

Interactions Between Social and Drug Reward on Stimulated Dopamine Release
in Male Prairie Voles

Curtis J. Austin

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Advisor: Dr. Brandon Aragona

Abstract

Positive social influences have been shown to provide a buffer against the reinforcing properties of drugs of abuse. Importantly, the neuroprotective effects of one type of positive social influence, selective attachment formation, can be studied in the socially monogamous prairie vole. Prairie voles form enduring pair bonds to their mating partner, which is linked to neuroplastic changes within reward processing regions of the brain. Specifically, there is an up-regulation of D1-like dopamine (DA) receptors in the nucleus accumbens (NAc) following pair bond formation and activation of these receptors increases dynorphin, the endogenous ligand for kappa-opioid receptors (KORs). Importantly, activation of these receptors decreases extracellular levels of DA. Given that DA transmission is a critical component of reward processing, an increase in D1-like DA receptor binding density after pair bond formation may act to decrease the rewarding properties of abused drugs through activation of the dynorphin/KOR system. To determine if exposure to amphetamine (AMPH) results in less DA release in pair bonded prairie voles compared to sexually naïve voles, we measured stimulated DA release in striatal slice preparations. In both groups, we show exposure to AMPH doubled the amount of DA released within the NAc shell compared to saline treated subjects. A similar increase in stimulated DA release in the nucleus NAc shell of sexually naïve and pair bonded prairie voles was surprising, but may be due to the fact that AMPH was administered in the subjects' home cage. Thus, similar experiments need to be conducted with the injections occurring in a novel environment, since this paradigm has been shown to increase behavioral sensitization to psychostimulants in rats.

Keywords: nucleus accumbens, dopamine, reward, prairie vole, amphetamine, kappa-opioid receptors, voltammetry, pair bond

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Although drugs of abuse have powerful reinforcing properties, only a small subset of the population that experiments with drugs of abuse actually becomes addicted (Anthony, Warner, & Kessler, 1994; Saunders & Robinson, 2013). Some individuals seem to be resilient to the addictive properties of drugs of abuse and recent data suggest that this resilience may in part be related to the quality of one's social world. Specifically, strong social bonds buffer against drug reward (Doremus-Fitzwater, Varlinskaya, & Spear, 2010; Kennedy, Panskepp, Runckel, & Lahvis, 2012; Young, Gobrogge, & Wang, 2011; Young, Liu, & Wang, 2008), while weak social ties or negative social environments can increase the risk for developing a psychological disorder, such as addiction (Bond, Butler, Thomas, Carlin, Glover, ... & Patton, 2007; Hamme Peterson, Buser, & Westburg, 2010; Koob & Le Moal, 2001; Lutz & Kieffer, 2013b). Given the devastating long-term effects that addiction imparts to both the affected individual and society, understanding the neurobiological underpinnings of socially induced attenuation of drug reward is crucial to reducing the prevalence of the disease.

An emerging animal model for investigating the neurological mechanism of social attachment in relation to drug reward is the socially monogamous prairie vole (*Microtus ochrogaster*) (Getz, Carter, & Gavish, 1981; Kenkel, Paredes, Yee, Pournajafi-Nazarloo, Bales, & Carter, 2012; McGraw & Young, 2009; Young, Gobrogge, Liu, & Wang, 2011). Unlike most mammalian species, prairie voles form enduring pair bonds to their mating partner (Carter & Getz, 1993; Kleiman, 1977) and the formation of a pair bond has recently been shown to protect against the reinforcing properties of drugs of abuse such as amphetamine (AMPH) (Liu, Aragona, Young, Dietz, Kabbaj, & Mazel-Robison, 2010). Thus, studying the neurobiological

mechanisms underlying these protective effects of pair bonding has the potential to provide critical new insight into possible therapeutic targets of addiction.

Pair bond formation induces a dramatic reorganization of reward circuitry, and consequently, an alteration in reward processing (Aragona, Liu, Curtis, Stephan, & Wang, 2003; Liu, et al., 2010). Of particular interest to reward processing is the up-regulation of D1-like dopamine (DA) receptors in the nucleus accumbens (NAc), a brain region that is important for reward and motivated behavior (Aragona, et al., 2006; Liu, Young, Curtis, Aragona, & Wang, 2011; Robbins & Everitt, 1996). Importantly, activation of these receptors increases the release of dynorphin (Gerfen, McGinty, & Young, 1991), the endogenous ligand for kappa-opioid receptors (KORs) (Chavkin & James, 1982) and activation of these receptors decreases synaptic levels of DA, as well as attenuates reward processing (Chefer, Czyzyk, Bolan, Moron, Pintar, & Shippenberg, 2005). Perhaps most importantly, these receptors have recently been implicated in mediating pair bond maintenance (Resendez, Kuhnmench, Kryzvosinski, & Aragona, 2012) and therefore may play a critical role in reducing drug reward by decreasing the extracellular release of DA.

Striatal DA mediates the rewarding properties of all drugs of abuse (Sulzer, 2011), including AMPH (Daberkow, Brown, Bunner, Kraniotis, Doellman, & Ragozzino, 2013; Schmitz, Lee, Schmauss, Gonon, & Sulzer, 2001; Sonders, Zahniser, Kavanaugh, & Amara, 1997). More specifically, administration of AMPH induces robust increases of DA release within the striatum, but this increase preferentially occurs in the NAc shell (Pontieri, Tanda, & Di Chiara, 1995). Such a large increase in DA is sufficient to activate the low-affinity D1-like DA receptors and corresponding downstream signaling cascades that lead to a decrease in DA, such as the dynorphin/KOR system. Given that pair bonded prairie voles have an up-regulation

of these receptors within the NAc, it is possible that these signaling cascades are more easily activated in pair bonded prairie voles and therefore result in an attenuation of DA release and the reinforcing properties of AMPH in comparison to sexually naïve prairie voles. To determine if AMPH exposure results in differential levels of DA release within the striatum of prairie voles, we measured stimulated DA release in striatal slice preparations of sexually naïve and pair bonded prairie voles exposed to either saline or AMPH. We predict that compared to sexually naïve prairie voles, we would see less DA release in pair bonded animals, after exposure to a rewarding dose of AMPH.

