2014

Caffeine's attenuation of cocaine-induced deficiency in acoustic startle response by inhibition of adenosine in a sex and dose dependent manner

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Masters Thesis Spring 2014

Caffeine's attenuation of cocaine-induced deficiency in acoustic startle response by inhibition of adenosine in a sex and dose dependent manner

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Abstract:

The dopaminergic system has been known to be involved in the process of sensorimotor gating and correspondingly pre-pulse inhibition (PPI). Disruption of the dopamine (DA) system causes deficits in PPI and these deficits in PPI reveal symptoms for many psychotic disorders. Likewise, cocaine induces a sharp increase of dopamine transmission that alters sensorimotor gating and decreases PPI responses (Geyer and Braff, 1987). Adenosine is one system that is associated with the sleep-wake cycle and most importantly in regulating neuronal activity. Thus, more and more evidence is pointing to its involvement in DA release. The current study set out to examine the role of adenosine in cocaine-induced PPI deficits. More specifically, acoustic startle responses were examined following cocaine, caffeine (a non-selective adenosine antagonist), and the combination of these two stimulants were used to measure PPI. The results of administering cocaine and caffeine simultaneously showed a dose-dependent neuroprotection of the cocaine-induced decrease in PPI. Thus, the data indicates an important role of adenosine in regulating DA transmission. In addition, sex differences were examined. Females showed sensitive responses to cocaine-induced PPI compared to males. Many studies show that sensitivity to cocaine reward varies during the estrus cycle (Kippin et al., 2005, Feltenstein et al., 2009, Zakharova et al., 2009). We observed that cocaine produced direct estrus cycle changes. Thus, these changes in estrus cycle may be the underlying cause of the sex differences in acoustic startle response for cocaine-administered animals. Overall, we propose that the blocking abilities of caffeine at specific doses suggests a strong interaction between DA and the adenosine system and may provide future treatment for psychotic disorders as these treatments may balance malfunctioning DA neural circuits.

Keywords: brain, acoustic startle response, PPI, caffeine, cocaine, adenosine, dopamine, sensorimotor gating, neuroprotection, nucleus accumbens, schizophrenia, psychostimulants, sex differences

Introduction:

Sensorimotor gating that occurs in the auditory neural circuit of the brain filters attention and response processing. This ability to precisely filter different stimuli allows the animal to adapt to non-threatening situations and it allows the inhibition of overstimulation of sensory stimuli (Geyer and Braff, 1987). Therefore, deficits in sensorimotor gating can lead to sensory overload and consequently cognitive disarray.

A valuable behavioral model to evaluate sensorimotor gating is the acoustic startle response (ASR). The ASR is provoked by sudden auditory stimuli and is composed of facial, neck, and limb muscle movement/twitches (Landis and Hunt, 1939). The process of "gating" different stimuli is reflected by the magnitude in which the reflex response is suppressed by a weaker prestimulus, also termed prepulse inhibition (PPI; (Graham, 1975)). PPI is used as a cross-species measure for examining processing deficits. Increased dopamine (DA) transmission has been shown to induce changes in PPI, as seen with the reduction of PPI after administration of DA agonists to rats (Swerdlow et al., 1986, Mansbach et al., 1988) or humans (Morton et al., 1995, Abduljawad et al., 1997, Hutchinson et al., 1997). Thus, DA is highly important in this sensory processing.

There are two different pathways involved in sensorimotor gating of the ASR. One is the simple response with the startle stimulus and the other is the auditory non-startle prepulse where PPI is achieved (Fendt et al., 2001). The midbrain was found to be important in this pathway because lesions to this area caused PPI deficits whereas lesions to other areas did not. The startling stimulus is processed through the cochlear nuclei that projects onto the caudal pontine reticular nucleus (PnC). The PnC is involved in movement and projects through interneurons and motorneurons to induce the startle response.

The non-startling tactile input is received through the cochlear nuclei as well but projects onto the inferior colliculus (IC) that relays the prepulse for PPI to the superior colliculus (SC). The IC is activated by the prepulse and elicits inhibitory mechanisms in the PPI response. The SC is a multi-modal processor where all external stimuli converge. These regions are essential because lesion studies to the IC (Leitner and Cohen, 1985) or SC (Meredith et al., 1992) caused PPI disruptions. The SC then mediates the auditory prepulse to the pedunculopontine tegmental nucleus (PPTg) and the laterodorsal tegmental nucleus (LDTg). The PPTg is involved in arousal and locomotion and the LDTg mediates prolonged attention or alerting responses.

The PPTg and LDTg receive modulatory input from the forebrain that associates the prepulse with the loud stimulus. This association is important in sensorimotor gating and psychosis disorders. The nucleus accumbens (Nac) and substantia nigra project onto the PPTg, LDTg, and PnC. These specific regions are highly DA dependent and involved in motivation and movement. The PPTg and LDTg then converge with the simple

response onto the PnC through cholinergic and GABAergic inputs to induce the startle response. Lesions to PPTg and LDTg caused significant PPI deficits indicating its crucial role (Leitner et al., 1981). Overall, the slow inhibitory pathway of the non-startling stimulus mediates the fast excitatory pathway of the startle stimulus to facilitate PPI. It is necessary that the occurrence of the prepulse is 20-500ms before the startling stimulus in order to achieve PPI.

Overactivity of the mesolimbic DA system and thus, disruptions in sensorimotor gating, are common in psychotic disorders. One such disorder that is characterized by a loss of PPI is schizophrenia (Perry and Braff, 1994, Braff et al., 1995). Schizophrenia involves an overabundance of DA transmission and difficulties in information processing. Patients have high vulnerability to stimuli inundation and cognitive problems. They cannot "gate" between extraneous thoughts or prevent sensory stimuli from invading their consciousness (Swerdlow et al., 1992). Therefore, sensorimotor gating is severely interrupted in schizophrenia and PPI responses are used to diagnose processing complications in patients.

Through its role as an indirect DA agonist, cocaine mimics a schizophrenic phenotype (Martinez et al., 1999a, Nunes and Broderick, 2007). Cocaine causes the euphoric increase of DA by inhibiting DA transporters (Koob GF, 1997). Therefore, there is an excess of DA in the mesolimbic system, the same system where sensorimotor deficits occur in schizophrenia. Cocaine has a linear dose-response curve, thus, higher doeses produce greater responses. Previous rodent studies showed that cocaine-induced deficits in PPI were caused by the activation of DA 1 receptors (D1R) and DA 2 receptors (D2R), and were reversed with the pretreatment of selective D1R and D2R antagonists (Doherty et al., 2008).

Given the highly addictive properties of cocaine, mechanisms to better understand cocaine abuse are being intensively examined. Animal studies have shown that pharmacologically altering adenosine aids in the locomotor, reinforcing, and sensitizing properties of cocaine (Bedingfield et al., 1998, Kuzmin et al., 2000, Filip et al., 2006, Marcellino et al., 2007). Adenosine antagonists facilitated self-administration of cocaine while agonists inhibited initiation at specific doses (Knapp et al., 2001). Thus, there are observed interactions between adenosine and dopamine that regulate behavior (Wang et al., 2003).

Adenosine is a ubiquitous purine molecule that plays a key modulatory role in both dorsal and ventral dopaminergic transmission (Daly, 1993, Fredholm et al., 1999). Adenosine has been shown to decrease neural activity. Inhibition of the release of excitatory neurotransmitters is potentiated by adenosine compared to any other inhibitory neurotransmitters. This results in decreased calcium entry and decreased cAMP formation (Fredholm and Dunwiddie, 1988). One substance that targets the adenosine system is coffee with the active ingredient of caffeine.

Caffeine is a psychostimulant that promotes wakefulness and increases locomotion by non-selectively antagonizing the adenosine A1 receptors (A1R) and A2A receptors (A2AR;(Lazarus et al., 2011)). There is a high prevalence of caffeine intake in schizophrenic patients making it a target of interest for potential treatment (Hughes et al., 1998, Lara, 2010). Caffeine has hydrophobic properties and easily passes the blood brain barrier. A1R are found throughout the brain but primarily in the hippocampus, cerebral cortex, NAc and caudate-putamen (Fredholm et al., 1999). A2AR are localized primarily in the striatum. The NAc, where both receptor types are located, is the DA dense brain area involved in reward and corresponds to motor response. Similarly, cocaine is strongly associated with the NAc. Therefore, the NAc is an important region of interest.

Previous findings showed that A1R's were co-expressed and formed heteromers with D1R, as well as A2AR with D2R (Ongini and Fredholm, 1996, Ferré et al., 2007a). Adenosine regulates DA by acting both presynaptically, on A1R to inhibit DA release, and postsynaptically, on A2AR by decreasing DA transmission and receptor susceptibility. A1R are mostly on glutamate and dopamine terminals, whereas, A2AR are concentrated on inhibitory GABAergic striatal neurons (Hettinger et al., 2001, Rosin et al., 2003). Thus, inhibiting adenosine facilitates DA release, increases turnover rates, and augments DA receptor stimulation (Fernstrom and Fernstrom, 1984, Bickford et al., 1985, Fredholm and Jonzon, 1988, Hadfield and Milio, 1989).

Importantly, caffeine-induced increase in locomotion and DA release is highly dose-dependent (Solinas et al., 2002, Karcz-Kubicha et al., 2003). Caffeine exhibits an inverted "U-shape" dose response curve with peak DA release and locomotor responses at 30mg/kg. The lowest dose that produced a weak response was 3mg/kg of caffeine. This same weak response was seen at the highest dose of 100mg/kg caffeine. Thus, at these low and high doses, caffeine does not elicit an increase in DA release nor its corresponding behavioral response. Previous studies have shown that other mechanisms of action are also at play at these doses, such as inhibition of phosphodiesterase and release of intracellular calcium (Daly, 1993, Ongini and Fredholm, 1996, Fredholm et al., 1999).

Previous studies in our laboratory directly examined DA release in the NAc following cocaine, caffeine and combination of cocaine and caffeine (Broderick et al., 2008, Malave and Broderick, 2014). Neuromolecular imaging data were measured using the BRODERICK PROBE® biosensor. Procedures and specificities are described previously (Broderick, 1988, 1989a, b, 1995, 1999, Broderick et al., 2008). Cocaine increased DA release linearly with dose, whereas, caffeine exhibited the inverted "U-Shape" curve (Broderick et al., 2007, Nesbitt et al., 2008). Combinations of cocaine and caffeine revealed a neuroprotection of the surge of DA release induced by cocaine. Importantly, this effect was dose-specific. Caffeine did not block cocaine-induced DA release at middle doses (25 mg/kg and 5 mg/kg, respectively) but was seen at high (50 mg/kg and 10 mg/kg, respectively) and low doses (12.5 mg/kg and 2.5 mg/kg,

respectively). Furthermore, 8-cyclopentyltheophylline (CPT; 4.8mg/kg), an adenosine A1 antagonist showed neuroprotection of cocaine (5mg/kg)-induced DA release, suggesting a direct role of A1R's in the regulating actions of cocaine (Broderick et al., 2007). CPT also exhibits an inverted "U-shape" dose response curve as caffeine but has peak activity at lower doses of 4.8mg/kg (Karcz-Kubicha et al., 2003).

