Amygdalar Efferents Initiate Auditory Thalamic Discriminative Training-Induced Neuronal Activity

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It is well known that neurons of the medial geniculate (MG) nucleus of the thalamus send axonal projections to the amygdala. It has been proposed that these projections supply information that supports amygdalar associative processes underlying acquisition of acoustically cued conditioning and learning. Here we demonstrate the reverse direction of influence. Temporary inactivation of the amygdala using the $GABA_A$ receptor agonist muscimol just before the onset of discriminative avoidance conditioning permanently blocked the development of training-induced discriminative neuronal activity in the MG nucleus of rabbits. No discriminative activity developed when the amygdala was inactivated or during later training to criterion without muscimol. Thus, amygdalar processing at the outset of training is necessary for the development of training-induced discriminative activity of neurons in the MG nucleus.

Key words: muscimol; $GABA_A$ agonist; temporary lesion; rabbits; associative conditioning; retention; multiunit neuronal activity

It is well established that neurons in the amygdala and the medial geniculate (MG) nucleus, the auditory region of the sensory thalamus, are importantly involved in mediating acoustically cued Pavlovian and instrumental aversive conditioning (Iwata et al., 1986; Jarrell et al., 1986; LeDoux et al., 1986; McEchron et al., 1995; Maren and Fanselow, 1996; Davis, 1997; Poremba and Gabriel, 1997a,b; Armony et al., 1998; Ferry et al., 1999). Yet controversy remains as to the separate and distinct contributions of these nuclei, and little is known about how their neurons interact in mediating learning and performance.

These issues could have been neatly resolved years ago had it been possible to confirm the hypothesis that neurons of the MG nucleus act simply to relay acoustic data to the amygdala via the direct axonal pathway documented by LeDoux et al. (1985). On this simple view, the function of MG nuclear neurons is sensory coding and transmission of acoustic signals. Interaction within the amygdala of the acoustic information with information concerning reinforcing stimuli would promote the development of plasticity at amygdalar synapses, which would thenceforth allow amygdalar neurons to respond uniquely to associatively significant acoustic cues, thus inducing the output of learned emotional responses and behaviors in other parts of the learning-relevant circuitry.

A finding not easily incorporated into the foregoing model is the occurrence of training-induced associative neuronal activity, not simply sensory transmission, in the MG nucleus itself. For example, conditioning-induced, brief-latency discriminative neuronal activity develops in the medial region of the MG nucleus, and this activity exhibits reversal, during acquisition and reversal learning of a discriminative avoidance response (for review, see Gabriel et al., 1982). In the discriminative avoidance task, rabbits learn to avoid a foot shock by locomoting in response to a tone, the positive conditional stimulus (CS+), and they ignore a different tone, the CS−, which is not predictive of the foot shock. Training-induced neuronal activity (TIA) is exhibited as development of enhanced neuronal firing in response to the CS+ and decreased firing in response to the CS−. Similar results have been found in studies using other learning paradigms (Supple and Kapp, 1989; Edeline, 1990; Edeline and Weinberger, 1992; Olds et al., 1972; Ryugo and Weinberger, 1978; Weinberger, 1982; McEchron et al., 1995; O'Connor et al., 1997). These associative neuronal responses of MG neurons raise a conundrum: If conditioning induces associative neuronal changes in the MG nucleus, what then is the additional and unique role of amygdala neurons in the conditioning process?

The present experiment resolves the conundrum, at least in the case of instrumental conditioning with rabbits. It demonstrates that amygdalar processes have precedence over the associative changes that occur in the MG nucleus. Rabbits given bilateral electrolytic lesions of the amygdala before discriminative avoidance training exhibited a severe avoidance learning deficit (Poremba and Gabriel, 1997a). To confirm this effect with fiber-sparing lesions, the amygdala was inactivated by microinjecting the $GABA_A$ receptor agonist muscimol before training. Neuronal activity was recorded in the MG nucleus during training with intra-amygdalar muscimol present and on subsequent days with no muscimol. Behavioral learning did not occur and no TIA developed in the MG nucleus during the initial training session with muscimol. Surprisingly, no TIA developed during later training without muscimol when rabbits exhibited significant although moderately impaired behavioral learning. Thus, amygdalar processes at the outset of training enable the development of MG nuclear TIA.

MATERIALS AND METHODS

Subjects, surgery, and data collection. The subjects were 26 male New Zealand White rabbits weighing 1.5–2.0 kg on delivery to the laboratory and maintained on *ad libitum* water and rabbit chow. After a minimum period of 48 hr for adaptation to living cages, each rabbit underwent

Received June 26, 2000; revised Oct. 3, 2000; accepted Oct. 12, 2000.

This research was supported by National Institutes of Health Grant NS26736 to M.G.

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surgery for implantation of guide cannulas for muscimol microinjection and electrodes for recording of extracellular, multiple-unit neuronal activity. Surgical anesthesia was induced by subcutaneous injection (1 ml/kg of body weight) of a solution containing 60 mg/ml ketamine-HCl and 8 mg/ml xylazine, followed by hourly injections of 1 ml of the solution.

Each rabbit was placed in a rabbit head holder (David Kopf Instruments, Inc.), and six intracranial multiunit recording electrodes were implanted, under stereotaxic guidance (Girgis and Shih-Chang, 1981), through burr holes (diameter, 0.5 mm) drilled through the skull over the target sites. Neuronal activity was monitored during advancement of the electrodes as an aid to placement. A stainless steel machine screw threaded into the frontal sinus served as the electrical reference for the recordings. Details regarding the procedures of electrode manufacture and recording are provided elsewhere (Gabriel et al., 1995).

The medial division of the MG nucleus constituted the target site of the recording electrodes (see below). The stereotaxic coordinates were as follows: anteroposterior (AP), 7.5; lateral (L), ±5.0; and ventral from brain surface (V), 9.0. In addition, recording electrodes were implanted in the dorsal division of the MG nucleus, the anterior ventral thalamic nucleus, the medial dorsal thalamic nucleus, and the anterior cingulate cortex. Because this paper concerns the relationship of neuronal activity in the medial MG and amygdala, the neuronal data of the other areas are to be reported elsewhere.

