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Blockade of α2-adrenergic receptors in prelimbic cortex: Impact on cocaine self-administration in adult Spontaneously Hypertensive Rats following adolescent atomoxetine treatment

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Abstract

Rationale—Research with the Spontaneously Hypertensive Rat (SHR) model of attention deficit/hyperactivity disorder demonstrated that chronic methylphenidate treatment during adolescence increased cocaine self-administration established during adulthood under a progressive ratio (PR) schedule. Compared to vehicle, chronic atomoxetine treatment during adolescence failed to increase cocaine self-administration under a PR schedule in adult SHR.

Objectives—We determined if enhanced noradrenergic transmission at α2-adrenergic receptors within prefrontal cortex contributes to this neutral effect of adolescent atomoxetine treatment in adult SHR.

Methods—Following treatment from postnatal day 28–55 with atomoxetine (0.3 mg/kg) or vehicle, adult male SHR and control rats from Wistar-Kyoto (WKY) and Wistar (WIS) strains were trained to self-administer 0.3 mg/kg cocaine. Self-administration performance was evaluated under a PR schedule of cocaine delivery following infusion of the α2-adrenergic receptor antagonist idazoxan (0 and 10–56 μg/side) directly into prelimbic cortex.

Results—Adult SHR attained higher PR breakpoints and had greater numbers of active lever responses and infusions than WKY and WIS. Idazoxan dose-dependently increased PR breakpoints and active lever responses in SHR following adolescent atomoxetine versus vehicle treatment. Behavioral changes were negligible after idazoxan pretreatment in SHR following adolescent vehicle or in WKY and WIS following adolescent atomoxetine or vehicle.

Conclusions—α2-Adrenergic receptor blockade in prelimbic cortex of SHR masked the expected neutral effect of adolescent atomoxetine on adult cocaine self-administration behavior.

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Conflict of Interest: Britahny Baskin, Bríd Nic Dhonnchadha, Linda Dwoskin, and Kathleen Kantak declare no conflicts of interest.

Compliance with Ethical Standards

Research Involving Animals: All procedures were approved by the Boston University Institutional Animal Care and Use Committee and were in accordance with the *National Institutes of Health Guide For the Care and Use of Laboratory Animals (8th Edition).*

Moreover, greater efficacy of acute idazoxan challenge in adult SHR after adolescent atomoxetine relative to vehicle is consistent with the idea that chronic atomoxetine may downregulate pre-synaptic α2A-adrenergic autoreceptors in SHR.

Keywords

Atomoxetine; Attention Deficit/Hyperactivity Disorder; Cocaine; Idazoxan; Norepinephrine; Prelimbic cortex; Self-administration

1. Introduction

Methylphenidate and atomoxetine are medications used globally for the treatment of attention deficit/hyperactivity disorder (ADHD) in pediatric patients (Scheffler et al. 2007). Methylphenidate is a dopamine and norepinephrine transporter (DAT and NET, respectively) inhibitor (Richelson and Pfenning 1984) and atomoxetine is a selective NET inhibitor (Bolden-Watson and Richelson 1993). Although methylphenidate and atomoxetine exert a variety of effects through influences on multiple brain regions, ADHD symptom relief is thought to be due to drug action in prefrontal cortex (Arnsten 2009), a site where NET is responsible primarily for dopamine clearance due to low DAT density (Moron et al. 2002). As doses of drugs that increase nucleus accumbens dopamine have abuse potential (Koob and Bloom 1988), it is essential to study low, pharmacologically relevant doses of ADHD medications in animal models of ADHD. Low doses of atomoxetine (≤3.0 mg/kg intraperitoneal) and methylphenidate (≤0.25 mg/kg intraperitoneal or ≤2.5 mg/kg oral) increase extracellular levels of dopamine in prefrontal cortex, but not in striatum or nucleus accumbens, and increase extracellular levels of norepinephrine in prefrontal cortex as well as in medial septal area and hippocampus (Berridge et al. 2006; Bymaster 2002; Kuczenski and Segal 2002).

Over the past several years, we have explored whether or not low doses of methylphenidate and atomoxetine had similar long-term consequences on cocaine self-administration after medication was discontinued. We used the inbred Spontaneously Hypertensive Rat (SHR) model of ADHD because this strain displays core characteristics of ADHD. Compared to controls, SHR are more hyperactive, inattentive, and impulsive (Sagvolden et al. 1992; Jentsch 2005; De Bruin et al. 2003; Hand et al. 2009; Somkuwar et al. 2016). SHR also have impaired working memory and show deficits in behavioral flexibility and habit learning (Nakamura-Palacios et al. 1996; De Bruin et al. 2003; Kantak et al. 2008; Wells et al. 2010; Harvey et al. 2013; Gauthier et al. 2014; Jordan et al. 2016b). Importantly, the ADHD-like phenotype of SHR is unrelated to hypertension (Gattu et al. 1997; Kantak et al. 2008; Wells et al. 2010). SHR also have several neurobiological abnormalities as are observed in patients with ADHD, such as greater striatal DAT density (Roessner et al. 2010; Silva et al. 2014). Relative to other rat models of ADHD, SHR is the only model that mimics ADHD combined subtype (Russell 2011), which is the most common subtype in children and teens (Nikolas and Nigg 2013). In studies with ADHD medications, chronic methylphenidate treatment (1.5 mg/kg; oral) during adolescence increased cocaine self-administration relative to vehicle in adult SHR under fixed ratio (FR), progressive ratio (PR), and second-order schedules of drug delivery (Baskin et al. 2015; Harvey et al. 2011; Jordan et al. 2014). In contrast,

chronic atomoxetine treatment (0.3 mg/kg; intraperitoneal) during adolescence did not increase cocaine self-administration relative to vehicle in adult SHR under these same cocaine delivery schedules (Jordan et al 2014; Somkuwar et al. 2013a).

Although both methylphenidate and atomoxetine increase extracellular levels of dopamine and norepinephrine in prefrontal cortex, subtle differences in their neurochemical profiles may underlie their differential effects on cocaine self-administration in SHR. Microdialysis studies in rat have demonstrated that 0.3 mg/kg atomoxetine produces relatively greater increases in prefrontal cortex norepinephrine (300% of baseline) than dopamine (150% of baseline), whereas 0.5 – 3.0 mg/kg methylphenidate produces relatively greater increases in prefrontal cortex dopamine (225 – 250% of baseline) compared to norepinephrine (200% of baseline) (Berridge et al. 2006; Bymaster et al. 2002). Also, atomoxetine has higher affinity than methylphenidate for inhibiting the reuptake of norepinephrine, and atomoxetine is more potent than methylphenidate for accumulating released norepinephrine (basal and stimulated) in rat frontal cortex (Easton et al. 2007). As the therapeutic effects of atomoxetine are mediated by enhanced noradrenergic transmission at α2A adrenergic receptors in prefrontal cortex (Gamo et al. 2010), we hypothesized that this same mechanism may explain why adolescent atomoxetine treatment does not increase cocaine self-administration in adult SHR.

