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Abstract

Previous work from our laboratories has shown that juvenile Fischer 344 (F344) rats are less playful than other strains and also appear to be compromised in dopamine (DA) functioning. To determine whether the dysfunctional play in this strain is associated with deficits in the handling and delivery of vesicular DA, the following experiments assessed the extent to which F344 rats are differentially sensitive to the effects of amphetamine. When exposed to amphetamine, striatal slices obtained from F344 rats showed a small increase in unstimulated DA release when compared with slices from Sprague–Dawley rats; they also showed a more rapid high K^+-mediated release of DA. These data provide tentative support for the hypothesis that F344 rats have a higher concentration of cytoplasmic DA than Sprague–Dawley rats. When rats were tested for activity in an open field, F344 rats presented a pattern of results that was consistent with either an enhanced response to amphetamine (3 mg/kg) or a more rapid release of DA (10 mg/kg). Although there was some indication that amphetamine had a dose-dependent differential effect on play in the two strains, play in F344 rats was not enhanced to any degree by amphetamine. Although these results are not consistent with our working hypothesis that F344 rats are less playful because of a deficit in vesicular release of DA, they still suggest that this strain may be a useful model for better understanding the role of DA in social behavior during the juvenile period.

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Abstract

Previous work from our laboratories has shown that juvenile Fischer 344 (F344) rats are less playful than other strains and also appear to be compromised in dopamine functioning. In order to determine whether the dysfunctional play in this strain may be associated with deficits in the handling and delivery of vesicular DA, the following experiments assessed the extent to which F344 rats may be differentially sensitive to the effects of amphetamine. When exposed to amphetamine, striatal slices obtained from F344 rats showed a small increase in unstimulated DA release when compared to slices taken from Sprague-Dawley (SD) rats and also showed a more rapid high K^+ mediated release of DA. These data provide tentative support for the hypothesis that F344 rats have a higher concentration of cytoplasmic DA than SD rats. When rats were tested for activity in an open field F344 rats presented a pattern of results that would be consistent with either an enhanced response to amphetamine (3 mg/kg) or a more rapid release of DA (10 mg/kg). While there was some indication that amphetamine was having a dose-dependent differential effect on play in the two strains, play in F344 rats was not enhanced to any degree by amphetamine. While these results are not consistent with our working hypothesis that F344 rats are less playful because of a deficit in vesicular release of DA, they still suggest that this strain may be a useful model for better understanding the role of DA in social behavior during the juvenile period.

Introduction

Engaging in positive social interactions is a major component of the behavioral repertoire of many, if not most, mammalian species. Before the onset of puberty, affectively positive social interactions often take the form of some type of social play. Play is one of the earliest social behaviors not directed towards the mother and occurs in varied forms in the young of most mammalian species and in a variety of other species (e.g., birds, reptiles, invertebrates) as well (Burghardt, 2005; Fagen, 1981; Pellis & Pellis, 2009) suggesting that this behavior pattern is well established in the mammalian brain. Play experience may be one way that young mammals use to learn the specific social nuances of their species (Siviy & Panksepp, 2011), thus providing considerable social benefits to those that engage in this early social behavior. Indeed, there is compelling evidence that removing the opportunity to play can have a number of adverse consequences on later behavior and social/emotional functioning (Pellis & Pellis, 2007; Spinka, Newberry, & Bekoff, 2001; Van den Berg et al., 1999) as well as cognitive functioning (Baarendse, Counotte, O'Donnell, & Vanderschuren, 2013). Gaining a better understanding of the neurobiological correlates of mammalian playfulness is likely to shed considerable light on the neural mechanisms associated with social/emotional and cognitive development.

Rats are particularly playful; bouts of rough-and-tumble activity begin shortly after independent locomotion has been attained, peaks at around 35 days of age, and then wanes around puberty (Panksepp, 1981). Play is easy to quantify and under fairly tight regulatory control (Pellis & Pellis, 2009; Siviy & Panksepp, 2011; Trezza, Baarendse, & Vanderschuren, 2010). In that sense, play can be approached empirically in a manner similar to other motivated behaviors. For example, the amount of play exhibited by pairs of young rats in a discrete period (e.g., 5 – 15 minute observation periods) can readily be titrated by isolating animals for periods

ranging from 4 to 24 hours. This makes play behavior in the rat particularly amenable to pharmacological research as one can easily obtain baseline levels of behavior that allow for both increases and decreases in playfulness to be readily observed with injections able to be timed to maximize the bioavailability for a particular drug. Indeed, considerable research has been directed towards the pharmacological modulation of playfulness and several neurochemical systems have been identified as being critical for play to unfold in the rat (Trezza et al., 2010).

With a considerable body of data suggesting that play is both pleasurable and highly motivated (Trezza, Campolongo, & Vanderschuren, 2011), there has often been an assumption that dopamine (DA) should have a powerful modulatory influence over playfulness. Indeed, DA antagonists reliably reduce playfulness (Beatty, Costello, & Berry, 1984; Niesink & Van Ree, 1989; Siviy, Fleischhauer, Kerrigan, & Kuhlman, 1996), neonatal 6-OHDA lesions disrupt patterning of play (Pellis, Casteneda, McKenna, Tran-Nguyen, & Whishaw, 1993), play increases DA utilization (Panksepp, 1993), and increases in play induced by alcohol, nicotine or by enhancing endocannabinoid tone can all be reversed by low doses of DA antagonists (Trezza, Baarendse, & Vanderschuren, 2009; Trezza & Vanderschuren, 2009). These data suggest that DA may have a positive influence on overall levels of playfulness. Psychomotor stimulants are extremely potent at reducing playfulness (Beatty et al., 1984; Beatty, Dodge, Dodge, White, & Panksepp, 1982; Sutton & Raskin, 1986) and while the reductions in play can be readily attributed to enhanced noradrenergic (NE) transmission (Achterberg et al., 2014; Vanderschuren, et al., 2008) a more recent study has shown that the psychomotor stimulant methylphenidate increases the motivation to play and this is dependent upon a dopaminergic mechanism (Achterberg et al., 2015a). These data suggest that psychomotor stimulants result in a neurochemical scenario whereby play is reduced through a NE mechanism while play motivation

is increased through a DA mechanism. Therefore, some aspects of play may be more sensitive to DAergic influences than others.

Given the widespread prevalence of social play among mammals in general and rats in particular (Burghardt, 2005; Fagen, 1981; Pellis & Pellis, 2009) and the potential for using rat play as a model to study neurodevelopmental disorders (Siviy & Panksepp, 2011), identifying a strain of rat that plays substantially less than other strains in the absence of any identifiable threat may then provide valuable insight into both the genetics and neurobiological substrates that modulate this complex social behavior. Use of strains with relatively unique neurobiological characteristics may also be particularly fruitful for gaining a better understanding of the neurochemical modulation of playfulness. Towards this end, we have identified the inbred Fischer 344 rat (F344) as a strain that is consistently less playful than other strains commonly used in behavioral research (Siviy, Baliko, & Bowers, 1997; Siviy, Crawford, Akopian, & Walsh, 2011; Siviy, Love, DeCicco, Giordano, & Seifert, 2003). F344 rats solicit less play and are less likely to respond to playful overtures with responses that would prolong a bout of play (Siviy et al., 1997; Siviy et al., 2011; Siviy et al., 2003). Low levels of play in F344 rats are relatively insensitive to cross-fostering (Siviy et al., 2003), further suggesting a genetic component to the dysfunctional play in this strain.

