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Oxytocin acts in nucleus accumbens to attenuate methamphetamine seeking and demand

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Abstract

Background—Evidence indicates that oxytocin, an endogenous peptide well known for its role in social behaviors, childbirth and lactation, is a promising addiction pharmacotherapy. We employed a within-session behavioral-economic (BE) procedure in rats to examine oxytocin as a pharmacotherapy for methamphetamine (meth) addiction. The BE paradigm was modeled after BE procedures used to assess motivation for drugs in human addicts. Importantly, the same BE variables assessed across species have been shown to predict later relapse behavior. Thus, the translational potential of preclinical BE studies is particularly strong.

Methods—We tested the effects of systemic and microinfused oxytocin on demand for self-administered i.v. meth and reinstatement of extinguished meth-seeking in male and female rats using a behavioral economics paradigm. Correlations between meth demand and meth seeking were assessed.

Results—Females showed greater demand (i.e., motivation) for meth compared to males. In both males and females, meth demand predicted reinstatement of meth-seeking, and systemic oxytocin decreased demand for meth and attenuated reinstatement to meth seeking. Oxytocin was most effective at decreasing meth demand and seeking in rats with the strongest motivation for drug. Finally, we found that these effects of systemic oxytocin were mediated by actions in the nucleus accumbens (NAc).

Discussion—Oxytocin decreases meth demand and seeking in both sexes, and these effects depend on oxytocin signaling in the NAc. Overall, these data indicate that development of oxytocin-based therapies may be a promising treatment approach for meth addiction in humans.

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Keywords

addiction; oxytocin; methamphetamine; self-administration; behavioral economics; reinstatement

Introduction

Oxytocin, an endogenous neuropeptide well known for its role in social behaviors and childbirth, is a potential pharmacotherapy for addiction (1–9). Clinical and preclinical research has shown that oxytocin can decrease addiction-related behaviors, including drug cravings in humans (10, 11) and self-administration and relapse-like behaviors in rodents (12–16). These effects of oxytocin may be caused by interactions within the mesocorticolimbic dopamine system, as this system mediates both social behaviors and addiction-like behaviors (17), including motivated drug seeking during relapse (18–24).

Behavioral-economic (BE) paradigms designed to assess addiction-like behaviors can be applied similarly in both humans and animals, giving them high translational potential. In this approach, demand for a drug is measured under varying price (or effort) conditions, providing demand curves that allow estimates of free consumption and motivation (16, 25). These analyses allow for direct comparison of the same BE variables across species or reinforcers (26, 27). Importantly, the variables measured with this translational approach (particularly α-demand elasticity, and Q_0 – free consumption) have been shown to predict addiction behaviors in both humans (28–30) and animals (16, 25, 31, 32), and may be an effective approach for developing and testing potential pharmacotherapies (16).

Previous methods to generate demand curves required subjects to stabilize responding at multiple different prices (numbers of lever presses per reward) over weeks of testing (31, 33), limiting the ability to assess underlying brain mechanisms or possible pharmacotherapies. This limitation has recently been overcome with the design of a within-session BE procedure for cocaine self-administration (25, 34, 35). However, no comparable within-session paradigm exists for meth self-administration. Here, we developed a new within-session BE method to accommodate the longer half-life of meth. We verified that demand curve variables (α and Q_0) obtained with our new method were similar to those obtained in a conventional, multi-day BE paradigm. This novel within-session paradigm now permits construction of demand curves for meth (or other long-acting rewards) within a single 105 min session.

Utilizing this BE procedure we find that there is a close relationship between the economic demand for meth and reinstatement of extinguished meth seeking. We also show that in both male and female rats oxytocin decreases motivation to seek meth and proportionally attenuates reinstatement behavior (16). Numerous studies have shown a crucial role of the nucleus accumbens (NAc) core in mediating oxytocin's effects on reward related behaviors (8, 9, 13, 17, 36–41). We show here that oxytocin decreased Fos activation in NAc core neurons associated with reinstated meth seeking, and that oxytocin actions in NAc are both sufficient and necessary to decrease meth demand and seeking.

Overall, this work indicates that oxytocin may be a promising treatment for meth addiction, and highlights the NAc as a region of interest for further assessment of the mechanism of oxytocin in drug addiction.

Methods and Materials

Subjects

Male and female Sprague Dawley rats (Harlan; initial weight of 200–275 g) were individually housed on a reversed 12:12 light-dark cycle in a temperature and humidity controlled vivarium. Water and food were available *ad libitum*. All experimental protocols were approved by the Institutional Animal Care and Use Committee, and were in accordance with the "Guide for the Care and Use of Laboratory Animals" (42).

Methamphetamine self-administration and BE procedures

Rats acquired i.v. meth self-administration in daily 2-hr sessions (fixed-ratio (FR) 1 schedule) for a minimum of 5 sessions (>20 infusions/session). The meth dose per infusion was adjusted for females (17.5 μg/50 μl) and males (20 μg/50 μl) to account for differences in average body weight (43, 44). In subsequent sessions, FR values were increased (i.e. FR3,10,32,100) and rats self-administered meth at each FR value until they reached <15% variability in the last 2 days and were then switched to the within-session BE paradigm. Rats experienced the within-session procedure (minimum of 5 sessions) until demand elasticity was stable (i.e., α values within 25% of the mean of the last 3 sessions). All testing during BE sessions was performed in a within-subject, counterbalanced manner, with responding re-stabilized between each test.

Following within-session BE tests, rats underwent a minimum of 7 extinction sessions (2-hr/daily sessions) to a criterion of <25 active lever presses on 2 consecutive days. After reaching criterion, rats underwent cue-induced reinstatement testing where one meth-associated cue (light+tone) was presented at the beginning of the session and subsequent responding on the active lever resulted in presentation of the cues along a FR1 schedule. Between all reinstatement tests, rats experienced a minimum of 2 extinction sessions, or until criterion was met. See supplement for surgery, drugs, standard self-administration procedures, estrous cycle monitoring, and statistical analysis.

Immunohistochemistry

Immediately following a final cue-induced reinstatement test or extinction, rats were sacrificed for Fos labeling. As the Fos protein takes 60–90 min to express this analysis captured the first 30 min of the session, and has been previously used to assess reinstatement-induced Fos (45). The total number of Fos+ neurons was quantified in the NAc core. See Supplemental Information for more specific immunohistochemical analysis.

Demand curve analysis

An exponential demand equation (25, 27) was used to fit a demand curve to the results from each session to determine economic demand parameters for meth, as prevously described in (25). Briefly, demand variables Q_0 (drug consumption at null cost, i.e., free consumption)

and α (rate of consumption decline with increasing price, i.e., demand elasticity) were extracted from the demand curves and used in subsequent analyses. Price is defined here as the number of responses needed to obtain one meth infusion. The primary measure of 'free consumption' was Q_0 (mg/kg/rat), corresponding to consumption at null effort. The primary measure of 'motivation' to self-administer drug was α (demand elasticity), which corresponds to the rate at which consumption decreases with increasing effort (25). Importantly, α scales inversely with motivation for meth. See Supplemental Information and (25, 27) for specifics of demand curve analysis.