This hypothesis was investigated by determining the dose-related effects of idazoxan, a relatively selective α2-adrenergic receptor antagonist, on self-administration studied under a PR schedule of cocaine delivery after local application of idazoxan into the prelimbic region of the prefrontal cortex. In rat brain, idazoxan binds to α2A-, α2B-, and α2C-adrenergic receptors and to imidazoline sites (MacDonald and Scheinin 1995; Mallard et al. 1992). Idazoxan is 5000-fold more selective for blocking α2 receptors than imidazoline sites, but its affinity does not differ among the α2 receptor subtypes. Notably, the α2A subtype predominates in prefrontal cortex (Aoki et al. 1998) where it is mainly located post-synaptically on perikarya and proximal dendrites of pyramidal neurons (Aoki et al. 1994). Locally applied idazoxan in rat prefrontal cortex results in concentration-dependent increases in norepinephrine release via action at pre-synaptic α2A autoreceptors (van Veldhuizen et al. 1994), while concurrently it blocks the effects of released norepinephrine on spontaneous firing of pyramidal neurons (Wang et al. 2011). In the current study, effects of idazoxan were determined in adult SHR as well as in inbred Wistar-Kyoto (WKY) and outbred Wistar (WIS) control strains that received atomoxetine or vehicle treatment during adolescence. Prelimbic cortex was selected as the site for idazoxan infusion because of its importance in regulating cocaine-seeking and cocaine-taking behavior (Di Pietro et al. 2006; Mashhoon et al. 2010; Stefanik et al. 2013) and in mediating clinically relevant effects of atomoxetine in rat (Bari et al. 2011; Bradshaw et al. 2016). Moreover, our previous research in SHR points to the importance of neurochemical changes within the medial prefrontal cortex for mediating the greater abuse potential of cocaine after adolescent methylphenidate treatment (Somkuwar et al. 2013b).

2. Materials and Methods

2.1 Subjects

A total of 42 male SHR/NCrl, WIS/NCrl, and WKY/NCrl rats (Charles River Laboratories, Wilmington, MA, USA) arrived on postnatal day (P) 25 in four separate cohorts (3–4 rats per stain in each cohort) spaced several months apart over a 1.5-year period. Upon arrival, rats were given 3 days to acclimate to their new individual housing conditions (08:00 lights on, 20:00 lights off) before experimental procedures began. Rats were housed individually as enriched housing (social and environmental) blocks acquisition of cocaine self-administration and blunts the ability of cocaine-paired cues to induce relapse behavior (Chauvet et al. 2009; Puhl et al. 2012; Thiel et al. 2010). A further consideration for using isolated housing was that enriched housing prevents ADHD-like symptoms in the SHR strain (Pamplona et al. 2009), an outcome that also may have undermined our ability to study how ADHD medications influence cocaine self-administration in an animal model of ADHD. From P28-P55, rats were given 10–16 g of food per day to maintain body weight at 85–90% of a growth-adjusted free-feeding body weight specific for each strain. Food restriction was implemented to keep conditions consistent with our past comparator studies determining the effects of adolescent atomoxetine treatment on cognitive performance during adolescence and on cocaine self-administration behavior studied under a PR schedule during adulthood (Harvey et al. 2013; Somkuwar et al. 2013a). Water was freely available throughout the study, and food was unrestricted after P55. Based on growth charts provided by Charles River Laboratories (www.criver.com) and the starting body weights on P28, rats from each strain were estimated to be 87–89% of ad-libitum body weight at the end of the food restriction period on P56 and to reach the 100% ad libitum body weight range on P77, when cocaine self-administration sessions began (Figure 1). Rats were maintained in accordance with the NIH Guide for Care and Use of Laboratory Animals and experimental protocols were approved by the Boston University Institutional Animal Care and Use Committee.

2.2 Drugs

Atomoxetine hydrochloride (Tocris Biosciences, Ellisville, MO, USA) was dissolved in 0.9% sterile saline and injected intraperitoneally in a volume of 2.0 ml/kg body weight. Rats were randomly assigned to treatment and received either 0.3 mg/kg atomoxetine (n= 9 WKY, 7 WIS, and 8 SHR) or an equal volume of vehicle (n= 5 WKY, 6 WIS, and 7 SHR) on weekdays from P28-P55 to mimic the clinical practice of drug-free holidays on weekends for youth with ADHD (Martins et al. 2004). P28-P55 in rat reflects early to late adolescence, based on adolescent-typical neurobehavioral characteristics during this period and maturation of prefrontal cortex at ~P60 (Schneider 2013; Spear 2000). Although a single drug dose design is a limitation, the selected dose of atomoxetine is pharmacologically relevant as an ADHD therapeutic because it increases extracellular concentrations of norepinephrine and dopamine preferentially in prefrontal cortex (Bymaster et al. 2002), and has pro-cognitive effects in SHR (Harvey et al. 2013) without producing an increase in locomotor activity (Turner et al. 2013). For the self-administration procedure, cocaine hydrochloride (National Institute on Drug Abuse, Bethesda, MD, USA) was dissolved in 0.9% sterile saline containing 3 IU heparin/ml saline. The cocaine-training dose was 0.3

mg/kg (a 0.8 mg/ml concentration infused at a rate of 1.8 ml/min for a duration of 1.2 s/100 g body weight) and was selected because it maintains moderate PR breakpoints from which increases or decreases can be detected in SHR, WKY and WIS (Harvey et al. 2011; Jordan et al. 2016a; Somkuwar et al. 2013a). To evaluate the effects of a locally applied α2-adrenergic receptor antagonist on cocaine self-administration behavior under a PR schedule, idazoxan (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 0.9% sterile saline to provide final concentrations of 10, 30, and 56 μg/0.5 μl for infusion into prelimbic cortex. In past studies, local infusion of a 10 μg/side dose of idazoxan into the bed nucleus of the stria terminalis or the subthalamic nucleus was behaviorally active (Delaville et al. 2012; Smith et al. 2012; Smith et al. 2013), and thus, was selected as the starting dose. Saline was used as the 0 μg idazoxan control.