Based on these neurochemical results, the current study set out to investigate the behavioral effects engendered by the combination of caffeine and cocaine using the ASR paradigm and its potential implications in the therapy for psychosis disorders. Cocaine alters sensorimotor gating because the increased DA causes inaccurate filtering between the different magnitudes of startle. Consequently, responses are similar between startles with a prepulse and startles without a prepulse, thereby, lowering PPI (Martinez et al., 1999b). On the other hand, caffeine does not alter PPI in previous human (Flaten and Elden, 1999, Swerdlow et al., 2000) or animal (Bakshi et al., 1995) studies.

Additionally, we examined the effects of sex on PPI responses following cocaine and caffeine administration. Females have shown increased vulnerability and respond to lower doses of cocaine compared to males (Quinones-Jenab et al., 2001, Quinones-Jenab and Jenab, 2012). In laboratory animals, females self-administer cocaine more rapidly, become dependent earlier, and relapse faster. Sex hormones, such as estrogen and progesterone, may be the underlying cause. The level of sex hormones fluctuates throughout the four stages of the estrus cycle, which alters sensitivity to cocaine (Roberts et al., 1989). Human studies showed higher cocaine response during the luteal phase (moderate estrogen and high progesterone levels) and not the follicular phase (low estrogen and progesterone levels) (Evans et al., 2002, Evans, 2007). Throughout the estrus cycle in rats, the highest estrogen levels are during the proestrus phase and drops during the estrus and diestrus phase (Carter, 1993). Additionally, ovariectomy (OVX) procedures significantly diminished these sex differences.

There is an abundance of research on the influence of sex hormones on cocaine addiction. Laboratory animals with higher estrogen levels were shown to have higher sensitivity to cocaine (Sircar and Kim, 1999, Becker and Hu, 2008, Quinones-Jenab and Jenab, 2012). Estradiol studies have shown an increase in the locomotor and motivational aspect of cocaine addiction (Roberts et al., 1989, Kippin et al., 2005). OVX rats treated with estradiol and progesterone had greater sensitivity to cocaine than OVX rats treated with progesterone alone or with vehicle (Sircar and Kim, 1999, Sell et al., 2000). On the other hand, progesterone attenuates cocaine effects by either blocking estrogen or counteracting the rewarding effects of cocaine (Jackson et al., 2006). Thus, the most assumed theory is that estrogen is essential in the reinforcement of cocaine. However, there are still conflicting results in the literature. Some report cocaine responses are increased during estrus phase (Hecht et al., 1999, Quiñones-Jenab et al., 1999), had the most self-administration during proestrus phase (Feltenstein and See, 2007), or exhibited no differences between the estrus or proestrus phase (Sell et al., 2000, Walker et al.,

2001). One explanation for this is that different hormones may be associated with different aspects of cocaine addiction, some affecting locomotion, while others altering motivation. The current study examined the interaction between cocaine and estrogen in sensorimotor gating.

We hypothesize that PPI results will reflect the observed DA release in our previous studies, that is, caffeine will neuroprotect against the cocaine-induced decrease of PPI in a dose dependent manner. We also hypothesize that PPI results for females following cocaine administration will be more sensitive caused by the fluctuation of sex hormones.

Methods:

Subjects:

Adult male and female Sprague-Dawley rats *(rattus norvegicus),* 2 months of age, weight matched for sex, were purchased from Charles River Laboratories, North Carolina, USA. Animals were housed in the Animal Care Facility under the auspices of the City College Institutional Animal Care and Use Committee (IACUC) in compliance with National Institute of Health (NIH) guidelines. Food (Purina Rat Chow) and water was available *ad libitum.* Males were housed separately from females in airtight seclusion, three or two per chamber.

Drugs and doses:

Studies were performed in three groups: Cocaine, Caffeine, or combination of cocaine and caffeine with low, middle and high doses. Each group had a sample size of 8. Within the three groups each animal received each dose as a within-subject control. The different doses were given at least 48 hours after the previous one to eliminate residual drug effects. Doses were given as described in table 1 in pseudo-random order. Solutions for cocaine HCl (Sigma-Aldrich, St. Louis, MO) and caffeine (Sigma-Aldrich, St. Louis, MO) were dissolved in distilled water and injected intraperitoneally (i.p). Saline (0.9% NaCl) solution was used as a control. Doses were chosen based on peak effects from the literature (Solinas et al., 2002, Karcz-Kubicha et al., 2003, Solinas et al., 2005) and previous NMI experiments done in this laboratory to correlate behavior with biochemistry (Broderick et al., 2007, Broderick et al., 2008, Malave and Broderick, 2014).

Group	**Dose**
Cocaine (n=8)	Saline
	LD: 2.5 mg/kg
	MD: 5 mg/kg
	HD: 10 mg/kg

Caffeine (n=8)	Saline
	LD: 12.5 mg/kg
	MD: 25 mg/kg
	HD: 50 mg/kg
Combination (n=8)	Saline
	LD: 2.5 mg/kg coc & 12.5 mg/kg caff
	MD: 5 mg/kg coc & 25 mg/kg caff
	HD: 10 mg/kg coc & 50 mg/kg caff

Table 1: Doses administered i.p. immediately before the injection trial. Coc=cocaine; Caff=caffeine; LD= low dose; MD= middle dose; HD= high dose.

ASR paradigm:

The acoustic startle chamber is an SR-LAB^{TM} model (San Diego Instruments, San Diego, CA) that is automated to record the startle response of small animals. The chamber consists of a cylindrical enclosure on a plexiglass base. A speaker is mounted 24cm above the animal, which provides the background noise, pre-pulse stimuli and startle stimuli in random order controlled by the SR-LAB^{TM} software. Whole body startle responses were recorded 200ms after the presentation of the stimulus. The startle responses were then transduced by a piezoelectric accelerometer mounted on a seismic sensor plate platform, located below the cylinder in which the animal is placed. The subject's movement generates an electrical current in the sensor that distinguishes voluntary movement from startle movement. The sensor detects the pressure pushed down by the animal on the platform due to the eliciting stimulus. The chambers were designed to minimize extraneous noise and vibrations to attenuate influences from external stimuli.

Startle response to auditory stimuli via muscle movements transduced electrically was studied by PPI. Data were recorded as potential in voltage and were concatenated for compatibility with Excel software. Vmax was used in calculations denoted by the highest voltage during the response window (i.e. the "peak" of the response). %PPI was calculated using the formula: %PPI=100-[[mean PPI Vmax/mean startle only Vmax] x100]. The 25 sessions in each of the trials (11 minute intervals per trial) consisted of 5 types of acoustic stimuli delineated in table 2.

Type	**No Stimulus**	**Prepulse**	**Pulse**
1.	75 decibels (broadband)	-	-
2.	-	-	120 decibels
3.	-	95 decibels	120 decibels
4.	-	105 decibels	120 decibels
5.	-	115 decibels	120 decibels

Table 2: Different magnitudes of acoustic stimuli throughout each trial, some with prepulse and some without.75 decibels was the lowest stimuli and 120 was considered the startle stimuli.

These types of sessions were randomly placed throughout the trial but each trial used the same format in terms of the arrangement of the sessions.

The ASR paradigm consisted of three trials: habituation, injection and post 1-hour. The habituation trial was preceded by a 5-minute acclimation period followed by an 11-minute test period. The habituation trial involved the simplest form of learning that conditions the animal to not respond to the pre-pulse stimulus. Pre-pulses were low-intensity stimuli used to decrease the magnitude of the startle-provoking stimulus response. The injection trial was preceded by acute injection of a specific dose of cocaine, caffeine or physiological saline relative to body weight and lasted 22 minutes (two 11-minute periods were averaged because no significant difference between them). The post 1-hour trial measured the lingering effect of the injected drug or saline 1 hour after administration and lasted 11 minutes. Animals were returned to their cage after the Injection trial was completed and until the Post-1 hour trial started.

Vaginal Smears:

Samples were collected pre- and post- the injection trial. Females were swabbed with a saline soaked cotton tip and dotted on a microscope slide. Samples were observed at 40x objective lenses. The vaginal smears were stained with a Diff staining kit (IMEB INC, San Marcos, CA) and analyzed according to four stages: proestrus, estrus, metestrus and diestrus (Marcondes et al., 2002). Samples were assessed based on the proportions of the type of cells to determine the appropriate stages. There were three types of cells: round nucleated epithelial cells, irregular shaped anucleated cornified cells and little round leukocyte cells. Proestrus phase had prominently epithelial cells. Estrus phase had cornified cells. Metestrus phase had an equal amount of all three cells. Finally, the diestrus phase consisted of predominantly leukocytes.

Statistics:

Repeated measures Analysis of Variance (ANOVA) as studied by the Statistica 6.0 Software program (Tulsa, OK) was used to determine statistically significant differences in behavioral responses after administration of drug(s) within and between sex, trial, drug and dose. *Fisher post hoc* tests performed pairwise statistical analyses within each individual group. Alpha significance was set at the $P<0.05$ level.

Results:

Female ASR

An overall 3-way Repeated Measures ANOVA analysis was used for the following results (Fig. 1-3). This ANOVA showed drug ($F(2,83)=11.9$, $p=0.00003$), dose ($F(3,83)=13.2$, $p<0.00001$), drug x dose ($F(6,83)=5.2$, $p=0.00015$), trial ($F(2,166)=28.2$, $p<0.00001$), trial x drug ($F(4,166)=5.9$, $p=0.000178$), and trial x dose ($F(6,166)=3.5$, $p=0.00264$) effects.

Female cocaine ASR

There were no changes in %PPI following saline injection or post 1 hour trials (Fig. 1). On the other hand, females decreased %PPI response after cocaine administration, regardless of dose. A *Fisher* pairwise post hoc test revealed significant decrease in %PPI between the cocaine group from the habituation trial to the injection trial for the LD ($p=0.00001$), MD ($p=0.00001$), and HD ($p=0.00001$) groups. During the injection trial, cocaine decreased %PPI compared to the saline group for the LD ($p=0.001$), MD ($p=0.00001$), and HD ($p=0.00001$) groups. Within the dose groups, HD had significantly lower %PPI compared to the LD group during the injection trial ($p=0.0304$). During the post 1-hour trial, the LD ($p=0.0432$), MD ($p=0.00001$), and HD ($p=0.00001$) groups had significantly lower %PPI than the saline group. Additionally during the post 1-hour trial, the LD ($p=0.0302$), MD ($p=0.00001$), and HD ($p=0.00001$) groups were significantly lower compared to their corresponding habituation trial. The LD group had significantly higher %PPI compared to the MD ($p=0.0255$) and HD ($p=0.0123$) groups during the post 1-hour trial. Both LD ($p=0.0166$) and HD ($p=0.0303$) groups increased %PPI from the injection trial to the post 1-hour trial but were still lower than the habituation trial.

