

Medial Dorsal Thalamic Lesions Impair Blocking and Latent Inhibition of the Conditioned Eyeblink Response in Rats

Daniel A. Nicholson and John H. Freeman Jr.
University of Iowa

The effects of lesions of the medial dorsal thalamic nucleus (MD) on blocking and latent inhibition (LI) of the rat eyeblink response were examined in the present study. Previous work has demonstrated that the cingulate cortex and related thalamic areas are involved in processing conditioning stimuli throughout training. The experiments in the present study tested the hypothesis that disruption of cingulothalamic stimulus processing produced by lesions of the MD would impair 2 types of associative learning that involve decremental changes in attention. In Experiment 1, MD lesions severely impaired blocking. In Experiment 2, MD lesions severely impaired LI. The results indicate that lesions of the MD impair incremental, decremental, or both types of changes in stimulus processing during learning.

Classical conditioning is a simple form of associative learning wherein animals presented with repeated pairings of a conditioned stimulus (CS; e.g., tone) with an unconditioned stimulus (US; e.g., airpuff) develop conditioned responses (CRs; e.g., eyeblink) to the CS. The behavioral and neural mechanisms of a variety of classically conditioned responses have been identified (e.g., Alkon, 1984; Davis, 1992; Disterhoft, Coulter, & Alkon, 1986; Domjan, 1998; Fanselow & LeDoux, 1999; Gormezano & Kehoe, 1981; Gormezano, Kehoe, & Marshall, 1983; Kapp, Wilson, Pascoe, Supple, & Whalen, 1990; LeDoux, 2000; Moyer, Thompson, & Disterhoft, 1996; Schneiderman et al., 1987; Schreurs, 1989, 2000; Stanton & Freeman, 2000; Thompson & Krupa, 1994; Woodruff-Pak & Steinmetz, 2000). Much research has also attempted to elucidate the neural and behavioral mechanisms of more complex forms of associative learning by using classical conditioning procedures (e.g., Falls & Davis, 1995, 1997; Freeman & Nicholson, 1999; Holland, 1997; Nicholson & Freeman, 2000b, 2002; Port & Patterson, 1984; Rescorla, 1988; Schmajuk & Holland, 1998; Solomon, 1987; Wasserman & Miller, 1997).

Kamin's blocking effect (Kamin, 1969) is a special case of associative learning, in that prior training involving paired presentations of CS_A and a US decreases the amount of conditioning to CS_B during subsequent pairings of a simultaneous compound stimulus CS_A-CS_B and the same US. The blocking effect has been attributed to decreases in the associability of the CS (Mackintosh, 1975; Pearce & Hall, 1980) or the US (Rescorla & Wagner, 1972). Another special form of associative learning is the CS preexposure

effect, or *latent inhibition* (LI; Lubow & Moore, 1959). LI is the impaired acquisition of CRs to CS_A during CS_A-US presentations that is attributable to prior CS_A presentations in the absence of the US. Pearce and Hall (1980) suggested that unreinforced presentations of CS_A decrease its associability, which impairs subsequent acquisition of CRs during CS_A-US pairings.

Holland, Gallagher, and colleagues suggested that both blocking and LI involve decrements in stimulus processing (attention), and through an elegant set of experiments, demonstrated a neural dissociation between incremental and decremental changes in attention (Baxter, Holland, & Gallagher, 1997; Bucci, Holland, & Gallagher, 1998; Chiba, Bucci, Holland, & Gallagher, 1995; Han, Gallagher, & Holland, 1995; Holland, 1997; Holland & Gallagher, 1993a, 1993b, 1999; Holland, Han, & Gallagher, 2000; Schoenbaum, Chiba, & Gallagher, 2000). In brief, incremental attention processes depend on the central nucleus of the amygdala and basal forebrain cholinergic system, and decremental attentional processes depend on the hippocampus (see also Romano, 1999).

Previous studies using eyeblink conditioning have demonstrated that damage to the hippocampal region impaired LI (Schmajuk, Christiansen, & Cox, 2000; Schmajuk, Lam, & Christiansen, 1994; Shohamy, Allen, & Gluck, 2000; Solomon & Moore, 1975) and blocking (Solomon, 1977). The impairment in the lesioned animals has been attributed to an inability to ignore irrelevant stimuli (Solomon, 1977, 1987), an impaired novelty-detection function (Buhusi, Gray, & Schmajuk, 1998; Schmajuk et al., 2000; Schmajuk et al., 1994), or an impairment in compression of cortical information produced by damage to the entorhinal cortex (Shohamy et al., 2000).

Schmajuk et al. (2000) demonstrated that impaired LI in rats with hippocampal lesions could be reversed with haloperidol administration. Schmajuk et al. (2000) and Buhusi et al. (1998) suggested that haloperidol decreased the influence of novelty on attention. According to this view, hippocampal lesions slow the rate at which the novelty of stimuli decreases, but the administration of haloperidol makes increases in attention to a CS more difficult, such that LI is reinstated in preexposed rats with hippocampal lesions. Buhusi et al. (1998) provided evidence for the view that hippocampal lesions slow, but do not prevent, decreases

Daniel A. Nicholson and John H. Freeman Jr., Department of Psychology, University of Iowa.

This work and preparation of this manuscript were supported by National Institute of Neurological Disorders and Stroke (NINDS) Grant NS38890 to John H. Freeman Jr. and NINDS Predoctoral Fellowship NS41713 to Daniel A. Nicholson. We thank Mark E. Stanton for the eyeblink conditioning equipment and Adam Muckler and Brian Nolan for help with data collection.

Correspondence concerning this article should be addressed to John H. Freeman Jr., Department of Psychology, University of Iowa, E-11 Seashore Hall, Iowa City, Iowa 52242. E-mail: john-freeman@uiowa.edu

in the novelty of a CS by using a neural network simulation (Schmajuk, Lam, & Gray, 1996). The computer simulations demonstrated that if given enough CS-only preexposures, even rats with hippocampal lesions should exhibit LI. Impairments in LI related to acquisition are consistent with theoretical accounts of LI (Lubow, 1973, 1989; Mackintosh, 1975) and empirical results demonstrating that the strength of LI is highly dependent on the number of CS preexposures (Clarke & Hupka, 1974; Lubow, 1965; Reiss & Wagner, 1972; Siegel, 1969, 1972). The amount of training during the first phase of conditioning has also been shown to determine whether or not blocking will occur. If conditioning has not reached asymptotic levels during Phase 1 training (CS_A -US), blocking will not occur during Phase 2 training (CS_A - CS_B -US; Marchant & Moore, 1973).

Gabriel and colleagues proposed that the cingulate cortex and related areas of the thalamus are also involved in associative attention to cues (Gabriel, 1993; Gabriel & Smith, 1999; Gabriel & Talk, 2001; Taylor, Freeman, Holt, & Gabriel, 1999). Gabriel (1993) suggested that the medial dorsal thalamus (MD) and interconnected anterior cingulate cortex (ACC, Brodmann's Area 24) function as a recency system. Lesions of the MD (Gabriel, Sparenborg, & Kubota, 1989) or ACC (Gabriel, Kubota, Sparenborg, Straube, & Vogt, 1991) impair initial acquisition of a discriminative avoidance response but do not affect asymptotic learning levels. Moreover, neuronal activity is greatest in the MD and ACC in the beginning stages of training, which suggests that the MD and ACC are involved in incremental attention during new learning situations (Beracochea, Jaffard, & Jarrard, 1989; Gabriel, 1993). Disruptions in eyeblink conditioning through the use of suboptimal conditioning parameters have been reported after MD lesions (Buchanan, Penney, Tebbutt, & Powell, 1997) or in utero cocaine exposure (Romano, Kachelries, Simansky, & Harvey, 1995), which alters the neurochemistry and morphology of ACC neurons (Jones, Fischer, & Levitt, 1996; Levitt, Harvey, Friedman, Simansky, & Murphy, 1997). It is possible that lesions of the ACC or MD, which abolish ACC learning-related activity (Gabriel et al., 1989), impair the acquisition of associative information about training stimuli by disrupting incremental attention processes that are normally recruited in new learning situations or learning situations that involve nonsalient stimuli (Gabriel & Talk, 2001; Sparenborg & Gabriel, 1990; Stolar, Sparenborg, Donchin, & Gabriel, 1989).