In addition to being less playful than other strains, F344 rats also appear to be compromised in dopaminergic functioning (Siviy et al., 2011). When compared to outbred Sprague-Dawley (SD) rats, F344 rats have higher DA levels in both striatum and frontal cortex, although DA turnover is lower in this strain. F344 rats also release less DA following local stimulation in both dorsal and ventral striatal slices. Finally, F344 rats exhibit less DA-dependent plasticity in cortical and striatal slices. Taken together, these data are consistent with a

working hypothesis that F344 rats have deficits in the handling and delivery of vesicular DA. Higher DA levels coupled with reduced turnover further suggests that rats of this strain may be sequestering DA in the cytoplasm. If this is the case and if F344 rats have higher levels of cytoplasmic DA, it is possible that some of the behavioral differences observed with F344 rats may be attributable to problems associated with DA handling and release. In this study, two sets of experiments were conducted to (1) further explore neurochemical differences in DA functioning in brain slices obtained from F344 and SD rats and (2) use the neurochemical data to inform behavioral hypotheses concerning the effects of amphetamine in these two strains.

In our earlier study (Siviy et al., 2011) DA release was induced by microelectrode stimulation. Microelectrode stimulation in striatal slices is more likely to increase synaptic DA primarily through vesicular release (Patel & Rice, 2006; Patel et al, 2003; Petzinger et al, 2007) so we set out in this experiment to introduce experimental conditions in our slice preparation that would tend to favor non-vesicular release of DA via reverse transport. Cytoplasmic non-vesicular accumulation of DA in presynaptic terminals of F344 should be detectible through treatment of brain slices with amphetamine resulting in reverse transport at the DA transporter (DAT) (Chen et al., 2004), so we predict that amphetamine should result in higher synaptic concentrations of DA in slices taken from F344 rats compared to those taken from SD rats. We also altered the ionic environment in ways that would be favorable for enhanced reverse transport. In particular, high K^+ has been shown to result in both vesicular and non-vesicular release of DA (Chen et al., 2004; Yamazaki et al., 1998), while elevating Zn^{2+} has been reported to enhance reverse transport of DA through DAT (Pifl et al, 2004; Loland et al, 2003). As was the case with amphetamine, we would also predict that increased accumulation of cytoplasmic

DA in F344 rats would lead to increased non-vesicular release of DA in response to these two ionic challenges.

With the results from our neurochemical experiments consistent with our hypothesis that striatal slices from F344 rats have a higher cytoplasmic pool of DA than SD rats and are more prone to non-vesicular release of DA in the presence of amphetamine, we next sought to determine whether this physiological response can translate into predictable behavioral consequences in these two strains. When removed from the isolated slice preparation, the effects of amphetamine on the behavior of an intact animal can be more varied. Nevertheless, systemic amphetamine is known to increase synaptic levels of DA via reverse transport (e.g., Fleckenstein, Volz, Riddle, Gibb, & Hanson, 2007; Sulzer, Sonders, Poulsen, & Galli, 2005) so if F344 rats are more prone to the effects of amphetamine on reverse transport then these rats should also be differentially sensitive to amphetamine in DA-sensitive behavioral models. Accordingly, the effects of amphetamine were initially assessed on open field activity in juvenile F344 and SD rats as this can be considered a fairly sensitive behavioral index of DA activity following systemic amphetamine (e.g., Kelly, Seviour, & Iversen, 1975; Kuczenski, Segal, & Aizenstein, 1991). It was predicted that amphetamine should result in higher levels of activity in F344 rats. Since we have hypothesized that lower levels of play in F344 rats may be due to an inability to package and release DA (Siviy et al., 2011) and since the effects of psychomotor stimulants have not yet been tested in F344 rats, we next examined the effects of amphetamine on play behavior in both juvenile F344 and SD rats. If F344 rats are less playful than other strains because of lower levels of synaptic DA we predict that amphetamine may increase play in this strain when compared to the SD strain.

Methods

Subjects and housing

For the neurochemical experiments, male SD and F344 rats were obtained from Charles River and housed at the University of Southern California in colony rooms that were maintained at 22° C with a 12/12 hr light/dark cycle (lights on at 06:00). All housing and testing was done in compliance with the NIH Guide for Care and Use of Laboratory Animals using protocols approved by the Institutional Animal Care and Use Committee at the University of Southern California.

For the behavioral experiments, male SD and F344 rats were obtained from Harlan Sprague-Dawley at approximately 25 days of age. Additional same-age Sprague-Dawley rats were also obtained to serve as target animals for the play experiments. Animals were housed in groups of four in solid bottom cages (48 X 27 X 20 cm) and periodically handled for a few days after arrival in order to acclimate to the laboratory. Food and water were always freely available. The colony room was maintained at 22° C with a 12/12 hr reversed light/dark cycle (lights off at 08:00), with all testing done during the dark phase of the light/dark cycle. All housing and testing was done in compliance with the NIH *Guide for Care and Use of Laboratory Animals* using protocols approved by the Institutional Animal Care and Use Committees at Gettysburg College.

Brain slice preparation

One-month old rats were anesthetized with halothane and decapitated. Brains were removed and placed in cooled (1-4° C), modified-oxygenated artificial cerebrospinal fluid (aCSF). In the modified aCSF, some of the sodium was replaced with sucrose to reduce tissue excitability during brain slice cutting (sucrose 124 mM, NaCl 62 mM). This solution maintained the osmotic balance found in normal aCSF. Normal aCSF contained (concentrations in mM)

NaCl 124, MgSO$_4$ 1.3, KCl 3.0, NaH$_2$PO$_4$ 1.25, NaHCO$_3$ 26, CaCl$_2$ 2.4, glucose 10.0,

equilibrated with a 95% O$_2$ - 5% CO$_2$ mixture to obtain a pH value of 7.3 - 7.4.

Hemi-coronal striatal slices were cut at a thickness of 400 µm using a Vibratome1000

(Vibratome Co., St. Louis, MO). The slices were immediately placed in an oxygenated aCSF

solution and were slowly brought to room temperature (23° C). Single slices were transferred to a

recording chamber (Haas ramp style gas interface chamber), and bathed continuously with the

oxygenated aCSF solution maintained at a temperature of 32° C. All recordings were performed

at the same dorsal-medial site in all experiments since DA release and DA-dependent forms of

synaptic plasticity varies within the dorsal-ventral and medial to lateral planes (Siviy et al, 2011;

Smith et al, 2001).

Fast scan cyclic voltammetry (FSCV) quantification of striatal DA release

Disc carbon fiber electrodes (CFE) were made from 7 mm unsized carbon fibers

(Goodfellow Corporation, Devon, PA) by electrophoretic anodic deposition of paint (ALA

Scientific Instruments, Inc., Westbury, NY) (Schulte and Chow, 1996). Extracellular DA was

monitored at the carbon fiber microelectrode every 100 msec by applying a triangular waveform

(-0.4 to +1.0 Volt vs. Ag/AgCl, 300 Volt/second). Currents were recorded with a modified VA-

10X Voltammetric and Amperometric Amplifier (NPI Electronic, Tamm, Germany). Data

acquisitions were controlled by Clampex 7.0 software (Axon Instruments, Foster City, CA).

Electrical stimulation of the brain slice surface across a twisted, bipolar, nichrome electrode was

used to evoke DA release. Single constant current pulses of 250 µA and 0.1 msec duration were

obtained by using an A360R Constant Current Stimulus Isolator (WPI, Sarasota, FL) and a

Master-8 pulse generator (A.M.P.I., Jerusalem, Israel). Stimulus intervals between pulses were

not less than 5 min. The CFE's were inserted 75 to 100 µm into brain slices at a position 100 to

200 µm from the stimulating electrode pair (Miles et al., 2002). DA was sampled in the dorsal striatum from brain slices in all experimental conditions. Changes in extracellular DA were determined by monitoring the current over a 200 mV window at the peak oxidation potential for DA (for review, see Patel & Rice, 2006). Subtracting the current obtained before stimulation from the current obtained in the presence of DA created background-subtracted cyclic voltammograms. Electrodes were calibrated with 5 µM DA solutions in aCSF following each experiment to convert the oxidation current to DA concentration. Previous work has demonstrated that this method causes action potential mediated DA release that is TTX sensitive (Patel, Mooslehner, Chan, Emson, & Stamford, 2003; Patel & Rice, 2006; Petzinger et al., 2007).

