce Δ FosB, a Fos family member, in the nucleus accumbens (NAc) (Wallace *et al.* 2008). Δ FosB, a truncated splice variant of FosB, is a unique member of the Fos family due to its greater stability (Carle *et al.* 2007; Ulery-Reynolds *et al.* 2008; Ulery *et al.* 2006) and plays a role in enhanced motivation and reward for drugs of abuse and the long-term neural plasticity mediating addiction (Nestler *et al.* 2001). Δ FosB forms a heteromeric transcription factor complex (activator protein-1 (AP-1)) with Jun proteins, preferably JunD (Chen *et al.* 1995; Hiroi *et al.* 1998). Through inducible over-expression of Δ FosB, primarily restricted to the striatum using bi-transgenic mice, a drug addicted-like behavioral phenotype is produced despite an absence of previous drug exposure (McClung *et al.* 2004). This behavioral phenotype includes a sensitized locomotor response to cocaine (Kelz *et al.* 1999), increased preference for cocaine (Kelz *et al.* 1999) and morphine (Zachariou *et al.* 2006), and increased cocaine self-administration (Colby *et al.* 2003).

Similar to drug reward, Δ FosB is upregulated by natural rewarding behaviors and mediates the expression of these behaviors. Over-expression of Δ FosB in the NAc using rodent models increases voluntary wheel running (Werme *et al.* 2002), instrumental responding for food (Olausson *et al.* 2006), sucrose intake (Wallace *et al.* 2008), and facilitates male (Wallace *et al.* 2008) and female (Bradley *et al.* 2005) sexual behavior. Thus, Δ FosB may be involved in mediating the effects of natural rewarding experiences. The current study expands on previous studies by specifically investigating the role of Δ FosB in the NAc in the long-term outcomes of sexual experience on subsequent mating behavior and neural activation in the mesolimbic system. First, it was established which brain regions implicated in the reward circuitry and sexual behavior express sex experience-induced Δ FosB. Next, the effect of sex experience-induced Δ FosB on mating-induced expression of *c-Fos*, a downstream target repressed by Δ FosB (Renthal *et al.* 2008), was investigated. Finally, the effect of manipulating Δ FosB activity in the NAc (gene over-expression and expression of a dominant negative-binding partner) on sexual behavior and experience-induced facilitation of sexual motivation and performance was determined using viral vector delivery technology.

METHODS

Animals

Adult male Sprague Dawley rats (200–225 grams) were obtained from Charles River Laboratories (Senneville, QC, Canada). Animals were housed in Plexiglas cages with a tunnel tube in same sex pairs throughout experiments. The colony room was temperature-regulated and maintained on a 12/12 hr light dark cycle with food and water available *ad libitum* except during behavioral testing. Stimulus females (210–220 grams) for mating sessions received a subcutaneous implant containing 5% estradiol benzoate and 95% cholesterol following bilateral ovariectomy under deep anaesthesia (0.35g ketamine/0.052g Xylazine). Sexual receptivity was induced by administration of 500 μ g progesterone in 0.1 mL sesame oil approximately 4 hours before testing. All procedures were approved by the Animal Care and Use Committees of the University of Western Ontario and conformed to CCAC guidelines involving vertebrate animals in research.

Sexual Behavior

Mating sessions occurred during the early dark phase (between 2–6 hours after onset of the dark period) under dim red illumination. Prior to experiment onset, animals were randomly divided into groups. During mating sessions male rats were allowed to copulate to ejaculation or 1 hour, and parameters for sexual behavior were recorded including: mount latency (ML; time from introduction of the female until the first mount), intromission latency (IL; time from introduction of the female until the first mount with vaginal penetration), ejaculation latency (EL; time from the first intromission to ejaculation), post ejaculation interval (PEI; time from ejaculation to first subsequent intromission), number of mounts (M; pelvic thrusting without vaginal penetration), number of intromissions (IM; mount including vaginal penetration) and copulation efficiency (CE = $IM/(M+IM)$) (Agmo 1997). Numbers of mounts and intromissions were not included in the analysis for animals that did not display ejaculation. Mount and intromission latencies are parameters indicative of sexual motivation, while ejaculation latency, number of mounts and copulation efficiency reflect sexual performance (Hull 2002).

Experiment 1: Expression of Δ FosB

Sexually naïve male rats were allowed to mate in clean test cages (60 × 45 × 50 cm) for 5 consecutive, daily mating sessions or remained sexually naïve. Supplementary Table 1 outlines the behavioral paradigm for experimental groups: naïve no sex (NNS; n=5), naïve sex (NS; n=5), experienced no sex (ENS; n=5) and experienced sex (ES; n=4). NS and ES animals were sacrificed 1 hour following ejaculation on the final day of mating to investigate mating-induced c-Fos expression. NNS animals were sacrificed concurrently with ENS animals 24 hours after final mating session to examine sex experience-induced Δ FosB. Sexually experienced groups were matched for sexual behavior before subsequent testing. No significant differences were detected between groups for any behavioral measures within the appropriate mating session and sex experience-induced facilitation of sexual behavior was displayed by both experienced groups (Supplementary Table 2). Controls included sexually naïve males handled concurrently with mating animals ensuring exposure to female odors and vocalizations without direct female contact.

