Published in final edited form as:
Neuroscience. 2010 March 31; 166(3): 771–784. doi:10.1016/j.neuroscience.2009.12.070.

Methamphetamine acts on subpopulations of neurons regulating sexual behavior in male rats

Karla S. Frohmader, B.Sc.[1], **Joost Wiskerke, M.Sc.**[2], **Roy A. Wise, Ph.D.**[3], **Michael N. Lehman, Ph.D.**[1], and **Lique M. Coolen, Ph.D.**[1,2]

[1] Department of Anatomy & Cell Biology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada, N6A 5C1 [2] Department of Physiology & Pharmacology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada, N6A 5C1 [3] Behavioral Neuroscience Branch, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, Baltimore, Maryland

Abstract

Methamphetamine (Meth) is a highly addictive stimulant. Meth abuse is commonly associated with the practice of sexual risk behavior and increased prevalence of Human Immunodeficiency Virus and Meth users report heightened sexual desire, arousal, and sexual pleasure. The biological basis for this drug-sex nexus is unknown. The current study demonstrates that Meth administration in male rats activates neurons in brain regions of the mesolimbic system that are involved in the regulation of sexual behavior. Specifically, Meth and mating co-activate cells in the nucleus accumbens core and shell, basolateral amygdala, and anterior cingulate cortex. These findings illustrate that in contrast to current belief drugs of abuse can activate the same cells as a natural reinforcer, i.e. sexual behavior, and in turn may influence compulsive seeking of this natural reward.

Keywords

nucleus accumbens; basolateral amygdala; prefrontal cortex; substance abuse; reproduction; addiction

Motivation and reward are regulated by the mesolimbic system, an interconnected network of the brain areas comprised by the ventral tegmental area (VTA) nucleus accumbens (NAc), basolateral amygdala, and medial prefrontal cortex (mPFC) (Kelley, 2004, Kalivas and Volkow, 2005). There is ample evidence that the mesolimbic system is activated in response to both substances of abuse (Di Chiara and Imperato, 1988, Chang et al., 1997, Ranaldi et al., 1999) and to naturally rewarding behaviors such as sexual behavior (Fiorino et al., 1997, Balfour et al., 2004). Male sexual behavior, and in particular ejaculation, is highly rewarding

Corresponding Author: Lique M Coolen, Phone: (519) 661 – 2111 ext. 80285, Fax: (519) 661-3827, lique.coolen@schulich.uwo.ca, Department of Anatomy & Cell Biology, Schulich School of Medicine & Dentistry, University of Western Ontario, Medical Sciences Building, Room 460, London, Ontario, Canada N6A 5C1.
Present address for JW: Department of Anatomy and Neurosciences, Neuroscience Campus Amsterdam, VU Medical Center, Amsterdam, The Netherlands.
Section Editor: Dr. Joan I. Morrell

and reinforcing in animals models (Pfaus et al., 2001). Male rodents develop a conditioned place preference (CPP) to copulation (Agmo and Berenfeld, 1990, Martinez and Paredes, 2001, Tenk, 2008), and will perform operant tasks to gain access to a sexually receptive female (Everitt et al., 1987, Everitt and Stacey, 1987). Drugs of abuse are also rewarding and reinforcing, and animals will learn to self-administer substances of abuse, including opiates, nicotine, alcohol, and psychostimulants (Wise, 1996, Pierce and Kumaresan, 2006, Feltenstein and See, 2008). Although it is known that both drugs of abuse and sexual behavior activate mesolimbic brain areas, it is currently unclear whether drugs of abuse influence the same neurons that mediate sexual behavior.

Electrophysiological studies have demonstrated that food and cocaine both activate neurons in the NAc. However, the two reinforcers do not activate the same cells within the NAc (Carelli et al., 2000, Carelli and Wondolowski, 2003). Moreover, food and sucrose self-administration do not cause long term alterations of electrophysiological properties as are induced by cocaine (Chen et al., 2008). In contrast, a collection of evidence suggests that male sexual behavior and drugs of abuse might indeed act on the same mesolimbic neurons. Psychostimulants and opioids alter the expression of sexual behavior in male rats (Mitchell and Stewart, 1990, Fiorino and Phillips, 1999a, Fiorino and Phillips, 1999b). Recent data from our lab showed that sexual experience alters the responsiveness to psychostimulants as evidenced by a sensitized locomotor responses and sensitized reward perception to d-amphetamine in sexually experienced animals (Pitchers et al., 2009). A similar response has previously been observed with repeated exposure to amphetamine or other drugs of abuse (Lett, 1989, Shippenberg and Heidbreder, 1995, Shippenberg et al., 1996, Vanderschuren and Kalivas, 2000). Together, these findings suggest that sexual behavior and responses to drugs of abuse are mediated by the same neurons in the mesolimbic system. Hence, the first objective of the present study is to investigate neural activation of the mesolimbic system by sexual behavior and drug administration in the same animal. In particular, we tested the hypothesis that the psychostimulant, methamphetamine (Meth), acts directly on neurons that normally mediate sexual behavior.

Meth is one of the most abused illicit drugs in the World (NIDA, 2006, Ellkashef et al., 2008) and it has been frequently linked to altered sexual behavior. Interestingly, Meth users report heightened sexual desire and arousal, as well as enhanced sexual pleasure (Semple et al., 2002, Schilder et al., 2005). Moreover, Meth abuse is commonly associated with sexually compulsive behavior (Rawson et al., 2002). Users often report having numerous sexual partners and are less likely to use protection than other drug abusers (Somlai et al., 2003, Springer et al., 2007). Unfortunately, studies indicating Meth use as a predictor of sexual risk behavior are limited as they rely on unconfirmed self-reports (Elifson et al., 2006). Therefore, an investigation into the cellular basis of Meth-induced changes in sexual behavior in an animal model is required for understanding this complex drug-sex nexus.

In view of the above outlined evidence suggesting that drugs of abuse, and particularly Meth, may act upon neurons normally involved in mediating sexual behavior, the objective of the present study was to investigate neural activation by sexual behavior and administration of the psychostimulant Meth. This study implemented a neuroanatomical technique, utilizing immunohistochemical visualization of the immediate early genes Fos and phosphorylated Map Kinase (pERK) to detect concurrent neural activation by sexual behavior and Meth respectively. Fos is only expressed within the nucleus of cells, with a maximal expression level 30–90 minutes after activation of the neuron. There is ample evidence that sexual activity induces Fos expression in the brain (Pfaus and Heeb, 1997, Veening and Coolen, 1998), including the mesocorticolimbic system (Robertson et al., 1991, Balfour et al., 2004). There is also evidence that drugs of abuse induce pERK expression within the mesocorticolimbic system (Valjent et al., 2000, Valjent et al., 2004, Valjent et al., 2005). In contrast to the

expression of Fos, phosphorylation of ERK is a highly dynamic process and only occurs 5–20 minutes after neuronal activation. The distinct temporal profiles of Fos and pERK makes them an ideal set of markers for subsequent neuronal activation by two different stimuli.

EXPERIMENTAL PROCEDURES

Subjects

Adult male Sprague Dawley rats (210–225 g) obtained from Charles River Laboratories (Montreal, QC, Canada) were housed two per cage in standard plexiglas cages (home cages). The animal room was maintained at a 12/12 h reversed light cycle (lights off at 10.00 h). Food and water were available *ad libitum.* All testing was performed during the first half of the dark phase under dim red illumination. Stimulus females used for sexual behavior were bilaterally ovariectomized under deep anaesthesia (13 mg/kg ketamine and 87 mg/kg xylazine) and received a subcutaneous implant containing 5% estradiol benzoate (EB) and 95% cholesterol. Sexual receptivity was induced by subcutaneous (s.c.) administration of 500 μg progesterone in 0.1 ml sesame oil 4 h prior to testing. All procedures were approved by the Animal Care Committee at the University of Western Ontario and conform to the guidelines outlined by the Canadian Council on Animal Care.

Experimental Designs

Experiments 1 and 2: Male rats (n=37) were allowed to mate with a receptive female to one ejaculation (E) or for 30 min, which ever came first in clean test cages (60 × 45 × 50 cm) during five twice-weekly pre-test mating sessions, to gain sexual experience. During the latter two sessions, all standard parameters for sexual performance 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), and number of intromissions (IM) (Agmo, 1997). All males received 1 ml/kg daily injection of 0.9% NaCl (saline; s.c.) 3 consecutive days prior to the test day, for habituation to handling and injections. One day before the test day, all males were single housed. In experienced males, Fos can be induced by conditioned contextual cues associated with prior sexual experience (Balfour et al, 2004). Therefore, all mating and control manipulations during the final tests were conducted in the home cage (avoid of predictive conditioned cues) to prevent conditioned-cue induced activation in the unmated control males. Males were distributed into eight experimental groups that did not differ in any measure of sexual performance during the last two mating sessions (data not shown). During the final test, males were either allowed to mate in their home cage until they displayed an ejaculation (sex) or did not receive female partner (no sex). All mated males were perfused 60 minutes following the onset of mating to allow for analysis of mating-induced Fos-expression. Males received an injection of 4 mg/kg Meth or 1 ml/kg saline (s.c) (n=4 each), either 10 (experiment 1) or 15 (experiment 2) min prior to perfusion, for analysis of drug-induced phosphorylation of MAP kinase. Dosage and time before perfusion were based on previous reports (Choe et al., 2002, Choe and Wang, 2002, Chen and Chen, 2004, Mizoguchi et al., 2004, Ishikawa et al., 2006). Control groups included males that did not mate, but received Meth 10 (n=7) or 15 (n=5) min prior to sacrifice, or saline injections 10 (n=5) or 15 (n=4) min prior to sacrifice. Following sacrifice, brains were processed for immunohistochemistry.

Experiment 3: Since a high dose of Meth was used in experiment 1 and 2, an additional neuroanatomical experiment was performed to investigate if sexual behavior and a lower dose of Meth induce dose dependent patterns of overlapping neural activation. This study was

carried out in an identical manner as experiments 1 and 2. However, on the final test, mated and unmated groups (n=6 each) received 1 mg/kg Meth (s.c) 15 min prior to sacrifice.

Experiment 4: To test if neural activation caused by sex and Meth is specific for Meth, this experiment investigated whether similar patterns of overlapping neural activation could be seen with the psychostimulant d-amphetamine (Amph). This experiment was carried out in an identical manner as experiments 1 and 2. However, on the final test, males were administered either Amph (5 mg/kg) or saline (1 mg/kg) (s.c) 15 min prior to sacrifice (n=5 each). Control unmated males received saline or Amph 15 minutes prior to sacrifice. An overview of the experimental groups utilized in experiments 1–4 is provided in Table 1.