Guide cannulas manufactured from 22 gauge stainless steel hypodermic tubing were implanted bilaterally in the dorsal aspect of the basolateral nucleus of the amygdala. The stereotaxic coordinates for positioning of the guide cannulas were AP, 7.7 mm; L, ±5.5 mm; and V, 14.0 mm. Injection cannulas to be inserted into the guide cannulas at the time of muscimol injection were manufactured from 28 gauge stainless steel hypodermic tubing. The injection cannulas extended 1 mm below the ends of the guide cannulas into the injection target site in the basolateral nucleus of the amygdala. The stereotaxic coordinates for the injection target site were AP, 0.7 mm; L, ±5.5 mm; and V, 16.0 mm.

Histology and assessment of injection size. After completion of testing, 0.5 μl of 0.2% cresyl violet dye was injected, as described above, to provide a means to visualize the approximate intracranial distribution of the muscimol. After the dye injection, killing was completed using an overdose of sodium pentobarbital followed by transcardial perfusion with normal saline and 10% formalin. The brains were frozen and sectioned at 40 μm, and the sections containing the electrode tracks were photographed while still wet (Fox and Eichman, 1959). Every fifth section through the areas containing the cannula tracks was saved to assess placement and the spread of the dye. All sections with electrode tracks were saved. After drying, the sections with electrode and cannula tracks were processed with a metachromatic Nissl and myelin stain (Donovick, 1974).

Avoidance conditioning. Discriminative avoidance learning was initiated after a 7–10 d postsurgical recovery period. Training was administered while the rabbits occupied a rotating wheel conditioning apparatus (Brogden and Culler, 1936) that was located in a chamber for acoustic and electrical shielding. The chamber occupied a room adjacent to that housing the equipment for data collection. An exhaust fan and a white noise source in the chamber produced a masking noise (70 dB re: 20 $\mu N/m^2$). Two pure tones of different acoustic frequency (1 or 8 kHz; duration, 500 msec; 85 dB re: 20 $\mu N/m^2$; rise time, 3 msec) served as the CS+ and CS−. The tones were assigned so that each acoustic frequency served equally often as CS+ and CS−. During conditioning, the tones were played through a loudspeaker attached to the chamber ceiling directly above the wheel. The presentation of the CS+ was followed after 5 sec by a foot shock unconditional stimulus (US), delivered through the grid floor of the wheel. The US was a constant AC current (1.5–2.5 mA). The rabbits learned to prevent US delivery by stepping in the rotating wheel apparatus in response to the CS+. The minimal effective locomotor conditioned response (CR) was defined as a wheel rotation of 2°. However, CRs of trained rabbits were typically robust locomotions. The average wheel rotation produced by CRs in a large group of trained rabbits was ~400°. The tone selected as the CS− was not followed by the US, and the rabbits learned to ignore the CS−.

Before training, each rabbit received two preliminary training sessions. In the first session, each tone was presented 60 times without the foot shock US. In the second session, the tones and the US were presented in an explicitly unpaired manner (Gabriel et al., 1995). The preliminary training sessions provided baseline data for CRs and neuronal responses induced by pairing of the CS and the US during conditioning. Each subject was trained and tested at approximately the same time each day.

Temporary inactivation of the amygdala and behavioral testing. All rabbits received intra-amygdalar microinjection of 0.5 μl of the $GABA_A$ agonist muscimol (concentration, 1.0 μmol, reconstituted with sterile 0.9% PBS). Controls received injections of 0.9% sterile PBS. The injections were given bilaterally at a rate of 0.4 μl/min, using a 28 gauge injection cannula attached through saline-filled polyethylene tubing to a 25 μl syringe held in a motor-driven infusion pump (Razel Instruments, Inc.). The injection solution was separated from the saline by a small air bolus. After the injection, the cannula remained in place for 1.5 min. All injections were given 20–30 min before the initiation of training. Available data indicate restoration of behavioral function 5–6 hr after CNS muscimol microinjection (Li et al., 1998).

The rabbits were assigned to a muscimol group or a saline (control) group. Approximately 24 hr after the second pretraining session, the rabbits were given the appropriate intra-amygdalar microinjection, followed by 240 trials of discriminative avoidance training, consisting of 120 CS+ presentations and 120 CS− presentations in an irregular sequence. The administration of 240 trials doubled the number of trials normally administered in a single session in these studies. This was done to obtain reliable discriminative learning in the control subjects during the first training session immediately after the microinjection of muscimol. To render the data comparable with the data of studies with 120-trial sessions, the 240-trial session was treated as two separate 120-trial sessions, labeled as session A and session B. Also, 240 trials were administered on the second day of training, and these trials were treated as separate 120-trial sessions (sessions C and D), but no injections were given before training on the second day. On subsequent days, standard training sessions consisting of 60 CS+ trials and 60 CS− trials were administered daily until a behavioral criterion of discriminative performance was reached. The criterion required that the percentage of CRs to the CS+ exceed the percentage of responses to the CS− by ≤60% in two consecutive sessions. Past experience has shown that asymptotic performance is attained with this criterion; i.e., performance levels yielded by the criterion are not exceeded significantly during postcriterial overtraining.

Recording and analysis of neuronal activity. Throughout behavioral training the multiunit neuronal records were fed into active bandpass filters (bandwidth, 600–8000 Hz) and subsequently to pulse height discriminators, set to detect the largest three or four action potentials. Outputs of the discriminators were fed to a computer that controlled task administration and sampled the neuronal data before and during CS presentation. The computer sampled the average frequency of multiunit firing in each of 100 consecutive 10 msec intervals, 30 before and 70 after CS onset. The firing frequencies in the intervals after CS onset were normalized with respect to the firing frequencies in the 30 consecutive 10 msec pre-CS (baseline) intervals, using the *Z* transformation. This normalization measures the frequency of CS elicited neuronal firing in units of pre-CS variability.