Female Caffeine ASR

After caffeine administration %PPI remained relatively stable from the habituation trial to the injection trial (Fig. 2). After post 1-hour, %PPI was significantly reduced for both LD ($p=0.0465$) and MD ($p=0.0199$) compared to saline. The LD group significantly decreased %PPI from the habituation to the post 1-hour trial ($p=0.0221$). The MD group had lower PPI responses compared to the HD group during the post 1-hour trial ($p=0.0274$). Across drugs, LD ($p=0.0060$), MD ($p=0.0010$) and HD ($p=0.00001$) groups had significantly lower %PPI for the cocaine group compared to the caffeine group during the injection trial. Cocaine HD group had significantly reduced %PPI compared to caffeine HD group during the post 1-hour trial ($p=0.00001$).

Female combination ASR

There were no changes in %PPI following administration of both LD and HD cocaine and caffeine across all trials (Fig. 3). The MD combination group had reduced

%PPI compared to saline (p=0.00001), LD (p=0.00001), and HD (p=0.00001) groups during the injection trial. The MD combination group had significantly lower %PPI during injection trial compared to habituation (p=0.00001) and post 1-hour (p=0.0031) trials. During the injection trial, the MD combination group had significantly lower PPI responses compared to the MD caffeine group (p=0.0002). Importantly, cocaine LD and HD groups had significantly reduced %PPI compared to combination LD (p=0.0008) and HD (p=0.00001) groups following the injection trial. PPI responses were significantly reduced in the post 1-hour trial for the cocaine HD group compared to the combination HD group (p=0.0010).

Male ASR Data

An overall 3-way Repeated Measures ANOVA analysis was used to examine the male data (Fig. 4-6). There were drug ($F(2,85)=13.6$, $p=0.00007$), dose ($F(3,85)=8.7$, $p<0.00004$), drug x dose ($F(6,85)=5.2$, $p=0.00013$), trial ($F(2,170)=58$, $p<0.00001$), trial x drug ($F(4,170)=23.7$, $p<0.00001$), trial x dose ($F(6,170)=9.8$, $p<0.00001$), and trial x drug x dose ($F(12,170)=7.2$, $p<0.00001$) effects.

Male cocaine ASR:

There were no significant changes in %PPI following saline administration for all trials (Fig. 4). During the injection trial, there were no changes in PPI responses for the LD or MD groups but %PPI decreased for the HD group compared to the habituation trial (p=0.0054). Male PPI responses following the LD of cocaine caused decreased PPI (p=0.0081), however, this was to a lower degree than females. During the post 1 hour trial, both MD and HD groups showed lower %PPI in a dose-dependent manner and showed significance to the habituation (MD: p=0.00001; HD: p=0.00001) and the injection (MD: p=0.00001; HD: p=0.00001) trials and to the saline group within the post 1-hour trial (MD: p=0.00001; HD: p=0.00001). The HD group during post 1-hour trial was significantly lower in PPI responses compared to both the LD (p=0.00001) and the MD (p=0.00001) groups.

Male caffeine ASR:

There were no differences in the male caffeine group following all trials despite drug or dose (Fig. 5).

Male combination ASR:

The LD or HD groups did not differ in PPI responses throughout the trials (Fig. 6). On the other hand, the MD group showed decreased %PPI following the injection trial compared to the saline (p=0.00001), LD (p=0.00001), HD (p=0.00001) groups and to the habituation trial (p=0.00001). The same was seen during the post 1-hour trial compared to the saline (p=0.0011), LD (p=0.0039), HD (p=0.0421) groups and to the habituation

trial (p=0.0001). Across drugs, MD combination group had significantly lower PPI responses compared to cocaine (p=0.00001) and caffeine (p=0.00001) MD groups during the injection trial. The combination MD group had lower %PPI compared to the caffeine MD group (p=0.0035) during the post 1-hour trial, while, it had higher %PPI compared to the cocaine MD group (p=0.0005). Importantly, the combination HD group had significantly higher PPI responses compared to the cocaine HD group during the post 1-hour trial (p=0.00001).

Male vs. Female ASR data:

An overall 4-way Repeated Measures ANOVA analysis was used to examine the female data compared to the male data (Fig. 1-6). There were sex ($F(1,168)=34.3$, $p=0.00001$), sex x dose ($F(3,168)=3.3$, $p=0.0215$), trial x sex ($F(2,336)=9.2$, $p=0.00013$), trial x sex x drug ($F(4,336)=13.7$, $p=0.00001$), trial x sex x dose ($F(6,336)=3.1$, $p=0.0057$), and trial x sex x drug x dose ($F(12,336)=2.0$, $p=0.0235$). During the injection trial following cocaine, females had significantly lower PPI responses compared to the male LD (p=0.00001), MD (p=0.00001) and HD (p=0.00001) groups (Fig. 1,4). During the post 1-hour trial, the male cocaine HD group had lower %PPI than the female group (p=0.00001). Within the caffeine groups, males had higher PPI responses than females during the post 1-hour trial for the LD (p=0.0172) and MD (p=0.0047) groups (Fig. 2,5). During the combination doses and throughout the trials, females and males exhibited similar behavior and did not significantly differ in PPI responses (Fig. 3,6).

Estrus cycle data

Female Vaginal Smears

Vaginal smears were assessed using a microscope and scored based on cells described in the methods section (Fig. 7a-h). For all doses of cocaine, female smears showed differences in estrus cycle phase between pre- and post-injection trials. Cocaine induced seemingly-random changes in the estrus cycle (Fig. 7 c-d). This was not seen following caffeine administration (Fig. 7e-f). Furthermore, combination of cocaine and caffeine did not alter the estrus cycle (Fig. 7g-h). The cocaine-induced changes were blocked by caffeine.

Female PPI vs. changes in estrus cycle correlations:

There was a negative correlation between PPI responses and number of changes in the estrus cycle, i.e. the more changes in the estrus cycle induced by cocaine the lower PPI responses (Fig. 8a,b). Past studies have shown that behavioral responses to cocaine are related to estrogen levels. On the other hand, we did not find a specific trend in one precise phase but in the number of phase fluctuations. There was no correlations between the lowest PPI response and highest estrogen levels (proestrus phase). Instead, cocaine

induced such changes that the post-injection smear was in 3 phases different from the pre-injection smear. The order of phases went as follows: proestrus-estrus-metestrus-diestrus. Thus, if the pre-injection smear was in estrus phase and the post-injection smear was in proestrus phase, the number of phase jumps was 3. This negative relationship was seen greater after the injection trial ($R(30)=0.58466$, $p<0.00044$) compared to the post 1-hour trial ($R(30)=0.45312$, $p<0.00920$), suggesting a time sensitive correlation based on the pharmacokinetics of cocaine. It is important to note that vaginal smears were only collected after the injection trial not the post 1-hour trial. Therefore, scores in Fig. 10b are correlated to the phase changes 1-hour prior to PPI testing.

Figures:

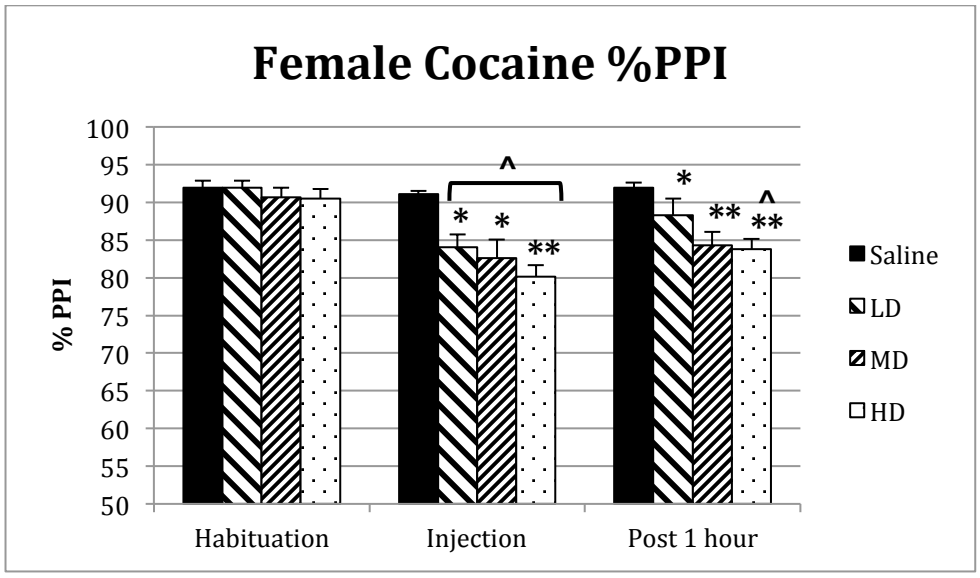


Figure 1: Female ASR data following cocaine administration, %PPI response decreased dose-dependently. Saline injection did not alter %PPI for all three trials. 1-hour after injection, %PPI increased for the LD and HD groups but did not reach the saline group levels. MD group remained relatively stable between injection trial and post-1 hour trial. Significance was denoted by $p<0.05$. LD= low dose; 2.5mg/kg, MD= middle dose; 5mg/kg, and HD= high dose; 10mg/kg. *: significant to saline group and habituation trial; **: significant to saline group, LD group, and habituation trial; ^: significant to respectful caffeine group.

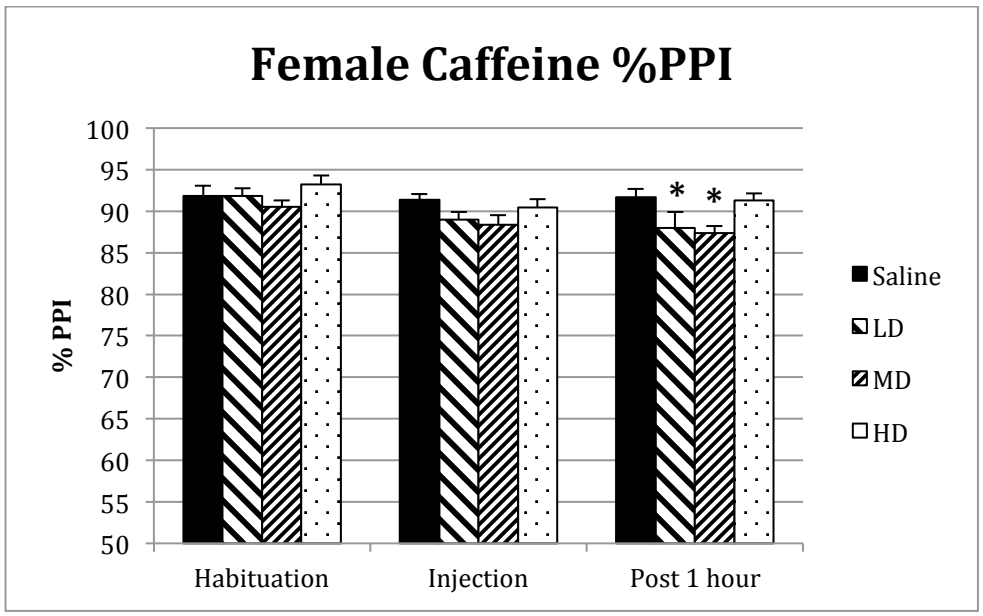


Figure 2: Female ASR data following various doses of caffeine administration, %PPI did not significantly change. Post 1-hour trial had significantly lower %PPI for the LD and MD groups from the saline group. The HD group did not. Significance was denoted by $p<0.05$. LD= low dose; 12.5mg/kg, MD= middle dose; 25mg/kg, and HD= high dose; 50mg/kg. *: significant to saline group, HD group, and habituation trial.