For sacrifice, animals were deeply anesthetized using sodium pentobarbital (270mg/kg; i.p.) and perfused intracardially with 50 mL of 0.9% saline, followed by 500 mL of 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Brains were removed and post-fixed for 1 h at room temperature in the same fixative, then immersed in 20% sucrose and 0.01% sodium azide in 0.1 M PB and stored at 4°C. Coronal sections (35 μ m) were cut with a freezing microtome (H400R, Micron, Germany), collected in four parallel series in cryoprotectant solution (30% sucrose and 30% ethylene glycol in 0.1 M PB) and stored at -20°C. Free floating sections were washed extensively with 0.1 M phosphate-buffered saline (PBS; pH 7.3–7.4) between incubations. Sections were exposed to 1% H₂O₂ for 10 min at room temperature to destroy endogenous peroxidases, then blocked in PBS+ incubation solution, which is PBS containing 0.1% bovine serum albumin (catalog item 005-000-121; Jackson ImmunoResearch Laboratories, West Grove, PA) and 0.4% Triton X-100 (catalog item BP151-500; Sigma-Aldrich) for 1 h. Sections were then incubated overnight at 4°C in a pan-FosB rabbit polyclonal antibody (1:5K; sc-48 Santa Cruz Biotechnology, Santa Cruz, CA, USA). The pan-FosB antibody was raised against an internal region shared by FosB and Δ FosB. The Δ FosB-IR cells were specifically Δ FosB-positive because at the post-stimulus time (24 hours) all detectable stimulus-induced FosB is degraded (Perrotti *et al.* 2004; Perrotti *et al.* 2008). In addition, in this experiment, animals mating on the final day (NS, ES) were sacrificed 1 h after mating, thus prior to FosB expression. Western blot analysis confirmed the detection of Δ FosB at approximately 37 kD. After primary antibody incubation, sections were incubated for 1 h in biotin-conjugated goat

anti-rabbit IgG (1:500 in PBS+; Vector Laboratories, Burlingame, CA, USA) and then 1 h in avidin-biotin-horseradish peroxidase (ABC elite; 1:1K in PBS; Vector Laboratories, Burlingame, CA, USA). Following this incubation sections were processed in one of the following ways:

1. Single peroxidase labeling—Sections of NNS and ENS animals were used for a brain analysis of sexual experience-induced Δ FosB accumulation. Following ABC incubation, the peroxidase complex was visualized following treatment for 10 minutes to a chromogen solution containing 0.02% 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma-Aldrich, St. Louis, MO) enhanced with 0.02% nickel sulfate in 0.1 M PB with hydrogen peroxide (0.015%). Sections were washed thoroughly in 0.1 M PB to terminate the reaction and mounted onto coded Superfrost plus glass slides (Fisher, Pittsburgh, PA, USA) with 0.3% gelatin in ddH₂O. Following dehydration, all slides were cover-slipped with DPX (dibutyl phthalate xylene).

2. Dual immunofluorescence—Sections from all four experimental groups containing NAc and mPFC were used for analysis of Δ FosB and c-Fos. Following ABC incubation, sections were incubated for 10 min with biotinylated tyramide (BT; 1:250 in PBS + 0.003% H₂O₂ Tyramid Signal Amplification Kit, NEN Life Sciences, Boston, MA) and for 30 min with Alexa 488-conjugated streptavidin (1:100; Jackson ImmunoResearch Laboratories, West Grove, PA). Sections were then incubated overnight with a rabbit polyclonal antibody specifically recognizing c-Fos (1:150; sc-52; Santa Cruz Biotechnology, Santa Cruz, CA), followed by a 30 min incubation with goat anti-rabbit Cy3-conjugated secondary antibody (1:200; Jackson ImmunoResearch Laboratories, West Grove, PA, USA). Following staining, the sections were washed thoroughly in 0.1 M PB, mounted onto coded glass slides with 0.3% gelatin in ddH₂O and cover-slipped with an aqueous mounting medium (Gelvatol) containing the anti-fading agent 1,4-diazabicyclo(2,2)octane (DABCO; 50 mg/ml, Sigma-Aldrich, St. Louis, MO). Immunohistochemical controls included omission of either or both primary antibodies, resulting in absence of labeling in the appropriate wavelength.

Data Analysis

Brain analysis of Δ FosB: Two experimenters blind to treatment performed the brain wide scan on coded slides. Δ FosB-immunoreactive (-IR) cells throughout the brain were semi-quantitatively analyzed using a scale to represent the number of Δ FosB-positive cells as outlined in Table 1. In addition, based on semi-quantitative findings, numbers of Δ FosB-IR cells were counted using standard areas of analysis in brain areas implicated in reward and sexual behavior using a camera lucida drawing tube attached to a Leica DMRD microscope (Leica Microsystems GmbH, Wetzlar, Germany): NAc (core (C) and shell (S); 400×600 μ m) analyzed at three rostral-caudal levels (Balfour *et al.* 2004); ventral tegmental area (VTA; 1000×800 μ m) analyzed at three rostral-caudal levels (Balfour *et al.* 2004) and VTA tail (Perrotti *et al.* 2005); prefrontal cortex (anterior cingulate area (ACA); prelimbic cortex (PL); infralimbic cortex (IL); 600×800 μ m each); caudate putamen (CP; 800×800 μ m); and medial preoptic nucleus (MPN; 400×600 μ m) (Supplementary Figures 1–3). Two sections were counted per subregion, and averaged per animal for calculation of the group mean. Sexually naïve and experienced group averages of Δ FosB-IR cells were compared for each subregion using unpaired t-tests.

Analysis of Δ FosB and c-Fos: Images were captured using a cooled CCD camera (Microfire, Optronics) attached to a Leica microscope (DM5000B, Leica Microsystems; Wetzlar, Germany) and Neurolucida software (MicroBrightfield Inc) with fixed camera settings for all subjects (using 10x objectives). Number of cells expressing c-Fos-IR or Δ FosB-IR in standard areas of analysis in NAc core and shell (400×600 μ m each;

Supplementary Figure 1) and ACA of the mPFC (600×800µm; Supplementary Figure 3) were manually counted by an observer blinded to the experimental groups, in 2 sections per animal using Neurolucida software (MBF Bioscience, Williston, VT) and averaged per animal. Group averages of c-Fos or ΔFosB cells were compared using two-way ANOVA (Factors: sexual experience and sex activity) and Fisher LSD for post hoc comparisons at a significance level of 0.05.