Intracerebroventricular (ICV) microinfusions

After rats were stabilized on the within-session BE procedure as described above, they received unilateral microinfusions of oxytocin into a lateral ventricle. An infusion pump delivered 1 μl/2 min, injectors were left in place for an additional 2 min. All rats received 4 microinfusions in a counterbalanced manner of desGly-NH_2,d(CH_2)$_5$[D-Tyr^2,Thr^4]OVT (oxytocin antagonist, OXA)(2 ug/infusion) or aCSF immediately followed by oxytocin (1 mg/kg, i.p.) or saline 30 min prior to testing. Responding was re-stabilized between tests.

NAc core microinfusions

On test days, rats received bilateral microinfusions (0.5 μl/side) over a 1 min period, and injectors were left in place for 1 min. To test whether oxytocin in the NAc core decreased meth seeking, rats received microinfusions of either oxytocin (0.6 μg/side) or aCSF vehicle (in a counter-balanced manner) 10 min prior to being tested on the BE paradigm and/or cue-induced reinstatement. To test if systemic oxytocin acted within the NAc to reduce meth seeking, rats received microinfusions of either OXA (1 ug/side) or aCSF vehicle into NAc immediately followed by a systemic injection of oxytocin (1 mg/kg) or saline 30 min prior to testing on the BE paradigm. Responding was re-stabilized between all tests. To show specificity of effect of oxytocin to NAc a separate group of rats with cannula placements outside of the NAc were also assessed.

Rats tested on cue-induced reinstatement received a maximum of 2 tests (oxytocin or vehicle, counterbalanced), and were returned to extinction criterion between tests. Each rat received a maximum of 6 NAc microinfusions.

Results

Demand elasticity predicts meth seeking

We compared results of the within-session BE paradigm in males and females with results from a conventional (multi-day) BE paradigm in which rats were stabilized at each FR value for multiple days (31). Following training on the conventional BE paradigm, rats were stabilized on the within-session paradigm. In both paradigms, females showed greater motivation to seek meth (lower α)(Figure 1C and 1D), and higher consumption at null cost (higher Q_0) than males (Figure 1E and 1F). We further validated our within-session BE paradigm by showing that α calculated during the traditional BE paradigm correlated with α assessed during the within-session paradigm in both sexes (Males $r=0.44$, $p<0.05$; Females $r=0.51$, $p<0.05$)(Figure 1G and 1H).

We next examined whether individual differences in baseline demand elasticity (α) or free consumption (Q_0) for meth predicted drug seeking during abstinence. Males and females were examined on the within-session BE paradigm and subsequently subjected to daily extinction sessions followed by cue-induced reinstatement sessions. α predicted meth seeking (number of active lever presses) on the first day of extinction (males: r=−0.49, p<0.05; females: r=−0.58, p<0.01) and during cue-induced reinstatement (males: r=−0.43, p=0.05; females: r=−0.47, p<0.05) (Figure 2). There was no relationship in either sex between baseline free consumption (Q_0) and meth seeking on the first day of extinction (males: r=0.07, p>0.05; females: r=0.43, p>0.05) or cue-induced reinstatement of meth seeking (males: r=0.20, p>0.05; females: r=0.19, p>0.05) (Supplemental Figure S2). These data indicate that free consumption (Q_0), which is closely related to FR1, is not a predictor of meth-seeking behavior, whereas demand elasticity (α, a measure of motivation) is a strong predictor of meth seeking during extinction and reinstatement.

The estrous cycle was monitored daily throughout the experiment. There was no significant effect of estrous cycle phase on BE variables during within-session stabilization (α: F(2,24)=0.24, p>0.05; Q0: F(2,24)=0.02, p>0.05) or cue-induced reinstatement (saline: F(2,14)=1.93, p>0.05; oxytocin: F(2,14)=0.68, p>0.05).

Oxytocin decreases motivation to seek meth similarly in males and females

We next assessed the effects of oxytocin on meth demand and cue-induced reinstatement. Rats were stabilized on the within-session and on subsequent BE sessions, rats were pretreated with oxytocin (1 mg/kg i.p.) or saline (counterbalanced) 30 min prior to testing. Oxytocin increased α (decreased motivation for meth) in both sexes (two-way ANOVA, treatment main effect $F_{(1,38)}$=52.12, p<0.0001)(Figure 3A), but had no effect on Q_0 (two-way ANOVA, treatment $F_{(1,38)}$=4.03, p=0.052)(Figure 3B). Thus, oxytocin greatly reduces the motivation to seek meth, but does not affect consumption at low effort. Rats were then extinguished and oxytocin was similarly tested on cue-induced reinstatement. Results showed that oxytocin decreased cue-induced reinstatement in both sexes (two-way ANOVA, treatment main effect, $F_{(1,42)}$=41.05, p<0.0001)(Figure 3C). There were no effects of sex for any measure.

In the next set of experiments, we examined mechanisms by which oxytocin decreased meth demand and seeking. Because the preceding experiments revealed no sex differences with oxytocin in either demand or reinstatement of meth seeking, the subsequent studies were conducted in male rats.

Oxytocin antagonist ICV blocks the effects of systemic oxytocin

Oxytocin administered systemically has been presumed to attenuate drug seeking via a central mechanism; however, this has not been previously demonstrated. To directly test this, responding by males was stabilized on the within-session BE paradigm. On subsequent sessions, rats received unilateral microinfusions of OXA (2 μg/1 μl) or aCSF vehicle into a lateral ventricle immediately followed by systemic oxytocin (1 mg/kg, i.p.) or saline; 30 min prior to BE testing.

Overall, ICV OXA blocked the ability of systemic oxytocin to decrease motivation (increase in α) for meth in the within-session BE paradigm (repeated measures ANOVA, $F_{(3,15)}$=20.57, p<0.0001)(Figure 4A). Specifically, systemic oxytocin significantly increased α (Tukey post hoc: aCSF/sal vs. aCSF/oxy, p<0.0001), and ICV OXA blocked this effect (Tukey: aCSF/oxy vs. OXA/oxy, p<0.001). There was no effect of ICV OXA in the absence of systemic oxytocin (Tukey: aCSF/sal vs. OXA/sal, p>0.05). There were no significant effects of any treatment on Q_0 (p>0.05)(Figure 4B). The ability of ICV OXA to prevent systemic oxytocin effects indicates that systemic oxytocin acts centrally to reduce meth seeking.

Systemic oxytocin decreases neuronal activation in NAc core

Oxytocin in NAc modulates reward behaviors (4, 8, 9, 13, 17, 36–41, 46). Thus, we examined whether oxytocin would reduce Fos induction in NAc core during cue-induced reinstatement. Rats were pretreated with oxytocin (1 mg/kg, i.p.) or saline 30 min prior to a final test of either extinction or cue-induced reinstatement. Compared to extinction, cue-induced reinstatement increased active lever presses (one-way ANOVA, $F_{(2,16)}$=20.82, p<0.0001; Tukey: EXT vs CUE SAL, p<0.0001), and oxytocin attenuated reinstatement of meth seeking (Tukey: CUE SAL vs. CUE OXY, p<0.001)(Figure 5A).

