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Medial Auditory Thalamic Input to the Lateral Pontine Nuclei is Necessary for Auditory Eyeblick Conditioning*

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Abstract

Auditory and visual conditioned stimulus (CS) pathways for eyeblink conditioning were investigated with reversible inactivation of the medial (MPN) or lateral (LPN) pontine nuclei. In Experiment 1, Long-Evans rats were given three phases of eyeblink conditioning. Phase 1 consisted of three training sessions with electrical stimulation of the medial auditory thalamic nuclei (MATN) paired with a periorbital shock unconditioned stimulus (US). An additional session was given with a muscimol (0.5 μ L, 10 mM) or saline infusion targeting the LPN followed by a recovery session with no infusions. The same training and testing sequence was then repeated with either a tone or light CS in phases 2 and 3 (counterbalanced). Experiment 2 consisted of the same training as Experiment 1 except that muscimol or saline was infused in the MPN during the retention tests. Muscimol infusions targeting the LPN severely impaired retention of eyeblink conditioned responses (CRs) to the MATN stimulation and tone CSs but only partially reduced CR percentage to the light CS. Muscimol infusions that targeted the MPN had a larger effect on CR retention to the light CS relative to MATN stimulation or tone CSs. The results provide evidence that the auditory CS pathway necessary for delay eyeblink conditioning includes the MATN-LPN projection and the visual CS pathway includes the MPN.

Keywords

Cerebellum; Eyeblink Conditioning; Medial Auditory Thalamus; Savings; Associative Learning

1. Introduction

Pavlovian eyeblink conditioning has been used with great success to delineate the neural mechanisms underlying associative learning and memory (Christian and Thompson 2003; Thompson 2005). Eyeblink conditioning is typically established by pairing a tone or light conditioned stimulus (CS) with an unconditioned stimulus (US) that elicits the eyeblink reflex. An eyeblink conditioned response (CR) emerges during paired training that precedes the onset of the US. The intermediate cerebellum, specifically the interpositus nucleus and cerebellar cortex, has been implicated as the site of learning-related plasticity necessary for the eyeblink CR (Freeman et al. 2005a; Garcia and Mauk 1998; Jirenhed et al. 2007; Krupa et al. 1993; Krupa and Thompson 1997; McCormick et al. 1982; McCormick and Thompson 1984a,b;

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Nicholson and Freeman 2002). Convergence of CS and US information in the cerebellum is necessary for establishing this learning-related plasticity (Thompson 1976). The dorsal accessory inferior olive provides the cerebellum with US input through climbing fibers in the inferior cerebellar peduncle that synapse on neurons in the interpositus nucleus and Purkinje cells in the cortex (Brodal 1981; Ito 1984; Mauk et al. 1986; McCormick et al. 1985; Mintz et al. 1994; Sugihara et al. 2001). The pontine nuclei (PN) provide the necessary and sufficient mossy fiber input to the cerebellum for eyeblink conditioning with auditory, somatosensory, and visual CSs (Bao et al. 2000; Campolattaro and Freeman 2008; Freeman and Rabinak 2004; Freeman et al. 2005b; Hesslow et al. 1999; Knowlton and Thompson 1988; Lewis et al. 1987; Steinmetz et al. 1986; Steinmetz et al. 1987; Steinmetz and Sengelaub 1992; Tracy et al. 1998). The PN show learning-related changes in activity that depend on feedback from the cerebellar nuclei and red nucleus (Clark et al. 1997; Cartford et al. 1997). Thus, pontine-cerebellar interactions may influence eyeblink conditioning.

The neural pathways for different modality CSs may be segregated within the PN. Bilateral electrolytic lesions of the lateral and dorsolateral pontine nuclei (LPN) impair CR retention to a tone CS, but have no effect on conditioning to a light CS in rabbits (Steinmetz et al. 1987). Click-evoked field potentials were also stronger in the lateral and dorsolateral PN relative to the medial pontine nuclei (MPN; Steinmetz et al. 1987). The findings of the Steinmetz et al. study suggest that CS inputs to the cerebellum are anatomically segregated at the level of the PN in rabbits. However, tone evoked neuronal activity is evident throughout the basilar pons in rats (Freeman and Muckler 2003). Axonal projections from auditory and visual areas also show a high degree of overlap in the rat PN (Campolattaro et al. 2007; Graybiel 1974). It is therefore possible that the auditory and visual pathways within the PN are not segregated in rats.