Experiment 2: ΔFosB expression manipulation

Viral Vector-Mediated Gene Transfer—Sexually naive male Sprague Dawley rats were randomly divided into groups prior to stereotaxic surgery. All animals received bilateral microinjections of recombinant adeno-associated viral (rAAV) vectors encoding GFP (control; n=12), wild-type ΔFosB (n=11) or a dominant-negative binding partner of ΔFosB termed ΔJunD (n=9) into the NAc. ΔJunD decreases ΔFosB mediated transcription by competitively heterodimerizing with ΔFosB before binding the AP-1 region within gene promoters (Winstanley *et al.* 2007). Virus titer was determined by qPCR and evaluated *in vivo* prior to study onset. Titer was $1-2 \times 10^{11}$ infectious particles per mL. rAAV vectors were injected in a volume of 1.5 µL/side over 7 minutes (coordinates: AP +1.5, ML +/-1.2 from Bregma; DV -7.6 from skull surface according to Paxinos and Watson, 1998) using a Hamilton syringe (5µL; Harvard Apparatus, Holliston, MA, USA). The vectors produce no toxicity greater than control infusions alone (Winstanley *et al.*, 2007; for details of AAV preparation, see Hommel *et al.*, 2003). Behavioral experiments started 3 weeks after vector injections allowing for optimal and stable viral infection (Wallace *et al.* 2008). Transgene expression in murine species peaks at 10 days and remains elevated for at least 6 months (Winstanley *et al.* 2007). At the end of the experiment, the animals were transcardially perfused and NAc sections were immuno-processed for GFP (1:20K; rabbit anti-GFP antibody; Molecular Probes) using an ABC-peroxidase-DAB reaction (as described above) to histologically verify injection sites using GFP as a marker (Supplementary Figure 4). ΔFosB and ΔJunD vectors also contain a segment expressing GFP separated by an internal ribosomal entry site, allowing for injection site verification by GFP visualization in all animals. Only animals with injection sites and spread of virus restricted to the NAc were included in statistical analyses. Spread of virus was generally limited to a portion of the NAc and did not spread rostral-caudally throughout the nucleus. Moreover, spread of virus appeared mostly restricted to either shell or core. However, the variation of injection sites and spread within the NAc did not influence effects on behavior. Finally, GFP injections did not affect sexual behavior or experience-induced facilitation of sexual behavior compared to non-surgery animals from previous studies (Balfour *et al.* 2004).

Sexual Behavior—Three weeks following viral vector delivery, animals mated to one ejaculation (or for 1 hour) for 4 consecutive, daily mating sessions to gain sexual experience (experience sessions) and were subsequently tested for long-term expression of experience induced facilitation of sexual behavior 1 and 2 weeks (test sessions 1 and 2) after the final experience session. Sexual behavior parameters were recorded during all mating sessions as described above. Statistical differences for all parameters during each mating session were compared within and between groups using two-way repeated measures ANOVAs (Factors: treatment and mating session) or one-way ANOVAs (ejaculation latency, number of mounts and intromissions; Factor: treatment or mating session) followed by Fisher LSD or Newman-Keuls tests for post hoc comparisons at a significance level of 0.05. Specifically, the facilitative effects of sexual experience on mating parameters were compared between experience session 1 (naïve) and experience sessions 2, 3, or 4 each, as well as between experimental groups within each experience session. Moreover, to analyze effects of treatment (vector) on long-term facilitation of sexual behavior, mating parameters were

compared between experience session 4 and test session 1 and 2 within each treatment group, and compared between experimental groups within each test session.

RESULTS

Sexual experience causes Δ FosB accumulation

Initially, a semi-quantitative investigation of Δ FosB accumulation throughout the brain in sexually experienced males compared to sexually naïve controls was conducted. A summary of overall findings is provided in Table 1. Δ FosB-IR analysis was furthered by determining the numbers of Δ FosB-IR cells in several limbic-associated brain regions using standard areas of analysis. Figure 1 demonstrates representative images of DAB-Ni staining the NAc of sexually naïve and experienced animals. Significant Δ FosB up-regulation was found in mPFC subregions (Figure 2A), NAc core and shell (2B), caudate putamen (2B) and VTA (2C). In NAc, significant differences existed at all rostral-caudal levels in the NAc core and shell, and data shown in Figure 2 is the average over all rostro-caudal levels. In contrast, there was no significant increase in Δ FosB-IR in the hypothalamic medial preoptic nucleus (NNS: Avg 1.8 +/- 0.26; ENS: Avg 6.0 +/- 1.86).

Sexual experience attenuates mating-induced c-Fos

The effect of sexual experience on Δ FosB levels in the NAc were confirmed using fluorescence staining techniques. In addition, effects of sexual experience on expression of c-Fos were analyzed. Figure 3 demonstrates representative images of Δ FosB- (green) and c-Fos (red)-IR cells in all experimental groups (A, NNS; B, NS; C, ENS; D, ES). Sexual experience significantly increased Δ FosB expression in NAc core (Figure 4A: $F_{1,15} = 12.0$; $p = 0.003$) and shell (Figure 4C: $F_{1,15} = 9.3$; $p = 0.008$). By contrast, mating 1 hour prior to perfusion, did not have an effect on Δ FosB expression (Figure 4A,C) and no interaction between sexual experience and mating immediately prior to perfusion was detected. There was an overall effect of mating prior to perfusion on c-Fos expression in both the NAc core (Figure 4B: $F_{1,15} = 27.4$; $p < 0.001$) and shell (Figure 4D: $F_{1,15} = 39.4$; $p < 0.001$). Moreover, an overall effect of sexual experience was detected in the NAc core (Figure 4B: $F_{1,15} = 6.1$; $p = 0.026$) and shell (Figure 4D: $F_{1,15} = 1.7$; $p = 0.211$) and an interaction between sexual experience and mating prior to perfusion was detected in the NAc core ($F_{1,15} = 6.5$; $p = 0.022$), with a trend in the shell ($F_{1,15} = 1.7$; $p = 0.211$; $F_{1,15} = 3.4$; $p = 0.084$). Post hoc analyses demonstrated mating-induced c-Fos expression in core and shell of sexually naïve males (Figure 4B,D). However, in sexually experienced males, c-Fos was not significantly increased in NAc core (Figure 4B) and significantly attenuated in the shell (Figure 4D). Thus, sexual experience caused a reduction of mating-induced c-Fos expression. P-values for specific pair-wise comparisons are in the figure legends.