Rats were sacrificed immediately after the 2-hr reinstatement session to examine neuronal activation in NAc core via Fos immunohistochemistry. Rats given systemic oxytocin had fewer Fos-activated cells after cue-induced reinstatement as compared to saline controls (one-way ANOVA: $F_{(2, 16)}$=4.076, p=0.037; Tukey: CUE SAL vs. CUE OXY, p<0.05) (Figure 5B).

Oxytocin infused into the NAc decreases meth seeking

To test whether oxytocin acts directly in the NAc to produce these behavioral effects, rats received counterbalanced microinfusions into NAc core of either oxytocin (0.6 μg/μl) or aCSF 10 min prior to being tested on the within-session BE paradigm or cue-induced reinstatement of extinguished meth seeking (Figure 6). Compared to aCSF, oxytocin microinjected into NAc increased α (t_{11}=2.274, p=0.04) but did not Q_0 (t_{11}=0.468, p=0.07). Oxytocin compared to aCSF in NAc also decreased cue-induced reinstatement bdhavior (t_{10}=2.940, p=0.015). Overall, intra-NAc oxytocin decreased meth demand and seeking in a manner highly similar to systemic oxytocin.

The effects of systemic oxytocin depend on actions in NAc

After stabilization on the BE paradigm, an additional group of rats received bilateral microinfusions of OXA (1 μg/side) or aCSF into NAc immediately followed by systemic oxytocin (1 mg/kg) or saline, 30 min prior to BE testing. Systemic oxytocin following intra-NAc aCSF significantly increased α (repeated measures ANOVA, $F_{(3,21)}$=6.690, p=0.002; Tukey post hoc: aCSF/sal vs. aCSF/oxy, p<0.05)(Figure 7A). However, OXA infusion into NAc immediately prior to systemic oxytocin blocked the oxytocin-induced decrease in demand for meth (α)(Tukey: aCSF/oxy vs. OXA/oxy, p<0.01). OXA had no effects on BE parameters when administered into NAc in the absence of systemic oxytocin (α, Q_0)(Tukey: aCSF/sal vs. OXA/sal, p>0.05). A repeated measures one-way ANOVA showed a significant

difference between group means for Q_0 ($F_{(3,21)}$=5.84, p=0.04)(Fig 7B); however, post hoc comparisons did not reveal any differences between groups (Tukeys, p>0.05) for all comparisons.

A separate group of 5 animals with placements outside of NAc were analyzed to serve as anatomical controls. Four were unilaterally only in NAc, with the contralateral cannula dorsal or lateral to the NAc for 3 rats; one rat was only injected unilaterally in the NAc. Both cannulae for the remaining subject were located rostral to NAc. Consistent with the above results, systemic oxytocin following microinfusion of aCSF significantly increased α (repeated measures ANOVA, $F_{(3,9)}$=4.66, p=0.03; Holm-Sidak post hoc: aCSF/sal vs. aCSF/oxy, p<0.05). However, in this group, OXA microinfusion outside of NAc immediately prior to systemic oxytocin did not block the oxytocin-induced decrease in demand for meth (α) (Holm-Sidak: aCSF/oxy vs. OXA/oxy, p>0.05). Additionally, oxytocin infused outside of NAc had no effect on demand compared to aCSF (t_3=0.84, p=0.46).

Discussion

Using a combination of economic demand curve analysis plus direct neural circuitry assessment, we found that oxytocin has promise as a pharmacotherapy for meth abuse via modulation of oxytocin receptor signaling in NAc. We developed a within-session BE paradigm that measures demand for meth and predicts reinstatement behaviors in both sexes. Although females had a greater demand for meth, systemic oxytocin attenuated meth demand and seeking similarly in both sexes. Importantly, oxytocin had the greatest effects in rats with the highest motivation (lowest demand elasticity, α) for meth, regardless of sex, indicating that it may be an effective pharmacotherapy in both males and females. We also found that systemic oxytocin acted primarily through a central mechanism to decrease meth demand and seeking, and that NAc was both necessary and sufficient for oxytocin to attenuate meth demand.

This newly adapted paradigm for meth allows for assessment of important economic variables within one session, which have previously only been assessed for drugs with a short half-life (i.e., cocaine (25, 34, 35) or remifentanil (47)). In both the multi-day and within-session BE paradigms, we confirmed previous findings that females take more meth than males during both low effort (e.g. FR1 schedule)(44) and high effort paradigms (e.g., PR schedule)(43, 48). Similar to what has been observed in both experimental animals (16, 31, 32, 47) and humans (28, 49), we found that demand elasticity (α) assessed with our within-session BE model accurately predicted meth seeking behaviors in both sexes. Specifically, α (but not Q_0) predicted meth seeking on the first day of extinction and during cue-induced reinstatement, which is analogous to what has been shown with cocaine (16).

Demand elasticity for meth predicted the efficacy of oxytocin to reduce cue-induced reinstatement of meth seeking in both sexes. Specifically, oxytocin reduced reinstatement most in rats with the strongest motivation (lowest elasticity, α) for meth. This phenomenon has also been observed for cocaine demand with orexin antagonists (32). Interestingly, studies in humans showed that economic demand in alcohol-dependent individuals predicted treatment outcomes (50), and pharmacological treatment of nicotine dependence both

increased α and the time to relapse (51). As α measured in a within-threshold procedure has predictive validity for addiction-like behavior in rats (16), studies in human psychostimulant addiction may also reveal that a similar procedure has predictive validity and serve as an indicator of those most susceptible to developing an addiction phenotype.

This BE paradigm demonstrated that oxytocin has similar efficacy to decrease meth self-administration in both sexes. This differs from previous studies assessing various social and sexual behaviors, which found that oxytocin has differential effects on males and females (4, 17, 36, 37, 52). However, these sex differences may be specific to social and sexual behaviors, as males and females have unique roles in these behaviors. Nevertheless, this does not preclude the possibility of sex differences in the effects of oxytocin on addiction-like behaviors for other drugs or in human addicts.

Our BE paradigm normalizes meth demand to independently measure demand elasticity (motivation; α) and free consumption (Q_0) within the same session (25). The normalization of α is important, as it provides a measure of motivation that is not confounded by amount of drug consumed at low effort (Q_0; which could change with tolerance or sensitization). We found that oxytocin reduced motivation (increased α) in both males and females, but did not affect free consumption (Q_0) in either sex. This result differs from our previous finding that oxytocin decreased meth seeking in males but not females during a progressive ratio (PR) schedule of reinforcement (43). However, meth intake is high at the start of the PR test and progressively decreases across the course of the session; the high blood levels of meth at the beginning of the PR session may confound subsequent responding for meth and measurement of motivation to seek meth.

Previous studies showed that systemic oxytocin decreases addiction-like behaviors in several drugs (1, 3, 5, 12–16, 43). Here, we showed that systemic oxytocin acts centrally to attenuate meth demand and seeking, as an ICV infusion of an oxytocin receptor antagonist (OXA) completely blocked these effects. A previous study reported that ICV OXA blocked sniffing behavior induced by an acute cocaine injection (53), indicating a central action of systemic oxytocin, but it was unclear whether this finding would extend to meth and/or seeking behavior after chronic self-administration.