2.3 Surgery

Beginning on P67, buprenorphine (0.05 mg/kg, subcutaneous; Butler Schein, Columbus, OH, USA) was administered as a preemptive analgesic. Catheters were surgically implanted into the right femoral vein under ketamine (90 mg/kg, intraperitoneal) and xylazine (10 mg/kg, intraperitoneal) anesthesia, as previously described in detail (Jordan et al. 2016b). During surgery, 22-gauge guide cannulae (Plastics One, Roanoke, VA, USA) were implanted bilaterally using the following Swanson (1992) atlas coordinates: anterior-posterior +2.8 mm, medial-lateral ±1.4 mm at a 15° angle, and dorsal-ventral −2.9 mm from bregma, with the guide cannulae tips inserted 1 mm above the intended site. Cannulae were angled to avoid breaching the medial wall of the cortex (Di Pietro et al. 2006). The catheter pedestal, two guide cannulae, and three stainless steel anchoring screws were embedded in dental acrylic to affix the instrumentation to the skull. Two 28-gauge stainless steel obturators were used to occlude cannulae when not in use. Post-surgical care, including the use of buprenorphine for postoperative pain relief, and catheter maintenance were as previously described in detail (Jordan et al. 2016b).

2.4 Cocaine Self-Administration Training and Progressive Ratio Testing

Operant chambers (model ENV-008CT, Med Associates, St. Albans, VT) were outfitted with two levers, a stimulus light above each lever, a food receptacle, a pellet dispenser, an infusion pump, and a house light as previously described (Somkuwar et al. 2013a). Each chamber was enclosed in a ventilated sound-attenuating box. After 1-week recovery from surgery, rats were trained to lever press for 45 mg food pellet reinforcement under a FR1 schedule to accelerate acquisition of cocaine self-administration in the three strains of rats. Without food pellet training or other external inducements for lever pressing, WKY and WIS typically require 3-fold more sessions than SHR to acquire cocaine self-administration to an established criterion (Harvey et al. 2011; Jordan et al 2016a; Somkuwar et al. 2013a). One day after earning 100 food pellets within 30 minutes, rats began cocaine self-administration training in daily (Monday-Friday) 2-hr sessions under a FR1 20-sec timeout schedule of cocaine delivery (0.3 mg/kg, intravenous) with the session contingencies as previously described (Somkuwar et al. 2013a). Rats continued self-administration training sessions under the FR1 schedule until responding stabilized in individual rats (<15% variation for 5 consecutive sessions and a ratio of 2:1 active to inactive lever responses). Next, rats proceeded to the PR schedule (Loh and Roberts 1990) using the 0.3 mg/kg cocaine unit

dose. This schedule involves a geometric increment in the number of lever presses required for each successive drug infusion (e.g., 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, etc.). Sessions terminated when a rat failed to complete the current FR requirement within 60 min of the last cocaine infusion or when 4.5 hr had elapsed, whichever occurred first. The last FR completed when the session ended was used as the PR breakpoint (Richardson and Roberts 1996).

Once PR breakpoints stabilized in individual rats (<15% variation for 5 consecutive sessions), test sessions with idazoxan pretreatment occurred twice each week (Tuesdays and Fridays) with washout sessions (no idazoxan pretreatment) on intervening days to allow any residual effects of idazoxan to subside. Idazoxan was infused bilaterally into the prelimbic cortex at a rate 0.59 μl/min, using a counterbalanced order for infusion of the 0, 10, 30 and 56 μg/0.5 μl concentrations across rats, beginning 20 min prior to the PR test sessions. A 20-min pretreatment time was selected because this is the amount of time needed for norepinephrine release to be increased significantly in rat medial prefrontal cortex during continuous perfusion with idazoxan through a dialysis probe (van Veldhuizen et al. 1993). The 28-gauge infusion cannula extended 1 mm beyond the tip of the guide cannula and was left in place for an additional 1 min following the completion of each infusion to allow for drug diffusion away from the infusion site. Prior to idazoxan pretreatment, intermittent sham infusions were provided to acclimate rats to this procedure. The last FR completed, number of cocaine infusions, and number of active and inactive lever responses during self-administration sessions were recorded and analyzed by analysis of variance (ANOVA; see below).

2.5 Histology

Three atomoxetine-treated WKY, two vehicle-treated SHR, and two atomoxetine-treated SHR died before completing behavioral tests. The remaining 35 rats were given an overdose of Euthasol (3 ml containing pentobarbital sodium and phenytoin sodium as the active ingredients) upon completion of the study and then were perfused intracardially with saline and 10% formalin solution. Brains were extracted, post-fixed in 10% formalin, and then cryoprotected in 30% sucrose at 4° C for 2–3 days before flash-freezing in 2-methyl butane and storing at −80° C until cryosectioning. Coronal sections (40 μm) targeting the prelimbic cortex and surrounding areas were collected and mounted onto slides. Slices were stained with thionin, and cannulae placements were verified by examination under a light microscope.

2.6 Data analyses

Dependent measures were analyzed by two-factor (FR and PR performance at acquisition), three-factor (breakpoint and number of infusions at test), or 4-factor (lever responses at test) ANOVA (SPSS version 20). Factors were strain, adolescent medication history, antagonist dose (repeated measure), and lever (repeated measure). The Tukey procedure was used for post-hoc testing following significant ANOVA factors. In addition, Dunnett t-tests were used to further clarify if idazoxan impacted cocaine self-administration differently in the three rat strains treated with either adolescent atomoxetine or vehicle, but only if the ANOVA revealed significant main effects of strain, medication history and antagonist dose, as well as

a significant interaction of medication history x antagonist dose. Dunnett t-tests control for type-1 error and permit multiple post-hoc comparisons regardless of the ANOVA outcome (Winer 1971). Effect sizes (partial eta squared; $\eta 2$) were computed for significant ANOVA main effects and interactions. As described by Cohen (1988), effect sizes can be characterized as small ($\eta 2 = 0.01$), medium ($\eta 2 = 0.06$), or large ($\eta 2 = 0.14$). Accordingly, all significant ANOVA factors in the current study had effect sizes that were in the medium to large range.

3. Results

3.1 Histology

Bilateral prelimbic cortex cannulae placements were confirmed for 33 of 35 rats that completed behavioral testing. Figure 2A illustrates that the midpoints of the idazoxan injection sites were within or close to the border of the prelimbic cortex along the dorsal-ventral and medial-lateral planes of both hemispheres in these 33 rats (n=6/strain for atomoxetine treatment and n=5/strain for vehicle treatment). The midpoints of the injection sites were no more than 1.9 mm anterior to the intended anterior-posterior stereotaxic coordinate used for cannulae placements. Behavioral data for the remaining two rats (one vehicle-treated and one atomoxetine-treated WIS) were not included in the statistical analyses, as the injection sites were not bilaterally positioned within or near the prelimbic cortex (not shown). The image depicted in Figure 2B is a representative photomicrograph of bilateral cannulae tracks in prelimbic cortex.