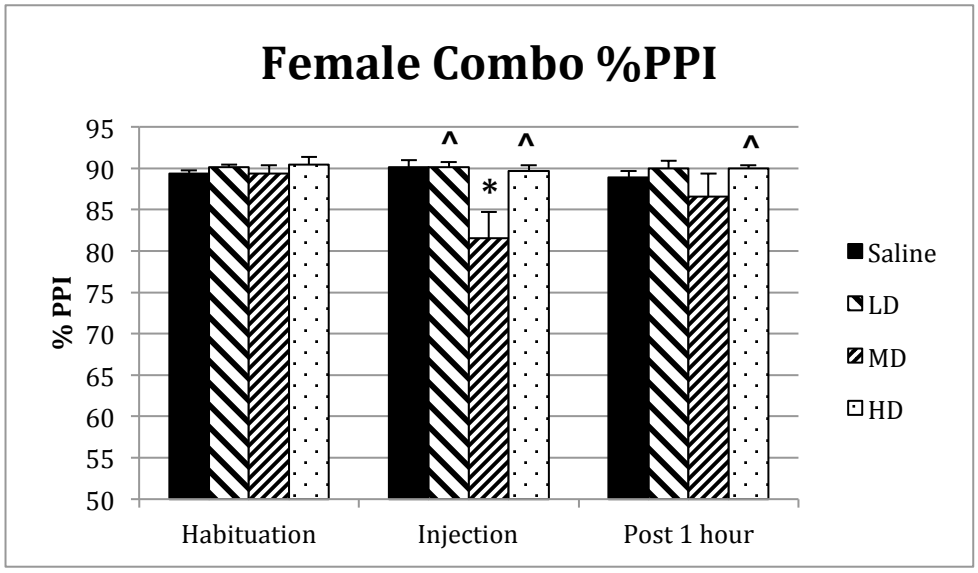


Figure 3: Combination of both cocaine and caffeine for female ASR data showed no differences between saline, LD or HD groups. All groups responded to acoustic startle the same way. However, the MD group significantly decreased %PPI during the injection trial and then increased post 1-hour. Significance was denoted by $p<0.05$. LD= low dose;

2.5mg/kg cocaine and 12.5mg/kg caffeine, MD= middle dose; 5mg/kg cocaine and 25mg/kg caffeine, and HD= high dose; 10mg/kg cocaine and 50mg/kg caffeine. ^: significant to cocaine group; *: significant to habituation trial, post injection trial, saline group, LD group, HD group, and respectful caffeine group.

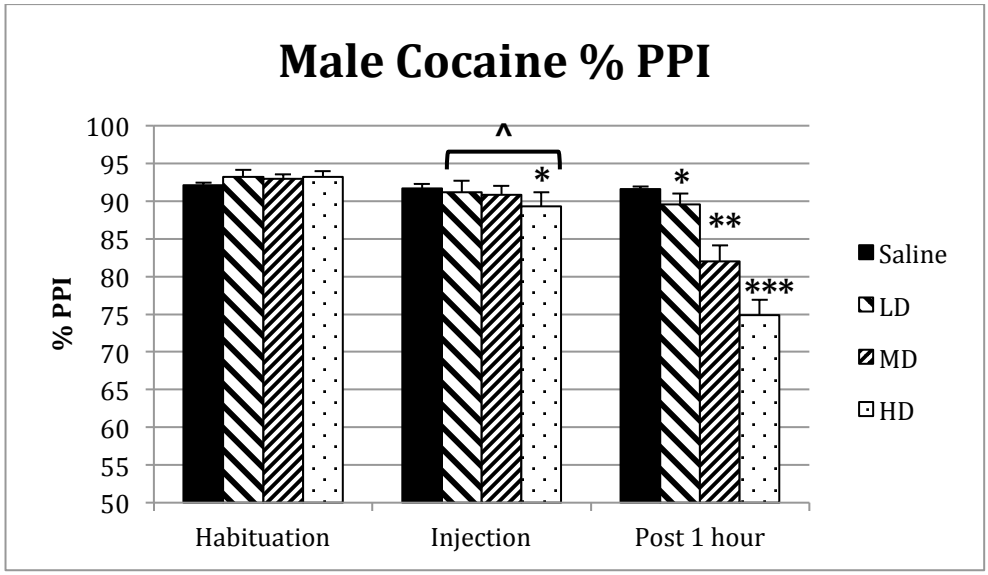


Figure 4: Following cocaine administration, PPI responses for males did not differ, however, post 1-hour %PPI response decreased dose-dependently. Saline injection did not alter %PPI for all three trials. Also, the LD group did not affect PPI as seen with females. Significance was denoted by $p<0.05$. LD= low dose 2.5mg/kg, MD= middle dose 5mg/kg, and HD= high dose 10mg/kg. *: significant to habituation trial; **: significant to habituation trial, injection trial, saline group, LD group, and respectful caffeine group; ***: significant to habituation trial, injection trial, saline group, LD group, MD group, and respectful caffeine and combination groups; ^: significant to respectful female group.

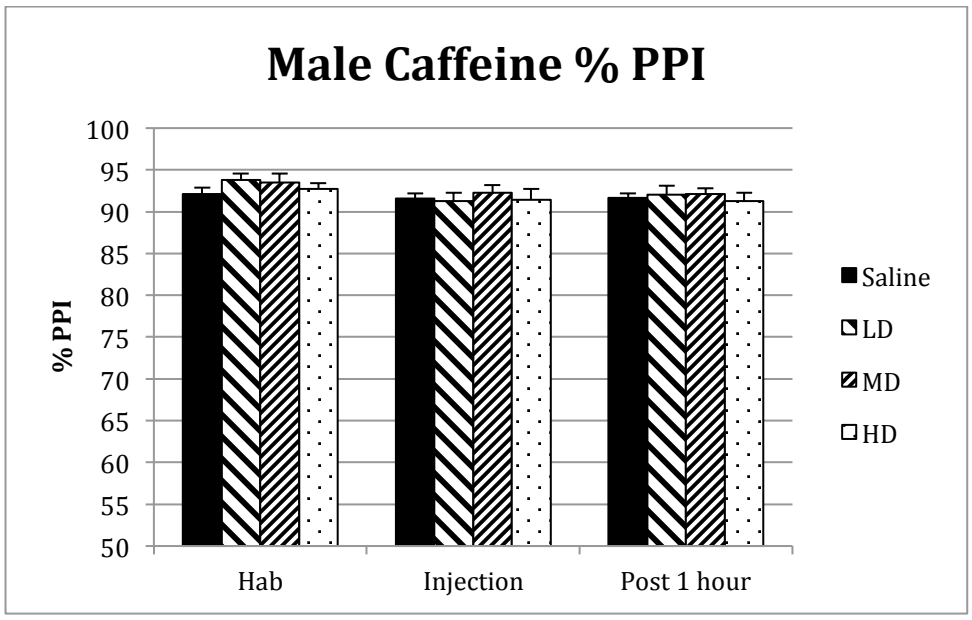


Figure 5: Male ASR data following various doses of caffeine administration, %PPI did not significantly change throughout the three trials. LD= low dose; 12.5mg/kg, MD= middle dose; 25mg/kg, and HD= high dose; 50mg/kg.

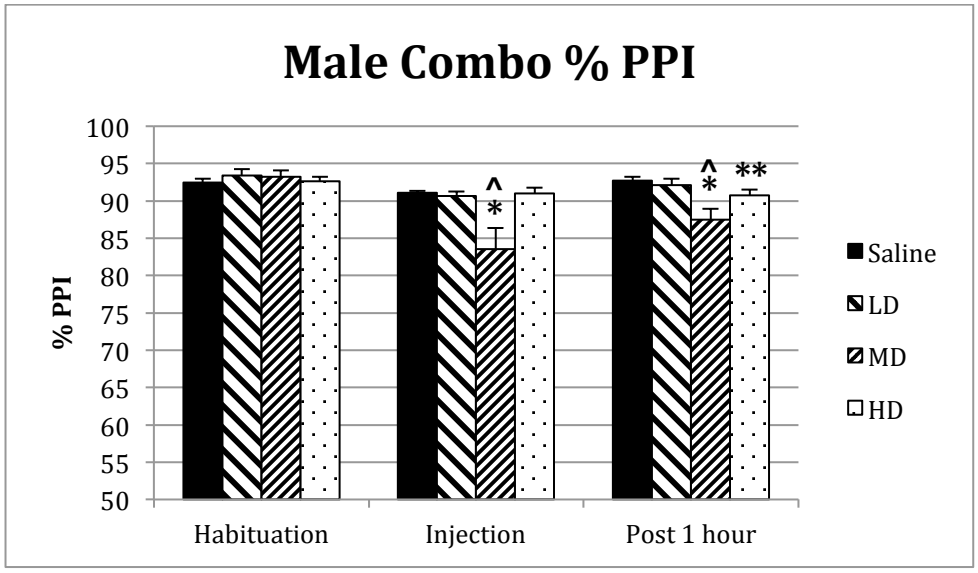
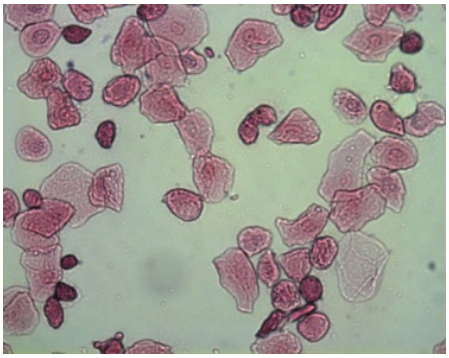


Figure 6: Throughout the three trials combination of both cocaine and caffeine for male ASR data showed no differences between saline, LD or HD groups. However, the MD group significantly decreased PPI responses during the injection trial and then increased slightly during the post 1-hour trial. Significance was denoted by $p<0.05$. LD= low dose; 2.5mg/kg cocaine and 12.5mg/kg caffeine, MD= middle dose; 5mg/kg cocaine and 25mg/kg caffeine, and HD= high dose; 10mg/kg cocaine and 50mg/kg caffeine. *: significant to habituation trial, saline group, LD group, and HD group; **: significant to respectful cocaine group; ^: significant to respectful cocaine and caffeine groups.

