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Conditioned Inhibition in a Rodent Model of Attention-Deficit/Hyperactivity Disorder

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Abstract

A deficit in inhibition may underlie some of the symptoms of Attention-deficit/hyperactivity disorder (ADHD), particularly impulsivity. However, the data on inhibitory deficits in children with ADHD are mixed. Moreover, there has been little characterization of inhibitory processes in animal models of ADHD. Pavlov's conditioned inhibition procedure allows a direct assessment of the inhibitory status of a stimulus via summation and retardation tests. Therefore, in the current study we examined conditioned inhibition in spontaneously hypertensive rats (SHRs), the most well-validated animal model of ADHD. SHRs and Wistar rats were trained in a simultaneous feature-negative discrimination in eyeblink conditioning. Each session consisted of a mixture of two trial types: a tone paired with a periocular stimulation (A+) or a tone and light presented simultaneously without a periocular stimulation (XA−). Both SHRs and Wistars were able to discriminate A+ from XA− trials. In subsequent summation (X presented simultaneously with a different conditioned excitator, B) and retardation (X paired with the periocular stimulation) tests, the presence of inhibition to X was confirmed in both SHRs and Wistars: X reduced responding to B and X was slow to develop excitation when paired with periocular stimulation. These results are the first to demonstrate Pavlovian conditioned inhibition in SHRs and to use a summation and a retardation test to confirm X as a conditioned inhibitor. The data indicate that conditioned inhibition is intact in SHRs, thus inhibitory processes that do not require prefrontal cortex or cerebellum may be normal in this strain.

Keywords

SHR; eyeblink conditioning; inhibition; feature-negative; ADHD

Attention-deficit/hyperactivity disorder (ADHD) is a neurobehavioral disorder with core symptoms of inattention and hyperactivity/impulsivity that must be present by age 7 and cause impairment in two or more settings (American Psychiatric Association, 2000). ADHD is more prevalent in boys than in girls and it is estimated to affect 3%-7% of children (American Psychiatric Association, 2000). Deficient inhibition has been hypothesized to be a core feature or endophenotype of ADHD (Barkley, 1997; Groman, James, & Jentsch, 2009; Nigg, 2001, 2006; Sergeant, 2005; but see Castellanos & Tannock, 2002). Nigg

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(2000) proposed a taxonomy of inhibition that includes executive (suppression of a response in the service of longer-term goals), motivational (suppression of a response to immediate novelty or punishment cues), and automatic inhibition (suppression of recently inspected stimuli or stimuli at unattended locations while attending elsewhere). Nigg (2001) reviewed evidence for a deficit in executive inhibition in ADHD and proposed that ADHD is associated with a deficit in executive motoric inhibition (measured with the antisaccade task), but not executive attentional inhibition (measured with the attentional blink task; e.g., Carr, Henderson, & Nigg, 2010; Carr, Nigg, & Henderson, 2006). Other measures of executive motoric inhibition (stop signal task; go/no-go task) also reveal deficiencies in executive inhibition in ADHD (see Nigg, 2001 for a review).

Spontaneously hypertensive rats (SHRs) are the most well-validated animal model of ADHD (Sagvolden, Russell, Aase, Johansen, & Farshbaf, 2005). Inhibition in SHRs mostly has been studied in appetitive operant tasks. These tasks could be argued to be most closely aligned with executive inhibition as defined by Nigg (2000). Several studies have used the differential reinforcement of low rates (DRL) operant task to examine timing and response inhibition processes in SHRs (Bull, Reavill, Hagan, Overend, & Jones, 2000; Ferguson et al., 2007; Orduna, Valencia-Torres, & Bouzas, 2009; Sanabria & Killeen, 2008; van den Bergh et al., 2006). While the results across these studies are somewhat mixed, the general conclusion from studies that have dissociated timing and inhibition processes is that SHRs show an inability to withhold responding in the DRL even while they are able to accurately time the intervals involved (Orduna et al., 2009; Sanabria & Killeen, 2008). Furthermore, this is not due to an abnormality in the ability to lever-press as the same dissociation is observed in a lever-holding task (Sanabria & Killeen, 2008). Finally, SHRs also show a heightened preference for a small, immediate reinforcer compared to a larger, delayed reinforcer (delay discounting; Bizot et al., 2007; Fox, Hand, & Reilly, 2008; Hand, Fox, & Reilly, 2006; Johansen, Sagvolden, & Kvande, 2005; Pardey, Homewood, Taylor, & Cornish, 2009), a possible indicator of deficient choice inhibition.

The main purpose of the current study was to examine Pavlovian conditioned inhibition (CI) in SHRs using a simultaneous feature-negative (FN) discrimination eyeblink conditioning procedure and to verify the net inhibitory status of the putative conditioned inhibitor via summation and retardation tests. CI procedures have been well-established in rat eyeblink conditioning by Freeman and colleagues and we based our stimuli and design on one of their studies that thoroughly documented the inhibitory status of the putative inhibitor (Campolattaro, Schnitker, & Freeman, 2008). An advantage of assessing CI using eyeblink conditioning, rather than other conditioning preparations, is that CI in eyeblink conditioning has the largest database of CI substrates (Berthier & Moore, 1980; Blazis & Moore, 1991; Campolattaro & Freeman, 2006; Freeman, Halverson, & Poremba, 2005; Freeman & Nicholson, 1999; Mis, 1977; Moore, Yeo, Oakley, & Russell, 1980; Nicholson & Freeman, 2002; Nolan & Freeman, 2005; Nolan, Nicholson, & Freeman, 2002; Solomon, 1977; Yeo, Hardiman, Moore, & Russell, 1983). Reliance on eyeblink conditioning for exploring CI thus narrows potential targets for manipulation in follow-up studies. Furthermore, because the stimuli used and the response measured are simple, eyeblink conditioning allows potential sensory and motor differences between groups to be easily measured and ruled out. In addition, because of the short intervals involved, the involvement of other executive processes such as working memory is minimized. Finally, eyeblink conditioning is conducted and measured in humans in much the same way as in non-human animals, facilitating cross-species comparisons.