Tissue Preparation—Animals were anesthetized with pentobarbital (270 mg/kg; i.p.) and perfused transcardially with 5 ml of saline followed by 500 ml 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 μm) were cut on 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 until further processing.

Immunohistochemistry—All incubations were performed at room temperature with gentle agitation. Free floating sections were washed extensively with 0.1 M Phosphate-buffered saline (PBS) between incubations. Sections were incubated in 1% H_2O_2 for 10 min, then blocked in incubation solution (PBS containing 0.1% bovine serum albumin and 0.4% Triton X-100) for 1 h.

pERK/Fos: Tissue was incubated overnight with a rabbit polyclonal antibody against p42 and p44 map kinases ERK1 and ERK2 (pERK; 1:400 experiment 1 lot 19; 1:4.000 experiment 2 and 3 lot 21; Cell Signaling Cat # 9101;), followed by a 1 h incubations with biotinylated donkey anti-rabbit IgG (1:500; Jackson Immunoresearch Laboratories, West Grove, PA) and avidin-horseradish peroxidase complex (ABC Elite; 1:1000; Vector Laboratories, Burlingame, CA). Then, the tissue was incubated for 10 min with biotinylated tyramide (BT; 1:250 in PBS + 0.003% H_2O_2; Tyramid Signal Amplification Kit, NEN Life Sciences, Boston, MA) and for 30 min with Alexa 488 conjugated strepavidin (1:100; Jackson Immunoresearch Laboratories, West Grove, PA). Next, tissue was incubated overnight with a rabbit polyclonal antibody against c-Fos (1:500; SC-52; Santa Cruz Biotechnology, Santa Cruz, CA), followed by a 30 min incubation with goat anti-rabbit Alexa 555 (1:200; Jackson Immunoresearch Laboratories, West Grove, PA). Following staining, the sections were washed thoroughly in 0.1 M PB, mounted onto glass slides with 0.3% gelatin in ddH_20 and coverslipped with an aqueous mounting medium (Gelvatol) containing 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

Sexual behavior: For all four experiments, standard parameters for sexual performance were recorded as described above and analyzed using analysis of variance (ANOVA). Data analysis of sexual behavior during the final test day revealed no significant differences between groups in any of the parameters of sexual performance.

pERK/Fos Cell Counts: Single and dual labeled cells for Fos and pERK were counted in the caudal levels of NAc core and shell subregions, basolateral amygdala (BLA), posterodorsal medial amygdala (MEApd), central amygdala (CeA), medial preoptic nucleus (MPN),

posteromedial and posterolateral bed nucleus of the stria terminalis (BNSTpm and BNSTpl), and the anterior cingulated area (ACA), prelimbic (PL), and infralimbic (IL) subregions of the mPFC. Images were captured using a cooled CCD camera (Microfire, Optronics) attached to a Leica microscope (DM500B, Leica Microsystems, Wetzlar, Germany) and Neurolucida software (MicroBrightfield Inc) with fixed camera settings for all subjects (using 10x objectives). Using neurolucida software, areas of analysis were defined based on landmarks (Swanson, 1998) unique for each brain region (see Figure 1). Standard areas of analysis were used in all areas except NAc core and shell. In the latter areas, pERK and Fos expression was not homogeneous and appeared in patch-like patterns. Therefore, the entire core and shell were outlined based on landmarks (lateral ventricle, anterior commisure, and islands of Calleja). The areas of analysis did not differ between experimental groups, and were 1.3 mm^2 in the NAc core and shell. Standard areas of analysis for the remaining areas were: 1.6 mm^2 in the BLA, 2.5 and 2.25 mm^2 in the MEApd and CeA respectively, 1.0 mm^2 in the MPN, 1.25 mm^2 in the BNST and mPFC subregions, and 3.15 mm^2 in the VTA. Two sections were counted bilaterally for each brain region per animal, and number of single and dual labeled cells for pERK and Fos as well as the percentages of pERK cells that expressed Fos marker were calculated. For experiments 1, 2, and 4, group averages were compared using two way ANOVA (factors: mating and drug) and Fisher's LSD for *post hoc* comparisons at a significance level of 0.05. For experiment 3, group averages were compared using unpaired t-tests at a significance level of 0.05.

Images—Digital images for Figure 3 were captured using CCD camera (DFC 340FX, Leica) attached to a Leica microscope (DM500B) and were imported into Adobe Photoshop 9.0 software (Adobe Systems, San Jose, CA). Images were not altered in any way except for adjustment of brightness.

RESULTS

Neural Activation of the Limbic System by Sexual Behavior and Meth Administration

Experiment 1: Analysis of single and dual labeled cells for mating-induced Fos and Meth-induced pERK in males that received Meth 10 minutes prior to sacrifice revealed mating-induced Fos in the MPN, BNSTpm, NAc core and shell, BLA, VTA, and all subregions of mPFC, consistent with prior studies demonstrating mating-induced Fos expression in these areas (Baum and Everitt, 1992, Pfaus and Heeb, 1997, Veening and Coolen, 1998, Hull et al., 1999). Meth administration 10 minutes prior to sacrifice induced pERK in NAc core and shell, BLA, MeApd, CeA, BNSTpl, and regions of mPFC, consistent with activation patterns induced by other psychostimulants (Valjent et al., 2000, Valjent et al., 2004, Valjent et al., 2005).

Moreover, three patterns of co-expression of neural activation by sexual behavior and Meth were observed: First, brain areas were identified where sex and drugs activated non-overlapping neural populations (Table 2). Specifically, in the CeA, MEApd, BNSTpl, and mPFC, significant increases in both drug-induced pERK ($F(1,16)=7.39–48.8$; $p=0.015-<0.001$) and sex-induced Fos ($F(1,16)=16.53–158.83$; $p<0.001$) were observed. However, in these regions there were no significant increases in dual labeled neurons in mated Meth-treated males. The only exception was the MEApd, where an effect of mating on numbers of dual labeled cells were found ($F(1,16)=9.991$; $p=0.006$). However, there was no overall effect of drug treatment and dual labeling in Meth treated groups was not significantly higher than in saline treated groups, thus was not caused by the drug (Table 2). Second, brain areas were identified where neural activation was only induced by mating (Table 3). Specifically, the MPN, BNSTpm, and VTA were activated only by mating, and contained significant increases in mating-induced Fos ($F(1,16)=14.99–248.99$; $p \leq 0.001$), but no Meth-induced pERK.

Finally, brain areas were found where sex and drugs activated overlapping populations of neurons (Figure 2 and 3). In the NAc core and shell, BLA, and ACA, there were overall effects of mating ($F(1,16)=7.87–48.43$; $p=0.013-<0.001$) and drug treatment ($F(1,16)=6.39–52.68$; $p=0.022-<0.001$), as well as an interaction between these two factors ($F(1,16)=5.082–47.27$; $p=0.04-<0.001$; no significant interaction in ACA) on numbers of cells expressing both mating-induced Fos and Meth-induced pERK. Post hoc analysis revealed that numbers of dual labeled neurons were significantly higher in mated Meth-injected males compared to unmated Meth-treated ($p=0.027-<0.001$), or mated saline-treated ($p=0.001-<0.001$) males (Figure 2 and 3). When data were expressed as the percentages of drug-activated neurons, 39.2 ± 5.3% in the NAc core, 39.2 ± 5.8% in the NAc shell, 40.9 ± 6.3% in the BLA, and 50.0 ± 5.3% of ACA neurons were activated by both mating and Meth.

An unexpected observation was that sexual behavior affected Meth-induced pERK. Although Meth significantly induced pERK levels in both mated and unmated Meth-injected groups, in the NAc, BLA, and ACA, pERK labeling was significantly lower in mated Meth-injected males when compared to unmated Meth-injected males (Figure 2b, e, h, k; $p=0.017-<0.001$). This finding may further support the hypothesis that sex and drugs act on the same neurons, but it may also be indicative of mating-induced alterations in drug uptake or metabolism that in turn cause altered neural responses to Meth. To investigate if sexual behavior causes a different temporal pattern of drug-induced activation, sections of the NAc, BLA, and ACA were stained for males sacrificed at a later time point (15 min) following drug administration (experiment 2).

Experiment 2: Analysis of single and dual labeled cells confirmed the findings described above that sexual behavior and subsequent exposure to Meth 15 minutes prior to sacrifice resulted in significant increases of Fos and pERK immunolabeling in the NAc core and shell, BLA, and ACA. In addition, significant co-expression of mating-induced Fos and Meth-induced pERK were again found in these areas (Figure 4; mating effect: $F(1,12)=15.93–76.62$; $p=0.002-<0.001$; drug effect: $F(1,12)=14.11–54.41$; $p=0.003-<0.001$). Number of dual labeled neurons in mated Meth-injected males was significantly higher compared to unmated Meth-treated ($p<0.001$) or mated saline-treated ($p<0.001$) males. When data was expressed as the percentages of drug-activated neurons, 47.2 ± 5.4% (NAc core), 42.7 ± 7.6% (NAc shell), 36.7 ± 3.7% (BLA), and 59.5 ± 5.1% (ACA) of neurons activated by mating were also activated by Meth. Moreover, drug-induced pERK did not differ between mated and unmated animals (Figure 4b, e, h, k), in all areas except for the ACA ($p<0.001$). These data indicate that sexual behavior indeed causes an alteration of the temporal pattern of pERK induction by Meth.

Neural Activation following Sexual Behavior and 1 mg/kg Meth

Thus far results revealed that sexual behavior and 4 mg/kg Meth activated overlapping populations of neurons in the NAc core and shell, BLA, and ACA. To investigate the influence of drug-dosage on this overlap in activation, patterns of neural activation were also studied using a lower dose of Meth. The NAc core and shell, BLA, and ACA were analyzed for activation induced by sex and Meth. Indeed, sexual behavior and subsequent exposure to Meth resulted in significant increases of Fos and pERK immunolabeling in the NAc core and shell subregions, the BLA, as well as neurons in the ACA region of the mPFC (Figure 5). Interestingly, the lower dose of Meth resulted in similar numbers of pERK labeled neurons as induced by 4 mg/kg Meth in the four brain regions analyzed. More importantly, the NAc core and shell, BLA, and ACA displayed significant increases in the number of dual labeled cells (Figure 5c, f, i, l) compared to unmated Meth-injected males ($p=0.003-<0.001$). When data was expressed as the percentages of drug-activated neurons, 21.1 ± 0.9% and 20.4 ± 1.8% in the NAc core and shell respectively, 41.9 ± 3.9% in the BLA, and 49.8 ± 0.8% of ACA neurons were activated by sex and Meth.

