Lesions of the thalamic reticular nucleus or the basal forebrain impair Pavlovian eyeblink conditioning and attenuate learning-related multiple-unit activity in the mediodorsal nucleus of the thalamus

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Rabbits received ibotenic acid lesions of the thalamic reticular nucleus or the basal forebrain, or sham lesions, and had multiple-unit recording electrodes implanted in the mediodorsal nucleus of the thalamus (MD). Animals were compared on four sessions of simple Pavlovian conditioning using a tone conditioned stimulus (CS) and periorbital electric shock unconditioned stimulus (US). Eyeblink (EB) and heart rate (HR) responses were recorded, as well as multiple-unit activity (MUA) from MD. MD MUA in sham-lesioned animals showed small but consistent increases in response to the CS. Lesions of either the basal forebrain or the thalamic reticular nucleus decreased the magnitude of the MUA changes recorded from MD and impaired acquisition of the EB conditioned response (CR). Reticular nucleus, but not basal forebrain, lesions slightly enhanced the magnitude of the HR CR. Since MD's efferent projections are to the prefrontal cortex, these results support the idea that MD, possibly due to its forebrain-reticular afferent connections, may participate in a "response selection" function of the prefrontal cortex.

The mediodorsal nucleus of the thalamus (MD) is a primary thalamic nucleus providing inputs to the prefrontal cortex (PFC) and has historically been implicated in many of the same behavioral functions as PFC. Previous studies from our laboratory have indicated that lesions of MD impair acquisition of the classically conditioned eyeblink (EB) response in the rabbit and, to some extent, bias the accompanying conditioned heart rate (HR) decelerations in a parasympathetic direction (Buchanan & Thompson, 1990). Lesions of PFC, on the other hand, dramatically impair the bradycardiac HR conditioned response (CR), but do not affect the EB CR (Buchanan & Powell, 1982; Powell, Watson, & Maxwell, 1994). Multiple- and single-unit activity in PFC is also correlated with acquisition and expression of the HR CR (Gibbs & Powell, 1988, 1991; Maxwell, Powell, & Buchanan, 1994). However, such neuronal activity recorded from MD during classical HR conditioning is also related to acquisition or expression

of the bradycardiac HR CR (Chachich, Buchanan, & Powell, in press; Powell, Watson, & Buchanan, 1990). Since the most dramatic effects of MD damage are on acquisition of the somatomotor EB response, one purpose of the present study was to assess multiple-unit activity (MUA) recorded from MD during acquisition of the classically conditioned EB response using stimulus parameters optimal for such acquisition.

The correlations of MD MUA with the decelerative HR CR are somewhat surprising, since MD has more frequently been implicated in sympathetic, rather than parasympathetic, function (Buchanan & Powell, 1986; Huang, Varner, Barman, & Gebber, 1988; Varner, Barman, & Gebber, 1988). Consistent with this are recent formulations suggesting that MD participates in a "response selection" (Devinsky, Morrell, & Vogt, 1995) function of PFC, perhaps through its connections with basal forebrain structures, such as the ventral pallidum (VP). Such a response-selection process presumably would include sympathetic activation in support of impending somatomotor activity. MD receives input from these forebrain structures both directly and indirectly through forebrain projections to the thalamic reticular nucleus (Groenewegen, Berendse, & Haber, 1993; Mogensen, Ciriello, Garland, & Wu, 1987). PFC, in turn, receives direct connections both from VP and from MD (Alexander, Crutcher, & DeLong, 1990). A second goal of the present experiment, then, was to determine the effect of lesions of these structures afferent to

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MD on the classically conditioned EB and HR responses and, further, to determine what, if any, effects such lesions would have on MUA recorded from MD. Thus, separate groups of animals received lesions of the basal forebrain, including VP, the thalamic reticular nucleus, or sham lesions, and received four sessions of Pavlovian conditioning training during which EB and HR responses were recorded, as well as MUA from MD.