The multiunit recording technique used combines the firing frequencies of several cells. With this approach it is possible to obtain a robust measure of localized learning-relevant neuronal activity, which remains stable over many days. Although the multiunit activity cannot document all relevant neuronal firing patterns in the sample, it has been shown to provide a reliable representation of the modal pattern of single-unit firing in many areas (Kubota et al., 1996).

A central feature of this and related studies is the use of discriminative neuronal activity for the assay learning-relevant brain processes. Discriminative neuronal activity is defined as significantly different neuronal firing in response to signals that have different learned meanings, such as the CS+ (which signals the occurrence of the aversive US) and the CS− (which predicts that no US will occur). Discriminative activity has the advantage that it is unambiguously associative in character; i.e., it cannot be attributed to nonassociative factors such as general arousal, pseudoconditioning, and motor preparation.

The neuronal and behavioral data were submitted to multifactor factorial repeated measures ANOVA (BMDP statistical software, program 2V). Factors of the analysis yielding significant overall *F* ratios were further analyzed using simple effect tests following procedures outlined by Winer (1962, chapter 7). Correction of the *F* test because of disconformity of the data with the sphericity assumption of these analyses was performed following the procedure of Huynh and Feldt (1976).

The analysis had a between-subject factor of group (two levels: muscimol and saline) and orthogonal repeated measures factors of training session (six levels as specified below), stimulus (two levels: CS+ and CS−), and poststimulus interval (40 10 msec intervals after CS onset).

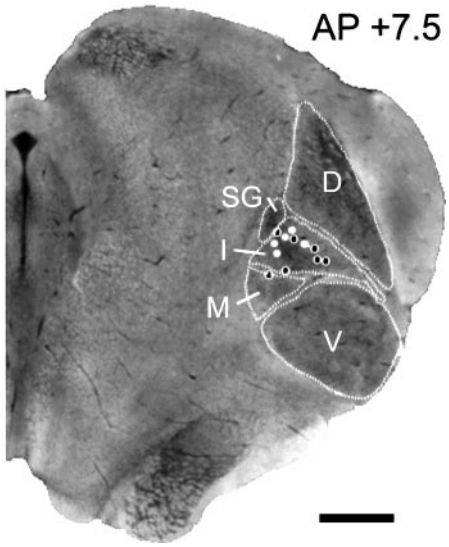

Figure 1. Sites of recording electrodes for the saline group (*solid white circles*) and the muscimol group (*black circles*) are shown on a coronal section through the right midrostral medial geniculate nucleus. Three and nine of the sites were in the left and right hemispheres, respectively, but all of the placements are shown in a single depiction of the right hemisphere. The coronal section shown is very similar to the section at 7.5 mm posterior to bregma in the stereotaxic atlas of Girgis and Shi-Chang (1981). This was the anteroposterior level used for stereotaxic placement of the MG recording electrodes. The indicated divisions of the MG nucleus are as defined by De Venecia et al. (1995). *M*, Medial geniculate nucleus; *D*, dorsal geniculate nucleus; *I*, internal nucleus; *SG*, suprageniculate. Scale bar, 1 mm.

The six sessions constituting the training session factor were (1) pretraining with unpaired presentations of the CSs and US; (2) sessions A and B, the first two 120-trial avoidance training sessions administered on the day after pretraining; (3) sessions C and D, the third and fourth 120-trial avoidance training sessions administered on the second day after pretraining; and (4) the session in which the acquisition criterion was attained.

RESULTS

Histology

The neuronal data were obtained from 10 rabbits that had cannula tips localized within the basolateral nucleus of the amygdala. In these rabbits 12 recording electrodes (7 in rabbits of the muscimol group and 5 in the saline group) were localized in the medial division of the MG nucleus (Fig. 1) as defined in the rabbit by Jones (1985). This area corresponds to the medial and internal divisions of the MG nucleus as defined by De Venecia et al. (1995). TIA was localized within these same areas in previous studies using the present procedures and in studies with other procedures (Gabriel et al., 1975, 1976; Supple and Kapp, 1989; Hocherman and Yirmiya, 1990; McEchron et al., 1995; O'Connor et al., 1997).

Assessment of the spread of dye injected through the cannulas during perfusion of the rabbits showed an approximately teardrop shape of the dye-stained areas, oriented dorsoventrally. The diameter was measured at the widest point. The maximum and minimum diameters were 0.5 and 1.5 mm. The average diameter was 0.9 mm. Injections were confined to the basolateral nucleus with very slight spread to the lateral and basomedial nuclei of the amygdala.

Behavior

The detailed behavioral results have been published in a separate report (Poremba and Gabriel, 1999). The focus of this paper is the learning-related neuronal activity of the MG nucleus. The following summary indicates the essential behavioral results.

Rabbits given injection of intra-amygdalar muscimol (the muscimol group) just before the first day of training failed to exhibit significant discriminative avoidance learning during the first day of training. Significant learning did not occur during the first 120 trials (session A) or during the second 120 trials (session B) that were administered on the first day of training. Rabbits given saline (the saline group) did exhibit significant learning during session B. The mean percentages of CRs performed by rabbits in each group for each session are shown in the top two rows of Table 1.

The foregoing results showed that the muscimol blocked the development of learned behavior during the first training day. It is possible that this effect was attributable to prevention of behavioral expression as a result of muscimol, not a true blockade of learning. Plasticity involved in coding of the association of the CSs with the US may have formed during the first session of training in the presence of muscimol. If present, such plasticity could have supported an enhancement of learned responding (i.e., savings) during the second day of training. However, the results showed that the performance on the second day (sessions C and D) of the rabbits of the muscimol group, although indicative of significant learning, was not significantly better than the first-day performance (sessions A and B) of saline group rabbits and thus did not indicate savings based on exposure to the conditioning contingencies during the first day of training. These results support the hypothesis that muscimol did not merely block the expression of learning but instead blocked the formation of neural plasticity necessary for learning.

