

Research report

Destruction of the inferior colliculus disrupts the production and inhibition of fear conditioned to an acoustic stimulus

Scott A. Heldt^{a,*}, William A. Falls^b

^a *Department of Psychology, Northern Illinois University, DeKalb, IL, USA*

^b *Department of Psychology, University of Vermont, Burlington, VT, USA*

Received 28 October 2002; received in revised form 26 February 2003; accepted 26 February 2003

Abstract

The inferior colliculus (IC) is the major source of auditory information involved in processing the behavioral significance of acoustic stimuli. In the current study, we assessed whether the IC is a critical source of information which mediates the expression of fear and the inhibition of fear conditioned to an auditory stimulus. Fear and the inhibition of fear were tested by measuring fear-potentiated startle. In Experiment 1, we demonstrated that rats which received electrolytic lesions of the IC failed to show fear-potentiated startle in the presence of a noise previously conditioned to elicit fear. In Experiment 2, we demonstrated that rats with similarly placed lesions of the IC failed to inhibit fear-potentiated startle in the presence of a noise previously conditioned to inhibit fear to a light. Thus, in both Experiments 1 and 2, lesions of the IC disrupted the behavioral significance of the noise stimulus. Together with previous findings, these results are consistent with the view that the IC is a common source of diverging auditory information used to mediate the fear eliciting and safety signal properties conditioned to auditory stimuli.

© 2003 Elsevier B.V. All rights reserved.

Keywords: Fear; Anxiety; Startle; Conditioned inhibition; Inferior colliculus; Feature-negative discrimination

1. Introduction

In recent years, considerable progress has been made in understanding the neurobiology of classical fear conditioning. Much of this knowledge comes from studies using simple Pavlovian conditioning procedures in which an auditory conditioned stimulus (CS) is repeatedly paired with an aversive unconditioned stimulus (US), such as a shock. By virtue of such pairing, the auditory stimulus acquires “fear-eliciting” properties which signal danger and elicits conditioned responses (CRs) that are indicative of a state of fear.

Auditory stimuli can also acquire the ability to inhibit fear by signaling the nonoccurrence of shock. For example, if rats are given training in which a light CS is repeatedly paired with a shock and a compound of a noise and the light is presented in the absence of shock (light+/noise&light–), the noise acquires the ability to inhibit the fear produced by the light CS, evident by the fact that the magnitude of fear in

the presence of the light is reduced when it is accompanied by the noise [18].

Considerable evidence indicates that the fear-eliciting properties of an auditory CS are mediated through the auditory thalamus [7,41,59]. Recently, we examined whether these same projections were also involved in the transmission of the safety properties of an auditory stimulus conditioned to inhibit fear [27]. In this experiment, rats were given bilateral lesions of the auditory thalamus followed by light+/noise&light– training. Our results showed that lesions of the auditory thalamus did not disrupt the ability of the noise to inhibit the expression of fear to the light CS. These same lesions did, however, disrupt conditioned fear when the noise was subsequently paired with shock. These results indicate that although auditory information deriving from the auditory thalamus may be important for conditioned fear to an auditory CS, it is not critical for the inhibition of fear that is conditioned to an auditory stimulus.

The fact that lesions of the auditory thalamus did not disrupt the safety properties of a noise inhibitor suggests that this information may be transmitted through auditory pathways that diverge below the level of the thalamus. One likely source of this information is the inferior colliculus (IC).

* Corresponding author. Present address: Emory University, Yerkes Primate Research Center, Atlanta, GA 30329, USA. Tel.: +1-404-727-8991; fax: +1-404-727-8070

The IC receives ascending auditory input from a number of brainstem nuclei and is critically involved in processing the behavioral significance of acoustic stimuli [15]. In turn, the IC sends efferent projections to the medial geniculate body and adjacent thalamic nuclei of the auditory thalamus [2,5,63]. Anatomical and behavioral data indicate that the fear-eliciting properties of an auditory CS are transmitted to the auditory thalamus by way of these IC efferent projections [37,39,40,42]. However, in addition to these primary projections, the IC also sends parallel ascending and descending projections to structures outside the auditory thalamus [29,64] which may be involved in the emotional and/or behavioral responses associated with the inhibition of fear [27]. Thus, in addition to mediating the fear-eliciting properties of an auditory CS, the IC may be a source of information which mediates the safety properties conditioned to auditory stimuli.

To examine this possibility, we first evaluated the effects of IC lesions on the expression of fear conditioned to an auditory stimulus. Next, we examined whether lesions of the IC would disrupt the inhibition of fear conditioned to the same auditory stimulus. Fear and the inhibition of fear was tested by measuring fear-potentiated startle, a paradigm for measuring conditioned fear and the inhibition of fear [10,19]. We presumed that lesions of the IC would disrupt auditory conditioned fear, as has been previously reported. If IC lesions also disrupt the inhibition of fear, it would provide evidence that the IC is also the source of auditory information that signals safety.

2. General methods

2.1. Subjects

The subjects were male Sprague–Dawley rats, approximately 60 days of age, bred in the Northern Illinois University Psychology rat colony from Charles-River derived parents (Harlan SD). Rats were individually housed in hanging wire cages (18 cm × 18 cm × 25 cm) with food and water available *ad libitum*. They were maintained on a 12 h/12 h light/dark schedule (lights on at 07:00 h), and behavioral procedures occurred during the light period. The present study complied with American Psychological Association ethical standards in the treatment of the rats and was approved by the Northern Illinois University Institutional Animal Care and Use Committee.

2.2. Apparatus

Rats were trained and tested in four identical stabilimeter devices that have been described previously [16]. Briefly, each stabilimeter consisted of an 8 cm × 15 cm × 15 cm Plexiglas and wire-mesh cage suspended between the compression springs within a Plexiglas and wood frame. The floor of each stabilimeter consisted of four 4.0-mm diameter stain-

less steel bars spaced 2 cm apart through which shock could be delivered. Each stabilimeter was housed in a ventilated, sound-attenuating chamber (Industrial Acoustics Co., model #105278, Bronx, NY). Ventilation fans produced a 55 dB (SPL) background noise. Cage movements resulted in displacement of an accelerometer (ICP Accelerometer, model #321A, PCB Piezotronics, Depew, NY) where the resultant voltage was proportional to the velocity of the cage displacement. Startle amplitude was defined as the peak-to-peak accelerometer voltage that occurred during a 200-ms period after the onset of the startle stimulus. The accelerometer output was amplified (Fintronics Accelerometer Amplifier, #FA-560, Orange, CT) and digitized (MacADIOS II Board, GW Instruments, Somerville, MA) on a 0–4096 unit scale. Data acquisitions were controlled by a Macintosh Power PC 7100/66 using SuperScope II software (GW Instruments).

2.3. Stimuli

The auditory stimulus used in both Experiments 1 and 2 was a band-passed filtered noise (Krohn-Hite, Model 3100A, Avon, MA) with both high and low passes set at 4 kHz. This noise was delivered through an 8 in full-range speaker (Radio Shack, #40-1286C) located 10 cm to the left of the stabilimeter at an intensity of 70 dB SPL. The same noise was conditioned to either elicit fear (Experiment 1) or inhibit fear (Experiment 2). The startle stimuli were 50 ms bursts of white noise with a 4-ms rise–fall time. The startle stimuli were provided by a high frequency speaker (Radio Shack Super Tweeter, Tandy Inc., Fort Worth, TX) located 5 cm from the rear of each stabilimeter. Three startle stimulus intensities were used: 95, 105, and 115 dB. Startle and auditory stimuli intensities were measured by a sound level meter (Radio Shack, #33-2055) directed inside of the stabilimeter. The light CS used in Experiment 2 was produced by an 8 W fluorescent light attached to the rear of each stabilimeter. The light CS was controlled by a specially designed control unit (Fintronics) that allowed near instantaneous rise time (100 μs) and control of light intensity (630 fL). The shock US was generated by four Lehigh Valley shock generators (model SGS-004) located outside the sound-attenuating chambers. Shock intensity was 0.6 mA. The presentation of all stimuli was controlled by the Macintosh Power PC 7100/66 using SuperScope II software.