The MD may also be involved in paradigms that require a decrease in stimulus processing, such as blocking and LI. The MD is interconnected with the hippocampal system (Gower, 1989; Russchen, Amaral, & Price, 1987; Steriade, Parent, Pare, & Smith, 1987) and the amygdala (Aggleton & Mishkin, 1984; Groenewegen, 1988), which are involved in decremental and incremental changes in attention, respectively. It is possible that the early increases in neuronal activity within the MD and ACC may represent early-stage increases in attention, and that the decreases in neuronal activity during later stages of training may represent decreases in attention.

The current study examined the effects of bilateral MD lesions on blocking and LI of delay eyeblink CRs in rats. Lesion-induced disruptions of more complex associative phenomena involving the eyeblink CR (e.g., blocking or LI) may be attributable to damage to the cingulothalamic system, as delay classical eyeblink conditioning was not impaired in rabbits with limbic thalamic lesions

(which included the MD; Gabriel et al., 1996). It is possible that MD lesions may impair blocking and LI by disruption of stimulus processing in the cingulothalamic system (Gabriel, 1993; Gabriel & Talk, 2001) or by disrupting interactions between the hippocampus and cingulothalamic system (Kang & Gabriel, 1998), which may impair the ability to decrease attention to redundant (e.g., blocking) or irrelevant (e.g., latent inhibition) stimuli (Baxter et al., 1997; Han et al., 1995; Holland, 1997).

Experiment 1

Blocking is commonly thought to be the result of decremental changes in the associability of the conditioning stimuli as a result of CS_A being a consistent predictor of the US (Holland, 1997; Pearce & Hall, 1980). If MD lesions impair cingulothalamic CS processing and attention has not waned by the end of Phase 1, then both CS_A and CS_B should be processed during Phase 2. Consequently, rats with MD lesions are expected to show less blocking than control rats.

Method

Subjects. The subjects were 41 male Long-Evans rats (250–350 g). The rats were housed in the animal colony in Spence Laboratories at the University of Iowa. All rats were maintained on a 12-hr light–dark cycle with light onset at 6:30 a.m. and were given ad-lib access to food and water.

Surgery. One week before training, rats were removed from their home cage, randomly assigned to a conditioning group (see below), and anesthetized by an intraperitoneal injection of sodium pentobarbital (60 mg/kg) and atropine sulfate (0.45 mg/kg). At the onset of anesthesia, the rats were fitted with differential electromyograph (EMG) electrodes that were implanted in the left upper eyelid muscle (orbicularis oculi); the ground electrode was attached to a stainless steel skull screw. The EMG electrode leads terminated in gold pins in a plastic connector, which was secured to the skull with dental acrylic. A bipolar stimulating electrode (Plastics One, Roanoke, VA) for delivering the shock US was implanted subdermally, caudal to the left eye. The bipolar electrode terminated in a plastic connector that was secured to the skull with dental acrylic.

One electrolytic lesion was made in each hemisphere. The coordinates relative to bregma for the skull holes were AP -2.9 , ML ± 0.6 , and DV -5.9 . Stainless steel insect pins (size 00), insulated except at the tips ($\sim 300 \mu\text{m}$), were lowered into the brain. In the lesion group, 1.3 mA DC was passed through the lesioning electrode for 30 s on each site. In the control group, the lesioning electrodes were lowered to the same coordinates, but no current was passed. Burr holes drilled in the skull for the lesion electrodes were filled with bone wax, and the surgical site was closed with sutures on both sides of the electrode connectors. The connectors for the EMG electrodes and bipolar stimulating electrode were connected to lightweight cables that allowed the rats to move freely during conditioning. All rats were given 1 week to recover from surgery before training began.

Apparatus. The conditioning apparatus consisted of four small-animal sound-attenuating chambers (BRS/LVE, Laurel, MD). Within each sound-attenuating chamber was a small-animal operant chamber (BRS/LVE) in which the rats were kept during conditioning. One wall of the operant chamber was fitted with two speakers that independently produced tones of up to 120 dB (SPL), with a frequency range of approximately 1000–9000 Hz. The back wall of the sound-attenuating chamber was equipped with a small light. A small fan on one of the walls provided a 65-dB masking noise. The electrode leads from the rat's headstage were connected to peripheral equipment and a desktop computer. Computer software controlled the delivery of stimuli and the recording of eyelid EMG activity. One circuit permitted the delivery of a shock stimulus (2–3 mA, depending

Table 1
Experimental Design

Behavioral group	Phase 1	Phase 2	Phase 3
Experiment 1: Blocking			
Blocking	T+ or L+	TL+	T-, L-
Sit control	Sit in chamber	TL+	T-, L-
Experiment 2: Latent inhibition			
Latent inhibition	450 T-	350 T+	—
Sit control	Sit in chamber	350 T+	—

Note. Dashes indicate that there was no third phase in Experiment 2. Numerals indicate the number of conditioned stimulus (CS) presentations. T = tone CS; L = light CS; + = reinforced; - = unreinforced.

on the threshold for eliciting an unconditioned response [UR]; 60 Hz; constant current) through a stimulus isolator (Model number 365A, World Precision Instruments, Sarasota, FL). EMG activity was recorded differentially, filtered, amplified, and integrated by equipment that was similar to that used in previous studies (Freeman & Nicholson, 1999; Nicholson & Freeman, 2000b; Skelton, 1988; Stanton, Freeman, & Skelton, 1992).

Conditioning procedures. All rats were given an initial 45-min session during which the EMG and bipolar shock electrodes were connected and the rat was allowed to adapt to the conditions of the training environment. Rats were also allowed to adapt to the training environment for 15 min before each conditioning session. All rats were given three phases of training (see Table 1). During Phase 1, rats in the blocking condition (control lesion rats, CTL-BLK; rats with MD lesions, LES-BLK) were given classical delay conditioning procedures in which a 300-ms CS coterminated with a 25-ms US (interstimulus interval = 275 ms). Rats in the blocking condition were given one session of 100 paired CS_A-US trials per day until a criterion of 80% CRs in a half-session was reached. The CS_A could be either a tone or a light (the number of rats receiving each was counterbalanced). Rats in the sit control group (control lesion rats, CTL-SIT; rats with MD lesions, LES-SIT) were yoked to a rat in the blocking condition and sat in the conditioning chamber until their yoked rat reached criterion (~65 min, typically one to three sessions). During Phase 2, all of the rats received three sessions (100 trials per day) in which a simultaneous compound stimulus CS_A-CS_B was paired with the US. The CS_A-CS_B compound was always a tone-light compound. During Phase 3, all rats received explicitly unpaired presentations of CS_A and CS_B. The US was not presented during Phase 3. Responses that crossed a threshold of 0.4 units (amplified and integrated arbitrary units) above baseline during the CS period were defined as CRs; responses that crossed the threshold after the onset of the US were defined as URs (Freeman & Nicholson, 1999; Nicholson & Freeman, 2000b; Stanton et al., 1992). During Phase 3, CRs were defined as responses that crossed the threshold during the period between CS onset and the end of the trial (700 ms; Nicholson & Freeman, 2000b).

Histology. After training, the rats were killed with a lethal injection of sodium pentobarbital (90 mg/kg) and transcardially perfused with 100 ml physiological saline, followed by 300 ml of 3% Formalin. After perfusion, the brains were postfixed in the same fixative for a minimum of 24 hr and subsequently sectioned at 50 μm with a sliding microtome. Sections were then stained with cresyl violet. The NIH Image program (Rasband, 1996) was used to quantify lesion size (Nicholson & Freeman, 2000b) by computing the size of the lesion at each of five different anterior-posterior coordinates (-1.8, -2.12, -2.56, -3.14, and -3.6 relative to bregma) from the atlas of Paxinos and Watson (1998). Rats with damage to the hippocampus or dentate gyrus were excluded from all analyses.