METHOD

Subjects and Surgery

The animals were male and female New Zealand albino rabbits obtained from a local USDA-licensed supplier. The animals were maintained in an AAALAC-accredited animal facility, with a 12:12-h light:dark cycle (lights on at 0700 h). Food and water were available ad lib. All behavioral testing was conducted during the daylight portion of the light:dark cycle. All USPHS regulations regarding animal welfare were followed. All surgery was performed under aseptic surgical conditions. The animals were anesthetized with ketamine hydrochloride (55 mg/kg, i.m.) supplemented by acepromazine maleate (2.2 mg/kg, i.m.) and xylazine (3.0 mg/kg, i.m.). In addition to the primary surgical procedure, chronic electrodes for presenting the unconditioned stimulus (US) and for measuring the HR CR (see below) were also implanted while the animal was anesthetized. All animals had a multiple-unit recording electrode implanted unilaterally in MD (stereotaxic coordinates 3.5 mm posterior to bregma, 1.5 mm lateral to the midline suture, and 8.5–9.5 mm ventral to the dura). Tungsten microelectrodes (25- μ m tip and impedance of 1–4 M Ω at 1000 Hz, Frederick Haer Co.) were stereotaxically implanted and held in place with dental acrylic and jeweler's screws. Two groups of animals received no further surgery. One group (sham control group, $n = 12$) received classical conditioning, as did the lesion animals (see below). The second group (unpaired control group, $n = 8$) received unpaired presentations of the conditioned stimulus (CS) and US as a control for pseudoconditioning (again, see below). Two additional groups of animals received lesions of structures afferent to MD, as well as implantation of an MUA recording electrode in MD. Bilateral ibotenic acid lesions were made in the reticular nucleus or the basal forebrain. Ibotenic acid (Sigma Chemical Co.) was dissolved in phosphate buffer pH 7.4, 10 mg/ml. Lesion injections were made through a Hamilton 1- μ l syringe. Each injection consisted of 0.5 μ l (i.e., 5 μ g) injected over a 20-min period. In the basal forebrain group ($n = 8$), the stereotaxic coordinates were 2 and 4 mm anterior to bregma, 2.0 mm lateral to the midline suture, and 11.5 mm ventral to the dura. In the reticular nucleus group ($n = 9$), the stereotaxic coordinates were 1.0 and 2.0 mm posterior to bregma, 3.0 and 4.0 mm lateral to the midline suture, and 10 mm ventral to the dura. After surgery, the animals were treated prophylactically with bicillin (penicillin G benzathine) and allowed a 2-week recovery period before behavioral testing began.

Apparatus and Procedure

Experimental contingencies were controlled by a PC-based data acquisition system (MACRO, Inc., Columbia, SC) supplemented by solid state transistor-transistor logic programming devices. HR and EB responses were recorded on a Grass Model 7 polygraph equipped with appropriate preamplifiers. During conditioning, the output of the polygraph was connected to the computer where A/D conversion was performed in real time. MUA was conventionally amplified with a WPI Model DAM 80 amplifier, band-passed (0.5–10 kHz) and notch (60 Hz) filtered, and fed into the A/D converter of the computer. During conditioning, the shock US was delivered by a Grass Model S88 stimulator equipped with constant current and stimulus isolation. During the experiment, the animals

were restrained in Plexiglas rabbit restrainers and ventilated, sound- and light-attenuating commercial animal enclosures (Industrial Acoustic Co., Bronx, NY).

The animals received 4 days of Pavlovian conditioning, during which a 1200-Hz, 75-dB tone (0.5 sec in duration) served as the CS and a 0.25-sec, 2-mA, ac electric shock train served as the US. The US was delivered periorbitally through previously implanted stainless steel wound clips (see above). The animals received two sessions of adaptation to the chamber and the restraining apparatus (Gormezano, 1966). Conditioning began on the next day. Immediately preceding the first conditioning session, the animals were presented five tone-alone presentations (i.e., not followed by the shock US) for assessment of the orienting response to novel stimuli. Each conditioning session consisted of 60 trials. The intertrial interval (ITI) was random, ranging from 30 to 90 sec ($M = 60$ sec). Tone duration was 0.5 sec, and tone offset coincided with US onset. To control for nonassociative effects, the unpaired control group received 60 tone and 60 shock presentations that were specifically unpaired and presented in a pseudorandom sequence, with a mean ITI of 30 sec (range, 15–45 sec). EB was measured via electrodes consisting of Truchrome dental wire acutely inserted over the eyelids before the beginning of each session. Insertion of these electrodes caused neither apparent discomfort nor any signs of infection or irritation (Buchanan & Thompson, 1990). Leads for the EB electrodes were connected to a Grass Model 7P3 preamplifier and integrator set in its integrator mode. The preamplifier was calibrated so that a 100 μ V change across the electrodes corresponded to a 1-mm deflection of the appropriate oscillograph pen. Stainless steel safety pins inserted subcutaneously over the left hind flank and right foreleg served as electrocardiographic EKG recording electrodes. Leads from these pins were connected to a Grass Model 7P4 EKG tachograph preamplifier. During conditioning, all EB and HR responses were recorded by the A/D converter of the computer, which sampled at 3000 Hz beginning 2 sec before tone onset and continuing for 1 sec after tone offset, except on 3 "test" trials (Trials 10, 30, and 50), on which the CS was presented but not followed by the shock US. During these test trials, HR was recorded beginning 2 sec before tone onset and continuing until 6 sec after tone onset. Heart period (the reciprocal of HR) was recorded for each interbeat (R–R) interval (IBI), in milliseconds, and converted to HR (expressed as beats per minute [BPM]). This allows for the description of the CR as HR change (in BPM) on a beat-by-beat basis. Particularly with the short ISI used, averaging across beats, or seconds, as is frequently done, can obscure rapidly occurring (in the first 2 or 3 beats) HR changes. These test trials were utilized to allow for measurement of the complete expression of the HR response, which is only possible in the absence of the shock US. After 4 days of training, all animals received 1 day of extinction testing, during which all parameters were identical to conditioning sessions except that no shocks were presented.