2.4. Surgery and histology

Surgery was performed 1–2 days following auditory fear conditioning (Experiment 1) or feature-negative discrimination training (FND; Experiment 2). Before surgery, rats were anesthetized by intraperitoneal injections of ketamine–xylazine (ketamine: 70 mg/kg; xylazine: 10 mg/kg). Rats were then mounted in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA) and the scalp was shaved and treated with a topical antiseptic. The incisor bar was adjusted to achieve a flat skull position and the skin was

retracted. Small holes were drilled in the skull above the lesion site, and a Kopf Model NE-300 electrode insulated within 0.5 mm of the tip was lowered to the following coordinates from bregma: anteroposterior = -8.2 , mediolateral = ± 2.7 , dorsoventral = -5.5 ; anteroposterior = -8.8 , mediolateral = ± 2.5 , dorsoventral = -5.5 . Lesions were made by passing 1-mA anodal current for 12 s. For the sham-operated rats, the electrode was lowered to the same coordinates but no current was passed. After surgery, the incision was closed with stainless steel suture clips. Rats were placed on a heated pad until fully recovered from anesthesia, then returned to home cages. All rats were given 6–7 days for recovery from surgery before testing. During this time, their body weight, eating, and drinking were monitored daily.

Following behavioral testing, lesioned rats were sacrificed by ketamine–xylazine overdose and perfused intracardially with 0.9% saline followed by 10% buffered formalin. The brains were stored in a solution of 20% sucrose in buffered formalin for at least 24 h. Coronal sections (60 μm) were cut on a cryostat. Every third section was mounted on a glass microscope slide and stained with cresyl violet. Lesion locations were transcribed onto copies of atlas plates [54] to determine the extent of the lesions.

3. Experiment 1: effects of lesions of the IC on auditory fear-potentiated startle

The purpose of Experiment 1 was to examine whether lesions of the IC would disrupt the expression of fear to an auditory stimulus. To this end, 15 rats were given paired noise + shock training followed by either lesions of the IC or sham surgery (Paired-IC group, $n = 8$; Paired-Shams group, $n = 7$). Because auditory stimuli can have unconditioned effects on behavior that may confound the measurement of conditioned fear, we included separate groups of lesion and sham rats that were given explicitly unpaired noise and shocks in training (Unpaired-IC group, $n = 7$; Unpaired-Shams group, $n = 7$). Fear-potentiated startle was assessed in all groups following a 6-day recovery period.

3.1. Method

3.1.1. Behavioral procedure

3.1.1.1. Acclimation. For 2 consecutive days, each rat was placed in the stabilimeter for approximately 30 min during which no stimuli were presented. After the 30-min exposure, they were returned to their home cage.

3.1.1.2. Training. On the following 2 consecutive days, rats were given either paired or unpaired noise + shock training. During training, rats were placed in the stabilimeter and 5 min later presented with the first of 10 training trials at an average intertrial interval of 4 min (range 3.5–4.5 min). Rats in the paired training group were given 10 noise + shock

training trials consisting of the 4-s noise co-terminating with a 0.5-s, 0.6-mA foot shock. Rats in the unpaired training group were given the same number of noise and shock presentations but the stimuli were explicitly unpaired with a variable interstimulus interval (CS onset to shock onset) that averaged 2 min and ranged from 1.5 to 2.5 min.

3.1.1.3. Testing. Fear-potentiated startle was assessed 6 days after surgery. In this test, rats were placed in the stabilimeter and after 5 min were given 10 startle stimuli at each of three different startle stimulus intensities (95, 105, and 115 dB). These initial 30 stimuli served to minimize the contribution of very high startle responses that often occur to the first few startle stimuli. Immediately after these startle stimuli, rats were presented with 10 startle-alone trials, 10 noise + startle test trials, at each of the three startle stimulus intensities. On noise + startle test trials, the 50-ms startle stimulus was presented 3.5 s after the onset of the noise (i.e. the time where the shock US occurred during paired training). The startle-alone and noise + startle test trials were presented in an irregular, balanced order across test session. All startle stimuli were presented at 30-s interstimulus intervals.

3.1.1.4. Statistical analysis and data reduction. Mean startle amplitudes for the startle-alone test trials and noise + startle test trials were calculated for each rat by averaging the startle amplitude of each trial type across the three different startle intensities. Mean startle amplitudes were analyzed with a three-way mixed-model analysis of variance (ANOVA) with training (Paired, Unpaired) and lesion (Sham, IC) as the between-subject factors and trial type (startle-alone, noise + startle) as the within-subject factor. Subsequent analyses were done with lower order ANOVAs, pairwise *t*-tests, and Bonferroni *t*-tests where appropriate.

3.2. Results and discussion

3.2.1. Histological results

One rat in the Paired-IC group was excluded from analysis because of substantial unilateral sparing of the IC. Fig. 1 shows a serial reconstruction of the smallest and largest lesions. Rats from both Paired-IC and Unpaired-IC groups sustained comparable damage to the IC. In general, rats sustained extensive bilateral damage to the entire caudolateral aspects of the central nucleus of the IC (CIC) and external cortex of the IC (ECIC). Less reliable damage was seen in the dorsal cortex of the IC (DCIC). In some cases, the caudal aspect of the lesion included limited damage to the cerebellum. In other cases (approximately 30%), lesions extended ventrally into the lateral lemniscus and encroached upon the dorsal nucleus of the lateral lemniscus (DNLL) and cuneiform nucleus (CnF). At rostral levels, lesions spared the DCIC and medial aspects of the CIC in most rats. Minimal damage was seen in the zone of transition between the rostral ECIC and lateral tegmentum below the superior colliculus (SC). In this