Stimulation of auditory structures can support eyeblink CRs, and many of these structures have a direct unilateral projection to the PN that may be important for eyeblink conditioning. The auditory cortex, cochlear nucleus (CN), inferior colliculus (IC), and medial auditory thalamic nuclei (MATN) all have direct projections to the PN (Campolattaro et al. 2007; Kawamura 1975; Knowlton et al. 1993; Steinmetz et al. 1987). Electrical stimulation of these auditory structures can be used as a CS to support acquisition of eyeblink conditioning (Campolattaro et al. 2007; Knowlton and Thompson 1992; Nowak et al. 1999; Patterson 1971). The direct PN projections from the auditory cortex are not necessary as decortication does not block CR acquisition or retention (Oakley and Russell 1972; Oakley and Russell 1977). In contrast, lesions of the MATN contralateral to the conditioned eye in rats completely block acquisition and retention of auditory eyeblink CRs, but do not prevent acquisition to a light CS, indicating that the MATN projection to the PN may be critical for eyeblink conditioning with an auditory CS (Halverson and Freeman 2006; Halverson et al. 2008). The current study was designed to determine whether the MATN-PN projection is necessary for auditory eyeblink conditioning in rats. Anatomical segregation of auditory and visual CS pathways within the PN was also examined.

2. Experiment 1

Experiment 1 was designed to determine whether the MATN projection to the LPN is necessary for auditory eyeblink conditioning in rats. Electrical stimulation of the MATN was used as a CS (Campolattaro et al. 2007) in the first phase of training. After three sessions of acquisition training with the stimulation CS, muscimol or saline was infused into the LPN during a retention test, followed by a recovery session with no infusions. The rats were then given the same sequence of training and testing with tone and light CSs (order counterbalanced). Additional training with tone and light CSs was used to assess the selectivity of LPN

inactivation and to determine whether MATN stimulation and auditory stimuli are projected to the cerebellum through the same region of the PN.

2.1. Materials and methods

2.1.1. Subjects—The subjects were 23 male Long-Evans rats (250–400 g). The rats were housed in the animal colony in Spence Laboratories of Psychology at the University of Iowa (Iowa City, IA). All rats were maintained on a 12 h light/dark cycle and given *ad libitum* access to food and water.

2.1.2. Surgery—One week before training, rats were removed from their home cages and anesthetized with isoflurane. After the onset of anesthesia, the rats were fitted with differential electromyograph (EMG) electrodes (stainless steel) implanted into the upper left orbicularis oculi muscle. The reference electrode was a silver wire attached to a stainless steel skull screw. The EMG electrode leads terminated in gold pins in a plastic connector. A bipolar stimulating electrode (Plastics One, Roanoke, VA) for delivering the shock US was implanted subdermally, caudal to the left eye. A 23 gauge guide cannula was implanted 2.0 mm dorsal to the right LPN. A 30 gauge stylet was inserted into the guide cannula and extended 1.0 mm from the end of the guide. A bipolar stimulating electrode was implanted into the right medial division of the medial geniculate (MGm). The stereotaxic coordinates for the LPN cannula, taken from bregma, were 7.4 mm posterior, 1.3 mm lateral, and 9.2 mm ventral to the skull surface for the LPN. The stereotaxic coordinates for the MGm electrode, taken from bregma, were 5.5 mm posterior, 3.1 mm lateral, and 6.3 mm ventral to the skull surface. The plastic connector housing the EMG electrode leads, bipolar stimulating electrodes, the guide cannula, and skull screws were secured to the skull with bone cement (Zimmer, Warsaw, IN). The rats were maintained on 0.006% Sulfatrim (Hi-Tech Pharmacal Co., Amityville, NY) in water for 4 d after surgery.