All of the rabbits were successful in reaching the learning criterion. The number of sessions required for the attainment of criterion by the rabbits of the muscimol group (8.90) was significantly greater than for the saline group (5.69; $p < 0.04$). However, the total number of sessions to criterion does not yield a meaningful comparison, because the rabbits of the muscimol group did not learn on the first day. When the first day performance of the muscimol group was eliminated from the analysis, no significant effect of the muscimol was found on the number of sessions required for criterion attainment ($p = 0.4105$). These results are in accord with the conclusion that the conditioning experience of the first day of training of rabbits in the muscimol group did not engender savings during subsequent training without muscimol. Nevertheless, the analysis did demonstrate a moderate but significant impairment of performance of the muscimol group during behavioral acquisition. These rabbits performed significantly fewer conditioned avoidance responses on average to the CS+ (67%) during the session of criterion attainment than the saline group (81%), whereas responding to the CS− was the same (12%) in both groups.

The foregoing analyses were performed for all subjects. However, neuronal data were also analyzed for the reduced sample of the subjects ($n = 10$) that had microinjection cannulas and recording electrodes placed accurately in the targeted areas (see Histology). Analyses were performed to determine whether the behavioral effects observed in the full sample also occurred in the reduced sample. As for the full sample, the analysis of the CR percentage data yielded a significant interaction of the group,

Table 1. Summary of behavioral results: percentage of conditioned responses

Group	Stimulus	Pretraining	Day 1		Day 2		Criterion	Sessions to criterion
			Acquisition A	Acquisition B	Acquisition C	Acquisition D		
Saline	CS+	6	48	57	74	73	81	
(all)	CS−	6	25	24	21	17	12	5.69
Muscimol	CS+	6	15	18	39	40	67	
(all)	CS−	7	10	8	19	12	12	8.60
Saline	CS+	5	49	59	74	72	82	
(reduced)	CS−	7	25	22	19	14	10	4.83
Muscimol	CS+	6	17	22	22	28	69	
(reduced)	CS−	7	8	12	11	12	18	7.75

stimulus, and session factors ($F_{(5,40)} = 3.74$; $p < 0.01$). The average percentages of CRs in response to the CS+ and CS− across training sessions for the reduced set of subjects are shown in the bottom portion of Table 1. Individual comparisons showed that the subjects in the muscimol group did not exhibit significant behavioral discrimination during sessions A–C but did discriminate significantly in session D. (Recall that the subjects in the full sample showed discrimination in sessions C and D but not in sessions A and B). The average CR percentages reached by the subjects of the reduced sample during the criterial session were identical to those of the full sample. As in the full sample the rabbits in the reduced sample did not exhibit behavioral savings as a result of their training with muscimol present. Finally, the muscimol and saline group rabbits reached the criterion after 7.75 and 4.83 sessions, respectively ($p < 0.01$), and, as for the full sample the difference fell below the significance threshold when the first day training was excluded from the muscimol group mean ($p < 0.06$).

Discriminative TIA

In replication of previous findings (for review, see Gabriel et al., 1982) significant neuronal discrimination between the CS+ and CS− developed in the MG nucleus in the saline group during the first training session. The discriminative TIA consisted of a significantly greater neuronal response to the CS+ than to the CS−. This effect first occurred in training session A and remained present during the remaining training sessions, including the criterial training session (Fig. 2, *top row*).

In contrast, neurons in the MG nucleus of the muscimol group exhibited virtually no discriminative TIA. No discriminative TIA was found during the first four training sessions (A–D) in these rabbits, except TIA in a single 10 msec interval during the criterial training session (Fig. 2, *bottom row*).

These conclusions were based on a significant interaction of the stimulus and group factors of the ANOVA ($F_{(1,10)} = 9.00$; $p < 0.02$) as well as a significant four-way interaction of the training session, stimulus, 10 msec interval, and group factors ($F_{(195,1950)} = 1.24$; $p < 0.02$). Simple effect tests of the two-way interaction means showed a significantly greater overall average neuronal response to the CS+ than to the CS− in rabbits of the saline group ($p < 0.01$), whereas the stimulus factor did not significantly affect the neuronal activity in the muscimol group. The 10 msec intervals in which discriminative TIA was exhibited for each training session in the saline and muscimol groups, as indicated by tests of simple effects among the four-way interaction means, are shown in Table 2. As can be seen from Table 2, discriminative TIA developed robustly and was exhibited in a large majority of poststimulus 10 msec intervals throughout training in the saline group, but only a single interval showed the effect during the criterial session in the rabbits of the muscimol group.

Additional analyses performed separately for the saline and muscimol groups again showed a significant interaction of the CS and training stage factors for the saline group data but not for the muscimol group data, thus corroborating the conclusion that discriminative TIA that developed robustly in the MG nucleus of the saline group did not develop in subjects of the muscimol group. Additional analyses were performed using four consecutive 100 msec intervals, rather than the customary 40 consecutive 10 msec intervals. Again, these analyses showed robust discrimination in the saline group but no discrimination in the muscimol group.

Neuronal firing increases during training measured relative to firing during pretraining with tone and unpaired foot shock US presentations

The average histograms shown in Figure 2 suggested that the discriminative TIA in the MG nucleus in rabbits of the saline group was attributable both to a significant increase in the neuronal response to the CS+ and to a decrease in the response to the CS− during training, relative to the response observed during pretraining. Increased responding to the CS+ during training was clearly shown by simple effect tests on the four-way interaction means, which compared average neuronal response magnitudes at each 10 msec interval during each training session with the magnitudes at corresponding intervals during the pretraining session. Increased responding to the CS+ was not found in control rabbits in any interval during the first training session (session A). However, the numbers of 10 msec intervals in which significantly increased responding to the CS+ was found were 10, 2, 21, and 8, respectively, during training sessions B–D and during the criterial session. Significant increases from pretraining to training in neuronal response to the CS+ in rabbits given muscimol occurred in a single 10 msec interval (the third interval, 30 msec after CS onset) in training sessions B and D. However, increases in response to the CS− were found in a total of five intervals in rabbits given muscimol. Note that the increased responding to the CS− serve to attenuate discriminative TIA (Table 3).