3.2 Acquisition under FR and PR schedules

Using the acquisition criteria outlined in section 2.4, Table 1 shows that there were strain differences in number of sessions needed to reach the FR 1 criterion ($F[2, 27]=6.9$, $p< 0.004$; $\eta 2 = 0.34$), number of infusions at the FR 1 criterion ($F[2, 27]=14.9$, $p< 0.001$; $\eta 2 = 0.53$), number of infusions at the PR criterion ($F[2, 27]=19.1$, $p< 0.001$; $\eta 2 = 0.59$), and breakpoints reached at the PR criterion ($F[2, 27]=22.0$, $p< 0.001$; $\eta 2 = 0.62$). There were no strain differences in number of sessions needed to reach the PR criterion ($F[2, 27]=0.5$, n.s.). Tukey post-hoc tests showed that SHR required fewer FR 1 sessions to reach criterion, and had a greater number of FR1 and PR infusions and higher PR breakpoints than WKY and WIS ($ps<0.02$) on the day the acquisition criterion was reached. There was no treatment main effect or strain x treatment interaction for any of these measures.

3.2 Progressive Ratio Performance after Idazoxan Pretreatment

ANOVA of breakpoints under the PR schedule (Figure 3) revealed significant main effects of strain ($F[2, 27]=37.3$, $p<0.001$; $\eta 2 = 0.73$), antagonist dose ($F[3, 81]=3.3$, $p<0.02$; $\eta 2 = 0.11$), and medication history ($F[1, 27]=6.2$, $p<0.02$; $\eta 2 = 0.19$) as well as a significant antagonist dose x medication history interaction ($F[3, 81]=3.1$, $p<0.03$; $\eta 2 = 0.10$). Tukey post-hoc tests of the strain main effect showed that SHR had higher PR breakpoints than either WKY or WIS ($p<0.001$), and that these two control strains did not differ significantly from each other. Tukey post-hoc tests of the antagonist dose x medication history interaction showed that 30 and 56 μg/side doses of idazoxan increased PR breakpoint in atomoxetine-treated compared to vehicle-treated rats ($p<0.05$). The antagonist dose x medication history

interaction within each strain was examined with Dunnett t-tests, despite a nonsignificant strain x antagonist dose x medication history interaction ($F[6, 81]=1.5$, $p<0.21$). SHR with adolescent atomoxetine treatment had higher PR breakpoints than SHR with adolescent vehicle treatment following 30 ($p<0.01$) and 56 ($p<0.01$) μg/side idazoxan (Figure 3, right panel). No doses of idazoxan significantly modified PR breakpoints in WKY or WIS treated with atomoxetine or vehicle during adolescence (Figure 3, left and middle panels).

Table 2 shows the additional performance measures under the PR schedule. For number of infusions earned, ANOVA revealed a significant main effect of strain ($F[2,27)=28.7$, $p<0.001$; $\eta 2 = 0.68$), but not of antagonist dose ($F[3,81)=1.9$ n.s.) or medication history ($F[1,27)=3.0$, n.s.). None of the interactions were significant. Tukey post-hoc tests of the strain main effect showed that SHR earned a greater number of cocaine infusions than either WKY or WIS ($p<0.001$), and that these two control strains did not differ significantly from each other. ANOVA for lever responding revealed significant main effects of strain ($F[2,27)=32.5$, $p<0.001$; $\eta 2 = 0.71$), medication history ($F[1,27)=6.1$, $p<0.02$; $\eta 2 = 0.19$), antagonist dose ($F[3,81)=3.2$, $p<0.03$; $\eta 2 = 0.11$), and lever ($F[1,27)=117$, $p<0.001$; $\eta 2 = 0.81$). Also interactions of strain x lever ($F[2,27)=30.4$, $p<0.001$; $\eta 2 = 0.69$) and lever x antagonist dose x mediation history ($F[3,81)=3.4$, $p<0.02$; $\eta 2 = 0.11$) were significant. Tukey post-hoc tests of the strain x lever interaction revealed that SHR made a greater number of responses than WKY and WIS on the active lever ($p<0.001$), but not on the inactive lever. Tukey post-hoc tests of the lever x antagonist dose x mediation history interaction revealed that 30 and 56 μg/side doses of idazoxan increased responding on the active lever in atomoxetine-treated compared to vehicle-treated rats ($p<0.05$). The lever x antagonist dose x medication history interaction within each strain was examined further with Dunnett t-tests, despite a nonsignificant strain x lever x antagonist dose x medication history interaction ($F[6, 81]=1.8$, $p<0.12$). SHR with adolescent atomoxetine treatment emitted a greater number of active lever responses than SHR with adolescent vehicle treatment following 30 ($p<0.01$) and 56 ($p<0.01$) μg/side idazoxan (Table 2, middle columns). No doses of idazoxan significantly modified the number of active lever responses emitted in WKY or WIS treated with atomoxetine or vehicle during adolescence.

4. Discussion

During the acquisition phase following lever press training for food reward, SHR reached stable responding faster and earned a greater number of infusions than WKY and WIS under the FR schedule and likewise earned a greater number of infusions and reached higher breakpoints than WKY and WIS under the PR schedule. These strain differences in FR and PR performance are consistent with our previous reports that did not use prior lever press training for food reward to directly study acquisition of cocaine self-administration in SHR and control strains (Harvey et al. 2011; Jordan et al. 2016b; Somkuwar et al. 2013a). Interestingly, the number of sessions needed to reach stable PR breakpoints did not differ by strain, indicating that the number of cocaine self-administration sessions under the PR schedule prior to acute idazoxan challenges was similar across SHR, WKY and WIS. Despite this similarity in PR session history, two factors to consider further apropos to cocaine self-administration behavior are the contribution of early life stress (stress due to

shipping, individual housing and food restriction) and the potential for strain differences in response to early life stress.