Results

Histology. All rats in the lesion group sustained bilateral damage to the MD nucleus (range = 40–100%; see Figure 1). In most cases, some bilateral damage was also sustained to the ventral portions of the medial and lateral habenula, stria medullaris of the thalamus, lateral portions of the anterior paraventricular and intermediodorsal thalamic nuclei, and dorsal portions of the central medial and paracentral thalamic nuclei. Within the MD nucleus, most lesions spared ventralmost portions of medial MD and lateralmost portions of lateral MD but still destroyed most of all

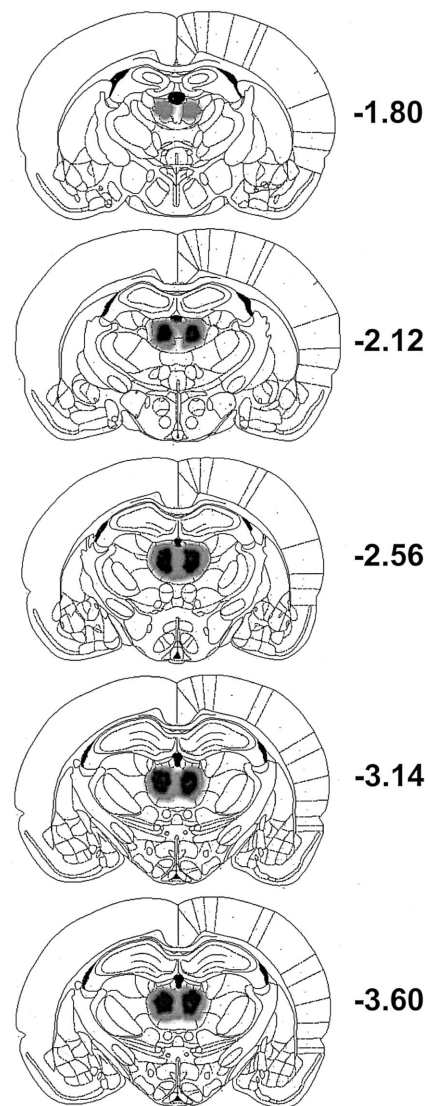


Figure 1. Drawing of five coronal sections of the rat brain, from the most rostral (top) to the most caudal (bottom) section. The smallest (black regions) and largest (gray regions) lesions are depicted. The numbers to the right of the drawings are the anterior-posterior coordinates relative to bregma (Paxinos & Watson, 1998). From *The Rat Brain in Stereotaxic Coordinates* (4th ed., Figures 26, 28, 30, 32, and 34), by G. Paxinos and C. Watson, 1998, New York: Academic Press. Copyright 1998 by Academic Press. Adapted with permission.

major subnuclei within the MD. There was no correlation between the extent of MD damage and behavior.

Behavioral data. There were no differences between CTL-BLK and LES-BLK rats in the percentage of CRs during the 1st day of Phase 1 training, the CR percentage on the day rats reached criterion, or the number of sessions required to reach criterion. These results suggest that the level of plasticity within the brainstem-cerebellum circuitry, and therefore delay eyeblink conditioning, was similar in the two groups.

CS_B will be considered the blocked stimulus, and CS_A will be considered the blocking stimulus. This is done in the interest of clarity, even though rats received paired presentations of the light or tone and US during Phase 1 (see Table 1 and *Conditioning procedures* in the *Method* section). The performance criterion of 80% CRs in a half-session was sustained in CTL-BLK and LES-BLK rats during Phase 2 training and was reached by all rats in the sit control groups by the last (3rd) day of Phase 2 training (CS_A-CS_B-US, data not shown). Rats with MD lesions responded more on the last day of Phase 2 training than rats with control lesions (MD lesions = 95.7% CRs, control lesions = 89.5% CRs), $F(1, 37) = 4.43, p < .05$. These results suggest that, although rats with MD lesions had a slightly higher CR percentage overall, all rats had reached similar levels of conditioning before being tested in Phase 3.

The data from the test session, which consisted of 40 unreinforced presentations of the CS_A alone and 40 presentations of the CS_B alone, were analyzed in three different ways. When separated into four 20-trial blocks, each consisting of 10 CS_A-alone and 10 CS_B-alone trials, an analysis of variance (ANOVA) found a Block \times Lesion \times Condition interaction, $F(3, 219) = 4.55, p < .05$. The rats with MD lesions responded at equally higher rates to both stimuli (see Figure 2a). Post hoc analysis (Tukey-Kramer, $ps < .05$) indicated that the interaction was due to higher rates of responding in Group LES-BLK compared with Group CTL-BLK in Blocks 3 and 4, and 4 and compared with Group LES-SIT in Blocks 3 and 4. It is interesting to note that only Group LES-BLK exhibited a resistance to extinction (see Figure 2a). An ANOVA on the total response percentage from all 80 test trials found only a

main effect of lesion, $F(1, 37) = 7.73, p < .05$, which was due to the low level of responding to the blocked stimulus in the control groups (perhaps due to low rate of responding to the blocked CS in Group CTL-BLK; see Figure 2b).

The relative rates of responding to CS_A were examined because of the apparent differences in the rates of extinction between groups (Annau & Kamin, 1961; Nicholson & Freeman, 2000b; Solomon, 1977). The relative rates of responding were determined by dividing the percentage of CRs to CS_B (the blocked stimulus) by the total percentage of CRs (Nicholson and Freeman, 2000b). A relative rate of responding equal to 0.5 means that rats responded equally often to CS_A and CS_B; a relative rate of responding of 0.0 means that rats never responded to CS_B. An ANOVA on the relative rates of responding yielded a Condition \times Lesion interaction, $F(1, 37) = 9.61, p < .05$. The Condition \times Lesion interaction was due to a lower relative rate of responding in Group CTL-BLK compared with Groups LES-BLK, CTL-SIT, and LES-SIT (Tukey-Kramer, $ps < .05$; see Figure 2c). There were no significant differences among Groups LES-BLK, CTL-SIT, or LES-SIT (see Figure 2c).

The three different analyses indicate that (a) MD lesions do not impair delay eyeblink conditioning in rats, (b) there was a slight resistance to extinction in rats with MD lesions in the blocking condition, (c) rats with MD lesions tended to respond at higher rates to both stimuli, (d) MD lesions did not impair conditioning to CS_A or CS_B during Phase 2 in the LES-SIT group, and (e) prior training with CS_A-US pairings impaired conditioning to CS_B during CS_A-CS_B-US pairings in rats with control lesions, but not in rats with MD lesions. It is possible that some or all of the effects in the lesion group are attributable to damage of fibers of passage. Experiment 2 used chemical lesions to more clearly illustrate the role of the MD in CS processing while minimizing the possible influences of damage to fibers of passage on behavior.

Experiment 2

It has been suggested that hippocampal lesions slow, but do not prevent, the decremental changes in CS associability that occur in

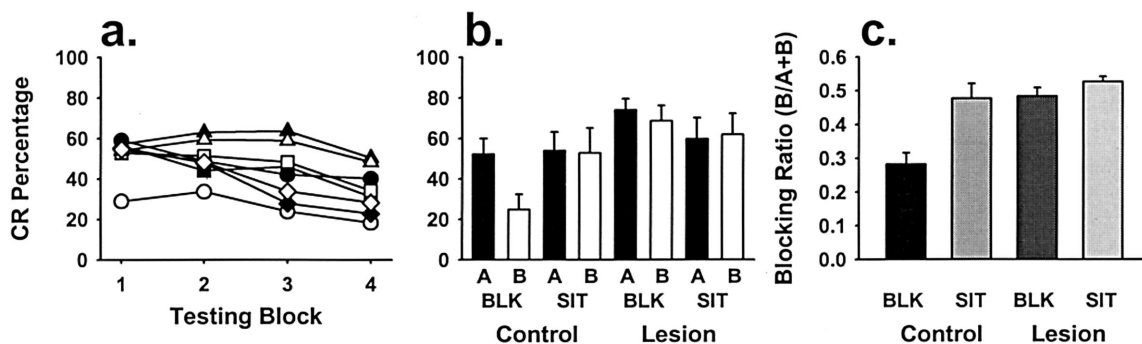


Figure 2. a: Mean percentage of conditioned responses (CRs) to conditioning stimuli (CS_A, filled symbols; CS_B, open symbols) during all four blocks of Phase 3 testing of blocking in Experiment 1 for groups control-blocking (circles), control-sit (squares), lesion-blocking (triangles), and lesion-sit (diamonds). Rats in the sit groups were yoked to rats in the blocking (BLK) condition and sat in the conditioning chamber until their yoked rats had reached criterion. b: Mean (\pm SEM) total percentage of CRs to CS_A and CS_B during Phase 3 of Experiment 1. c: Mean (\pm SEM) relative rate of responding to CS_B during Phase 3, in the form of a blocking ratio.

LI (Buhusi et al., 1998). Similarly, MD lesions slow, but do not prevent, learning in a discriminative avoidance task (Gabriel, 1993; Gabriel et al., 1989). If cingulothalamic CS processing is impaired by MD lesions, then CS processing may continue into Phase 2 CS_A-US training. As a result, LI would be expected to be present in rats with control lesions but to be impaired in rats with MD lesions.

Method

Subjects. Subjects were 30 male Long-Evans rats (250–350 g). The rats were housed in the animal colony in Spence Laboratories at the University of Iowa. All rats were maintained on a 12-hr light–dark cycle with light onset at 6:30 a.m. and given ad-lib access to food and water.