The average *Z* scores associated with presentations of the CS− during training in rabbits of the saline group had primarily negative values, indicating that the CS− reduced the firing rate of MG neurons to below-baseline levels during training. The negative scores did not occur during the pretraining session. Nevertheless, comparisons similar to those reported above failed to

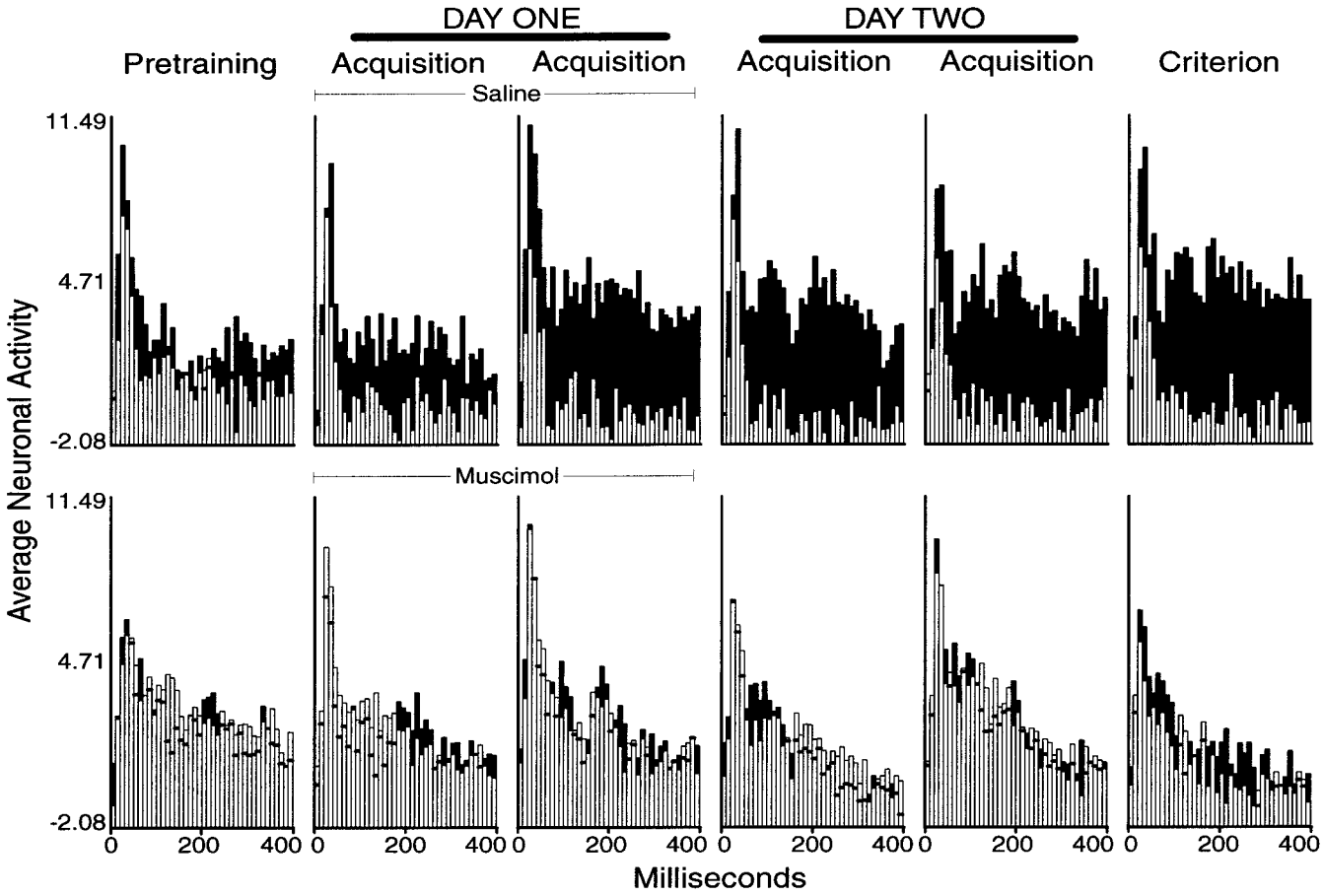

Figure 2. Average neuronal firing frequency of neurons in the medial geniculate nucleus recorded in rabbits given intra-amygdaloid injections of saline on the first day of training (*top row*) or injection of muscimol on the first day of training (*bottom row*). The data are in the form of *Z* scores normalized with respect to a 300 msec pre-CS baseline period as detailed in Materials and Methods. Two values, one the average neuronal response to the CS+ (*black bars*), the other the average neuronal response to the CS− (*white bars*), are plotted for each panel showing discharge frequency during the first 40 consecutive 10 msec intervals after CS onset. Across each row are panels showing the neuronal responses for six training sessions: pretraining with the CSs and unpaired foot shock, two acquisition sessions on the first day of training (sessions A and B), two acquisition sessions on the second day of training (sessions C and D), and the session in which the acquisition criterion was attained.

Table 2. Results of analysis of discriminative neuronal activity in the MG nucleus: comparison of CS+ and CS− by training sessions

		Day 1		Day 2		
Group	Pretraining	Acquisition A	Acquisition B	Acquisition C	Acquisition D	Criterion
Saline	2, 7, 28	4, 7, 15, 17–19, 22, 26, 33	2–5, 7–16, 18–40	4–5, 7–34, 38–40	4–6, 8–31, 33–40	3–4, 6–7, 9–40
Muscimol	14	14	None	None	None	22

show a significant training-induced reduction of firing to the CS− in the saline group. Yet such an effect was indicated by between-group simple effect tests, which showed a significantly reduced neuronal response to the CS− in rabbits of the saline group compared with the response in the rabbits of the muscimol group (session B, 10 msec intervals 7 and 8; and session D, 10 msec intervals 10 and 16). At no interval during any training session did the rabbits in the saline group exhibit a neuronal response to the CS− that was significantly greater than in the muscimol group.