Shipping transportation can produce short-term physiological changes in individual or group housed rats (decreased heart rate, body weight, plasma corticosterone, and activity level) that can last up to 3 days (Capdevila et al. 2007; van Ruiven et al. 1998). Our design incorporated a 3-day acclimation period to allow rats to stabilize and adjust to their new individual housing conditions. Although individual housing of juvenile rats can result in greater impulsivity during adulthood than group housing (Kirkpatrick et al. 2014), individual housing of juvenile or adolescent rats does not result in greater cocaine self-administration compared to group housing during adulthood over a range of doses under a FR 1 schedule and at 0.2 mg/kg cocaine under a PR schedule (Boyle et al. 1991; Westenbroek et al. 2013). Regarding food restriction, a previous study showed that individually housed adult rats maintained at 90% ad libitum body weight had an initial increase in locomotor response to cocaine, but this effect was not sustained after repeated cocaine exposure (Stamp et al. 2008). In the current study, rats were maintained at 87–89% ad libitum body weight during adolescence, but were at the 100% ad libitum body weight range when cocaine self-administration sessions began 3 weeks later. Thus, a greater sensitivity to cocaine in adulthood due to the stress of adolescent food restriction was not likely an issue. However, strain differences in stress vulnerability may have influenced the magnitude of cocaine self-administration, as stress is known to increase cocaine self-administration (Mantsch et al. 2014). Previous studies demonstrated that WKY have greater stress vulnerability (Nam et al. 2014) and anxiogenic responses to chronic stress (Roman et al. 2004, Sterley et al. 2011) than SHR and/or WIS. If strain differences in stress vulnerability were a factor, then the similar magnitude of cocaine self-administration in WKY and WIS and the greater magnitude of cocaine self-administration in SHR compared to WKY and WIS would not be expected. Collectively, the equivalent exposure across strains to early life stress due to shipping, isolated housing, and food restriction did not appear to systematically account for the strain and treatment differences in cocaine self-administration assessed herein during adulthood.

Our previous research demonstrated that relative to vehicle, methylphenidate treatment in adolescent SHR further enhanced the speed to acquire cocaine self-administration as well as reinforcing efficacy and PR breakpoints for cocaine in adult SHR (Baskin et al. 2015; Harvey et al. 2011). These findings contrast with the effects of adolescent atomoxetine treatment, which failed to increase these three indices of cocaine self-administration behavior relative to vehicle in adult SHR (Somkuwar et al. 2013a). Also, adolescent atomoxetine treatment decreased cue-induced cocaine seeking during reinstatement testing in adult SHR (Jordan et al. 2014). These latter findings with chronic administration of atomoxetine (0.3 mg/kg) in adolescent SHR complement previous studies showing that acute administration of atomoxetine (1.5 and 3.0 mg/kg, respectively) reduced cue-induced cocaine seeking in high impulsive Wistar rats (Ziebnik and Carroll 2015) and high impulsive Listar Hooded rats (Economidou et al. 2009). Moreover, subchronic administration of atomoxetine (1 mg/kg) reduced context-induced cocaine seeking during reinstatement testing in adult WIS rats 10 days after treatment discontinuation (Broos et al. 2015). Another report showed that acute administration of 1.0–3.0 mg/kg of the selective NET inhibitors

desipramine and nisoxetine did not alter cocaine self-administration in adult Sprague-Dawley rats (Tella 1995). In the present study, chronic administration of a low dose (0.3 mg/kg) of atomoxetine during adolescence did not alter PR breakpoints for cocaine in adult SHR, WKY and WIS pretreated with the 0 μg idazoxan control dose in prelimbic cortex. The subsequent findings with idazoxan (10–56 μg) suggest that enhanced noradrenergic transmission at post-synaptic α2-adrenergic receptors may explain why adolescent atomoxetine treatment does not increase cocaine self-administration in SHR (see section 4.1 below) and that adolescent atomoxetine treatment may downregulate pre-synaptic α2A-adrenergic autoreceptors in SHR (see section 4.2 below). The equal distribution of cannula placements across superficial and deep layers of prelimbic cortex in each of the six groups of rats argues against differential idazoxan effects based on layer targeted. Idazoxan effects were observed only in atomoxetine-treated SHR, whether or not superficial or deep layers were targeted. Although the present study did not include a control brain region for idazoxan pretreatment, negligible effects of idazoxan on inactive lever responses in all strains and on PR breakpoints in vehicle-treated SHR and in atomoxetine- and vehicle-treated WKY and WIS serves as a control for potential non-specific effects of idazoxan in the prelimbic cortex.

4.1. Enhanced noradrenergic transmission at post-synaptic α2-adrenergic receptors may explain why adolescent atomoxetine treatment does not increase cocaine self-administration in SHR

In prefrontal cortex, norepinephrine exerts physiological and behavioral effects by binding at pre- and post-synaptic α2-adrenergic receptors (Aoki 1998). When norepinephrine is released from the pre-synaptic terminal and binds to pre-synaptic α2A-adrenergic autoreceptors, the subsequent release of norepinephrine is inhibited (Invernizzi and Garattini 2004). Norepinephrine released into the synapse also binds to post-synaptic α2-adrenergic receptors located on pyramidal dendrites, the stimulation of which improves cognitive function (Hains et al. 2015; Wang et al. 2007). Importantly, therapeutic actions of atomoxetine for ADHD are mediated by enhanced noradrenergic transmission at the post-synaptic α2A adrenergic receptor subtype in prefrontal cortex (Gamo et al. 2010). We suggest that enhanced noradrenergic transmission at post-synaptic α2A-adrenergic receptors in prelimbic cortex may explain why adolescent atomoxetine treatment does not increase cocaine self-administration in adult SHR. Consistent with this view are findings showing that α2-adrenergic receptor agonists attenuate drug-seeking behavior in monkeys trained to self-administer cocaine (Lee et al. 2004).

Current results with idazoxan pretreatment during PR test sessions support the above hypothesis regarding atomoxetine. Specifically, adult SHR that received chronic treatment with atomoxetine (0.3 mg/kg) during adolescence exhibited dose-dependent increases in PR breakpoints and active lever responses relative to chronic vehicle treatment after blockade of α2-adrenergic receptors in prelimbic cortex. Thus, when pre- and post-synaptic α2-adrenergic receptors in prelimbic cortex were blocked concurrently, the neutral effects of adolescent atomoxetine in adult SHR were masked. While antagonism of pre-synaptic α2-adrenergic receptors in prefrontal cortex results in increased norepinephrine release (van Veldhuizen et al. 1994), concurrent antagonism of post-synaptic α2-adrenergic receptors blocks the effects of released norepinephrine on post-synaptic prefrontal pyramidal cells