Surgery. The surgical methods were identical to those used in Experiment 1, except that rats were given bilateral MD lesions with ibotenic acid rather than electrolysis. Ibotenic acid (10 mg/ml, 0.1 μ l) was infused through a 28-gauge infusion cannula at a rate of 6 μ l/hr. The infusion cannula remained in position for 5 min after infusion. Pilot experiments indicated that these infusion parameters optimized the probability of a complete lesion and reduced the number of deaths caused by the putative diffusion of ibotenic acid into the ventricles (Beracochea et al., 1989; glass micropipettes also work and may form a tighter seal with the periventricular tissue, L. Jarrard, personal communication, December 7, 1999). Burr holes for the infusion cannula were filled with bone wax, and the EMG and bipolar shock electrode for delivering the shock US were implanted and attached to the skull with dental acrylic. The surgical site was closed with sutures on both sides of the electrode connectors.

Apparatus. The conditioning apparatus was the same as that used in Experiment 1.

Conditioning procedures. All rats were given an initial 45-min session during which the EMG and bipolar shock electrodes were connected and the rat was allowed to adapt to the conditions of the training environment. Pilot studies indicated that enhancing the salience of the context by applying ammonium hydroxide to the cage liner dramatically increased the reliability and strength of LI. Also, all rats were run at exactly the same time, by the same experimenter, with special care taken to ensure that no extraneous odors or noises were present. Rats were also allowed to adapt to the training environment for 15 min before each conditioning session.

All rats were given two phases of training (see Table 1). During Phase 1, rats in the preexposed condition (control lesions, CTL-PRE; MD lesions, LES-PRE) were given 450 unreinforced presentations of CS_A (a 300-ms, 2-kHz tone) over 4.5 sessions (100 trials per day for 4 days, 50 trials on the 5th day). As in Experiment 1, rats in the sit control group, CTL-SIT and LES-SIT, were yoked to a rat in the preexposed condition and sat in the conditioning chamber. Fifty paired presentations of CS_A-US were given to all rats on the 5th day. Rats in the preexposed condition received 50 CS_A-alone trials, followed by 50 CS_A-US trials. Rats in the CTL-SIT group sat in the chamber for ~ 0.5 hr, after which they were given 50 CS_A-US trials. The 300-ms CS_A coterminated with a 25-ms US, yielding an interstimulus interval of 275 ms. After this session, all rats were given 300 CS_A-US presentations (100 trials per day over 3 days), for a total of 350 CS_A-US presentations (see Table 1). The definition of CRs and URs was identical to that of Experiment 1.

Histology. Histological procedures and method of analysis of lesion size were identical to those of Experiment 1.

Results

Histology. Neuronal degeneration, loss in the volume of MD, and gliosis were used as evidence of ibotenic acid lesions in the MD (Beracochea et al., 1989; Buchanan et al., 1997; Krazem, Beracochea, & Jaffard, 1995). The extent of damage to the MD

caused by ibotenic acid lesions tended to be slightly smaller than that caused by electrolytic lesions in Experiment 1. A Lesion Size \times Coordinate ANOVA between Experiments 1 and 2 confirmed this by yielding a main effect of experiment, $F(1, 155) = 10.16, p < .05$ (electrolytic damage = 86.023 %, ibotenic damage = 77.873 %). There was also a main effect of coordinate, $F(4, 155) = 5.79, p < .05$, which was due to smaller lesions in the anterior portions of the MD in both Experiment 1 and Experiment 2. Like the electrolytic lesions, ibotenic acid lesions tended to spare the ventralmost portions of the medial MD and the lateralmost portions of the lateral MD but still destroyed most of all major subnuclei within the MD. Most lesions produced collateral damage to the same structures as in Experiment 1. There was no correlation between MD damage and behavior.

Behavioral data. The CR-percentage data from the 350 CS_A-US presentations were analyzed in two different ways (all post hoc analyses are Tukey–Kramer, $ps < .05$). When separated into blocks of 10 trials (35 blocks for each rat), there was a between-subjects Lesion \times Condition interaction, $F(1, 26) = 12.85, p < .05$. The interaction was due to the persistently low rate of responding in Group CTL-PRE, but not Group LES-PRE. That is, unreinforced presentations of CS_A impaired conditioning during subsequent CS_A-US training in Group CTL-PRE, but not in Group LES-PRE (see Figure 3a). There were within-subjects interactions of Block \times Condition, $F(34, 884) = 2.72, p < .05$, and Block \times Lesion \times Condition, $F(34, 884) = 1.71, p < .05$. The Block \times Lesion \times Condition interaction was due to lower responding in Group CTL-PRE compared with (a) all groups during Blocks 7–15 and Blocks 17–25; (b) Group LES-PRE during Blocks 5, 27, and 32; (c) Group CTL-SIT during Blocks 16 and 26; and (d) Group LES-SIT during Blocks 16, 26, and 28. There were no significant differences among Groups LES-PRE, CTL-SIT, and LES-SIT. In general, the impaired conditioning in group CTL-PRE was most obvious between Blocks 7 and 28 (see Figure 3a). Presentations of CS_A in the absence of the US impaired conditioning in CTL-PRE in a long-lasting, but reversible, manner (see Figure 3a). Presentations of CS_A in the absence of the US did not impair conditioning in rats with MD lesions (see Figure 3a).

An ANOVA of the total percentage of CRs for each session yielded a Session \times Lesion \times Condition interaction, $F(3, 78) = 3.34, p < .05$. Post hoc analyses (Tukey–Kramer, $ps < .05$) indicated that Group CTL-PRE exhibited significantly fewer CRs than all other groups during Sessions 2 and 3 and significantly fewer than Group LES-SIT during Session 4 (see Figure 3b). There were no significant differences during Session 1, nor were there any significant differences among Groups LES-PRE, CTL-SIT, and LES-SIT.

The two different analyses indicate that MD lesions did not impair delay eyeblink conditioning in rats and that CS_A presentations in the absence of the US impaired conditioning during subsequent CS_A-US trials in rats with control lesions, but not rats with MD lesions.

Discussion

The present study investigated the effects of MD lesions on two forms of associative learning that involve decremental changes in stimulus processing: blocking and LI (Holland, 1997). In Experiment 1, prior conditioning with CS_A-US presentations impaired

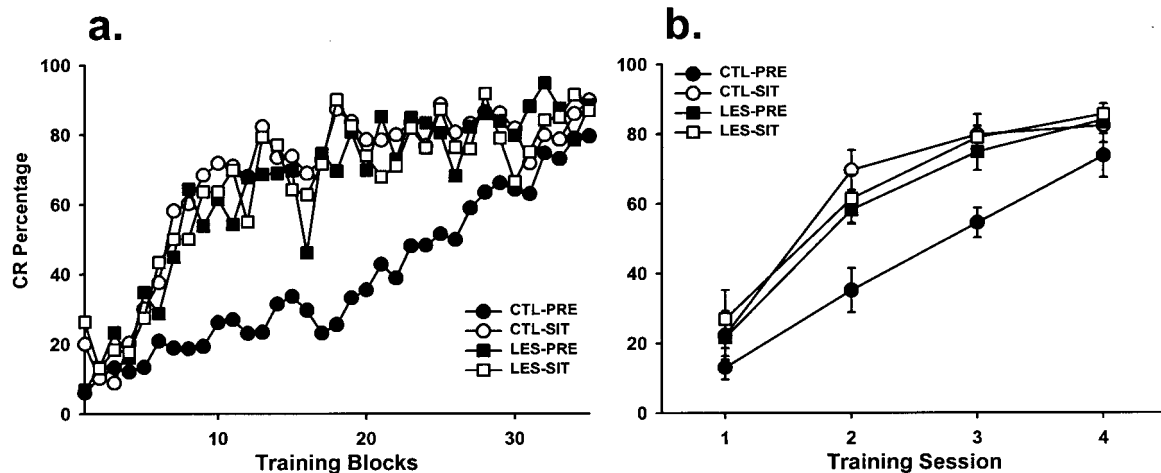


Figure 3. a: Mean percentage of conditioned responses (CRs) during 35 blocks of conditioned stimulus–unconditioned stimulus (CS_A –US) training in Phase 2 of Experiment 2 for groups control–preexposed (CTL–PRE), control–sit (CTL–SIT), lesion–preexposed (LES–PRE), and lesion–sit (LES–SIT). Rats in the sit groups were yoked to rats in the preexposed condition and sat in the conditioning chamber until their yoked rats had reached criterion. b: Mean (\pm SEM) percentage of CRs across four sessions of CS_A –US training (Phase 2, Experiment 2).

conditioning to CS_B during subsequent paired presentations of a simultaneous compound CS_A – CS_B and the same US (i.e., blocking) in rats with control lesions, but not in rats with MD lesions. In Experiment 2, presentations of CS_A in the absence of the US impaired conditioning during subsequent CS_A –US training (i.e., LI) in rats with control lesions, but not in rats with MD lesions.