It is unclear how systemic oxytocin activates central oxytocin neurons, as negligible amounts of peripherally-administered oxytocin cross the blood brain barrier (54–56) and the half-life of oxytocin in the periphery is only minutes (55). However, systemically administered oxytocin activates oxytocin neurons in the paraventricular nucleus (PVN)(46), and oxytocin neuronal activation results in local dendritic release of oxytocin and a subsequent positive-feedback effect that allows for prolonged activation of the oxytocin system (57, 58) and oxytocin release throughout the brain. It has been hypothesized that systemic oxytocin may stimulate the release of central oxytocin in the PVN via vagus nerve stimulation (59), as the nucleus of the solitary tract (which receives vagal afferents) has a direct projection to PVN (60). Alternatively, oxytocin could enter the brain via circumventricular organs, as these regions lack a blood brain barrier. Further studies are needed to examine the mechanisms by which systemically administered oxytocin decreases meth seeking.

Several studies have examined the effects of oxytocin in NAc on reward and drug related behaviors (4, 8, 9, 13, 17, 37, 41). We found that oxytocin robustly decreased meth demand and seeking, and also decreased the number of Fos+ neurons in NAc core during reinstatement behavior. Our results in an animal model of relapse expand on previous work that showed oxytocin decreased Fos induced by an acute injection of meth (46).

In previous studies, oxytocin infused into NAc core blocked conditioned place preference for meth (41) and decreased meth-primed reinstatement (13). Here, we demonstrated that intra-NAc core microinfusions of oxytocin increased demand elasticity (reduced motivation) and reduced cue-induced reinstatement of meth seeking. Importantly, intra-NAc core oxytocin attenuated meth demand and seeking in a manner similar to systemic treatment, indicating a common mechanism. As a final assessment, we observed that OXA infused into NAc blocked the ability of systemic oxytocin to increase meth demand elasticity. Although previous studies have proposed that NAc is a key region where oxytocin acts to reduce drug seeking (13, 41, 46), the current study is the first to demonstrate that oxytocin in NAc is both necessary and sufficient to reduce meth demand and seeking.

As oxytocin plays a distinct role in the modulation of neural circuits involved in stress and social behaviors (2, 3), it has been hypothesized that oxytocin may also decrease addiction behaviors through these mechanisms. Oxytocin has recently been shown to decrease anxiety triggered by cue-induced reinstatement of cocaine seeking (15). Thus, as stress is a major contributor to drug relapse (61, 62), the anxiolytic effects of oxytocin (63, 64) may help prevent relapse. Also, oxytocin facilitates social behavior, and it has been suggested that increased social interaction in human drug addicts (e.g., group treatment programs) may contribute to reduced addiction behavior (3). A recent study showed that oxytocin mediates social reward through actions in the NAc core (38). Similarly, we showed that the reduction of meth seeking by systemic oxytocin depends upon NAc. Thus, a significant overlap may exist for the mechanisms that modulate social reward and addiction behaviors (17).

Although we only included rats with bilateral cannula placements in NAc core (Supplemental Figure 3) and employed infusion parameters used previously for this region (65), oxytocin or antagonist infusions may have spread to the NAc shell. Thus, our findings indicate that effects seen here primarily involve oxytocin signaling in the NAc core, however, we cannot rule out potential contributions of NAc shell. Additionally, our analysis showing lack of effect of microinjections outside of NAc strengthens the evidence for NAc-mediated effects.

The molecular site of action for oxytocin in attenuating meth seeking is unknown. Dolen et al. (2013) found that postsynaptic oxytocin receptors in mice are found in NAc only on parvalbumin-positive (Parv+) interneurons and glia (GFAP+)(38). Therefore, oxytocin may act on Parv+ interneurons, and as oxytocin receptors are Gq-coupled such activation would be expected to inhibit GABAergic medium spiny neurons. Selective inhibition of neurons in the NAc core (which is composed of >90% medium spiny neurons) can decrease cocaine seeking (66). Additionally, oxytocin may also act on astrocytes (GFAP+ glia cells) in the NAc core. These astrocytes contribute extensively to glutamate regulation, which is known to be disrupted after drug self-administration and during reinstatement (67). Thus, oxytocin

may increase glial glutamate release and restore glutamatergic tone, a mechanism that has been shown to decrease reinstatement of cocaine seeking of rats (68).

Although a clear synaptic mechanism remains to be determined, it is evident that oxytocin acts within the NAc to reduce meth demand and seeking. Future studies are needed to determine if oxytocin attenuates meth demand and seeking via a pre- or postsynaptic mechanism in NAc, and if these effects extend to other addictive drugs. Overall, these data indicate that BE paradigms may help predict the efficacy of pharmacotherapies and support development of oxytocin-based therapies for meth addiction in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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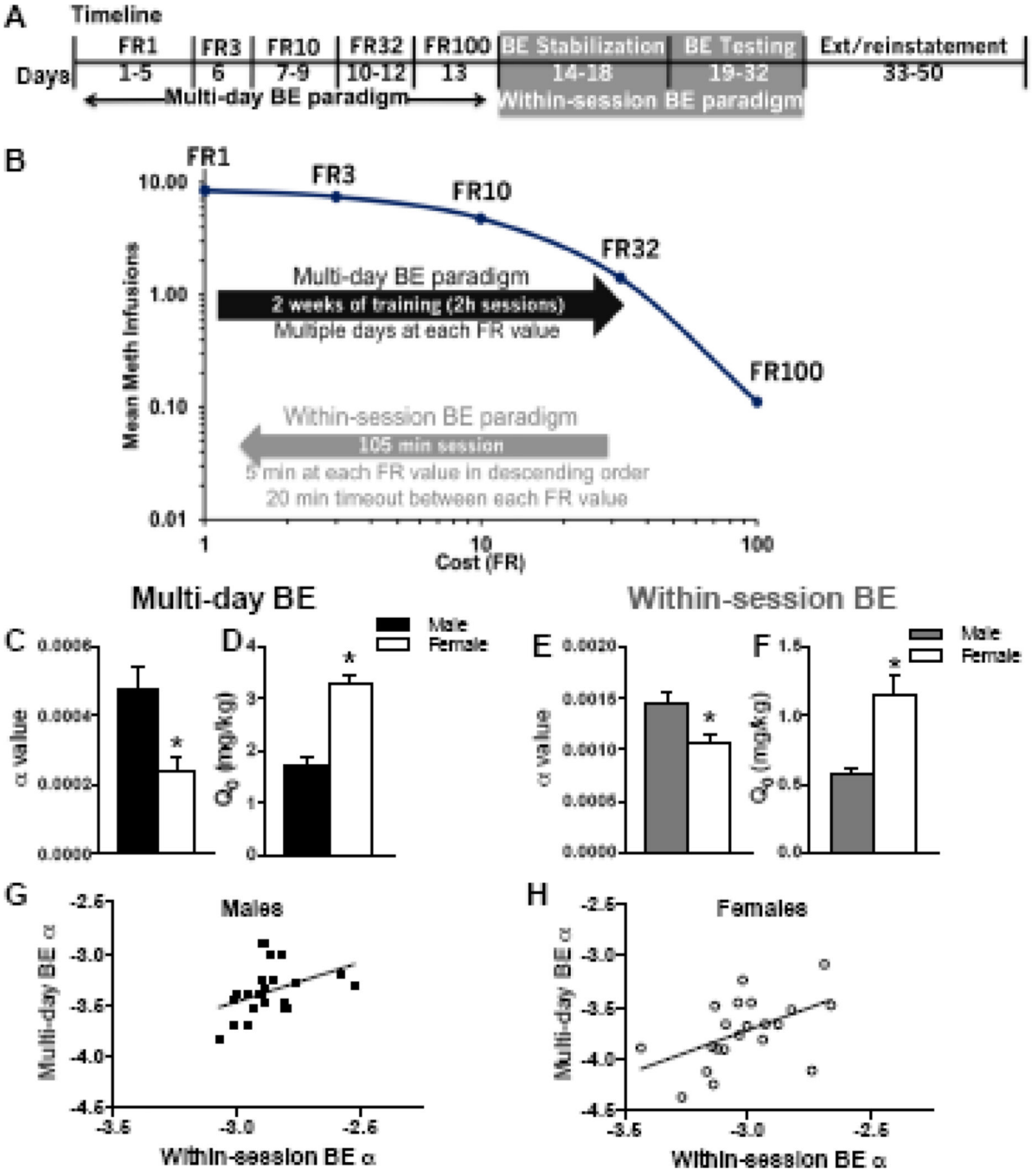