Group differences in MG nuclear responses during pretraining

Inspection of Figure 2 indicates that the stimulus to be used as the CS+ during training elicited a somewhat larger neuronal response than the CS− during pretraining in the MG nucleus of saline group subjects. Indeed, simple effect tests of the four-way interaction means indicated that a significantly greater response occurred to the prospective CS+ than to the prospective CS− during pretraining in 3 of the 40 10 msec intervals in the saline group, whereas a significantly greater response to the CS− than to the CS+ was found in a single 10 msec interval in the muscimol group (Table 2). Although the foregoing analysis showed that significant discriminative TIA developed during training in the saline group but essentially no TIA developed in the muscimol group, it is possible that the preexisting discriminative responses determined whether TIA developed during training. In the extreme case it is possible that large discriminative TIA in MG nucleus only develops in neurons that are predisposed to respond to the CS+.

Table 3. Results of analysis of elicited neuronal activity in the MG nucleus: comparison of changes during the training sessions measured relative to pretraining

		Day 1		Day 2		
Group	Stimulus	Acquisition A	Acquisition B	Acquisition C	Acquisition D	Criterion
Saline	CS+	None	15, 16, 19, 21–27	21, 25	13, 17–36	11, 13, 18–19, 21–22, 25, 36
	CS−	None	None	None	None	None
Muscimol	CS+	None	3	None	3	None
	CS−	3	3	None	3	11, 22

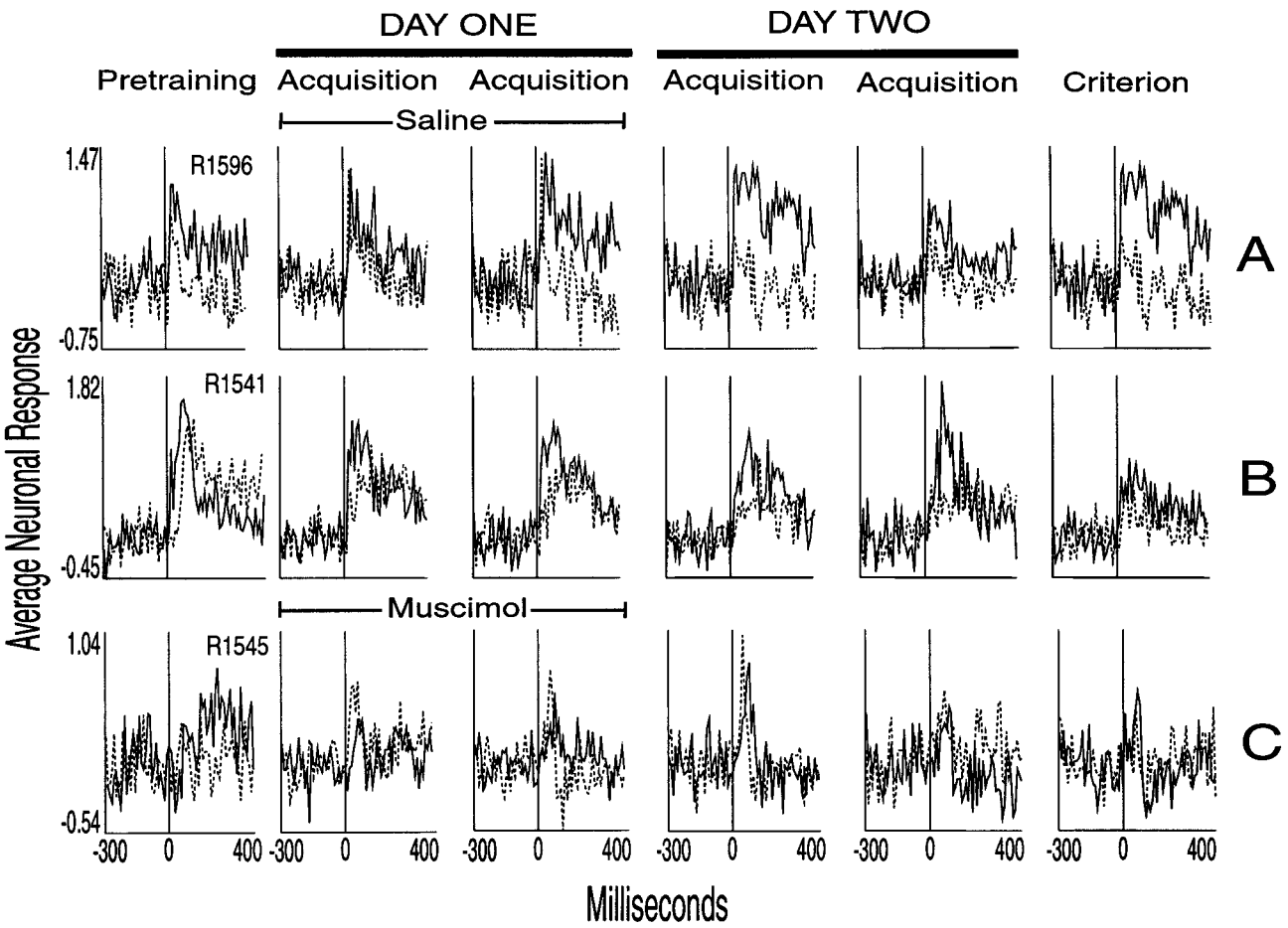

Figure 3. Each row shows the average MG nuclear multiple-unit firing frequency of an individual subject during pretraining with the CSs and unpaired foot shock, two acquisition sessions on the first day of training (sessions A and B), two acquisition sessions on the second day of training (sessions C and D), and the session in which the acquisition criterion was attained. Data are plotted in consecutive 10 msec intervals for 300 msec before and 400 msec after CS onset. The *solid* and *dashed lines* show the neuronal response to the CS+ and the CS−, respectively. The records plotted in *A* and *B* were obtained from subjects in the saline group. The record plotted in *C* was obtained from a subject in the muscimol group. See Results for further explanation.