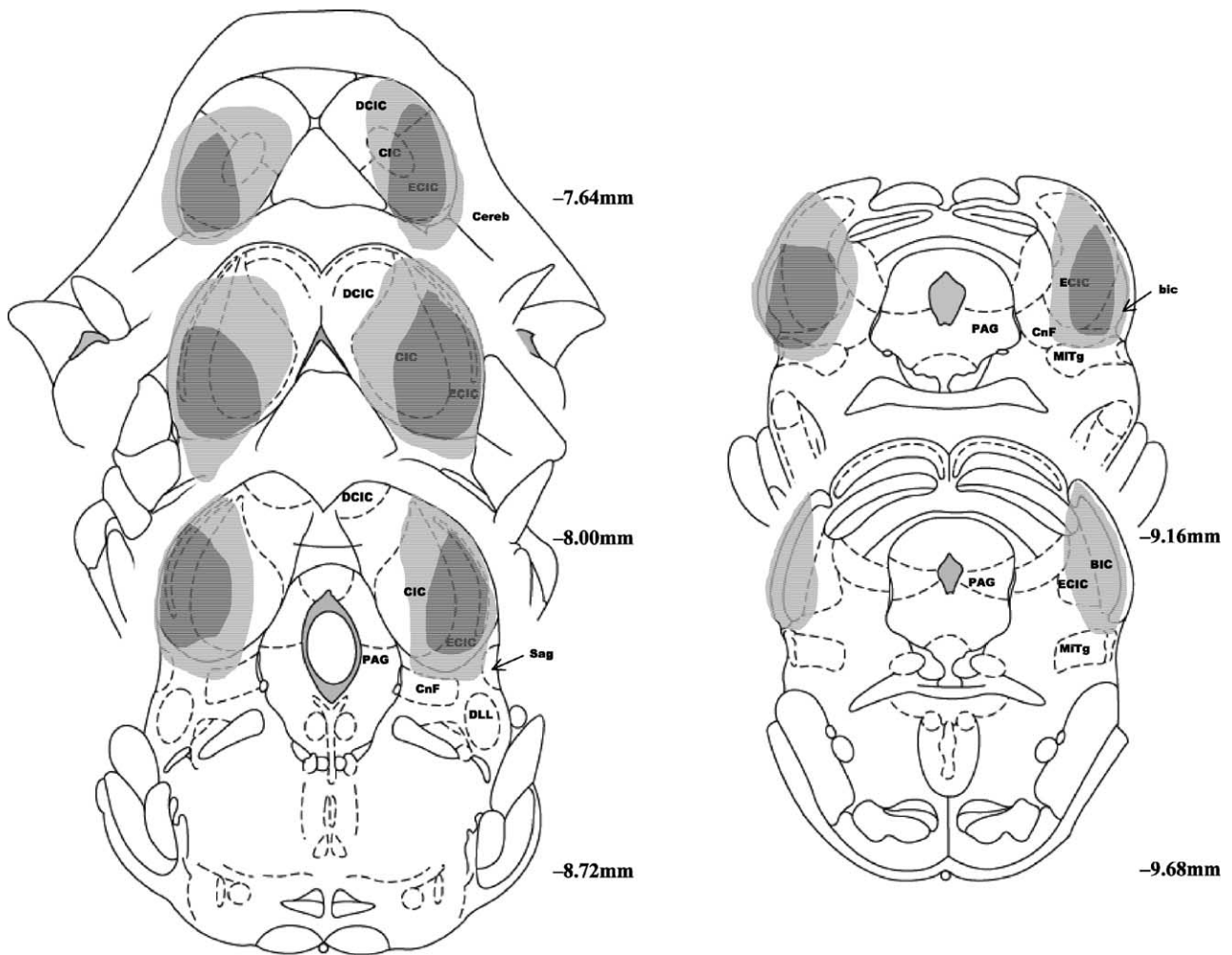


Fig. 1. Serial histological reconstruction of electrolytic lesions of the IC in rats included in Experiments 1 and 2. Reconstructions have been transcribed onto modified plates 48, 50, 52, 54, and 56 as adapted from Paxinos and Watson [54] and illustrate the largest (light grey) and smallest (dark grey) lesions included in statistical analyses. Numbers indicate rostrocaudal levels relative to bregma. Abbreviations: central nucleus of the IC, CIC; external cortex of the IC, ECIC; dorsal cortex of the IC, DCIC; dorsal nucleus of the lateral lemniscus, DNLL; cuneiform nucleus, CnF; nucleus of the brachium of the IC, NBIC; the brachium of the IC, bic.

transitional zone, damage was usually limited to lateral margins of the ECIC and included damage to the nucleus of the brachium of the IC (NBIC) and the brachium of the IC (bic).

3.2.2. Behavioral results

Fig. 2 shows the mean startle amplitude on the startle-alone and the noise + startle stimulus test trials and the mean difference scores between these two trial types for Paired-IC ($n = 7$), Paired-Sham ($n = 7$), Unpaired-IC ($n = 7$), and Unpaired-Sham ($n = 7$) groups. Lesions of the IC significantly impaired the expression of conditioned fear to the noise CS. Rats in the Paired-Sham group, which were given noise + shock training followed by sham lesions of the IC, showed significantly more fear-potentiated startle than rats in the Paired-IC group which were given the same training followed by lesions of the IC.

In support of these observations, the three-way ANOVA with training (Paired, Unpaired) and lesion (Sham, IC) as

the between-subject factors and trial type (startle-alone, noise + startle) as the within-subject factor yielded a reliable Training \times Lesion interaction, $F(1, 24) = 7.63$, $P = 0.011$, but no main effects of lesion, $F(1, 24) = 3.47$, $P = 0.08$, or training, $F(1, 24) = 0.84$, $P = 0.37$. All tests involving the within-subject factor of trial type were significant, $F_s(1, 24) > 5.50$, $P_s < 0.05$. Most important was the significant Training \times Lesion \times Trial type interaction, $F(1, 24) = 6.09$, $P < 0.05$.

To evaluate the three-way interaction, lower order lesion by trial type ANOVAs were conducted at each level of training. For rats in the Paired training groups, this lower order ANOVA revealed significant main effects of lesion, $F(1, 12) = 8.21$, $P < 0.05$, and trial type, $F(1, 12) = 42.99$, $P < 0.01$. More importantly, the Lesion \times Trial type interaction was significant, $F(1, 12) = 11.34$, $P < 0.01$, indicating that lesions of the IC significantly disrupted the expression of fear-potentiated startle to the noise CS. Both groups of

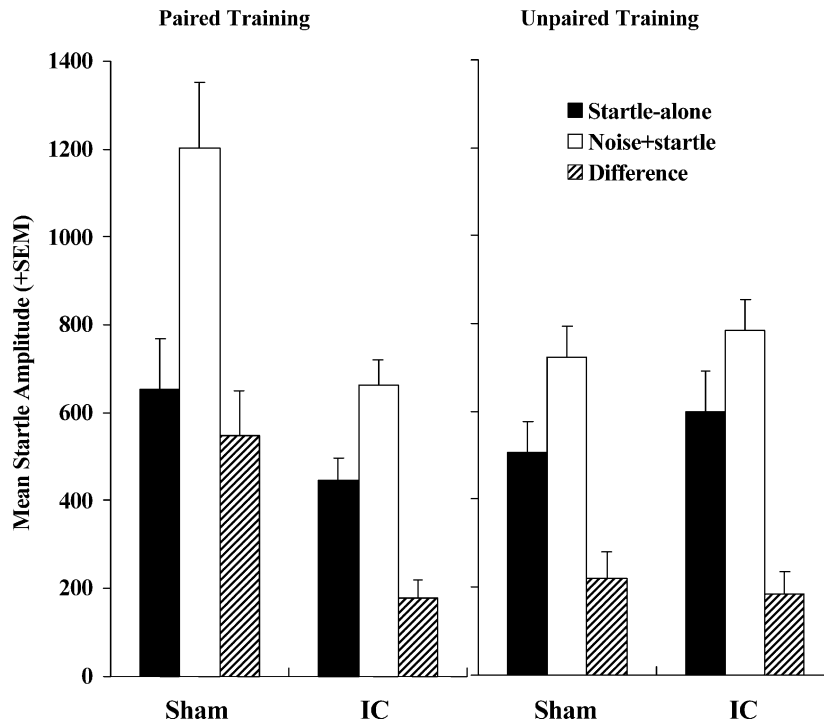


Fig. 2. Mean startle amplitudes on the startle-alone and the noise + startle stimulus test trials and the mean difference scores between these two trial types for groups in Experiment 1. Rats in the Paired groups received noise + shock training followed by either sham surgery (Paired-Sham, $n = 7$) or lesions of the IC (Paired-IC, $n = 7$). Rats in the Unpaired groups received explicitly unpaired noise and shocks in training followed by sham surgery (Unpaired-Sham, $n = 7$) or lesions of the IC (Unpaired-IC, $n = 7$). Error bars denote one standard error of the mean (+S.E.M.).

rats, however, displayed greater responding on noise + startle than on startle-alone trials as revealed by pairwise t -tests, (Paired-Sham, $t(6) = 5.41$, $P < 0.01$; Paired-IC groups, $t(6) = 3.99$, $P < 0.01$). Thus, although both Paired groups displayed some fear-potentiated startle, rats with lesions of the IC displayed significantly less than rats in the Paired-IC group.