Histology

After testing, a marker lesion was made through the recording electrode (20–30 μ A anodal current). Approximately 24 h later, the animals were sacrificed with pentobarbital and perfused with physiological saline and 10% formalin. Forty-micrometer sections throughout the lesions and electrode tip were stained with thionin. Extent of the lesion and electrode placement were then charted relative to stereotaxic plates (Urban & Richard, 1972), using a Leitz drawing tube and microscope.

Data Reduction and Analysis

The criterion for an EB CR was a 200- μ V change from baseline during CS presentation. This is equivalent to a 2-mm deflection of the appropriate polygraph pen and approximately 1 mm of EB or nictitating membrane extension. EB amplitude was recorded as the maximum amplitude change in microvolts from baseline during

the 0.5-sec CS period. HR was recorded only during the 3 test trials (see above). HR was measured during the 2-sec pre- or baseline period and the 6-sec period after tone onset on a beat-by-beat basis and was converted to BPM. MUA was assessed by compiling peri-stimulus time histograms in bins of 20 msec over the 200-msec period immediately preceding and following tone onset. Such MUA was compiled in 10-trial blocks throughout conditioning. Z-score equivalents for each of the tone bins were calculated by subtracting the mean baseline discharge from the discharge evoked by the tone and dividing this difference by the standard deviation of the baseline discharge as previously described (e.g., Maxwell et al., 1994). Offline data reduction software counted as discriminable unit activity any activity above a preselected voltage threshold, which resulted in only the largest 3 or 4 spikes being included in the analysis. This procedure resulted in baseline activity of approximately 20–50 Hz. As a control for movement or other artifact, the software eliminated from Z-score calculation any trial on which more than 3 pre-CS bins demonstrated more than 5 discriminable unit responses (cf. Gabriel et al., 1983). All data were analyzed by repeated measures analysis of variance (ANOVA). Significant effects were further analyzed by post hoc application of Duncan's Multiple Range Test. This approach to the making of multiple com-

parisons adjusts the critical difference depending upon the number and relative ranking of means being tested (Edwards, 1964).

RESULTS

Histology

Histological analysis revealed that 8 animals had bilateral lesions of the anterior portion of the reticular nucleus and 9 had bilateral damage in the basal forebrain. Damage to the reticular nucleus was, for the most part, confined to the anterior regions, which are interconnected with MD (Tai, Yi, Ilinsky, & Kultas-Ilinsky, 1995). In all 8 animals, large portions of this part of the reticular nucleus were destroyed bilaterally. In addition, minor damage to the external medullary lamina and the more lateral nuclei of the thalamus, including VL and VP, also occurred. The damage common to all animals was, however, centered on the reticular nucleus, as illustrated in Figure 1. Basal forebrain damage included large portions

Figure 1. Minimal and maximal extent of damage in animals with ibotenic acid lesions of the thalamic reticular nucleus. MD, mediodorsal nucleus; RET, reticular nucleus; RE, reuniens nucleus; AM, anteromedial nucleus; AV, anteroventral nucleus; MV, medioventral nucleus; VA, ventroanterior nucleus; PT, paratenial nucleus; PAV, paraventricular nucleus; AD, anterodorsal nucleus; LD, laterodorsal nucleus; VM, ventromedial nucleus; VP, ventroposterior nucleus; VL, ventrolateral nucleus; IMD, intermediodorsal nucleus.

Figure 2. Minimal and maximal extent of damage in animals with ibotenic acid lesions of the basal forebrain.

of the VP, portions of nucleus basalis, and more dorsolateral portions of the substantia innominata. On the basis of earlier work in the rat (Heimer, Switzer, & Van Hoesen, 1982; Young, Alheid, & Heimer, 1984), which defined the VP-MD projections via retrograde tracing studies, these lesions destroyed most of the more dorsolateral portions of VP, but left the ventromedial portions intact. However, on the basis of our own unpublished observations (Powell & Maxwell, 1993) in the rabbit, as well as those described by Young et al. (1984) in the rat, the lesions also clearly destroyed other, non-VP basal forebrain projections to MD (referred to by Heimer et al., 1982, as *ventrostriatal* projections). However, for the most part, areas dorsal to the anterior commissure, including the nucleus accumbens, were intact in most animals. The maximal and minimal extent of these lesions is shown in Figure 2.