Method

Subjects

Male prairie voles (80-110 days of age) bred at the University of Michigan were used for all experiments. Female prairie voles were used as stimulus animals. All animals were housed in a 14:10 LD cycle and given food and water *ad libitum*. Male subjects were divided into one of two groups: they either remained housed with their male cage mate (sibling housed group) or were housed with an intact female partner for two weeks prior to testing (paired group).

AMPH Injection Procedures

Sibling housed or paired males were randomly divided into two treatment groups: saline (control) or 1 mg/kg AMPH (drug). Saline or AMPH intraperitoneal injections were administered in the home cage of the test subject. Injections were given for three consecutive days, and testing began 24 hours following the final injection. This drug dosage and injection regimen were chosen because the administration of 1 mg/kg of AMPH for three consecutive days induces a reliable conditioned place preference in prairie voles, an indication that this injection regimen of AMPH is rewarding in this species (Liu, et al., 2010).

Electrode Fabrication and Calibration

Glass capillary tubes (1.2 mm in diameter) were filled with a single carbon fiber strand and placed in a Narishige (Model PE-22) heated-coil vertical puller to create a seal between the glass and carbon fiber. The carbon fiber was then cut using a scalpel under a microscope, at a length of 125-150 μm past the seal. Finally, the electrode was filled with an ionic solution of 0.15 M KCl, and a 30 gauge silver wire was inserted into the open end of the electrode.

Brain Slice Preparation

On the day of testing, animals were weighed then sacrificed via live and rapid decapitation. Brains were quickly extracted and submerged in ice-cold, pre-oxygenated high sucrose artificial cerebrospinal fluid (aCSF; 180 mM sucrose, 30 mM NaCl, 4.5 mM KCl, 1 mM MgCl_2 , 26 mM NaHCO_3 , 1.2 mM NaH_2PO_4 , 10 mM D-glucose in deionized H_2O , pH 7.4). Using a vibratome, the brain was sectioned into 400 μm thick coronal slices containing the dorsal and ventral striatum. Sections were immediately transferred to a holding chamber containing oxygenated aCSF (176.13 mM ascorbate, 180.16 mM D-glucose, 84.01 mM NaHCO_3 , 58.44 mM NaCl, 156 mM NaH_2PO_4 , 74.56 mM KCl, 147.01 mM CaCl_2 , and 203.30 mM MgCl_2 in deionized H_2O , pH 7.4) and incubated at room temperature for a minimum of one hour before testing.

For males in the pair bonded group, female partners were checked for pregnancy by examining the uterus. If pregnant, fetuses were removed and weighed. Females with an average fetus weight of > 0.30 g were considered optimally pregnant (Resendez et al., 2012).

Fast-scan Cyclic Voltammetry (FSCV)

Following incubation, the slice was transferred to the testing chamber perfused with oxygenated aCSF. A two-pronged Tungsten bipolar stimulating electrode was lowered onto the

surface of the striatum and the recording electrode was implanted into the slice approximately 100-200 μm away from the stimulating electrode, see Figure 1 A (Jones, Gainetdinov, Wightman, & Caron, 1998). The three regions of the striatum tested for this study were the dorsal striatum, the NAc core, and the NAc shell. These regions were chosen because they have been demonstrated to mediate reward (Pecina, Smith, & Berridge, 2006) as well as the neurobiology of pair bonding in prairie voles (Young et al., 2011).

Tarheel CV software (University of North Carolina; Chapel Hill, NC) written in LABVIEW (National Instruments; Austin, TX) was used to apply a triangular ramp to the carbon fiber electrode, from -0.4 V to +1.2 V and back at a frequency of 10 Hz. Every 5 minutes, a 15-second measurement of DA efflux was made. At 5 seconds into each recording, a depolarizing stimulation was applied to the slice, resulting in a synaptic increase in DA concentration (Figure 1 B,C). After three single pulse stimulations, the level of stimulation was increased to 5 pulses (at a frequency of 20 Hz) for an additional three readings, and finally 20 depolarizing pulses (at 20 Hz) for three more readings. Thus, nine readings were recorded from each region (three recordings at each of the three pulse levels). Following the completion of the experiment, the carbon fiber electrode was calibrated and standardized with a 3 μM DA solution.

Results

Previous studies in prairie voles have determined that administration of 1 mg/kg of AMPH for three consecutive days reliably induces a conditioned place preference (a behavioral indicator of reward) in sexually naïve, but not pair bonded prairie voles (Liu, et al., 2010). Moreover, the rewarding properties of AMPH have been attributed to robust increases of DA in the NAc shell, a region of the striatum that is important for both drug reward processing and pair bonding. We therefore hypothesized that exposure to rewarding levels of DA (three consecutive

daily injections of 1 mg/kg AMPH) would result in a much more robust increases in DA in the NAc shell of sexually naïve prairie voles compared to pair bonded voles (i.e., those that have mated with the female with whom they have been paired for two weeks).

Effects of AMPH Exposure in Sexually Naïve and Pair Bonded Subjects

We first compared stimulated DA release in the striatum of sexually naïve prairie voles that were treated with either saline or AMPH for three consecutive days. In sexually naïve males, exposure to AMPH significantly increased stimulated DA release within the NAc shell compared to saline controls, $F(2,18) = 6.79$, $p = 0.02$ (Figure 2 E,F), but post hoc test did not reveal any significant differences between specific pulses. There were no significant differences between saline and AMPH treated animals at higher pulse stimulations within the dorsal striatum, $F(2,27) = 3.23$, $p = 0.08$ (Figure 2 A,B) or NAc core, $F(2,21) = 0.82$, $p = 0.38$ (Figure 2 C,D). Therefore, exposure to AMPH robustly increases DA release within the NAc shell, but not the dorsal striatum or NAc core, of sexually naïve males.