For rats in the Unpaired training groups, the lower order ANOVA revealed a significant within-subject effect of trial type, $F(1, 12) = 24.46$, $P < 0.01$, but no main effect of lesion, $F(1, 12) = 0.58$, $P = 0.46$, or Lesion \times Trial type interaction, $F(1, 12) = 0.17$, $P = 0.69$. As seen in Fig. 2, animals in the Unpaired training groups demonstrated modest but reliably higher startle responses on noise + startle trials compared to startle-alone trials ($P_s < 0.02$). This finding is consistent with past research showing nonassociative facilitatory effects of noises on startle [6,17,24,30].

To examine whether groups differed in the magnitude of fear-potentiated startle, analyses were performed on mean startle difference scores. These difference scores were computed for each rat by subtracting the mean response obtained on startle stimulus alone test trials from the mean response on noise + startle stimulus test trials. A one-way ANOVA which compared the mean startle difference scores of all groups revealed significant differences in the magnitude of fear-potentiated startle among groups, $F(3, 24) = 6.80$, $P < 0.01$. Post hoc Bonferroni t -tests showed that magnitude of fear-potentiated startle in the Paired-Sham group was signif-

icantly higher than all other groups ($P_s < 0.01$). No differences were found among the Paired-IC, Unpaired-IC, and Unpaired-Sham groups, ($P_s > 0.05$). Thus, the greater responding on noise + startle trials displayed by the Paired-IC group was similar to the nonassociative enhancement displayed by the Unpaired groups. A one-way ANOVA revealed no group differences on startle-alone trials, $F(3, 24) = 1.21$, $P = 0.33$. Hence, our results were not confounded by difference in responding to the startle stimulus in the absence of the noise CS.

4. Experiment 2: effects of lesions of the IC on the inhibition of fear-potentiated startle by an auditory stimulus

In Experiment 1, we demonstrated that posttraining lesions of the IC disrupted the ability of a noise CS to elicit fear. In Experiment 2, we evaluated whether similar lesions would disrupt the safety properties of a noise conditioned to inhibit fear. To accomplish this, a group of 17 naïve male albino Sprague–Dawley rats was trained for FND in which the noise stimulus was conditioned to inhibit fear to a light CS [18]. To assess any nonassociative effects of the noise on the light CS, a separate group of 16 rats received contrasting control training which does not produce associative inhibition of fear. Twenty-four to 48 h after training, rats were given either lesions of the IC or sham surgery.

4.1. Method

4.1.1. Behavioral procedure

4.1.1.1. Acclimation. For 2 consecutive days, each rat was placed in the stabilimeter for approximately 30 min during which no stimuli were presented. After the 30-min exposure, they were returned to their home cage.

4.1.1.2. Feature-negative discrimination. FND training consisted of two phases and was started 1 day after acclimation. In Phase 1, rats were given pairings of the light and foot shock to establish the light as a CS. On each of 2 consecutive days, rats were placed in the stabilimeter, and after 5 min were presented with 10 pairings of a 4-s light that co-terminated with and a 0.6-mA, 0.5-s foot shock. The average intertrial interval was 4 min (range 3.5–4.5 min). Phase 2 began 1 day after the completion of Phase 1 and was designed to establish the noise as an inhibitor of fear. On each of 5 consecutive days, rats were placed in stabilimeter and 5 min later presented with five light + shock trials identical to those given in Phase 1, intermixed with 15 nonreinforced, serial noise&light compound trials. On noise&light compound trials, the offset of the 4-s noise coincided with the onset of the 4-s light CS. The two trial types were presented in pseudo-random order with an average intertrial interval of 2 min (range 1.5–2.5 min).

4.1.1.3. Contrasting control training. Training of the Control rats consisted of two phases and was started 1 day after acclimation. Phase 1 training was identical to Phase 1 FND training. In Phase 2, rats were presented, on each of 5 consecutive days, with five light + shock trials intermixed with 15 nonreinforced noise trials and 15 nonreinforced light trials (i.e. light + shock, noise – no shock, light – no shock).

4.1.1.4. Testing. FND of fear-potentiated startle was assessed 6 days after surgery. In this test, rats were placed in the stabilimeter and after 5 min received 10 startle stimuli at each of three different startle stimulus intensities (95, 105, and 115 dB). After these initial startle stimulus-alone trials, rats were presented with five additional startle stimulus-alone trials, five light test trials, and five noise&light test trials at each of the three startle stimulus intensities. On light test trials, the startle stimulus was presented 3.5 s after the onset of the light (i.e. the time where the foot shock would have occurred). On noise&light test trials, the startle stimulus was presented 3.5 s after the onset of the light. All trials were presented in a pseudo-random order with the constraint that each trial type occurred only once in each consecutive nine trial block. The intertrial interval, defined as the interval between startle stimuli, was 30 s.

4.1.1.5. Statistical analysis and data reduction. Mean startle amplitudes for the startle-alone trials, light test trials, and noise&light test trials were calculated for each rat

by averaging the startle amplitude of each trial type at the three different startle intensities. Two difference scores were then computed for each rat by subtracting the mean startle amplitude of the startle-alone trial from the mean startle amplitude of the light test trial (L-Diff) and noise&light test trial (NL-Diff). Each difference score reflected the magnitude of fear-potentiated startle in the presence of light or noise&light compound.

The L-Diff and NL-Diff difference scores were analyzed with a mixed-model ANOVA with training (FND, Control) and lesion (Sham, IC) as the between-subject factors and trial type (light, noise&light) as the within-subject factor. This analysis was followed up with lower order ANOVAs, pairwise *t*-tests, and Bonferroni *t*-tests where appropriate.

4.2. Results and discussion

4.2.1. Histological results

One rat in the Control-IC group was excluded from analysis because of unilateral sparing of the IC. In all other rats, damage to the IC was essentially identical to those of Experiment 1 (see Fig. 1). Rats from both FND-IC and Control-IC groups sustained comparable damage.

4.2.2. Behavioral results

Mean startle amplitudes on startle-alone test trials were analyzed separately to determine if there were any group differences in startle amplitude. A two-way ANOVA with training (FND, Control) and lesion (Sham, IC) as the between-subject factors showed no effect of training, $F(1, 28) = 0.65$, $P = 0.43$; lesion, $F(1, 28) = 0.05$, $P = 0.83$; or Training \times Lesion interaction, $F(1, 28) = 0.05$, $P = 0.47$. Thus, the interpretation of the difference scores was not confounded by group differences in startle response on the startle-alone trials. The mean startle amplitudes on startle-alone trials were 892 for FND-Sham, 968 for FND-IC, 882 for Control-Sham, and 744 for Control-IC groups.