Location of the electrode sites within MD is illustrated in Figure 3. Sites for recording electrodes in each of the four groups are indicated separately. As can be seen in this figure, electrode sites were located throughout the anterior-posterior and mediolateral extent of MD. There were no discernable differences in locations associated with the four groups.

Orienting

During orienting, the HR response was a deceleration from pretone baseline, which declined somewhat across the five trials. Difference scores were obtained by averaging the HR associated with each IBI during the pretone baseline period and subtracting this mean from the HR associated with each posttone IBI. An ANOVA conducted on this data utilized as dimensions group (4 levels), trial (5 levels), and posttone IBI (10 levels). There were sig-

Figure 3. MUA recording sites in MD. Electrode sites are shown for animals with lesions of the thalamic reticular nucleus (X) or the basal forebrain (Δ) and for sham-lesioned animals receiving either Pavlovian conditioning (paired CS-US ($\bullet$)) or unpaired CS-US presentations ($\circ$). Abbreviations as in Figure 1.

nificant trial and IBI effects and a significant trial $\times$ IBI interaction [smallest $F(36,800) = 1.88, p < .001$, for trial $\times$ IBI]. There were no significant group or group interaction effects on this measure. There was minimal tone-evoked MUA during orienting; there were no significant effects on this measure, indicating that any changes taking place were random and unrelated to the presentation of the tone.

Acquisition

Eyeblink. Acquisition of the EB response was analyzed by an ANOVA of percent EB CRs per session, using as dimensions group (4 levels) and session (4 levels). These data are shown in Figure 4. There was a significant session effect [$F(3,93) = 67.53, p < .0001$], reflecting the acquisition of the response across sessions. There were also significant effects for group [$F(3,32) = 14.44, p < .0001$] and group $\times$ session [$F(9,93) = 6.48, p < .0001$]. Post-tests (Duncan's) revealed that the group effect was due solely to significantly lower responding by the unpaired group than by any of the other three groups throughout training ($p < .05$, for all comparisons). The significant group $\times$ session effect was due primarily to higher levels of responding by the sham group, particularly during the second and third sessions. The unpaired group showed minimal responding throughout training. The responding of all other groups was significantly greater than that of

these animals throughout Sessions 2–4 ($p < .05$, for all comparisons). On the second session, the sham animals also showed higher levels of responding than did either the basal forebrain or reticular nucleus groups ($p < .05$, for each comparison). On the third session, the sham group

Figure 4. Mean percentage conditioned eyeblink responses in animals with basal forebrain or reticular nucleus lesions and sham-lesioned animals receiving paired or unpaired CS-US presentations. Data are shown for four acquisition and one extinction session.

Figure 5. Mean heart rate change from baseline across four acquisition sessions for animals with basal forebrain or reticular nucleus lesions and sham-lesioned animals receiving paired or unpaired CS-US presentations, as indicated. Data are shown in beats per minute (BPM) and represent the first 10 interbeat intervals after tone conditioned stimulus onset during nonshock test trials.

showed higher responding than did the reticular group ($p < .05$). Examination of Figure 4 indicates that the response of the sham animals increased significantly between Session 1 and Session 2 and continued to increase from Session 2 to Session 3, by which time they had reached asymptotic levels of responding. The reticular lesion animals showed significantly lower levels of responding throughout Sessions 2–4 than did the sham animals ($p < .05$, for all sessions). However, the group with basal forebrain lesions behaved more like the sham group, although, as mentioned, their response levels were significantly lower than those of the shams on Session 2 ($p < .05$). These animals thus showed a slightly slower acquisition function, but reached asymptotic levels comparable to those of the sham animals. EB amplitude measures fairly consistently followed the percent EB measures, with increases in amplitude appearing across sessions, particularly from Session 1 to Session 2. This was reflected in a significant session effect [$F(3,55) = 3.97$, $p < .01$]. No group effect or group interaction was, however, significant for this variable.

Heart rate. During acquisition, the HR CR evoked by the tone in the paired groups was in all cases the normally obtained deceleration from baseline, which became somewhat smaller across sessions. However, in the unpaired group, only small and variable changes were obtained. These data are depicted in Figure 5, which shows HR change from pre-CS baseline as a function of post-CS IBIs averaged across sessions and trials. Again, these difference scores were obtained by averaging the HR associated with each IBI during the pre-CS baseline period and subtracting this mean from the HR associated with each post-CS IBI. An ANOVA revealed significant effects for session, IBI, and session $\times$ trial (smallest $F = 2.45$, $p < .03$, for session $\times$ trial). All of these effects re-