Figure 1. Validation of within-session BE paradigm

Male and female rats were trained on a traditional BE paradigm (multiple days at each fixed ratio (FR) value) and subsequently stabilized on the within-session BE paradigm. Meth demand (α) and preferred levels of intake (Q_0) were assessed from both paradigms to test whether individual variability in these variables predicted reinstatement behavior. A&B) Timeline and description of the behavioral paradigm. C&D) α and Q_0 values for males and females during the multi-day BE paradigm. E&F) α and Q_0 values during the within-session BE paradigm. In both paradigms, females (grey bars) had lower α values (higher motivation)

and higher Q_0 values (higher free consumption) than males (black bars) (*$p<0.05$). G&H) Demand elasticity (α) calculated from the traditional BE paradigm correlated significantly with the same variables assessed during the within-session BE paradigm in both sexes (males $r=0.44$, $p<0.05$; Females $r=0.51$, $p<0.05$; for all data: males: $n=22$; females $n=21$).

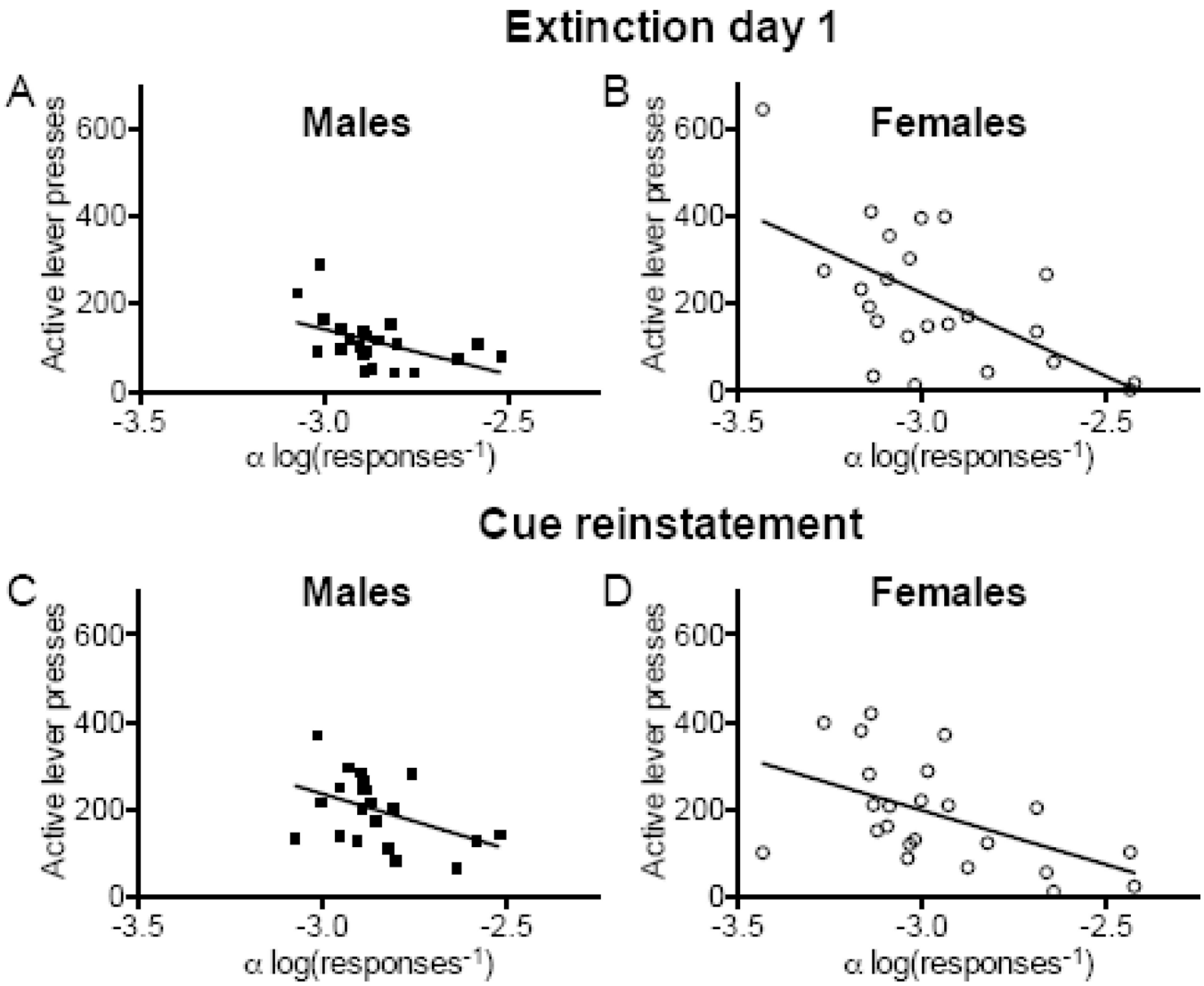

Figure 2. Demand elasticity predicts meth seeking behaviors

A&B) Demand elasticity predicts meth seeking in both males (panel A) and females (panel B) on the first day of extinction (males: $r = -0.49$, $p<0.05$; females: $r = -0.51$, $p<0.05$). C&D) Following extinction, α predicted cue-induced reinstatement of meth seeking in both sexes (males: $r = -0.56$, $p<0.05$; females: $r = -0.44$, $p<0.05$). For all data: males n=22; females n=21.

