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Shortened Conditioned Eyeblink Response Latency in Male but not Female Wistar-Kyoto Hyperactive Rats

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Abstract

Reductions in the volume of the cerebellum and impairments in cerebellar-dependent eyeblink conditioning have been observed in attention-deficit/hyperactivity disorder (ADHD). Recently, it was reported that subjects with ADHD as well as male spontaneously hypertensive rats (SHR), a strain that is frequently employed as an animal model in the study of ADHD, exhibit a parallel pattern of timing deficits in eyeblink conditioning. One criticism that has been posed regarding the validity of the SHR strain as an animal model for the study of ADHD is that SHRs are not only hyperactive but also hypertensive. It is conceivable that many of the behavioral characteristics seen in SHRs that seem to parallel the behavioral symptoms of ADHD are not solely due to hyperactivity but instead are the net outcome of the interaction between hyperactivity and hypertension. We used Wistar-Kyoto Hyperactive (WKHA) and Wistar-Kyoto Hypertensive (WKHT) rats (males and females), strains generated from recombinant inbreeding of SHRs and their progenitor strain, Wistar-Kyoto (WKY) rats, to compare eyeblink conditioning in strains that are exclusively hyperactive or hypertensive. We used a long-delay eyeblink conditioning task in which a tone conditioned stimulus was paired with a periorbital stimulation unconditioned stimulus (750-ms delay paradigm). Our results showed that WKHA and WKHT rats exhibited similar rates of conditioned response (CR) acquisition. However, WKHA males displayed shortened CR latencies (early onset and peak latency) in comparison to WKHT males. In contrast, female WKHAs and WKHTs did not differ. In subsequent extinction training, WKHA rats extinguished at similar rates in comparison to WKHT rats. The current results support the hypothesis of a relationship between cerebellar abnormalities and ADHD in an animal model of ADHD-like symptoms that does not also exhibit hypertension, and suggest that cerebellar-related timing deficits are specific to males.

Keywords

WKHA; eyeblink classical conditioning; cerebellum; ADHD; timing

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Attention-deficit/hyperactivity disorder (ADHD) is a neurobehavioral disorder with a typical onset around the age of 7 years old (American Psychiatric Association, 2000). ADHD is more prevalent in boys than in girls and it affects 3%–7% of children (DSM-IV TR, 2000). The core symptoms of the disorder are inattention, hyperactivity, and impulsivity, usually manifested in the form of distractibility, difficulty in sustaining attention, and failure to appropriately control motor responses.

Much neurochemical research has demonstrated alterations in catecholamine synthesis and reuptake in children and adolescents with ADHD (Arnsten, 2006). In terms of neuroanatomy, structural magnetic resonance imaging has revealed that in children and adolescents with ADHD, through age 19, the total cerebral volume, particularly the right hemisphere, is 3%–5% smaller (Castellanos et al., 2002; Kates et al., 2002; Mostofsky, Cooper, Kates, Denckla, & Kaufman 2002; Hill, Yeo, Campbell, Hart, Vigil, & Brooks 2003). Also, smaller gray and white matter volumes over the entire brain have been reported (Castellanos et al., 2002; Mostofsky et al., 2002) as well as smaller volumes in the right and left dorsolateral prefrontal cortex (Castellanos, et al., 2002; Durnston, et al., 2004, Kates, et al., 2002; Mostofsky, et al., 2002). Abnormalities in the corpus callosum and particularly the posterior regions linked to temporal and parietal cortices in the splenium have been reported in a number of morphometric studies of children with ADHD (Baumgardner, Singer, Denckla, Rubin, Abrams, Colli, & Reiss, 1996; Hill et al., 2003; Lyoo, Lee, Lee, Kennedy, & Renshaw, 1996). Furthermore, children with ADHD have been shown to have smaller volumes of the striatum (Castellanos et al., 1996, 2002; Overmeyer, Bullmore, Suckling, Simmons, Williams, Santosh, & Taylor, 2001) further strengthening the long held association between ADHD symptoms and striatum abnormalities. To this effect, striatal lesions in animals produce hyperactivity and poorer performance in response inhibition tasks (Alexander, DeLong, & Strick, 1986). Moreover, the striatum is rich in dopaminergic synapses and dopamine function appears to be impaired in children and adolescents with ADHD (Arnsten, 2006).

The cerebellum is an area that has been receiving increasing attention in the study of ADHD pathophysiology. In addition to smaller overall volume of the cerebellum, children and adolescents with ADHD appear to have volume reductions in the posterior-inferior lobules (VIII to X) of the cerebellar vermis (Bussing, Grudnik, Mason, Wasiak, & Leonard, 2002, Castellanos et al., 1996, Castellanos et al., 2001; Hill et al., 2003) even after controlling for stimulant medication (Castellanos et al., 2002) that is the most commonly used type of medication in this population.

Motor output relies on the effective representation of time in the brain. Both time processing and coordination are subserved by a predominantly right frontostriatocerebellar network (Smith, Taylor, Lidzba, & Rubia, 2003). Interestingly, ADHD subjects are impaired in a range of neuropsychological procedures that examine time perception skills, such as the discrimination of different durations (Smith, Taylor, Rogers, Newman, & Rubia, 2002) or the estimation of time intervals (Rubia, Noorloos, Smith, Gunning, & Sergeant, 2003). Similarly, ADHD subjects exhibit poorer performance in finger tapping (Pitcher, Piek, & Barret, 2002; Rubia, Taylor, Taylor, & Sergeant, 1999), the reproduction of time intervals

(Barkley, Edwards, Laveri, Fletcher, & Metevia, 2001; McInerney & Kerns, 2003), and the synchronization of motor responses to sensory stimuli (Rubia et al., 1999, 2003).

Another procedure that requires the cerebellum and involves precisely timed motor responses is classical eyeblink conditioning. In the simplest form of eyeblink conditioning, a tone conditioned stimulus (CS) precedes a mild electrical stimulation to the eyelid or an airpuff to the cornea. The eyelid or cornea stimulation unconditioned stimulus (US) causes an eye blink unconditioned response (UR). After a number of CS-US pairings, the organism learns to blink to the tone in anticipation of the eyelid stimulation (the conditioned response; CR). As training progresses, CRs become increasingly well-timed with respect to the US onset. The acquisition of eyeblink CRs depends upon the relay of CS and US information to the interpositus nucleus, one of the deep cerebellar nuclei (Thompson, 2005), while the timing of eyeblink CRs depends, at least partially, upon the cerebellar cortex (see Perrett, 1998, for a review). Intriguingly, alterations of eyeblink CR timing have been reported in children with ADHD (Coffin, Baroody, Schneider & O'Neill, 2005).