Amphetamine, extracellular stimulation, high K^+ mediated DA release

Amphetamine: Artificial CSF was delivered alone for 10 minutes and it was followed by aCSF containing amphetamine (20 µM) for 30 minutes. 20 µM amphetamine was added directly to the perfusate and FSCV sampled DA release. This approach was used to monitor 1) the direct release of DA by perfused amphetamine, 2) the effect amphetamine had on microelectrode stimulation-induced DA release and 3) the effect amphetamine had on high K^+ (20 mM) mediated DA release.

Extracellular stimulation: A monopolar stimulating electrode was positioned 1 mm from the DA sampling carbon fiber electrode. A single stimulus (0.1 msec) was delivered to the monopolar electrode every 5 minutes before and during the addition of amphetamine. Microelectrode stimulation induced DA release was compared during the same recording for aCSF versus aCSF + 20 µM amphetamine. The same 250 µA, 0.1 msec duration stimulus was used to sample action potential mediated DA release in all experiments.

High K$^+$ stimulation: Brain slices were perfused with normal aCSF containing 20 mM K$^+$ (high K$^+$) for 30 seconds to induce DA release. The High K$^+$ aCSF solution was made by adding KCl to achieve the final desired concentration of 20 mM. High K$^+$ aCSF-mediated DA release was measured in separate slices bathed in normal aCSF, aCSF + amphetamine, or aCSF + amphetamine + elevated Zn^{2+}.

Zn^{2+} containing aCSF: 20 μM ZnCl was added to the amphetamine (20 μM) plus aCSF solution to see if Zn^{2+} would enhance rat strain differences in the effect amphetamine had on DA release in brain slices.

Open field activity

Open field activity was monitored using a commercially available system (Med Associates; St. Albans, VT) that used infrared (IR) beams to detect locomotor activity and rearing in an open field (43.2 x 43.2 x 30.5 cm) placed within a sound-attenuated chamber. Locomotor activity was monitored by 2 sets of 16 beam IR arrays placed 3 cm above the floor of the test chamber and quantified by distance traveled (cm). Vertical activity (rearing) was monitored by a single array of 16 IR beams placed 10 cm above the floor of the test chamber and quantified by total number of beam breaks.

One set of rats (8 F344, 8 SD) were tested for the effects of 3 mg/kg amphetamine and another set (8 F344, 8 SD) were tested for the effects of 10 mg/kg amphetamine. These doses were chosen primarily on the basis of preliminary data from our lab showing minimal strain differences with lower doses. Rats were individually placed in the open field for an initial 60 minute baseline period. After the 60 minute baseline, all rats were injected with saline (1 ml/kg IP) and returned to the open field for an additional 60 minutes. Rats were then injected (IP) with their respective dose of amphetamine and returned to the open field for an additional 60 minutes.

Data (distance traveled, rearing) were collected in 10 minute blocks. Since the two experiments were conducted at separate times, they were analyzed separately.

Play behavior

Play behavior was assessed in a clear Plexiglas chamber (40 x 40 x 50 cm) that was enclosed within a sound-attenuated wooden chamber illuminated by a single 25W red light bulb. The floor of the testing chamber was covered with approximately 3 cm of Aspen pine shavings. Play bouts were recorded as digital video files and scored later using behavioral observation software (Noldus XT: Noldus Information Technology) by an observer unaware of the strain of the animal and the treatment.

One week after arrival in the laboratory all of the rats were acclimated to the testing chamber by being placed individually in the testing chamber for 5 minutes. Rats were tested for the effects of amphetamine on 4 separate test days. On each of these days, the rats were isolated for 4 hours before being given a 5 minute opportunity to play with a novel SD rat that had also been acclimated and isolated in the same manner. Three doses of amphetamine (0.1, 0.3, 1.0 mg/kg) plus vehicle were tested in 8 SD rats and 8 F344 rats. The novel SD partner was not treated. Injections were given intraperitoneally (IP) 30 minutes before testing. Each rat was tested with each dose in a counterbalanced manner. At least 48 hours separated each test. Play was quantified by counting the frequency of contacts directed by the test rat towards the nape of the target SD rat (nape contacts) and the likelihood that a nape contact directed by the target SD rat to the test rat resulted in that rat rotating completely to a supine position (probability of a complete rotation). Nape contacts were quantified by frequency of occurrence, while complete rotations were quantified in probabilistic terms by calculating the probability of a complete rotation occurring in response to a nape contact. These two measures of playfulness have been

commonly used in this lab and are also thought to be controlled by independent motivational and neural substrates (Pellis & Pellis, 1991; Pellis & Pellis, 1987; Siviy et al., 1997; Siviy & Panksepp, 1987). Rats were re-housed socially after all testing was completed for that day.

An additional experiment was conducted to assess the effects of a higher dose of amphetamine. Housing and acclimation was the same as the initial experiment. SD and F344 rats were either injected with vehicle (n = 4/strain) or 10 mg/kg amphetamine (n = 4/strain) immediately before a 15 minute test session. As in the initial experiment, rats were paired with a novel untreated SD partner.

Results

Fast scan cyclic voltammetry (FSCV) analysis of strain differences in dopamine release caused by perfusion of amphetamine in brain slices

Amphetamine-induced DA release was compared between strains over 40 minutes of exposure (Figure 1). A representative voltammogram trace can be seen in Figure 1A. DA release evoked by intra-striatal electrical stimulation was also sampled in the same experiment every five minutes during the 40 minute perfusion with amphetamine. Amphetamine caused a small gradual increase in unstimulated DA release (Figure 1B). Comparing F344 (n = 8) with SD (n = 9) slices revealed differences near the end of the 40 minute sampling period. When comparing DA concentration between F344 and SD slices at times 35 and 40 minutes using t-tests with a Bonferroni correction, amphetamine resulted in more DA being released from F344 slices than SD slices at 40 minutes after application of amphetamine, $t(15) = 2.98$, $p < .05$.

The effect of amphetamine on microelectrode stimulation-induced DA release was also assessed. Electrical stimulation-induced DA release was monitored every five minutes before and after 40 minutes exposure to 50 µM amphetamine. The amphetamine mediated change in

stimulation-induced DA release was calculated as a percentage of the release measured prior to amphetamine exposure (Figure 1C). Amphetamine caused a rapid and equal reduction in evoked DA in both F344 and SD rats.

FSCV analysis of strain differences in dopamine release caused by perfusion of high K^+ aCSF, amphetamine, and Zn^{2+} in brain slices.

For control slices (F344 n = 5; SD n = 5), high K^+ (20 mM) aCSF was perfused through the slices for 30 seconds and DA release was monitored. To assess the effects of amphetamine on high K^+ induced DA release, separate brain slices (F344 n = 5; SD n = 3) were perfused with 50 µM amphetamine and then perfused with 50 µM amphetamine plus high K^+ aCSF for 30 seconds, and then back to 50 µM amphetamine alone. In order to assess the ability of extracellular Zn^{2+} to augment amphetamine's effect on high K^+ aCSF-mediated DA release, separate brain slices (F344 n = 3; SD n =3) were perfused with 50 µM amphetamine containing 20 µM Zn^{2+} and then perfused with 50 µM amphetamine-Zn^{2+} plus High K^+ aCSF for 30 seconds, and then back to 50 µM amphetamine + Zn^{2+} alone.

Comparisons were made on three measures of high K^+ mediated DA release – (1) time to onset of DA release, (2) time to peak DA concentration, and (3) maximum DA output. A representative voltammogram trace showing the effect of high K^+ alone on DA release in an SD and F344 slice can be seen in Figure 2A. The data from each measure were analyzed by a 2 x 3 Analysis of Variance (ANOVA) with 2 factors for strain (F344 and SD) and 3 for condition (high K^+ aCSF + control, high K^+ aCSF + amphetamine, and high K^+ aCSF + amphetamine + Zn^{2+}).