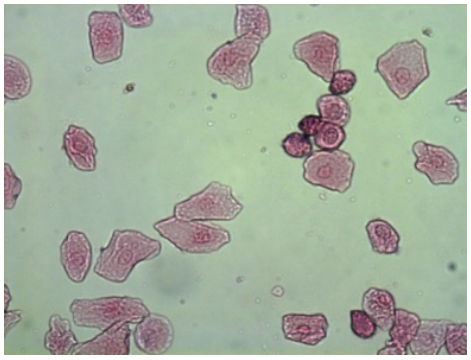
7 a, b

Pre-Saline



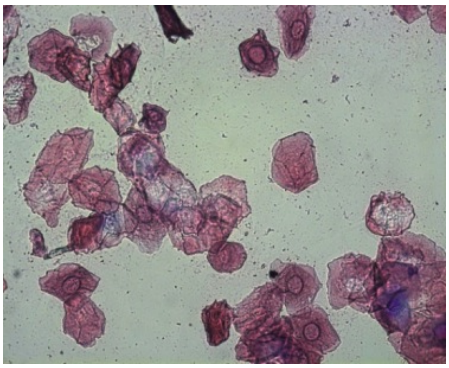
Proestrus → Proestrus

Post-Saline



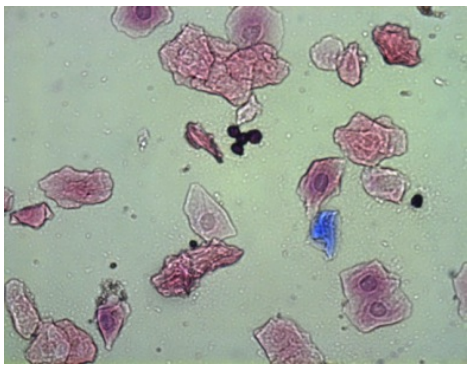
7 c, d

Pre-2.5 mg/kg Cocaine



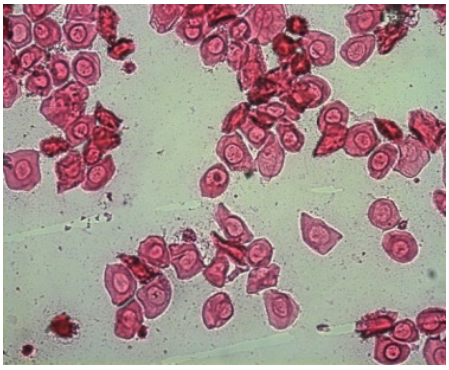
Estrus → Metestrus

Post-2.5 mg/kg Cocaine



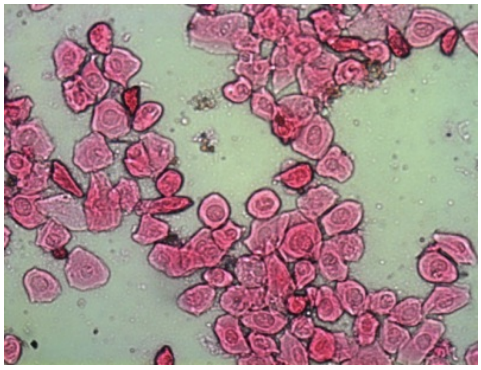
7 e, f

Pre-12.5 mg/kg Caffeine



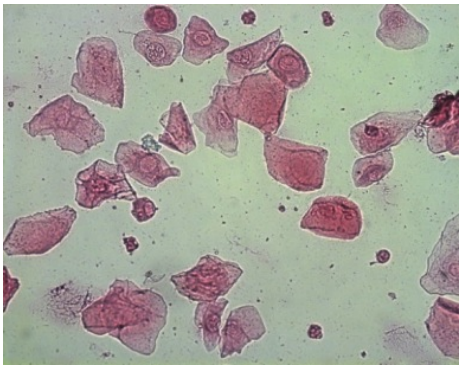
Proestrus → Proestrus

Post-12.5 mg/kg Caffeine



7 g, h

Pre-2.5/12.5 combo



Metestrus → Metestrus

Post- 2.5/12.5 combo

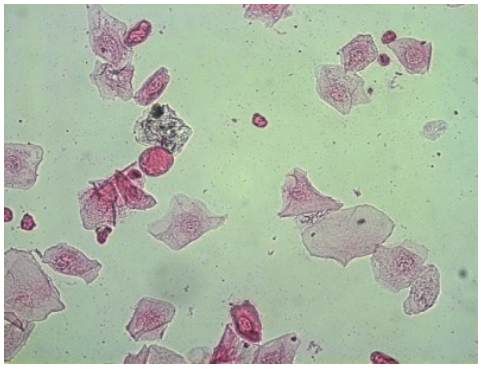


Fig 7 a-h: Studies on the estrus cycle in females show that cocaine induces changes in the estrus cycle that is not seen with caffeine, combination of cocaine and caffeine or saline. These changes are even expressed at the low dose as seen above.

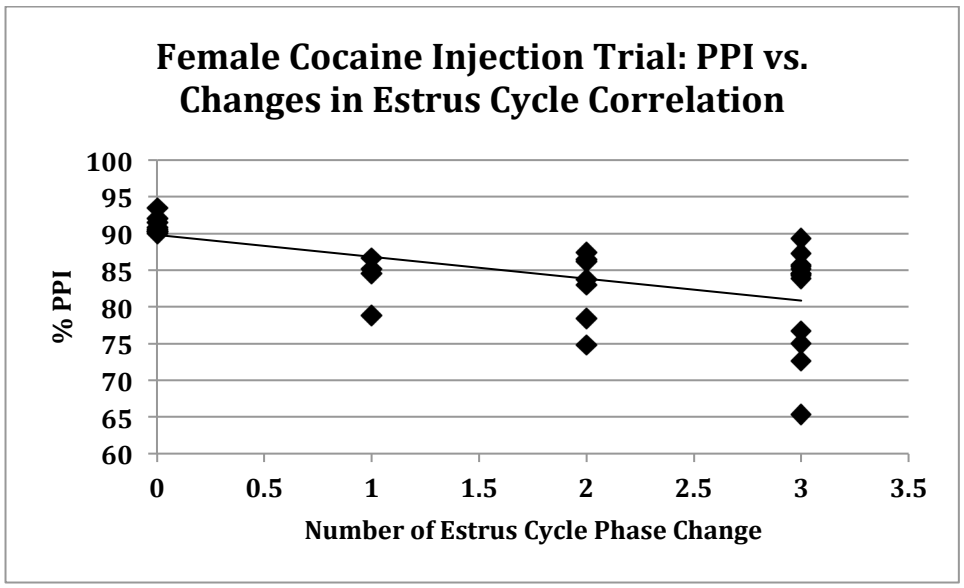


Fig. 8a: ASR responses were correlated with number of estrus cycle phase changes following the cocaine injection trial. Cocaine induced randomized changes in the estrus cycle that were correlated to their %PPI scores. There was an indirect relationship between the number of phase changes to PPI responses, i.e. if the animal's estrus cycle jumped from estrus to proestrus the phase change would be "3". The order of phases in the estrus cycle went from proestrus-estrus-metestrus-diestrus. R value= 0.58466.

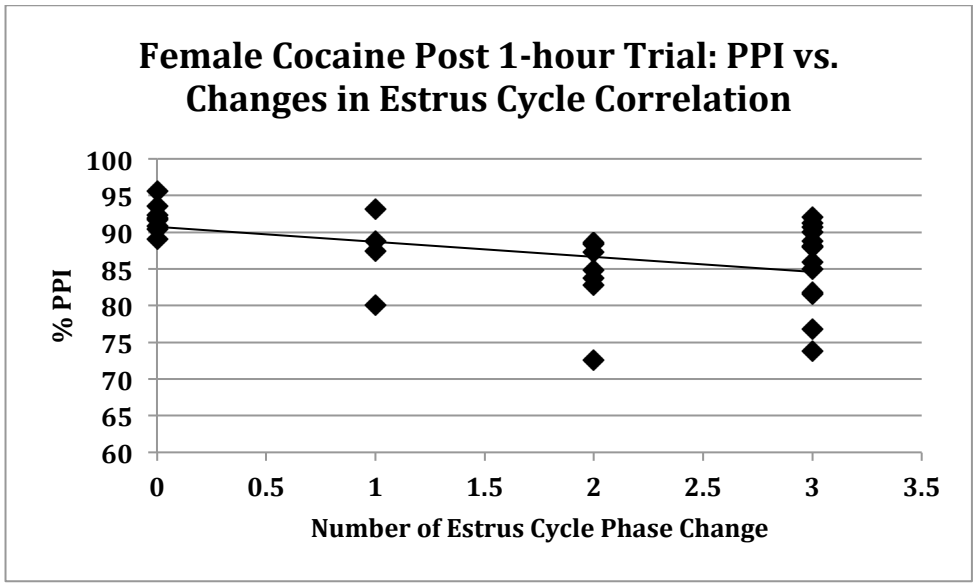


Fig. 8b: ASR responses were correlated as described in Fig. 8a, but for the cocaine post 1-hour trial. There is a negative correlation. Correlation was not as strong during the post 1-hour trial compared to the injection trial, which may be a result of the time gap. R value=0.45312.

Discussion:

The major findings of this study are 1) females showed exacerbated PPI responses, that is deficits in sensorimotor gating, to cocaine compared to males, 2) caffeine neuroprotected against cocaine-induced deficits in sensorimotor gating, reflecting similar patterns in DA release and estrus cycle changes, 3) this neuroprotection was highly dose-dependent at low and high doses, and 4) cocaine caused rapid changes in the estrus cycle that was blocked with the combination of caffeine, indicating an interaction between estrogen and DA with a regulatory role of adenosine.

Sensorimotor gating deficit responses were detected at a higher degree for females compared to males during the low dose of cocaine. The effects of the low dose of cocaine were minimal in the males, whereas, the females showed significant disruption in sensorimotor gating. This is consistent with the literature on cocaine sensitivity, acquisition, dependence and relapse being stronger in females than males at lower doses (Griffin et al., 1989, Becker and Hu, 2008, Quinones-Jenab and Jenab, 2012). Females were found to associate cocaine use to contextual cues and conditioned stimuli more so than males (Quinones-Jenab and Jenab, 2012).

There are sex differences observed throughout all stages of drug abuse. Our results show that females were more sensitive to the aversive cognitive effects of cocaine. Decreased PPI reflects a schizophrenic phenotype, therefore, this may provide insight as to why females have different symptoms than males and are more sensitive to cognitive disarray (Häfner, 2003). In general, males have a quicker onset than females, however, females experience more positive symptoms and males experience more negative symptoms (Castle and Murray, 1993, Castle, 1999). Positive symptoms include delusions, thought disorder, and hallucinations. These respond well to medication because they involve a chemical imbalance in the brain associated with DA. Negative symptoms are more behavior and social developmental problems that do not respond well to treatment. With schizophrenia, females are more vulnerable to sensorimotor deficits than males. Thus, our results indicate greater sensitivity to ASR consistent with female psychosis symptoms.

Corresponding with the literature, cocaine administration produced a linear decrease in PPI for the increasing doses. This finding was regardless of sex. However, females responded at a faster rate than males for the same dose. In the literature, female locomotor responses were double-fold or higher to male response at the same dose and lasted longer (Quinones-Jenab and Jenab, 2012). Previous studies in this laboratory on the release of DA in the NAc *in vivo* following low, middle, and high doses of cocaine showed this linear effect as well (Broderick et al., 2006, Broderick et al., 2007). Additionally, preliminary data in this laboratory showed that females had higher release of DA at lower doses compared to males. Thus, males needed a higher dose to reach equivalent levels of DA release and locomotor effects as females (Quinones-Jenab and

Jenab, 2012). These sex differences were more substantial at lower doses, whereas, the gap between sexes decreased with higher doses. Importantly, this increased vulnerability to cocaine in females was not caused by the pharmacokinetic differences of cocaine between sexes (Evans and Foltin, 2010). Females had similar benzoylecgonine (a cocaine metabolite) plasma levels to males, indicating similar metabolism of cocaine. Also, peak cocaine plasma levels did not change between different estrus cycle phases. Therefore, estrogen did not change the rate of metabolism.