The most frequently used animal model of ADHD is the spontaneously hypertensive rat (SHR) that possesses a hypertensive and hyperactive phenotype, a behavioral profile suggestive of ADHD-like symptomatology (Johansen & Sagvolden, 2004; Sagvolden, Hendley & Knardhal, 1992), and a reduced volume of the cerebellum (Li et al., 2007). Recently, Chess and Green (2008) examined male SHRs and males of a control strain, Wistar, on a long-delay eyeblink conditioning task in which a tone CS was paired with a periorbital stimulation US in a 750-ms delay paradigm. Their results showed that male SHRs exhibited faster acquisition of eyeblink CRs and displayed mistimed CR latencies in the form of both early onset and peak latency in comparison with Wistar rats. In subsequent extinction training, SHRs were slower to extinguish eyeblink CRs.

One criticism that has been posed regarding the validity of the SHR strain as an animal model for the study of ADHD is that SHRs are not only hyperactive but also hypertensive. Although hypertension develops later in life in SHRs (8–12 weeks of age; Hendley, & Ohlsson, 1991; Sagvolden, Russel, Aase, Johansen, & Farshbaf, 2005) elevated blood pressure is evident even in young SHRs (4 weeks of age) (Okamoto & Aoki, 1963). It is thus possible that the behaviors of SHRs that seem to parallel the behavioral symptoms of ADHD are not due solely to hyperactivity, but instead are the net outcome of the interaction between hyperactivity and hypertension. Since the diagnosis of ADHD is largely behavioral, the face validity of a proposed animal model for the study of the particular clinical condition becomes an essential goal.

The purpose of this study was to examine cerebellar-dependent learning and timing in an animal model of ADHD-like symptoms that does not also exhibit hypertension, and to determine whether any abnormalities in eyeblink conditioning are present in both males and females. To this effect, we trained two inbred strains, the Wistar-Kyoto Hyperactive rat (WKHA) and the Wistar-Kyoto Hypertensive rat (WKHT) in eyeblink conditioning. These two strains have been bred from the SHR strain and its progenitor Wistar-Kyoto (WKY) strain with recombinant inbreeding to isolate the hyperactive from the hypertensive genetic component, since these two components are not genetically linked (Hendley, Atwater,

Myers, & Whitehorn, 1983). In addition, we examined possible differences between males and females since ADHD appears to be more prevalent in boys (DSM-IV TR, 2000). In Experiment 1, we compared male and female WKHA and WKHT rats in a long-delay eyeblink conditioning procedure with a 750-ms CS-US interval. Experiment 2 consisted of explicitly unpaired presentations of the tone and the eyelid stimulation to rule out the possibility that nonassociative responding to the stimuli was different between the two strains.

Experiment 1

The purpose of Experiment 1 was to compare male and female WKHAs and WKHTs on the acquisition and extinction of eyeblink conditioning using a 750-ms CS-US interval. A longer, nonoptimal CS-US interval (750 ms) was used to slow the development of eyeblink CRs, such that any potential differences between the strains in the learning and timing of CRs would be more easily revealed.

Method and Materials

Subjects—The subjects were 95–115 day old adult male ($n = 7$; 250–320 g) and female ($n = 7$; 150–220 g) WKHAs, and male ($n = 7$; 320–380 g), and female ($n = 8$; 220–280 g) WKHTs obtained from Dr. Edith Hendley at the University of Vermont. The strains achieved homozygosity (20 successive inbreedings) in 1990 (for WKHA) and 1992 (for WKHT) and offspring of both strains were phenotyped until 2006 (Edith Hendley, personal communication). Activity levels in the open field and blood pressure constitute the main strain classification criteria (Deschepper, Prescott, Hendley, & Reudelhuber, 1997; Hendley, 2000; Palmer, Chen, Lachapelle, Hendley, & LeWinter, 2006). Rats were housed in groups of 2 to 4 per cage 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).

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 eye blinks 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 μm) 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 60–65 dB sound pressure level (SPL). A speaker was mounted in each corner of the rear wall and a light (off during testing) 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 was controlled by an IBM PC-compatible computer running custom software (Chen & Steinmetz, 1998). A 765 ms, 2800 Hz, 80 dB tone, delivered through the left speaker of the sound-attenuating chamber, served as the CS. 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 US. Recording of eyelid EMG activity was controlled by a computer interfaced with a Power 1401 (sampling rate = 2 kHz) high-speed data acquisition unit and running Spike2 software (Version 5, 2003). 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 (see Figure 4).

Procedure—All rats were given 6–7 days after surgery for recovery and then submitted to two phases of training (eyeblink conditioning, extinction).

For each day of conditioning, 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). On Days 2–11 (conditioning), 100 trials per day were delivered, at an average inter-trial interval (ITI) of 30-sec (range = 20–40 sec). Trials consisted of a 765-ms tone (CS) which coterminated with a 15-ms, 4.0 mA eyelid stimulation (US). On Days 12–15 (extinction), 100 trials per day were delivered at an average ITI of 30-sec (range = 20–40 sec), each consisting of a 765-ms tone CS.

Behavioral Data Analysis—For conditioning sessions, paired 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, 81–750 ms after CS onset (i.e., CS-US interval); and (4) a US period, 65–165 ms after US onset. Eye blinks that exceeded mean baseline activity by 0.5 arbitrary units (where these units had a range of 0.0–5.0) during the CR period were scored as CRs. This time point was scored as CR onset. The difference in time between CS onset and CR onset represents CR onset latency. The time of maximum amplitude of the eyeblink CR is CR peak latency. Trials in which eyeblinks exceeded 1.0 arbitrary unit during the baseline period were discarded. Comparable scoring intervals and criteria were used to evaluate spontaneous blink rate during the initial adaptation day when

no stimuli were administered. Dependent measures of CR acquisition and extinction were percentage of CRs during the CR period as well as the magnitude of the responses emitted during the CR period across all trials within each session. Response magnitude was scored whether a CR was emitted or not and was equal to less than 0.5 above baseline on trials in which the response did not meet the criteria to be considered a CR. The topography of the CR was measured by CR amplitude, CR onset latency, and CR peak latency during the CR period across trials in which a CR was emitted. Because CR amplitude was measured only on trials in which a CR was emitted, it was always at least 0.5 units above baseline.

We computed all statistical analyses using SPSS 12.0. We conducted a repeated measures analysis of variance (ANOVA) using strain (WKHA vs. WKHT) and gender (males vs. females) as the between-subjects factors and session as the repeated measure. For instances where Mauchly's test of sphericity indicated nonhomogeneity of variance, Greenhouse-Geisser corrections were used to adjust the degrees of freedom. An alpha level of .05 was set as the rejection criterion for all statistical tests. We made estimates of effect size for each significant factor using partial eta squared. A partial eta squared of .14 corresponds to $f = 0.40$ which is considered a large effect (Cohen, 1992).

Results

Adaptation—A 2 (strain) X 2 (gender) ANOVA was conducted to evaluate the rates of spontaneous blinking during the initial adaptation day. The analysis revealed a significant effect of strain, $F(1, 25) = 7.243$, $p < .05$. All other main and interaction effects were not significant ($p's > .05$). Separate independent samples t-tests were conducted to follow up the significant main effect of strain; there were no significant differences in spontaneous blinking between strains in WKHA and WKHT male or female rats ($p's > .05$) (Figure 1; "sit").