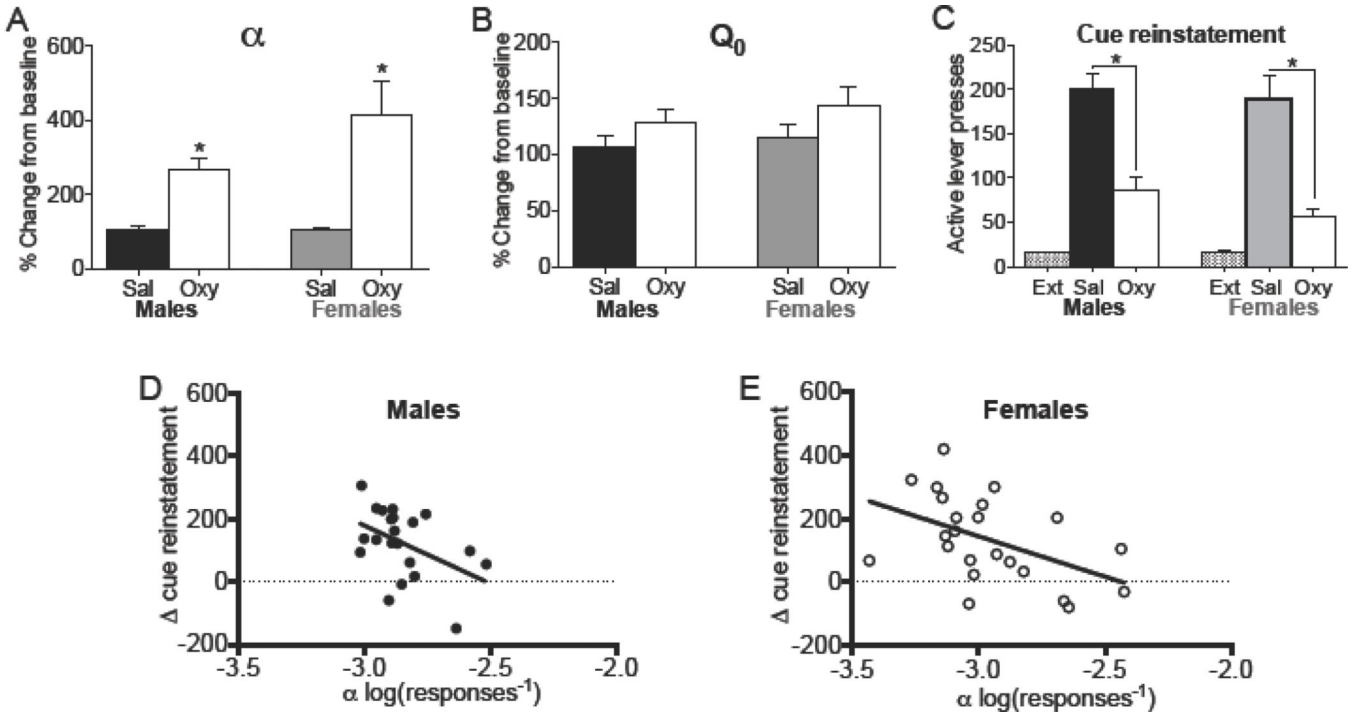

Figure 3. Oxytocin decreases meth seeking similarly in both sexes

Male and female rats were stabilized on the within-session BE paradigm to generate baseline demand elasticity (α) and free consumption (Q0) values for meth, and then these were were measured again after vehicle (saline, sal) or oxytocin administration (oxy, 1 mg/kg i.p.). Subsequently, responding was extinguished and tested on cue-induced reinstatement of meth seeking after vehicle or oxytocin administration. For A–C, significant treatment effects are noted, *p<0.05. A) Percent change in α from baseline after vehicle or oxytocin administration. In both sexes (male n=22, female n=21), oxytocin robustly increased α (decreased motivation). B) Percent change in Q_0 from baseline after vehicle or oxytocin administration. Oxytocin did not significantly affect Q_0 in either sex (male n=22, female n=21). C) Number of active lever presses during cue-induced reinstatement of meth seeking after vehicle or oxytocin administration. Oxytocin decreased cue-induced reinstatement responding in both sexes (male n=21, female n=23). Overall, a two-way ANOVA showed no effects of sex in any of these measures. D&E) Baseline demand elasticity (α) predicted the efficacy of oxytocin to reduce cue-induced reinstatement (change in cue reinstatement from saline test) in both sexes (males n=21: r= −0.45, p<0.05; female n=20: −0.50, p<0.05).