To our knowledge, there is only a single published study examining inhibition in SHRs in a Pavlovian conditioning task. Bucci and colleagues tested male and female SHRs in a serial FN discrimination appetitive conditioning procedure (Bucci, Hopkins, Keene, Sharma, &

Orr, 2008). Female SHR_s showed less robust discrimination than male SHR_s, in terms of the number of food cup entries and time spent in the food cup. Both sexes did discriminate, however, and appeared to show somewhat stronger discrimination (in terms of food cup entries) than Wistar-Kyoto (WKY) rats tested in a separate experiment. However, the serial FN discrimination used in Bucci et al. (2008) does not pass the standard versions of the summation or the retardation tests (Holland, 1984, 1989), the accepted indices of Pavlovian conditioned inhibition (Papini & Bitterman, 1993; Rescorla, 1969; Savastano, Cole, Barnett, & Miller, 1999; Williams, Overmier, & LoLordo, 1992). Instead, serial FN discriminations may bias the feature stimulus towards functioning as a negative occasion setter, which activates a CS-US inhibitory association (Holland, 1984). The feature in a serial FN discrimination would not pass the standard summation or retardation tests because the standard version of these tests does not activate a CS-US inhibitory association established by a serial FN discrimination (Holland, 1984, 1989; Holland & Lamarre, 1984; Lamarre & Holland, 1987). In contrast, simultaneous FN discriminations may bias the feature stimulus towards functioning as a conditioned inhibitor, which inhibits a US representation (Holland, 1984, 1989; Holland & Lamarre, 1984; Lamarre & Holland, 1987). The feature in a simultaneous FN discrimination would pass both tests because its inhibitory power extends to any CS associated with the US. Thus, in the current study we examined whether SHR_s could learn a simultaneous FN discrimination in eyeblink conditioning and, if so, whether this was due to detectable inhibition to the feature cue.

Method

Subjects

Subjects were 20 male Wistar rats and 20 male SHR_s (SHR/NHsd) from Harlan (Indianapolis, IN). We chose to use Wistars as the comparison strain rather than WKY rats to both match our previous eyeblink conditioning work with SHR_s (Chess & Green, 2008) and because there is some disagreement in the literature about whether the WKY strain itself shows abnormalities in behavior. For example, Sagvolden and colleagues (Sagvolden, Johansen, Woien, Walaa, Storm-Mathisen, Bergersen et al., 2009) argue that WKYs from Charles River and Harlan are not genetically identical and that WKYs from Harlan are the appropriate control for SHR_s. However, WKYs from Harlan have been shown to exhibit depressive-like behaviors, such as increased immobility in a forced swim test, as well as enhanced pain sensitivity (e.g., Burke, Hayes, Calpin, Kerr, Moriarty, Finn, & Roche, 2010).

Rats were between 59 and 63 days old when they arrived in the colony. After arrival, the rats were housed individually for approximately one week prior to surgery with ad libitum chow and water. The colony was maintained on a 12 hour light-dark cycle (lights on at 7 am). All procedures were approved by the Institutional Animal Care and Use Committee at the University of Vermont.

Surgery

Rats were anesthetized using 3% isoflurane in oxygen and, using aseptic surgical procedures, each rat was surgically prepared with differential electromyographic (EMG) recording wires for recording eyeblinks and a bipolar periocular stimulation electrode for delivering the stimulation US. In addition, a ground wire was connected to three stainless steel skull screws.

The EMG wires for recording activity of the external muscles of the eyelid, the orbicularis oculi, were constructed of two strands of ultra-thin (75 μ m) Teflon-coated stainless steel wire soldered at one end to a mini-strip connector. The other end of each wire was passed subdermally to penetrate the skin of the upper eyelid of the left eye and a small amount of

the insulation was removed. The bipolar stimulation electrode (Plastics One, Roanoke, VA) was positioned subdermally immediately dorsocaudal to the left eye. The mini-strip connector and the bipolar stimulation electrode were cemented to the skull with dental cement. The wound was salved with antibiotic ointment (Povidone), and an analgesic (buprenorphine) was administered (s.c.) immediately after surgery and twice the following day. Rats were given 6-7 days to recover prior to eyeblink conditioning.

Apparatus

Eyeblink conditioning took place in one of four identical testing chambers (30.5 × 24.1 × 29.2 cm; Med-Associates, St. Albans, VT), each with a grid floor. The top of each chamber was modified so that a 25-channel tether/commutator could be mounted to it. Each testing chamber was housed within an electrically-shielded, sound-attenuating chamber (45.7 × 91.4 × 50.8 cm; BRS-LVE, Laurel, MD). A fan in each sound-attenuating chamber provided background noise of approximately 60 dB sound pressure level (SPL). A speaker was mounted in each corner of the rear wall and a houselight (off during testing, except when used as a conditioned stimulus) was mounted in the center of the rear wall of each sound-attenuating chamber. The sound-attenuating chambers were housed within a walk-in sound-proof chamber.

Stimulus delivery and recording of eyelid EMG activity were controlled by a computer interfaced with a Power 1401 high-speed data acquisition unit and running Spike2 software (CED, Cambridge, UK). All conditioned stimuli were 490 ms in duration. A 2 kHz, 80 dB tone served as conditioned stimulus A (CSA) and an 8 kHz, 80 dB tone served as conditioned stimulus B (CSB). A 25 lux houselight served as conditioned stimulus X (CSX). A 15 ms, 4.0 mA unipolar periorbital stimulation, delivered from a constant current stimulator (model A365D; World Precision Instruments, Sarasota, FL), served as the unconditioned stimulus (US). The eyelid EMG signals were amplified (10k) and bandpass filtered (100-1000 Hz) prior to being passed to the Power 1401 and from there to a computer running Spike2. Spike2 was used to full-wave rectify, smooth (10 ms time constant), and time shift (10 ms, to compensate for smoothing) the amplified EMG signal.