Acquisition

Measures of Nonassociative Responding: The amplitude of the UR was analyzed during Session 1 to address the possibility that the WKHA and WKHT rats differed in terms of sensitivity to the periorbital stimulation US. UR amplitude was measured 65–165 ms after the onset of the 15-ms US (the first 65 ms after US onset was obscured by the US artifact). A 2 (strain) X 2 (gender) ANOVA was conducted to evaluate UR amplitude. The analysis did not reveal any significant main or interaction effects ($p's > .05$).

To investigate whether WKHA and WKHT rats differed in terms of startle responses to the CS, we analyzed the percentage of eyeblink responses exceeding 0.5 in amplitude above baseline that occurred during the first 80 ms following CS onset across all sessions using a repeated measures ANOVA. A 2 (strain) X 2 (gender) X 10 (session) ANOVA was conducted but there were no significant main or interaction effects ($p's > .05$). These results suggest that both strains and genders showed similar levels of startle responses to the CS throughout training.

Measures of Conditioning: Figure 1 presents the percentage of CRs as a function of session for WKHA and WKHT male and female rats. A 2 (strain) X 2 (gender) X 10

(session) ANOVA was conducted to analyze percentage of CRs. The analysis revealed a significant effect for session, $F(5, 116) = 22.334$, $p < .001$, partial $\eta^2 = .472$, a significant Session X Strain interaction, $F(5, 116) = 2.745$, $p < .01$, partial $\eta^2 = .099$, and a significant Strain X Gender interaction, $F(5, 116) = 4.639$, $p < .05$, partial $\eta^2 = .157$. All other main effects and interactions were not significant (p 's $> .05$). Separate analyses were conducted to further analyze the significant interactions. Paired samples-t-tests conducted to analyze the Session X Strain interaction revealed that statistically significant differences between the two strains occurred only during acquisition day 1, $t(12) = 3.069$, $p < .05$. Independent samples-t-tests conducted to analyze the Strain X Gender interaction did not reveal any strain differences within each gender or any gender differences within each strain (p 's $> .05$). These results suggest that male and female WKHA and WKHT rats acquired equal levels of conditioned responding and they did not show any significant differences in their rate of acquisition in terms of percentage of CRs. We also analyzed response magnitude as an index of conditioning. A 2 (strain) X 2 (gender) X 10 (session) ANOVA revealed a significant effect for session, $F(4, 102) = 7.218$, $p < .001$, partial $\eta^2 = .224$. All other main and interaction effects were not significant (p 's $> .05$).

Measures of CR Topography: Figure 2A presents the mean onset latency of CRs as a function of session for WKHA and WKHT male and female rats. Figure 2B presents the mean onset latency as a function of session for WKHA and WKHT male rats and for male SHR and male Wistar rats from our previous study (Chess & Green, 2008) for comparison purposes. A 2 (strain) X 2 (gender) X 10 (session) ANOVA was conducted to analyze CR onset latencies across sessions in WKHA and WKHT male and female rats. The analysis revealed a significant main effect of session, $F(5, 132) = 22.768$, $p < .001$, $\eta^2 = .477$, and strain, $F(1, 25) = 7.972$, $p < .001$, $\eta^2 = .242$, and a significant Session X Gender interaction effect, $F(5, 132) = 2.883$, $p < .05$, $\eta^2 = .102$, as well as a Strain X Gender interaction effect, $F(5, 132) = 7.649$, $p < .05$, $\eta^2 = .234$. All other main and interaction effects were not significant (p 's $> .05$).

Separate analyses were conducted to follow up the significant interaction effects. Paired sample-t-tests conducted to analyze the Session X Gender interaction effect revealed that male and female rats were different in acquisition day 3, $t(13) = 2.477$, $p < .05$, in acquisition day 6, $t(13) = 2.277$, $p < .05$, and acquisition day 7, $t(13) = 2.254$, $p < .05$. Independent-t-tests conducted to analyze the Strain X Gender interaction revealed significant differences only between WKHA and WKHT male rats, $t(12) = 3.345$, $p < .01$, as well as between WKHA male and female rats, $t(12) = 2.932$, $p < .05$. These results suggest that WKHA males (mean = 270.96 ms, SEM = 19.035) exhibited earlier onset CR latency (i.e., CRs were initiated earlier following CS onset) compared to WKHT males (mean = 353.25 ms, SEM = 19.035), WKHA (mean = 352.25 ms, SEM = 19.035) and WKHT (mean = 353.33 ms, SEM = 17.806) females.

Figure 3 illustrates the total number of CRs with onset latencies occurring in each of fifteen 50-ms bins across the 750-ms CS US interval for WKHA and WKHT males. Figures 3A, 3B, and 3C illustrate the distribution of these onset latencies for Sessions 1, 5, and 10, respectively. In Session 5, three out seven male WKHAs exhibited a very high number of CRs with onset latencies during the 101–150 ms bin. In Session 1 that trend was not nearly

as evident, suggesting that the high number of CRs with onset latencies during the 101–150 ms bin in Session 5 reflect associative processes instead of a longer startle period in male WKHAs. Figure 4 depicts an amplified raw and processed (full-wave rectified, smoothed with a 10-ms time constant, and time-shifted 10-ms to compensate for smoothing) eyelid EMG from an individual conditioning trial from Session 5 for a male WKHA (Figure 4A) and a male WKHT (Figure 4B). Further arguing against the possibility that these short latency eyeblinks are actually long latency startle responses is that fact that these eyeblinks were not present in Experiment 2 during explicitly unpaired stimulus presentations (see below).

Figure 5 illustrates the total number of CRs with onset latencies occurring in each of fifteen 50-ms bins across the 750-ms CS US interval for WKHA and WKHT females. Figures 5A, 5B, and 5C illustrate the distribution of these onset latencies for Sessions 1, 5, and 10, respectively. As training progressed, the distribution of CR onset latencies across the CS US interval remained concentrated in the middle of the CS US interval for both strains.

We also analyzed peak latency of CRs (i.e., time point after CS onset of maximum eye closure). A 2 (strain) X 2 (gender) X 10 (session) ANOVA revealed significant main effects of strain, $F(1, 25) = 12.505$, $p < .01$, $\eta^2 = .333$, and session, $F(5, 124) = 5.846$, $p < .001$, $\eta^2 = .190$, and a significant Session X Gender interaction, $F(5, 124) = 2.547$, $p < .05$, $\eta^2 = .092$. All other main and interaction effects were not significant (p 's $> .05$). Paired samples- t -tests conducted to analyze the Session X Gender interaction revealed that male and female rats significantly differed only during acquisition day 1, $t(13) = 2.623$, $p < .05$. These results suggest that WKHA males (mean = 439.5 ms, SEM = 13.391) exhibited earlier peak CR latency compared to WKHT males (mean = 502.87 ms, SEM = 13.391) and WKHT females (mean = 481.96 ms, SEM = 12.53) but approximately equal to WKHA females (mean = 451.9 ms, SEM = 13.391). Our 3-way ANOVA analysis of CR amplitude revealed no significant main or interaction effects (p 's $> .05$).