2.1.3. Muscimol infusion procedure—Before the muscimol infusions, the stylet was removed from the guide cannula and replaced with a 30 gauge infusion cannula that extended 2.0 mm beyond the guide cannula. The infusion cannula was connected to polyethylene tubing (PE 10; 110–120 cm), which was connected to a 10 μ l gas tight syringe (Hamilton, Reno, NV). The syringe was placed in an infusion pump (Harvard Apparatus, Holliston, MA), and 0.5 μ l of muscimol (10 mM, pH = 7.4) or saline was infused over 2 minutes at a rate of 15 μ l/h. After the infusion, the tubing connected to the infusion cannula was sealed. The infusion cannula remained in place for the duration of the session and was replaced by the stylet after each session.

2.1.4. Apparatus—The apparatus has been described in detail in previous publications (Halverson & Freeman, 2006; Halverson et al. 2008). Briefly, the conditioning apparatus consisted of two 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. The stimulation CS used in training was a 200 Hz train of biphasic pulses (50–150 μ A). The stimulation intensity for each rat was set before training by increasing the test current until a behavioral response was observed. The current was then turned down in 5 μ A increments until there was no observable behavioral response (Campolattaro et al. 2007). Typical behavioral responses observed from the test stimulation included head turns, orienting responses, and ear movements. The tone CS used in training was a 2000 Hz pure tone (85 dB; range in conditioning chamber, 83–87 dB). A 4W light mounted on the back wall of the sound-attenuating chamber provided a whole field white light illumination CS. Computer software controlled the delivery of stimuli and eyelid EMG recording (JSA Designs, Raleigh, NC). The intensity of the shock US was set to two times the threshold for eliciting a discrete eyeblink (range = 2–4 mA).

2.1.5. Conditioning procedure—The rats were allowed to adapt to the context for 5 min before each training session. All rats were initially given three sessions of paired delay eyeblink conditioning with the MATN stimulation CS and US. The rats were then given saline ($n = 7$) or muscimol ($n = 16$) infusions into the LPN 40 min before a retention test with the MATN stimulation CS followed by a recovery session with no infusions. The 40 min interval between infusion and the retention test was used to maximize the effectiveness of muscimol during the entire 1-hour session (Martin 1991). Rats were then given the same sequence of training with tone and light CSs, with the order of stimulus type counterbalanced. Each training session consisted of 100 trials per day, with a pseudorandom distribution of intertrial intervals between 18 and 42 s that averaged 30 s. The 300 ms CS coterminated with a 25 ms shock US, yielding an interstimulus interval of 275 ms. Daily training sessions consisted of 10 blocks of 9 paired CS-US presentations and 1 CS alone probe trial. The values relayed to the computer software from the EMG integrator were voltage values of integrated EMG activity. The CR threshold was set to 0.4 V above the amplified and integrated EMG activity during the pre-CS baseline period. The EMG baseline was zero except for an offset. The 0.4 V threshold was approximately 10% of the peak amplitude of the average CR (4.2 V) in well trained rats. No adjustments of the EMG activity were made for individual rats to equalize the magnitude of the signal relative to the threshold. Integrated EMG responses that exceeded the threshold during the first 80 ms of the CS period were defined as startle responses to the CS; responses that exceeded the threshold during the last 195 ms of the CS before the US were defined as CRs; responses that exceeded the threshold after US onset were defined as URs.

2.1.6. Histology—After training, the rats were euthanized with a lethal injection of sodium pentobarbital (150 mg/kg) and transcardially perfused with ~100 mL of physiological saline followed by ~300 mL of 10% buffered formalin. After perfusion, the brains were postfixed in the same fixative for a minimum of 24 h, cryoprotected in a 30% sucrose in formalin solution, and subsequently sectioned at 50 μ m with a sliding microtome. Sections were then stained with thionin. The location of the cannula and electrode placements was verified using a light microscope (Leica DMLS, Wetzlar, Germany) and a stereotaxic brain atlas (Paxinos and Watson, 1998).