Procedure

The procedure was adapted, with slight modifications, from Freeman and colleagues (Campolattaro et al., 2008). At the beginning of each session, each rat was plugged in, via the connectors cemented to its head, to the 25-channel tether/commutator, which carried leads to and from peripheral equipment and allowed the rat to move freely within the testing box. On Day 1 (adaptation), rats were plugged in but no stimuli were delivered. They remained in the chamber for 60 min (the approximate length of a training session). Spontaneous eyelid EMG activity was sampled for the same duration and at the same time points as during the sessions in which stimuli were delivered (i.e., 2 sec samples with an average intertrial interval of 30 sec and a range of 20-40 sec). The purpose of this session was to adapt the rats to the chamber as well as to get a measure of baseline eyelid activity in the absence of the CS and US. On Days 2-11 (conditioning), rats in Group Conditioned Inhibition (CI; 10 SHRs, 10 Wistars) received 100 trials per day, with an average inter-trial interval (ITI) of 30 sec (range = 20-40 sec). Fifty of these trials consisted of CSA coterminating with the periorbital stimulation US. The other fifty of these trials consisted of CSA and CSX presented simultaneously with no US. The two trial types were intermixed such that there were three or fewer consecutive trials of a particular type. Rats in Group Control (10 SHRs, 10 Wistars) also received 100 trials per day at an average ITI of 30 sec. Half of these trials were CSA paired with the US and the other half of these trials were CSA alone. On Day 12, all rats received 100 trials at an average ITI of 30 sec. Each of these trials consisted of CSB coterminating with the US. On Day 13 (summation), rats in Group CI

received 30 trials of CSB alone and 30 trials of CSB and CSX presented simultaneously. The two trial types were intermixed such that there were three or fewer consecutive trials of a particular type. Rats in Group Control received 60 trials of CSB alone. On Days 14 and 15 (retardation), all rats received 100 trials per day at an average ITI of 30 sec. Each of these trials consisted of CSX coterminating with the US.

Data Analysis

Trials were subdivided into four time periods: (1) a “baseline” period, 280 ms prior to CS onset; (2) a “startle” period, 0-80 ms after CS onset; (3) a CR period, 81-475 ms after CS onset; and (4) a UR period, 65-165 after US onset (the first 65 ms was obscured by the shock artifact). Eye blinks that exceeded mean baseline activity by 0.5 arbitrary units (range = 0.0 to 5.0) during the CR period were scored as CRs. This time point was also defined as CR onset. The difference in time (in milliseconds) between CS onset and CR onset represents CR onset latency. Eyeblinks that met the response threshold during the startle period were scored as startle responses (SRs). Data were analyzed using SPSS 17.0.2. An alpha level of 0.05 was used as the rejection criterion for all statistical tests, except where noted. We made estimates of effect size for each significant factor using partial eta squared (η^2), which is equal to sum of squares of an effect / (sum of squares of an effect + sum of squares of the error term of an effect).

Results

Training

One Wistar in Group CI was removed because he was unresponsive to the periorbital stimulation US, leaving 9 Wistars in Group CI and 10 Wistars in Group Control. Wistars in Group CI responded significantly more on A+ trials compared to XA- trials, indicating that Wistars, like Long-Evans rats (Campolattaro et al., 2008), learn this FN discrimination (Figure 1A). In contrast, Wistars in Group Control did not respond significantly more on A+ trials compared to A- trials (Figure 1B). These observations were confirmed by a 2 (Group) $\times$ 2 (Trial Type) $\times$ 10 (Session) repeated-measures ANOVA which revealed a significant main effect of Trial Type, $F(1,17) = 34.28$, $p < 0.01$, partial $\eta^2 = 0.67$, a significant main effect of Session, $F(9,153) = 28.77$, $p < 0.01$, partial $\eta^2 = 0.63$, a significant Trial Type $\times$ Session interaction effect, $F(9,153) = 7.27$, $p < 0.01$, partial $\eta^2 = 0.30$, and a significant Group $\times$ Trial Type $\times$ Session interaction effect, $F(9,153) = 8.21$, $p < 0.01$, partial $\eta^2 = 0.33$. Follow-up simple effects tests using paired-samples t-tests and a Bonferroni-corrected alpha of 0.005 for 10 contrasts revealed that Wistars in Group CI responded significantly more on A+ trials compared to XA- trials in Sessions 4-10 (p 's ≤ 0.004). In contrast, Wistars in Group Control did not respond differentially between the two trial types except in Session 9 when they responded more on A- trials compared to A+ trials ($p = 0.002$ in Session 9).

SHRs in Group CI also learned the FN discrimination (Figure 2A). In contrast, SHRs in Group Control did not respond significantly more on A+ trials compared to A- trials (Figure 2B). These observations were confirmed by a 2 (Group) $\times$ 2 (Trial Type) $\times$ 10 (Session) repeated-measures ANOVA which revealed a significant main effect of Trial Type, $F(1,18) = 58.05$, $p < 0.01$, partial $\eta^2 = 0.76$, a significant main effect of Session, $F(9,162) = 86.11$, $p < 0.01$, partial $\eta^2 = 0.83$, a significant Trial Type $\times$ Session interaction effect, $F(9,162) = 2.10$, $p < 0.04$, partial $\eta^2 = 0.10$, and a significant Group $\times$ Trial Type $\times$ Session interaction effect, $F(9,162) = 1.95$, $p < 0.05$, partial $\eta^2 = 0.10$. Follow-up simple effects tests using paired-samples t-tests and a Bonferroni-corrected alpha of 0.005 for 10 contrasts revealed SHRs in Group CI responded significantly more on A+ trials compared to XA- trials in Sessions 2-9 (p 's ≤ 0.005). In contrast, SHRs in Group Control did not respond differentially between the two trial types in any Session.

As a further measure of discrimination, we computed a discrimination ratio (percentage of CRs to A+ / [(percentage of CRs to A+) + (percentage of CRs to XA-)]) for each rat in Group CI in each session. Thus, a discrimination ratio of 0.5 means that there was no discrimination and responding was equal between the two trial types. We then computed a series of one-sample t-tests comparing the discrimination ratio for each session in each group to 0.5 and using a Bonferroni-corrected alpha of 0.005 for 10 contrasts. For Wistars in Group CI, the discrimination ratio was significantly greater than 0.5 in Sessions 4, 5, 7, and 10 (p 's ≤ 0.001). For SHRs in Group CI, the discrimination ratio was significantly greater than 0.5 in Sessions 2-7 (p 's ≤ 0.005), with Sessions 8 and 9 approaching significance (p 's = 0.006).