Extinction

Measures of Extinction: Extinction data for percentage of CRs for WKHA and WKHT males are depicted in Figure 1 (the last four data points). A 2 (strain) X 2 (gender) X 4 (session) ANOVA revealed significant main effects of session, $F(2, 43) = 25.319$, $p < .001$, partial $\eta^2 = .503$, and gender, $F(1, 25) = 5.743$, $p < .05$, partial $\eta^2 = .187$, and a significant Strain X Gender X Session interaction, $F(2, 43) = 5.581$, $p < .01$, partial $\eta^2 = .183$. All other main and interaction effects were not significant (p 's $> .05$). Separate analyses conducted to further analyze the 3-way interaction revealed no significant differences between strains within each gender at any extinction day. These results suggest that males and females in both strains extinguished CRs, and that there were no significant differences in their rate of extinction in terms of percentage of CRs. We also analyzed response magnitude as an index of extinction. A 2 (strain) X 2 (gender) X 4 (session) ANOVA revealed a significant main effect of session, $F(2, 47) = 18.182$, $p < .001$, partial $\eta^2 = .421$, and a significant Strain X Gender X Session, $F(2, 47) = 3.3825$, $p < .05$, partial $\eta^2 = .119$. All other main and interactions effects were not significant (p 's $> .05$). Separate analyses conducted to analyze

the 3-way interaction revealed no differences between strains for male or female rats (p 's > .05).

Measures of CR Topography: Figure 2 presents the mean onset latency of CRs as a function of extinction session for WKHA and WKHT male and female rats (last four data points). A 2 (strain) X 2 (gender) X 4 (session) ANOVA revealed only a significant Session X Gender interaction, $F(3, 75) = 4.850$, $p < .01$, $\eta^2 = .162$. All other main and interaction effects were not significant (p 's > .05). Paired samples- t -tests conducted to follow up the latter significant interaction revealed that male and female rats significantly differed only during extinction day 1, $t(13) = 3.086$, $p < .01$. A 2 (strain) X 2 (gender) X 4 (session) ANOVA conducted to analyze peak CR latencies revealed a significant Session X Gender interaction, $F(3, 75) = 5.584$, $p < .01$, $\eta^2 = .183$. All other main and interaction effects were not significant (p 's > .05). Paired samples- t -tests conducted to follow up the significant interaction revealed that male and female rats significantly differed only during extinction day 1, $t(13) = 2.361$, $p < .05$. Analysis of CR amplitude revealed no main or interaction effects (p 's > .05).

Discussion

Experiment 1 revealed that WKHA and WKHT rats conditioned and extinguished equivalently, exhibiting similar levels of CRs. This was true for both male and females. Nevertheless, eyeblink CRs were generated earlier following CS onset for WKHA male rats during acquisition, suggesting abnormal timing of the eyeblink CR. It is likely that no differences in CR timing were observed in extinction because CR onset latency in male WKHAs was beginning to lengthen at the end of acquisition (e.g., Session 10 in Figure 1). Abnormal timing of eyeblink CRs (i.e., CRs occurring early during the CS-US interval) has been previously reported in children with ADHD (Coffin, et al., 2005) and in male SHRs, another hyperactive rat strain employed as an animal model for ADHD (Chess & Green, 2008). Although nonassociative indexes such as UR amplitude and startle responses did not differ between the two groups we decided to exclude the possibility that our effects were due to sensitization or pseudoconditioning. Experiment 2 was designed directly to address that possibility.

Experiment 2

The purpose of Experiment 2 was to determine whether the number of eyeblink responses of WKHA and WKHT rats would differ during an explicitly unpaired procedure in which the tone and eyelid stimulation were pseudorandomly presented during each of 10 daily sessions. The development of significant eyeblink responses to the tone would indicate either sensitization or pseudoconditioning, while very low levels of eyeblink responses would suggest that nonassociative processes do not likely explain the results of Experiment 1.

Method and Materials

Subjects, Surgery and Apparatus—The subjects were 95–115 day old adult male ($n = 4$; 250–320 g) and female ($n = 3$; 150–220 g) WKHAs, and male ($n = 4$; 320–380 g), and

female ($n = 4$; 220–280 g) WKHTs. All housing and care was identical to those of Experiment 1. The surgical procedures, apparatus and data analysis for Experiment 2 were also identical to those described for Experiment 1.

Conditioning Procedures—Following the first day of adaptation (Day 1) where rats were only exposed to the apparatus without any stimulus delivery, rats were given 10 days of explicitly unpaired presentations of the tone and periorbital eyelid stimulation for a total of 200 trials (i.e., 100 presentations of each stimulus) each day (Days 2–11). Stimuli were delivered in a pseudorandom order such that the same stimulus was never administered more than three times in a row. The average ITI was 15 s (range = 10–20 s) in order to maintain a total session duration of 1 hour, equivalent to the session length in Experiment 1.

Results

Of the 15 animals that were trained, data from one WKHT female rat had to be excluded after day 7 due to equipment malfunction. For analysis purposes, we filled in the missing cases (sessions 8–10) for that particular animal with a mean calculated based on the performance of all WKHTs for the remaining training days.

Measures of Nonassociative Responding—The amplitude of the UR was analyzed during Session 1 to address the possibility that the WKHA and WKHT rats differed in terms of sensitivity to the periorbital stimulation US. UR amplitude was measured 65–165 ms after the onset of the 15-ms US (the first 65 ms after US onset was obscured by the US artifact). A 2 (strain) X 2 (gender) ANOVA was conducted to evaluate UR amplitude. The analysis revealed a significant main effect of gender, $F(1, 11) = 9.248$, $p < .05$, $\eta^2 = .457$, and a significant Strain X Gender interaction, $F(1, 11) = 7.273$, $p < .05$, $\eta^2 = .398$. The main effect of strain was not significant. Independent t-tests were conducted to follow up the significant interaction. WKHA males (mean = 1.000, SEM = .316) were significantly different from WKHA females (mean = 1.833, SEM = .352), $t(5) = 3.300$, $p < .05$. All other comparisons (i.e., WKHA males vs. WKHT males (mean = 1.450, SEM = .238), WKHA females vs. WKHT females (mean = 1.500, SEM = .216), WKHT males vs. WKHT females) were not significant (p 's $> .05$). Figure 6 depicts an example of a UR during Session 1 of unpaired training in each of the 4 groups.

To investigate whether WKHA and WKHT rats differed in terms of startle responses to the CS, we analyzed the percentage of eyeblink responses exceeding 0.5 in amplitude above baseline that occurred during the first 80 ms following CS onset across all sessions using a repeated measures ANOVA. A 2 (strain) X 2 (gender) X 10 (session) ANOVA was conducted but there were no significant main or interaction effects (p 's $> .05$). These results suggest that both strains showed similar levels of startle responses to the CS throughout training.