The impairment in blocking and LI of the conditioned eyeblink response is similar to results of previous studies that investigated the effects of hippocampal (Schmajuk et al., 1994, 2000; Solomon & Moore, 1975) or entorhinal cortical (Shohamy et al., 2000) lesions in that delay eyeblink conditioning, which requires only brainstem–cerebellum circuitry (Thompson & Krupa, 1994), was not affected. Moreover, Gabriel et al. (1996) demonstrated that limbic thalamic lesions, which included the MD, did not disrupt delay eyeblink conditioning in rabbits.

The MD and ACC may function in at least one of three roles during blocking and LI. First, the MD may be involved in both incremental and decremental changes in attention. Early increases in neuronal activity within the MD and ACC may represent early-stage increases in attention to the conditioning stimuli (Gabriel & Talk, 2001). Moreover, the decreases in neuronal activity during later training stages may represent decremental changes in attention, attributable to associative strength accruing to the conditioning stimuli (Pearce & Hall, 1980). The MD receives input from the perirhinal cortex and entorhinal cortex (Gower, 1989; Russchen, Amaral, & Price, 1987; Steriade, Parent, Pare, & Smith, 1987), which likely interact with the hippocampus to subservise a variety of functions (Cohen & Eichenbaum, 1995; Eichenbaum, Otto, & Cohen, 1994), one of which may be decremental changes in attention. The MD also receives input from the amygdala (Aggleton & Mishkin, 1984; Groenewegen, 1988), which is involved in incremental changes in attention (Holland et al., 2000; Holland & Gallagher, 1993a, 1993b, 1999). If the MD is involved in attentional changes (i.e., both incremental and decremental), then MD

lesions in the present study may have impaired the ability to change attention to stimuli as a function of learning. That is, the absence of attentional changes may have resulted in associative strength accruing to conditioning stimuli at all points in training, thereby impairing blocking and LI.

Second, the MD may be involved primarily in either incremental or decremental changes in attention. If the MD is involved primarily in incremental changes in attention, then MD lesions may retard stimulus processing in the cingulothalamic system such that the eyeblink CR is established before the cingulothalamic system can completely encode the significance of CS_A during Phase 1 in blocking and LI. Protracted CS processing during the second phase of blocking and LI may impair blocking and LI by affecting the enhanced cingulothalamic neuronal responses to nonsalient or new conditioning stimuli (e.g., CS_A), which Gabriel and Talk (2001) proposed increases the probability that a cue will capture the subject's attention. Impaired cingulothalamic neuronal responses to CS_A in Phase 1 of blocking and LI may have reduced the probability of the cue capturing the attention of rats with MD lesions, resulting in residual CS processing in rats with MD lesions in Phase 2 of blocking and LI. Residual CS processing during Phase 2 may have been directed toward CS_A and CS_B during blocking and toward the CS_A –US relationship during LI, thereby impairing both blocking and LI. If the MD is involved primarily in decremental changes in attention, then the impairments in blocking and LI are likely attributable to a failure to decrease attention to well-learned stimuli. Neurons throughout the cingulothalamic axis (including the ACC) exhibit “neuronal habituation” during pre-training CS-alone presentations (Gabriel & Saltwick, 1977), and a substantial proportion of neurons in the MD show increases in firing to an unreinforced CS during discriminative avoidance conditioning (Kubota, Wolske, Poremba, Kang, & Gabriel, 1996). Lesions to the MD may have selectively obstructed decremental changes in attention by damaging MD neurons that show neuronal

habituation, which may be important during the first phase of LI, as well as MD neurons that exhibit increases in firing during presentations of a CS-, which may represent active and persistent decremental processing responsible for the blocking effect.

An alternative possibility is that MD lesions impaired blocking and LI by delaying or slowing the transition between increases and decreases in attention during conditioning by slowing, but not preventing, CS processing, which Buhusi et al. (1998) and Schmajuk et al. (2000) suggested occurs in rats with hippocampal lesions. The susceptibility of blocking and LI to lesions that impair acquisition rate is consistent with disruptions in blocking (Marchant & Moore, 1973) or LI (Clarke & Hupka, 1974; Lubow, 1965; Reiss & Wagner, 1972; Siegel, 1969) after insufficient training in Phase 1. If blocking and LI depend on decremental changes in CS processing that occur during Phase 1 (Holland, 1997), then residual CS processing during Phase 2 would be severely disruptive.

Third, MD lesions may have disrupted interactions between the hippocampus and the cingulothalamic system, so that only decremental changes in CS processing were impaired. Stolar et al. (1989) demonstrated that subicular lesions impaired cingulothalamic neuronal responses to rare CSs and suggested that an intact hippocampus is essential for normal cingulothalamic neuronal responses to rare CSs. Kang and Gabriel (1998) demonstrated that the hippocampal system modulates cingulate cortical and limbic thalamic neuronal activity by means of excitatory input from the subiculum. It is possible that MD lesions, which disrupt neuronal activity in the ACC (Gabriel et al., 1989), altered the responsiveness of the cingulothalamic system to inputs from the hippocampus. Lesions of the MD may have impaired blocking and LI by interfering with information flow from the hippocampus and retrosplenial cortex to the pontine nuclei (Berger, Weikart, Bassett, & Orr, 1986; Nicholson & Freeman, 2000b).

The preceding possible roles for the MD in blocking and LI are consistent with theories of learning that propose finite amounts of associability for the conditioning stimuli. For example, MD lesions may slow the rate at which CS information is acquired by impairing incremental changes in attention. If Phase 1 conditioning in blocking is insufficient to produce asymptotic learning, then the remaining associative strength that the CSs or US can support will be shared between CS_A and CS_B during Phase 2 (CS_A - CS_B -US), reducing or eliminating the blocking effect of CS_A on CS_B . Alternatively, MD lesions may disrupt the ability to decrease attention to stimuli that are consistent predictors of an outcome. Blocking and LI may be impaired if attention to conditioning stimuli remains high through all phases of training (e.g., high values for α_A^n and α_B^n throughout Phases 1 and 2 of blocking; Pearce & Hall, 1980). Kim, Krupa, and Thompson (1998) reported that pharmacological blockade of GABAergic receptors within the inferior olive impair blocking. Kim et al. (1998) suggested that nucleo-olivary inhibition represents active decremental processing of US information (see also Nicholson & Freeman, 2000a; Sears & Steinmetz, 1991), which is consistent with some theories of learning (e.g., Albus, 1971; Bartha, Thompson, & Gluck, 1991; Donegan, Gluck, & Thompson, 1989; Ito, 1984; Rescorla & Wagner, 1972). It is possible that the MD lesions impaired blocking by disrupting nucleo-olivary inhibitory feedback, so that the inferior olive (the putative US pathway) continues to provide the cerebellum with US information throughout Phases 1 and 2. However, the

impairments in blocking and LI induced by MD lesions and the impairment in blocking induced by pharmacological blockade of nucleo-olivary feedback (Kim et al., 1998) may represent similar outcomes induced by disrupting parallel systems (e.g., MD lesions may affect CS processing systems, but not US processing systems).

How do MD lesions impair blocking and LI without impairing acquisition of the eyeblink CR? Delay eyeblink conditioning requires only brainstem-cerebellum circuitry (Thompson & Krupa, 1994). Therefore, lesions outside of this circuitry that affect eyeblink CRs in more complex forms of associative learning (e.g., blocking and LI) must somehow interact with brainstem-cerebellum circuitry (Berger et al., 1986; Mauk & Donegan, 1997; Nicholson & Freeman, 2000b; but see Kim et al., 1998 for a within-eyeblink-circuit effect on blocking). The MD lesion-induced disruption of blocking and LI is likely a result of disrupting cingulate or retrosplenial cortical input to the brainstem-cerebellum circuitry underlying the eyeblink CR (Berger et al., 1986; Nicholson & Freeman, 2000b). Excitatory projections to the pontine nuclei from the prefrontal cortex (e.g., Allen & Hopkins, 1998; Schmahmann & Pandya, 1997; Vilensky & van Hoesen, 1981) and retrosplenial cortex (e.g., Azkue et al., 1995; Vilensky & van Hoesen, 1981) may provide routes of communication for the hippocampus, cingulate gyrus, and retrosplenial cortex to the cerebellum. The pontine nuclei are the primary source of CS information to the cerebellum (Solomon, Lewis, LoTurco, Steinmetz, & Thompson, 1986; Steinmetz, Lavond, & Thompson, 1985, 1989; Steinmetz et al., 1987). Thus, blocking or LI may occur through modifications of pontine nuclear afferents of the cerebellum (Mauk & Donegan, 1997), such that limbic circuitry may have a descending influence on pontine inputs to the cerebellum. CS information beyond Phase 1 may be gated by corticopontine projections. As MD lesions impaired CS processing, the descending influence likely included information from Phase 2 training. As a result, the descending influence underlying blocking and LI in rats with control lesions would not have been present in rats with MD lesions, resulting in impaired performance.