We next compared stimulated DA release within the striatum of pair bonded prairie voles treated with either saline or AMPH. Although AMPH exposure also appeared to double stimulated DA release within the NAc shell of pair bonded prairie voles, there was no significant difference in DA release at any pulse stimulation within this region, $F(2,18) = 0.24$, $p = 0.63$ (Figure 3 E,F). There were also no significant differences in stimulated DA release within the dorsal striatum, $F(2,27) = 0.30$, $p = 0.59$ (Figure 3 A,B). In the NAc core, it appeared as though AMPH exposure resulted in a decrease in stimulated DA release, but this decrease was not significant, $F(2,18) = 2.72$, $p = 0.12$ (Figure 3 C,D). Overall, AMPH exposure increased DA release within the NAc shell, but decreased DA release within the NAc core of pair bonded prairie voles, although these differences were not significant.

Effects of Social Interaction on Stimulated DA Release

We next directly compared stimulated DA release between sexually naïve and pair bonded prairie voles that were exposed to either saline (control) or AMPH (drug treated). Previous research from our laboratory has determined that the establishment of a pair bond results in robust increases in stimulated DA release within the NAc shell of male and female prairie voles compared to sexually naïve prairie voles (unpublished data). Moreover, in males, this increase in DA release is correlated with the pregnancy status of the female, in that stimulated DA release is greater in males paired with females that became pregnant soon into the pairing period and were therefore farther along in gestation at the time of testing. Additionally, this increase in stimulated DA release is specific to the NAc shell as there is no difference in stimulated DA release between the dorsal striatum or NAc core between sexually naïve and paired males (unpublished data). Similar to previous findings from our laboratory, there was no difference in stimulated DA release following a one pulse stimulation in the dorsal striatum, $F(2,19) = 1.24$, $p = 0.28$ (Figure 4 A,D), or the NAc core, $F(2,19) = 1.24$, $p = 0.28$ (Figure 4 B,E) of sexually naïve and pair bonded prairie voles (Figure 4).

There was also no significant difference in stimulated DA release in the NAc shell $F(2,19) = 1.24$, $p = 0.28$ (Figure 4 C,F), of sexually naïve and pair bonded prairie voles, nor in the dorsal striatum or NAc core (Figure 4 G). This might seem surprising at first, but not upon inspection of the pregnancy status of the females. In the present study, fewer than half of the females, on average, were optimally pregnant (pregnancy occurring as soon as possible into the pairing period) with the remaining females having sub-optimal pregnancies (having a delay in pregnancy) or no pregnancy at all. Importantly, we have previously demonstrated that the robust increase in stimulated DA release only occurs in NAc shell of males that are paired with

optimally pregnant females, and there is no difference in stimulated DA release between sexually naïve males and males paired with sub-optimally pregnant females. Therefore, data from the present study are consistent with previous data from our lab in that increases in stimulated DA release in pair bonded males are directly related to the pregnancy status of the female. To further assess this relationship, we directly compared stimulated DA release following a one pulse depolarizing stimulation to the average weight of the fetus at testing. Although there was no significant relationship between stimulated DA release and neonatal weight within the dorsal striatum $R^2 = 0.39$, $p = 0.18$ (Figure 4 H), NAc core $R^2 = 0.62$, $p = 0.11$ (Figure 4 I), or NAc shell $R^2 = 0.78$, $p = 0.11$ (Figure 4 J), the correlation trend in the NAc shell was similar to previous findings from our lab. Importantly, the lack of significance in the correlation is likely due to low sample sizes in the present study.

Given that pair bonded prairie voles do not find AMPH rewarding, we hypothesized that exposure to AMPH would result in much lower levels in stimulated DA release in pair bonded animals treated with AMPH compared to sexually naïve treated animals. However, in contrast to our hypothesis, stimulated DA release following a one pulse depolarizing stimulation resulted in similar levels of peak DA release between sexually naïve and pair bonded prairie voles exposed to AMPH in all regions of the striatum $F(2,19) = 1.24$, $p = 0.28$ (Figure 5 A-G). In pair bonded prairie voles, we also looked at the relationship between stimulated DA release following a one pulse depolarizing stimulation and neonatal weight within all regions in the striatum. There was no relationship between stimulated DA release and neonatal weight within the dorsal striatum, $R^2 = 0.08$, $p = 0.46$ (Figure 5 H), NAc core, $R^2 = 0.19$, $p = 0.33$ (Figure 5 I), or NAc shell, $R^2 = 0.15$, $p = 0.51$ (Figure 5 J). This finding is consistent with recent data from our lab demonstrating that pair bonded males whose females are either sub-optimally or optimally pregnant both do not

find AMPH rewarding (unpublished data). In addition to a lack of a significant difference in 1 pulse DA release within all regions of the striatum, there was no difference in stimulated DA release at higher pulses within the dorsal striatum, $F(2,27) = 0.30$, $p = 0.59$ (Figure 6 A,B), NAc core, $F(2,18) = 2.72$, $p = 0.12$ (Figure 6 C,D), or NAc shell, $F(2,18) = 0.24$, $p = 0.63$ (Figure 6 E,F) between sexually naïve and pair bonded prairie voles that were treated with AMPH. Finally, it is important to mention that for the AMPH group, like the saline group, there was an extremely low rate of optimal pregnancy. Fewer than half of the females in each group were optimally pregnant, indicating a delay or absence of mating which suggests those males did not form a true pair bond (Resendez et al., 2012).

Although many of the results presented above were not significant, we believe the overall trends in the data provide critical insight into how AMPH exposure alters reward processing regions of the brain in sexually naïve and pair bonded prairie voles. Importantly, these data are preliminary and we believe the addition of more animals to the study will yield more significant findings. Moreover, the lack of the expected difference between stimulated DA release in sexually naïve and pair bonded prairie voles may be due to the fact that AMPH was administered in the home cage of all subjects.