For time to onset of DA release (Figure 2B), there was found to be a significant main effect of condition, $F(2,18) = 4.12$, $p = .034$. Pairwise comparisons indicated that the slices

treated with amphetamine had a quicker time to onset than control slices (p = .011). Slices treated with amphetamine + Zn^{2+} did not differ from either control slices or amphetamine slices. There was no significant main effect of strain nor was there a strain x condition interaction.

For time to peak DA concentration (Figure 2C), there was a significant main effect of strain, $F(2,19) = 72.92$, $p < .001$, with F344 slices reaching peak DA concentration much sooner than SD slices. There was a significant main effect of condition, $F(2,19) = 81.95$, $p < .001$, with the two amphetamine conditions significantly different from control slices but not from each other. Those slices treated with amphetamine took longer to reach peak DA concentration than control slices. There was also a significant strain x condition interaction $F(2,19) = 20.39$, $p < .001$. Further analysis of this interaction indicated that F344 slices reached peak DA concentration sooner than SD slices in each of the two amphetamine conditions, but not in the control (high K^+ only) condition. This suggests that amphetamine led to a more rapid release of DA in F344 slices.

For maximum DA concentration (Figure 2D), there was a significant main effect of condition, $F(2,30) = 9.23$, $p = .001$, with both amphetamine conditions having higher DA concentrations than the control condition. Adding Zn^{2+} to the amphetamine in the perfusate appeared to increase maximum DA concentration over that of amphetamine alone although this only approached significance (p = .078). There was also a significant strain x condition interaction, $F(2,30) = 9.23$, $p = .001$. Further analysis of this interaction indicated that F344 slices had a higher DA concentration than SD slices in the control (high K^+) condition, but not in the two amphetamine conditions. This strain difference in maximal DA release caused by high K^+ aCSF is opposite to what we reported for microelectrode stimulation evoked DA release in brain slices (Siviy et al, 2011).

Open field activity

Two separate experiments were conducted to assess the effects of 3 mg/kg and 10 mg/kg amphetamine on open field activity in SD and F344 rats. In each experiment, rats were initially placed in the open field for a 60 minute baseline session. Rats were then injected with saline (1 ml/kg IP) and returned to the open field for an additional 60 minutes. Rats were then injected (IP) with their respective dose of amphetamine and returned to the open field for an additional 60 minutes. The data for distance traveled and vertical counts (rears) for each 60 minute test were analyzed by repeated measures ANOVA with strain as a between-subjects factor.

For the baseline period of the first experiment (3 mg/kg; Figure 3), there was a significant strain x block interaction for both distance traveled, $F(5,70) = 3.79$, $p = .004$, and for vertical counts, $F(5,70) = 6.08$, $p < .001$. Further analysis of these interactions indicated that F344 rats were less active and reared less than SD rats but this was limited to the first 10-minute block. After being injected with saline, there was found to be a significant main effect of strain for both distance traveled, $F(1,14) = 12.66$, $p = .003$, and vertical counts, $F(1,14) = 10.66$, $p = .006$. F344 rats continued to be less active and reared less than SD rats. The strain x block interaction was not significant for either measure indicating that the strain difference was consistent across the 60 minute test session. After amphetamine (3 mg/kg), the only significant effects were main effects of block for distance traveled, $F(5,70) = 10.6$, $p < .001$, and vertical counts, $F(5,70) = 12.23$, $p < .001$. Both measures of activity peaked during the fourth 10-minute block and began to decline afterwards.

Although these data suggest that both strains were affected to a comparable extent, it is worth noting that F344 rats were consistently less active on both measures over the entire post-saline testing period prior to administration of amphetamine. In order to take this pre-existing

strain difference into account, the data after amphetamine were converted into a percentage of the average movement (distance traveled and vertical activity) after saline and re-analyzed (Figure 4). For distance traveled, there was found to be a marginal main effect of strain, $F(1,14) = 3.84$, $p = .07$, with amphetamine having a marginally greater effect on locomotor activity in F344 rats. For vertical counts, there was a significant strain x block interaction, $F(5,70) = 2.38$, $p = .047$, which can be accounted for by amphetamine having a greater effect on rearing in F344 rats during the second half of the 1 hour test session.

The results from the experiment assessing the effects of 10 mg/kg can be seen in Figure 5. For the baseline period, there was a marginal effect of strain on distance traveled, $F(1,14) = 4.44$, $p = .054$, and a significant effect of strain on vertical counts, $F(1,14) = 8.10$, $p = .013$. As was seen with the first experiment, F344 rats were less active than SD rats during the baseline period, although differences were more constant throughout the session in this experiment. After saline, there was a significant strain x block interaction for distance traveled, $F(5,70) = 3.80$, $p = .004$, and for vertical activity, $F(5,70) = 3.61$, $p = .006$. Further analysis of these interactions indicated that F344 rats were less active and reared less than SD rats during the first 10 minute block but not afterwards. After amphetamine (10 mg/kg) there was a significant strain x block interaction for distance traveled, $F(5,70) = 6.56$, $p < .001$, and for vertical counts, $F(5,70) = 3.49$, $p = .007$. For distance traveled, further analysis of the data indicated that F344 rats were more active than SD rats during the first 10 minute block but then less active during the last four 10 minute blocks. A similar pattern emerged with vertical counts, with F344 rats rearing more during the first two 10-minute blocks and then significantly less than SD rats during the last four 10-minute blocks.

Play behavior

We initially tested a moderate dose range (0.1, 0.3, 1.0 mg/kg) of amphetamine in both SD and F344 rats (Figure 6). As expected, F344 rats were significantly less likely than SD rats to direct nape contacts towards an untreated partner, $F(1,14) = 20.41$, $p < .001$, and were less likely to respond with a complete rotation when solicited by the untreated partner, $F(1,14) = 13.66$, $p = .002$. Amphetamine had a significant effect on nape contacts, $F(3,42) = 15.26$, $p < .001$, with the highest dose significantly reducing this measure of play solicitation. Amphetamine reduced nape contacts to a comparable extent in both SD and F344 rats, as indicated by a non-significant dose x strain interaction, $F(3,42) = 1.75$. Although there was a visible trend for amphetamine to also reduce the likelihood of responding to a nape contact with a complete rotation this was not found to be significant, $F(3,42) = 1.48$, nor was there found to be a significant dose x strain interaction, $F(3,42) < 1.0$.

When a higher dose (10 mg/kg) was used (Figure 7) amphetamine significantly reduced both nape contacts, $F(1,12) = 8.59$, $p = .013$, and the likelihood of a complete rotation, $F(1,12) = 18.03$, $p = .001$. For nape contacts, there was also a significant effect of strain, $F(1,12) = 8.59$, $p = .013$, with F344 rats soliciting less play than SD rats, as well as a significant drug x strain interaction, $F(1,12) = 11.21$, $p = .006$. Further analysis of the interaction indicated that the higher dose of amphetamine significantly reduced nape contacts in SD rats but had no effect in the F344 rats. There was also a significant strain effect for playful responsiveness, $F(1,12) = 7.38$, $p = .019$, with F344 rats significantly less likely to respond to nape contacts with complete rotations than SD rats. There was also a marginal drug x strain interaction, $F(1,12) = 4.64$, $p = .052$, suggesting that amphetamine may be having a differential effect between the 2 strains on this measure of play as well.