Fig. 3 shows the mean difference scores on light and noise&light test trials and the difference scores between these two trial types scores for FND-Sham ($n = 8$), FND-IC ($n = 9$), Control-Sham ($n = 8$), and Control-IC ($n = 7$) groups. Within the FND groups, rats in the Sham group displayed greater fear-potentiated startle on light test trials than on noise&light compound trials, indicating the noise significantly inhibited fear to the light CS. In contrast, rats in the IC group showed similar magnitudes of fear-potentiated startle on light and noise&light compound test trials, indicating that lesions of the IC interfered with the inhibition of fear. Likewise, both Control groups demonstrated no inhibition of fear in the presence of the noise.

These observations were supported by the three-way ANOVA with training (FND, Control) and lesion (Sham, IC), as the between-subject factors and trial type (light, noise&light), as the within-subject factor. This analysis yielded a reliable main effect of lesion, $F(1, 28) = 4.76$,

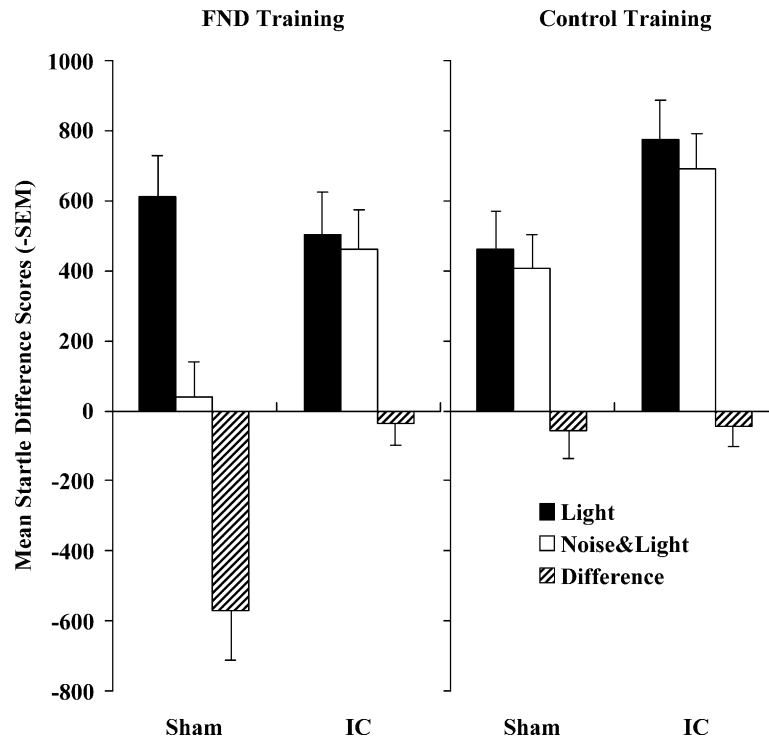


Fig. 3. Mean startle difference scores on light and noise&light test trials in Experiment 2. Rats in the FND groups received FND training followed by either sham surgery (FND-Sham, $n = 8$) or lesions of the IC (FND-IC, $n = 9$). Rats in the Control groups received contrasting control training followed by sham surgery (Control-Sham, $n = 8$) or lesions of the IC (Control-IC, $n = 7$). The mean difference scores were computed by subtracting the startle amplitude on the startle-alone test trials from the startle amplitude on light test trials and noise&light test trials. Also shown are the differences between the two trial types. A negative difference represents inhibition of fear-potentiated startle. Error bars denote one standard error of the mean (+S.E.M.).

$P < 0.05$, but no main effect of training, $F(1, 28) = 2.88$, $P = 0.10$, or Training \times Lesion interaction, $F(1, 28) = 0.38$, $P = 0.54$. All tests involving the within-subject factor of trial type were significant, $F_s(1, 28) = 7.90$, $P_s < 0.01$, including the Training \times Lesion \times Trial type interaction, $F(1, 28) = 8.18$, $P < 0.01$.

To determine the source of the three-way interaction, lower order lesion by trial type ANOVAs were conducted at each level of training. For rats in the FND training groups, this analysis revealed a significant main effect of trial type, $F(1, 15) = 17.27$, $P < 0.01$, but no reliable lesion effect, $F(1, 15) = 1.34$, $P = 0.65$. More importantly, there was a significant Lesion \times Trial type interaction, $F(1, 15) = 13.27$, $P < 0.01$. Pairwise t -tests revealed that the FND-Sham group displayed lower levels of fear-potentiated startle on noise&light than on light trials, $t(7) = 4.07$, $P < 0.01$, indicating fear was inhibited in the presence of the noise. In contrast, rats in the FND-IC group did not show a difference in responding across trial type, $t(8) = 0.63$, $P = 0.55$, indicating that they did not display inhibition of fear.

For rats in the Control training groups, the lower order ANOVA revealed no main effects of lesion, $F(1, 13) = 3.57$, $P = 0.08$, trial type, $F(1, 13) = 0.96$, $P = 0.35$, or a Lesion \times Trial type interaction, $F(1, 13) = 0.02$, $P = 0.90$. Thus, both Control-Sham and Control-IC groups responded similarly on light and noise&light test trials.

An analysis among all groups revealed no differences in the magnitude of fear-potentiated startle on light test trials, $F(3, 28) = 0.15$, $P = 0.93$. Thus, all groups showed comparable levels of fear-potentiated startle in the presence of the light CS.

To examine whether groups differed in the magnitude of inhibition of fear-potentiated startle, analyses were also performed on FND difference scores (FN-Diff). These FN-Diff scores were calculated for each rat by subtracting the mean noise&light difference score from the mean light difference score and reflected the magnitude of inhibition of fear-potentiated startle to the light CS when it was preceded by the noise inhibitor. The magnitude of inhibition differed among groups, $F(3, 28) = 8.47$, $P < 0.01$. Post hoc Bonferroni t -tests showed that magnitude of inhibition in the FND-Sham group was significantly higher than all other groups ($P_s < 0.01$). No differences were found among the FND-IC, Control-Sham, and Control-IC groups ($P_s > 0.05$).

5. Discussion

The results of this study demonstrate the importance of the IC in the expression of conditioned fear and conditioned safety to an auditory stimulus. In Experiment 1, we

demonstrated that rats which received electrolytic lesions of the IC failed to show fear-potentiated startle in the presence of a noise previously conditioned to elicit fear. In Experiment 2, we demonstrated that rats with similarly placed lesions of the IC failed to inhibit fear-potentiated startle in the presence of a noise previously conditioned to inhibit fear. Thus, in both Experiments 1 and 2, lesions of the IC disrupted the behavioral significance of the noise stimulus. Together, these results are consistent with the view that the IC is a common source of auditory information used to mediate the fear eliciting and safety signal properties conditioned to auditory stimuli.

5.1. *The effects of IC lesions on the expression of auditory fear-potentiated startle*

Both anatomical and behavioral data indicate that the IC is a critical source of auditory information to the forebrain. Tracing studies show that the IC provides the major source of afferent projections to the auditory thalamus, including the MGB and associated posterior intralaminar nuclei [37,39]. In turn, the auditory thalamus provides both direct and indirect input to the lateral nucleus of the amygdala which is a critical locus known to be involved in the production of a number of behavioral and physiological responses associated with fear [7,36,59]. Previous studies have demonstrated that pretraining lesions of the auditory thalamus [7,27,31,35,38,41,55] and posttraining lesions of the auditory thalamus [7] disrupt conditioned fear to an auditory CS. Using conditioned freezing as an index of fear, LeDoux et al. [42] have shown that lesions of the IC also disrupt conditioned fear to an auditory CS when lesions are performed before training. However, because the IC has been implicated in somatosensory as well as auditory processing [1,9,44], it is unclear whether deficits obtained from pretraining lesions of the IC are caused by a disruption of CS auditory information, nociceptive US-shock information, or both. The fact that that posttraining lesions of the IC are also effective in disrupting the expression of auditory fear (Experiment 1) indicates that IC lesions disrupt the processing of CS information. Furthermore, the finding that lesions had no effect on the expression of fear to the light CS (Experiment 2) indicates that its effect was specific to auditory information and not a general sensory or performance deficit. These data do not, however, exclude the possibility that deficits caused by pretraining lesions of the IC are a product of the disruption of US somatosensory processing.