Pseudoconditioning—A 2 (strain) X 2 (gender) X 10 (session) ANOVA revealed significant main effects of session, $F(9, 99) = 2.644$, $p < .05$, $\eta^2 = .194$, and gender, $F(1, 11) = 9.736$, $p < .05$, $\eta^2 = .470$, and a significant Strain X Gender X Session interaction, $F(9, 99) = 2.397$, $p < .05$. The main effect of strain and all other interactions were not

significant (p 's > .05) (Figure 7). Paired samples- t -tests conducted to follow up the significant interaction did not reveal any significant differences between strains in male or female rats across sessions (p 's > .05). These results showed that males and females of the two strains did not differ in nonassociative responding and that nonassociative responding is not likely to account for the results of the males reported in Experiment 1.

General Discussion

Collectively, our data demonstrate that WKHA male rats exhibited shorter onset and peak latency of the eyeblink CR compared to WKHT male rats, and female WKHA and WKHT rats. Acquisition of the eyeblink CR for both strains and genders was at a similar rate. It is unlikely that these effects were attributable to nonassociative processes, such as sensitization or pseudoconditioning, since neither strains nor genders differed in the explicitly unpaired procedure used in Experiment 2 or in any other index of nonassociative responding (UR amplitude, percentage of startle responses to the CS), with the exception of a larger UR amplitude in female WKHA rats compared to male WKHA rats.

Like male SHRs, male WKHA rats exhibit abnormal timing of CR onset latencies compared to an outbred strain, the Wistars. WKHA males (selectively bred from SHRs and their progenitor strain, WKYs, for hyperactivity) exhibit even shorter CR onset latencies compared to SHR males (Chess & Green, 2008) which are both hyperactive and hypertensive (see Figure 2B). Interestingly, male WKHTs (also selectively bred from SHRs and WKYs but for hypertension) in the current study exhibit approximately the same CR onset latencies as male SHRs (both hyperactive and hypertensive) (see Figure 2B). Although caution is warranted when comparisons are made across studies, the timing deficits exhibited by these three inbred strains (WKHAs, WKHTs, SHRs) compared to an outbred strain, such as Wistar rats, are quite compelling. Moreover, it is rather intriguing that SHRs (both hyperactive and hypertensive) may exhibit the same CR latencies as WKHTs (only hypertensive) suggesting the possibility that hypertension itself contributes to shortened cerebellar-dependent timing. Examining cerebellar-dependent learning in WKHAs provides an alternative model of ADHD-like symptoms that eliminates hypertension as a confounding variable. In the current study we chose to compare WKHAs to WKHTs, who are genetically closest to WKHAs but are not hyperactive. Future studies should include an outbred strain as a second control group since WKHT rats may themselves show some abnormalities in cerebellar-dependent behavior. Importantly, however, male WKHA show shortened CR latencies even compared to WKHTs. Finally, our pattern of CR latency results parallels those observed in ADHD subjects. Coffin et al. (2005) reported that the rate of acquisition of the eyeblink CR was unaltered in ADHD subjects while the execution of these CRs was significantly earlier during the CS-US interval than it was in control subjects. Shortened timing of the eyeblink CR is consistent with a large body of literature documenting timing deficits in a variety of tasks in subjects with ADHD, including the cerebellar-dependent finger tapping task (Toplak, Dockstader, & Tannock, 2006).

Our current data indicating that male WKHAs display mistimed CRs suggest possible deficits in parts of the cerebellar circuit that subserve appropriate timing of eyeblink CRs. It is possible, for example, that alterations in Purkinje cells (in terms of number or physiology)

in the cerebellar cortex of male WKHAs could cause disinhibition of neurons in the interpositus nucleus early after CS onset, leading to a mistimed CR. Posttraining destruction of the cerebellar cortex or pharmacological disconnection of the cerebellar cortex from the deep cerebellar nuclei results in fixed, short-latency eyeblink CRs but does not affect the percentage of CRs (Bao, Chen, Kim, & Thompson, 2002; Garcia & Mauk, 1998; Medina, Garcia, Nores, Taylor, & Mauk, 2000; Ohshima, Nores, Medina, Riusech, & Mauk, 2006; Perrett, Ruiz, & Mauk, 1993). Thus, alterations in Purkinje cell number or firing properties in WKHA males may account for our results. For example, it is conceivable that the early onset of CRs in male WKHAs is the result of fewer Purkinje cells. To this effect, reduced cerebellar volume has been reported in ADHD (Bussing, Grudnik, Mason, Wasiak, & Leonard, 2002; Castellanos et al., 1996; Castellanos et al., 2001; Hill et al., 2003) and it is possible that a decrease in cerebellar neuronal populations is one of the contributing factors to these volume reductions.

Another interesting possibility based on cerebellar circuitry is that differences in cerebellar granule cells, the main excitatory relay from pontine nuclei (which process the CS) to Purkinje cells, are responsible for the timing effects seen in male WKHAs. The tone CS projects bilaterally via pontine nuclei to granule cells in cerebellar cortex and neurons in the deep nuclei along glutamatergic mossy fibers (Steinmetz, Logan, Rosen & Thompson, 1987; Steinmetz, Rosen, Chapman, Lavond, & Thompson, 1986; Steinmetz, 1990; Freeman & Rabinak, 2004; Tracy, Thompson, Krupa, & Thompson, 1998). In turn, granule cell axons (parallel fibers) project to Purkinje cells. Each Purkinje cell receives ~200,000 synapses from parallel fibers (Hansel, Linden & D'Angelo, 2001). The confinement of CRs to the later parts of the CS with training that occurs in eyeblink conditioning has been hypothesized to be due to slight variations in subsets of cerebellar granule cells that are active at different times during the CS (Mauk & Donegan, 1997). This temporal code may create the potential for differential responding at different times during the CS. Early during the CS and prior to generation of the eyeblink CR, Purkinje cells increase their firing rate (Green & Steinmetz, 2005; Hesslow & Ivarson, 1994) (hypothesized to be due to long term potentiation [LTP] at granule cell-to-Purkinje cell synapses) inhibiting activity in the interpositus nucleus cells that generate the CR (Hansel, Linden, & DiAngelo, 2001; Medina et al., 2000). At later parts of the CS, Purkinje cells are hypothesized to show activity decreases (hypothesized to be due to long term depression [LTD] at granule cell-to-Purkinje cell synapses from convergent climbing fiber input activated by the US) thus contributing to the expression of a well-timed conditioned response via disinhibition of the interpositus nucleus neurons that generate the eyeblink CR (Green & Steinmetz, 2005; Jirenbek et al., 2007; Koekkoek et al., 2003; Medina et al., 2000; Thompson, 2005;). Fewer granule cells may lead to changes in the ratio of LTP and LTD at synapses formed with Purkinje cells, resulting in the timing deficits seen in male WKHAs. Last but not least, differences in the physiological properties (e.g., induction or maintenance of LTP and/or LTD) of granule cell-to-Purkinje cell synapses (or in the inferior olive; see Van Der Giessen et al., 2008) in male WKHAs, rather than differences in numbers of cells of these neuronal populations may account for the timing deficits seen in these two strains during eyeblink conditioning.