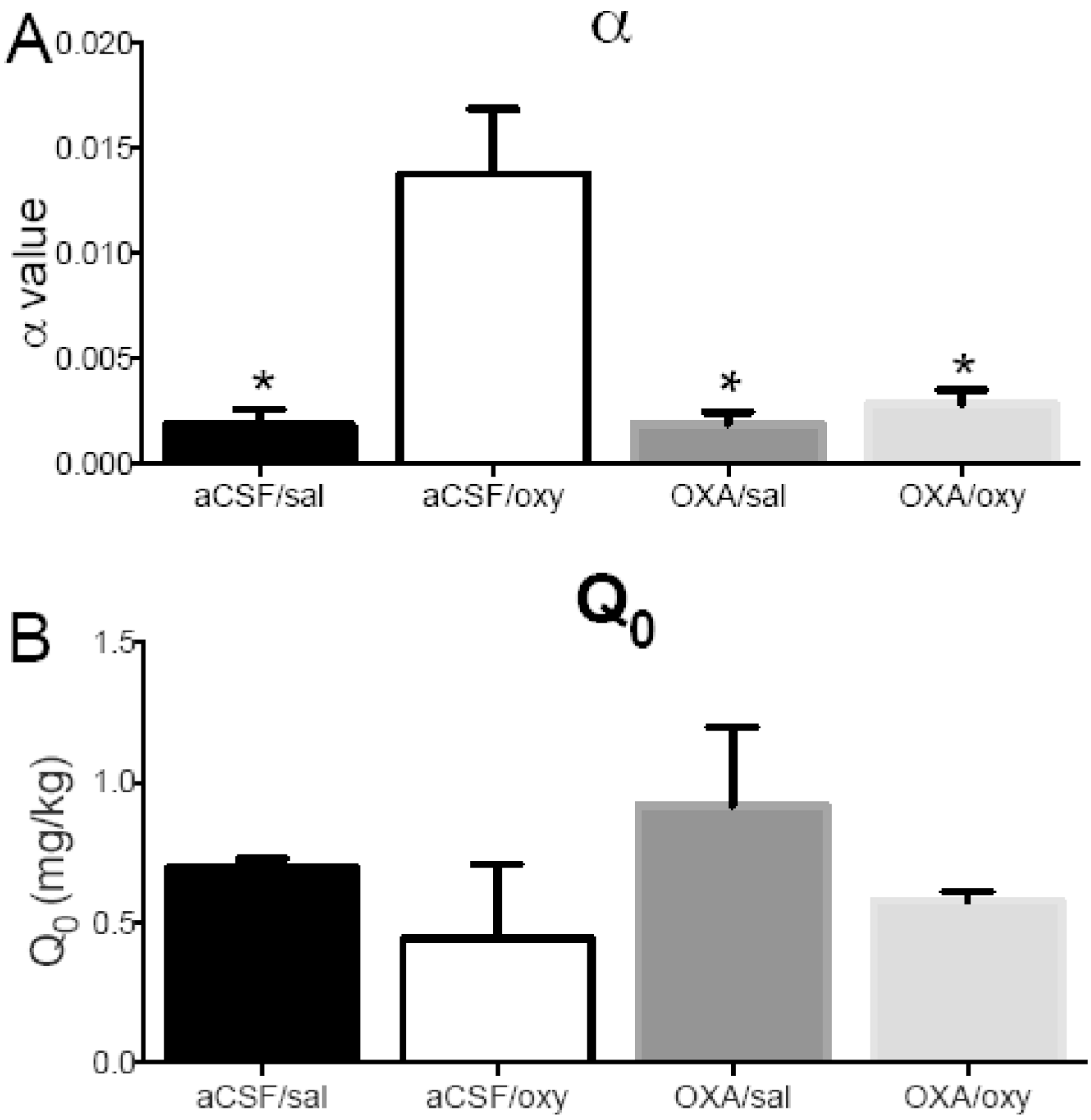

Figure 4. Central oxytocin antagonist blocks systemic oxytocin effects on meth demand

Rats stabilized on the within-session BE paradigm for meth were tested with either ICV microinfusions of OXA or aCSF immediately followed by administration of i.p. oxytocin or saline in a counterbalanced manner. A) α values assessed during the within-session BE paradigm. Systemic oxytocin (aCSF/oxy, white bar) increased α (decreased motivation) compared to controls (aCSF/sal, black bar). ICV administration of OXA blocked this effect of systemic oxytocin (OXA/oxy, light grey bar), although it did not affect behavior when administered with i.p. saline vehicle (OXA/sal, dark grey bar). *p<0.05 compared with

aCSF/oxy. B) Q_0 values assessed during the within-session BE paradigm. There were no effects on Q_0 for any of the treatments.

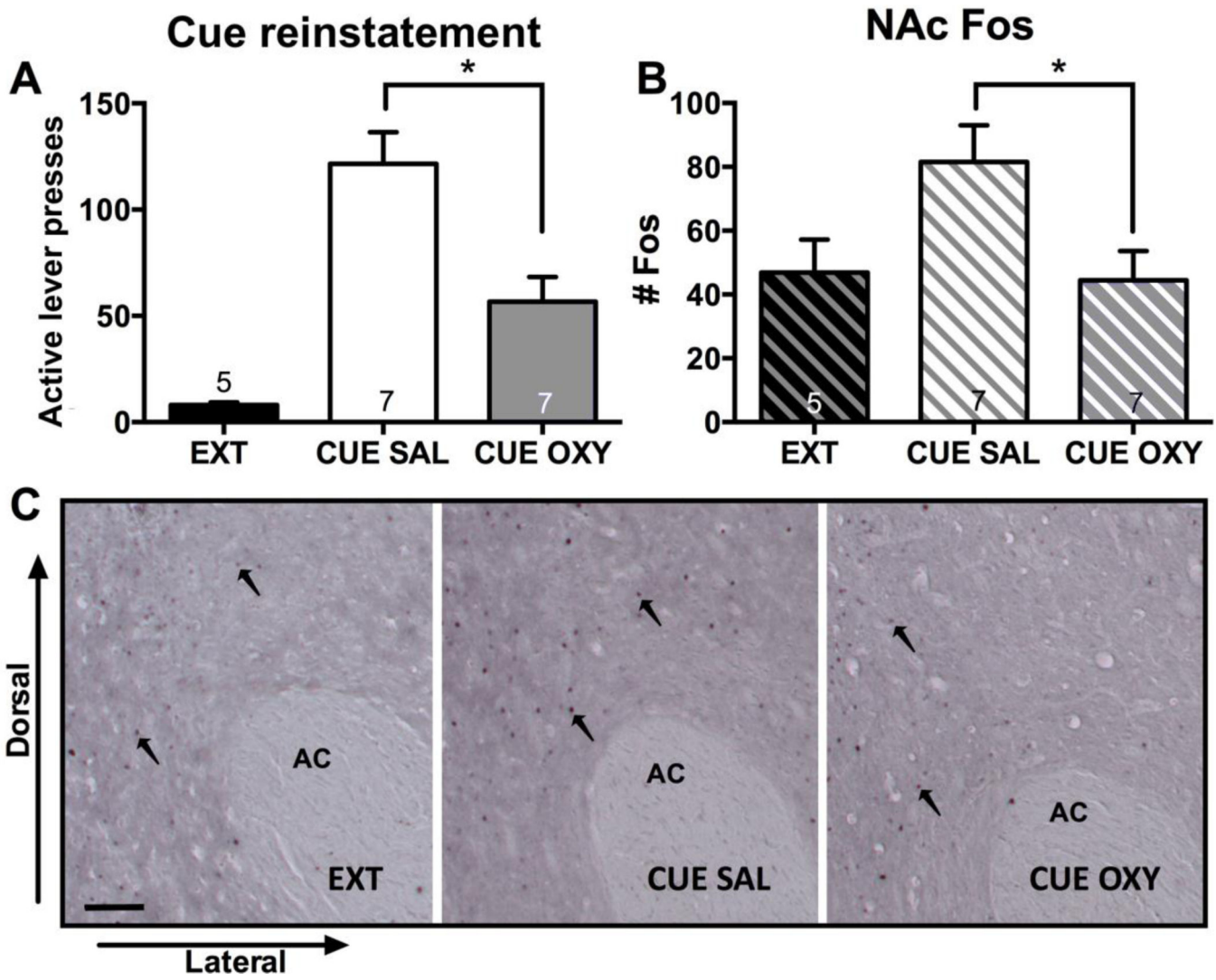

Figure 5. Oxytocin decreases reinstatement-induced Fos in NAc core

A) Number of active lever presses during extinction (EXT, black bar) or cue-induced reinstatement of meth seeking with pretreatment of saline (CUE SAL, white bar) or oxytocin (1 mg/kg i.p.; CUE OXY, grey bar). Oxytocin attenuated cue-induced reinstatement of meth seeking (*p<0.05). B) Rats were sacrificed immediately after the session to examine Fos expression in the NAc core after extinction (black hashed bar), cue-induced reinstatement with saline (white hashed bar), or with oxytocin (grey hashed bar). Rats treated with oxytocin prior to cue-induced reinstatement had fewer Fos-positive neurons in the NAc core than saline controls (one way ANOVA *p<0.05). C) Example of Fos staining in each treatment group (AC: anterior commissure; scale bar= 100 μm)