Although lesions of the IC disrupted fear-potentiated startle to the auditory CS, the nonassociative response to the auditory CS was unaffected by IC lesions. This was indicated by the fact that both Unpaired-Sham and Unpaired-IC groups, which received unpaired noise + shock training, displayed modestly higher startle responses in the presence of the noise during testing. The magnitude of this effect was similar in both groups but significantly smaller than the associative response seen in the Paired-Sham group. Past

research indeed has shown that noises can produce nonassociative enhancement of startle at moderate intensities [6,17,24,30]. Furthermore, data from our lab (Heldt and Falls, unpublished data) indicate that untrained naïve rats and unoperated rats which receive unpaired noise + shock training display a similar nonspecific enhancement; thus, this effect does not appear to be a weak associative effect or the result of either sham or IC surgery. Because this nonassociative response remained after IC lesions, this response is probably mediated by neural structures below the level of the IC. In Experiment 2, we found no facilitatory effect of the noise on light fear-potentiated startle. In this experiment, startle was elicited 3.5 s after the offset of the noise. This finding is consistent with data from our lab in which we found no augmentation of startle when it was elicited 3.5 s after the offset of a conditioned or unconditioned noise (Heldt and Falls, unpublished data).

The present study also found no evidence that lesions of the IC influence baseline startle amplitude. In both Experiments 1 and 2, sham-operated groups and IC-lesioned groups showed similar startle amplitudes on startle-alone trials. These findings support the view that the primary startle circuit is mediated by subcollicular pathways [11]. However, other studies have shown that damage to the IC and associated structures can either increase [32,35,43] or decrease startle amplitude [25]. One key factor in determining whether lesions affect the startle reflex is the timing of lesions with respect to testing. For example, Parham and Willott [53] reported IC lesions were associated with attenuated startle amplitudes 1 day after recovery but with increased amplitudes 7 and 14 days postoperatively. Their results are consistent with other data showing that short intervals between surgery and testing are associated with reduced startle amplitudes whereas longer intervals are associated with increased startle. In both experiments of the current study, testing was conducted after 6 days of post-surgery recovery. Our data suggest that this interval between surgery and testing may be sufficient for recovery of baseline startle. It is not known if startle in the present study would have been facilitated, had a longer surgery-to-test interval been used.

5.2. *The effects of IC lesions on the inhibition of fear-potentiated startle by an auditory stimulus*

In addition to disrupting the fear-eliciting properties of an auditory stimulus, the current study demonstrated that lesions of the IC are also effective in disrupting safety signal properties of a noise conditioned to inhibit fear. Rats which received FND training followed by sham lesions of the IC displayed reliable inhibition of fear-potentiated startle on trials with included the noise inhibitor. In contrast, rats which received FND training followed by lesions of the IC failed to show a reduction of fear-potentiated startle in the presence of the noise inhibitor. Importantly, both groups of rats displayed equivalent levels of fear-potentiated startle to the light CS,

indicating that the IC is not essential for fear-potentiated startle to a visual CS.

In a previous study, we demonstrated that lesions of the auditory thalamus followed by FND training had no effect on inhibition of fear-potentiated startle to the noise inhibitor [27]. These lesions did, however, disrupt the acquisition of fear-potentiated startle to the noise when the noise was subsequently paired with shock. In a similar fashion, lesions of the perirhinal cortex do not disrupt the ability of the noise to inhibit the expression of fear to the light [16], but blocks the expression of fear when the same noise serves as a CS [7]. Thus, the available evidence, combined with the results of the current study, suggest that a noise inhibitor may use different pathways than a noise CS. In the case of a noise CS, the normal pathways underlying the fear-eliciting properties include lemniscal projections from the IC to the auditory thalamus. From the thalamus, the noise CS is normally relayed to the amygdala via the temporal cortex [7]. In contrast, the neural pathways subserving the safety properties of a noise inhibitor may include extralemniscal efferents that project from the IC and diverge before reaching the auditory thalamus. Under this hypothesis, lesions of the IC disrupt both a noise CS and noise inhibitor (current study), by disrupting the flow of auditory information through both of these pathways. Lesions of lemniscal structures efferent to the IC (e.g. auditory thalamus, temporal cortex) selectively disrupt a noise CS and leave intact extralemniscal pathways that support a noise inhibitor.

At present, it is unclear which extralemniscal structure(s) may mediate the safety properties of a noise inhibitor. In addition to auditory structures within the main lemniscal pathway, the IC sends efferents to a number of other structures which may be directly or indirectly responsible for the reduction of fear. One prime candidate is the SC. Prior anatomical work in both the cat and rat has shown that peripheral regions of the IC send auditory efferents to the middle and deep layers of the SC [14,26,29]. In the case of a noise inhibitor, this intermodal pattern of connectivity may play a key functional role because the SC is an integral part of the sensory pathway involved in the transmission of visual CS information to the amygdala [60]. Thus, input from the IC to this pathway may allow a noise inhibitor to reduce the expression of fear by altering the processing associative value of the light CS. The suggestion that an inhibitor acts directly on the sensory processing of a fearful stimulus is supported by fluorodeoxyglucose (FDG) studies that demonstrate a modification in the sensory pathways of an auditory CS in the presence of a light conditioned to inhibit fear [50,51].

The auditory projections from the IC to the SC may also provide a transmission pathway used to relay information about the emotional significance of a noise inhibitor to the forebrain. The SC convey auditory as well as light sensory information rostrally to thalamic nuclei which have direct access to limbic structures [62] including the amygdala [13,46,47,64]. Thus, using pathways that run outside the

lemniscal system, the IC may relay emotionally significant auditory information to limbic areas known to be involved in the regulation of fear. It is also worth noting that both the IC and SC are believed to be critical components of network mediating the brain's ability to gate disruptive sensory, motor, and cognitive information. This phenomenon is often measured by prepulse inhibition (PPI) which is the suppression of the startle reflex when a startling stimulus is preceded by a weak stimulus at lead time of 20–500 ms. Studies investigating the neural mechanisms involved in PPI have shown that destruction of either the IC or SC interfere with PPI [21,43]. Conversely, stimulation of the IC and SC produces a strong, long-lasting inhibitory effect on the startle response [45]. The inhibition of startle seen in PPI is mediated by descending efferents from the tectum to the primary startle pathway by way of the pedunculo-pontine tegmental nucleus (PPTg) [22,33,34]. Interestingly, Campeau et al. [8] have demonstrated that PPTg showed high levels of c-fos induction in the presence of a noise inhibitor of fear-potentiated startle. Given the importance of the IC and SC in the inhibition of startle seen in PPI, descending efferents originating from these tectal structures may also play a key role in the inhibition of potentiated startle by a noise inhibitor.