In conclusion, the present study provides evidence for a role of the MD in blocking and LI of the classically conditioned eyeblink response. The results are consistent with current views of the role of the MD and ACC as being involved in incremental changes in attention during new learning situations (Beracochea et al., 1989; Buchanan & Powell, 1982; Devinsky, Morrell, & Vogt, 1995; Gabriel, 1993; Gabriel & Talk, 2001; Orona & Gabriel, 1983; Powell, Buchanan, & Gibbs, 1990), but they also provide a possible new role for the MD in decremental changes in attention. Specifically, MD lesions may impair the rate at which information about CS_A is acquired by impairing incremental attentional processes within cingulothalamic circuitry, or MD lesions may impair the ability to decrease attention to stimuli as a function of training. It is possible that MD lesions disrupted information flow between the hippocampus and cingulothalamic circuitry, thereby affecting hippocampal influence on pontine efferents to the cerebellum. Pharmacological inactivation of the MD, in combination with reducing or extending Phase 1 training, may clarify whether MD lesions impair or prevent CS processing during blocking and LI.

References

- Aggleton, J. P., & Mishkin, M. (1984). Projection of the amygdala to the thalamus in the cynomolgus monkey. *Journal of Comparative Neurology*, *222*, 56–68.
- Albus, J. S. (1971). A theory of cerebellar function. *Mathematical Biosciences*, *10*, 25–61.
- Alkon, D. L. (1984). Calcium-mediated reduction of ionic currents: A biophysical memory trace. *Science*, *226*, 1037–1045.
- Allen, G. V., & Hopkins, D. A. (1998). Convergent prefrontal cortex and mammillary body projections to the medial pontine nuclei: A light and electron microscopy study in the rat. *Journal of Comparative Neurology*, *398*, 347–358.
- Annau, Z., & Kamin, L. J. (1961). The conditioned emotional response as a function of intensity of the US. *Journal of Comparative and Physiological Psychology*, *54*, 428–432.
- Azkue, J., Bidaurrazaga, A., Mayteos, J. M., Sarria, R., Streit, P., & Grandes, P. (1995). Glutamate-like immunoreactivity in synaptic terminals of the posterior cingulo-pontine pathway: A light and electron microscopy study in the rabbit. *Journal of Chemical Neuroanatomy*, *9*, 261–269.
- Bartha, G. T., Thompson, R. F., & Gluck, M. A. (1991). Sensorimotor learning and the cerebellum. In M. E. Arbib & T. Ewert (Eds.), *Visual structures and integrated functions* (pp. 381–396). Berlin: Springer.
- Baxter, M. G., Holland, P. C., & Gallagher, M. (1997). Disruption of decrements in conditioned stimulus processing by selective removal of hippocampal cholinergic input. *Journal of Neuroscience*, *17*, 5230–5236.
- Beracochea, D. J., Jaffard, R., & Jarrard, L. E. (1989). Effects of anterior or dorsomedial thalamic ibotenic lesions on learning and memory in rats. *Behavioral and Neural Biology*, *51*, 374–376.
- Berger, T. W., Weikart, C. L., Bassett, J. L., & Orr, W. B. (1986). Lesions of the retrosplenial cortex produce deficits in reversal learning of the rabbit nictitating membrane response: Implications for potential interactions between hippocampal and cerebellar systems. *Behavioral Neuroscience*, *100*, 802–809.
- Bucci, D. J., Holland, P. C., & Gallagher, M. (1998). Removal of cholinergic input to rat posterior parietal cortex disrupts incremental processing of conditioned stimuli. *Journal of Neuroscience*, *18*, 8038–8046.
- Buchanan, S. L., Penney, J., Tebbutt, D., & Powell, D. A. (1997). Lesions of the mediodorsal nucleus of the thalamus and classical eyeblink conditioning under less-than-optimal stimulus conditions: Role of partial reinforcement and interstimulus interval. *Behavioral Neuroscience*, *111*, 1075–1085.
- Buchanan, S. L., & Powell, D. A. (1982). Cingulate cortex: Its role in Pavlovian conditioning. *Journal of Comparative and Physiological Psychology*, *96*, 755–774.
- Buhusi, C. V., Gray, J. A., & Schmajuk, N. A. (1998). The perplexing effects of hippocampal lesions on latent inhibition: A neural network solution. *Behavioral Neuroscience*, *112*, 316–351.
- Chiba, A. A., Bucci, D. J., Holland, P. C., & Gallagher, M. (1995). Basal forebrain cholinergic lesions disrupt increments but not decrements in conditioned stimulus processing. *Journal of Neuroscience*, *15*, 7315–7322.
- Clarke, M. E., & Hupka, R. B. (1974). The effects of stimulus duration and frequency of daily preconditioning stimulus exposures on latent inhibition in Pavlovian conditioning of the rabbit nictitating membrane response. *Bulletin of the Psychonomic Society*, *4*, 225–228.
- Cohen, N. J., & Eichenbaum, H. (1995). *Memory, amnesia, and the hippocampal system*. Cambridge, MA: MIT Press.
- Davis, M. (1992). The role of the amygdala in conditioned fear. In J. P. Aggleton (Ed.), *The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction* (pp. 255–305). New York: Wiley-Liss.
- Devinsky, O., Morrell, M. J., & Vogt, B. A. (1995). Contributions of anterior cingulate cortex to behavior. *Brain*, *118*, 279–306.
- Disterhoft, J. F., Coulter, D. A., & Alkon, D. L. (1986). Conditioning-specific membrane changes of rabbit hippocampal neurons measured *in vitro*. *Proceedings of the National Academy of Sciences, USA*, *83*, 2733–2777.
- Domjan, M. (1998). *The principles of learning and behavior*. Pacific Grove, CA: Brooks/Cole.
- Donegan, N. H., Gluck, M. A., & Thompson, R. F. (1989). Integrating behavioral and biological models of classical conditioning. In R. D. Hawkins & G. H. Bower (Eds.), *Computational models of learning in simple neural systems* (pp. 109–156). New York: Academic Press.
- Eichenbaum, H., Otto, T., & Cohen, N. J. (1994). Two functional components of the hippocampal memory system. *Behavioral and Brain Sciences*, *17*, 449–472.
- Falls, W. A., & Davis, M. (1995). Lesions of the central nucleus of the amygdala block conditioned excitation, but not conditioned inhibition of fear as measured with the fear-potentiated startle effect. *Behavioral Neuroscience*, *109*, 379–387.
- Falls, W. A., & Davis, M. (1997). Inhibition of fear-potentiated startle can be detected after the offset of a feature trained in a serial feature-negative discrimination. *Journal of Experimental Psychology: Animal Behavior Processes*, *23*, 3–14.
- Fanselow, M., & LeDoux, J. E. (1999). Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. *Neuron*, *23*, 229–232.
- Freeman, J. H., Jr., & Nicholson, D. A. (1999). Neuronal activity in the cerebellar interpositus and lateral pontine nuclei during inhibitory classical conditioning of the eyeblink response. *Brain Research*, *833*, 225–233.
- Gabriel, M. (1993). Discriminative avoidance learning: A model system. In M. Gabriel & B. Vogt (Eds.), *Neurobiology of cingulate cortex and limbic thalamus* (pp. 478–523). Toronto, Canada: Birkhauser Publishers.
- Gabriel, M., Kang, E., Poremba, A., Kubota, Y., Allen, M. T., Miller, D. P., & Steinmetz, J. E. (1996). Neural substrates of discriminative avoidance learning and classical eyeblink conditioning in rabbits: A double dissociation. *Behavioural Brain Research*, *82*, 23–30.
- Gabriel, M., Kubota, Y., Sparenborg, S., Straube, K., & Vogt, B. A. (1991). Effects of cingulate cortical lesions on avoidance learning and training-induced unit activity in rabbits. *Experimental Brain Research*, *86*, 585–600.
- Gabriel, M., & Saltwick, S. E. (1977). Effects of unpaired footshock on rabbit limbic and auditory neuronal responses to tone stimuli. *Physiology & Behavior*, *19*, 29–34.
- Gabriel, M., & Smith, D. M. (1999). What does the limbic memory circuit actually do? *Behavioral and Brain Sciences*, *22*, 451.
- Gabriel, M., Sparenborg, S., & Kubota, Y. (1989). Anterior and medial thalamic lesions, discriminative avoidance learning, and cingulate cortical neuronal activity in rabbits. *Experimental Brain Research*, *76*, 441–457.
- Gabriel, M., & Talk, A. C. (2001). A tale of two paradigms: Lessons learned from parallel studies of discriminative instrumental learning and classical eyeblink conditioning. In J. E. Steinmetz, M. A. Gluck, & P. R. Solomon (Eds.), *Model systems and the neurobiology of associative learning: A festschrift in honor of Richard F. Thompson* (pp. 149–185). Hillsdale, NJ: Erlbaum.
- Gormezano, I., & Kehoe, E. J. (1981). Classical conditioning and the law of contiguity. In P. Harzem & M. D. Zeiler (Eds.), *Advances in the analysis of behavior: Predictability, correlation, and contiguity* (pp. 1–45). Chichester, England: Wiley.
- Gormezano, I., Kehoe, E. J., & Marshall, B. S. (1983). Twenty years of classical conditioning research with the rabbit. *Progress in Psychobiology and Physiological Psychology*, *10*, 197–275.
- Gower, E. C. (1989). Efferent projections from limbic cortex of the