Previously, we reported that SHRs displayed a shorter CR onset latency than Wistars during a 750 ms delay procedure (Chess & Green, 2008). We compared CR onset latencies on A+ trials (475 ms delay) between SHRs and Wistars. Three Wistars were eliminated from this analysis because they failed to show CRs in one or more of Sessions 2-10. We chose to analyze Sessions 2-10 so as to include two other Wistars who failed to show CRs in Session 1. In addition, we combined Groups CI and Control within each strain for this analysis. As in our previous study (Chess & Green, 2008), SHRs showed a significantly shorter onset latency than Wistars ($M = 223.9 \text{ ms} \pm 6.76 \text{ SEM}$ for SHRs; $M = 291.2 \text{ ms} \pm 7.56 \text{ SEM}$ for Wistars). This was confirmed by a 2 (Strain) $\times$ 9 (Session) repeated-measures ANOVA which revealed a significant main effect of Strain, $F(1,34) = 44.04$, $p < 0.01$, partial $\eta^2 = 0.56$, a significant main effect of Session, $F(8,272) = 9.23$, $p < 0.01$, partial $\eta^2 = 0.56$, and a significant Strain $\times$ Session interaction effect, $F(8,272) = 2.19$, $p < 0.03$, partial $\eta^2 = 0.06$. Follow-up simple effects tests of the significant interaction effect using independent-samples t-tests revealed no significant simple effects at a Bonferroni-corrected alpha of 0.006 (0.05/9). Thus, SHRs showed a shorter CR onset latency than Wistars overall.

Finally, we compared UR amplitude between strains in Session 1, before the development of a significant number of CRs could distort this measure. There were no differences between strains or groups in UR amplitude. This was confirmed by a 2 (Strain) $\times$ 2 (Group) ANOVA (p 's > 0.13). Mean UR amplitude was $2.17 (\pm 0.24 \text{ SEM})$ for SHRs and $2.42 (\pm 0.21 \text{ SEM})$ for Wistars.

Summation Test

Data from 9 Wistars in Group CI and 10 Wistars in Group Control were analyzed. The Light CS (X) passed the summation test for a conditioned inhibitor in Wistars in Group CI (Figure 3A); Wistars in Group CI responded less on XB- trials compared to B- trials. For Group Control, there was no difference between B- trials presented at comparable time points to B- versus XB- trials in Group CI. These observations were confirmed by a 2 (Group) $\times$ 2 (Trial Type) repeated-measures ANOVA which revealed a significant Group $\times$ Trial Type interaction effect, $F(1,17) = 6.60$, $p = 0.02$, partial $\eta^2 = 0.28$. Follow-up simple effects tests using one-way repeated-measures ANOVAs revealed a significant difference between trial types in Group CI, $F(1,8) = 7.66$, $p < 0.03$, partial $\eta^2 = 0.49$ but not in Group Control, $F(1,9) = 0.95$, $p > 0.35$.

One SHR in Group CI lost its headstage prior to the Summation Test and one SHR in Group CI had poor EMG data due to a faulty tether. Therefore, data from 8 SHRs in Group CI and 10 SHRs in Group Control were analyzed. The Light CS (X) passed the summation test for a conditioned inhibitor in SHRs in Group CI (Figure 3B); SHRs in Group CI responded less on XB- trials compared to B- trials. For Group Control, there was no difference between B- trials presented at comparable time points to B- versus XB- trials in Group CI. These observations were confirmed by a 2 (Group) $\times$ 2 (Trial Type) repeated-measures ANOVA which revealed a significant Trial Type effect, $F(1,16) = 12.09$, $p = 0.01$, partial $\eta^2 = 0.43$.

and a significant Group $\times$ Trial Type interaction effect, $F(1,16) = 7.62$, $p < 0.02$, partial $\eta^2 = 0.32$. Follow-up simple effects tests using one-way repeated-measures ANOVAs revealed a significant difference between trial types in Group CI, $F(1,7) = 11.59$, $p < 0.02$, partial $\eta^2 = 0.62$ but not in Group Control, $F(1,9) = 0.48$, $p > 0.50$.

Retardation Test

Data for the two sessions of the retardation test were blocked into 50 trials corresponding to the first and second halves of each session. Each session was analyzed separately with a 2 (Group) $\times$ 2 (Block) repeated-measures ANOVA.

Data from 9 Wistars in Group CI and 10 Wistars in Group Control were analyzed for both Retardation Session 1 and Retardation Session 2. The Light CS (X) passed the retardation test for a conditioned inhibitor in Wistars (Figure 4A). Both groups showed learning across the two 50 trial blocks in each retardation session. However, Wistars in Group CI responded significantly less to X compared to Wistars in Group Control in Retardation Session 1. These observations were confirmed by a pair of 2 (Group) $\times$ 2 (Block) repeated-measures ANOVAs. For Retardation Session 1, there was a significant Group main effect, $F(1,17) = 4.94$, $p = 0.04$, partial $\eta^2 = 0.22$ and a significant Block main effect, $F(1,17) = 7.11$, $p < 0.02$, partial $\eta^2 = 0.29$. For Retardation Session 2, there was a significant Block main effect, $F(1,17) = 14.64$, $p < 0.01$, partial $\eta^2 = 0.46$, but the Group main effect was not significant, $F(1,17) = 2.35$, $p > 0.14$.