Discussion

Perhaps one of the lesser appreciated aspects of behavioral pharmacology is the extent to which individual differences can modify the behavioral consequences of pharmacological treatments. One approach towards assessing individual differences within the framework of an animal model is to take advantage of strains of rats that differ systematically on certain physiological and/or neurochemical dimensions in order to generate testable hypotheses concerning the behavioral consequences of various psychoactive compounds. As one example, such a strategy has been quite useful in understanding the neurobiology of those mechanisms that underlie addiction (Haile, Kosten, & Kosten, 2007; Self & Nestler, 1995). In the present context, we have been looking at the inbred F344 rat as an interesting and potentially useful model for examining the neurobiological substrates of social play in the juvenile rat. Rats of this strain are consistently less playful than other inbred and outbred strains (Siviy et al., 1997; Siviy et al., 2011; Siviy et al., 2003) so it is likely that this behavioral phenotype could be accounted for by underlying systematic differences in those neural substrates that modulate playfulness. In addition to the dysfunctional play of F344 rats, we also previously found that F344 rats release less DA in response to electrode stimulation of brain slices in the nucleus accumbens and in the dorsal striatum using FSCV (Siviy et al., 2011). In that study, HPLC showed that F344 rats had higher DA content in the striatum and prefrontal cortex, but in both sites F344 rats showed less DA turnover relative to SD rats. These data suggested that F344 rats may have a deficit in the packaging of DA into vesicles and consequently that a build-up of DA exists in the cytoplasm. We reasoned that cytoplasmic non-vesicular accumulation of DA in presynaptic terminals of F344 rats should be detectible through treatment of brain slices with amphetamine, which causes cytoplasmic DA release via reverse transport at DA transporters (DAT) (Chen et al., 2004).

The neurochemical results from the present study show that amphetamine caused greater release of DA in striatal slices from F344 rats (Figure 1) providing tentative support for our hypothesis that F344 rats have a higher concentration of cytoplasmic DA than SD rats. This complements our earlier work showing less DA release in striatal slices obtained from F344 rats when elicited by electrical stimulation, a preparation that would be more likely to tap into vesicular mechanisms of release. Interestingly, a similar neurochemical profile has been reported for the Spontaneously Hypertensive Rat (SHR) when compared to the normotensive Wistar-Kyoto (WKY) strain (Carboni, Silvagni, Valentini, & diChiara, 2003; Russell, de Villiers, Sagvolden, Lamm, & Taljaard, 1998). For example, electrical stimulation-based DA release is reduced in SHR brain slices, but amphetamine caused greater release of DA in SHR brain slices. It was concluded that SHR rats experienced a deficit in vesicular packaging of DA (Russell et al, 1998) and we would argue that a similar deficit is present in F344 rats.

Previous work has shown that high K^+ aCSF causes DA release through both vesicular release and reverse transport at monoamine transporters (Chen et al, 2004; Yamazaki et al, 1998). We found high K^+ (20 mM) aCSF caused greater DA release in brain slices from F344 rats than in brain slices from SD rats (Figure 2). This trend is the complete opposite from that observed for DA release mediated by electrical stimulation (Siviy et al, 2011). A possible explanation for High K^+ aCSF producing greater DA release in F344 rats is that this approach tapped into the greater cytoplasmic DA content seen in DA neurons from F344 rats and did so by activating reverse DA transport at DAT. Thirty seconds of exposure to 20 mM K^+ aCSF would produce a sustained depolarization and consequently sustained intracellular elevation of Na^+ (Somjen and Muller, 2000). Elevating intracellular Na^+ is a key mechanism for triggering reverse transport of DA through DAT (Chen and Reith, 2004; Khoshbouei et al, 2003; Sitte and

Freissmuth, 2010; for review Sitte and Freissmuth, 2015). Another important ionic mechanism for enhancing reverse transport at DAT is raising intracellular Cl^- concentration (Sitte and Freissmuth, 2010). High K^+ aCSF was made by adding 20 mM KCl, which thus raised extracellular Cl^- concentration from 5.4 mM in normal aCSF to 22.4 mM. This elevation in extracellular Cl^- could have also contributed to DA release via reverse transport at DAT and differences observed in DA release between F344 and SD rats.

We further examined possible strain difference in DA pools by using amphetamine as a tool to sample cytoplasmic DA available through reverse transport via DAT. Amphetamine increased high K^+-mediated DA release in both strains. We also found, as have others that amphetamine slowed the kinetics of DA release in both strains. DA synapses from F344 rats, however, showed more rapid DA release when amphetamine was present (Figure 2) (Schmitz et al, 2001). These data suggest DA is more readily available for reverse transport through DAT in dopaminergic terminals from F344 rats. No strain differences were found in the maximum concentration of DA release under amphetamine conditions. This might be explained by amphetamine having equal action on DA release via reverse transport and vesicular release (Daberkow et al, 2013).

Zn^{2+} has also been reported to enhance reverse transport of DA though DAT (Pifl et al, 2004; Loland et al, 2003). We looked for strain differences in high K^+ aCSF-mediated DA release in slices incubated in amphetamine plus Zn^{2+} and again, found the same strain difference in the rate of high K^+-mediated DA release. Similarly, we did not see a strain difference in the peak concentration of DA release in slices incubated in amphetamine plus Zn^{2+}. Within strain comparison of high K^+-mediated DA under amphetamine versus amphetamine plus Zn^{2+} did not

reveal differences, indicating that elevating Zn^{2+} did not enhance DA release via reverse transport beyond that produced by amphetamine alone.

Overall, these data are consistent with our earlier data (Siviy et al., 2011) and extends our earlier work by providing further evidence that F344 rats may have a higher pool of cytoplasmic DA than SD rats and this is more readily available for release by reverse transport following treatment with amphetamine. To initially test this hypothesis in a behavioral model we assessed the effectiveness of amphetamine to increase locomotor activity in an open field. Upon initial analysis, a moderate to high dose of amphetamine (3 mg/kg) yielded a comparable increase in both measures of activity between the two strains. However, baseline levels of activity after saline differed between the strains in this experiment, with F344 rats being less active than SD rats after saline across the entire 60 minute test session. When this was taken into account by converting both distance traveled and rears after amphetamine to a percentage of average movement after saline, there was evidence for this dose of amphetamine to be more effective in F344 rats (Figure 4). A higher dose (10 mg/kg) also resulted in a pattern of results that would be consistent with a more rapid release of DA in F344 rats. In particular, F344 rats displayed a considerable amount of activity during the first 20 minutes after injection and then became fairly inactive, relative to SD rats. Comparing rearing activity in the two strains is especially illuminating. After the higher dose of amphetamine, SD rats displayed an elevated but stable amount of rearing throughout the 60 minute post-injection period. F344 rats, on the other hand, exhibited roughly twice as much rearing as SD rats during the first 20 minutes and then showed almost no rearing during the last 40 minutes of the test. Taken together, these data suggest that F344 rats are more susceptible to the stimulant properties of amphetamine and are consistent

with our working hypothesis that rats of this strain are more susceptible to non-vesicular release of DA via amphetamine-mediated reverse transport.

An important caveat to these data is that the measures used in the present study to assess locomotor activity (distance traveled and rearing) do not fully capture the richness of amphetamine's effect on movement. For example, amphetamine results in more locomotor activity at low to moderate doses and this is thought to be associated with increased DA activity in the ventral striatum (nucleus accumbens). On the other hand, higher doses lead to increased stereotypy and this is thought to be associated with increased DA activity in the dorsal striatum (Joyce & Iversen, 1984; Kelly et al., 1975; Sahakian, Robbins, Morgan, & Iversen, 1975). Although we utilized fairly high doses, we did not independently assess stereotypy in this study. A more detailed behavioral assessment of strain differences to a range of amphetamine doses would be a fruitful avenue for further investigation and could also help further illuminate if the behavioral differences being observed between F344 and SD rats following treatment with amphetamine also reflect putative strain differences in anatomical substrate (e.g., dorsal vs. ventral striatum).