A parsimonious explanation for the disruptive effects of IC lesions on the expression/production and inhibition of fear can be based on the assumed disconnection of the IC from efferent structures which play a more central role in assessing the emotional significance of the noise stimulus. In the case of auditory fear conditioning, it is generally assumed that the IC is merely a component of the sensory pathway that passively relays information about the physical properties of auditory stimuli. During conditioning, this information along with US somatosensory information is sent to the amygdala where the associative neural plasticity that underlie the behavioral significance of acoustic stimuli takes place [20,48,57,58]. There is, however, considerable evidence which indicates that neurons within sensory systems also demonstrate learning related plasticity as a consequence of conditioning. In the case of the IC, electrophysiological studies have showed changes in behavioral response and the responses of collicular neurons after conditioning with acoustic stimuli [12,23]. Likewise, FDG and cytochrome oxidase metabolic studies have also demonstrated experience-dependent changes (increases) in the activity of the IC related to the behavioral significance of an acoustic stimulus [49,56]. As is the case in many regions of the brain, the associative plasticity in the IC appears to be mediated by *N*-methyl-D-aspartate receptors [65]. Thus, we can not rule out the possibility that the IC plays a more central role in the associative processes which underlie the safety properties of a noise inhibitor.

The disruptive effects of IC lesions seen in the current study suggest that the IC is also the source of information that signals conditioned fear or safety to a noise stimulus. However, this view is based on the results of axon-damaging electrolytic lesions of the IC; thus, we can not completely

rule out the possibility that these findings may be attributed to damage of fibers which originate outside and pass through the IC. For instance, anatomical studies have revealed the existence of direct projections from the dorsal nucleus of the lateral lemniscus to the SC which presumably pass through the external nucleus of the IC [3,14,61]. In addition, the partial damage of cells in the lateral lemniscus raises the possibility that our results were a consequence of damage to ascending subcollicular projections which by-pass the IC (e.g. [4,28,52]) However, careful examination of IC groups revealed damage extending outside the boundaries of the IC in only about 30% of animals. The behavior of these animals did not differ from animals with more restricted lesions confined to the IC. Nevertheless, to address these concerns, we are currently performing reversible lesions of the IC after training by infusion of muscimol, a GABA_A receptor agonist. If inactivation of the IC interferes with the inhibition of fear, we can conclude that cells in the IC are important for processing the auditory inhibitor.

References

- [1] Aitkin LM, Dickhaus H, Schult W, Zimmermann M. External nucleus of inferior colliculus: auditory and spinal somatosensory afferents and their interactions. *J Neurophysiol* 1978;41:837–47.
- [2] Andersen RA, Roth GL, Aitkin LM, Merzenich MM. The efferent projections of the central nucleus and the pericentral nucleus of the inferior colliculus in the cat. *J Comp Neurol* 1980;194:649–62.
- [3] Appell PP, Behan M. Sources of subcortical GABAergic projections to the superior colliculus in the cat. *J Comp Neurol* 1990;302:143–58.
- [4] Bajo VM, Merchan MA, Lopez DE, Rouiller EM. Neuronal morphology and efferent projections of the dorsal nucleus of the lateral lemniscus in the rat. *J Comp Neurol* 1993;334:241–62.
- [5] Calford MB, Aitkin LM. Ascending projections to the medial geniculate body of the cat: evidence for multiple, parallel auditory pathways through thalamus. *J Neurosci* 1983;3:2365–80.
- [6] Campeau S, Davis M. Fear-potentiation of the acoustic startle reflex in rats using noises of various spectral frequencies. *Anim Learn Behav* 1992;20:177–86.
- [7] Campeau S, Davis M. Involvement of subcortical and cortical afferents to the lateral nucleus of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. *J Neurosci* 1995;15:2312–27.
- [8] Campeau S, Falls WA, Cullinan WE, Helmreich DL, Davis M, Watson SJ. The elicitation and reduction of fear: behavioral and endocrinological indices and brain induction of the immediate-early gene *c-fos*. *Neuroscience* 1997;78:1087–104.
- [9] Coleman JR, Clerici WJ. Sources of projections to subdivisions of the inferior colliculus in the rat. *J Comp Neurol* 1987;262:215–26.
- [10] Davis M, Campeau S, Kim M, Falls WA. Neural systems of emotion: the amygdala's role in fear and anxiety. In: McGaugh JL, Weinberger NM, Lynch G, editors. *Brain and memory: modulation and mediation of neuroplasticity*. New York: Oxford University Press; 1995. p. 3–40.
- [11] Davis M, Gendelman DS, Tischler MD, Gendelman PM. A primary acoustic startle circuit: lesions and stimulation studies. *J Neurosci* 1982;6:791–805.
- [12] Disterhoft JF, Stuart DK. Differentiated short latency response increases after conditioning in inferior colliculus neurons of alert rat. *Brain Res* 1977;130:315–33.
- [13] Doron NN, LeDoux JE. Organization of projections to the lateral amygdala from auditory and visual areas of the thalamus in the rat. *J Comp Neurol* 1999;412:383–409.
- [14] Druga R, Syka J. Projections from auditory structures to the superior colliculus in the rat. *Neurosci Lett* 1984;45:247–52.
- [15] Ehret G. The auditory midbrain, a “shunting yard” of acoustical information processing. In: Ehret G, Romand R, editors. *The central auditory system*. New York: Oxford University Press; 1997. p. 259–316.
- [16] Falls WA, Bakken K, Heldt S. Lesions of the perirhinal cortex block conditioned excitation but not conditioned inhibition of fear. *Behav Neurosci* 1997;111:476–86.
- [17] Falls WA, Davis M. Fear-potentiated startle using three conditioned stimulus modalities. *Anim Learn Behav* 1994;22:379–83.
- [18] Falls WA, Davis M. 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. *Behav Neurosci* 1995;109:379–87.
- [19] Falls WA, Davis M. Inhibition of fear-potentiated startle can be detected after the offset of a feature trained in a serial feature-negative discrimination. *J Exp Psychol Anim Behav Process* 1997;23:3–14.
- [20] Fanselow MS, LeDoux JE. Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. *Neuron* 1999;23:229–32.
- [21] Fendt M, Koch M, Schnitzler HU. Sensorimotor gating deficit after lesions of the superior colliculus. *Neuroreport* 1994;5:1725–8.
- [22] Fendt M, Li L, Yeomans JS. Brain stem circuits mediating prepulse inhibition of the startle reflex. *Psychopharmacology (Berl)* 2001;156:216–24.
- [23] Gao E, Suga N. Experience-dependent plasticity in the auditory cortex and the inferior colliculus of bats: role of the corticofugal system. *Proc Natl Acad Sci USA* 2000;97:8081–6.
- [24] Gerrard RL, Ison JR. Spectral frequency and the modulation of the acoustic startle reflex by background noise. *J Exp Psychol Anim Behav Process* 1990;16:106–12.
- [25] Groves PM, Wilson CJ, Boyle RD. Brain stem pathways, cortical modulation, and habituation of the acoustic startle response. *Behav Biol* 1974;10:391–418.
- [26] Harting JK, Van Lieshout DP. Projections from the rostral pole of the inferior colliculus to the cat superior colliculus. *Brain Res* 2000;881:244–7.
- [27] Heldt SA, Falls WA. Destruction of the auditory thalamus disrupts the production of fear but not the inhibition of fear conditioned to an auditory stimulus. *Brain Res* 1998;813:274–82.
- [28] Henkel CK. Evidence of sub-collicular auditory projections to the medial geniculate nucleus in the cat: an autoradiographic and horseradish peroxidase study. *Brain Res* 1983;259:21–30.
- [29] Huffman RF, Henson Jr OW. The descending auditory pathway and acousticomotor systems: connections with the inferior colliculus. *Brain Res Brain Res Rev* 1990;15:295–323.
- [30] Ison JR, Hammond GR. Modification of the startle reflex in the rat by changes in the auditory and visual environments. *J Comp Physiol Psychol* 1971;75:435–52.
- [31] Iwata J, LeDoux JE, Meeley MP, Arneric S, Reis DJ. Intrinsic neurons in the amygdala field projected to by the medial geniculate body mediate emotional responses conditioned to acoustic stimuli. *Brain Res* 1986;383:195–214.
- [32] Jordan WP, Leaton RN. Startle habituation in rats after lesions in the brachium of the inferior colliculus. *Physiol Behav* 1982;28:253–8.
- [33] Koch M, Kungel M, Herbert H. Cholinergic neurons in the pedunculopontine tegmental nucleus are involved in the mediation of prepulse inhibition of the acoustic startle response in the rat. *Exp Brain Res* 1993;97:71–82.
- [34] Koch M, Schnitzler HU. The acoustic startle response in rats—circuits mediating evocation, inhibition and potentiation. *Behav Brain Res* 1997;89:35–49.