Neural Activation following sexual behavior and administration of d-Amphetamine

To test whether the above results were specific for Meth, an additional experiment was conducted to study mating- and Amph-induced neural activation. Analysis of single and dual labeled cells for pERK and Fos showed that sexual behavior and subsequent exposure to Amph resulted in significant increases of Fos and pERK immunolabeling in the NAc core and shell and BLA (Figure 6; mating effect: $F(1,15)=7.38–69.71$; $p=0.016-<0.001$; drug effect: $F(1,15)=4.70–46.01$; $p=0.047-<0.001$). Moreover, the numbers of dual labeled neurons were significantly higher in mated Amph-treated compared to unmated Amph-treated ($p=0.009-<0.001$), or mated saline-treated ($p=0.015-<0.001$) males (Figure 6c, f, i). When data was expressed as the percentages of drug-activated neurons, 25.7 ± 2.8% and 18.0 ± 3.2% in the NAc core and shell respectively, and 31.4 ± 2.0% of BLA neurons were activated by both mating and Amph. The ACA region of the mPFC displayed significant levels of mating-induced Fos (Figure 6j; $F(1,15)=168.51$; $p<0.001$). However, unlike Meth, Amph did not result in significant increases in drug-induced pERK levels in the ACA (Figure 6k) or numbers of dual labeled neurons in the ACA (Figure 6l) when compared to both mated and unmated saline-injected males.

DISCUSSION

The current study demonstrates at a cellular level an overlap between neural activation by the natural reinforcer sexual behavior and the psychostimulant Meth. Therefore, these data show that not only do drugs act on the same brain regions that regulate natural reward, but in fact, drugs activate the same cells involved in the regulation of natural reward. Specifically, it was shown here that sexual behavior and Meth co-activated a population of neurons in the NAc core and shell, BLA, and ACA region of the mPFC, identifying potential sites where Meth may influence sexual behavior.

The current finding that sexual behavior and administration of Meth activate overlapping populations of neurons in the NAc, BLA, and ACA is in contrast to findings from other studies showing that different populations of NAc neurons encode drug and natural reward. Specifically, electrophysiological studies that compared neural activation during self-administration of natural rewards (food and water) and intravenous cocaine have indicated that cocaine self-administration activated a differential, non-overlapping population of neurons that was generally not responsive during operant responding for water and food reinforcement (92%). Only 8% of accumbal neurons showed activation by both cocaine and natural reward (Carelli et al., 2000). In contrast, a majority (65%) of cell in the NAc showed activation by different natural rewards (food and water), even if one reinforcer was more palatable (sucrose) (Roop et al., 2002). Several factors may have contributed to the discrepancy with the current results. First, different technical approaches were used to investigate neural activity. The current study utilized a neuroanatomical method for detection of concurrent neural activation by two different stimuli using dual fluorescencent immunocytochemisty for Fos and pERK, allowing for investigation of single cell activation over large spans of brain areas. In contrast, the studies by Carelli and co-workers used electrophysiological recordings restricted to the NAc of behaving animals to address whether self-administration of drugs of abuse activate the same neural circuitry used by natural rewards. Second, the current study investigated a different natural reward i.e. sexual behavior compared to previous studies, which used food and water in restricted rats (Carelli, 2000). Food and water might have lesser rewarding value than mating. Sexual behavior is highly rewarding and rats readily form CPP to copulation (Agmo and Berenfeld, 1990, Martinez and Paredes, 2001, Tenk, 2008). Although, diet restricted rats do form CPP for water (Agmo et al., 1993, Perks and Clifton, 1997) and food (Perks and Clifton, 1997), diet unrestricted rats preferably consume and form CPP for more palatable foods (Jarosz et al., 2006, Jarosz et al., 2007). Third, our studies included different drugs of abuse compared

to previous studies, utilizing methamphetamine and amphetamine instead of cocaine. The present results demonstrate that specifically Meth, and to a lesser extent amphetamine, resulted in activation of neurons also activated by sexual behavior. Drug experience may have also played a factor in our findings. The current studies utilized animals that were sexually experienced, but drug naïve. In contrast, the electrophysiological studies of Carelli and co-workers used "well-trained" animals that received repeated exposures to cocaine.

Hence, it is possible that the Meth-induced activation of neurons activated by sexual behavior is altered in drug experienced rats. However, preliminary studies from our lab suggest that drug experience is unlikely to be a major factor as sexual behavior and Meth treatment in males chronically treated with Meth co-activated similar percentages of drug-activated neurons as reported in the current study (20.3 ± 2.5 % in NAc core and 27.8 ± 1.3 % in NAc shell; Frohmader and Coolen, unpublished observations). Finally, the current study investigated the "direct" action of drugs utilizing passive administration. Therefore, the current analysis does not reveal information regarding neural circuits involved in drug seeking or cues associated to drug reward, but rather reveals neural activity caused by the pharmacological action of the drug. In the previous electrophysiological studies, NAc neural activity occurring within seconds of reinforced responses are not the result of the pharmacological action of cocaine, but is greatly dependent on associative factors within the self-administration paradigm (Carelli, 2000, Carelli, 2002). Specifically, NAc neural activity is influenced by response-independent presentations of stimuli associated with intravenous cocaine delivery as well as by instrumental contingencies (i.e., lever pressing) inherent in this behavioral paradigm (Carelli, 2000, Carelli and Ijames, 2001, Carelli, 2002, Carelli and Wightman, 2004). In summary, our findings of co-activation by natural and drug reward may be specific for activation by sexual behavior and passively administered Meth and Amph.

Meth and sex activated overlapping populations of neurons in the NAc core and shell in a dose-dependent manner. The co-activated neurons in the NAc may mediate potential effects of Meth on the motivation and rewarding properties of sexual behavior as lesions of the NAc disrupt sexual behavior (Liu et al., 1998, Kippin et al., 2004). In addition, these neurons are potentially a locus for dose-dependent drug effects on mating, since the lower Meth dose (1 mg/kg) reduced the number of dual labeled cells by 50% compared to the higher dose of Meth (4 mg/kg). Although this study does not identify the chemical phenotype of co-activated neurons, previous studies have shown that drug-induced pERK and Fos expression in the NAc is dependent on both dopamine (DA) and glutamate receptors (Valjent et al., 2000, Ferguson et al., 2003, Valjent et al., 2005, Sun et al., 2008). Although it is not clear if mating-induced neural activation in the NAc is dependent on these receptors, this has been demonstrated on other brain regions, particularly in the medial preoptic area (Lumley and Hull, 1999, Dominguez et al., 2007). Thus, Meth may act on neurons also activated during sexual behavior via activation of dopamine and glutamate receptors. The role of NAc glutamate in sexual behavior is currently unclear, but it is well established that DA plays a critical role in the motivation for sexual behavior (Hull et al., 2002, Hull et al., 2004, Pfaus, 2009). Microdialysis studies reported increases in NAc DA efflux during appetitive and consummatory phases of male sexual behavior (Fiorino and Phillips, 1999a, Lorrain et al., 1999) and mesolimbic DA efflux has been correlated to facilitation of the initiation and maintenance of rat sexual behavior (Pfaus and Everitt, 1995). Furthermore, DA manipulation studies show DA antagonists in the NAc inhibit sexual behavior, while agonists facilitate the initiation of sexual behavior (Everitt et al., 1989, Pfaus and Phillips, 1989). Thus, Meth may affect motivation for sexual behavior via activation of DA receptors.

In contrast to the NAc, the number of dual labeled cells in the BLA and ACA remained relatively unchanged regardless of the Meth dose. The BLA is critical for discrete associative learning and is strongly involved in conditioned reinforcement and reward evaluation during

instrumental responding (Everitt et al., 1999, Cardinal et al., 2002, See, 2002). BLA lesioned rats display decreased lever pressing for conditioned stimuli paired with food (Everitt et al., 1989) or sexual reinforcement (Everitt et al., 1989, Everitt, 1990). In contrast, this manipulation does not affect the consummatory phase of feeding and sexual behavior (Cardinal et al., 2002). The BLA also plays a key role in memory of conditioned stimuli associated with drug stimuli (Grace and Rosenkranz, 2002, Laviolette and Grace, 2006). Lesions or pharmacological inactivations of the BLA block the acquisition (Whitelaw et al., 1996) and expression (Grimm and See, 2000) conditioned-cued cocaine reinstatement, while not affecting the process of drug administration. Furthermore, Amph infused directly into the BLA results in a potentiated drug reinstatement in the presence of the conditioned cues (See et al., 2003). Therefore, it is possible that psychostimulant-enhanced DA transmission in the BLA results in potentiated emotional salience and seeking (Ledford et al., 2003) of sexual reward, thus contributing to the enhanced sexual drive and desire reported by Meth abusers (Semple et al., 2002, Green and Halkitis, 2006).

In the ACA, neural activation of sex-activated neurons was dosage-independent and specific for Meth, as it was not observed with Amph. Although Meth and Amph have similar structural and pharmacological properties, Meth is a more potent psychostimulant than Amph with longer lasting effects (NIDA, 2006). Studies by Goodwin et al. showed that Meth generates a greater DA efflux and inhibits the clearance of locally applied DA more effectively in the rat NAc than Amph. These characteristics could contribute to the addictive properties of Meth compared to Amph (Goodwin et al., 2009) and perhaps the neural activation differences observed between the two drugs. However, it is not clear whether the different patterns of results are due to efficacy differences between the drugs or potency issues related to the doses employed and further investigation is required.

Co-activation by Meth and sex was not observed in other subregions of the mPFC (IL and PL). In the rat, the ACA has been extensively studied using appetitive tasks, supporting a role in stimulus–reinforcer associations (Everitt et al., 1999, See, 2002, Cardinal et al., 2003). There is ample evidence that the mPFC is involved in drug craving and relapse to drug-seeking and drug-taking behavior in both humans and rats (Grant et al., 1996, Childress et al., 1999, Capriles et al., 2003, McLaughlin and See, 2003, Shaham et al., 2003, Kalivas and Volkow, 2005). In line with this, it has been proposed that mPFC dysfunctioning caused by repeated exposure to drugs of abuse might be responsible for reduced impulse control and increased drug-directed behavior as observed in many addicts (Jentsch and Taylor, 1999). Recent data from our laboratory demonstrated that mPFC lesions result in continued seeking of sexual behavior when this was associated with an aversive stimulus (Davis et al., 2003). Even though this study did not investigate the ACA, it supports the hypothesis that the mPFC (and the ACA specifically) mediates the effects of Meth on a loss of inhibitory control over sexual behavior as reported by Meth abusers (Salo et al., 2007).