Pavlov (1927) was the first to propose the term “inhibition of delay” to characterize a systematic observation he and his colleagues had made in his seminal studies on conditioned

digestive reflexes, namely that the timing of conditioned responses could be appropriately delayed via differential conditioning within each trial. In Pavlov's view, the suppression of CR expression seen early in the CS could be achieved by a brain mechanism that subserved inhibition of CRs during the early part of the CS, confining them to the latter part of the CS, the part that was contiguous with US reinforcement. According to Rescorla (1967), the phenomenon of inhibition of delay consists of three separate observations: a) a reduction in the CR in the early portion of the CS as training progresses, b) the confinement of the CR to the later part of the CS, and c) the ability of the early part of the CS to inhibit CRs elicited by other stimuli. The confinement of CRs to the later parts of the CS with training occurs in eyeblink conditioning only with relatively long CS-US intervals and lengthy training (Vogel, Brandon, & Wagner, 2003), and is potentially facilitated by connectivity within the cerebellar cortex producing slight variations in subsets of cerebellar granule cells that are active at different times during the CS (Mauk & Donegan, 1997; Medina et al., 2000). The pattern of results, collectively, in our work with WKHAs, WKHTs, SHRs and Wistars, suggests the possibility that the mistimed CRs generated by male WKHAs, especially, may be due to a deficit in neural mechanisms underlying inhibition of delay.

The hypothesized LTP induced at granule cell-to-Purkinje cells synapses, resulting in the inhibition of activity of the interpositus nucleus cells, and hence, in the suppressed expression of conditioned eyeblink responses early in the CS, may underlie either of two behavioral types of inhibition accrued in this part of the CS. Thus, it is possible that either conditioned inhibition develops to the early part of the CS (i.e., the early part of the CS becomes a conditioned inhibitor suppressing conditioned responding to any CS during this part of the interval; cf. Rescorla, 1967), or extinction of eyeblink CRs occurs in the early part of the CS (i.e., the early part of the CS has both residual excitatory and newer inhibitory components; cf. Medina et al., 2000). Determining the form of inhibition underlying inhibition of delay in eyeblink conditioning will allow us to characterize the impaired behavioral processes involved in the mistimed eyeblink CRs in male WKHAs.

Studies of eyeblink conditioning in rodent models of ADHD may further facilitate our understanding of timing deficits associated with ADHD by providing us with an experimental preparation suited to investigate timing mechanisms and their dependence on the cerebellum. Because of the precise control over stimulus delivery and measurement of responses, as well as the existence of detailed quantitative theories of the learning process, classical conditioning procedures have provided the best template to study processes in learning, memory and emotion. The major advantage of the eyeblink preparation is a fully mapped neural circuit for the simple CS-US association, including stimulus input and response output pathways. Moreover, the conservation of cerebellar mechanisms involved in eyeblink conditioning across species and the parallel timing deficits seen in children with ADHD and hyperactive rodents such as WKHAs support the use of the eyeblink preparation as a way to investigate timing deficits and abnormalities in cerebellar processing which may underlie some of the symptoms of ADHD.

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References

- Alexander GE, DeLong MR, Strick PL. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annual Review of Neuroscience*. 1986; 9:357–381.
- American Psychiatric Association. *Diagnostic and Statistical Manual of Mental disorders*. 4. Washington, DC: American Psychiatric Association; 2000. Text Revision
- Arnsten AFT. Stimulants: Therapeutic actions in ADHD. *Neuropsychopharmacology*. 2006; 31:2376–2383. [PubMed: 16855530]
- Bao S, Chen L, Kim JJ, Thompson RF. Cerebellar cortical inhibition and classical eyeblink conditioning. *Proceedings of the National Academy of Sciences, USA*. 2002; 99:1592–1597.
- Barkley RA, Edwards G, Laneri M, Fletcher K, Metevia L. Executive functioning, temporal discounting, and sense of time in adolescents with attention deficit hyperactivity disorder (ADHD) and oppositional defiant disorder (ODD). *Journal of Abnormal Child Psychology*. 2001; 29:541–556. [PubMed: 11761287]
- Baumgardner TL, Singer HS, Denckla MB, Rubin MA, Abrams MT, Colli MJ, Reiss AL. Corpus callosum morphology in children with Tourette syndrome and attention deficit hyperactivity disorder. *Neurology*. 1996; 47:1–6. [PubMed: 8710059]
- Bussing R, Grudnik J, Mason D, Wasiak M, Leonard C. ADHD and conduct disorder: An MRI study in a community sample. *World Journal of Biological Psychiatry*. 2002; 3:216–220. [PubMed: 12516313]
- Castellanos FX, Giedd JN, Marsh WL, Hamburger SD, Vaituzis AC, Dickstein DP, et al. Quantitative brain magnetic resonance imaging in attention deficit hyperactivity disorder. *Archives of General Psychiatry*. 1996; 53:607–616. [PubMed: 8660127]
- Castellanos FX, Lee PP, Sharp W, Jeffries NO, Greenstein DK, Clasen LS, et al. Developmental trajectories of brain volume abnormalities in children and adolescents with attention-deficit/hyperactivity disorder. *Journal of the American Medical Association*. 2002; 288:1740–1748. [PubMed: 12365958]
- Castellanos FX, Tannock R. Neuroscience of attentiondeficit/hyperactivity disorder: The search for endophenotypes. *Nature Review Neuroscience*. 2002; 3:617–628.
- Chen G, Steinmetz JE. A general-purpose computer system for behavioral conditioning and neural recording experiments. *Behavior Research Methods, Instruments, & Computers*. 1998; 30:384–391.
- 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]
- Coffin JM, Baroody 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]
- Cohen J. A power primer. *Psychological Bulletin*. 1992; 112:155–159. [PubMed: 19565683]
- Douglas VI. Stop, look and listen: The problem of sustained attention and impulse control in hyperactive and normal children. *Canadian Journal of Behavioral Science*. 1972; 4:259–282.
- Durston S, Hulshoff Pol HE, Schnack HG, Buitelaar JK, Steenhuis MP, Minderaa RB, et al. Magnetic resonance imaging of boys with attention- deficit/hyperactivity disorder and their unaffected siblings. *Journal of American Academy of Child and Adolescent Psychiatry*. 2004; 43:332–340.
- Deschepper CF, Prescott G, Hendley ED, Reudelhuber TL. Genetic characterization of novel strains of rats derived from crosses between Wistar-Kyoto and spontaneously hypertensive rats, and comparisons with their parental strains. *Laboratory Animal Science*. 1997; 47:638–646. [PubMed: 9433701]