2.2. Results

2.2.1. Cannula and electrode placements—Electrode placements in the MATN were also verified by examining a series of coronal sections. All electrode placements were in either the medial division of the medial geniculate nucleus (MGm) ($n = 18$) or supragenicular (SG) ($n = 5$). No differences in CR acquisition rate were observed between rats with electrode placements in MGm or SG. Figure 1A is an image of a representative electrode placement in the MATN.

Cannula placements in the PN were verified by examining a series of coronal sections. Placements were in the right LPN ($n = 16$), dorsal to the LPN ($n = 4$), or medial to the LPN ($n = 3$). Dorsal and medial cannula placements in the LPN were within 0.2 mm of the target. Figure 1B is an image of a representative cannula placement in the LPN and Figure 1C shows reconstructions of all of the cannula placements.

2.2.2. Eyeblink conditioning in Experiment 1—Rats received two training sequences to counterbalance the presentation order of stimuli during phases 2 and 3, after receiving stimulation of the auditory thalamus in phase 1. One group of rats ($n = 15$) received MATN stimulation in phase 1, followed by a tone CS in phase 2, and a light CS in phase 3 (STL). Another group ($n = 8$) received the light CS in phase 2, and tone CS in phase 3 (SLT). CR percentage did not differ significantly during any session between the rats receiving STL or SLT training (Fig. 2). As reported previously, acquisition with thalamic stimulation was very

rapid relative to conditioning with a tone or light CS (Campolattaro et al. 2007). Rats typically show 20% CRs during the first training session with a tone or light CS (Campolattaro and Freeman 2009), whereas MATN stimulation produced approximately 40% CRs on the first session. Savings was observed on the first session of phase 2 for the tone and light CSs relative to initial acquisition with peripheral CSs (Campolattaro and Freeman 2009). An increase in the amount of savings was observed for both tone and light CSs during session 1 of phase 3 relative to performance on the first session of phases 1 and 2.

Acquisition data for rats with LPN cannula that received the two training sequences (STL and SLT) was examined with a repeated measures ANOVA for the three phases (sessions 1–3, 6–8, and 11–13) (Fig. 2) for both sequences of training (STL and SLT), which yielded an interaction of the phase and session factors $F(4,128) = 9.15, p < 0.001$. Post-hoc tests (Tukey HSD) indicated that all rats showed more CRs on the first session of phase 3 (session 11) than on the first session of phases 1 and 2 (sessions 1 and 6), $p < 0.05$. All rats showed more CRs on the final 2 sessions of each phase than on the first session, $p < 0.05$. The order of training did not produce any group differences in CRs. Data from each CS were then pooled across sequences for analysis during the different muscimol retention tests.

All rats given muscimol infusions into the LPN walked slowly counter-clockwise in a circle within the home cage and training apparatus. No other motor abnormalities were observed. A repeated measures ANOVA on the CR percentage data for the muscimol retention tests, the sessions prior to the muscimol tests, and the sessions after the muscimol tests (sessions 3–5, 8–10, and 13–15) during each CS revealed an interaction of the stimulus, session, and group factors $F(4,84) = 3.75, p < 0.007$ (Fig. 3). Post-hoc tests indicated that rats receiving muscimol showed fewer CRs during each CS relative to rats receiving saline on the infusion sessions (sessions 4, 9, and 14), $p < 0.05$. Rats receiving muscimol in LPN also showed fewer CRs on sessions with infusions than the final acquisition session (sessions 3, 8, and 13) and the recovery session (sessions 5, 10, and 15) for each CS, $p < 0.05$ (Fig. 4). However, muscimol infusions into the LPN significantly reduced CR retention to MATN stimulation and tone CSs relative to the light CS, $p < 0.05$.

3. Experiment 2

Experiment 2 examined whether inactivation of the MPN would produce differential effects on retention of CRs elicited by MATN stimulation, tone, or light CSs. Electrolytic lesions of the middle cerebellar peduncle, which is the primary pontine mossy fiber input into the cerebellum, block retention of light evoked CRs, indicating the pontine nuclei relay visual CS information into the cerebellum for eyeblink conditioning as well as tone CS information (Lewis et al. 1987). Neural tracing studies indicate convergent projections from many visual areas including visual cortex, superior colliculus and the ventral division of the lateral geniculate within the MPN (Graybiel 1974; Redgrave et al. 1987; Wells et al. 1989). Combined bilateral lesions of the visual cortex, superior colliculus, and pretectal nuclei block acquisition of eyeblink CRs to a light CS in rabbits suggesting that the MPN may be part of the visual CS pathway (Koutalidis et al. 1988).