Since males require higher doses of cocaine to elicit similar biochemical and behavioral responses to females, one drawback of this study is that doses did not exceed 10mg/kg of cocaine. However, previous studies in this laboratory used doses 10-40mg/kg of cocaine during the ASR for males and females (Broderick and Rosenbaum, 2013). Results showed that both males and females exhibited decreased PPI at these doses and were more exacerbated in females. In the current study, males administered 10mg/kg started to show a decrease in PPI during the injection trial compared to the habituation trial that was not seen during the low or middle dose groups. Thus, higher doses of cocaine can produce greater responses earlier in trials and the doses used in this experiment were too low to affect sensorimotor gating to a worse degree.

Alternatively, these sex differences in ASR were abolished following caffeine administration. Caffeine is showing more and more clinical relevance, caused by its antagonistic effects on adenosine, including protecting against Parkinson's Disease and aiding in stroke (Ross et al., 2000, Rivera-Oliver and Díaz-Ríos, 2014). It is well established that caffeine has biphasic properties and is behaviorally active at specific doses (Fredholm et al., 1999). Extremely high doses of caffeine show aversive effects. The LD_{50} in rodents for caffeine is 200mg/kg (Eichler, 1976). Caffeine studies must adjust for metabolic body weight to accurately compare rodents to humans. For instance, 10mg/kg of caffeine injected into a rat is equivalent to 250mg for a human weighing 70kg or 3.5mg/kg dose, roughly 2-3 cups of coffee (Morgan et al., 1982). The route of entry is also different; rodents are given i.p. or i.v. injections while humans orally consume caffeine periodically throughout the day. However, in this study we were testing the immediate response of specific doses of caffeine and their interaction with cocaine.

Regardless of dose, caffeine did not alter PPI responses for either males or females during the injection phase. On the other hand, females started to show a minimal decrease in PPI responses post-1 hour after the caffeine injection for the low and middle dose groups. This effect was not seen following the high dose. Therefore, caffeine had minor residue effects that caused decreased PPI responses for low and middle doses. It could be postulated that this decrease is caused by the "crash" associated after caffeine intake. More research is needed to determine this effect and to explain why it was not seen after the high dose. There is little research on the residual effects of caffeine on ASR (Fredholm et al., 1999).

Interestingly, cocaine and caffeine studies have shown very dose-specific effects. Conditioned place preference (CPP) and drug discrimination studies showed biphasic results: increasing effects of cocaine at first and then decreasing effects, that of which were reflective of the caffeine dose (Harland et al., 1989, Bedingfield et al., 1998, Fredholm et al., 1999). Previous studies in this laboratory found that males following the high dose paradigm (10mg/kg cocaine and 50mg/kg caffeine) showed cocaine CPP, caffeine CPP aversion, and CPP levels similar to baseline in combination (Nesbitt et al., 2008). Thus, at the high dose caffeine blocked the CPP abilities of cocaine. Self-administration studies showed caffeine-induced reinstatement of extinguished cocaine seeking behavior. However, doses did not exceed 20mg/kg (Green and Schenk, 2002). Indeed, caffeine has been shown to have aversive effects at doses exceeding 30mg/kg (Brockwell et al., 1991). Additionally, results from our laboratory showed biochemical and behavioral evidence in this dose-specific response of caffeine (Broderick et al., 2008, Malave and Broderick, 2014). Based on this we propose that behaviorally active doses of caffeine potentiate cocaine's actions, whereas, low and high doses of caffeine neuroprotect.

In the present results, this neuroprotective behavior (i.e. the reversal of cocaine-induced PPI deficits after combination with caffeine) was seen at low doses and high doses for both males and females. On the other hand, neuroprotection was not seen for the middle dose group. These results parallel the previous neuromolecular imaging data from this laboratory on DA release. Also, this is consistent with the inverted "U-shaped" curve observed with peak caffeine responses at the 30mg/kg dose. There is a synergic effect at the peak middle dose of caffeine when combined with cocaine. Low and high doses of caffeine were shown to release DA at a lower rate than the middle dose, and thus did not contribute to this further disruption in PPI with cocaine but acted to reverse it.

The lack of neuroprotection at the peak middle dose following combination of both cocaine and caffeine could be dependent on the interaction between the two-receptor types. Since caffeine is non-selective the activation and the communication of both A1R and A2AR may be involved. On GABAergic neurons DA and adenosine receptors form heteromers that cross talk and are highly dependent on each other (Ferré et al., 2007a, Ferré et al., 2007b). On Glutamatergic neurons, A1R and A2ARs form heteromers and indirectly influence DA release (Ciruela et al., 2006). Previous data on A2AR antagonists showed the importance of blocking A2AR in the stimulatory effects of caffeine (Ledent et al., 1997, El Yacoubi et al., 2000), and that A1R inhibition potentiated A2AR effects (Karcz-Kubicha et al., 2003). Without blocking A1R, the stimulatory effects of A2AR antagonists were lower, indicating an important role for inhibiting both to induce increased stimulatory response (Halldner et al., 2004). Thus, the interaction of both receptors may potentiate cocaine's response leading to the synergistic effect for both DA release and PPI deficits observed at the combination middle dose.

On the other hand, the neuroprotective behavior of caffeine may be caused by the low and high doses activating different receptors. Past studies have found that A1R are activated first by caffeine because of their higher affinity (Ferré et al., 2013). Then at increasing doses A2AR are activated which consequently decreases A1R activity. Therefore, at low doses, A1Rs are predominately active, eliminating the A1R-A2AR heteromer stimulatory interaction. Previous data in this laboratory using A1R antagonist, CPT, showed the ability of CPT (4.8mg/kg) to decrease DA release of 5mg/kg cocaine (Broderick et al., 2006, Malave and Broderick, 2014). Similarly, at high doses of caffeine A1Rs have been shown to be predominately involved in the depressant locomotor effects of caffeine (El Yacoubi et al., 2000). Thus, A1R selective antagonism may play a crucial role in this neuroprotection. Alternatively, interaction with other mechanisms may be the underlying cause of neuroprotection seen at the high dose, such as regulation of phosphodiesterase and calcium (Daly, 1993, Fredholm et al., 1999). Additional selective adenosine antagonists, specifically A2AR, on DA release in combination with cocaine and during ASR studies are needed.

Duration of caffeine exposure can be a factor as well. Acute vs. chronic studies using an i.p injection vs. freely drinking access of caffeine showed attenuation in cocaine self-administration in chronic caffeine use than in acute low dose use (Kuzmin et al., 2000). Chronic caffeine drinking increased c-*fos* and NGFI-A mRNA in the cerebral cortex following administration of cocaine. c-*fos* and NGFI-A are transcription factors characterized as immediate early genes (IEG). IEGs are activated in response to first round of stimuli at the transcription level and regulate cell growth and differentiation signaling. Increased neuronal activity cause increased IEG expression (Fredholm et al., 1999). Late response genes are activated based on IEGs. Thus, chronic caffeine use changed the expression of proteins in the brain that are shown to regulate cocaine use (Kuzmin et al., 2000). Additionally, repeated exposure to caffeine was shown to eliminate the reinstatement of cocaine extinction that is present during the acute dose (Schenk et al., 1996).

Furthermore, there are beneficial aspects to chronic caffeine use. Long-term use was shown to be associated with decreased seizure susceptibility (Von Lubitz et al., 1993b), decreased ischemic brain damage (Rudolphi et al., 1989) and increased spatial learning capacity (Von Lubitz et al., 1993a). Daily caffeine exposure at a low dose could cause characteristics similar to an acute high dose of caffeine shown to decrease cocaine-induced effects. Indeed, IEGs are increased following doses exceeding 50mg/kg of caffeine (Svenningsson et al., 1995). Therefore, high doses of caffeine may facilitate important adaptive transcriptional or neuropeptide protein expression in the brain inducing neuroprotective behavior.

Expanding on this concept, patients with schizophrenia have increased intake of caffeine suggesting a form of self-medication (Hughes et al., 1998, Lara, 2010). Although we state that behaviorally active doses of caffeine potentiate cocaine-induced disruption

in PPI, this is at an acute dose. Caffeine intake is habitually consumed throughout the day with multiple servings on a daily basis. Therefore, the chronic intake of caffeine may aid in psychosis symptoms inducing protein expression, via IEGs, protecting against the aversive effects of schizophrenia. This is a fairly new concept and more preclinical and clinical research is needed.

The sex differences observed in this study were highly dependent on sex hormones. We observed that cocaine induced phase changes in the estrus cycle upon injection at all doses, whereas, caffeine did not produce such changes. Interestingly, the cycle altering effects of cocaine were blocked in the combination of caffeine and cocaine. This pattern of neuroprotection via caffeine was similar to responses in PPI and DA release studies suggesting that DA, adenosine, and estrogen strongly interact with each other causing increased sensitivity to cocaine that was reversed when the adenosine system was antagonized.

Next, we looked at correlating the PPI responses with the estrus cycle changes. Since PPI is DA dependent, we predicted higher levels of estrogen (during proestrus) would induce greater PPI deficiencies. However, our correlations did not conclude concrete evidence for a specific phase causing the most vulnerability. Instead we found a negative relationship between PPI scores and the number of phase changes in the estrus cycle. For instance, pre-cocaine injection smears in the estrus phase changing to proestrus phase in post-cocaine injection of the same animal would be considered 3 phase jumps based on the succession of phases in the cycle: proestrus-estrus-metestrus-diestrus. Therefore, the increased sensitivity of sensorimotor gating from cocaine appeared to be caused by the change in phase itself and the impact of the fluctuation in hormone levels instead of one specific phase.

Sex differences are seen throughout the stages of drug abuse. Thus, this is a complex problem and may have a combination of factors. More studies are needed to extensively examine this concept in ASR. Ovariectomy with supplemental hormone treatment can help determine exact interactions between PPI responses and sex hormone levels. Blood samples can be taken to quantify concentrations of circulating sex hormones with ELISA kits in addition to vaginal smears.

There is another method of analyzing the estrus cycle phase changes. This method interestingly reads the electrical resistance of the vaginal mucosa (EC40, Fine Science Tools, Foster City, CA). Preliminary data in this laboratory shows a correlation between estrus cycle phase and voltage (Nesbitt et al., 2008). Using a volt reader to swab the same way as a vaginal smear elicits a voltage that varies between the four estrus phases. The preliminary results show that cocaine changes the electrical resistance (kilohm) at low, middle, and high doses, whereas, caffeine does not significantly change the electrical resistance. Additionally, combination of cocaine with caffeine does not change the electrical resistance. The electrical resistance voltage and vaginal smear exfoliate cytology show paralleling results and reflect the DA release and PPI results. The volt

reader gives a conclusive number immediately after, whereas a vaginal smear is subjectively scored. Therefore, the electrical resistance of the vaginal mucosa recording is potentially a new tool to better test/evaluate estrus cycle changes.