In conclusion, together these studies form a critical first step towards a better understanding of how drugs of abuse act on neural pathways that normally mediate natural rewards. Moreover, these findings illustrate that in contrast to the current belief that drugs of abuse do not activate the same cells in the mesolimbic system as natural reward, Meth, and to a lesser extent Amph, activate the same cells as sexual behavior. In turn, these co-activated neural populations may influence seeking of natural reward following drug exposure. Finally, the results of this study may significantly contribute to our understanding of the basis of addiction in general. Comparisons of the similarities and differences, as well alterations in neural activation of the mesolimbic system elicited by sexual behavior versus drugs of abuse may lead to a better understanding of substance abuse and associated alterations in natural reward.

Acknowledgments

This research was supported by grants from the National Institutes of Health R01 DA014591 and Canadian Institutes of Health Research RN 014705 to LMC.

ABBREVIATIONS

ABC	avidin-biotin–horseradish peroxidase complex
ACA	anterior cingulate area
Amph	d-amphetamine
BLA	basolateral amygdala
BNSTpl	posterolateral bed nucleus of the stria terminalis
BNSTpm	posteromedial bed nucleus of the stria terminalis
BT	biotinylated tyramide
CeA	cental amygdala
CPP	conditioned place preference
E	ejaculation
EL	ejaculation latency
IF	infralimbic area
IL	intromission latency
IM	intromission
M	mount
MAP Kinase	mitogen-activated protein kinase
MEApd	posterodorsal medial amygdala
Meth	methamphetamine
ML	mount latency
mPFC	medial prefrontal cortex
MPN	medial preoptic nucleus
NAc	nucleus Accumbens
PB	phosphate buffer
PBS	phosphate buffered saline
PEI	post ejaculatory interval
pERK	phosphorylated MAP Kinase
PL	prelimbic area
VTA	ventral tegmental area

References

Agmo A. Male rat sexual behavior. Brain Res Brain Res Protoc 1997;1:203–209. [PubMed: 9385085]

Agmo A, Berenfeld R. Reinforcing properties of ejaculation in the male rat: role of opioids and dopamine. Behav Neurosci 1990;104:177–182. [PubMed: 2156520]

Agmo A, Federman I, Navarro V, Padua M, Velazquez G. Reward and reinforcement produced by drinking water: Role of opioids and dopamine receptor subtypes. Pharmacol Biochem Behav 1993;46

Balfour ME, Yu L, Coolen LM. Sexual behavior and sex-associated environmental cues activate the mesolimbic system in male rats. Neuropsychopharmacology 2004;29:718–730. [PubMed: 14694350]

Baum MJ, Everitt BJ. Increased expression of c-fos in the medial preoptic area after mating in male rats: Role of afferent inputs from the medial amygdala and midbrain central tegmental field. Neuroscience 1992;50:627–646. [PubMed: 1436507]

Capriles N, Rodaros D, Sorge RE, Stewart J. A role for the prefrontal cortex in stress- and cocaine-induced reinstatement of cocaine seeking in rats. Psychopharmacology (Berl) 2003;168:66–74. [PubMed: 12442201]

Cardinal RN, Parkinson JA, Hall J, Everitt BJ. Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. Neuroscience & Biobehavioral Reviews 2002;26:321–352. [PubMed: 12034134]

Cardinal RN, Parkinson JA, Marbini HD, Toner AJ, Bussey TJ, Robbins TW, Everitt BJ. Role of the anterior cingulate cortex in the control over behavior by Pavlovian conditioned stimuli in rats. Behavioral Neuroscience 2003;117:566–587. [PubMed: 12802885]

Carelli RM. Activation of accumbens cell firing by stimuli associated with cocaine delivery during self-administration. Synapse 2000;35:238–242. [PubMed: 10657032]

Carelli RM. Nucleus accumbens cell firing during goal-directed behaviors for cocaine vs. ‘natural’ reinforcement. Physiology & Behavior 2002;76:379–387. [PubMed: 12117574]

Carelli RM, Ijames SG. Selective activation of accumbens neurons by cocaine-associated stimuli during a water/cocaine multiple schedule. Brain Research 2001;907:156–161. [PubMed: 11430899]

Carelli RM, Ijames SG, Crumling AJ. Evidence that separate neural circuits in the nucleus accumbens encode cocaine versus “natural” (water and food) reward. J Neurosci 2000;20:4255–4266. [PubMed: 10818162]

Carelli RM, Wightman RM. Functional microcircuitry in the accumbens underlying drug addiction: insights from real-time signaling during behavior. Current Opinion in Neurobiology 2004;14:763–768. [PubMed: 15582381]

Carelli RM, Wondolowski J. Selective encoding of cocaine versus natural rewards by nucleus accumbens neurons is not related to chronic drug exposure. J Neurosci 2003;23:11214–11223. [PubMed: 14657180]

Chang JY, Zhang L, Janak PH, Woodward DJ. Neuronal responses in prefrontal cortex and nucleus accumbens during heroin self-administration in freely moving rats. Brain Res 1997;754:12–20. [PubMed: 9134954]

Chen BT, Bowers MS, Martin M, Hopf FW, Guillory AM, Carelli RM, Chou JK, Bonci A. Cocaine but Not Natural Reward Self-Administration nor Passive Cocaine Infusion Produces Persistent LTP in the VTA. Neuron 2008;59:288–297. [PubMed: 18667156]

Chen P-C, Chen J-C. Enhanced Cdk5 Activity and p35 Translocation in the Ventral Striatum of Acute and Chronic Methamphetamine-Treated Rats. Neuropsychopharmacology 2004;30:538–549. [PubMed: 15536496]

Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M, O’Brien CP. Limbic activation during cue-induced cocaine craving. Am J Psychiatry 1999;156:11–18. [PubMed: 9892292]

Choe ES, Chung KT, Mao L, Wang JQ. Amphetamine increases phosphorylation of extracellular signal-regulated kinase and transcription factors in the rat striatum via group I metabotropic glutamate receptors. Neuropsychopharmacology 2002;27:565–575. [PubMed: 12377393]

Choe ES, Wang JQ. CaMKII regulates amphetamine-induced ERK1/2 phosphorylation in striatal neurons. Neuroreport 2002;13:1013–1016. [PubMed: 12060798]

Davis, JF.; Loos, M.; Coolen, LM. Society for Behavioral Neuroendocrinology. Vol. 44. Cincinnati, Ohio: Hormones and Behavior; 2003. Lesions of the medial prefrontal cortex do not disrupt sexual behavior in male rats; p. 45

Di Chiara G, Imperato A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci U S A 1988;85:5274–5278. [PubMed: 2899326]

Dominguez JM, Balfour ME, Lee HS, Brown HJ, Davis BA, Coolen LM. Mating activates NMDA receptors in the medial preoptic area of male rats. Behavioral Neuroscience 2007;121:1023–1031. [PubMed: 17907833]

Elifson KW, Klein H, Sterk CE. Predictors of sexual risk-taking among new drug users. Journal of sex research 2006;43:318–327. [PubMed: 17599253]

Ellkashef A, Vocci F, Hanson G, White J, Wickes W, Tiihonen J. Pharmacotherapy of Methamphetamine Addiction: An update. Substance Abuse 2008;29:31–49. [PubMed: 19042205]

Everitt BJ. Sexual motivation: a neural and behavioural analysis of the mechanisms underlying appetitive and copulatory responses of male rats. Neurosci Biobehav Rev 1990;14:217–232. [PubMed: 2190121]

Everitt BJ, Cador M, Robbins TW. Interactions between the amygdala and ventral striatum in stimulus-reward associations: Studies using a second-order schedule of sexual reinforcement. Neuroscience 1989;30:63–75. [PubMed: 2664555]

Everitt BJ, Fray P, Kostarczyk E, Taylor S, Stacey P. Studies of instrumental behavior with sexual reinforcement in male rats (Rattus norvegicus): I. Control by brief visual stimuli paired with a receptive female. J Comp Psychol 1987;101:395–406. [PubMed: 3691062]

Everitt BJ, Parkinson JA, Olmstead MC, Arroyo M, Robledo P, Robbins TW. Associative Processes in Addiction and Reward The Role of Amygdala-Ventral Striatal Subsystems. Annals of the New York Academy of Sciences 1999;877:412–438. [PubMed: 10415662]

Everitt BJ, Stacey P. Studies of instrumental behavior with sexual reinforcement in male rats (Rattus norvegicus): II. Effects of preoptic area lesions, castration, and testosterone. J Comp Psychol 1987;101:407–419. [PubMed: 3691063]

Feltenstein MW, See RE. The neurocircuitry of addiction: an overview. Br J Pharmacol 2008;154:261–274. [PubMed: 18311189]

Ferguson SM, Norton CS, Watson SJ, Akil H, Robinson TE. Amphetamine-evoked c-fos mRNA expression in the caudate-putamen: the effects of DA and NMDA receptor antagonists vary as a function of neuronal phenotype and environmental context. Journal of Neurochemistry 2003;86:33–44. [PubMed: 12807422]

Fiorino DF, Coury A, Phillips AG. Dynamic changes in nucleus accumbens dopamine efflux during the Coolidge effect in male rats. J Neurosci 1997;17:4849–4855. [PubMed: 9169543]

Fiorino DF, Phillips AG. Facilitation of Sexual Behavior and Enhanced Dopamine Efflux in the Nucleus Accumbens of Male Rats after D-Amphetamine-Induced Behavioral Sensitization. J Neurosci 1999a; 19:456–463. [PubMed: 9870973]

Fiorino DF, Phillips AG. Facilitation of sexual behavior in male rats following d-amphetamine-induced behavioral sensitization. Psychopharmacology 1999b;142:200–208. [PubMed: 10102773]

Goodwin JS, Larson GA, Swant J, Sen N, Javitch JA, Zahniser NR, De Felice LJ, Khoshbouei H. Amphetamine and Methamphetamine Differentially Affect Dopamine Transporters in Vitro and in Vivo. J Biol Chem 2009;284:2978–2989. [PubMed: 19047053]

Grace AA, Rosenkranz JA. Regulation of conditioned responses of basolateral amygdala neurons. Physiology & Behavior 2002;77:489–493. [PubMed: 12526988]

Grant S, London ED, Newlin DB, Villemagne VL, Liu X, Contoreggi C, Phillips RL, Kimes AS, Margolin A. Activation of memory circuits during cue-elicited cocaine craving. Proc Natl Acad Sci U S A 1996;93:12040–12045. [PubMed: 8876259]

Green AI, Halkitis PN. Crystal methamphetamine and sexual sociality in an urban gay subculture: An elective affinity. Culture, Health & Sexuality 2006;8:317–333.

