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Basolateral amygdaloid multi-unit neuronal correlates of discriminative avoidance learning in rabbits

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Basolateral (BL) amygdaloid multi-unit activity was recorded as male albino rabbits learned to avoid a foot-shock unconditioned stimulus (US) by stepping in an activity wheel to an acoustic (pure tone) warning stimulus (CS+). A second tone (CS–) of different auditory frequency than the CS+ was presented in an irregular order on half of the conditioning trials but was never followed by the US. BL amygdaloid neurons developed, in the first session of conditioning, enhanced CS–elicited discharges relative to discharges recorded during pretraining with tones and noncontingent US presentations (excitatory plasticity), and greater discharges to the CS+ than to the CS– (discriminative plasticity). The discriminative plasticity attained maximal magnitude as the rabbits reached the asymptote of behavioral discrimination, and persisted during post-asymptotic training. Peak excitatory plasticity occurred in the session of the first significant behavioral discrimination and declined during the asymptotic and post-asymptotic stages of training. Similar patterns of excitatory and discriminative plasticity in structures directly interconnected with the BL nucleus (anterior cingulate cortex; medial dorsal thalamic nucleus) and effects of lesions suggest that the neurons in these areas participate in a circuit involved in mediation of avoidance learning.

Neurons comprising the amygdaloid nuclei have been implicated in the mediation of several learned behaviors (see Sarter and Markowitsch[32], for review), including active and passive avoidance learning[5,19,34], classical conditioning of bradycardia[1,20,21], recognition learning in primates[26,27,33], appetitive Pavlovian conditioning[15], and fear conditioning[18,23,24]. The present study is part of an ongoing analysis of the neural mediation of discriminative avoidance learning in rabbits. Past studies of this project indicate involvement of the anterior cingulate cortex and medial dorsal (MD) thalamic nucleus in the mediation of this learning[8]. Neurons in these areas are directly interconnected with basolateral (BL) amygdaloid neurons[22,29,30], suggesting that all 3 brain areas are nodes of a functionally unified learning-relevant brain circuit. Here we describe BL amygdaloid multi-unit correlates of discriminative avoidance learning as a step toward assessment of the role of this area in discriminative avoidance learning and the interactions of BL amygdaloid neurons with neurons in interconnected cortical and thalamic areas. Preliminary results have been presented in an abstract[25].

The subjects were 9 male New Zealand White rabbits weighing 1.5–2.0 kg on delivery from a professional supplier. They were maintained in individual living cages on ad libitum water and rabbit chow on a 12 h light/dark cycle. All testing was conducted during the light phase.

Six multi-unit recording electrodes (500–2000 kΩ impedance) made from stainless-steel pins insulated with Epoxylite were implanted in each rabbit using the stereotaxic atlas of Girgis and Shih-Chang[16]. Surgical anesthesia was induced by a subcutaneous injection (1 ml/kg) of a solution containing ketamine HCl (60 mg/ml) and the muscle relaxant xylazine (8 mg/ml). Anesthesia was maintained with hourly injections (1 ml) of the solution. The rabbits received either unilateral (n = 6) or bilateral electrode placements (n = 3) in the basolateral amygdaloid nucleus at stereotaxic coordinates of 1.5 mm posterior, 5.0 mm lateral, and 14.2–16.2 mm ventral to bregma. Other target sites were the nucleus accumbens, bed nucleus of the stria terminalis, anterior cingulate cortex and the MD thalamic nucleus.

After a minimum interval of 10 days following surgery, training was given and unit activity recorded while the rabbits occupied a Brogden–Culler running wheel apparatus contained within an aluminum chamber that provided electrical and acoustical shielding[2]. The conditional stimuli were pure tones (1 or 8 kHz, 85 dB re 20 N/m^2, 500 ms in duration, rise time of 3 ms), played through a speaker located directly above the wheel. The tone

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frequency chosen as CS+ was counterbalanced. Onset of the positive conditional stimulus (CS+) was followed after 5 s by onset of the unconditional stimulus (US), a constant AC current of 1.5–2.5 mA delivered through the grid floor of the wheel. The negative conditional stimulus (CS–) was never followed by footshock. Both CSs and the US were terminated by behavioral responses, defined as wheel rotations exceeding 2 degrees. Responses prior to US onset prevented US delivery. The maximum duration of the US in the absence of locomotion was 1 s. The intertrial interval (from the end of the CS or of wheel rotation when locomotion occurred) to the onset of a new CS was 5, 10, 15, or 20 s. These values occurred in an irregular sequence. Intertrial responses reset this interval.