The effect of sexual experience on mating-induced c-Fos levels was not restricted to the NAc. A similar attenuation of c-Fos expression was observed in the ACA in sexually experienced animals compared to sexually naïve controls. Sexual experience had a significant effect on Δ FosB expression in the ACA (Figure 4E: $F_{1,15} = 154.2$; $p < 0.001$). Mating prior to perfusion did not have an effect on Δ FosB expression (Figure 4C) but significantly increased c-Fos (Figure 4F: $F_{1,15} = 203.4$; $p < 0.001$) in the ACA. Moreover, mating-induced c-Fos expression in the ACA was significantly decreased by sexual experience (Figure 4F: $F_{1,15} = 15.8$; $p = 0.001$). A two-way interaction between sexual experience and mating prior to perfusion was detected for c-Fos expression (Figure 4F: $F_{1,15} = 15.1$; $p < 0.001$). P-values for specific pair-wise comparisons are in the figure legends. Finally, there was no significant reduction in mating-induced c-Fos expression in the medial preoptic nucleus (NS: Avg 63.5 +/- 4.0; ES: Avg 41.4 +/- 10.09), an area where mating

experience did not cause a significant increase in Δ FosB expression, indicating that mating-induced c-Fos expression was not affected in all brain areas.

Δ FosB in the NAc mediates reinforcement of sexual behavior

To explore a potential molecular mechanism for reinforcement of sexual behavior as demonstrated by experience-induced facilitation of sexual behavior, effects of local manipulation of Δ FosB levels and its transcriptional activity were determined. Sexual experience during the four consecutive experience sessions had a significant effect on mount latency (Figure 5A: $F_{1,23} = 13.8$; $p = 0.001$), intromission latency (Figure 5B: $F_{1,23} = 18.1$; $p < 0.001$), and ejaculation latency (Figure 5C: GFP, $F_{11,45} = 3.8$; $p = 0.006$). GFP control animals displayed the expected experience-induced facilitation of sexual behavior and displayed significantly lower latencies to first mount, first intromission and ejaculation during experience session 4 compared to experience session 1 (Figure 5A–C; see figure legend for p-values). This experience-induced facilitation of sexual behavior was also observed in the Δ FosB group for mount and intromission latencies, but there was not a significant difference detected in the ejaculation latency (Figure 5A–C). In contrast, Δ JunD animals displayed a stunted facilitation; even though latencies for mounts, intromissions, and ejaculations did decrease with repeated mating sessions, none of these parameters reached statistical significance when compared between experience sessions 1 and 4 (Figure 5A–C). Between group comparisons for each experience session show that Δ JunD had significantly longer latencies to mount, intromit and ejaculate during experience sessions compared to Δ FosB and GFP (Figure 5A–C). In addition, both sexual experience and treatment had significant effects on copulation efficiency (Figure 5F: sexual experience, $F_{1,12} = 22.5$; $p < 0.001$; treatment, $F_{1,12} = 3.3$; $p = 0.049$). Δ FosB males had increased copulation efficiency during experience session 4 compared to experience session 1 (Figure 5F). In addition, Δ FosB animals had significantly fewer mounts preceding ejaculation during experience session day 4, compared to experience session 1 (Figure 5D: $F_{10,43} = 4.1$; $p = 0.004$), and that Δ JunD males had significantly more mounts preceding ejaculation, thus significantly decreased copulation efficiency, than either of the other two groups (Figure 5D and F). Thus, GFP and Δ FosB animals displayed experience-induced facilitation of initiation of sexual behavior and sexual performance, whereas Δ JunD animals did not.

To test the hypothesis that Δ FosB expression is critical for long-term expression of experience-induced facilitation of sexual behavior, animals were tested 1 week (test session 1) and 2 weeks (test session 2) after the final experience session. Indeed, facilitated sexual behavior was maintained in both GFP and Δ FosB groups as none of the behavioral parameters differed between test sessions 1 or 2 and the final experience session 4, within GFP and Δ FosB groups (Figure 5A–C; except for ejaculation latency and copulation efficiency in test session 1 for Δ FosB animals). Significant differences between Δ JunD animals and GFP or Δ FosB groups were detected in both test sessions for all sexual behavior parameters (Figure 5A–F). There were no differences detected between or within groups when comparing numbers of intromissions, PEI, or percentages of animals that ejaculated (100% of males in all groups ejaculated during last four mating sessions).

DISCUSSION

The current study demonstrated that sexual experience causes an accumulation of Δ FosB in several limbic-associated brain regions, including the NAc core and shell, mPFC, VTA and caudate putamen. In addition, sexual experience attenuated mating-induced expression of c-Fos in the NAc and ACA. Finally, Δ FosB in the NAc was shown to be critical in mediating facilitation of mating during acquisition of sexual experience and the long-term expression of experience-induced facilitation of sexual behavior. Specifically, reducing Δ FosB-mediated transcription attenuated experience-induced facilitation of sexual motivation and

performance, while over-expression of Δ FosB in the NAc caused an enhanced facilitation of sexual behavior, in terms of increased sexual performance with less experience. Together, the current findings support the hypothesis that Δ FosB is a critical molecular mediator for the long-term neural and behavioral plasticity induced by sexual experience.