Grimm JW, See RE. Dissociation of primary and secondary reward-relevant limbic nuclei in an animal model of relapse. Neuropsychopharmacology 2000;22:473–479. [PubMed: 10731622]

Hull EM, Lorrain DS, Du J, Matuszewich L, Lumley LA, Putnam SK, Moses J. Hormone-neurotransmitter interactions in the control of sexual behavior. Behavioural Brain Research 1999;105:105–116. [PubMed: 10553694]

Hull, EM.; Meisel, RL.; Sachs, BD. Male sexual behavior. In: Pfaff, DW., et al., editors. Hormones Brain and Behavior. San Diego, CA: Elsevier Science (USA); 2002. p. 1-138.

Hull EM, Muschamp JW, Sato S. Dopamine and serotonin: influences on male sexual behavior. Physiology & Behavior 2004;83:291–307. [PubMed: 15488546]

Ishikawa K, Nitta A, Mizoguchi H, Mohri A, Murai R, Miyamoto Y, Noda Y, Kitaichi K, Yamada K, Nabeshima T. Effects of single and repeated administration of methamphetamine or morphine on neuroglycan C gene expression in the rat brain. The International Journal of Neuropsychopharmacology 2006;9:407–415. [PubMed: 16146580]

Jarosz PA, Kessler JT, Sekhon P, Coscina DV. Conditioned place preferences (CPPs) to high-caloric "snack foods" in rat strains genetically prone vs. resistant to diet-induced obesity: Resistance to naltrexone blockade. Pharmacology Biochemistry and Behavior 2007;86:699–704.

Jarosz PA, Sekhon P, Coscina DV. Effect of opioid antagonism on conditioned place preferences to snack foods. Pharmacology Biochemistry and Behavior 2006;83:257–264.

Jentsch JD, Taylor JR. Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. Psychopharmacology (Berl) 1999;146:373–390. [PubMed: 10550488]

Kalivas PW, Volkow ND. The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry 2005;162:1403–1413. [PubMed: 16055761]

Kelley AE. Memory and addiction: shared neural circuitry and molecular mechanisms. Neuron 2004;44:161–179. [PubMed: 15450168]

Kippin TE, Sotiropoulos V, Badih J, Pfaus JG. Opposing roles of the nucleus accumbens and anterior lateral hypothalamic area in the control of sexual behaviour in the male rat. European Journal of Neuroscience 2004;19:698–704. [PubMed: 14984420]

Laviolette SR, Grace AA. Cannabinoids Potentiate Emotional Learning Plasticity in Neurons of the Medial Prefrontal Cortex through Basolateral Amygdala Inputs. J Neurosci 2006;26:6458–6468. [PubMed: 16775133]

Ledford CC, Fuchs RA, See RE. Potentiated Reinstatement of Cocaine-Seeking Behavior Following D-amphetamine Infusion into the Basolateral Amygdala. Neuropsychopharmacology 2003;28:1721–1729. [PubMed: 12865896]

Lett BT. Repeated exposures intensify rather than diminish the rewarding effects of amphetamine, morphine, and cocaine. Psychopharmacology (Berl) 1989;98:357–362. [PubMed: 2546170]

Liu YC, Sachs BD, Salamone JD. Sexual behavior in male rats after radiofrequency or dopamine-depleting lesions in nucleus accumbens. Pharmacol Biochem Behav 1998;60:585–592. [PubMed: 9632244]

Lorrain DS, Riolo JV, Matuszewich L, Hull EM. Lateral Hypothalamic Serotonin Inhibits Nucleus Accumbens Dopamine: Implications for Sexual Satiety. J Neurosci 1999;19:7648–7652. [PubMed: 10460270]

Lumley LA, Hull EM. Effects of a D1 antagonist and of sexual experience on copulation-induced Fos-like immunoreactivity in the medial preoptic nucleus. Brain Research 1999;829:55–68. [PubMed: 10350530]

Martinez I, Paredes RG. Only self-paced mating is rewarding in rats of both sexes. Horm Behav 2001;40:510–517. [PubMed: 11716580]

McLaughlin J, See RE. Selective inactivation of the dorsomedial prefrontal cortex and the basolateral amygdala attenuates conditioned-cued reinstatement of extinguished cocaine-seeking behavior in rats. Psychopharmacology (Berl) 2003;168:57–65. [PubMed: 12845418]

Mitchell JB, Stewart J. Facilitation of sexual behaviors in the male rat in the presence of stimuli previously paired with systemic injections of morphine. Pharmacology Biochemistry and Behavior 1990;35:367–372.

Mizoguchi H, Yamada K, Mizuno M, Mizuno T, Nitta A, Noda Y, Nabeshima T. Regulations of Methamphetamine Reward by Extracellular Signal-Regulated Kinase 1/2/ets-Like Gene-1 Signaling Pathway via the Activation of Dopamine NIDA (Research Report Series: Methamphetamine abuse and addiciton. 2006 NIH Publication number 06-4210.

Perks SM, Clifton PG. Reinforcer revaluation and conditioned place preference. Physiology & Behavior 1997;61:1–5. [PubMed: 8976526]

Pfaus JG. Pathways of Sexual Desire. Journal of Sexual Medicine 2009;6:1506–1533. [PubMed: 19453889]

Pfaus, JG.; Everitt, BJ. The psychopharmacology of sexual behavior. In: Bloom, FE.; Kupfer, DJ., editors. Psychopharmacology: the fourth generation of progress. New York: Raven; 1995. p. 743-758.

Pfaus JG, Heeb MM. Implications of Immediate-Early Gene Induction in the Brain Following Sexual Stimulation of Female and Male Rodents. Brain Research Bulletin 1997;44:397–407. [PubMed: 9370204]

Pfaus JG, Kippin TE, Centeno S. Conditioning and sexual behavior: a review. Horm Behav 2001;40:291–321. [PubMed: 11534994]

Pfaus JG, Phillips AG. Differential effects of dopamine receptor antagonists on the sexual behavior of male rats. Psychopharmacology 1989;98:363–368. [PubMed: 2568656]

Pierce RC, Kumaresan V. The mesolimbic dopamine system: The final common pathway for the reinforcing effect of drugs of abuse? Neuroscience & Biobehavioral Reviews 2006;30:215–238. [PubMed: 16099045]

Pitchers KK, Balfour ME, Lehman MN, Richtand NM, Yu L, Coolen LM. Sexual experience induces functional and structural plasticity in the mesolimbic system. Biological Psychiatry. 2009 In press.

Ranaldi R, Pocock D, Zereik R, Wise RA. Dopamine fluctuations in the nucleus accumbens during maintenance, extinction, and reinstatement of intravenous D-amphetamine self-administration. J Neurosci 1999;19:4102–4109. [PubMed: 10234038]

Rawson RA, Washton A, Domier CP, Reiber C. Drugs and sexual effects: role of drug type and gender. Journal of Substance Abuse Treatment 2002;22:103–108. [PubMed: 11932136]

Robertson GS, Pfaus JG, Atkinson LJ, Matsumura H, Phillips AG, Fibiger HC. Sexual behavior increases c-fos expression in the forebrain of the male rat. Brain Res 1991;564:352–357. [PubMed: 1810635]

Roop RG, Hollander RJ, Carelli RM. Accumbens activity during a multiple schedule for water and sucrose reinforcement in rats. Synapse 2002;43:223–226. [PubMed: 11835516]

Salo R, Nordahl TE, Natsuaki Y, Leamon MH, Galloway GP, Waters C, Moore CD, Buonocore MH. Attentional Control and Brain Metabolite Levels in Methamphetamine Abusers. Biological Psychiatry 2007;61:1272–1280. [PubMed: 17097074]

Schilder AJ, Lampinen TM, Miller ML, Hogg RS. Crystal methamphetamine and ecstasy differ in relation to unsafe sex among young gay men. Canadian journal of public health 2005;96:340–343.

See RE. Neural substrates of conditioned-cued relapse to drug-seeking behavior. Pharmacology Biochemistry and Behavior 2002;71:517–529.

See RE, Fuchs RA, Ledford CC, McLaughlin J. Drug Addiction, Relapse, and the Amygdala. Annals of the New York Academy of Sciences 2003;985:294–307. [PubMed: 12724166]

Semple SJ, Patterson TL, Grant I. Motivations associated with methamphetamine use among HIV men who have sex with men. Journal of Substance Abuse Treatment 2002;22:149–156. [PubMed: 12039618]

Shaham Y, Shalev U, Lu L, De Wit H, Stewart J. The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology (Berl) 2003;168:3–20. [PubMed: 12402102]

Shippenberg TS, Heidbreder C. Sensitization to the conditioned rewarding effects of cocaine: pharmacological and temporal characteristics. J Pharmacol Exp Ther 1995;273:808–815. [PubMed: 7752084]

Shippenberg TS, Heidbreder C, Lefevour A. Sensitization to the conditioned rewarding effects of morphine: pharmacology and temporal characteristics. Eur J Pharmacol 1996;299:33–39. [PubMed: 8901005]

Somlai AM, Kelly JA, McAuliffe TL, Ksobiech K, Hackl KL. Predictors of HIV sexual risk behaviors in a community sample of injection drug-using men and women. AIDS and behavior 2003;7:383–393. [PubMed: 14707535]

Springer A, Peters R, Shegog R, White D, Kelder S. Methamphetamine Use and Sexual Risk Behaviors in U.S. High School Students: Findings from a National Risk Behavior Survey. Prevention Science 2007;8:103–113. [PubMed: 17318422]

Sun W-L, Zhou L, Hazim R, Quinones-Jenab V, Jenab S. Effects of dopamine and NMDA receptors on cocaine-induced Fos expression in the striatum of Fischer rats. Brain Research 2008;1243:1–9. [PubMed: 18822274]

Swanson, LW., editor. Brain Maps: Structure of the Rat Brain. Amsterdam: Elsevier Science; 1998.