As mentioned earlier, there are striking similarities in the neurochemical and behavioral profile between F344 and SHR strains. Amphetamine causes a greater striatal release of DA from both SHR and F344 rats, both seem to have deficits in vesicular packaging of DA, and the SHR rat has also been reported to play less than WKY and SD rats (Ferguson & Cada, 2004). If the dysfunctional play of F344 rats reflects impaired vesicular DA release then it seemed reasonable to hypothesize that amphetamine-induced non-vesicular release of DA may lead to an increase in play of this strain. To test this hypothesis we assessed a range of amphetamine doses in both F344 and SD rats when paired with untreated playful SD partners. At the low dose range

(0.1 – 1.0 mg/kg) amphetamine had a comparable dose-dependent effect on play in both SD and F344 rats, with a significant reduction in nape contacts seen after 1.0 mg/kg in both strains. When given a higher dose of amphetamine (10 mg/kg), an even further reduction in both measures of play was observed in SD rats, although there was no further reduction seen with play solicitation in F344 rats. It is possible that any further reduction in play solicitation in F344 rats may have been unlikely due to a "floor effect". However, it is worth noting that the mean number of nape contacts for F344 rats in the vehicle condition was virtually identical in the two experiments and while 1.0 mg/kg amphetamine reduced nape contacts by a further 61% in that study, there was no further reduction after 10 mg/kg. Therefore, the inability of 10 mg/kg amphetamine to further reduce play in F344 rats may not be easily attributable to low baseline levels of play in this strain. Whether this can be related to the dysfunctional neurochemical profile of this strain remains to be determined.

The reduction in play following psychomotor stimulants such as amphetamine and methylphenidate can be attributed primarily to increased release of norepinephrine since these reductions in play can be blocked by selective antagonists at alpha-2 receptors and mimicked by selective noradrenergic reuptake inhibitors (Achterberg et al., 2015a, 2015b; Vanderschuren et al., 2008). But when play is made contingent on an operant response methylphenidate increases responding (e.g., lever pressing) when an opportunity to play is the reward, suggesting that dopamine increases the motivation to engage in play (Achterberg et al., 2015a). As such, F344 rats may play less because they are less motivated to do so and, if this is the case, a more specific assessment of play motivation may be needed to test the effects of amphetamine and/or other psychomotor stimulants in this model. In the present behavioral model used to assess play it may not be possible to overcome the NE-induced reduction in play following amphetamine.

The results of this series of experiments provide further insight into the neurochemical and behavioral phenotype associated with the F344 strain. While it is difficult to draw a specific connection between an *in vitro* neurochemical effect and an *in vivo* behavioral effect, we believe that these data highlight how these two approaches may complement one another and how results from neurochemical experiments can be used to inform behavioral hypotheses. In particular, these data provide further evidence that F344 rats have deficits in the handling and delivery of vesicular DA. This was demonstrated in the results of neurochemical experiments and reflected in at least one of the behavioral assays where we noted an exaggerated response to a high dose of amphetamine in F344 rats that would be consistent with more rapid (i.e., non-vesicular) release in this strain. On the other hand, play in F344 rats was not enhanced to any degree by amphetamine as might have been predicted. Nevertheless, it is still particularly intriguing that the F344 rat has a similar neurochemical profile to the SHR rat and that both of these strains are less playful than comparison strains. The SHR strain has been established as a rodent model for ADHD (Sagvolden et al., 1992; Sagvolden, Russell, Aase, Johansen, & Farshbaf, 2005; Wultz, Sagvolden, Moser, & Moser, 1990) so the F344 rat may be useful for testing specific neurochemical and behavioral hypotheses that could then provide converging evidence towards understanding the underlying deficits associated with neurodevelopmental disorders such as ADHD.

Acknowledgements

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Figure captions

Figure 1. Amphetamine effects on basal and electrically evoked DA release. A: Voltammogram of DA release during application of amphetamine (arrow). A single electrical stimulus was delivered to the surface of the brain slice 1 mm from the carbon fiber recording electrode. Electrical stimuli were delivered every 5 minutes (250 μA, 0.1 msec duration). Example recordings from Fischer 344 and SD rats are shown. B: Plot of amphetamine-induced DA release.in brain slices from Fischer 344 and SD rats (F344 n= 9, SD n=8) (* indicates $p<0.05$). C: Plot of amphetamine-induced change in electrical stimulation evoked DA release in same brain slices shown in B.

Figure 2. High K^+ aCSF-mediated DA release: Effect of amphetamine and Zn^{2+}. A: Example voltammogram traces taken from F344 and SD striatal brain slices for response to 30 second perfusion of High K^+ aCSF. B: Graph of time to onset of DA release in response to high K^+ challenge under each brain slice incubation condition indicated. C: Graph of time to peak after onset of DA release under each brain slice incubation condition (* indicates $p<0.005$ for comparison between F344 and SD responses for that condition). D: Graph of peak DA concentration release in response to high K^+ aCSF under each incubation condition (⧧ indicates $p<0.05$ for control F344 vs control SD; # indicates $p<0.01$ for amphetamine and amphetamine plus Zn^{2+} compared to control.

Figure 3. Effects of 3 mg/kg amphetamine on mean (± SEM) distanced traveled (top) and rears (bottom) in both SD and F344 rats.

Figure 4. Effects of 3 mg/kg amphetamine on distance traveled (top) and rears (bottom) when calculated as a mean (± SEM) percentage of average distance traveled and rears after saline.

Figure 5. Effects of 10 mg/kg amphetamine on mean (± SEM) distanced traveled (top) and rears (bottom) in both SD and F344 rats.