- temporal pole to the magnocellular medial dorsal nucleus in the rhesus monkey. *Journal of Comparative Neurology*, 277, 343–358.
- Groenewegen, H. J. (1988). Organization of the afferent connections of the mediodorsal thalamic nucleus in the rat, related to mediodorsal–prefrontal topography. *Neuroscience*, 24, 379–431.
- Han, J.-S., Gallagher, M., & Holland, P. (1995). Hippocampal lesions disrupt decrements but not increments in conditioned stimulus processing. *Journal of Neuroscience*, 15, 7323–7329.
- Holland, P. (1997). Brain mechanisms for changes in processing of conditioned stimuli in Pavlovian conditioning: Implications for behavior theory. *Animal Learning & Behavior*, 373–399.
- Holland, P. C., & Gallagher, M. (1993a). Amygdala central nucleus lesions disrupt increments, but not decrements, in conditioned stimulus processing. *Behavioral Neuroscience*, 107, 246–253.
- Holland, P. C., & Gallagher, M. (1993b). Effects of amygdala central nucleus lesions on blocking and unblocking. *Behavioral Neuroscience*, 107, 235–245.
- Holland, P. C., & Gallagher, M. (1999). Amygdala circuitry in attentional and representational processes. *Trends in Cognitive Sciences*, 3, 65–73.
- Holland, P. C., Han, J.-S., & Gallagher, M. (2000). Lesions of the amygdala central nucleus alter performance on a selective attention task. *Journal of Neuroscience*, 20, 6701–6706.
- Ito, M. (1984). *The cerebellum and neural control*. New York: Raven Press.
- Jones, L., Fischer, I., & Levitt, P. (1996). Nonuniform alteration of dendritic development in the cerebral cortex following prenatal cocaine exposure. *Cerebral Cortex*, 6, 431–445.
- Kamin, L. J. (1969). Predictability, surprise, attention, and conditioning. In B. A. Campbell & R. M. Church (Eds.), *Punishment and aversive behavior* (pp. 279–296). New York: Appleton-Century-Crofts.
- Kang, E., & Gabriel, M. (1998). Hippocampal modulation of cingulothalamic neuronal activity and discriminative avoidance learning in rabbits. *Hippocampus*, 8, 491–510.
- Kapp, B. S., Wilson, A., Pascoe, J. P., Supple, W. F., & Whalen, P. J. (1990). A neuroanatomical systems analysis of conditioned bradycardia in the rabbit. In M. Gabriel & J. W. Moore (Eds.), *Neurocomputation and learning: Foundations of adaptive networks* (pp. 53–90). New York: Bradford Books.
- Kim, J. J., Krupa, D. J., & Thompson, R. F. (1998, January 23). Inhibitory cerebello–olivary projections and blocking effect in classical conditioning. *Science*, 279, 570–573.
- Krazem, A., Beracochea, D., & Jaffard, R. (1995). Effects of mammillary bodies and mediodorsal thalamic nucleus lesions on the acquisition and retention of a learning set in mice: Paradoxical effect of the intersession interval. *Behavioural Brain Research*, 67, 51–58.
- Kubota, Y., Wolske, M., Poremba, A., Kang, E., & Gabriel, M. (1996). Stimulus-related and movement-related single-unit activity in rabbit cingulate cortex and limbic thalamus during performance of discriminative avoidance behavior. *Brain Research*, 721, 22–38.
- LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience*, 23, 155–184.
- Levitt, P., Harvey, J. A., Friedman, E., Simansky, K., & Murphy, E. H. (1997). New evidence for neurotransmitter influences on brain development. *Trends in Neurosciences*, 20, 269–274.
- Lubow, R. E. (1965). Latent inhibition: Effects of nonreinforced preexposure to the CS. *Journal of Comparative and Physiological Psychology*, 60, 454–457.
- Lubow, R. E. (1973). Latent inhibition. *Psychological Bulletin*, 79, 398–407.
- Lubow, R. E. (1989). *Latent inhibition and conditioned attention theory*. Cambridge, England: Cambridge University Press.
- Lubow, R. E., & Moore, A. U. (1959). Latent inhibition: The effect of nonreinforced preexposure to the conditional stimulus. *Journal of Comparative and Physiological Psychology*, 52, 415–419.
- Mackintosh, N. J. (1975). A theory of attention: Variations in the associability of stimuli with reinforcement. *Psychological Review*, 82, 276–298.
- Marchant, H. G., III, & Moore, J. W. (1973). Blocking of the rabbit's conditioned nictitating membrane response in Kamin's two-stage paradigm. *Journal of Experimental Psychology*, 101, 155–158.
- Mauk, M. D., & Donegan, N. H. (1997). A model of Pavlovian eyelid conditioning based on the synaptic organization of the cerebellum. *Learning and Memory*, 3, 130–158.
- Moyer, J. R., Thompson, L. T., & Disterhoft, J. F. (1996). Trace eyeblink conditioning increases CA1 excitability in a transient and learning-specific manner. *Journal of Neuroscience*, 16, 5536–5546.
- Nicholson, D. A., & Freeman, J. H., Jr. (2000a). Developmental changes in eyeblink conditioning and neuronal activity in the inferior olive. *Journal of Neuroscience*, 20, 8218–8226.
- Nicholson, D. A., & Freeman, J. H., Jr. (2000b). Lesions of the perirhinal cortex impair sensory preconditioning in rats. *Behavioural Brain Research*, 112, 69–75.
- Nicholson, D. A., & Freeman, J. H., Jr. (2002). Neuronal correlates of conditioned inhibition of the eyeblink response in the anterior interpositus nucleus. *Behavioral Neuroscience*, 116, 22–36.
- Orona, E., & Gabriel, M. (1983). Multiple-unit activity of the prefrontal cortex and mediodorsal thalamic nucleus during acquisition of discriminative avoidance behavior in rabbits. *Brain Research*, 263, 295–312.
- Paxinos, G., & Watson, C. (1998). *The rat brain in stereotaxic coordinates* (4th ed.). New York: Academic Press.
- Pearce, J. M., & Hall, G. (1980). A model for Pavlovian learning: Variations in the effectiveness of conditioned but not of unconditioned stimuli. *Psychological Review*, 87, 532–552.
- Port, R. L., & Patterson, M. M. (1984). Fimbrial lesions and sensory preconditioning. *Behavioral Neuroscience*, 98, 584–589.
- Powell, D. A., Buchanan, S. L., & Gibbs, C. M. (1990). Role of the prefrontal–thalamic axis in classical conditioning. In H. B. M. Uylings, C. G. Van Eden, J. P. C. De Bruin, M. A. Corner, & M. G. P. Feenstra (Eds.), *Progress in brain research* (pp. 433–466). Amsterdam: Elsevier Science.
- Rasband, W. (1996). NIH Image (Version 1.62) [Computer software]. Bethesda, MD: National Institutes of Health.
- Reiss, S., & Wagner, A. R. (1972). CS habituation produces a “latent inhibition effect,” but no active “conditioned inhibition.” *Learning and Motivation*, 3, 237–245.
- Rescorla, R. A. (1988). Pavlovian conditioning: It's not what you think it is. *American Psychologist*, 43, 151–160.
- Rescorla, R. A., & Wagner, A. R. (1972). A theory of Pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinforcement. In A. H. Black & W. F. Prokasy (Eds.), *Classical conditioning II: Current research and theory* (pp. 64–99). New York: Appleton-Century-Crofts.
- Romano, A. G. (1999). Variations in CS associability and multiple unit hippocampal activity in the rabbit. *Behavioural Brain Research*, 103, 163–173.
- Romano, A. G., Kachelries, W. J., Simansky, K. J., & Harvey, J. A. (1995). Intrauterine exposure to cocaine produces a modality-specific acceleration of classical conditioning in adult rabbits. *Pharmacology Biochemistry and Behavior*, 52, 415–420.
- Russchen, F. T., Amaral, D. G., & Price, J. L. (1987). The afferent input to the magnocellular division of the mediodorsal thalamic nucleus in the monkey, *Macaca fascicularis*. *Journal of Comparative Neurology*, 256, 175–210.
- Schmahmann, J. D., & Pandya, D. N. (1997). Anatomic organization of the basilar pontine projections from the prefrontal cortices in rhesus monkeys. *Journal of Neuroscience*, 17, 438–458.
- Schmajuk, N. A., Christiansen, B., & Cox, L. (2000). Haloperidol rein-