The design of Experiment 2 was the same as Experiment 1 except that muscimol was infused into the MPN rather than the LPN.

3.1. Materials and methods

The conditioning apparatus, surgical procedure, conditioning procedure, muscimol infusion procedure, and histological methods used in Experiment 2 were the same as Experiment 1 except where indicated below.

3.1.1. Surgery—Rats received the same surgery as in Experiment 1 except the cannula was implanted into the MPN. Stereotaxic coordinates taken from bregma for the cannula were 7.4 mm posterior, 0.6 mm lateral, and 9.2 mm ventral to the skull surface.

3.1.2. Conditioning procedure—Rats were given training with the MATN stimulation CS initially, followed by training with a light and then a tone CS. The rats were then given saline ($n = 6$) or muscimol ($n = 5$) infusions into the medial pontine nucleus 40 min before the retention test for each CS followed by a recovery session with no infusions.

3.2. Results

3.2.1. Cannula and electrode placements—Cannula placements in the pontine nuclei were verified by examining coronal sections. Placements were in the right MPN ($n = 9$) or dorsal to the MPN ($n = 2$). Dorsal cannula placements to the MPN were within 0.2 mm of the target. Figure 5A shows an image of a representative cannula placement in the MPN and Figure 5B shows reconstructions of all of the cannula placements.

Electrode placements in the MATN were also verified by examining coronal sections. All electrode placements were in either the medial division of the medial geniculate nucleus (MGm) ($n = 9$) or supragenulate (SG) ($n = 2$). Similar to Experiment 1, no differences in CR acquisition rate were observed between rats with electrode placements in MGm or SG.

3.2.2. Eyblink conditioning in Experiment 2—Repeated measures ANOVA on the CR percentage data for the three acquisition phases (sessions 1–3, 6–8, and 11–13) for the muscimol and saline groups revealed a main effect of the session factor $F(2,18) = 112.84, p < 0.0001$. Post-hoc tests indicated that rats in both groups showed more CRs on the first session of phase 3 than the first session of phase 2, $p < 0.05$. This indicates savings on session 1 during phase 3 relative to the phase 2. A separate repeated measures ANOVA on the CR percentage for the three retention tests (sessions 3–5, 8–10, and 13–15) revealed an interaction of the stimulus, session, and group factors $F(4,36) = 8.98, p < 0.0001$ (Fig. 6). Post-hoc tests indicated different retention effects for the three stimuli. Retention of CRs to MATN stimulation was less impaired than retention to the tone CS, and retention to both MATN stimulation and tone CSs were less impaired than retention to the light CS, $p < 0.05$. Muscimol infusions into the MPN, therefore, had a larger effect on CR retention to a visual CS than to an auditory stimulus.

4. Retention Tests

4.1. Combined retention test analyses

Additional analyses were run to determine possible differences in CR retention between rats receiving muscimol or saline infusions into the LPN or MPN in Experiments 1 and 2. Repeated measures ANOVA on the CR percentage data for the muscimol retention tests, the sessions prior to the muscimol tests, and the sessions after the muscimol tests (sessions 3–5, 8–10, and 13–15) (Fig. 3, Fig. 6) for the LPN, MPN, and saline groups revealed an interaction of the stimulus, session, and group factors $F(8,124) = 12.78, p < 0.001$. Post-hoc tests indicated that rats with cannula in the LPN showed fewer CRs than rats with cannula in MPN and rats receiving saline infusions during the MATN stimulation retention test, $p < 0.05$. During the tone retention test, rats with cannula in the MPN showed fewer CRs than rats in the saline groups, but rats with LPN cannula showed fewer CRs than both MPN and saline groups, $p < 0.05$. During the light retention test, rats with cannula in LPN showed fewer CRs than rats in the saline groups, but rats with MPN cannula showed fewer CRs than both LPN and saline groups, $p < 0.05$.