Figure 6. Effects of amphetamine (0.1 – 1.0 mg/kg) on nape contacts and probability of complete rotation in SD and F344 rats. Rats were paired with an untreated SD partner.
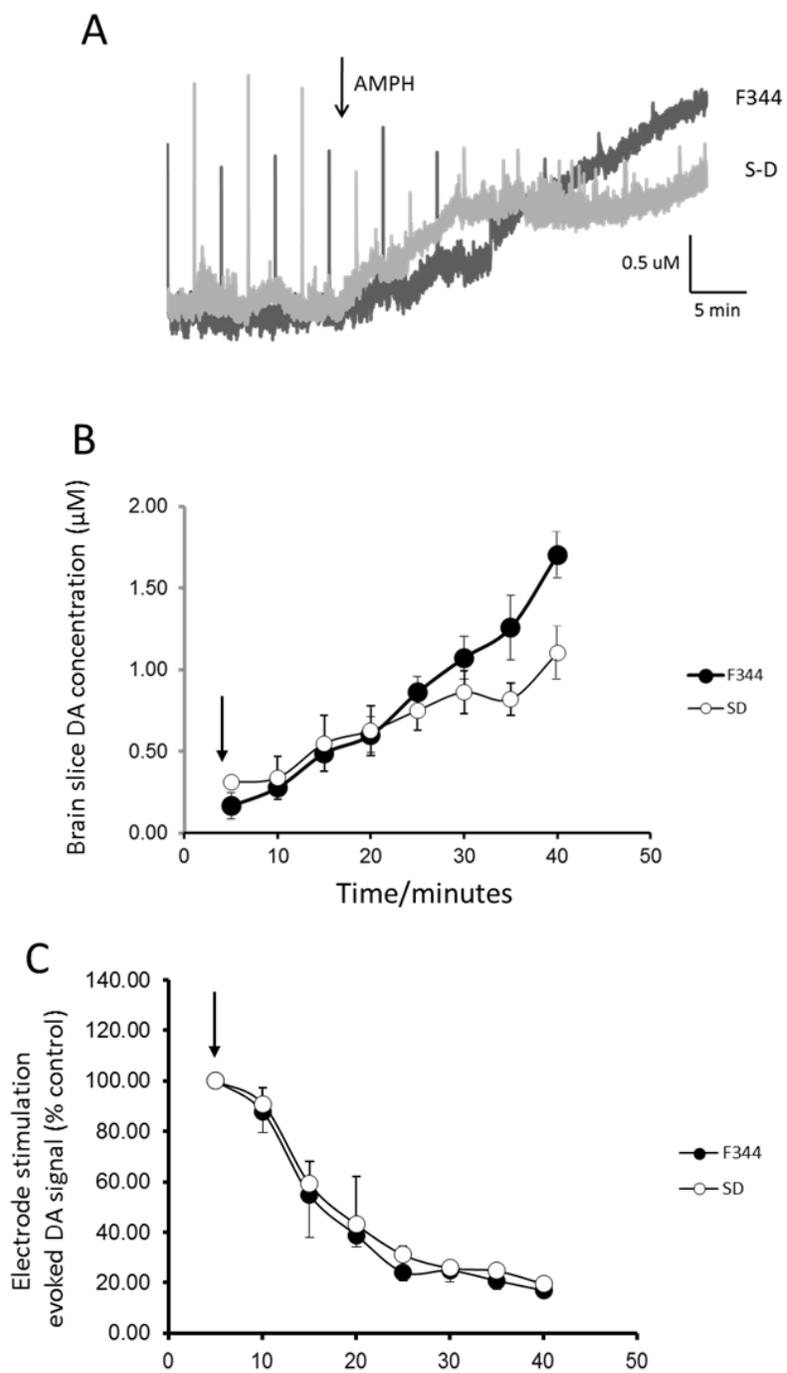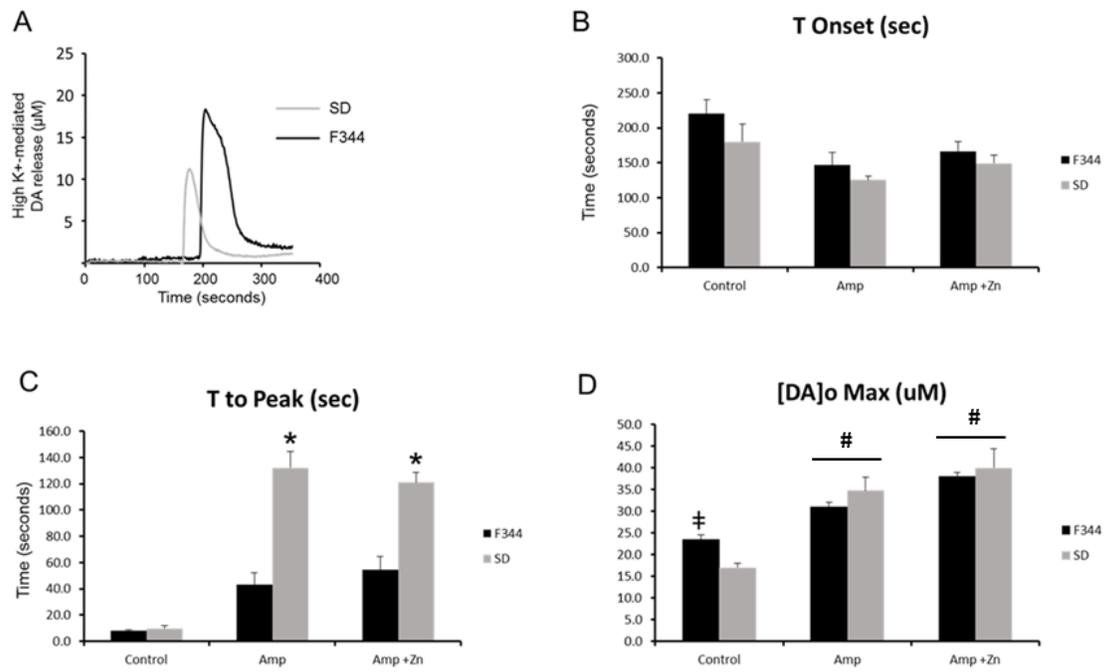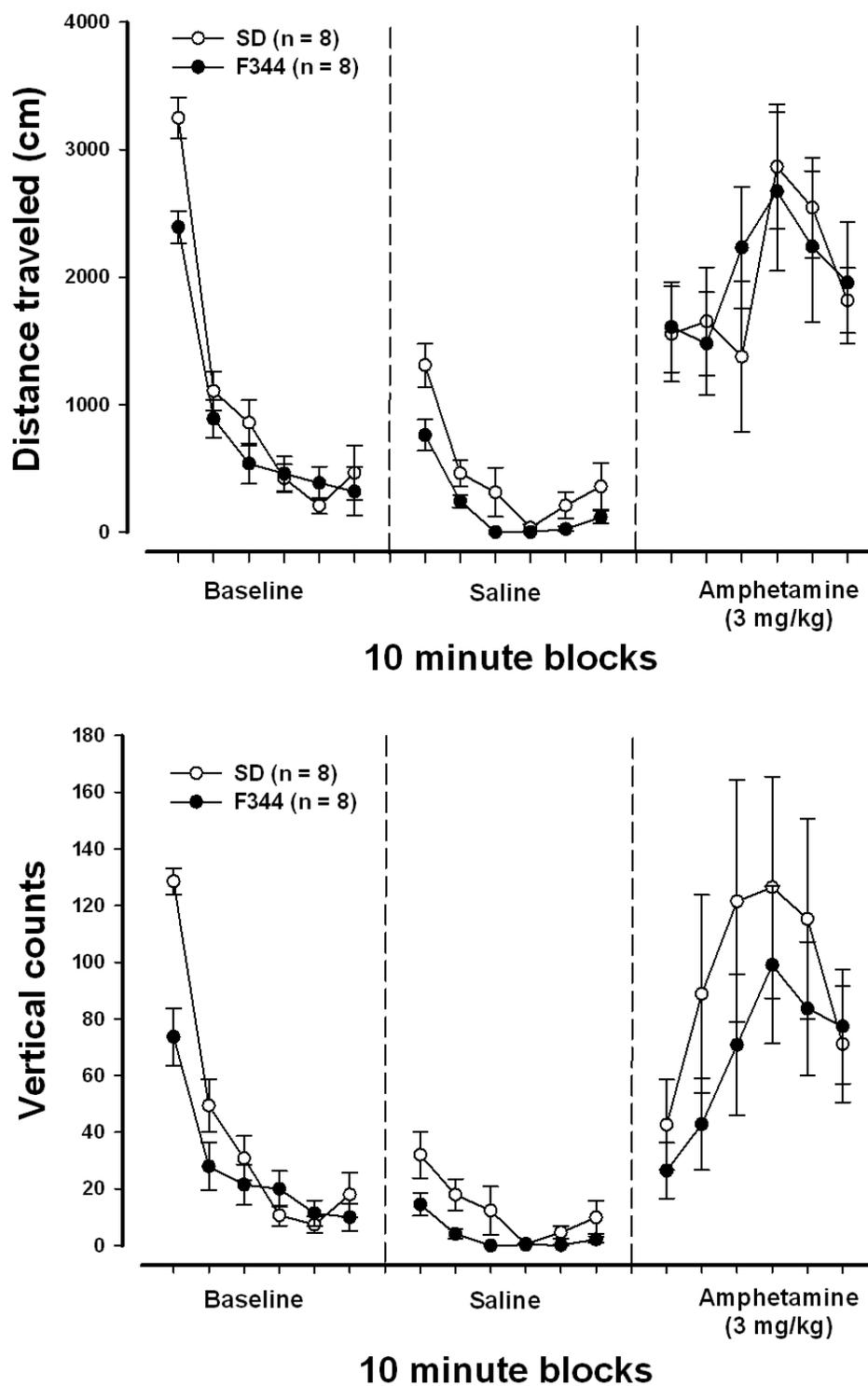