The current findings extend previous studies showing sex experience-induced Δ FosB in the NAc in male rats (Wallace *et al.* 2008) and female hamsters (Hedges *et al.* 2009). Wallace *et al.* (2008) showed that rAAV- Δ FosB over-expression in the NAc enhanced sexual behavior in sexually naïve animals during the first mating session, as evidenced by fewer intromissions to ejaculation and shorter post-ejaculatory intervals, but had no effect in sexually experienced males (Wallace *et al.* 2008). In contrast, the current study demonstrated no effects of Δ FosB over-expression in sexually naïve males during the first test, but rather during and following the acquisition of sexual experience. Δ FosB over-expressors demonstrated increased sexual performance (increased copulation efficiency) compared to GFP animals. In addition, the current study tested the role of Δ FosB by blocking Δ FosB-mediated transcription using a Δ JunD-expressing viral vector. Prevention of experience-induced increase in Δ FosB expression inhibited experience-induced facilitation of sexual motivation (increased mount and intromission latencies) as well as sexual performance (increased ejaculation latency and number of mounts) and subsequent long-term expression of facilitated sexual behavior. Hence, these data are the first to indicate an obligatory role for Δ FosB in the acquisition of experience-induced facilitation of sexual behavior. Moreover, these data show that Δ FosB is also critically involved in the long-term expression of experience-induced facilitated behavior. We propose that this long-term expression of facilitated behavior represents a form of memory for natural reward, hence Δ FosB in NAc is a mediator of reward memory. Sexual experience also increased Δ FosB levels in the VTA and mPFC, areas implicated in reward and memory (Balfour *et al.* 2004; Phillips *et al.* 2008). Future studies are required to elucidate a potential significance of Δ FosB up-regulation in these areas for reward memory.

Δ FosB expression is highly stable, thus it has great potential as a molecular mediator of persistent adaptations of the brain following chronic perturbations (Nestler *et al.* 2001). Δ FosB has been shown to gradually increase in the NAc over multiple cocaine injections and persist for up to several weeks (Hope *et al.* 1992; Hope *et al.* 1994). These changes in NAc Δ FosB expression are associated with drug reward sensitization and addiction (Chao & Nestler 2004; McClung & Nestler 2003; McClung *et al.* 2004; Nestler 2004, 2005, 2008; Nestler *et al.* 2001; Zachariou *et al.* 2006). In contrast, the role of Δ FosB in mediating natural reward has been understudied. Recent evidence has surfaced suggesting that Δ FosB induction in the NAc is involved in natural reward. Δ FosB levels are similarly increased in the NAc following sucrose intake and wheel running. The over-expression of Δ FosB in the striatum using bitransgenic mice or viral vectors in rats causes an increase in sucrose intake, enhanced motivation for food and increased spontaneous wheel running (Olausson *et al.* 2006; Wallace *et al.* 2008; Werme *et al.* 2002). The current data substantially add to these reports and further support the notion that Δ FosB is a critical mediator for reward reinforcement and natural reward memory.

Δ FosB may mediate experience-induced reinforcement of sexual behavior via induction of plasticity in the mesolimbic system. Indeed, sexual experience causes a number of long-lasting changes to the mesolimbic system (Bradley & Meisel 2001; Frohmader *et al.* 2009; Pitchers *et al.* 2010). At the behavioral level, a sensitized locomotor response to amphetamine and an enhanced amphetamine reward have been shown in sexually experienced male rats (Pitchers *et al.* 2010); an altered locomotor response to amphetamine has also been observed with female hamsters (Bradley & Meisel 2001). Furthermore, increases in number of dendritic spines and complexity of dendritic arbors have been found

following an abstinence period from sexual experience in male rats (Pitchers *et al.* 2010). The current study suggests Δ FosB may be a specific molecular mediator of the long-term outcomes of sexual experience. In agreement, Δ FosB has recently been shown to be important for inducing dendritic spine changes in response to chronic cocaine administration (Dietz *et al.* 2009; Maze *et al.* 2010).

It is not clear which upstream neurotransmitter(s) is responsible for inducing Δ FosB in the NAc, but DA has been proposed as a candidate (Nye *et al.* 1995). Virtually all drugs of abuse, including cocaine, amphetamine, opiates, cannabinoids, and ethanol, as well as natural rewards, increase Δ FosB in the NAc (Perrotti *et al.* 2005; Wallace *et al.* 2008; Werme *et al.* 2002). Both drugs of abuse and natural rewards increase synaptic DA concentration in the NAc (Damsma *et al.* 1992; Hernandez & Hoebel 1988a, b; Jenkins & Becker 2003). Δ FosB induction by drugs of abuse has been shown in DA receptor containing cells and cocaine-induced Δ FosB is blocked by a D1 DA receptor antagonist (Nye *et al.* 1995). Hence, DA release is hypothesized to stimulate Δ FosB expression and thereby mediate reward-related neuroplasticity. Further supporting the idea that Δ FosB levels are DA-dependent is the finding that brain areas where sexual experience altered Δ FosB levels do receive strong dopaminergic input from the VTA, including the medial prefrontal cortex and basolateral amygdala. However, in contrast, Δ FosB is not increased in the medial preoptic area even though this area receives dopaminergic input, albeit from hypothalamic sources (Miller & Lonstein 2009). Future studies are needed to test if mating-induced Δ FosB expression and the effects of sexual experience on sexual motivation and performance are dependent on DA action. The role for DA in sexual reward in male rats is currently not completely clear (Agmo & Berenfeld 1990; Pfau 2009). There is ample evidence that DA is released in the NAc during exposure to a female or mating (Damsma *et al.* 1992) and DA neurons are activated during sexual behavior (Balfour *et al.* 2004). However, systemic injections of DA receptor antagonist do not prevent sexual reward-induced conditioned place preference (Agmo & Berenfeld 1990) and the hypothesis that DA is critical for experience-induced reinforcement of mating is untested.