Sixty trials with each conditional stimulus were given in each daily training session. CS+ and CS– trials were presented in an irregular order. Prior to avoidance conditioning each rabbit received 2 pretraining sessions. In the first of these, the tones to be used as conditional stimuli were presented alone and in the second the tones were presented with the US interspersed in a noncontingent, explicitly unpaired manner[31]. The pretraining sessions provided baseline (control) values of the behavioral and neuronal responses under conditions of stress comparable to the training situation but without the CS–US contingency critical for learning. After pretraining, the subjects were trained in daily acquisition sessions to a performance criterion which required that the proportion of trials with behavioral responses to the CS+ exceed the proportion of trials with responses to the CS– by 0.60 or more in 2 consecutive sessions. Past experience with this task has shown that the average performance of rabbits does not improve with training beyond this criterion. After criterion attainment 3 additional training sessions (overtraining) were administered.

Neuronal records were fed into field-effect transistors (FETs) that served as high impedance source-followers located about 2.5 cm from the recording sites within the brain. The FET outputs fed via shielded cable were split, one limb entering single-ended preamplifiers with bandwidth appropriate for unit recording (gain = 8000, 1/2 amplitude cutoffs at 500 and 8000 Hz), the other limb entering preamplifiers for electroencephalographic (EEG)

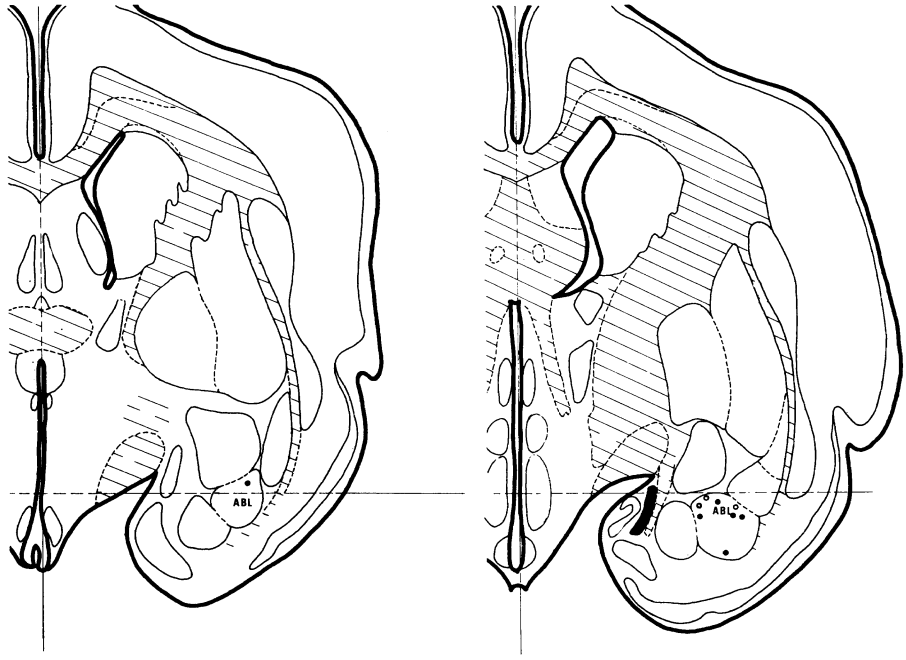

Fig. 1. Schematic representation of electrode placements in the basolateral amygdaloid nucleus. Closed circles represent placements in the left hemisphere and open circles represent those in the right hemisphere.