Discussion

The present study is the first to examine the effects of AMPH administration on stimulated DA release throughout the striatum of sexually naïve and pair bonded prairie voles. Although the findings presented here are preliminary, they provide critical insight into how AMPH exposure differentially regulates reward-processing regions of the brain in sexually naïve and pair bonded prairie voles. Specifically, we showed that AMPH increases stimulated DA release in the NAc shell of both pair bonded and sexually naïve males. However, stimulated DA

release was attenuated in the NAc core of AMPH exposed pair bonded males, but not sexually naive males. Finally, AMPH exposure had no effect in stimulated DA release in the dorsal striatum of sexually naive or pair bonded voles. Thus, data from the present study provides the first insight into how AMPH experience in the home cage environment of prairie voles impacts three critical regions of motivational circuitry that are important for reward processing.

Following the formation of a pair bond, the rewarding properties of AMPH are attenuated and this attenuation is partially controlled by an up-regulation of D1-like DA receptors within the NAc shell. Importantly, activation of these receptors increases dynorphin release, the endogenous ligand for KORs. Given that activation of KORs (which are also important for pair bond maintenance) decreases DA release and attenuates reward processing, we hypothesized that pair bonded prairie voles would have lower DA release within the NAc shell following AMPH exposure. However, our study found that pair bonded prairie voles exposed to AMPH had almost double the amount of DA release compared to pair bonded prairie voles treated with saline. Additionally, and perhaps most importantly, there was no difference in stimulated DA release between sexually naive and pair bonded prairie voles that were treated with AMPH. This is again likely a result of the low rates of optimal pregnancy, which suggests the males in those groups did not completely form a pair bond, and therefore may be neurobiologically comparable to sexually naïve prairie voles.

Another possible explanation for a lack of a difference in stimulated DA release between sexually naïve prairie voles, which normally find AMPH rewarding, and pair bonded prairie voles that do not find AMPH rewarding may be due to the fact that in the present study, AMPH was administered in the home cage of all the subjects and in the presence of their cage mate. Importantly, although social bonds can buffer the rewarding properties of drugs of abuse,

experience drugs in a social environment can also enhance drug reward (Lutz & Kieffer, 2013b). For example, same-sex pairs of prairie voles drink greater amounts of alcohol when housed with a social partner compared to voles given alcohol in isolation (Anacker, Loftis, Kaur, & Ryabinin, 2011). Moreover, in other rodent species, experiencing nicotine (Thiel, Sanabria, & Neisewander, 2009), cocaine (Acosta, Thiel, Sanabria, Browning, & Neisewander, 2008), or AMPH (Watanabe, 2011) with a social partner enhances the reward properties of all of these drugs. Together, these data suggest that social factors modify the reinforcing properties of abused drugs and data from the present study provides the first evidence that a neural site of interaction between AMPH and social reward may occur in the NAc shell.

In addition to social experience, environmental context also plays a critical role in drug reward processing (Badiani, Camp, & Robinson, 1997). Specifically, administration of AMPH in the home cage of rats resulted in a moderate increase in neural activation within the dorsal striatum, while administration of AMPH in a novel environments resulted in a robust increase in neural activation within this same brain region (Ferguson, Norton, Watson, Akil, & Robinson, 2003). Additionally, the administration of AMPH in a novel environment causes a great increase in locomotor sensitization compared to when it is administered in the home cage (Badiani, Anagnostaras, & Robinson, 1995; Crombag, Badiani, & Robinson, 1996; Robinson, Browman, Crombag, & Badiani, 1998) and higher levels of *c-fos* expression (Badiani, Oates, Day, Watson, Akil, & Robinson, 1999). Thus, it is possible that the moderate effect on stimulated DA release observed in the present study is due to the administration of AMPH in the test subject's home cage. As drugs of abuse are administered in a variety of social and environmental context, it will be important that future studies examine the effects of AMPH on reward circuitry when

administered in both the home cage and a novel environment as well as with and without the cage mate/partner in highly social species.

Our results show that AMPH had opposite effect (although not significant) in the NAc core and shell of paired animals. The animals exposed to AMPH had a trend of lower DA release levels than saline-exposed animals in the NAc core, but higher DA release trends in the NAc shell. This decrease in DA release in response to AMPH exposure was not seen in the sexually naïve prairie voles, suggesting perhaps something about the neurobiological response of pair bonding elicits this effect. This idea could be explored further by analyzing locomotor response in subjects, since the NAc core has been proposed to mediate this effect (Sellings & Clarke, 2003; Weiner, Gal, Rawlins, & Feldon, 1996). If pair bonded voles exposed to AMPH have lower locomotor activity compared to AMPH-exposed sexually naïve voles, it would suggest that the neural mechanisms of pair bonding attenuate NAc core-mediated increases in locomotor activity.

Conclusion

Both social and drug rewards act on motivational circuitry and therefore exposure to either one of these environmental or pharmacological manipulations can impact the consequent reward processing of the other (Burkett & Young, 2012; Lutz & Kieffer, 2013a). Specifically, exposure to either of the rewards can attenuate or enhance the processing of the other depending on the timing of the interaction. Drug reward can be attenuated if given during critical social periods such as following the formation of a bond with a mating/parenting partner (Liu et al., 2011) or during the rearing of offspring (i.e., social interactions that are critical for the fitness of a species) (Mattson, Williams, & Rosenblatt, 2001). On the other hand, social interactions with an adult nonspecific can enhance drug reward (Anacker et al., 2011). Finally, negative social

environments at any developmental time point can enhance drug reward (Bond et al., 2007; Hamme Peterson et al., 2010; Zakharova, Miller, Unterwald, Wade, & Izenwasser, 2009). Thus, the interactions between social and drug reward are incredibly complex and more studies are necessary to tease apart how social experience across the lifetime of an individual impacts drug reward processing.