flect the significant change from baseline and the decline in the magnitude of the response across sessions. In addition, there were significant effects for group [$F(3,31) = 3.58$, $p < .02$] and group $\times$ IBI [$F(27,272) = 2.73$, $p < .0001$]. The group effect reflects the smaller decelerations by the unpaired group; changes in this group were significantly smaller than in any of the other three groups ($p < .05$, for all comparisons). In general, the animals in the reticular group showed larger HR decelerations than those in any of the other groups. The magnitude of the response in this group increased from Session 1 to Session 2 and then remained relatively large, whereas, in the other groups, there was a consistent decline in magnitude of the response across Sessions 1–4 (not shown in Figure 5). These effects were reflected in the group $\times$ IBI effect. Posttests (Duncan's) showed that the reticular animals showed larger decelerations during IBIs 4, 5, 6, and 7 than did any of the other groups ($p < .05$, for all comparisons). In addition, during the early IBIs, the animals in the sham group tended to show larger changes than did the remaining groups; these differences were significant during IBIs 1 and 2 ($p < .05$). Most of these effects had disappeared by the end of the trials so that IBIs 8, 9, and 10 showed a significant group effect only for the comparison of the unpaired group versus the other three groups ($p < .05$, for all comparisons).

Multiple-unit activity. Overall, MUA changes recorded from MD during conditioning were small. However, they were reliable and systematically related to the classical conditioning contingencies. These data are shown in Figure 6, which shows mean multiple-unit discharge, in Z-score equivalents, for the sham group compared with each of the other three groups. These data are shown for ten 20-msec bins after CS onset, for each of the four sessions. Asterisks indicate significant comparisons (Duncan's, $p < .05$). There was a significant bin effect [$F(9,270) = 4.13$, $p < .0001$], reflecting increases in MUA across the ten 20-msec bins. There was also a significant trial-block effect [$F(5,145) = 4.24$, $p < .001$] and a significant session $\times$ trial-block $\times$ bin interaction [$F(135,3010) = 1.25$, $p < .03$]; these effects are not shown in Figure 6. However, the significant trial-block effect reflects larger responding during the early trial blocks (Trial Blocks 1 and 2, i.e., early during each session), with somewhat smaller responding later within each session. The session $\times$ trial-block $\times$ bin effect reflects the fact that, on Session 1, there was less decrease across trial blocks than on later sessions. This difference was significant only for the last trial block (Trial Block 6) of the first session, and only during Bins 2, 3, 6, 9, and 10 ($p < .05$, for all comparisons).

There was also a small but significant effect for group $\times$ session $\times$ bin [$F(81,702) = 1.48$, $p < .005$]. Posttests conducted on this effect indicated that, in general, the sham group showed significantly larger CS-evoked MUA increases than did the other three groups (see Figure 6). The unpaired control group showed only small and variable changes. Indeed, this group showed changes that were significantly different from baseline (i.e., $Z > 1.96$) on only 2 or 3 bins per session. In contrast, the paired group

Figure 6. Mean CS-evoked multiple-unit activity recorded from MD. Data are plotted as a function of 20-msec intervals after CS onset in Z-score units normalized with respect to pre-CS (baseline) discharge. Data are shown for each of the four acquisition sessions, separately comparing the sham-lesioned animals receiving paired CS-US presentations (i.e., conditioning) with sham-lesioned animals receiving unpaired CS-US presentation (top), animals with reticular nucleus lesions (middle), and animals with basal forebrain lesions (bottom). *Significant comparisons, $p < .05$.

showed significant changes on 38 of the 40 bins during acquisition (nonsignificant changes on the 2nd and 3rd bins of Session 2). This latter finding also suggests that the associative changes were rapidly acquired, probably during the first few trials. The reticular group was not significantly different from the unpaired group at any bin on any session and, in fact, actually showed significantly lower levels of responding than did the unpaired group on isolated bins (Bins 9 and 10 on Session 2; Bin 1 on Session 3; Bins 1, 7, and 8 on Session 4). As can be seen in Figure 6, the reticular lesion group also showed significantly lower levels of responding than did the sham group virtually throughout training. The basal forebrain animals tended to show smaller changes than did the shams, particularly in the first 2 sessions. On the last two sessions, however, these animals differed from the shams only on isolated bins, as shown in Figure 6. As with the reticular lesion group, these animals were significantly different from the unpaired animals only on isolated bins (Bins 6–10 on Session 3; Bins 4 and 10 on Session 4).

It has been reported that MUA recorded from lateral MD is more robust and appears earlier than that recorded from medial MD (Orona & Gabriel, 1983a, 1983b). A com-

parison of lateral versus medial placements in the present study, however, suggested the opposite (i.e., that medial placements yielded larger MUA than did lateral placements). Thus, an ANOVA showed a significant subdivision (medial vs. lateral) $\times$ session $\times$ trial-block effect [$F(15,68) = 1.89, p < .04$], due to larger responding in the medial group on all but one trial block during Session 4 (Duncan's, $p < .05$, for Trial Blocks 1, 2, 3, 5, and 6). However, due to the small and unequal n s in this analysis, these results must be interpreted with extreme caution.