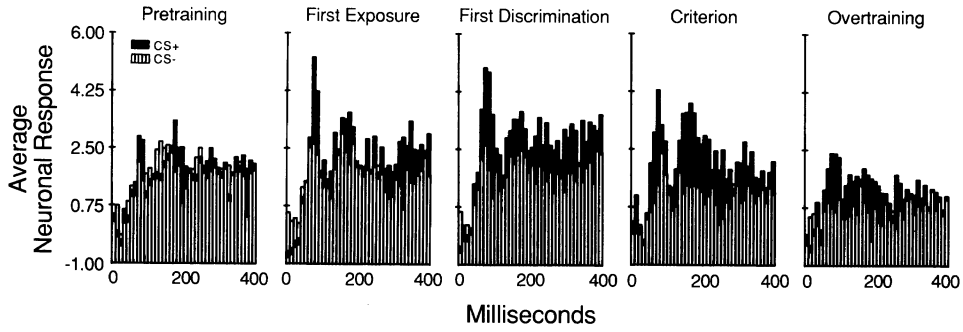

Fig. 2. Each panel represents the average spike frequency elicited in the amygdaloid basolateral nucleus in response to the CS+ (black bars) and the CS– (white bars), plotted for 40 consecutive 10 ms intervals following conditioned stimulus (CS) onset. The ordinate values are in standard deviations (z-score units), reflecting response magnitude relative to the baseline (pre-CS) period. The values on the abscissa represent elapsed 10 ms intervals following CS onset. Each panel represents the data for one behavioral stage of training. The behavioral training stages are: pretraining (Pretraining), the first session of conditioning (First Exposure), the first significant behavioral discrimination (First Discrimination), the criterial session (Criterion), and the third session of overtraining (Overtraining).

recording (gain = 4000, 1/2 amplitude cutoffs at 0.2 and 60 Hz). The unit activity was subjected to a second stage of active bandpass filtering (1/2 amplitude cutoffs at 600 and 8000 Hz, rolloff = 18 dB/octave) to remove all slow EEG frequencies. The unit records were then fed to Schmitt triggers. The triggering thresholds were automatically adjusted under computer control during testing so that they were exceeded by the largest 3 or 4 spikes on each record. In addition, the bandpass filter outputs were half-wave rectified and integrated. The time constants for the rise and the fall of the integrators were 15 and 75 ms, respectively. The Schmitt trigger outputs reflected the discharge frequency of the largest spikes on each record, whereas the integrated activity measured the oscillations of the entire multi-unit record, including activity below the triggering thresholds. The Schmitt trigger pulses were counted and the integrator and macropotential signals digitized during each trial (CS presentation) for a 1 s interval, 0.3 s before CS onset and 0.7 s after CS onset. A digital value was stored for each measure and electrode, every 10 ms during the sampling interval. Individual trial data were stored on digital magnetic tape and subsequently averaged and subjected to statistical analysis.

Neuronal and behavioral data of 5 training stages shared by all subjects were analyzed. Each stage was constituted by the data of a single training session. The sessions were: (a) pretraining with the CSs and noncontingent US; (b) the first session of training; (c) the session of the first significant behavioral discrimination (the first session in which the proportion of trials with behavioral responses to the CS+ exceeded the proportion of trials with responses to the CS– by 0.25 or more); (d) the session in which the criterion of learning was attained (see above); and (e) the third session of overtraining. Peristimulus histograms were compiled for each training stage. The firing frequencies and integrated discharges in 40 10 ms intervals after CS onset were normalized relative to the 30 (baseline) 10 ms pre-CS intervals. The neuronal data in the form of z-scores were submitted to

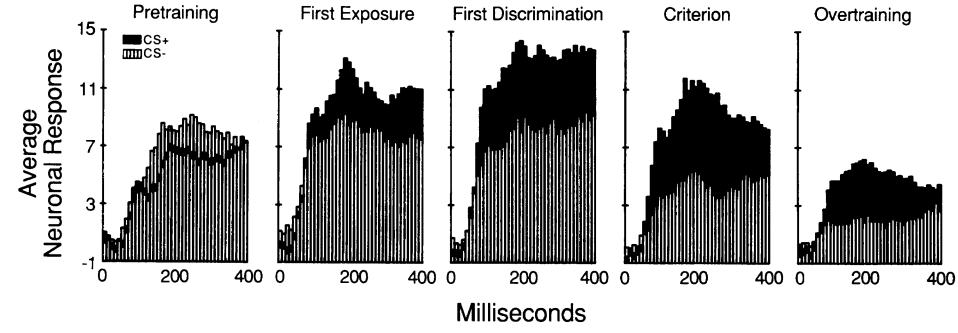

Fig. 3. Each panel represents the average integrated unit activity elicited in the amygdaloid basolateral nucleus in response to the CS+ (black bars) and the CS– (white bars), plotted for 40 consecutive 10 ms intervals following conditioned stimulus (CS) onset. The ordinate values are in standard deviations (z-score units), reflecting response magnitude relative to the baseline (pre-CS) period. The values on the abscissa represent elapsed 10 ms intervals following CS onset. Each panel represents the data for one behavioral stage of training. The behavioral training stages are: pretraining (Pretraining), the first session of conditioning (First Exposure), the first significant behavioral discrimination (First Discrimination), the criterial session (Criterion), and the third session of overtraining (Overtraining).

a factorial repeated measures analysis of variance (mixed model) with orthogonal factors of subject (9 levels), training stage (5 levels as described previously), conditional stimulus (2 levels: CS+ and CS–), and interval (40 levels, the consecutive 10 ms intervals after CS onset). Individual comparisons in the form of two-tailed *t*-tests (alpha = 0.05, see Winer[35]) were carried out following significant *F*-tests to assess excitatory and discriminative neuronal plasticity in each 10 ms interval after CS onset.