One SHR in Group Control lost its headstage prior to Retardation Session 1 and two SHRs in Group Control lost their headstages prior to Retardation Session 2. Data from 9 SHRs in Group CI and 9 SHRs in Group Control were analyzed for Retardation Session 1 and data from 9 SHRs in Group CI and 7 SHRs in Group Control were analyzed for Retardation Session 2. The Light CS (CSX) passed the retardation test for a conditioned inhibitor in SHRs (Figure 4B). Both groups showed learning across the two 50 trial blocks in each retardation session and SHRs in Group CI responded significantly less to CSX compared to SHRs in Group Control in both retardation sessions. These observations were confirmed by a pair of 2 (Group) $\times$ 2 (Block) repeated-measures ANOVAs. For Retardation Session 1, there was a significant Group main effect, $F(1,16) = 4.76$, $p < 0.05$, partial $\eta^2 = 0.23$ and a nearly significant Block main effect, $F(1,16) = 4.14$, $p = 0.059$, partial $\eta^2 = 0.21$. For Retardation Session 2, there was a significant Group main effect, $F(1,14) = 5.98$, $p < 0.03$, partial $\eta^2 = 0.30$ and a significant Block main effect, $F(1,14) = 5.83$, $p = 0.03$, partial $\eta^2 = 0.29$.

Discussion

Our results indicate that, like Wistars, SHRs discriminate between A+ and XA- trials in a simultaneous FN discrimination. Importantly, our results suggest that both strains discriminate A+ from XA- by the development of inhibition to X, as confirmed by a summation test and a retardation test. Our results extend the findings of Bucci et al. (2008) who, in the sole published report on Pavlovian inhibition and SHRs, showed that male and female SHRs can learn a serial FN discrimination in appetitive conditioning. We further extended those findings to show that male SHRs can learn a simultaneous FN discrimination and they most likely do so by developing inhibition to the feature, X. Finally, we replicated our previous finding of a shortened eyeblink CR onset latency in SHRs (Chess & Green, 2008).

A small note of caution is warranted regarding the summation and retardation tests; because we had only one control group, our results do not completely rule out the possibility that Wistars and/or SHRs passed the summation test due to a generalization decrement (from

compounding B with X) and passed the retardation test due to latent inhibition (because the CI group received preexposure to X during discrimination training and the summation test while the control group did not). However, the most parsimonious interpretation of our results is that both Wistars and SHRs passed the summation and retardation tests due to the development of inhibition to X. Furthermore, Campolattaro et al. (2008), whose methods and results were very similar to ours, included additional control groups that helped to rule out these alternative explanations.

Recently, it has been argued that temporal processing, inhibitory control, and tolerance of delay are all impaired in ADHD, although some children may have deficits in only one of these constructs (Sonuga-Barke, Bitsakou, & Thompson, 2010). SHRs may model ADHD-related deficits in all three of these factors. For example, delay eyeblink conditioning may draw heavily upon temporal processing (Mauk & Donegan, 1997; Thompson & Steinmetz, 2009), and possibly some form of inhibition (i.e., inhibition of delay) as conditioning progresses (Thanellou & Green, 2009; Vogel, Brandon, & Wagner, 2003). We have shown (current study; Chess & Green, 2008) that SHRs display an abnormally short eyeblink CR onset latency; others have shown the same thing in children with ADHD (Coffin, Barody, Schneider, & O'Neill, 2005; Frings, Gaertner, Buderath, Gerwig, Christiansen, Schoch et al., 2010). The DRL task may depend upon both temporal processing and inhibitory control throughout training (cf. Sanabria & Killeen, 2008); both SHRs (Orduna et al., 2009; Sanabria & Killeen, 2008) and children with ADHD (Avila, Cuenca, Felix, Parcet, & Miranda, 2004) have difficulty withholding responding in the DRL. Finally, it has been suggested that the delay discounting task is dissociable from temporal processing and inhibitory control, making it a relatively pure measure of tolerance of delay (Sonuga-Barke et al., 2010). Both SHRs (Bizot et al., 2007; Fox et al., 2008; Hand et al., 2006; Johansen et al., 2005; Pardey et al., 2009) and children with ADHD (e.g., Marco, Miranda, Schlotz, Melia, Mulligan Muller et al., 2009) show a heightened preference for a small, immediate reward over a larger, delayed reward (impulsive choice). Thus, SHRs may have deficits in temporal processing, inhibitory control, and tolerance of delay while children with ADHD may have deficits in only one of these constructs.

If SHRs model inhibitory control deficiencies in ADHD, why were we able to demonstrate intact inhibition in SHRs in the current study? One possibility is that the inhibition we tapped into with the simultaneous FN discrimination does not depend upon either the cerebellum or the medial prefrontal cortex, making it different from inhibitory processing in simple delay eyeblink conditioning and the DRL. Withholding responses in the DRL requires medial prefrontal cortex (e.g., Cho & Jeantest, 2010) and SHRs have well-documented abnormalities in frontal and striatal catecholamine systems (Linthorst, Van Den Buuse, De Jong, & Versteeg, 1990; Russell, de Villiers, Sagvolden, Lamm, & Taljaard, 1995, 1998; Russell & Wiggins, 2000; Russell, Allie, & Wiggins, 2000). Similarly, delaying eyeblink CRs until the time of the US requires cerebellar cortex (e.g., Medina, Garcia, Nores, Taylor, & Mauk, 2000) and at least one study reported that SHRs have a smaller cerebellum than control strains (Li, Lu, Antonio, Mak, Rudd, Fan, & Yew, 2007). Simultaneous FN discrimination does not require either medial prefrontal cortex (Gewirtz, Falls, & Davis, 1997; Rhodes & Killcross, 2007) or cerebellum (Freeman et al., 2005). Thus, it is possible that inhibition is abnormal in ADHD only when it depends on prefrontal or cerebellar processing. Unfortunately, to our knowledge simultaneous FN discrimination has never been evaluated in children or adolescents with ADHD.