In addition to hormone effects, there may be other factors involved in these sex differences (Quinones-Jenab and Jenab, 2012). Differences in signaling caused by sexually dimorphic regulation of DA or other neuronal regulators may be the origin of these differences. Also, differences caused by the sex chromosomes on the nigrostriatal or mesocorticolimbic system could play a role.

Moreover, there were no changes in the estrus cycle for females following the middle dose in combination. However, there were differences in PPI and DA release, indicating that these neuroprotective differences were not a result of estrogen levels. These differences reflect the male data further proving that estrogen is not involved in the behavioral and biochemical differences at the middle dose because it is present for both sexes.

For future studies, the BRODERICK PROBE® biosensor will be placed in the NAc imaging DA release *in vivo* while simultaneously in the ASR paradigm to better correlate DA release to behavior. Another important area of interest in the ASR pathway is the PnC (Fendt and Koch, 1999). As described earlier the PnC is strongly involved in PPI. Therefore, the BRODERICK PROBE® biosensor can detect neurotransmitter activity in the PnC as a direct result of cocaine and caffeine activation in the NAc and its effect on ASR.

In conclusion, we propose evidence that disruptions in PPI are directly contingent on interactions between DA and the adenosine system, providing a close cause and effect relationship between brain and behavior that is strongly dependent on dose to produce neuroprotective behavior. Additionally, these results provide insight on sex differences in cocaine abuse pointing to the strong interaction between estrogen and DA in concert with modulation from the adenosine system. Furthermore, we suggest potential treatment options for mental disorders involving the malfunction of the DA circuit via the adenosine system.

Acknowledgements:

Lauren Malave has performed the above research in Dr. Patricia Broderick's laboratory in the Department of Physiology, Pharmacology and Neuroscience at the Sophie Davis School of Biomedical Education, CCNY, CUNY and Neurol., NYU Langone Med. Ctr., New York, NY. Dr. Broderick is a Principal Investigator, Medical Professor and Mentor.

The author wishes to thank the Broderick Brain Foundation, the F.M. Kirby Foundation, the Center for Advanced Technology CUNY, the City College of New York, and the MacKenzie Foundation for funding support during these studies.

References:

Abduljawad KA, Langley RW, Bradshaw CM, Szabadi E (1997) Effects of clonidine and diazepam on the acoustic startle response and on its inhibition by 'prepulses' in man. J Psychopharmacology 11:29-34.

Bakshi VP, Geyer MA, Taaid N, Swerdlow NR (1995) A comparison of the effects of amphetamine, strychnine and caffeine on prepulse inhibition and latent inhibition. Behavioral Pharmacology 6:801-809.

Becker JB, Hu M (2008) Sex differences in drug abuse. Frontiers in Neuroendocrinology 29:36-47.

Bedingfield JB, King DA, Holloway FA (1998) Cocaine and caffeine: conditioned place preference, activity, and additivity. . Pharmacology Biochemistry and Behavior 61:291-296.

Bickford PC, Fredholm BB, Dunwiddie TV, Freedman R (1985) Inhibition of Purkinje cell firing by systemic administration of phenylisopropyl adenosine: effect of central noradrenaline depletion by DSP4. Life Sci 37:289-297.

Braff DL, Swerdlow NR, Geyer MA (1995) Prepulse intensity of the startle response in schizophrenia: the effects of varying prepulse intensity and gender. Proc Am Coll Neuropsychopharmacology 107.

Brockwell NT, Eikelboom R, Beninger RJ (1991) Caffeine-induced place and taste conditioning: Production of dose-dependent preference and aversion. Pharmacology Biochemistry and Behavior 38:513-517.

Broderick P, Ho H, Wat K, Murthy V (2008) Laurate Biosensors Image Brain Neurotransmitters In Vivo: Can an Antihypertensive Medication Alter Psychostimulant Behavior? Sensors 8:4033-4061.

Broderick PA (1988) Distinguishing in vitro electrochemical signatures for norepinephrine and dopamine. Neurosci Lett 95.

Broderick PA (1989a) Cathodic Electrochemical Current Arrangement with Telemetric Application. US Patent #4 883, 057.

Broderick PA (1989b) Characterizing stearate probes in vitro for the electrochemical detection of dopamine and serotonin. . Brain Res 495:115-121.

Broderick PA (1995) Microelectrodes and their use in cathodic electrochemical current arrangement with telemetric application. . US Patent #5 433, 710.

Broderick PA (1999) Microelectrodes and their use in an electrochemical arrangement with telemetric application. . US Patent #5 938, 710.

Broderick PA, Ho H, Huang J, Wat K, Shahidullah J, Murthy V, Wenning L, Buchheim L (2007) Caffeine may neuroprotect against cocaine-induced dopamine release in accumbens of female rats. Society for Neuroscience, San Diego.

Broderick PA, Murthy V, Forrester LW (2006) Adenosine A1 antagonist 8-cyclopentyltheophylline) mediates cocaine interactions; a view from the ventrolateral nucleus accumbens. Society for Neuroscience abstr, Atlanta, GA 32.

Broderick PA, Rosenbaum T (2013) Sex-Specific Brain Deficits in Auditory Processing in an Animal Model of Cocaine-Related Schizophrenic Disorders. Brain Sci 3:504-520.

Carter CS (1993) Neuroendocrinology of sexual behavior in the female. Behavioral Endocrinology 71-95.

Castle DJ (1999) Gender and age at onset in schizophrenia. Wrightson Biomedical Hampshire 147-164.

Castle DJ, Murray RM (1993) The Epidemiology of Late-onset Schizophrenia. Schizophrenia Bulletin 19:691-700.

Ciruela F, Casado V, Rodriques RJ, Lujan R, Burgueno J, Canals M, Borycz J, Rebola N, Goldberg SR, Mallol J, Cortes A, Canela E, Lopez-Gimenez JF, Milligan G, Lluis C, Cunha RA, Ferré S, Franco R (2006) Presynaptic control of striatal glutamatergic neurotransmission by adenosine A1-A2A receptor heteromers. J Neurosci 26:2080-2087.

Daly JW (1993) Mechanism of action of caffeine. Caffeine, Coffee, and Health 97-150.

Doherty JM, Masten VL, Powell SB, Ralph RJ, Klamer D, Low MJ, Geyer MA (2008) Contributions of Dopamine D1, D2, and D3 Receptor Subtypes to the Disruptive Effects of Cocaine on Prepulse Inhibition in Mice. Neuropharmacology 33:2648-2656.

Eichler O (1976) Zentrale Wirkung. Kaffee und Coffein 65-102.

El Yacoubi M, Ledent C, Ménard J-F, Parmentier M, Costentin J, Vaugeois J-M (2000) The stimulant effects of caffeine on locomotor behaviour in mice are mediated through its blockade of adenosine A2A receptors. British Journal of Pharmacology 129:1465-1473.

Evans S (2007) The role of estradiol and progesterone in modulating the subjective effects of stimulants in humans. Exp Clin Psychopharmacol 15:418-426.

Evans S, Haney M, Foltin R (2002) The effects of smoked cocaine during the follicular and luteal phases of the menstrual cycle in women. Psychopharmacology 159:397-406.

Evans SM, Foltin RW (2010) Does the response to cocaine differ as a function of sex or hormonal status in human and non-human primates? Hormones and Behavior 58:13-21.

Feltenstein MW, Byrd EA, Henderson AR, See RE (2009) Attenuation of cocaine-seeking by progesterone treatment in female rats. Psychoneuroendocrinology 34:343-352.

Feltenstein MW, See RE (2007) Plasma progesterone levels and cocaine-seeking in freely cycling female rats across the estrous cycle. Drug and Alcohol Dependence 89:183-189.

Fendt M, Koch M (1999) Cholinergic modulation of the acoustic startle response in the caudal pontine reticular nucleus of the rat. European Journal of Pharmacology 370:101-107.

Fendt M, Li L, Yeomans J (2001) Brain stem circuits mediating prepulse inhibition of the startle reflex. Psychopharmacology 156:216-224.

Fernstrom JD, Fernstrom MH (1984) Effects of caffeine on monoamine neurotransmitters in the central and peripheral nervous system. Caffeine: Perspectives from Recent Research 107-118.

Ferré S, Agnati LF, Ciruela F, Lluis C, Woods AS, Fuxe K, Franco R (2007a) Neurotransmitter receptor heteromers and their integrative role in 'local modules': The striatal spine module. Brain Research Reviews 55:55-67.

Ferré S, Ciruela F, Woods AS, Lluis C, Franco R (2007b) Functional relevance of neurotransmitter receptor heteromers in the central nervous system. Trends in Neurosciences 30:440-446.

Ferré S, Orrú M, Guitart X (2013) Paraxanthine: Connecting Caffeine to Nitric Oxide Neurotransmission. Journal of Caffeine Research 3:72-78.

Filip M, Frankowska M, Zaniewska M, Przegalinski E, Muller CE, Agnati L, Franco R, Roberts DC, Fuxe K (2006) Involvement of adenosine A2A and dopamine receptors in the locomotor and sensitizing effects of cocaine. Brain Res 1077:67-80.

Flaten MA, Elden Å (1999) Caffeine and prepulse inhibition of the acoustic startle reflex. Psychopharmacology 147:322-330.

Fredholm BB, Bättig K, Holmén J, Nehlig A, Zvartau EE (1999) Actions of Caffeine in the Brain with Special Reference to Factors That Contribute to Its Widespread Use. Pharmacological Reviews 51:83-133.

Fredholm BB, Dunwiddie TV (1988) How does adenosine inhibit transmitter release? Trends in Pharmacological Sciences 9:130-134.

Fredholm BB, Jonzon B (1988) Adenosine receptors: Agonists and antagonists,. Role of Adenosine in Cerebral Metabolism and Circulation 17-46.

Geyer MA, Braff DL (1987) Startle Habituation and Sensorimotor Gating in Schizophrenia and Related Animal Models. Schizophrenia Bulletin 13:643-668.

Graham F (1975) The more or less startling effects of weak prestimuli. Psychophysiology 12:238-248.

Green TA, Schenk S (2002) Dopaminergic mechanism for caffeine-produced cocaine seeking in rats. Neuropsychopharmacology 26:422-430.

Griffin ML, Weiss RD, Mirin SM, Lange U (1989) A comparison of male and female cocaine abusers. Arch Gen Psychiatry 46:122-126.

Hadfield MG, Milio C (1989) Caffeine and regional brain monoamine utilization in mice. Life Sci 45:2637-2644.

Häfner H (2003) Gender differences in schizophrenia. Psychoneuroendocrinology 28, Supplement 2:17-54.

Halldner L, Ådén U, Dahlberg V, Johansson B, Ledent C, Fredholm BB (2004) The adenosine A1 receptor contributes to the stimulatory, but not the inhibitory effect of caffeine on locomotion: a study in mice lacking adenosine A1 and/or A2A receptors. Neuropharmacology 46:1008-1017.

Harland RD, Gauvin DV, Michaelis RC, Carney JM, Seale TW, Holloway FA (1989) Behavioral interaction between cocaine and caffeine: a drug discrimination analysis in rats. Pharmacol Biochem Behav 32:1017-1023.