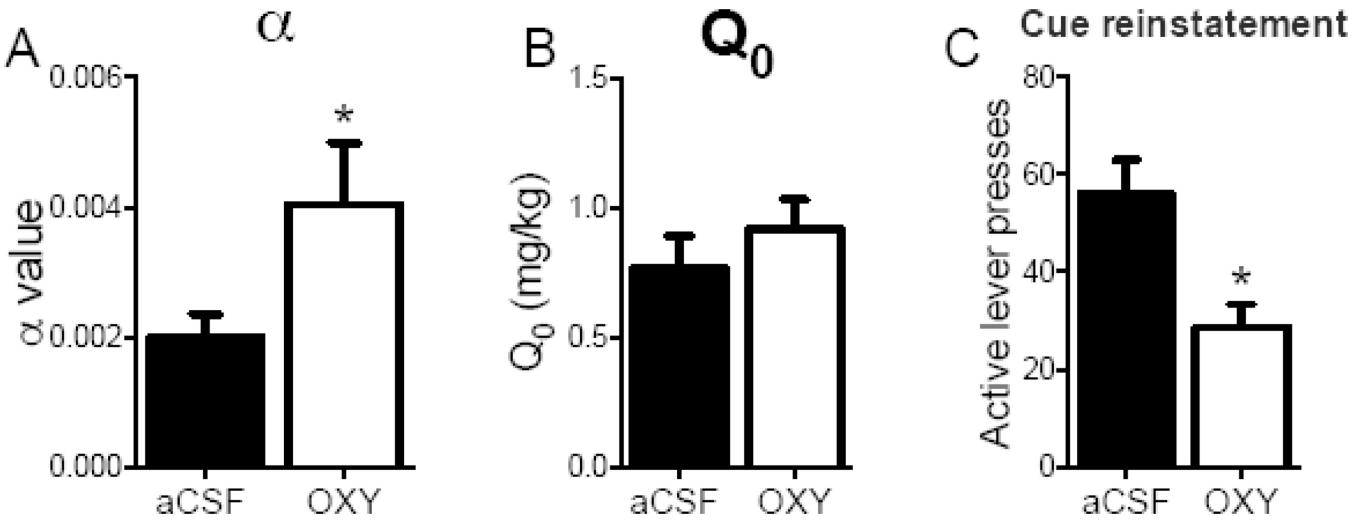

Figure 6. Microinfusions of oxytocin into NAc core decreases meth demand and seeking

A) Demand elasticity (α) was increased (decreased motivation) following oxytocin microinfusions into NAc as compared to aCSF in NAc. B) Oxytocin microinfusions in NAc had no effect on Q_0 compared to aCSF in NAc. C) Oxytocin in NAc decreased the number of active lever presses during cue-induced reinstatement of meth seeking (*p<0.05).

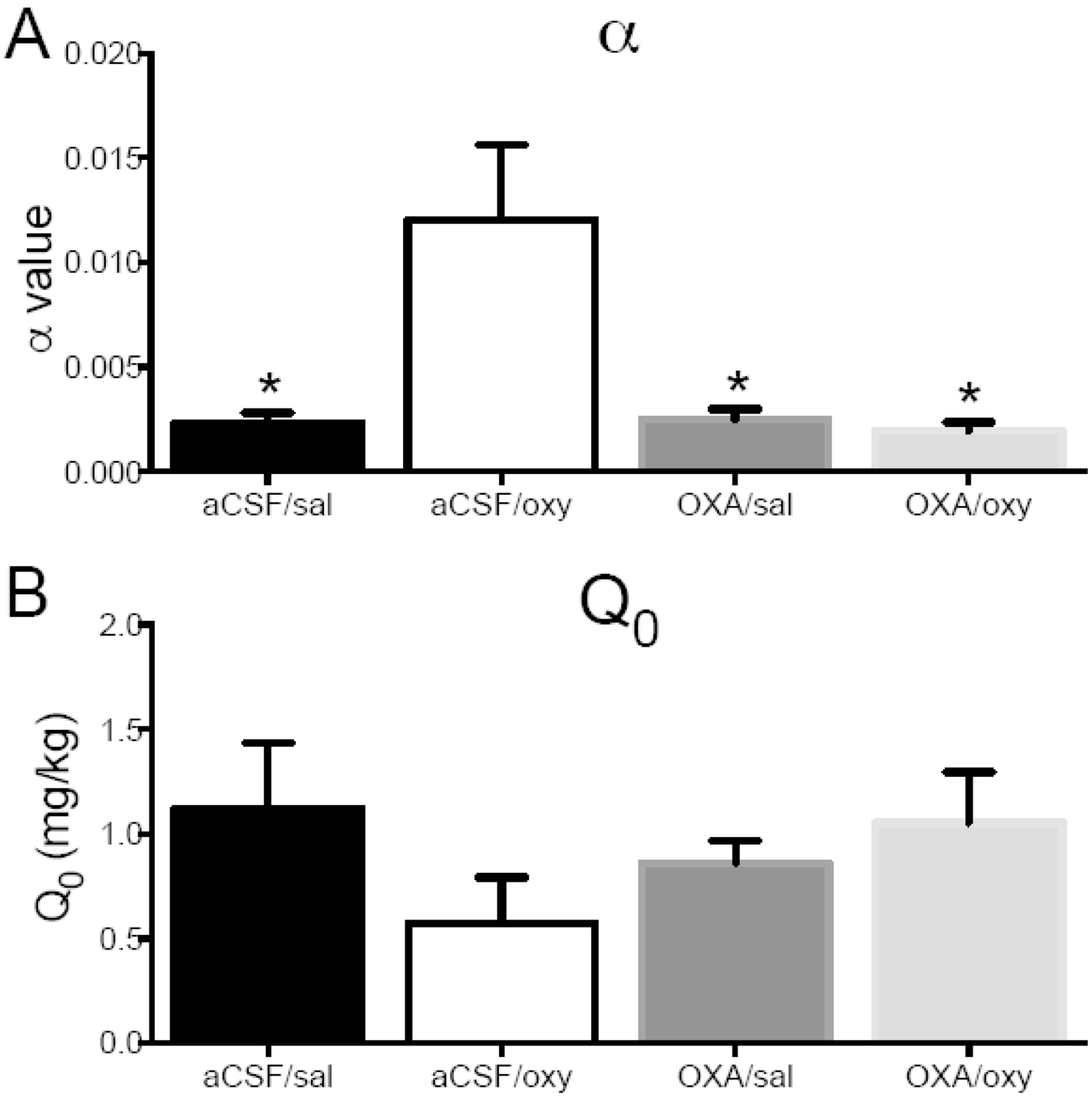

Figure 7. Oxytocin antagonist infused into NAc core blocks effect of systemic oxytocin on meth demand

Rats stabilized on the within-session BE paradigm were tested with either microinfusions of OXA or aCSF into the NAc core immediately followed by i.p. administration of oxytocin or saline in a counterbalanced manner. A) α values assessed during the within-session BE paradigm. Systemic oxytocin (aCSF/oxy, white bar) increased α (decreased motivation) compared to controls (aCSF/sal, black bar). Intra-NAc administration of OXA blocked this effect of systemic oxytocin (OXA/oxy, light grey bar), although it did not affect behavior when administered with systemic saline vehicle (OXA/sal, dark grey bar). *p<0.05

compared with aCSF/oxy. B) Q_0 values assessed during the within-session BE paradigm. There were no significant effects on Q_0.