Tenk CM, Wilson H, Zhang Q, Pitchers KK, Coolen LM. Sexual reward in male rats: Effects of sexual experience on conditioned place preference associated with ejaculations and intromissions. Horm Behav. 2008

Valjent E, Corvol JC, Pages C, Besson MJ, Maldonado R, Caboche J. Involvement of the extracellular signal-regulated kinase cascade for cocaine-rewarding properties. J Neurosci 2000;20:8701–8709. [PubMed: 11102476]

Valjent E, Pages C, Herve D, Girault JA, Caboche J. Addictive and non-addictive drugs induce distinct and specific patterns of ERK activation in mouse brain. Eur J Neurosci 2004;19:1826–1836. [PubMed: 15078556]

Valjent E, Pascoli V, Svenningsson P, Paul S, Enslen H, Corvol JC, Stipanovich A, Caboche J, Lombroso PJ, Nairn AC, Greengard P, Herve D, Girault JA. Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. Proc Natl Acad Sci U S A 2005;102:491–496. [PubMed: 15608059]

Vanderschuren LJ, Kalivas PW. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology (Berl) 2000;151:99–120. [PubMed: 10972458]

Veening JG, Coolen LM. Neural activation following sexual behavior in the male and female rat brain. Behavioural Brain Research 1998;92:181–193. [PubMed: 9638960]

Whitelaw RB, Markou A, Robbins TW, Everitt BJ. Excitotoxic lesions of the basolateral amygdala impair the acquisition of cocaine-seeking behaviour under a second-order schedule of reinforcement. Psychopharmacology 1996;127:213–224. [PubMed: 8912399]

Wise RA. Neurobiology of addiction. Current Opinion in Neurobiology 1996;6:243–251. [PubMed: 8725967]

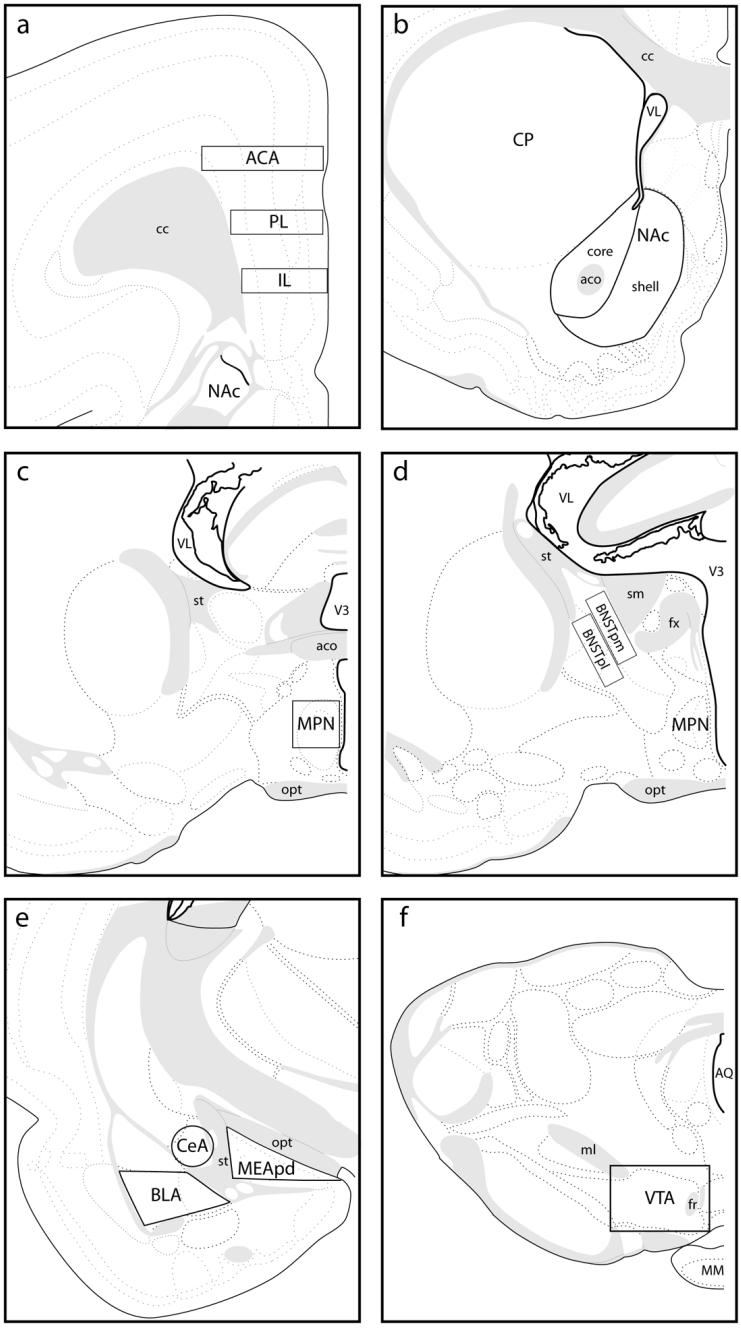

Figure 1.

Schematic drawings and images illustrating brain areas of analysis. Areas of analysis indicated were based on landmarks unique for each brain region, did not differ between experimental groups, and were 1.25 mm^2 in mPFC subregions (a), 1.3 mm^2 in the NAc core and shell (b), 1.0 mm^2 in the MPN (c), 1.25 mm^2 in the BNST subregions (d), 1.6, 2.25, and 2.5 mm^2 in the BLA, CeA, and MEApd respectively (e), and 3.15 mm^2 in the VTA (f). Abbreviations: aco, anterior commisure; AQ, cerebral aqueduct; cc, corpus callosum; CP, caudate putamen; fr, fasciculus retroflexus; fx, fornix; ml, medial lemniscus; MM, medial mammillary nucleus; opt, optic tract; V3, third ventricle; sm, stria medullaris; st, stria terminalis VL, lateral ventricle. Brain drawings were modified from Swanson (1998).

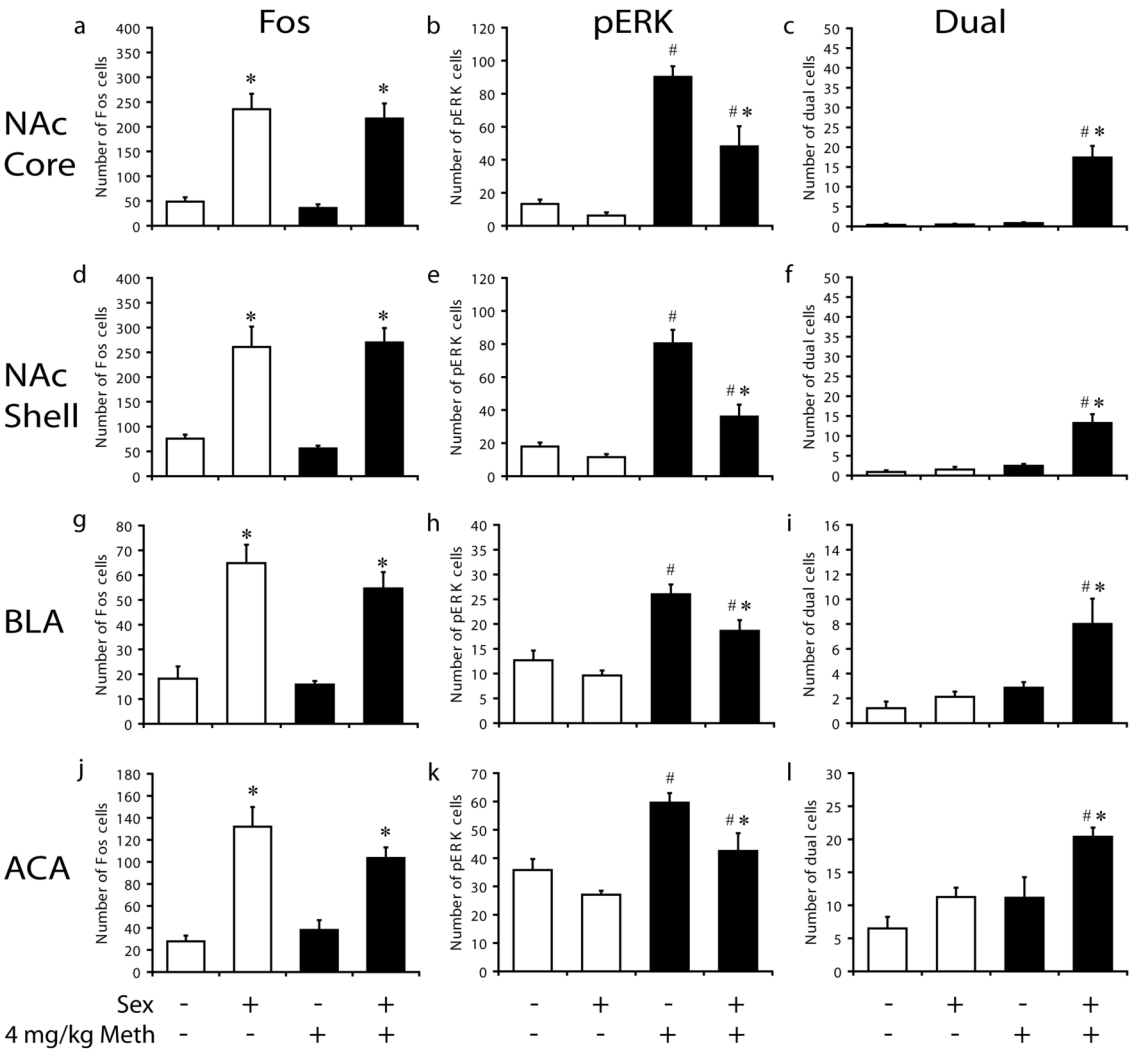

Figure 2.

Sex-induced Fos and Meth-induced pERK expression in NAc, BLA, and ACA neurons 10 min following administration of 4 mg/kg Meth. Mean numbers ± s.e.m. of Fos (a, d, g, j), pERK (b, e, h, k), and dual (c, f, i, l) labeled cells in the NAc core (a, b, c) and shell (d, e, f), the BLA (g, h, i), and ACA (j, k, l). * indicate significant differences from unmated males of the same saline or Meth injected groups ($p < 0.05$); # indicates significant differences from saline injected groups of the same sex or no sex treatment ($p < 0.05$).