It is also unclear as to what are the downstream mediators of Δ FosB effects on sexual behavior. Δ FosB has been shown to act as both a transcriptional activator and repressor through an AP-1 dependent mechanism (McClung & Nestler 2003; Peakman *et al.* 2003). Numerous target genes have been identified, including the immediate early gene *c-fos* (Hope *et al.* 1992; Hope *et al.* 1994; Morgan & Curran 1989; Renthal *et al.* 2008; Zhang *et al.* 2006), *cdk5* (Bibb *et al.* 2001), dynorphin (Zachariou *et al.* 2006), sirtuin-1 (Renthal *et al.* 2009), NF κ B subunits (Ang *et al.* 2001), and the AMPA glutamate receptor GluR2 subunit (Kelz *et al.* 1999). The current results demonstrate that mating-induced *c-Fos* levels were reduced by sexual experience in brain areas with increased Δ FosB (NAc and ACA). The suppression of *c-Fos* appears dependent on the period since last mating and repeated mating sessions, as in previous studies, such a decrease in *c-Fos* was not detected in male rats tested 1 week following the final mating session (Balfour *et al.* 2004) or after sexual experience consisting of only a single mating session (Lopez & Ettenberg 2002). Moreover, the current finding is consistent with the evidence that Δ FosB represses the *c-fos* gene after chronic amphetamine exposure (Renthal *et al.* 2008). In line with these findings, the induction of several immediate early gene mRNAs (*c-fos*, *fosB*, *c-jun*, *junB*, and *zif268*) was reduced following repeated cocaine injections in comparison to acute drug injections (Hope *et al.* 1992; Hope *et al.* 1994), and amphetamine-induced *c-fos* was suppressed following withdrawal from chronic amphetamine administration (Jaber *et al.* 1995; Renthal *et al.* 2008). The functional relevance of the down-regulation of *c-Fos* expression after chronic drug treatment or sexual experience remains unclear, and has been suggested to be an important homeostatic mechanism to regulate an animal's sensitivity to repeated reward exposure (Renthal *et al.* 2008).

In conclusion, the current study demonstrates that Δ FosB in the NAc plays an integral role in sexual reward memory, supporting the possibility that Δ FosB is important for general reward reinforcement and memory. The findings from the current study further elucidate our understanding of cellular and molecular mechanisms that mediate sexual reward and motivation, and add to a body of literature showing that Δ FosB is an important player in development of addiction, by demonstrating a role for Δ FosB in natural reward reinforcement.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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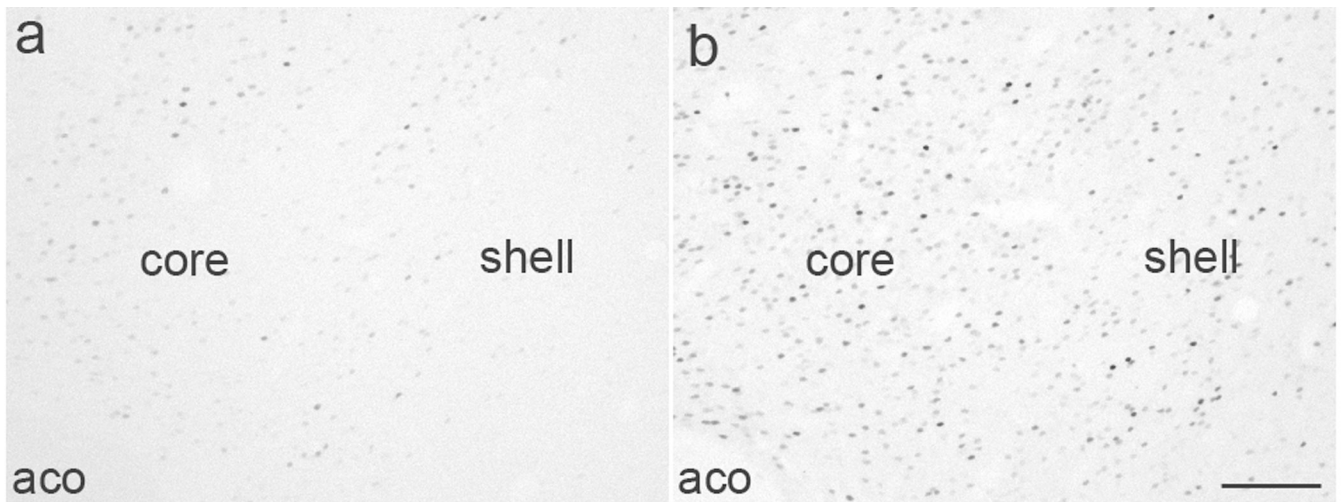


Figure 1. Representative images showing Δ FosB-IR cells (black) in the NAc of naïve no sex (A) and experience no sex (B) groups. aco: anterior commissure Scale bar indicates 100 μ m.