In summary, the sham-lesioned animals showed increases in MUA in response to the classical conditioning contingencies. These responses were small but reliable and were consistently larger than the responses of the other three groups. Either lesion decreased the magnitude of the response.

Extinction

Eyeblink. All animals showed a decrease in EB responding during extinction (see Figure 4). There was a significant group effect [$F(3,32) = 9.06, p < .0002$]. However, this effect was due solely to the lower levels of responding in the unpaired group, relative to those of the

other three groups ($p < .05$, for all comparisons). As would be expected, these animals showed much the same level of responding as they had throughout training. All other groups showed decreases in responding. Examination of Figure 4 indicates that, during extinction, in the sham and basal forebrain lesion groups, responding decreased to the levels of the group with reticular nucleus lesions. It can be seen from this figure that the basal forebrain animals showed slightly higher levels of responding during extinction than did the other groups, but this was not significant, except in comparison with the unpaired group.

Heart rate. Although there were some decreases in responding (i.e., smaller magnitude decelerations) during extinction, most animals continued to show HR decelerations during this session. There were significant effects of IBI [$F(9,269) = 3.96, p < .0001$] and trial $\times$ IBI [$F(18,460) = 1.63, p < .05$], reflecting this continued responding. Furthermore, there was a significant group $\times$ IBI effect [$F(27,269) = 2.04, p < .002$]; as during acquisition, this was almost solely due to larger magnitude decelerations in the reticular nucleus lesion group, relative to those in the remaining three groups. This was particularly apparent for the middle IBIs (i.e., 5, 6, and 7; $p < .05$), when the response of all animals was at its greatest.

Multiple-unit activity. As with the other responses, CS-evoked MUA declined during extinction. There was, however, still a significant bin effect [$F(9,243) = 4.47, p < .0001$], suggesting that there was still differential responding across bins, even during extinction. There was also a significant group $\times$ bin effect [$F(27,243) = 1.90, p < .006$] due to continued responding by the basal forebrain group during this session, relative to that of the other three groups. The basal forebrain lesion group showed higher response levels than did the unpaired group during Bins 7–10, than did the reticular nucleus group during Bins 8–10, and than did the sham group during Bins 8 and 9 ($p < .05$, for all comparisons). In fact, only the basal forebrain group demonstrated any significant MUA responding (i.e., $Z > 1.96$) during extinction, and only on Bins 7–10. During all bins of extinction, the sham group was virtually identical to the unpaired group, indicating extinction of this response, along with the EB and HR responses.

Baseline measures. Separate analyses were conducted on baseline HR and baseline MUA during each of the three parts of training (i.e., orienting, acquisition, and extinction). There were no significant group or group interaction effects on baseline HR or MUA at any stage of training.

DISCUSSION

The results of this experiment may be summarized as follows. MUA recorded from MD in otherwise intact animals showed small but consistent CS-evoked increases, which were associated with execution of the classically conditioned EB and HR responses. These CS-evoked MUA increases were significantly greater than in an identical sham lesion group receiving unpaired CS and US presentations, were nonsignificant in response to

nonreinforced tone presentations during orienting, and declined to nonsignificant levels during extinction. These findings all suggest that these increases in neuronal activity were associatively produced. Lesions of the thalamic reticular nucleus or of the basal forebrain decreased the magnitude of the MUA changes recorded from MD. These lesions also impaired acquisition of the EB response and, in the case of reticular lesions, decreased asymptotic responding. These effects are similar to those seen after lesions of MD, which also retard acquisition of the EB response but have no effect on asymptotic responding (Buchanan & Thompson, 1990). A similar effect of MD lesions was reported on acquisition of a discriminative running wheel avoidance task, although, on this task, MD lesions had little effect on acquisition of the response but significantly impaired asymptotic responding (Gabriel, Sparenborg, & Kubota, 1989).