- states latent inhibition impaired by hippocampal lesions: Data and theory. *Behavioral Neuroscience*, *114*, 659–670.
- Schmajuk, N. A., & Holland, P. C. (1998). *Occasion setting: Associative learning and cognition in animals*. Washington, DC: American Psychological Association.
- Schmajuk, N. A., Lam, Y.-W., & Christiansen, B. A. (1994). Latent inhibition of the rat eyeblink response: Effect of hippocampal aspiration lesions. *Physiology & Behavior*, *55*, 597–601.
- Schmajuk, N. A., Lam, Y.-W., & Gray, J. A. (1996). Latent inhibition: A neural network approach. *Journal of Experimental Psychology: Animal Behavioral Processes*, *22*, 321–349.
- Schneiderman, N., McCabe, P. M., Haselton, J. R., Ellenberger, H. H., Jarrell, T. W., & Gentile, C. G. (1987). Neurobiological bases of conditioned bradycardia in rabbits. In I. Gormezano, W. F. Prokasy, & R. F. Thompson (Eds.), *Classical conditioning* (3rd ed., pp. 37–63). Hillsdale, NJ: Erlbaum.
- Schoenbaum, G., Chiba, A. A., & Gallagher, M. (2000). Changes in functional connectivity in orbitofrontal cortex and basolateral amygdala during learning and reversal training. *Journal of Neuroscience*, *20*, 5179–5189.
- Schreurs, B. G. (1989). Classical conditioning of model systems: A behavioral review. *Psychobiology*, *17*, 145–155.
- Schreurs, B. G. (2000). Cellular correlates of eyeblink classical conditioning. In D. S. Woodruff-Pak & J. E. Steinmetz (Eds.), *Eyeblink classical conditioning: Animal* (pp. 179–204). Amsterdam: Kluwer.
- Sears, L. L., & Steinmetz, J. E. (1991). Dorsal accessory inferior olive activity diminishes during acquisition of the rabbit classically conditioned eyelid response. *Brain Research*, *545*, 114–122.
- Shohamy, D., Allen, M. T., & Gluck, M. (2000). Dissociating entorhinal and hippocampal involvement in latent inhibition. *Behavioral Neuroscience*, *114*, 867–874.
- Siegel, S. (1969). Effect of CS habituation on eyelid conditioning. *Journal of Comparative and Physiological Psychology*, *2*, 245–248.
- Siegel, S. (1972). Latent inhibition and eyelid conditioning. In A. H. Black & W. F. Prokasy (Eds.), *Classical conditioning II: Current theory and research* (pp. 231–247). New York: Appleton-Century-Crofts.
- Skelton, R. W. (1988). Bilateral cerebellar lesions disrupt conditioned eyelid responses in unrestrained rats. *Behavioral Neuroscience*, *102*, 586–590.
- Solomon, P. R. (1977). Role of the hippocampus in blocking and conditioned inhibition of the rabbit's nictitating membrane response. *Journal of Comparative and Physiological Psychology*, *91*, 407–417.
- Solomon, P. R. (1987). Neural and behavioral mechanisms involved in learning to ignore irrelevant stimuli. In I. Gormezano, W. F. Prokasy, & R. F. Thompson (Eds.), *Classical conditioning* (3rd ed., pp. 117–150). Hillsdale, NJ: Erlbaum.
- Solomon, P. R., Lewis, J. L., LoTurco, J. J., Steinmetz, J. E., & Thompson, R. F. (1986). The role of the middle cerebellar peduncle in acquisition and retention of the rabbit's classically conditioned nictitating membrane response. *Bulletin of the Psychonomic Society*, *24*, 74–78.
- Solomon, P. R., & Moore, J. W. (1975). Latent inhibition and stimulus generalization of the classically conditioned nictitating membrane response in rabbits (*Oryctolagus cuniculus*) following dorsal hippocampal ablation. *Journal of Comparative and Physiological Psychology*, *89*, 1192–203.
- Sparenborg, S., & Gabriel, M. (1990). Neuronal encoding of conditional stimulus duration in the cingulate cortex and the limbic thalamus of rabbits. *Behavioral Neuroscience*, *100*, 729–744.
- Stanton, M. E., & Freeman, J. H., Jr. (2000). Developmental studies of eyeblink conditioning in a rat model. In D. S. Woodruff-Pak & J. E. Steinmetz (Eds.), *Eyeblink classical conditioning: Animal* (pp. 105–134). Amsterdam: Kluwer.
- Stanton, M. E., Freeman, J. H., Jr., & Skelton, R. W. (1992). Eyeblink conditioning in the developing rat. *Behavioral Neuroscience*, *106*, 657–665.
- Steinmetz, J. E., Lavond, D. G., & Thompson, R. F. (1985). Classical conditioning of the rabbit eyelid response with mossy fiber stimulation as the conditioned stimulus. *Bulletin of the Psychonomic Society*, *23*, 245–248.
- Steinmetz, J. E., Lavond, D. G., & Thompson, R. F. (1989). Classical conditioning in rabbits using pontine nucleus stimulation as a conditioned stimulus and inferior olive stimulation as an unconditioned stimulus. *Synapse*, *3*, 225–233.
- Steinmetz, J. E., Logan, C. G., Rosen, D. J., Thompson, J. K., Lavond, D. G., & Thompson, R. F. (1987). Initial localization of the acoustic conditioned stimulus projection system to the cerebellum essential for classical eyelid conditioning. *Proceedings of the National Academy of Sciences USA*, *84*, 3531–3535.
- Steriade, M., Parent, A., Pare, D., & Smith, Y. (1987). Cholinergic and non-cholinergic neurons of cat basal forebrain project to reticular and mediodorsal thalamic nuclei. *Brain Research*, *408*, 372–376.
- Stolar, N., Sparenborg, S., Donchin, E., & Gabriel, M. (1989). Conditional stimulus probability and activity of hippocampal, cingulate cortical, and limbic thalamic neurons during avoidance conditioning in rabbits. *Behavioral Neuroscience*, *103*, 919–934.
- Taylor, C. L., Freeman, J. H., Jr., Holt, W., & Gabriel, M. (1999). Impairment of cingulothalamic learning-related neuronal coding in rabbits exposed to cocaine *in utero*: General and sex-specific effects. *Behavioral Neuroscience*, *113*, 62–77.
- Thompson, R. F., & Krupa, D. J. (1994). Organization of memory traces in the mammalian brain. *Annual Review of Neuroscience*, *17*, 519–549.
- Vilensky, J. A., & van Hoesen, G. W. (1981). Corticopontine projections from the cingulate cortex in the rhesus monkey. *Brain Research*, *205*, 391–395.
- Wasserman, E. A., & Miller, R. R. (1997). What's elementary about associative learning? *Annual Review of Psychology*, *48*, 573–607.
- Woodruff-Pak, D. S., & Steinmetz, J. E. (2000). *Eyeblink classical conditioning: Animal*. Amsterdam: Kluwer.

Received June 25, 2001

Revision received September 4, 2001

Accepted September 21, 2001 ■