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Author Note

Curtis J. Austin, Department of Psychology, University of Michigan, Ann Arbor

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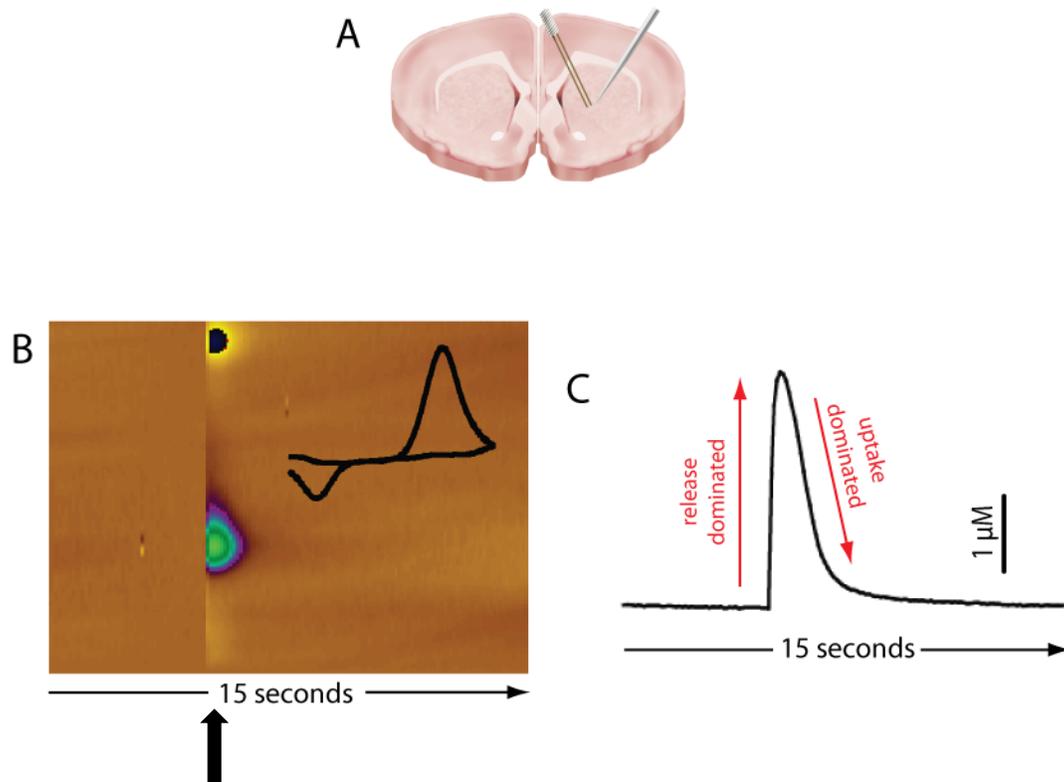


Figure 1. Striatal slice FSCV methods. A) The stimulating and recording electrodes are placed into the brain slice, in either the dorsal striatum, NAc core, or NAc shell. A depolarizing electric stimulation evokes DA release, which, along with uptake, is measured by the carbon fiber electrode. B) A representative color plot shows changes in current for the entire measurement period. The stimulation is applied 5 seconds into the measurement (designated by arrow). The cyclic voltammogram (inset) shows the oxidation and reduction peaks for the DA molecule, serving as its chemical signature. C) Following electrode calibration, current is converted into DA concentration.