To examine this issue the neuronal data of individual subjects were plotted. Records of three subjects that represented the variety of outcomes found are shown in Figure 3. Two of the plotted records are from rabbits in the saline group. The record shown in Figure 3*A* had a neuronal response during pretraining that favored the CS+, whereas the record in Figure 3*B* had an initial response that favored the CS−. In both cases robust discriminative TIA (a greater neuronal response to the CS+ than to the CS−) developed during training. The preexisting difference favoring the CS+ (Fig. 3*A*) increased greatly during training. The record in Figure 3*B* is critical in showing development of discriminative TIA despite a greater response to the CS− than to the CS+ during pretraining. The third case shown is for a subject in the muscimol group (Fig. 3*C*). This subject developed no discriminative TIA during training despite a greater initial response to the CS+ than to the CS−. Thus discriminative TIA can develop in individual subjects whether the neuronal population response favors the prospective CS+ or the CS−. Moreover, TIA does not develop when intra-amygdalar muscimol is administered at the outset of training, even when the initial neuronal response favors the CS+. Also, the abolition of TIA in the MG nucleus found here, attributable to a single muscimol injection at

the onset of discriminative avoidance training, has been replicated in an independent study (Talk et al., 2000).

DISCUSSION

This report concerns the neuronal activity of the MG nucleus in rabbits given inactivating intra-amygdalar microinjection of muscimol before the onset of discriminative avoidance training. TIA in the form of greater neuronal firing in response to the positive conditional stimulus (CS+) than to the negative conditional stimulus (CS−) did not develop in rabbits subjected to amygdalar inactivation with muscimol, whereas robust TIA developed in the MG nucleus of the control subjects. Moreover, no TIA developed subsequently during later training sessions (without muscimol) during the completion of behavioral acquisition to a criterion by the rabbits subjected to a single inactivation of the amygdala before the first training session. These findings indicate that a single muscimol-induced inactivation of the amygdala at the outset of training was sufficient to block MG nuclear TIA development while the amygdala was inactivated and also during later training to a criterion, when the amygdala was no longer inactivated by muscimol.

It is important to note that the loss of discriminative TIA in the muscimol group was not attributable to continuing operation of muscimol at amygdalar synapses during the course of behavioral learning. Available data indicate restoration of behavioral function 5–6 hr after microinjection of muscimol in the CNS (Li et al., 1998). Our data showed an absence of significant discriminative TIA throughout training in the MG nucleus of rabbits given a single muscimol injection before the first training day. TIA was absent on the first day (with muscimol present), on the second day (with muscimol absent), and on subsequent days of training to criterion (days 3–8 depending on the learning rate of the particular rabbit) with muscimol absent. Thus, TIA did not develop during the full, multiday course of behavioral learning in rabbits given intra-amygdalar muscimol before the first training day. On the basis of these results, we conclude that amygdalar activity at the outset of training is essential for the development of discriminative TIA in the MG nucleus.

We showed recently that bilateral electrolytic lesions of the amygdala blocked discriminative avoidance learning and TIA development in cingulate cortex and in the limbic (anterior and medial dorsal) thalamic nuclei (Poremba and Gabriel, 1997a). These results indicated that the involvement of the amygdala in the development of TIA extends to structures other than the MG nucleus and that a general function of the amygdala may be to initiate TIA development in multiple areas of the learning-relevant circuitry. These conclusions are intriguingly convergent with the notion that the amygdala is involved in the modulation of memory storage processes in nonamygdalar brain areas (Cahill et al., 1999).

Although our results support the notion that amygdalar neurons initiate learning-relevant change in nonamygdalar brain areas, we hasten to add that our data in no way exclude the possibility that the amygdala is a primary site of fear-conditioning processes, as argued by Fanselow and LeDoux (1999). Indeed, the idea that the TIA exhibited by amygdala neurons is not intrinsic to the amygdala but is rather synaptically driven by TIA in the MG nucleus is not supported by our data. Quite different forms of TIA develop in the amygdala and in the MG nucleus. Amygdalar TIA is primarily a result of increased firing to the CS+ and little or no change in response to the CS− (Maren et al., 1991), whereas TIA in the MG nucleus results from increased firing to the CS+ and decreased firing to the CS−, as found here. These results are compatible with the idea that amygdalar TIA and MG nuclear TIA are based on distinct and separate instances of synaptic plasticity.

The finding that the amygdala plays an essential role in relation to MG nuclear TIA development is surprising. The MG nucleus is positioned upstream from the amygdala with respect to the afferent flow of information from the periphery. Indeed, the MG nucleus is a component of the auditory sensory projection system, whereas the amygdala is not a sensory nucleus. The MG nucleus is also the origin of a direct axonal pathway to amygdalar and periamygdalar areas, yet there is no known direct pathway from the amygdala to the MG nucleus. The latencies of auditory stimulus-elicited neuronal responses and discriminative TIA in the MG nucleus are shorter than in the amygdala (compare the results shown in Fig. 2 with those of Maren et al., 1991). Finally, MG nuclear lesions severely impaired behavioral learning, and they blocked all auditory cue-elicited neuronal firing in the basolateral nucleus of the amygdala (Poremba and Gabriel, 1997b). All of these findings are compatible with feed-forward influence from the MG nucleus to the amygdala. Yet, to our knowledge, there has been no indication in the literature that amygdalar processes influence the MG nucleus, as demonstrated by the present results.