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References

- American Psychiatric Association. Diagnostic and statistical manual of mental disorders. Author; Washington, DC: 2000.
- Avila C, Cuenca I, Felix V, Parcet M-A, Miranda A. Measuring impulsivity in school-aged boys and examining its relationship with ADHD and ODD ratings. *Journal of Abnormal Child Psychology*. 2004; 32:295–304. [PubMed: 15228178]
- Barkley R. Behavioral inhibition, sustained attention, and executive functions: Constructing a unifying theory of ADHD. *Psychological Bulletin*. 1997; 121:65–94. [PubMed: 9000892]
- Berthier NE, Moore JW. Disrupted conditioned inhibition of the rabbit nictitating membrane response following mesencephalic lesions. *Physiology & Behavior*. 1980; 25:667–673. [PubMed: 7443828]
- Bizot J-C, Chenault N, Houze B, Herpin A, David S, Pothion S, et al. Methylphenidate reduces impulsive behaviour in juvenile Wistar rats, but not in adult Wistar, SHR and WKY rats. *Psychopharmacology*. 2007; 193:215–223. [PubMed: 17406857]
- Blazis DEJ, Moore JW. Conditioned inhibition of the nictitating membrane response in rabbits following hypothalamic and mesencephalic lesions. *Behavioural Brain Research*. 1991; 46:71–81. [PubMed: 1786114]
- Bucci DJ, Hopkins ME, Keene CS, Sharma M, Orr LE. Sex differences in learning and inhibition in spontaneously hypertensive rats. *Behavioural Brain Research*. 2008; 187:27–32. [PubMed: 17904233]
- Bull E, Reavill C, Hagan JJ, Overend P, Jones DNC. Evaluation of the spontaneously hypertensive rat as a model of attention deficit hyperactivity disorder: Acquisition and performance of the DRL-60s test. *Behavioural Brain Research*. 2000; 109:27–35. [PubMed: 10699655]
- Burke NN, Hayes E, Calpin P, Kerr DM, Moriarity O, Finn DP, Roche M. Enhanced nociceptive responding in two rat models of depression is associated with alterations in monoamine levels in discrete brain regions. *Neuroscience*. 2010; 171:1300–1313. [PubMed: 20955767]
- Campolattaro MM, Freeman JH. Perirhinal cortex lesions impair feature-negative discrimination. *Neurobiology of Learning and Memory*. 2006; 86:205–213. [PubMed: 16617027]
- Campolattaro MM, Schnitker KM, Freeman JH. Changes in inhibition during differential eyeblink conditioning with increased training. *Learning & Behavior*. 2008; 36:159–165. [PubMed: 18543716]
- Carr LA, Henderson J, Nigg JT. Cognitive control and attentional selection in adolescents with ADHD versus ADD. *Journal of Clinical Child and Adolescent Psychology*. 2010; 39:726–740. [PubMed: 21058121]
- Carr LA, Nigg JT, Henderson JM. Attentional versus motor inhibition in adults with attention-deficit/hyperactivity disorder. *Neuropsychology*. 2006; 20:430–441. [PubMed: 16846261]
- Castellanos FX, Tannock R. Neuroscience of attention-deficit/hyperactivity disorder: The search for endophenotypes. *Nature Reviews Neuroscience*. 2002; 3:617–628.
- Chess AC, Green JT. Abnormal topography and altered acquisition of conditioned eyeblink responses in a rodent model of attention-deficit/hyperactivity disorder. *Behavioral Neuroscience*. 2008; 122:63–74. [PubMed: 18298250]
- Cho YH, Jeantet Y. Differential involvement of prefrontal cortex, striatum, and hippocampus in DRL performance in mice. *Neurobiology of Learning and Memory*. 2010; 93:85–91. [PubMed: 19703576]
- Coffin JM, Barody S, Schneider K, O'Neill J. Impaired cerebellar learning in children with prenatal alcohol exposure: A comparative study of eyeblink conditioning in children with ADHD and dyslexia. *Cortex*. 2005; 41:389–398. [PubMed: 15871603]
- Ferguson SA, Paule MG, Cada A, Fogle CM, Gray EP, Berry KJ. Baseline behavior, but not sensitivity to stimulant drugs, differs among Spontaneously Hypertensive, Wistar-Kyoto, and Sprague-Dawley rat strains. *Neurotoxicology and Teratology*. 2007; 29:547–561. [PubMed: 17689921]
- Fox AT, Hand DJ, Reilly MP. Impulsive choice in a rodent model of attention-deficit/hyperactivity disorder. *Behavioural Brain Research*. 2008; 187:146–152. [PubMed: 17950930]