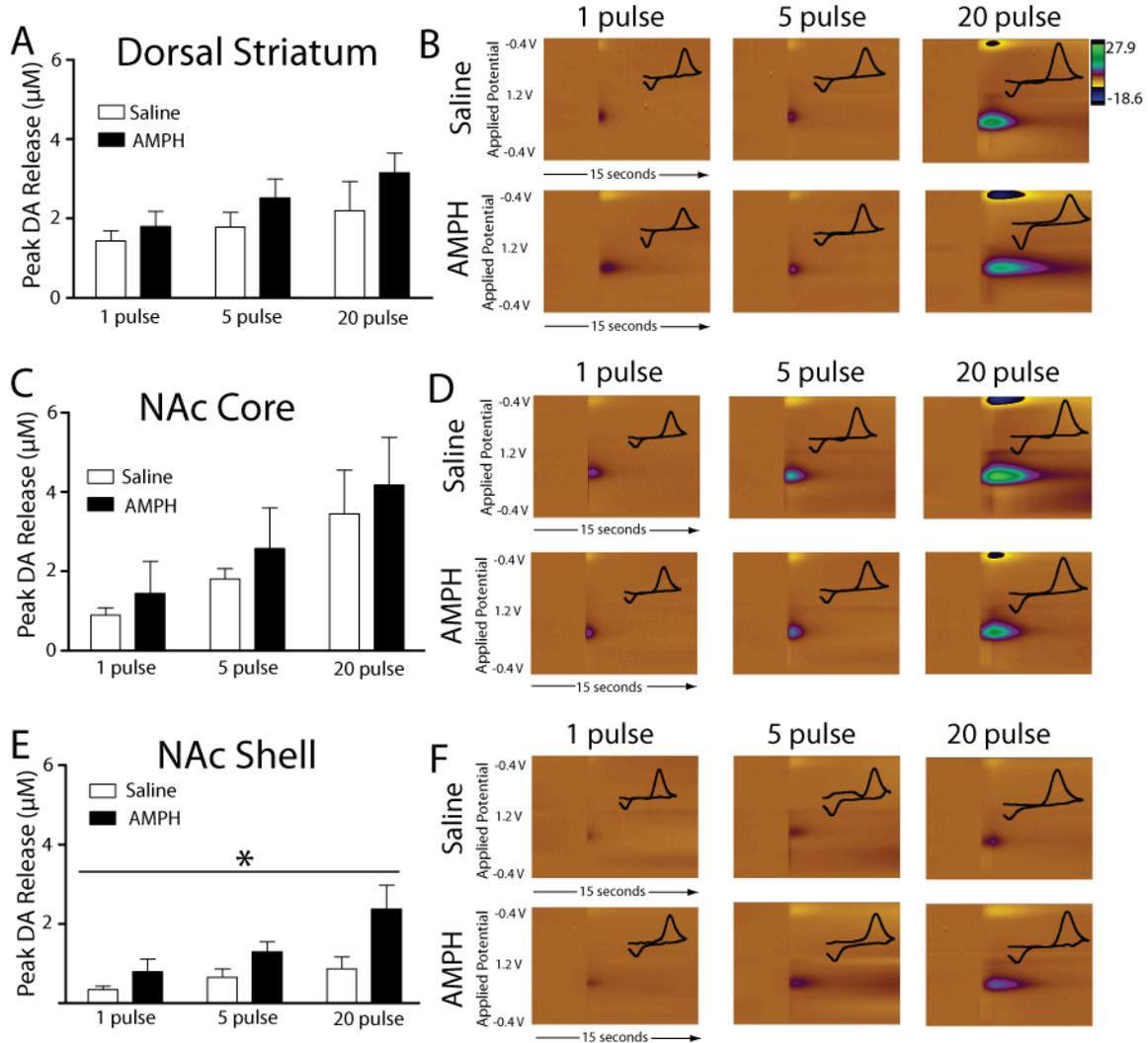


Figure 2. Note. * = $p < 0.05$

Effect of AMPH exposure on sibling housed prairie voles. A and B) There is no difference in DA release in the dorsal striatum between animals exposed to saline or AMPH. C and D) Similarly, there is no difference between the two groups when DA is measured in the NAc core. E and F) Sibling housed male prairie voles exposed to AMPH have significantly higher stimulated DA release in the NAc shell than those exposed to saline when all pulse levels are compared

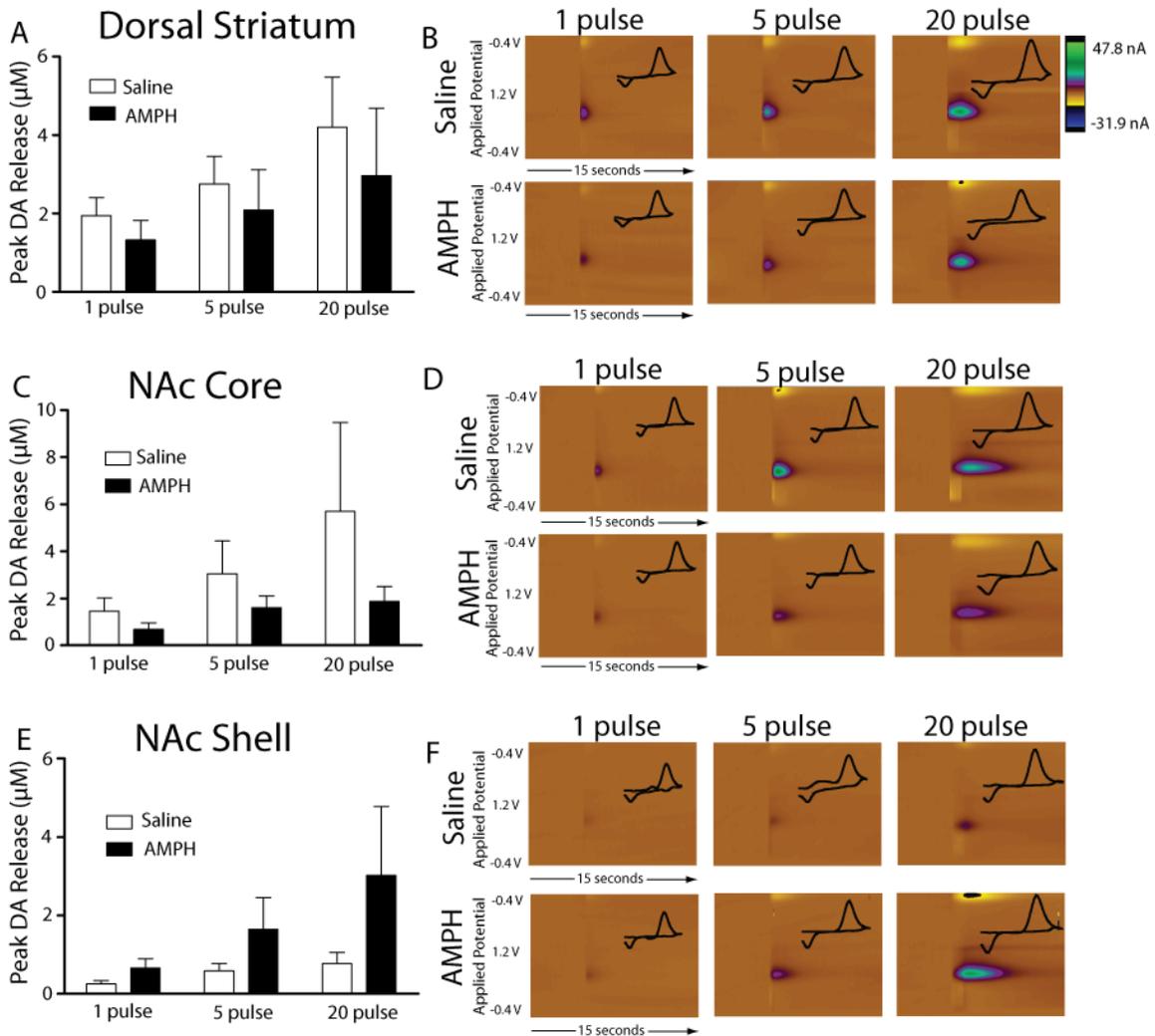


Figure 3. Effect of AMPH exposure on paired prairie voles. A and B) There is no difference in stimulated DA release within the dorsal striatum between pair bonded voles exposed to AMPH or saline. C and D) There is a prominent but insignificant attenuation in stimulated DA release within the NAc core in animals that have been exposed to AMPH. This difference is heightened when the number of pulses is increased. E and F) Conversely to NAc core, AMPH enhances stimulated DA release in the NAc shell in pair bonded voles. This difference is also more prominent at higher pulse levels.