5. Discussion

Acquisition of delay eyeblink conditioning with unilateral MATN stimulation as the CS was rapid, as previously reported (Campolattaro et al. 2007). Savings was observed when the CS was switched from MATN stimulation to a peripheral CS relative to *de novo* acquisition with a peripheral CS (Campolattaro and Freeman 2009). Retention of eyeblink conditioning with MATN stimulation, tone, or light CSs was severely impaired by muscimol inactivation of the PN contralateral to the conditioned eye. There were differences in CR retention between rats that received muscimol infusions into the LPN or MPN. Muscimol infusions into the LPN (Experiment 1) had a larger effect on CR retention with MATN stimulation and tone CSs than retention with the light CS. In contrast, MPN inactivation (Experiment 2) had a larger effect on CR retention with the light CS relative to retention with the MATN stimulation or tone CSs.

The results suggest that the ipsilateral MATN projection to the LPN (Campolattaro et al. 2007) may be necessary for conveying auditory CS information to the cerebellum during delay eyeblink conditioning in rats. Evoked auditory field potentials in the PN show strong responses in the lateral and dorsolateral nuclei and weak responses in the medial nuclei in rabbits (Steinmetz et al. 1987). Moreover, lesions or inactivation of the rabbit LPN block CR retention to a tone CS (Bao et al. 2000; Steinmetz et al. 1987). The field potential and lesion data suggest that the LPN conveys necessary auditory stimulation to the cerebellum during eyeblink conditioning in rabbits. The current results suggest that auditory CS information is also projected to the LPN in rats. Moreover, a primary source of auditory input to the LPN during delay eyeblink conditioning is the MATN. The roles of the MATN and thalamopontine projection in rabbit eyeblink conditioning have not been examined.

As in previous studies with rabbits, inactivation of the PN in rats impairs CRs with auditory and visual CSs (Bao et al. 2000; Knowlton and Thompson 1988). Muscimol infusions in Experiment 1, targeting the LPN, had a larger effect on CR retention to MATN stimulation and tone CSs relative to the light CS. In contrast, muscimol infusions into the MPN in Experiment 2 had a larger effect on retention to the light CS relative to the MATN stimulation and tone CSs. However, partial impairments were observed with the visual CS following LPN inactivation and with the auditory CS following MPN inactivation. Muscimol may have spread beyond the targets and could have partially inactivated inputs from other sensory modalities, which might account for the partial impairments. An alternative interpretation is that auditory and visual pathways are not completely segregated. Indeed, axonal projections from auditory and visual areas project to both the LPN and MPN (Campolattaro et al. 2007; Graybiel, 1974; Wells et al. 1989). The anatomical data, therefore, indicate that there is very little segregation of auditory and visual inputs to the PN. Why then is partial functional segregation observed following muscimol infusions or lesions in the LPN or MPN? There may be differences in the efficacy or density of synaptic inputs from different modalities within the LPN and MPN. Another possibility is that there are modality-specific differences in the efficacy of mossy fibers synapses from the LPN and MPN to the cerebellum.

Rats in the current study did not show immediate generalization between the MATN stimulation CS and the tone or light CSs but showed substantial savings. Failure to observe immediate generalization from a stimulation CS to a peripheral CS is not surprising because immediate Wells generalization is not commonly observed during studies in which animals are transferred from one peripheral CS to another, from one stimulation CS to another, or from a stimulation CS to a peripheral CS (Campolattaro & Freeman 2009; Campolattaro, Schnitker, & Freeman, 2008; Garcia et al. 2003; Kehoe & Holt 1984; Kehoe et al. 1984; Ohyama et al. 2003; Schreurs & Kehoe 1987; Steinmetz et al. 1986; Steinmetz 1990). The majority of animals in these studies showed savings rather than immediate generalization after being switched from one CS to another. Thus, unless the thalamic stimulation provides a close approximation of

the transfer tone CS at the correct frequency, there should be little immediate generalization to a tone CS, as seen in the current study.