Figure 7. Effects of amphetamine (10 mg/kg) on nape contacts and probability of complete rotation in SD and F344 rats. Rats were paired with an untreated SD partner.
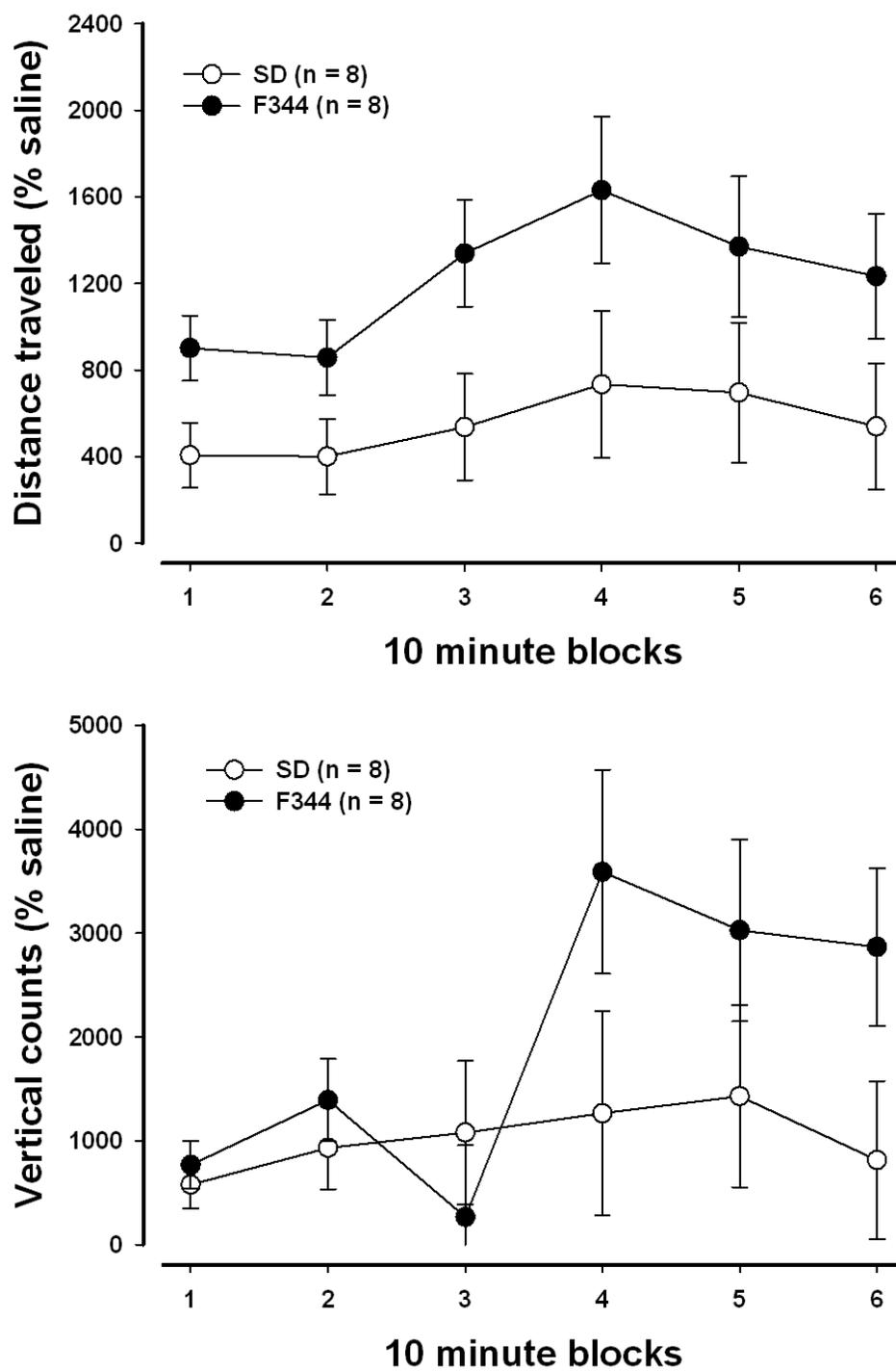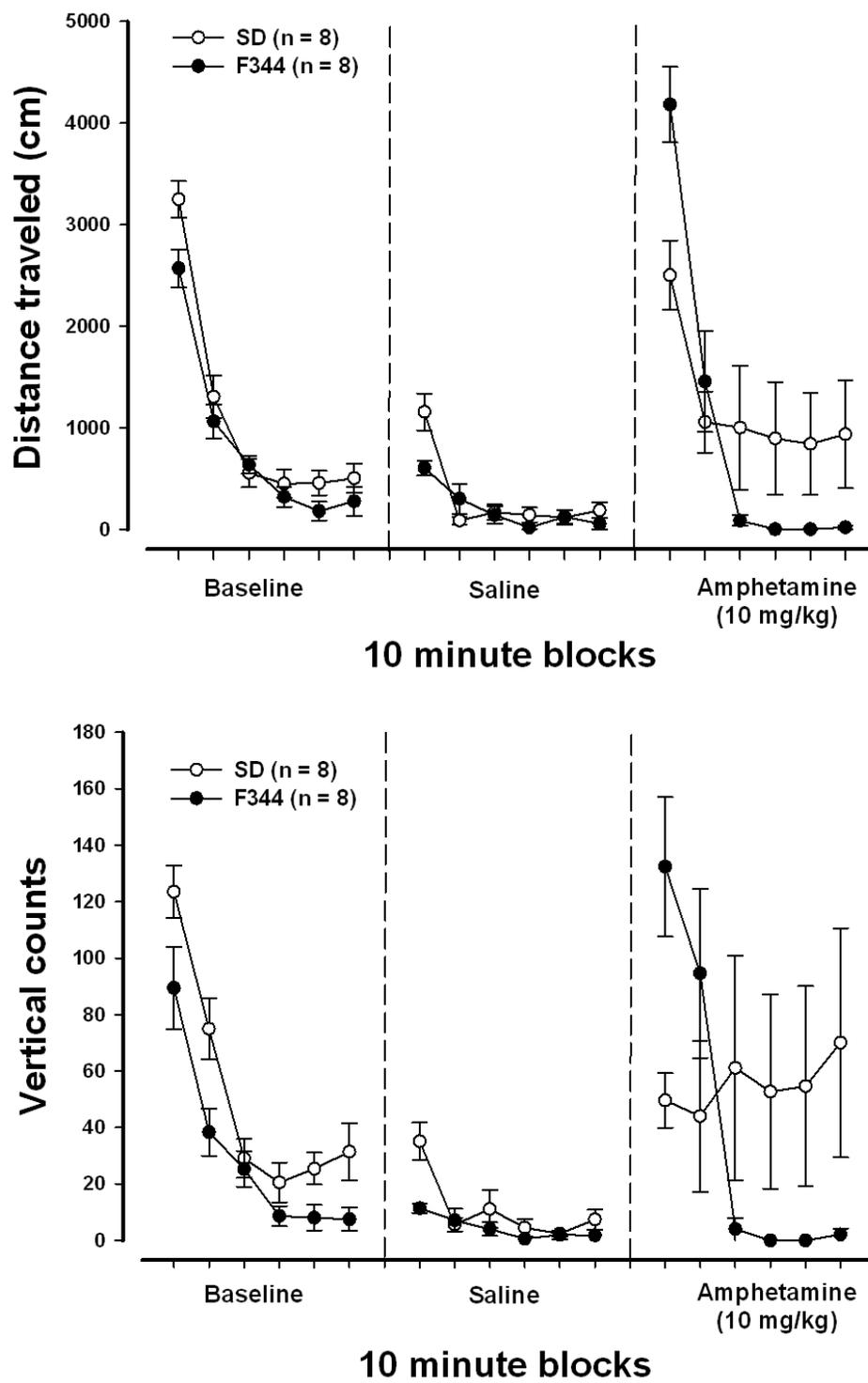
Figure 1

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