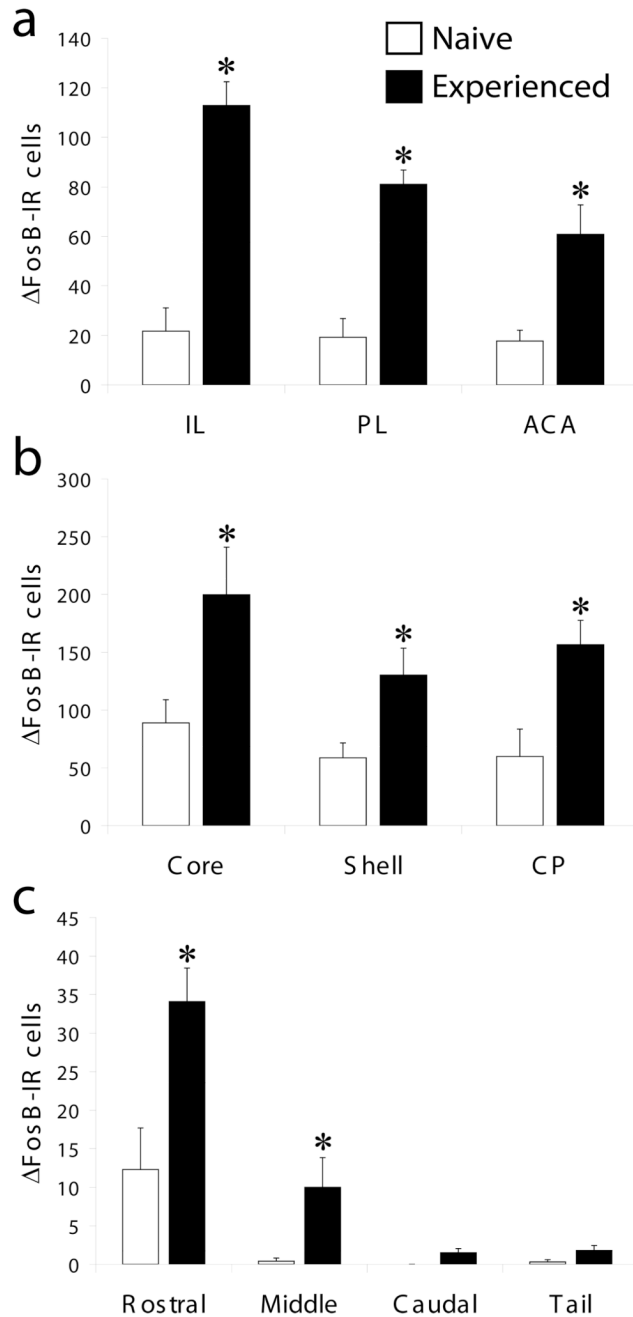


Figure 2.

Number of Δ FosB-IR cells in: A. infralimbic (IL), prelimbic (PL) and anterior cingulate cortex (ACA) subregions of the medial prefrontal cortex; B. Nucleus accumbens core and shell, and caudate putamen (CP); C. Rostral, middle, caudal and tail sections from the ventral tegmental area. Data are expressed as group means (\pm SEM). * indicates significant difference from sexually naïve animals ($p=0.037-0.001$) ($n=5$ each group).

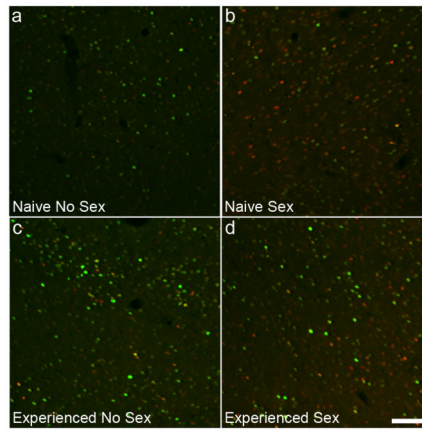


Figure 3. Representative images showing Δ FosB (green) and c-Fos (red) in NAc for each experimental group. Scale bar indicates 100 μ m.

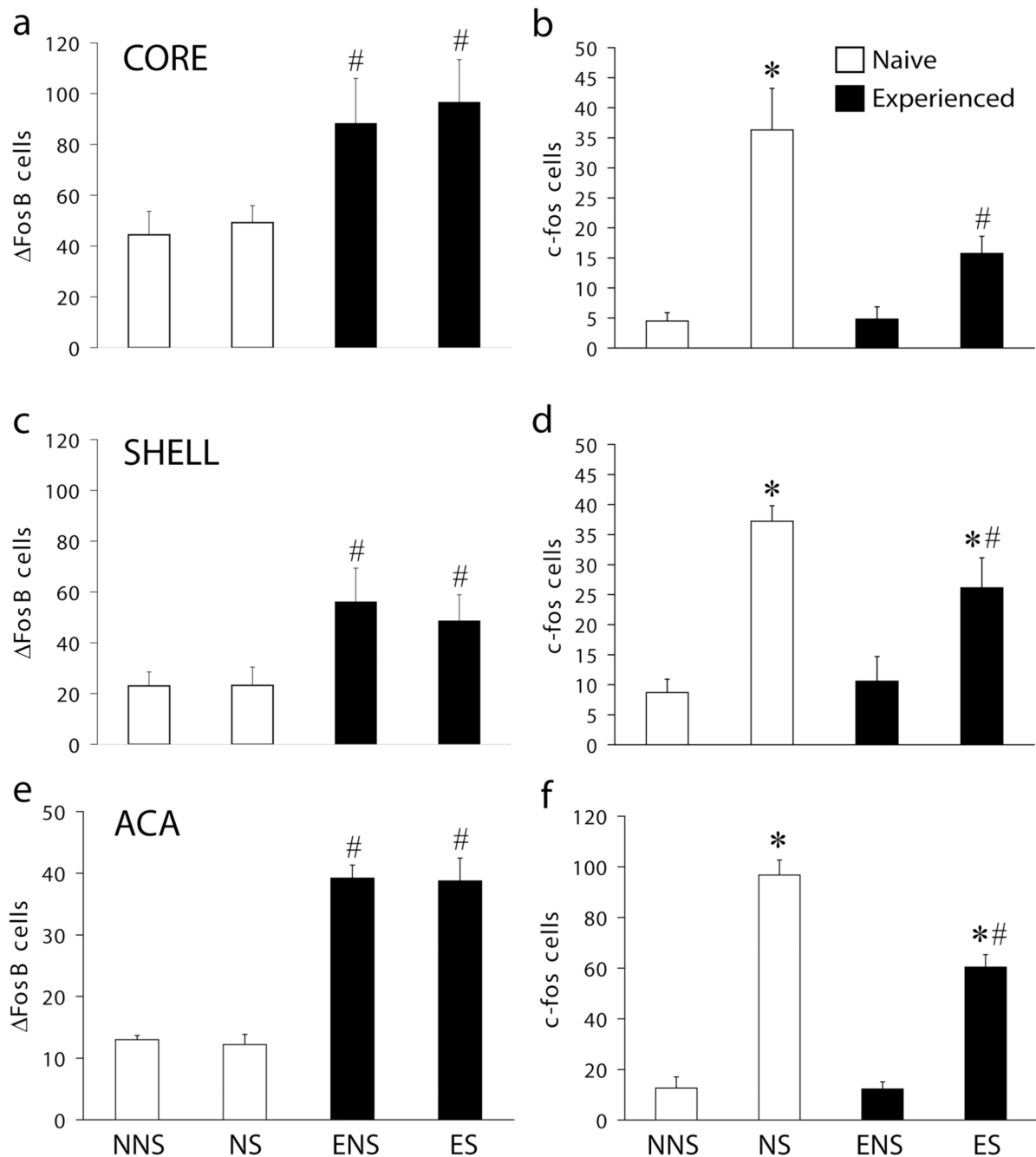


Figure 4.