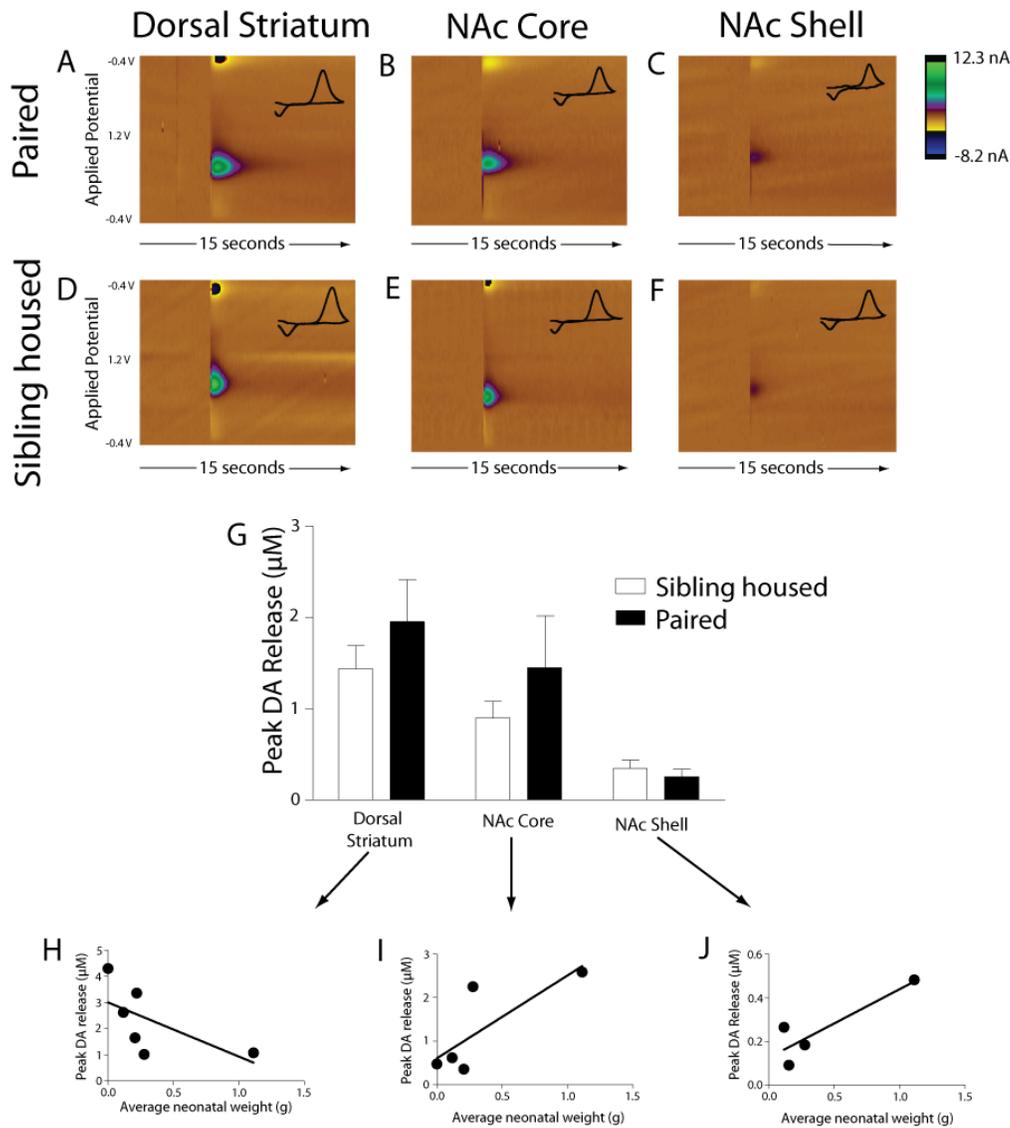


Figure 4. Effect of pairing on DA release in saline-exposed prairie voles. A-G) There is no significant difference in representative color plots or peak DA release of 1-pulse stimulations between paired and sibling housed animals in any region studied. H) There was no relationship in the dorsal striatum between DA release levels and average fetus weight of female partner. I and J) Although failing to reach statistical significance, there is a positive correlation between peak male DA release in the NAc (both core and shell) and average neonatal weight of female partners.