firing (Wang et al. 2011). These later findings suggest that enhanced noradrenergic transmission at post-synaptic α2-adrenergic receptors in prelimbic cortex after atomoxetine may have prevented adolescent atomoxetine treatment from increasing cocaine self-administration in adult SHR. Under idazoxan pretreatment, adolescent atomoxetine produced an effect on PR breakpoints similar to that produced by adolescent methylphenidate treatment (Harvey et al. 2011; Baskin et al. 2015). The behavioral profile for atomoxetine in SHR receiving idazoxan pretreatment may be related to a dramatic increase in extracellular dopamine, as is observed in rat medial prefrontal cortex after co-administration of the selective NET inhibitor reboxetine and the α2-adrenergic receptor antagonist RX821002 (Masana et al. 2011) and in rat occipital cortex after the selective NET inhibitors reboxetine and desipramine are co-administered with idazoxan (Valentini et al. 2006). Adolescent methylphenidate treatment may enhance cocaine self-administration in adult SHR because of its greater influence on dopamine relative to norepinephrine (Berridge et al. 2006; Bymaster et al. 2002) and ability to increase DAT function in medial prefrontal cortex of SHR, which is an adaptation not observed after chronic adolescent atomoxetine treatment (Somkuwar et al. 2013b). It is noteworthy that adolescent d-amphetamine treatment, like atomoxetine, does not increase cocaine self-administration in the SHR model of ADHD (Jordan et al. 2016a; 2016b) and is more potent for enhancing extracellular norepinephrine than dopamine in rat prefrontal cortex (Berridge and Stalnaker 2002; Easton et al. 2007).

4.2. Adolescent atomoxetine treatment may downregulate pre-synaptic α2A-adrenergic autoreceptors in SHR

A comparison of idazoxan efficacy in vehicle vs. atomoxetine treated SHR offers a partial answer to the question of how adolescent atomoxetine treatment might enhance noradrenergic neurotransmission in SHR to yield a neutral effect on subsequent cocaine self-administration. Although our experimental design cannot dissociate whether the effects of idazoxan are pre- or post-synaptic, idazoxan pretreatment resulted in higher PR breakpoints in adult SHR that had received adolescent atomoxetine treatment compared to vehicle, indicating greater efficacy of the α2-adrenergic receptor antagonist in adult SHR after adolescent atomoxetine treatment. Notably, chronic treatment with another selective NET inhibitor, desipramine, downregulated pre-synaptic α2A-adrenergic autoreceptors and concurrently enhanced the efficacy of an α2-adrenergic receptor antagonist after an acute challenge (Cottingham et al. 2011; Garcia et al. 2004). Given the greater efficacy of acute idazoxan in atomoxetine-treated SHR, these findings suggest that adolescent atomoxetine treatment may downregulate pre-synaptic α2A-adrenergic autoreceptors in prelimbic cortex of SHR. As norepinephrine release from nerve terminals is greater with diminished negative feedback, this mechanism may underlie the observation that atomoxetine does not increase cocaine self-administration in SHR. Future receptor expression studies are needed to confirm this interpretation.

4.3. Differential strain sensitivity to idazoxan after adolescent atomoxetine treatment

In rats that received adolescent vehicle treatment, idazoxan pretreatment had no effects on PR performance, although robust strain differences in PR breakpoints were evident. Results from previous neurochemical studies assessing noradrenergic transmission in prefrontal

cortex of SHR and WKY may explain why the effect of idazoxan is found only in SHR that received adolescent atomoxetine. One study showed that the norepinephrine system is under less inhibitory control (less α2 agonist-induced inhibition of potassium-stimulated norepinephrine release) in non-medicated SHR than WKY (Russell et al. 2000), an effect that is consistent with the compensatory increase in the rate of norepinephrine uptake or clearance in frontal cortex of non-medicated SHR vs. WKY (Myers et al. 1981) or in orbitofrontal cortex of non-medicated SHR vs. WKY and WIS (Somkuwar et al. 2015). If norepinephrine clearance is faster in SHR, then the consequent low extracellular noradrenergic tone in cortex of non-medicated SHR compared to the control strains may contribute to the robust strain differences in PR breakpoints, as reduced noradrenergic transmission at post-synaptic sites within prefrontal cortex may enhance cocaine self-administration (see section 4.1 above). Furthermore, increases in potassium-stimulated norepinephrine release in prefrontal cortex did not differ between non-medicated SHR and WKY after the addition of idazoxan (Russell et al. 2000) or injection of atomoxetine (Ago et al 2014). These neurochemical findings may explain why idazoxan pretreatment in vehicle-treated rats and why saline pretreatment in atomoxetine-treated rats did not have differential effects on PR breakpoints within each of the three strains. If chronic adolescent atomoxetine downregulated pre-synaptic α2A-adrenergic autoreceptors only in SHR (see section 4.2 above), then there is less negative feedback and greater norepinephrine release from nerve terminals. However, the concurrent blockade of postsynaptic α2-adrenergic receptors would negate the impact of this pre-synaptic effect and this action of idazoxan might mask the expected neutral effect of atomoxetine on cocaine self-administration in SHR.

5. Conclusions

The results demonstrate increased motivation to self-administer cocaine in adult SHR that received chronic atomoxetine during adolescence combined with acute idazoxan challenge in prelimbic cortex during adulthood. This finding suggested that enhanced noradrenergic transmission at post-synaptic α2-adrenergic receptors in prelimbic cortex might explain why adolescent atomoxetine treatment does not increase cocaine self-administration in adult SHR (Somkuwar et al. 2013a). Furthermore, the greater efficacy of an acute idazoxan challenge under chronic atomoxetine vs. vehicle treatment conditions in adult SHR suggests that chronic atomoxetine may downregulate pre-synaptic α2A-adrenergic autoreceptors in prelimbic cortex of SHR (Garcia et al. 2004) Such adaptation after chronic atomoxetine would result in enhanced noradrenergic transmission at post-synaptic α2-adrenergic receptors in prelimbic cortex, which supports the explanation for why adolescent atomoxetine treatment does not increase cocaine self-administration in adult SHR.

This preclinical work has important clinical implications. While it is known that atomoxetine is not the gold standard for reducing ADHD symptoms, several clinical studies suggest that it may be a safer choice than methylphenidate for newly medicated adolescents due to abuse potential, diversion, and side effects of methylphenidate (Klein-Schwartz and McGrath 2003; Garnier et al. 2010; Prasad and Steer 2008; Efron et al. 1997; Stein et al. 2003). The present preclinical research supports the use of atomoxetine over methylphenidate for treatment of adolescent ADHD to avoid an increase in cocaine use in adulthood after discontinuing medication (Harvey et al. 2011; Somkuwar et al. 2013a).