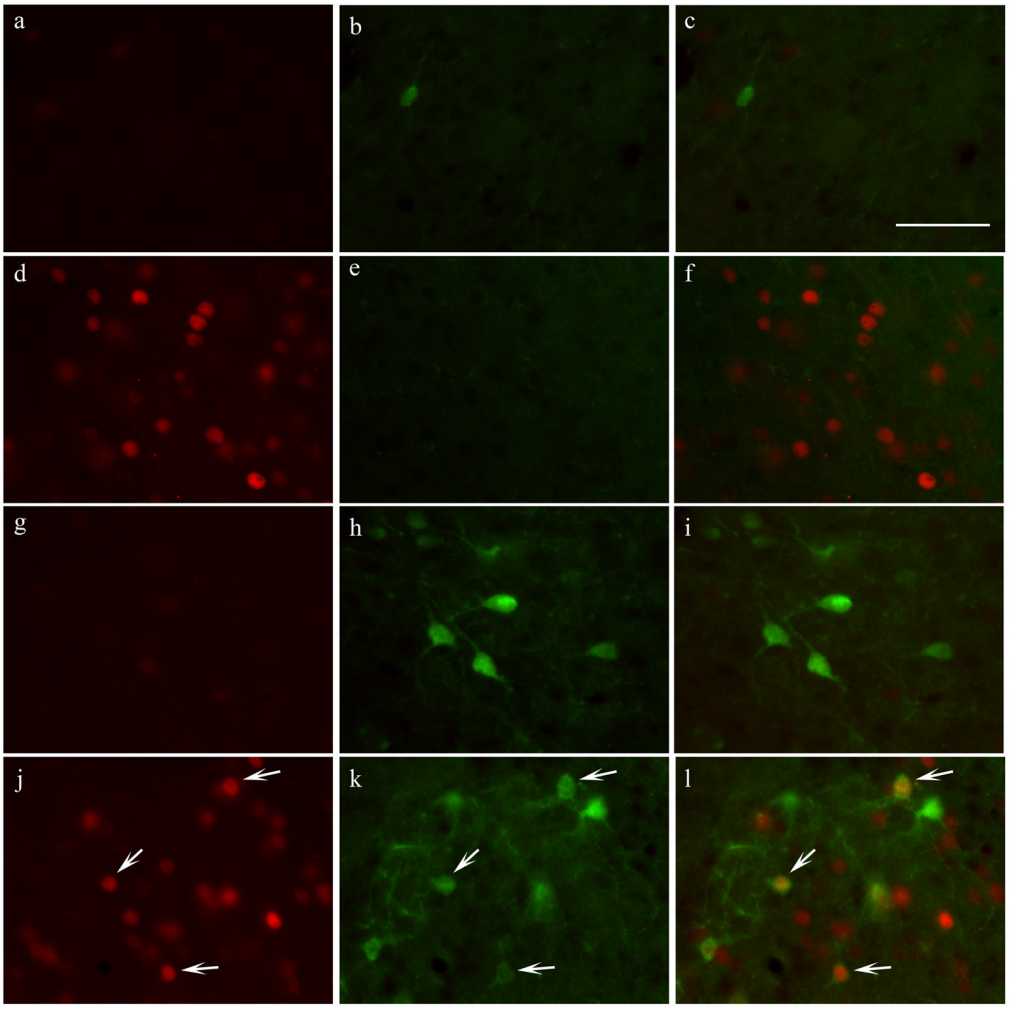

Figure 3.
Representative images of NAc sections immunostained for Fos (red; a, d, g, j) and pERK (green; b, e, h, k) of animals of each experimental group: No Sex+Sal (a, b, c), Sex+Sal (d, e, f), No Sex+Meth (g, h, i), and Sex+Meth (j, k, l). Right panels are merged images illustrating co-localization of Fos and pERK. Arrows indicate dual labeled cells. Scale bar indicates 50 μm.

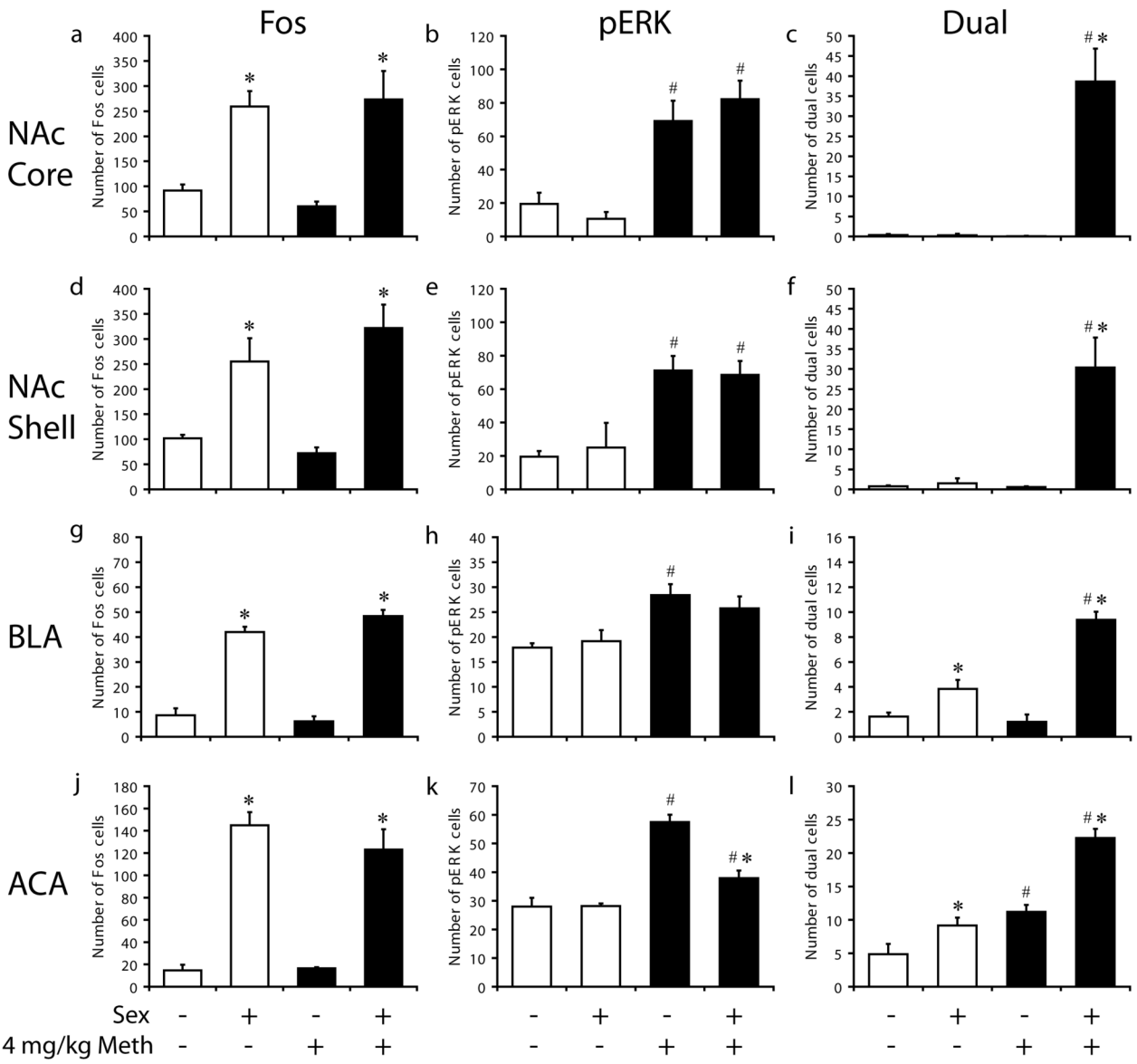

Figure 4.

Sex-induced Fos and Meth-induced pERK expression in NAc, BLA, and ACA neurons 15 min following administration of 4 mg/kg Meth. Mean numbers ± s.e.m. of Fos (a, d, g, j), pERK (b, e, h, k), and dual (c, f, i, l) labeled cells in the NAc core (a, b, c) and shell (d, e, f), the BLA (g, h, i), and ACA (j, k, l). * indicate significant differences from unmated males of the same saline or Meth injected groups ($p < 0.05$); # indicates significant differences from saline injected groups of the same sex or no sex treatment ($p < 0.05$).

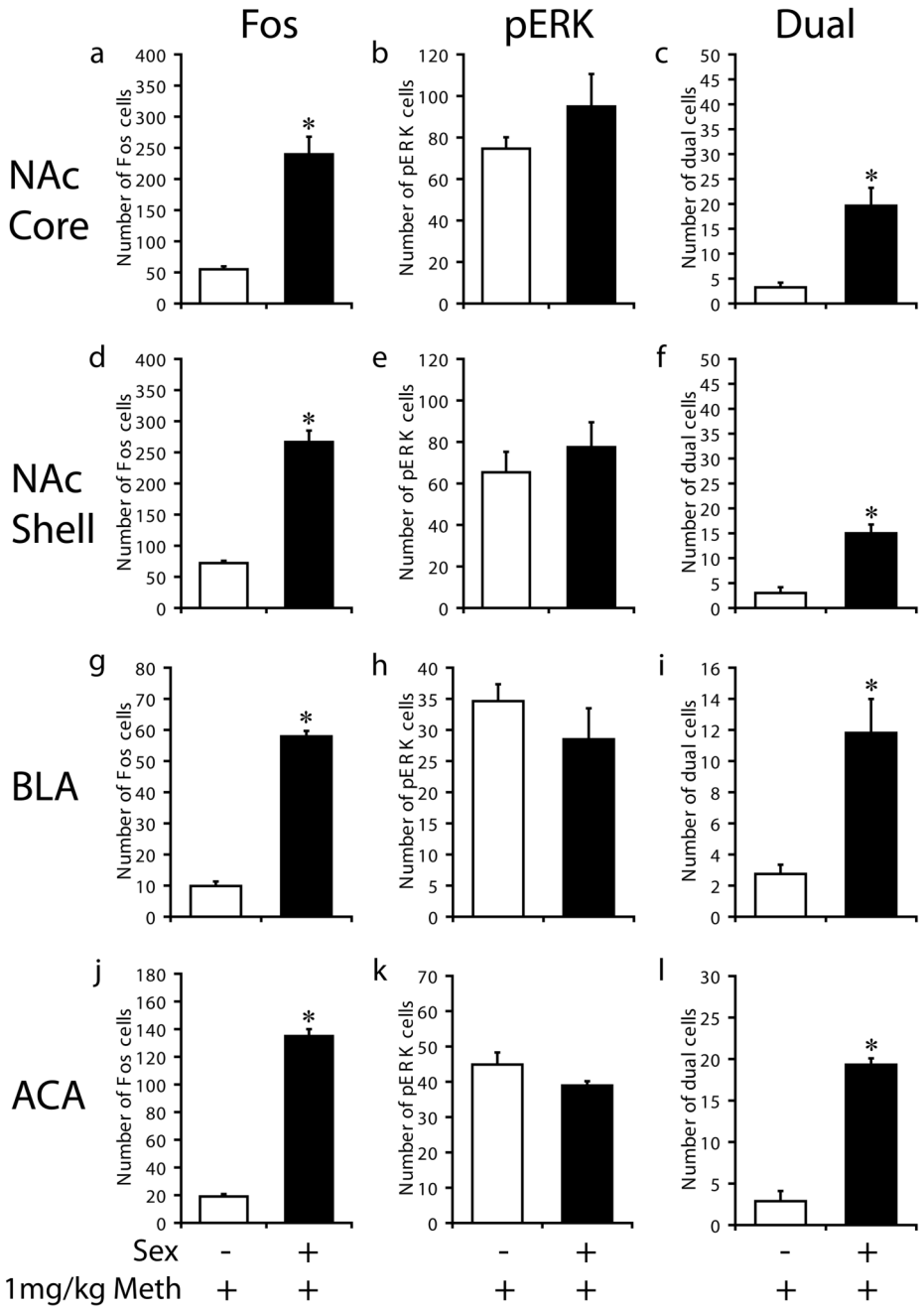

Figure 5.