The HR CR was little affected by either lesion, although reticular nucleus lesions caused slightly larger overall HR decelerations due to a failure of this group to show smaller changes as training progressed, both within and across sessions, as was seen in the other conditioning groups. This may reflect an actual lesion effect on the HR CR, or it may reflect a smaller sympathetic bias associated with EB CR execution. In normal animals, as EB CRs increase, the HR CR becomes less decelerative and may actually assume an accelerative component by the end of training, presumably due to increased sympathetic demand produced by increased somatomotor activation (Powell & Joseph, 1974). The HR response in the reticular lesion group resembles that seen after lesions to MD (i.e., an apparent bias in a parasympathetic direction as evidenced by larger than normal HR decelerations). It is also possible that the exaggerated HR CR could represent, at least in part, an enhanced orienting response (OR) to the omission of the shock US, since HR was measured only on such shock-omission test trials, due to presence of shock artifact after US presentation. The complete expression of the HR CR on nonshock test trials frequently includes such an orienting component, coincident with tone offset, when the shock is expected. With a 0.5-sec CS, as was used in the present experiment, tone offset occurs after 2 or 3 heartbeats (IBIs). As can be seen in Figure 5, there is, in the reticular lesion group, a large continued deceleration after this time, supporting the idea that the enhanced HR CR contains at least some component of an increased OR to shock omission. However, direct assessment of the HR OR to nonreinforced tones prior to conditioning showed no lesion effect. It would be expected that lesion effects on the OR would be manifest here, as well as during conditioning. It is also possible that the larger HR CR in the reticular lesion group represents sensitization to the tone as a result of US exposure. This possibility cannot be eliminated without examination of responding in a reticular lesion group given unpaired CS–US presentations, which was not done in the present study.

Devinsky et al. (1995) recently suggested that the MD projection cortex (i.e., the medial prefrontal cortex [mPFC]) is important for visceromotor control and may

also participate in a response-selection process, particularly in situations requiring novel response choices. These authors suggest that this area of the cortex is involved in early stimulus processing (as would be reflected in the HR CR) and in preparation for motor responses (such as the EB CR) but is not involved in long-term storage of information. These authors further suggest that these two functions (i.e., visceromotor control and response selection) may be "co-activated" when appropriate, as in learning situations. In the rabbit EB and HR classical conditioning model utilized here, mPFC appears necessary for acquisition of conditioned HR changes (e.g., Buchanan & Powell, 1982; Powell et al., 1994), but it does not appear necessary for EB CR acquisition. Damage to MD, on the other hand, impacts minimally on HR CR acquisition but significantly impairs EB CR acquisition (Buchanan & Thompson, 1990). We have previously suggested that these results implicate MD in the response-selection function of mPFC, but less directly in the visceromotor function (Buchanan & Thompson, 1990; Chachich et al., in press; Powell et al., 1990).

The present results support the idea that basal forebrain-reticular nucleus-MD connections may participate in such a response-selection function of PFC. It has been suggested that such connections are particularly relevant to initiation of motor behavior in response to salient environmental stimuli (Churchill, Zahm, Duffy, & Kalivas, 1996). Recent studies have demonstrated an inhibitory GABAergic input from the basal forebrain (specifically VP) to MD (Churchill, Zahm, & Kalivas, 1996), as well as to the reticular nucleus. It has also been reported that VP stimulation inhibits firing of MD cells (Mogensen et al., 1987; Vives & Mogensen, 1985). However, MD cells can also show a longer latency excitatory response after VP stimulation (Lavin & Grace, 1994), probably due to disinhibition of reticular-MD connections (Churchill, Zahm, & Kalivas, 1996). The reticular nucleus plays an important role in control of thalamocortical activity in a variety of dorsal thalamic nuclei (Steriade, Contreras, Amzica, & Timofeev, 1996). During brain-activated states (waking and REM sleep), reticular neurons discharge tonically and produce short-lasting inhibition of dorsal thalamic relay nuclei. During resting sleep, reticular neurons show prolonged spike bursts and produce long-lasting inhibition in relay nuclei (Steriade et al., 1996). The fast oscillations in reticular cells appear to result from interactions among neocortical and other thalamic nuclei rather than from some intrinsic "pacemaker" properties (Steriade, McCormick, & Sejnowski, 1993). It has been suggested that the role of tonically active reticular neurons, as during waking, is to enhance signal-to-noise ratio in dorsal thalamic nuclei, by reducing background activity relative to activity to specific input (Warren & Jones, 1994). Such input may be direct sensory input, in the case of the primary relay nuclei, or more nonspecific (e.g., mesopontine input), in the case of the medial and intralaminar nuclei (Spreafico, Amadeo, Angoscini, Panzica, & Battaglia, 1993). It is tempting to speculate that the reticular nucleus' regulation of MD assumes a similar form (i.e.,

the reduction of background activity in MD relative to specific activity). In the case of MD, such specific activity could well be relevant in learning situations such as the present experiment such that removal of reticular inputs to MD lowers the signal-to-noise ratio, thus making appropriate response selection more difficult.