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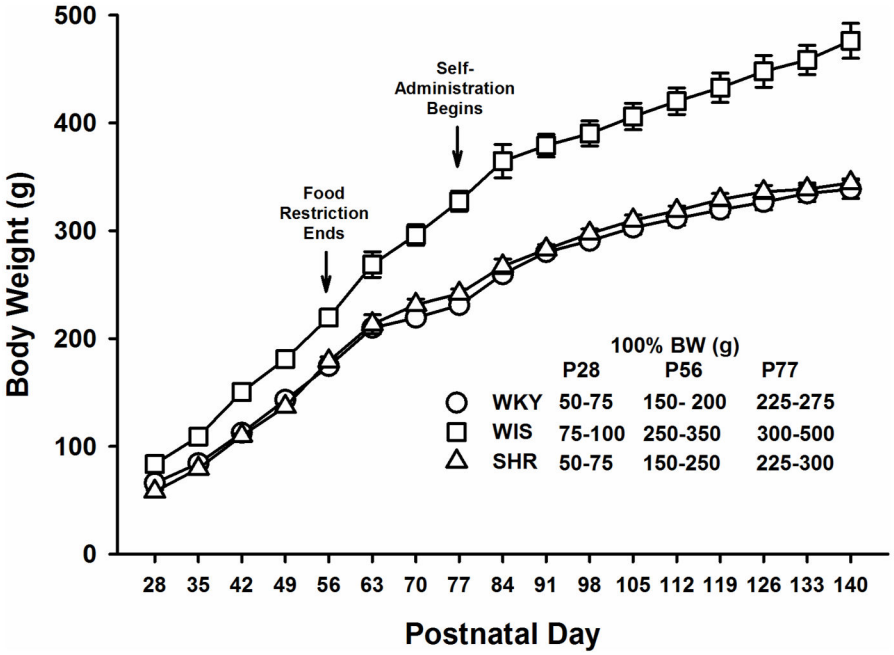

Figure 1.

Mean ± SEM weekly body weight (in grams) recorded from P28 through P140 in WKY, WIS, and SHR. Arrows indicate the time point for the end of food restriction (P56) and beginning of cocaine self-administration sessions (P77). The inset illustrates the 100% ad libitum body weigh ranges for WKY, WIS, and SHR on P28, P56, and P77 and is based on growth charts provided by Charles River Laboratories (www.criver.com).

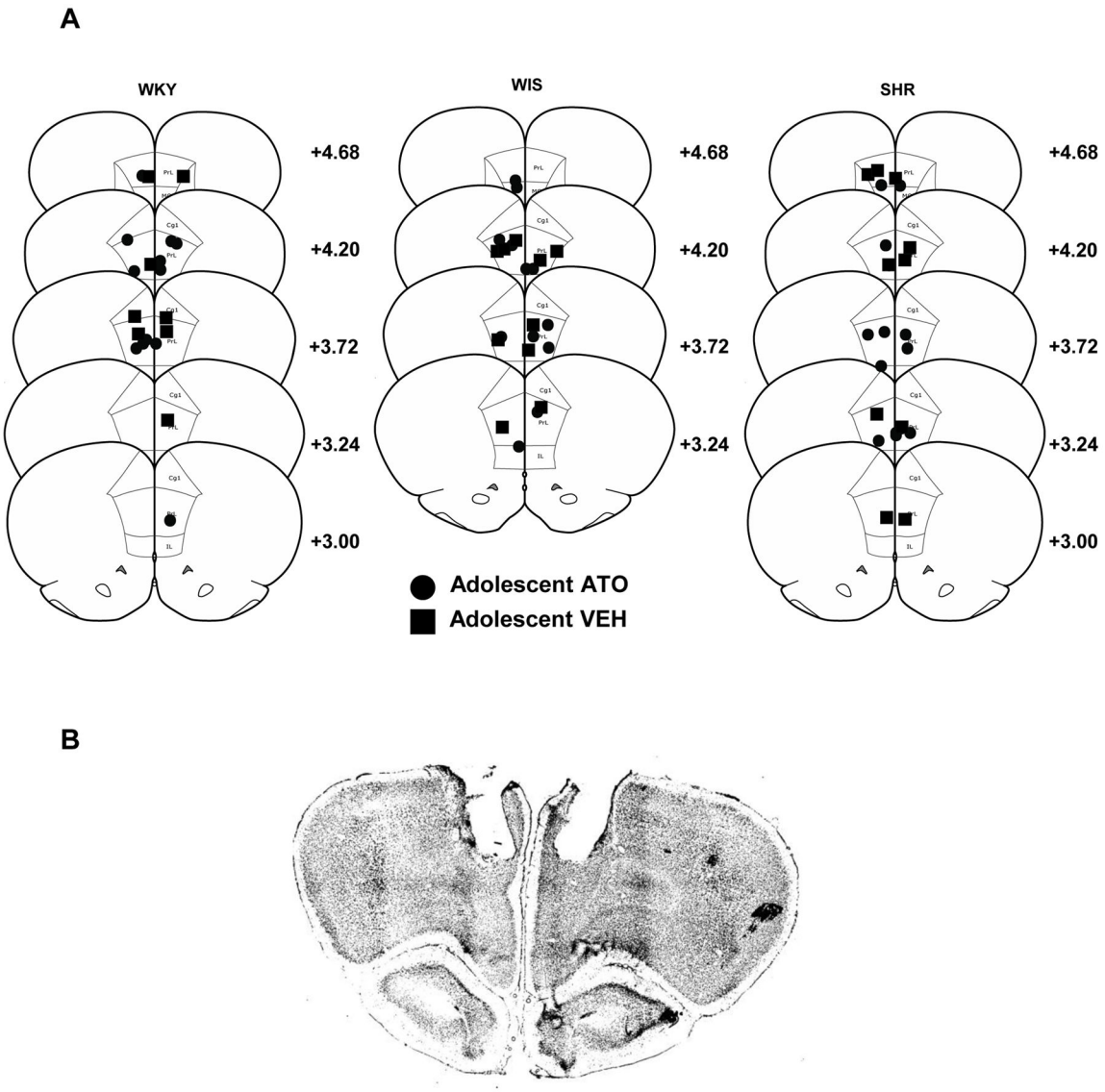

Figure 2.

(A) Schematic drawings of coronal sections depicting idazoxan injection sites in WKY, WIS, and SHR. Sections are arranged from anterior (+4.68 mm) to posterior (+3.00 mm) relative to bregma. Black circles represent placements from animals that had received adolescent atomoxetine (ATO) treatment and black squares represent animals that had received adolescent vehicle (VEH) treatment. (B) Representative photomicrograph of bilateral cannulae tracks in prelimbic cortex.