Sex-induced Fos and Meth-induced pERK expression in NAc, BLA, and ACA neurons 15 min following administration of 1 mg/kg Meth. Mean numbers ± s.e.m. of Fos (a, d, g, j), pERK (b, e, h, k), and dual (c, f, i, l) labeled cells in the NAc core (a, b, c) and shell (d, e, f), the BLA (g, h, i), and ACA (j, k, l). * indicate significant differences from unmated males of the same Meth injected groups ($p < 0.05$).

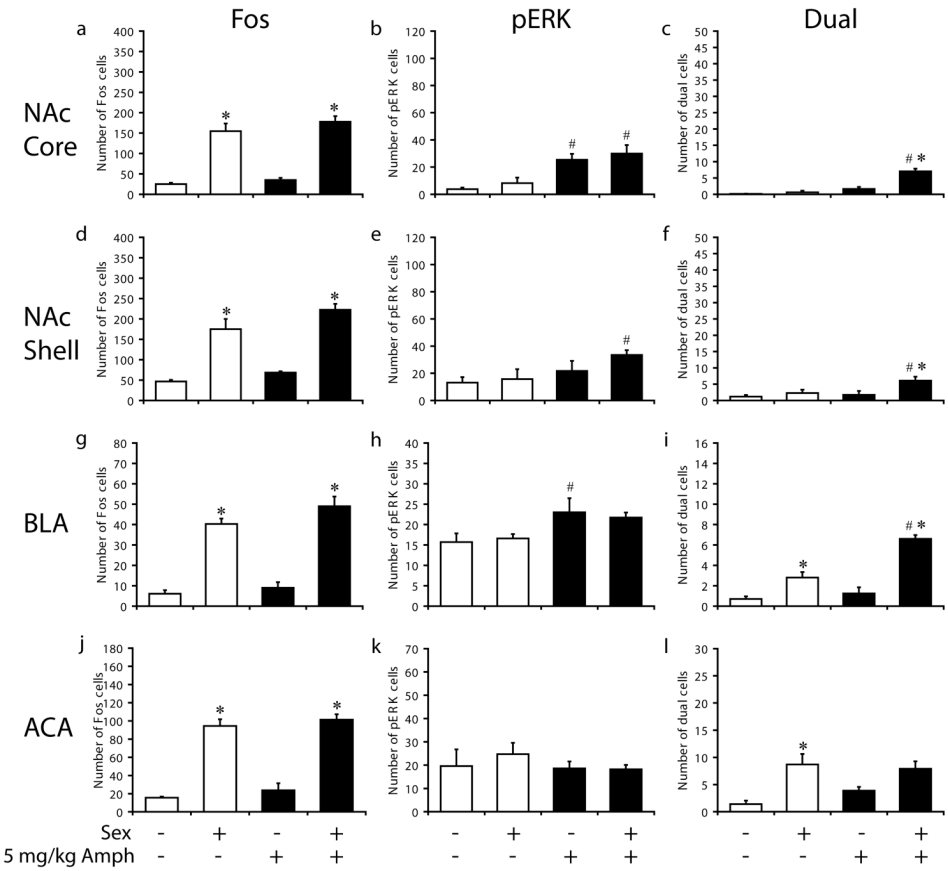

Figure 6.

Sex-induced Fos and Amph-induced pERK expression in NAc, BLA, and ACA neurons 15 min following administration of 5 mg/kg Amph. Mean numbers ± s.e.m. of Fos (a, d, g, j), pERK (b, e, h, k), and dual (c, f, i, l) labeled cells in the NAc core (a, b, c) and shell (d, e, f), the BLA (g, h, i), and ACA (j, k, l). * indicate significant differences from unmated males of the same saline or Meth injected groups ($p < 0.05$); # indicates significant differences from saline injected groups of the same sex or no sex treatment ($p < 0.05$)

Table 1

Overview of experimental groups included in experiments 1–4.

Sexual Behavior	Treatment	Dose	Time point	Group size
Experiment 1				
No Sex	Saline	1 mg/kg	10	n=5
Sex	Saline	1 mg/kg	10	n=4
No Sex	Meth	4 mg/kg	10	n=7
Sex	Meth	4 mg/kg	10	n=4
Experiment 2				
No Sex	Saline	1 mg/kg	15	n=4
Sex	Saline	1 mg/kg	15	n=4
No Sex	Meth	4 mg/kg	15	n=5
Sex	Meth	4 mg/kg	15	n=4
Experiment 3				
No Sex	Meth	1 mg/kg	15	n=6
Sex	Meth	1 mg/kg	15	n=6
Experiment 4				
No Sex	Saline	1 mg/kg	15	n=5
Sex	Saline	1 mg/kg	15	n=5
No Sex	Amph	5 mg/kg	15	n=5
Sex	Amph	5 mg/kg	15	n=5

Table 2

Overview of mating-induced Fos and Meth-induced pERK expression in brain areas where sex and drugs activate non-overlapping neural populations.

Brain areas	Group	Fos	pERK	Dual	% Dual/pERK
Amygdala					
CeA	Sal10	8.1 ± 2.0	33.7 ± 6.1	3.3 ± 0.8	11.1 ± 3.1
	Sex+Sal10	22.9 ± 2.7*	31.0 ± 5.7	13.9 ± 2.1*	43.2 ± 4.3
	Meth10	10.3 ± 2.2	95.3 ± 8.3#	5.1 ± 1.1	5.5 ± 1.6
	Sex+Meth10	21.6 ± 5.9*	77.6 ± 6.6#	17.3 ± 4.6*	20.7 ± 4.6
MEApd	Sal10	72.1 ± 13.5	40.2 ± 7.2	5.1 ± 1.9	11.4 ± 2.7
	Sex+Sal10	295.3 ± 48.1*	47.3 ± 6.7	13.8 ± 2.5*	29.4 ± 1.1
	Meth10	99.1 ± 6.4	84.5 ± 11.1#	9.1 ± 1.4	12.2 ± 2.2
	Sex+Meth10	403.5 ± 3.5*#	56.5 ± 7.1	11.4 ± 0.5	21.3 ± 3.9
BNST					
BNSTpl	Sal10	19.9 ± 3.3	13.9 ± 5.9	2.5 ± 1.0	15.2 ± 5.4
	Sex+Sal10	116.8 ± 14.9*	6.8 ± 1.1	3.3 ± 0.8	49.3 ± 9.6
	Meth10	23.1 ± 1.7	35.4 ± 4.1#	2.9 ± 0.6	8.6 ± 1.2
	Sex+Meth10	113.3 ± 16.5*	20.6 ± 4.7*	4.9 ± 1.1	24.5 ± 10.5
mPFC					
PL	Sal10	69.5 ± 13.4	31.5 ± 2.2	5.9 ± 2.1	17.9 ± 6.1
	Sex+Sal10	168.6 ± 32.4*	28.3 ± 1.6	12.0 ± 1.4*	41.7 ± 4.9
	Meth10	70.9 ± 11.9	49.7 ± 2.4#	8.1 ± 1.6	16.4 ± 3.3
	Sex+Meth10	148.1 ± 9.6*	32.3 ± 3.5*	13.0 ± 1.4	41.4 ± 5.9
IL	Sal10	59.8 ± 7.9	31.9 ± 2.0	5.2 ± 1.6	15.8 ± 4.4
	Sex+Sal10	129.8 ± 20.5*	23.5 ± 2.9	9.3 ± 2.3	39.4 ± 3.6
	Meth10	62.9 ± 9.0	47.6 ± 2.7#	6.1 ± 1.5	12.8 ± 3.3
	Sex+Meth10	108.5 ± 12.1*	30.1 ± 2.6*	8.3 ± 1.6	27.8 ± 3.7

Mean numbers ± of single and dual labeled neurons for pERK and Fos, as well as percentage of Meth activated neurons co-activated by sexual behavior are listed for each brain area for all four experimental groups.

* indicate significant differences from unmated males of the same saline or Meth injected group ($p < 0.05$);

# indicates significant differences from saline injected groups of the same sex or no sex treatment ($p < 0.05$).

Table 3

Overview of mating-induced Fos and Meth-induced pERK expression in brain areas where neural activation was induced only by mating.

Brain areas	Group	Fos	pERK	Dual	% Dual/pERK
MPN					
	Sal10	14.1 ± 3.2	7.4 ± 4.2	4.8 ± 3.6	52.7 ± 10.8
	Sex+Sal10	214.3 ± 36.8*	8.4 ± 3.2	7.3 ± 3.1	83.1 ± 10.2
	Meth10	10.3 ± 4.6	11.8 ± 1.5	2.7 ± 0.9	20.0 ± 5.7
	Sex+Meth10	211.8 ± 10.0*	10.4 ± 3.3	8.7 ± 3.0	73.7 ± 12.5
BNST					
BNSTpm	Sal10	13.3 ± 1.3	13.3 ± 1.3	0.9 ± 0.3	7.3 ± 2.9
	Sex+Sal10	216.8 ± 8.4*	4.6 ± 1.6*	2.4 ± 1.0	36.5 ± 15.0
	Meth10	15.2 ± 1.1	13.3 ± 2.1	1.8 ± 0.5	13.1 ± 4.0
	Sex+Meth10	171.1 ± 27.3*	9.1 ± 2.9	4.9 ± 1.1*	49.0 ± 1.3
VTA					
	Sal10	35.6 ± 4.6	37.2 ± 11.2	14.9 ± 3.6	39.7 ± 7.7
	Sex+Sal10	114.3 ± 39.9*	46.3 ± 9.6	23.6 ± 7.0	24.2 ± 4.9
	Meth10	42.6 ± 4.7	50.2 ± 5.5	14.9 ± 3.5	34.1 ± 4.9
	Sex+Meth10	101.0 ± 16.7*	42.0 ± 8.5	19.5 ± 2.5	22.8 ± 4.2

Mean numbers ± s.e.m. of single and dual labeled neurons for pERK and Fos, as well as percentage of Meth activated neurons co-activated by sexual behavior are listed for each brain area or all four experimental groups.

* indicate significant differences from unmated males of the same saline or Meth injected group ($p < 0.05$);

# indicates significant differences from saline injected groups of the same sex or no sex treatment ($p < 0.05$).