The similarity of effects seen after lesions of the basal forebrain to those after reticular nucleus lesions suggest that the direct basal forebrain-MD connections may also be involved in the present task. However, it should be noted that the basal forebrain lesions in the present study were not restricted to VP but also destroyed cholinergic cells, which have direct connections with cortex and which have themselves been implicated in various aspects of learning and memory (Dekker, Connor, & Thal, 1991). Previous work using the present preparation indicated that nucleus basalis lesions attenuated the HR CR but had no effect on the EB CR (Ginn & Powell, 1992). These effects are, of course, exactly opposite from those obtained in the present study. It therefore seems unlikely that these cholinergic connections are responsible for the present results, although the present data do not allow for a firm conclusion in this regard.

It is important to note that the proposed "response selection" occurs well before overt movement and represents some level of processing that determines whether or not a response is necessary and the correct response if one is required (Devinsky et al., 1995). It has also been suggested that this process is particularly important in situations involving motivationally significant stimuli (Heimer et al., 1982), such as the CS and US in classical conditioning. The proposed involvement of these forebrain-reticular-MD structures in a response-selection process is therefore independent of any direct skeletomotor function. Thus, in the present study, while no direct quantification of the EB unconditioned response (UR) was made (due to the presence of shock artifact), there was no observational evidence of any impairment of the lesioned animals' ability to execute the EB response. This is further supported by the absence of lesion effects on EB amplitude, again suggesting that, when the response occurs, it is normal in form. Indeed, given that forebrain and reticular inputs to MD are inhibitory (Churchill, Zahm, & Kalivas, 1996), it would be expected that, if anything, removal of these structures would increase MD activity and perhaps behavioral responding as well. Thus, while further studies specifically examining the UR following forebrain or reticular lesions will be required to definitively answer this question, it appears unlikely that the present results are due to deficits in the performance of the EB response.

For the most part, the results obtained here in sham animals are compatible with those of earlier studies in rat and rabbit. Orona and Gabriel (1983a, 1983b), for example, reported CS-evoked differential short latency increases in MUA in both the mPFC and the MD in rabbits during a CS+ tone signaling footshock during a running wheel avoidance response. Such increases occurred during both pretraining and conditioning sessions and were

significantly greater than changes recorded during a CS– tone that never signaled footshock. In an earlier study in the rat, Disterhoft and Olds (1972) also reported significant differential MUA increases in a variety of thalamic and cortical structures, including MD, in response to a tone CS+ that predicted food, relative to those in response to a second tone CS– that was never paired with food. Also compatible with the present results, these investigators reported that CS-evoked MUA in MD was small relative to that obtained from other thalamic and cortical areas. Delacour (1984) has also reported that MD cells, unlike thalamic midline or hippocampal pyramidal cells, show significantly greater activity in response to a reinforced CS+ relative to that in response to an unreinforced CS–. As mentioned above, we have also found that MD multiple- and single-unit activity is correlated with discriminative HR CRs (Chachich et al., in press; Powell et al., 1990). The changes recorded in the present study were smaller than in our earlier studies and showed no clear acquisition function. However, this may be due to the different parameters employed (i.e., the 0.5-sec CS, which is optimal for EB conditioning, relative to the 4.0-sec CS used earlier, which is optimal for HR conditioning). A second difference between the present study and earlier ones, including those from other investigators, is the use of a simple conditioning paradigm, as opposed to a discrimination task. We have previously suggested that MD may participate in the postulated response-selection process differentially, such that its participation is more important under situations of greater stimulus uncertainty or ambiguity (Buchanan, 1991). The simple classical conditioning task studied here is relatively unambiguous (i.e., a less difficult task). MD activity may thus be minimal under such circumstances. Consequently, MD lesion effects on this task are relatively small, and MUA changes accompanying the task are also small. Orona and Gabriel (1983a, 1983b) also reported differences in MUA responding of lateral versus medial MD cells during acquisition and reversal of the running wheel avoidance response. Lateral MD demonstrated earlier developing and more robust discriminative responding than did medial MD (Orona & Gabriel, 1983a, 1983b). These effects mimicked those obtained from the prefrontal cortical subdivisions interconnected with medial versus lateral MD (Orona & Gabriel, 1983a, 1983b). In the present study, the response of MD cells appeared to be more homogeneous, although there were too few placements in each subdivision for a truly valid comparison.

In summary, the present findings show that CS-evoked increases in MD MUA are rapidly acquired during classical EB conditioning and that damage to the reticular nucleus or the basal forebrain, both of which provide inputs to MD, dramatically affect these neuronal changes. Since changes in MD neuronal activity evoked by classical conditioning contingencies also occur when no overt somatomotor response is learned (Chachich et al., in press; Powell et al., 1990), we have previously suggested that MD processes information necessary for optimum operation

of an mPFC-mediated response-selection mechanism but does not directly participate in such a mechanism. The finding that lesions of the reticular nucleus and basal forebrain impair EB conditioning, while decreasing CS-evoked MD neuronal activity, are clearly compatible with this hypothesis.

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