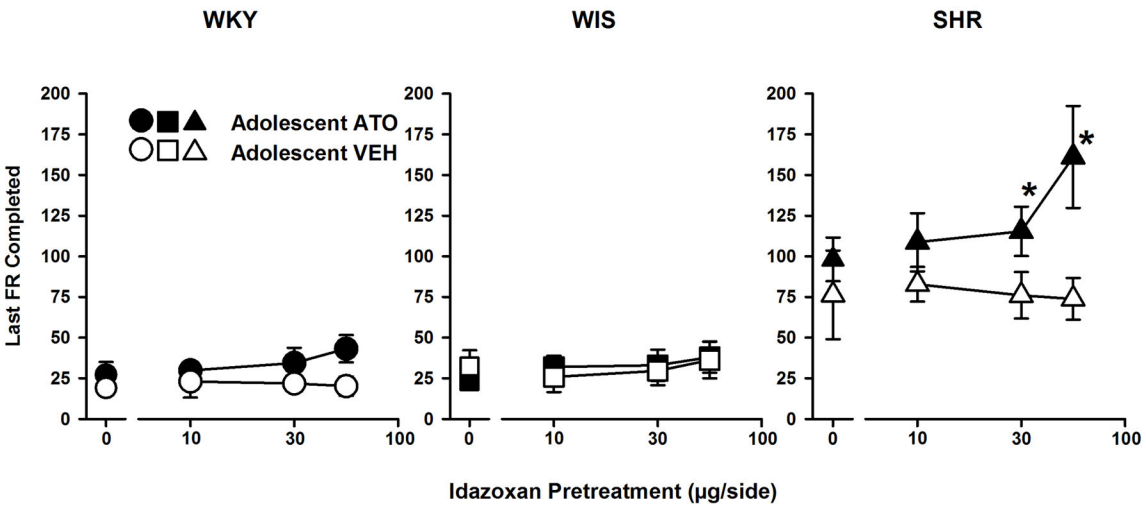

Figure 3.

Effects of idazoxan pretreatment (0 – 56 µg/side; x-axis log scale) in prelimbic cortex on progressive ratio breakpoint for 0.3 mg/kg self-administered cocaine. Values are the mean ± SEM last FR completed in adult WKY, WIS, and SHR that received either 0.3 mg/kg/day atomoxetine (ATO; black symbols; n=6/strain) or vehicle (VEH; white symbols; n=5/strain) treatment during adolescence. * $p<0.01$ compared to VEH-treated SHR that received the same idazoxan pretreatment dose.

Table 1

Mean ± SEM number of sessions to the acquisition criterion, number of infusions at the acquisition criterion, and breakpoints at the acquisition criterion under fixed ratio 1 (FR1) and progressive ratio (PR) schedules of responding maintained by 0.3 mg/kg cocaine

		FR1 Acquisition		PR Acquisition		
Strain	**Tx**	**Sessions to Criterion**	**Infusions at Criterion**	**Sessions to Criterion**	**Infusions at Criterion**	**Breakpoint at Criterion**
WKY	ATO	11.3 (± 0.5)	39.5 (± 7.4)	15.5 (± 1.1)	9.3 (± 1.2)	32.8 (± 9.7)
	VEH	11.8 (± 0.6)	31.8 (± 3.0)	17.2 (± 2.5)	8.0 (± 1.1)	22.2 (± 5.9)
WIS	ATO	10.7 (± 0.7)	49.3 (± 4.9)	17.7 (± 1.3)	9.0 (± 1.4)	31.7 (± 10.3)
	VEH	11.6 (± 0.7)	30.2 (± 4.2)	18.0 (± 0.9)	10.2 (± 0.6)	34.4 (± 4.8)
SHR *	ATO	9.0 (± 0.7)	67.3 (± 6.0)	17.7 (± 1.5)	15.7 (± 0.8)	116.5 (± 18.3)
	VEH	9.4 (± 0.8)	66.6 (± 9.4)	16.4 (± 1.5)	13.6 (± 0.5)	72.2 (± 7.6)

* $p<0.01$ compared to atomoxetine- and vehicle-treated WKY and WIS on all measures, except for sessions to criterion under the PR schedule.

Table 2

Mean ± SEM number of infusions and active and inactive lever responses maintained by 0.3 mg/kg cocaine during progressive ratio testing with idazoxan pretreatment (0–56 μg/side) in prelimbic cortex in WKY, WIS, and SHR that received atomoxetine (ATO) or vehicle (VEH) treatment during adolescence.

		Infusions				Active Lever Responses				Inactive Lever Responses			
		Idazoxan Pretreatment (μg/side)				Idazoxan Pretreatment (μg/side)				Idazoxan Pretreatment (μg/side)			
Strain	**Tx**	**0**	**10**	**30**	**56**	**0**	**10**	**30**	**56**	**0**	**10**	**30**	**56**
WKY	ATO	8.6 (± 1.1)	9.3 (± 0.9)	9.7 (± 1.1)	10.8 (± 1.1)	139 (± 54.9)	142 (± 33.7)	172 (± 63.2)	227 (± 49.6)	12.7 (± 3.9)	12.2 (± 3.8)	9.8 (± 1.9)	17.7 (± 9.6)
	VEH	7.7 (± 0.5)	7.6 (± 1.4)	8.2 (± 0.7)	7.4 (± 1.4)	80.3 (± 11.4)	115 (± 64.2)	96.4 (± 18.4)	86.6 (± 28.5)	10.6 (± 2.7)	8.2 (± 2.1)	7.0 (± 2.6)	8.2 (± 2.1)
WIS	ATO	8.0 (± 1.1)	9.5 (± 1.0)	9.2 (± 1.4)	9.8 (± 1.6)	108 (± 25.2)	155 (± 37.0)	173 (± 58.7)	197 (± 52.7)	16.1 (± 12.7)	16.7 (± 8.8)	20.8 (± 14.8)	30.5 (± 17.6)
	VEH	9.3 (± 1.1)	8.4 (± 1.2)	9.0 (± 1.3)	9.8 (± 1.4)	165 (± 64.4)	119 (± 54.2)	146 (± 54.5)	186 (± 77.9)	6.0 (± 1.9)	6.0 (± 2.7)	6.6 (± 3.3)	16.2 (± 11.2)
SHR	ATO	14.9 (± 0.7)	15.3 (± 0.8)	15.7 (± 0.7)	17.0 (± 1.1)	576 (± 92.7)	642 (± 132.3)	696 (± 97.7) *	1008 (± 207) *	61.8 (± 21.7)	63.3 (± 30.8)	59.7 (± 19.9)	103 (± 42.9)
	VEH	14.1 (± 0.5)	14.3 (± 0.8)	13.0 (± 0.9)	13.3 (± 1.4)	444 (± 63.0)	478 (± 83.0)	454 (± 105)	421 (± 83.7)	43.0 (± 6.2)	29.0 (± 6.0)	25.5 (± 5.0)	24.0 (± 4.6)

* $p<0.01$ compared to VEH-treated SHR that received the same idazoxan pretreatment dose.