To account for our findings, it is proposed that a combination of the shock US and the novel prediction of the US by the CS+ activate amygdalar neurons at the outset of training. On activation by novel and painful inputs, amygdalar neurons initiate synaptic changes that give rise to discriminative TIA in the MG nucleus. We have recently found that lesions of the auditory cortex eliminated TIA development in the MG nucleus and significantly retarded behavioral acquisition of the discriminative avoidance response (A. Duvel, D. Smith, A. Talk, and M. Gabriel, unpublished results). These results raise the possibility that a portion of the amygdalar influence on MG nuclear TIA is relayed via amygdalar projections to the auditory cortex (McDonald and Jackson, 1987).

It has been proposed elsewhere that MG nuclear TIA is a product of the convergence of subcortical acoustic (CS-related) and somatic sensory (US-related) input to the medial division of the MG nucleus (LeDoux et al., 1987; see also Cruikshank et al., 1992; Bordi and LeDoux, 1994). How can our account above be reconciled with this account?

The notion that convergence of subcortical acoustic and nociceptive afferents accounts for synaptic plasticity in the MG nucleus has been applied to studies of artificially induced synaptic plasticity and neuronal changes in nondiscriminative conditioning paradigms in which neuronal responses emerge as a result of pairing a single acoustic stimulus with shock (Edeline, 1990; Edeline and Weinberger, 1993). The convergence of CS and US information on MG and related neurons may be sufficient to explain these instances of neuronal plasticity. However, in addition to convergent subcortical CS and US information, a contribution that operates via amygdalar projections to the auditory cortex may be particularly important for the production of discriminative TIA, whereby synaptic changes enhance transmission of CS+ frequencies and diminish transmission of CS− frequencies. In this instance, amygdalar afferents could trigger frequency-specific plasticity mechanisms of the auditory cortex that could in turn act via corticothalamic feedback to predispose MG neurons to develop frequency-selective (discriminative) plasticity. This hypothesis is consistent with the conditioning-induced plasticity

of single auditory cortical neuron frequency response profiles elegantly documented by Edeline et al. (1993) and Weinberger and Bakin (1998). Moreover, the results are consistent with earlier findings that the very first conditioning-related changes in neuronal firing occurred in the auditory cortex and were followed later by changes in the MG nucleus (Disterhoft and Stuart, 1976). Of course, the possibility exists that in addition to CS–US convergence, amygdalar modulation is also necessary for the establishment of MG nuclear plasticity during conditioning with just a single CS. To our knowledge, no extant data negate this possibility.

Although MG nuclear TIA did not develop in rabbits subjected to amygdalar inactivation before training, these rabbits did learn as a result of the daily training sessions administered after the initial training session with muscimol. Yet the performance levels exhibited during these later sessions and the levels reached in the criterial session by the rabbits in the muscimol group were significantly reduced compared with the performance levels of controls (Poremba and Gabriel, 1999). In addition, learning-relevant TIA did develop in the cingulate cortex and in the limbic (anterior and mediodorsal) thalamic nuclei during training on the day after amygdalar inactivation (Poremba, 1996). However, just as behavioral performance was impaired, the cingulothalamic TIA was significantly attenuated relative to the TIA in the controls. We offer the suggestion that the reduced performance efficiency and the attenuation of cingulothalamic TIA may have been consequences of the absence of MG nuclear TIA in the rabbits subjected to amygdalar inactivation before the initiation of training. These results suggest that the MG nuclear TIA is one of several discriminative processes that contribute to discriminative avoidance learning. Its removal noticeably impairs but does not prevent behavioral learning. We would not, however, draw the inference that the contribution of MG nuclear TIA to behavioral learning is unimportant. The importance of this TIA to learning and performance could become more substantial in learning tasks characterized by more challenging acoustic processing demands than are imposed by our discriminative avoidance task.

The finding that MG nuclear TIA was blocked throughout training, whereas cingulothalamic TIA was blocked only while muscimol was present in the amygdala, indicates two distinct modes whereby the amygdala modulates plasticity development in nonamygdalar areas. That is, amygdalar efferents trigger MG nuclear TIA during a brief period at the outset of training but are involved in a more sustained manner in maintaining cingulothalamic plasticity during training. This distinction is in keeping with the ever-growing body of evidence that distinct functional processes are mediated by different populations of amygdalar neurons (Hatfield et al., 1996; Killcross et al., 1997; Pitkanen et al., 1997; Da Cunha et al., 1999; Dayas et al., 1999; Parkinson et al., 2000). Although our muscimol injections were well confined to the basolateral nucleus of the amygdala (see Results), these distinct effects could have arisen from possibly different functional characteristics of the separate efferent populations within the basolateral nucleus that project to the auditory cortex (McDonald and Jackson, 1987) and to the cingulothalamic areas (Krettek and Price, 1978; Porrino et al., 1981; Price et al., 1987).

Given that 240 training trials with amygdalar inactivation permanently blocked MG nuclear TIA, it is of interest to consider how much training is needed with muscimol present to block TIA. Such information would delineate the "training window" for the effect, thus helping focus future studies of the specific cellular and molecular influences that promote TIA in the MG nucleus. Recent results indicate that the effect can be obtained with 120 training trials but not with 60 training trials (Talk et al., 2000).

Also, given that training with amygdalar inactivation permanently blocked TIA, it follows that the training experience must have been encoded in some manner despite the inactivation of the amygdala during training. Unless such encoding is assumed, it becomes difficult to explain how the training experience renders the task events less effective later, when the amygdala is operative. We offer the suggestion that the CS+, CS−, shock US, and contingencies among these stimuli are encoded in parahippocampal areas such as the perirhinal and entorhinal cortices in animals that have been trained with an inactivated amygdala. These areas are involved in novelty and familiarity coding of stimuli (Zhu et al., 1997; Xiang and Brown, 1998; Wan et al., 1999). As a consequence of this coding, the task events may be rendered less novel. Later when the amygdala is back on line, the task events now coded as familiar fail to activate the amygdalar processes that engender MG nuclear TIA.

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