- Freeman JH, Rabinak CA. Eyeblink conditioning in rats using pontine stimulation as a conditioned stimulus. *Integrative Physiological & Behavioral Science*. 2004; 39:180–191. [PubMed: 15929500]
- Green JT, Steinmetz JE. Purkinje cell activity in the cerebellar anterior lobe after rabbit eyeblink conditioning. *Learning & Memory*. 2005; 12:260–269. [PubMed: 15897252]
- Garcia KS, Mauk MD. Pharmacological analysis of cerebellar contributions to the timing and expression of conditioned eyelid responses. *Neuropharmacology*. 1998; 37:471–480. [PubMed: 9704988]
- Hansel C, Linden DJ, D'Angelo E. Beyond parallel fiber LTD: the diversity of synaptic and non-synaptic plasticity in the cerebellum. *Nature Neuroscience*. 2001; 4:467–475.
- Harvey WJ, Reid G. Attention-deficit/hyperactivity disorder: A review of research on movement skill performance and physical fitness. *Adapted Physical Activity Quarterly*. 2003; 20:1–25.
- Hendley ED. WKHA rats with genetic hyperactivity and hyperreactivity to stress: a review. *Neuroscience Biobehavioral Review*. 2000; 24:41–44.
- Hendley ED, Atwater DG, Myers MM, Whitehorn D. Dissociation of genetic hyperactivity and hypertension in SHR. *Hypertension*. 1983; 5:211–217. [PubMed: 6681804]
- Hesslow G, Ivarson M. Suppression of cerebellar Purkinje cells during conditioned responses in ferrets. *Neuroreport*. 1994; 5:649–652. [PubMed: 8025262]
- Hill DE, Yeo RA, Campbell RA, Hart B, Vigil J, Brooks W. Magnetic resonance imaging correlates of attention-deficit/hyperactivity disorder in children. *Neuropsychology*. 2003; 17:496–506. [PubMed: 12959515]
- Jirenhed DA, Bengtsson F, Hesslow G. Acquisition, extinction, and reacquisition of a cerebellar cortical memory trace. *Journal of Neuroscience*. 2007; 27:2493–2502. [PubMed: 17344387]
- Johansen EB, Sagvolden T. Response disinhibition may be explained as an extinction deficit in an animal model of attention-deficit/hyperactivity disorder (ADHD). *Behavioural Brain Research*. 2004; 149:183–196. [PubMed: 15129781]
- Kates WR, Frederikse M, Mostofsky SH, Folley BS, Cooper K, Mazur-Hopkins P, et al. MRI parcellation of the frontal lobe in boys with attention deficit hyperactivity disorder or Tourette syndrome. *Psychiatry Research*. 2002; 116:63–81. [PubMed: 12426035]
- Koekkoek SKE, Hulscher HC, Dortland BR, Hensbroek RA, Elgersma Y, Ruigrok TJH, De Zeeuw CI. Cerebellar LTD and learning-dependent timing of conditioned eyelid responses. *Science*. 2003; 301:1735–1739.
- Kuntsi J, Eley TC, Taylor A, et al. Co-occurrence of ADHD and low IQ has genetic origins. *American Journal of Medical Genetics*. 2004; 124B:41–47. [PubMed: 14681911]
- 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]
- Lyoo I, Noam G, Lee C, Lee H, Kennedy B, Renshaw P. The corpus callosum and lateral ventricles in children with attention-deficit hyperactivity disorder: A brain magnetic resonance imaging study. *Biological Psychiatry*. 1996; 40:1060–1063. [PubMed: 8915567]
- Mauk MD, Donegan NH. A model of Pavlovian eyelid conditioning based on the synaptic organization of the cerebellum. *Learning & Memory*. 1997; 4:130–158. [PubMed: 10456059]
- McInerney RJ, Kerns KA. Time reproduction in children with ADHD: motivation matters. *Child Neuropsychology (Neuropsychology, Development and Cognition: Section C)*. 2003; 9:91–108.
- 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]
- Medina JF, Mauk MD. Computer simulation of cerebellar information processing. *Nature Neuroscience*. 2000; 3:1205–1211.
- Mostofsky SH, Cooper KL, Kates WR, Denckla MB, Kaufmann WE. Smaller prefrontal and premotor volumes in boys with attentiondeficit/hyperactivity disorder. *Biological Psychiatry*. 2002; 52:785–94. [PubMed: 12372650]

- Ohshima T, Norez WL, Medina JF, Riusech FA, Mauk MD. Learning-induced plasticity in deep cerebellar nucleus. *Journal of Neuroscience*. 2006; 26:12656–12663. [PubMed: 17151268]
- Okamoto K, Aoki K. Development of a strain of spontaneous hyperactive rats. *Japanese Circulation Journal*. 1963; 27:282–293. [PubMed: 13939773]
- Overmeyer S, Bullmore ET, Suckling J, Simmons A, Williams SC, Santosh PJ, Taylor E. Distributed grey and white matter deficits in hyperkinetic disorder: MRI evidence for anatomical abnormality in an attentional network. *Psychological Medicine*. 2001; 31:1425–1435. [PubMed: 11722157]
- Palmer BM, Chen Z, Lachapelle RL, Hendley ED, LeWinter MM. Cardiomyocyte function associated with hyperactivity and/or hypertension in genetic models of LV hypertrophy. *American Journal of Physiology-Heart and Circulatory Physiology*. 2005; 290:463–473.
- Pavlov, IP. Conditioned reflexes. Oxford University Press; London: 1927.
- Perrett SP. Temporal discrimination in the cerebellar cortex during conditioned eyelid responses. *Experimental Brain Research*. 1998; 121:115–124. [PubMed: 9696380]
- Perrett SP, Ruiz BP, Mauk MD. Cerebellar cortex lesions disrupt learning-dependent timing of conditioned eyelid responses. *Journal of Neuroscience*. 1993; 13:1708–1718. [PubMed: 8463846]
- Pitcher TM, Piek JP, Barrett NC. Timing and force control in boys with attention deficit hyperactivity disorder: subtype differences and the effect of comorbid developmental coordination disorder. *Human Movement Science*. 2002; 21:919–945. [PubMed: 12620726]
- Rapoport M. The cerebellum in psychiatric disorders. *International Review of Psychiatry*. 2001; 13:295–301.
- Rescorla RA. Inhibition of delay in pavlovian fear conditioning. *Journal of Comparative and Physiological Psychology*. 1967; 64:114–120. [PubMed: 6064413]
- Rosenbaum M, Baker E. Self-control behavior in hyperactive and nonhyperactive children. *Journal of Abnormal Child Psychology*. 1984; 12:303–317. [PubMed: 6725787]
- Rubia K, Noorloos J, Smith A, Gunning B, Sergeant J. Motor timing deficits in community and clinical boys with hyperactive behavior: the effect of methylphenidate on motor timing. *Journal of Abnormal Child Psychology*. 2003; 31:301–313.
- Rubia K, Taylor A, Taylor E, Sergeant JA. Synchronization, anticipation, and consistency in motor timing of children with dimensionally defined attention deficit hyperactivity behavior. *Perceptual Motor Skills*. 1999; 89:1237–1258. [PubMed: 10710774]
- Sagvolden T, Hendley ED, Knardahl S. Behavior of hypertensive and hyperactive rat strains: Hyperactivity is not unitarily determined. *Physiology & Behavior*. 1992; 52:49–57. [PubMed: 1529013]
- 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]
- Sergeant JA, Scholten CA. A stages-of-information approach to hyperactivity. *Journal of Child Psychology and Psychiatry*. 1983; 24:49–60. [PubMed: 6826676]
- Sergeant JA, Scholten CA. On data limitations in hyperactivity. *Journal of Child Psychology and Psychiatry*. 1985; 26:111–124. [PubMed: 3972927]
- Smith A, Taylor E, Lidzba K, Rubia K. A right hemispheric frontocerebellar network for time discrimination of several hundreds of milliseconds. *Neuroimage*. 2003; 20:344–350. [PubMed: 14527594]
- Smith A, Taylor E, Rogers JW, Newman S, Rubia K. Evidence for a pure time perception deficit in children with ADHD. *Journal of Child Psychology and Psychiatry*. 2002; 43:529–542. [PubMed: 12030598]
- Spike2 (Version 5) [Computer software]. Cambridge, United Kingdom: Cambridge Electronic Design Limited; 2003.
- Steinmetz JE. Neuronal activity in the rabbit interpositus nucleus during classical NM-conditioning with a pontine-nucleus-stimulation CS. *Psychological Science*. 1990; 1:378–382.
- Steinmetz JE, Logan CG, Rosen DJ, Thompson RF. Initial localization of the acoustic conditioned stimulus projection system to the cerebellum essential for classical eyelid conditioning. *Proceedings of the National Academy of Sciences*. 1987; 84:3531–3535.