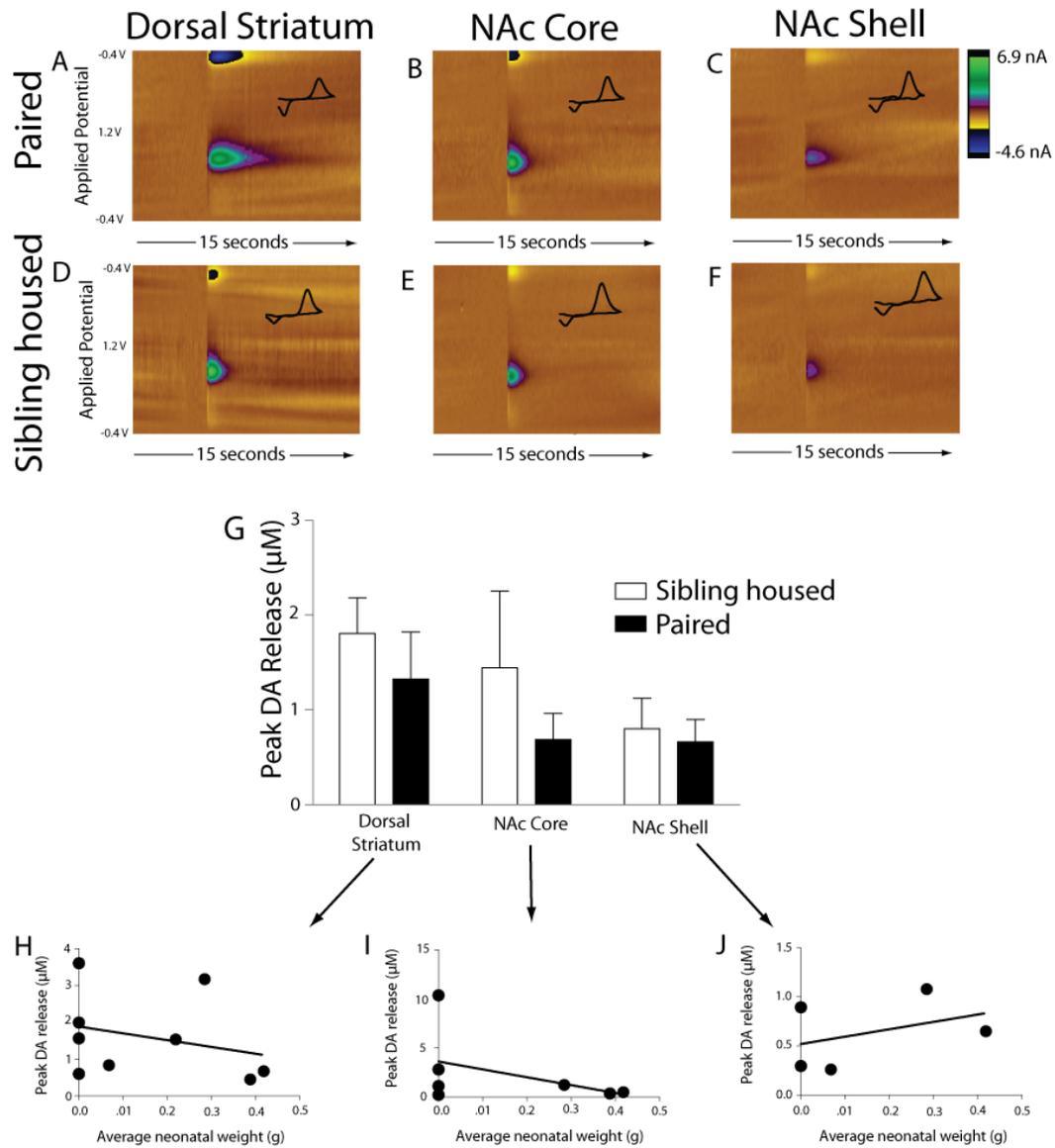


Figure 5. Effect of pairing on 1-pulse stimulated DA release in AMPH-exposed prairie voles. A-F) There is no significant difference in representative color plots of 1-pulse stimulations between paired and sibling housed animals in any region studied. G) 1-pulse stimulated DA release is approximately equal for sibling housed and paired animals in all striatal brain regions studied. H-J) There is no correlation between pregnancy status of the female and her mate's peak DA release in any region studied.

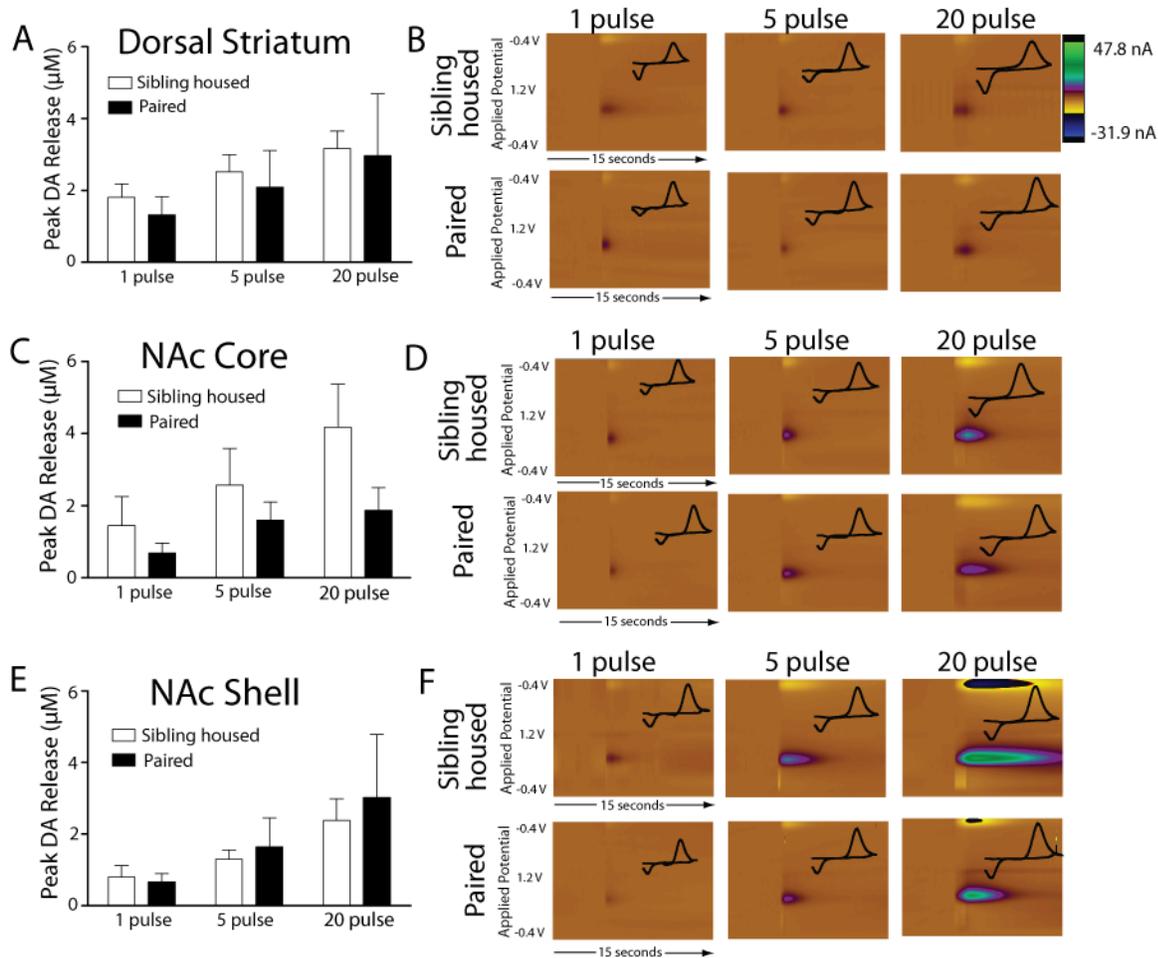


Figure 6. Effect of pairing on 5 and 20-pulse stimulated DA release in AMPH-exposed prairie voles. A and B) There is no significant difference in peak DA release in the dorsal striatum or representative color plots at any pulse level between sibling housed or paired animals exposed to AMPH. C) Within the NAc core, there was a noticeable but not significant trend that sibling housed animals had higher peak DA release compared to their paired cohorts. D) This trend in the NAc core is also visible in the representative color plots, displaying the largest difference between the groups at 1 and 20-pulse stimulations. E and F) No significant differences in stimulated DA release were found in the NAc shell when comparing AMPH-exposed sibling housed and paired prairie voles.