- Freeman JH, Halverson HE, Poremba A. Differential effects of cerebellar inactivation on eyeblink conditioned excitation and inhibition. *Journal of Neuroscience*. 2005; 25:889–895. [PubMed: 15673669]
- Freeman JH, Nicholson DA. Neuronal activity in the cerebellar interpositus and lateral pontine nuclei during inhibitory classical conditioning of the eyeblink response. *Brain Research*. 1999; 833:225–233. [PubMed: 10375698]
- Frings M, Gaertner K, Buderath P, Gerwig M, Christiansen H, Schoch B, Gizewski ER, Hebebrand J, Timmann D. Timing of conditioned eyeblink responses is impaired in children with Attention-Deficit/Hyperactivity Disorder. *Experimental Brain Research*. 2010; 201:167–176.
- Gewirtz JC, Falls WA, Davis M. Normal conditioned inhibition and extinction of freezing and fear-potentiated startle following electrolytic lesions of medial prefrontal cortex in rats. *Behavioral Neuroscience*. 1997; 111:712–726. [PubMed: 9267649]
- Groman SM, James AS, Jentsch JD. Poor response inhibition: At the nexus between substance abuse and attention deficit/hyperactivity disorder. *Neuroscience and Biobehavioral Reviews*. 2009; 33:690–698. [PubMed: 18789354]
- Hand DJ, Fox AT, Reilly MP. Response acquisition with delayed reinforcement in a rodent model of attention-deficit/hyperactivity disorder (ADHD). *Behavioural Brain Research*. 2006; 175:337–342. [PubMed: 17034874]
- Holland PC. Differential effects of reinforcement of an inhibitory feature after serial and simultaneous feature negative discrimination training. *Journal of Experimental Psychology: Animal Behavior Processes*. 1984; 10:461–475. [PubMed: 6491607]
- Holland PC. Transfer of negative occasion setting and conditioned inhibition across conditioned and unconditioned stimuli. *Journal of Experimental Psychology: Animal Behavior Processes*. 1989; 15:311–328. [PubMed: 2794868]
- Holland PC, Lamarre J. Transfer of inhibition after serial and simultaneous feature negative discrimination training. *Learning and Motivation*. 1984; 15:219–243.
- Johansen EB, Sagvolden T, Kvande G. Effects of delayed reinforcers on the behavior of an animal model of attention-deficit/hyperactivity disorder (ADHD). *Behavioural Brain Research*. 2005; 162:47–61. [PubMed: 15922066]
- Lamarre J, Holland PC. Transfer of inhibition after serial feature negative discrimination training. *Learning and Motivation*. 1987; 18:319–342.
- Li Q, Lu G, Antonio GE, Mak YT, Rudd JA, Fan M, Yew DT. The usefulness of the spontaneously hypertensive rat to model attention-deficit/hyperactivity disorder (ADHD) may be explained by the differential expression of dopamine-related genes in the brain. *Neurochemistry International*. 2007; 50:848–857. [PubMed: 17395336]
- Linthorst ACE, Van Den Buuse M, De Jong W, Versteeg DHG. Electrically stimulated [^3H]dopamine and [^{14}C]acetylcholine release from nucleus caudatus slices: Differences between spontaneously hypertensive rats and Wistar-Kyoto rats. *Brain Research*. 1990; 509:266–272. [PubMed: 2138926]
- Marco R, Miranda A, Schlotz W, Melia A, Mulligan A, Muller U, Andreou P, Butler L, Christiansen H, Gabriels I, Medad S, Albrecht B, Uebel H, Asherson P, Banaschewski T, Gill M, Kuntsi J, Mulas F, Oades R, Roeyers H, Steinhausen H-C, Rothenberger A, Faraone SV, Sonuga-Barke EJS. Delay and reward choice in ADHD: An experimental test of the role in delay aversion. *Neuropsychology*. 2009; 23:367–380. [PubMed: 19413450]
- Mauk MD, Donegan NH. A model of Pavlovian eyelid conditioning based on the synaptic organization of the cerebellum. *Learning & Memory*. 1997; 3:130–158. [PubMed: 10456059]
- Medina JF, Garcia KS, Nores WL, Taylor NM, Mauk MD. Timing mechanisms in the cerebellum: Testing predictions of a large-scale computer simulation. *Journal of Neuroscience*. 2000; 20:5516–5525. [PubMed: 10884335]
- Mis FW. A midbrain-brain stem circuit for conditioned inhibition of the nictitating membrane response in the rabbit (*Oryctolagus cuniculus*). *Journal of Comparative and Physiological Psychology*. 1977; 91:975–988.
- Moore JW, Yeo CH, Oakley DA, Russell IS. Conditioned inhibition of the nictitating membrane response in decorticate rabbits. *Behavioural Brain Research*. 1980; 1:397–409. [PubMed: 7236350]

- Nicholson DA, Freeman JH. Neuronal correlates of conditioned inhibition of the eyeblink response in the anterior interpositus nucleus. *Behavioral Neuroscience*. 2002; 116:22–36. [PubMed: 11895180]
- Nigg JT. On inhibition/disinhibition in developmental psychopathology: Views from cognitive and personality psychology and a working inhibition taxonomy. *Psychological Bulletin*. 2000; 126:200–246.
- Nigg JT. Is ADHD a disinhibitory disorder? *Psychological Bulletin*. 2001; 127:571–598. [PubMed: 11548968]
- Nigg, JT. What causes ADHD?. Guilford; New York: 2006.
- Nolan BC, Freeman JH. Purkinje cell loss by OX7-saporin impairs excitatory and inhibitory eyeblink conditioning. *Behavioral Neuroscience*. 2005; 119:190–201. [PubMed: 15727524]
- Nolan BC, Nicholson DA, Freeman JH. Blockade of GABA_A receptors in the interpositus nucleus modulates expression of conditioned excitation but not conditioned inhibition of the eyeblink response. *Integrative Physiological and Behavioral Science*. 2002; 37:293–310. [PubMed: 12645845]
- Orduna V, Valencia-Torres L, Bouzas A. DRL performance of spontaneously hypertensive rats: Dissociation of timing and inhibition of responses. *Behavioural Brain Research*. 2009; 201:158–165. [PubMed: 19428629]
- Papini MR, Bitterman ME. The two-test strategy in the study of inhibitory conditioning. *Journal of Experimental Psychology: Animal Behavior Processes*. 1993; 19:342–352. [PubMed: 8228833]
- Pardey MC, Homewood J, Taylor A, Cornish JL. Re-evaluation of an animal model for ADHD using a free-operant choice task. *Journal of Neuroscience Methods*. 2009; 176:166–171. [PubMed: 18835408]
- Rescorla RA. Pavlovian conditioned inhibition. *Psychological Bulletin*. 1969; 72:77–94.
- Rhodes SEV, Killcross AS. Lesions of rat infralimbic cortex result in disrupted retardation but normal summation test performance following training on a Pavlovian conditioned inhibition procedure. *European Journal of Neuroscience*. 2007; 26:2654–2660. [PubMed: 17970744]
- Russell VA, Allie S, Wiggins T. Increased noradrenergic activity in prefrontal cortex slices of an animal model for attention-deficit/hyperactivity disorder – the spontaneously hypertensive rat. *Behavioural Brain Research*. 2000; 117:69–74. [PubMed: 11099759]
- Russell VA, de Villiers A, Sagvolden T, Lamm M, Taljaard J. Altered dopaminergic function in the prefrontal cortex, nucleus accumbens and caudate-putamen of an animal model of Attention-Deficit Hyperactivity Disorder – the spontaneously hypertensive rat. *Brain Research*. 1995; 676:343–351. [PubMed: 7614004]
- Russell VA, de Villiers A, Sagvolden T, Lamm M, Taljaard J. Differences between electrically-, ritalin-, and D-amphetamine-stimulated release of [³H]dopamine from brain slices suggest impaired vesicular storage of dopamine in an animal model of Attention-Deficit/Hyperactivity Disorder. *Behavioural Brain Research*. 1998; 94:163–171. [PubMed: 9708847]
- Russell VA, Wiggins T. Increased glutamate-stimulated norepinephrine release from prefrontal cortex slices of spontaneously hypertensive rats. *Metabolic Brain Disease*. 2000; 15:297–304. [PubMed: 11383554]
- Sagvolden T, Johansen EB, Woien G, Walaas SI, Storm-Mathisen J, Bergersen LH, et al. The spontaneously hypertensive rat model of ADHD -- The importance of selecting the appropriate reference strain. *Neuropharmacology*. 2009; 57:619–626. [PubMed: 19698722]
- Sagvolden T, Russell VA, Aase H, Johansen EB, Farshbaf M. Rodent models of attention-deficit/hyperactivity disorder. *Biological Psychiatry*. 2005; 57:1239–1247. [PubMed: 15949994]
- Sanabria F, Killeen PR. Evidence for impulsivity in the Spontaneously Hypertensive Rat drawn from complementary response-withholding tasks. *Behavioral and Brain Functions*. 2008; 4:1–17. [PubMed: 18173840]
- Savastano HI, Cole RP, Barnet RC, Miller RR. Reconsidering conditioned inhibition. *Learning and Motivation*. 1999; 30:101–127.
- Sergeant JA. Modeling attention-deficit/hyperactivity disorder: A critical appraisal of the cognitive-energetic model. *Biological Psychiatry*. 2005; 57:1248–1255. [PubMed: 15949995]