Hecht GS, Spear NE, Spear LP (1999) Changes in progressive ratio responding for intravenous cocaine throughout the reproductive process in female rats. Developmental Psychobiology 35:136-145.

Hettinger BD, Lee A, Linden J, Rosin DL (2001) Ultrastructural localization of adenosine A2A receptors suggests multiple cellular sites for modulation of

GABAergic neurons in rat striatum. The Journal of Comparative Neurology 431:331-346.

Hughes JR, McHugh P, Holtzman S (1998) Caffeine and schizophrenia. Psychiatr Serv 49:1415-1417.

Hutchinson WD, Levy R, Dostrovsky JO, Lozano AM, Lang AE (1997) Effects of apomorphine on globus pallidus neurons in parkinsonian patients. . Neurology 42:767-775.

Jackson LR, Robinson TE, Becker JB (2006) Sex differences and hormonal influences on acquisition of cocaine self-administration in rats. Neuropsychopharmacology 31:129-138.

Karcz-Kubicha M, Antoniou K, Terasmaa A, Quarta D, Solinas M, Justinova Z, Pezzola A, Reggio R, Muller C, Fuxe K, Goldberg S, Popoli P, Ferre S (2003) Involvement of Adenosine A1 and A2A Receptors in the Motor Effects of Caffeine after its Acute and Chronic Administration. Neuropsychopharmacology 28:1281-1291.

Kippin T, Fuchs R, Mehta R, Case J, Parker M, Bimonte-Nelson H, See R (2005) Potentiation of cocaine-primed reinstatement of drug seeking in female rats during estrus. Psychopharmacology 182:245-252.

Knapp CM, Foye MM, Cottam N, Ciraulo DA, Kornetsky C (2001) Adenosine agonists CGS 21680 and NECA inhibit the initiation of cocaine self-administration. Pharmacology Biochemistry and Behavior 68:797-803.

Koob GF NE (1997) The neurobiology of drug addiction. J Neuropsychiatry Clin Neurosci 9:482-497.

Kuzmin A, Johansson B, Semenova S, Fredholm BB (2000) Differences in the effect of chronic and acute caffeine on self-administration of cocaine in mice. European Journal of Neuroscience 12:3026-3032.

Landis C, Hunt WA (1939) The Startle Pattern. Farrar and Rinehart, New York.

Lara DR (2010) Caffeine, mental health, and psychiatric disorders. J Alzheimers Dis 20:S239-248.

Lazarus M, Shen H-Y, Cherasse Y, Qu W-M, Huang Z-L, Bass CE, Winsky-Sommerer R, Semba K, Fredholm BB, Boison D, Hayaishi O, Urade Y, Chen J-F (2011) Arousal Effect of Caffeine Depends on Adenosine A2A Receptors in the Shell of the Nucleus Accumbens. The Journal of Neuroscience 31:10067-10075.

Ledent C, Vaugeois JM, Schiffmann SN, Pedrazzini T, El Yacoubi M, Vanderhaeghen JJ, Costentin J, Heath JK, Vassart G, Parmentier M (1997) Aggressiveness, hypoalgesia and high blood pressure in mice lacking the adenosine A2a receptor. Nature 388:674-678.

Leitner DS, Cohen ME (1985) Role of the inferior colliculus in the inhibition of acoustic startle in the rat. Physiology & Behavior 34:65-70.

Leitner DS, Powers AS, Sititt CL, Hoffman HS (1981) Midbrain reticular formation involvement in the inhibition of acoustic startle. Physiology & Behavior 26:259-268.

Malave L, Broderick PA (2014) Caffeine's attenuation of cocaine-induced dopamine release. . Journal of Caffeine Research in press.

Mansbach RS, Geyer MA, Braff DL (1988) Dopaminergic stimulation disrupts sensorimotor gating in the rat. Psychopharmacology 94:507-514.

Marcellino D, Roberts DC, Navarro G, Filip M, Agnati L, Lluis C, Franco R, Fuxe K (2007) Increase in A2A receptors in the nucleus accumbens after extended cocaine self-administration and its disappearance after cocaine withdrawal. . Brain Res 1143:208-220.

Marcondes FK, Bianchi FJ, Tanno AP (2002) Determination of the estrous cycle phases of rats: some helpful considerations. Braz J Biol 62:609-614.

Martinez ZA, Ellison GD, Geyer MA, Swerdlow NR (1999a) Effects of sustained cocaine exposure on sensorimotor gating of startle in rats. Psychopharmacology 142:253-260.

Martinez ZA, Ellison GD, Geyer MA, Swerdlow NR (1999b) Effects of Sustained Phencyclidine Exposure on Sensorimotor Gating of Startle in Rats. Neuropsychopharmacology 21:28-39.

Meredith MA, Wallace MT, Stein BE (1992) Visual, auditory and somatosensory convergence in output neurons of the cat superior colliculus: multisensory properties of the tecto-reticulo-spinal projection. Exp Brain Res 88:181-186.

Morgan KJ, Stults VJ, Zabik ME (1982) Amount and dietary sources of caffeine and saccharin intake by individuals ages 5 to 18 years. Regul Toxicol Pharmacol 2:296-307.

Morton N, Ellis C, Gray NS (1995) The effects of apomorphine and L-dopa challenge on prepulse inhibition in patients with Parkinson's disease. . Schizophr Res 15.

Nesbitt J, Raju C, Vidal L, Alimova A, Steiner J, Broderick PA, Friedman E (2008) Sexual dimorphism studies of cocaine and caffeine in conditioned place preference: estrous cycle stage and open-field behaviors. Society for Neuroscience, San Diego.

Nunes J, Broderick PA (2007) Novel research translates to clinical cases of schizophrenia and cocaine
psychosis. Neuropsychiatric Disease and Treatment 3.

Ongini E, Fredholm BB (1996) Pharmacology of adenosine A2A receptors. Trends in Pharmacological Sciences 17:364-372.

Perry W, Braff DL (1994) Information-processing deficits and thought disorder in schizophrenia. Am J Psychiatry 151:363-367.

Quiñones-Jenab V, Ho A, Schlussman SD, Franck J, Kreek MJ (1999) Estrous cycle differences in cocaine-induced stereotypic and locomotor behaviors in Fischer rats. Behavioural Brain Research 101:15-20.

Quinones-Jenab V, Jenab S (2012) Influence of Sex Differences and Gonadal Hormones on Cocaine Addiction. ILAR Journal 53:14-22.

Quinones-Jenab V, Perrotti LI, Fabian SJ, Chin J, Russo SJ, Jenab S (2001) Endocrinological Basis of Sex Differences in Cocaine-Induced Behavioral Responses. Annals of the New York Academy of Sciences 937:140-171.

Rivera-Oliver M, Díaz-Ríos M (2014) Using caffeine and other adenosine receptor antagonists and agonists as therapeutic tools against neurodegenerative diseases: A review. Life Sciences 101:1-9.

Roberts DC, Bennet SA, Vickers GJ (1989) The estrous cycle affects cocaine self-administration on a progressive ratio schedule in rats. Psychopharmacology 3:408-411.

Rosin DL, Hettinger BD, Lee A, Linden J (2003) Anatomy of adenosine A2A receptors in brain: morphological substrates for integration of striatal function. Neurology 61.

Ross GW, Abbott RD, Petrovitch H, White LR, Tanner CM (2000) Relationship between caffeine intake and parkinson disease. JAMA 284:1378-1379.

Rudolphi KA, Keil M, Fastbom J, Fredholm BB (1989) Ischaemic damage in gerbil hippocampus is reduced following upregulation of adenosine (A1) receptors by caffeine treatment. Neurosci Lett 103:275-280.

Schenk S, Worley CM, McNamara C, Valadez A (1996) Acute and repeated exposure to caffeine: effects on reinstatement of extinguished cocaine-taking behavior in rats. Psychopharmacology 126:17-23.

Sell SL, Scalzitti JM, Thomas ML, Cunningham KA (2000) Influence of Ovarian Hormones and Estrous Cycle on the Behavioral Response to Cocaine in Female Rats. Journal of Pharmacology and Experimental Therapeutics 293:879-886.

Sircar R, Kim D (1999) Female Gonadal Hormones Differentially Modulate Cocaine-Induced Behavioral Sensitization in Fischer, Lewis, and Sprague-Dawley Rats. Journal of Pharmacology and Experimental Therapeutics 289:54-65.

Solinas M, Ferré S, Antoniou K, Quarta D, Justinova Z, Hockemeyer J, Pappas L, Segal P, Wertheim C, Müller C, Goldberg S (2005) Involvement of adenosine A1 receptors in the discriminative-stimulus effects of caffeine in rats. Psychopharmacology 179:576-586.

Solinas M, Ferré S, You Z-B, Karcz-Kubicha M, Popoli P, Goldberg SR (2002) Caffeine Induces Dopamine and Glutamate Release in the Shell of the Nucleus Accumbens. The Journal of Neuroscience 22:6321-6324.

Svenningsson P, Nomikos GG, Fredholm BB (1995) Biphasic changes in locomotor behavior and in expression of mRNA for NGFI-A and NGFI-B in rat striatum following acute caffeine administration. J Neurosci 15:7612-7624.

Swerdlow NR, Braff DL, Geyer MA, Koob GF (1986) Central dopamine hyperactivity in rats mimics abnormal acoustic startle response in schizophrenics. Biological psychiatry 21:23-33.

Swerdlow NR, Caine SB, Braff DL, Geyer MA (1992) The neural substrates of sensorimotor gating of the startle reflex: a review of recent findings and their implications. J Psychopharmacology 6:176-190.

Swerdlow NR, Eastvold A, Gerbranda T, Uyan KM, Hartman P, Doan Q, Auerbach P (2000) Effects of caffeine on sensorimotor gating of the startle reflex in normal control subjects: impact of caffeine intake and withdrawal. . Psychopharmacology 151:368-378.

Von Lubitz DK, Paul IA, Bartus RT, Jacobson KA (1993a) Effects of chronic administration of adenosine A1 receptor agonist and antagonist on spatial learning and memory. European Journal of Pharmacology 249:271-280.

Von Lubitz DK, Paul IA, Carter M, Jacobson KA (1993b) Effects of N6-cyclopentyl adenosine and 8-cyclopentyl-1,3-dipropylxanthine on N-methyl Daspartate induced seizures in mice. European Journal of Pharmacology 249:265-270.

Walker QD, Francis R, Cabassa J, Kuhn CM (2001) Effect of Ovarian Hormones and Estrous Cycle on Stimulation of the Hypothalamo-Pituitary-Adrenal Axis by

Cocaine. Journal of Pharmacology and Experimental Therapeutics 297:291-298.

Wang JH, Short J, Ledent C, Lawrence AJ, Buuse Mvd (2003) Reduced startle habituation and prepulse inhibition in mice lacking the adenosine A2A receptor. Behavioural Brain Research 143:201-207.

Zakharova E, Wade D, Izenwasser S (2009) Sensitivity to cocaine conditioned reward depends on sex and age. Pharmacology Biochemistry and Behavior 92:131-134.