- Steinmetz JE, Rosen DT, Chaptman PF, Lavond DG, Thompson RF. Classical conditioning of the rabbit eyelid response with mossy-fiber stimulation CS: Pontine nuclei and middle cerebellar peduncle stimulation. *Behavioral Neurosci.* 1986; 100:878–887.
- Toplak ME, Dockstader C, Tannock R. Temporal information processing in ADHD: Findings to date and new methods. *Journal of Neuroscience Methods.* 2006; 151:15–29. [PubMed: 16378641]
- Tracy JA, Thompson JK, Krupa DJ, Thompson RF. Evidence of plasticity in the pontocerebellar conditioned stimulus pathways during classical conditioning of the eyeblink response in the rabbit. *Behavioral Neuroscience.* 1998; 112:267–285. [PubMed: 9588477]
- Thompson RF. In search of memory traces. *Annual Review of Psychology.* 2005; 56:1–23.
- Van Der Giessen RS, Koekkoek SK, Van Dorp S, Gruijl JR, Cupido A, et al. Role of olivary electrical coupling in cerebellar motor learning. *Neuron.* 2008; 58:599–612. [PubMed: 18498740]
- Van Der Meere J, van Baal M, Sergeant J. The additive factor method: a differential diagnostic tool in hyperactivity and learning disability. *Journal of Abnormal Child Psychology.* 1989; 17:409–422. [PubMed: 2794254]
- Vogel EH, Brandon SE, Wagner AR. Stimulus representation in SOP. II. An application to inhibition of delay. *Behavioral Processes.* 2003; 62:27–48.

Figure 1.

Percentage of conditioned responses did not differ between male Wistar Kyoto Hyperactive (WKHAs) and Wistar Kyoto Hypertensive (WKHTs) rats during acquisition and extinction sessions (p 's > .05). All data are means $\pm$ standard errors of the mean.

Figure 2A

Figure 2B

Figure 2.

Onset of conditioned responses in Wistar Kyoto Hyperactive (WKHA) and Wistar Kyoto Hypertensive (WKHT) male and female rats as well as in male Spontaneously Hyperactive (SHR) and male Wistar rats (published in Chess & Green, 2008). Male WKHAs exhibited earlier conditioned response onset latencies compared to male WKHTs ($p < .01$) and female WKHAs ($p < .05$) during acquisition. All data are means $\pm$ standard errors of the mean.

Figure 3.

Conditioned response (CR) onset latencies distributed in fifteen 50-ms bins across the 750-ms conditioned stimulus (CS) – unconditioned stimulus (US) interval for Session 1 (A), Session 5 (B), and Session 10 (C). Male Wistar Kyoto Hyperactive rats (WKHAs) exhibited CR onsets primarily in the earlier 50-ms bins, whereas Wistar Kyoto Hypertensive rats (WKHTs) rats exhibited CR onsets concentrated during the later 50-ms bins. This difference in the distribution of the onset latency of CRs between WKHA and WKHT male rats became particularly pronounced during later training sessions. Each bin is a sum of CRs with a particular onset latency for rats of a group across an entire conditioning session.

Figure 4.

Amplified raw eyelid EMG and full-wave rectified, smoothed (10-ms time constant) and timed-shifted (10-ms to compensate for smoothing) eyelid EMG of a CR from a male WKHA rat (A) and a male WKHT rat (B) during a trial in Session 5 of conditioning. CR onset latency of this trial was 116-ms for the male WKHA rat (session average = 147-ms) and 325-ms for the male WKHT rat (session average = 294-ms). Threshold for scoring an eyeblink was 0.5 units (rectified and smoothed EMG) above baseline measured 280-ms prior to tone CS onset.

Figure 5.

Conditioned response (CR) onset latencies distributed in fifteen 50-ms bins across the 750-ms conditioned stimulus (CS) – unconditioned stimulus (US) interval for Session 1 (A), Session 5 (B), and Session 10 (C). Both female Wistar Kyoto Hyperactive and Wistar Kyoto Hypertensive rats exhibited CR onsets primarily in the middle of the CS-US interval. Each bin is a sum of CRs with a particular onset latency for rats of a group across an entire conditioning session.

Figure 6.

Amplified raw eyelid EMG and full-wave rectified, smoothed (10-ms time constant) and timed-shifted (10-ms to compensate for smoothing) eyelid EMG of a CR from a female WKHA rat (A) and a female WKHT rat (B) during a trial in Session 5 of conditioning. CR onset latency of this trial was 319-ms for the female WKHA rat (session average = 359-ms) and 380-ms for the female WKHT rat (session average = 356-ms). Threshold for scoring an eyeblink was 0.5 units (rectified and smoothed EMG) above baseline measured 280-ms prior to tone CS onset.

Figure 7.

Amplified raw eyelid EMG and full-wave rectified, smoothed (10-ms time constant) and timed-shifted (10-ms to compensate for smoothing) eyelid EMG of a male WKHA rat (A), a male WKHT rat (B), a female WKHA rat (C), and a female WKHT rat (D) during a US trial in Session 1 of unpaired training. The gray rectangle represents the time of electrical artifact from the 15-ms US (0–64 ms after US onset). UR amplitude was measured 65–165 ms after US onset. Female WKHA rats had a significantly greater UR amplitude than the other 3 groups. There is no clear relationship between UR amplitude in unpaired training (which was greatest in WKHA females) and CR onset latency during conditioning (which was shortest in WKHA males). Mean Session 1 UR amplitude was 0.8 units for this male WKHA, 1.6 units for this male WKHT, 2.2 units for this female WKHA, and 1.2 units for this female WKHT.

Figure 8.

A: Percentage of eyeblinks during unpaired stimulus presentations in Wistar Kyoto Hyperactive (WKHAs) and Wistar Kyoto Hypertensive (WKHTs) male and female rats. There were no significant differences between strains in nonassociative responding. All data are means $\pm$ standard errors of the mean.