- [35] Leaton RN, Kelso JM. The auditory pathways: startle amplitude and fear in an acoustic startle response paradigm in rats. *Psychobiology* 2000;28:492–506.
- [36] LeDoux JE. The amygdala and emotion: a view through fear. In: Aggleton JP, editor. *The amygdala: a functional analysis*. Oxford, New York: Oxford University Press; 2000. p. 289–310.
- [37] LeDoux JE, Farb C, Ruggiero DA. Topographical organization of neurons in the acoustic thalamus that project to the amygdala. *J Neurosci* 1990;10:1043–54.
- [38] LeDoux JE, Iwata I, Pearl D, Reis DJ. Disruption of auditory but not visual learning by destruction of intrinsic neurons in the rat medial geniculate body. *Brain Res* 1986;371:395–9.
- [39] LeDoux JE, Ruggiero DA, Forest R, Stornetta R, Reis DJ. Topographical organization of convergent projections to the thalamus from the inferior colliculus and spinal cord in the rat. *J Comp Neurol* 1987;264:123–46.
- [40] LeDoux JE, Ruggiero DA, Reis DJ. Projections of the subcortical forebrain from anatomically defined regions of the medial geniculate body in the rat. *J Comp Neurol* 1985;242:182–213.
- [41] LeDoux JE, Sakaguchi A, Iwata J, Reis DJ. Interruption of projections from the medial geniculate body to an arch-neostriatal field disrupts the classical conditioning of emotional responses to acoustic stimuli. *Neuroscience* 1986;17:615–27.
- [42] LeDoux JE, Sakaguchi A, Reis DJ. Subcortical efferent projections of the medial geniculate nucleus mediate emotional responses conditioned to acoustic stimuli. *J Neurosci* 1984;4:683–98.
- [43] Leitner DS, Cohen ME. Role of the inferior colliculus in the inhibition of acoustic startle in the rat. *Physiol Behav* 1985;34:65–70.
- [44] Li H, Mizuno N. Single neurons in the spinal trigeminal and dorsal column nuclei project to both the cochlear nucleus and the inferior colliculus by way of axon collaterals: a fluorescent retrograde double-labeling study in the rat. *Neurosci Res* 1997;29:135–42.
- [45] Li L, Yeomans JS. Using intracranial electrical stimulation to study the timing of prepulse inhibition of the startle reflex. *Brain Res Brain Res Protoc* 2000;5:67–74.
- [46] Linke R. Differential projection patterns of superior and inferior collicular neurons onto posterior paralamina nuclei of the thalamus surrounding the medial geniculate body in the rat. *Eur J Neurosci* 1999;11:187–203.
- [47] Linke R, De Lima AD, Schwegler H, Pape HC. Direct synaptic connections of axons from superior colliculus with identified thalamo-amygdaloid projection neurons in the rat: possible substrates of a subcortical visual pathway to the amygdala. *J Comp Neurol* 1999;403:158–70.
- [48] Maren S. Long-term potentiation in the amygdala: a mechanism for emotional learning and memory. *Trends Neurosci* 1999;22:561–7.
- [49] McIntosh AR, Gonzalez-Lima F. Network analysis of functional auditory pathways mapped with fludeoxyglucose: associative effects of a tone conditioned as a Pavlovian excitator or inhibitor. *Brain Res* 1993;627:129–40.
- [50] McIntosh AR, Gonzalez-Lima F. Functional network interactions between parallel auditory pathways during Pavlovian conditioned inhibition. *Brain Res* 1995;683:228–41.
- [51] McIntosh AR, Gonzalez-Lima F. Large-scale functional connectivity in associative learning: interrelations of the rat auditory, visual, and limbic systems. *J Neurophysiol* 1998;80:3148–62.
- [52] Morest DK. The lateral tegmental system of the midbrain and medial geniculate body: study with Golgi and Nauta methods in cat. *J Anat* 1965;99:611–34.
- [53] Parham K, Willott JF. Effects of inferior colliculus lesions on the acoustic startle response. *Behav Neurosci* 1990;104:831–40.
- [54] Paxinos G, Watson C. *The rat brain in stereotaxic coordinates*. 4th ed. San Diego, CA: Academic Press; 1998.
- [55] Poremba A, Gabriel M. Medial geniculate lesions block amygdalar and cingulothalamic learning-related neuronal activity. *J Neurosci* 1997;17:8645–55.
- [56] Poremba A, Jones D, Gonzalez-Lima F. Metabolic effects of blocking tone conditioning on the rat auditory system. *Neurobiol Learn Mem* 1997;68:154–71.
- [57] Rogan MT, LeDoux JE. LTP is accompanied by commensurate enhancement of auditory-evoked responses in a fear conditioning circuit. *Neuron* 1995;15:127–36.
- [58] Romanski LM, Clugnet MC, Bordi F, LeDoux JE. Somatosensory and auditory convergence in the lateral nucleus of the amygdala. *Behav Neurosci* 1993;107:444–50.
- [59] Romanski LM, LeDoux JE. Equipotentiality of thalamo-amygdala and thalamo-cortico-amygdala circuits in auditory fear conditioning. *J Neurosci* 1992;12:4501–9.
- [60] Shi C, Davis M. Visual pathways involved in fear conditioning measured with fear-potentiated startle: behavioral and anatomic studies. *J Neurosci* 2001;21:9844–55.
- [61] Taylor AM, Jeffery G, Lieberman AR. Subcortical afferent and efferent connections of the superior colliculus in the rat and comparisons between albino and pigmented strains. *Exp Brain Res* 1986;62:131–42.
- [62] Thompson SM, Robertson RT. Organization of subcortical pathways for sensory projections to the limbic cortex. II. Afferent projections to the thalamic lateral dorsal nucleus in the rat. *J Comp Neurol* 1987;265:189–202.
- [63] Winer JA. The medial geniculate body of the cat. *Adv Anat Embryol Cell Biol* 1985;86:1–97.
- [64] Yasui Y, Kayahara T, Nakano K, Mizuno N. The subparafascicular thalamic nucleus of the rat receives projection fibers from the inferior colliculus and auditory cortex. *Brain Res* 1990;537:323–7.
- [65] Zhang Y, Wu SH. Long-term potentiation in the inferior colliculus studied in rat brain slice. *Hear Res* 2000;147:92–103.