- Solomon PR. Role of the hippocampus in blocking and conditioned inhibition of the rabbit's nictitating membrane response. *Journal of Comparative and Physiological Psychology*. 1977; 91:407–417. [PubMed: 404342]
- Sonuga-Barke E, Bitsakou P, Thompson M. Beyond the dual pathway model: Evidence for the dissociation of timing, inhibitory, and delay-related impairments in Attention-Deficit/Hyperactivity Disorder. *Journal of the American Academy of Child & Adolescent Psychiatry*. 2010; 49:345–355. [PubMed: 20410727]
- Thanellou, AG.; Green, JT. Timing of rat conditioned eyeblink responses and inhibition of delay. 2009 Neuroscience Meeting Planner; Chicago, IL: Society for Neuroscience; 2009. Program No. 283.172009. Online
- Thompson RF, Steinmetz JE. The role of the cerebellum in classical conditioning of discrete behavioral responses. *Neuroscience*. 2009; 162:732–755. [PubMed: 19409234]
- van den Bergh FS, Bloemarts E, Chan JSW, Groenink L, Olivier B, Oosting RS. Spontaneously hypertensive rats do not predict symptoms of attention-deficit hyperactivity disorder. *Pharmacology, Biochemistry and Behavior*. 2006; 83:380–390.
- Vogel EH, Brandon SE, Wagner AR. Stimulus representation in SOP: II. An application to inhibition of delay. *Behavioural Processes*. 2003; 62:27–48. [PubMed: 12729967]
- Williams DA, Overmier JB, LoLordo VM. A reevaluation of Rescorla's early dictums about Pavlovian conditioned inhibition. *Psychological Bulletin*. 1992; 111:275–290.
- Yeo CH, Hardiman MJ, Moore JW, Russell IS. Retention of conditioned inhibition of the nictitating membrane response in decorticate rabbits. *Behavioural Brain Research*. 1983; 10:383–392. [PubMed: 6661284]

Figure 1.

Percentage of eyeblink conditioned responses as a function of conditioning session in Wistar rats: (A) Group CI received 50 A+ trials and 50 XA- trials per session, pseudorandomly intermixed. A+ trials consisted of a 490-ms, 2000-Hz, 80-dB tone that coterminated with a 15-ms, 4-mA periorbital stimulation. XA- trials consisted a 25-lux light presented simultaneously with A. There was significantly greater responding to A+ than XA- by Session 4; (B) Group Control received 50 A+ trials and 50 A- trials per session, pseudorandomly intermixed. Error bars are standard error of the mean (SEM).

Figure 2. Percentage of eyeblink conditioned responses as a function of conditioning session in SHRs: (A) Group CI received 50 A+ trials and 50 XA- trials per session, pseudorandomly intermixed. A+ trials consisted of a 490-ms, 2000-Hz, 80-dB tone that coterminated with a 15-ms, 4-mA periorbital stimulation. XA- trials consisted a 25-lux light presented simultaneously with A. There was significantly greater responding to A+ than XA- by Session 2; (B) Group Control received 50 A+ trials and 50 A- trials per session, pseudorandomly intermixed. Error bars are SEM.

Figure 3.

Percentage of eyeblink conditioned responses as a function of group and trial type in the summation test session: (A) Wistars in Group CI received 30 B- trials and 30 XB- trials, pseudorandomly intermixed. B- trials consisted of a 490-ms, 8000-Hz, 80-dB tone. XB- trials consisted of a 25-lux light presented simultaneously with B. Wistars in Group Control received 60 B- trials. There was significantly greater responding to B- than to XB- in Group CI, indicating that inhibition to X had developed during conditioning in Wistars; (B) SHRs in Groups CI and Control were treated identically to Wistars in comparable groups. As for Wistars, in SHRs there was significantly greater responding to B- than to XB- in Group CI, indicating that inhibition to X had developed during conditioning in SHRs. Error bars are SEM.

Figure 4.

Percentage of eyeblink conditioned responses as a function of group and 50-trial block in the retardation test sessions: (A) Wistars in Groups CI and Control received 100 X+ trials in each session. X+ trials consisted of a 490-ms, 25-lux light that coterminated with a 15-ms, 4-mA periorbital stimulation. There was significantly greater responding in retardation session 1 in Group Control compared to Group CI, indicating that inhibition to X had developed during conditioning in Wistars; (B) SHRs in Groups CI and Control were treated identically to Wistars in comparable groups. As for Wistars, there was significantly greater responding in retardation session 1 in Group Control compared to Group CI, indicating that inhibition to X had developed during conditioning in SHRs. There was also significantly greater responding in retardation session 2 in Group Control compared to Group CI. Error bars are SEM.