The results of the current experiments replicate a previous study which showed that stimulation of the MATN contralateral to the trained eye is a sufficient CS to support eyeblink conditioning (Campolattaro et al. 2007). The current experiments also provide evidence that auditory information from the MATN to the cerebellum is projected primarily through the LPN. It is likely that CS inputs into the PN are not strictly segregated anatomically or functionally in rats. Differences between sensory pathways in the PN may be more quantitative than qualitative, with most of the necessary auditory input to the LPN and most of the necessary visual input to the MPN. Smaller muscimol infusions or discrete lesions in the PN are needed to determine conclusively whether the incomplete segregation of CS modalities observed in the current study was due to partially distributed CS inputs within the PN or to the spread of muscimol beyond the LPN and MPN. Indeed, it is likely that muscimol spread to nuclei dorsal to targets as seen in previous studies (Campolattaro and Freeman 2009), which probably included the ventral nucleus of the lateral lemniscus for LPN infusions and the reticulotegmental pontine nucleus for MPN infusions. Muscimol infusions into the MPN probably spread to the contralateral MPN as well. Thus, the differential effects of MPN versus LPN muscimol infusions in the current study could be related to differences in inactivation of extra-pontine structures or to the extent of bilateral inactivation.

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Figure 1.

(**A**) Coronal sections of the PN showing representative cannula placement in the lateral pontine nuclei (LPN). MPN: medial pontine nuclei, CT: cannula tip. Magnification is 2.5x. (**B**) Coronal section of the medial auditory thalamic nuclei showing a representative electrode placement in the medial division of the medial geniculate (MGm). APT, anterior pretectal nucleus; MGd, dorsal division of the medial geniculate; MGv, ventral divisions of the medial geniculate; PIN, posterior intralaminar nucleus; SG, supragenulate; SN, substantia nigra. ET: electrode tip. Magnification is 2.5x. (**C**) Drawings of coronal sections depicting cannula placements in the LPN for the muscimol (black) and saline (gray) groups. Numbers represent distance from bregma. (Drawings adapted from Paxinos and Watson 1998).

Figure 2.

Mean $\pm$ SE conditioned response (CR) percentage for rats given training with a medial auditory thalamic stimulation conditioned stimulus (CS) (phase1) followed by a tone CS (phase 2) and then a light CS (phase 3)(STL), or followed by a light CS and then a tone CS (SLT) in Experiment 1. Rats in both groups showed similar acquisition rates during phases 2 and 3 indicating that there were no differences between the two training sequences. Data for the muscimol infusion sessions (sessions 4, 9, and 14) are presented in Figure 3 and Figure 6.

Figure 3.

Mean $\pm$ SE conditioned response (CR) percentage for rats given training with a medial auditory thalamic stimulation conditioned stimulus (CS)(sessions 1–5), a tone CS (sessions 6–10), and a light CS (sessions 11–15) in Experiment 1. Rats received muscimol (black arrows) or saline infusions into the lateral pontine nuclei on sessions 4, 9, and 14.

Figure 4.

Orbicularis oculi EMG activity recorded from a rat on CS-alone test trials during the conditioning sessions before (Session 3), during (Session 4), and after (Session 5) pontine inactivation. Dashed lines indicate the onset and offset times of the 300 ms CS.

Figure 5.

(A) Coronal section of the PN showing a representative cannula placement in the medial pontine nucleus (MPN). LPN: lateral pontine nuclei, CT: cannula tip. Magnification is 2.5x. (B) Drawings of coronal sections depicting cannula placements in the LPN for the muscimol (black) and saline (gray) groups. Numbers represent distance from bregma. (Drawings adapted from Paxinos and Watson 1998).

Figure 6.

Mean $\pm$ SE conditioned response (CR) percentage for rats given training with a medial auditory thalamic stimulation conditioned stimulus (CS)(sessions 1–5), a light CS (sessions 6–10), and a tone CS (sessions 11–15) in Experiment 2. Rats received muscimol (black arrows) or saline infusions into the MPN on sessions 4, 9, and 14.