Sex experience-induced Δ FosB and mating-induced c-Fos. Numbers of Δ FosB (Core, A; Shell, C; ACA, E) or c-Fos (Core, B; Shell, D; ACA, F) immunoreactive cells for each group: NNS (n=5), NS (n=5), ENS (n=5) or ES (n=4). Data are expressed as group means (\pm SEM). * indicates significant difference between sexually naïve or experienced sex groups with the appropriate no sex control group (NS vs NNS, ES vs ENS; all p-values $p < 0.001$; except H: ES vs ENS, $p = 0.008$); # indicates significant difference between sexually naïve and experienced sex or no sex groups resp. (ES vs NS; $p = 0.049 - < 0.001$; ENS vs NNS; $p = 0.028 - < 0.001$).

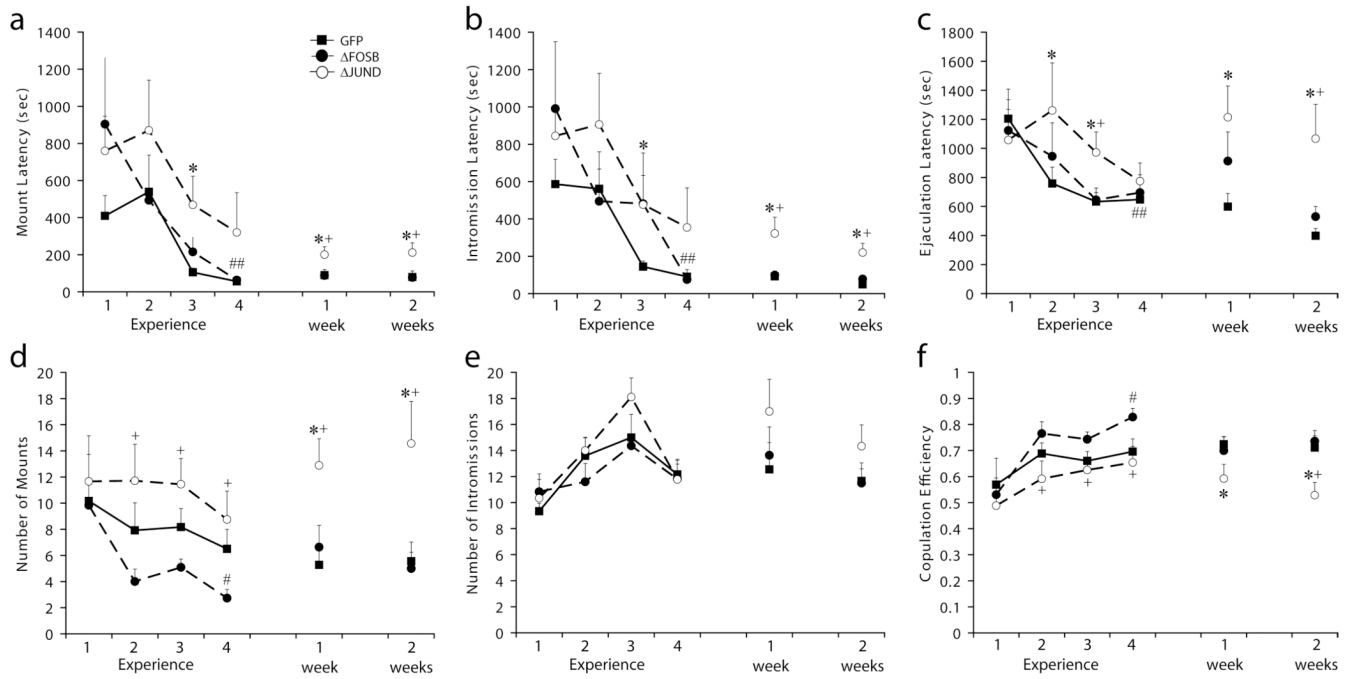


Figure 5. Sexual behavior of GFP (n=12), Δ FosB (n=11) and Δ JunD (n=9) animals: mount latency (A), intromission latency (B), ejaculation latency (C), number of mounts (D), number of intromissions (E) and copulation efficiency (F). Data are expressed as group means (\pm SEM). * indicates significant difference from GFP ($p=0.047-0.007$); + indicates significant difference from Δ FosB ($p=0.036-0.005$); and # indicates a statistical difference between mating sessions 1 and 4 (GFP: $p=0.025-0.001$; Δ FosB: $p=0.036-0.001$).

Table 1
Summary of Δ FosB expression in sexually naïve and experienced animals

Semi-quantitative analysis of Δ FosB expression in sexually naïve and experienced animals (n=5 each group). Numbers of Δ FosB-IR cells were rated using the following scale: - (absent; 0–1 cell), + (low; 2–10 cells), ++ (10–50 cells), +++ (50–100 cells) and ++++ (highest; >100 cells).

Brain Region	Control	Sexual Experience
Prefrontal Cortex		
Anterior Cingulate Area	++	+++
Prelimbic Area	++	+++
Infralimbic Area	++	++++
Nucleus Accumbens		
Core	++	++++
Shell	++	++++
Caudate Putamen	++	++++
Ventral Pallidum	-	-
Globus Pallidum	-	-
Olfactory Tubercle	+	++
Piriform Cortex	+	++
Bed Nucleus of the Stria Terminalis, Principal Nucleus	-	+
Medial Preoptic Area	+	+
Hypothalamus		
Anterior	-	+++
Lateral	-	+
Posterior	+	++
Suprachiasmatic Nucleus	+	++
Paraventricular Nucleus of the Hypothalamus	-	-
Amygdala		
Basolateral	-	++
Central	-	+
Medial	-	+
Hippocampus		
Dentate Gyrus	+	++
CA1	-	-
CA3	+	++
Ventral Tegmental Area	++	+++
Substantia Nigra	-	-
Subparafascicular Thalamic Nucleus, Parvocellular	-	+
Periaqueductal Grey	-	+