Following behavioral training, the rabbits received an overdose of sodium pentobarbital and transcardiac perfusion with normal saline followed by 10% formalin. The brains were removed, frozen, and sectioned at 40 μm. The sections were photographed while still wet[7]. Histological confirmation of electrode placements was carried out by examination of the photographs. After drying, the sections were treated with a metachromatic nissl and myelin stain using formolthionin[6]. Nine electrodes were confirmed in the BL amygdaloid nucleus (Fig. 1). Further details of all procedures are provided in other reports[14].

Acquisition of the discriminative avoidance response was essentially identical to that reported previously[28]. The mean number of sessions to the completion of the acquisition criterion was 4.5. At criterion the mean percentage of avoidances was 84.7% and the mean percentage of responses to the CS– was 14.6%.

The spike frequency (SF) and integrated unit activity (IUA) data are shown in Figs. 2 and 3, respectively. The ANOVA revealed significant *F* ratios for the main effect of training stage [SF: $F_{4,32} = 9.28$, $P < 0.0001$; IUA: $F_{4,32} = 9.09$, $P < 0.0001$], indicating that the neuronal activity was significantly altered during the course of behavioral acquisition. In addition, the interactions of training stage and post-CS interval [SF: $F_{156,1248} = 1.55$, $P < 0.0001$; IUA: $F_{156,1248} = 3.78$, $P < 0.0001$], CS type and interval [SF: $F_{39,312} = 1.84$, $P < 0.0025$; IUA: $F_{39,312} = 2.89$, $P < 0.0001$], training stage, CS type and interval [SF: $F_{156,1248} = 1.21$, $P < 0.0489$; IUA: $F_{156,1248} = 2.36$, $P < 0.0001$] were significant. The latter interaction indicated that the training-induced change in neuronal activity covaried with the specific post-CS interval and the CS type.

For brevity, only individual comparisons carried out on the IUA data will be reported. These comparisons indicated that significant discrimination (i.e. greater neuronal firing to the CS+ than to the CS–) occurred in the first training session (110, and 140–400 ms after CS onset), the session of the first significant behavioral discrimination (80–400 ms after CS onset), the session of asymptotic behavioral discrimination (70–400 ms after CS onset), and the overtraining session (80–350 ms after CS onset). There was no significant discrimination in the pretraining sessions.

The average latency of neuronal discrimination (85 ms) observed in the BL nucleus was noticeably larger than that previously observed in the anterior cingulate cortex (60 ms) and the MD nucleus (30 ms)[9,12]. Thus, the ordering of discrimination latency in these areas from brief to long was: MD nucleus > anterior cingulate > BL nucleus.

Further comparisons indicated the development of excitatory training-induced plasticity in the BL nucleus, i.e. increased neuronal activity during conditioning relative to the pretraining session with tones and noncontingent shocks. Significant excitatory plasticity occurred in response to the CS+ in the first session of conditioning (60–400 ms after CS onset) and in the session of the first significant behavioral discrimination (120–160, and 250–400 ms after CS onset). No significant firing increments in response to the CS+ were found during the criterial and overtraining stages, indicating that the peak of excitatory plasticity occurred during the session of the first significant behavioral discrimination and a decline of excitatory plasticity occurred during training after this stage. No significant increments of discharges elicited by the CS– were found during training, relative to pretraining.

In summary, BL amygdaloid neurons developed discriminative activity rapidly (in the first conditioning session) and this form of training-induced plasticity persisted at a fairly constant magnitude throughout acquisition and overtraining. Excitatory plasticity also developed in the first conditioning session, reached a peak value as behavioral discrimination first occurred, and declined thereafter during training. This same pattern of results, for the 2 forms of plasticity, has been observed in several studies[12,14,28] to occur in the anterior cingulate cortex, a region that has direct and reciprocal interconnection with the BL nucleus. Neurons of the MD thalamic nucleus also exhibit this pattern, with one exception: the peak of excitatory plasticity in the MD nucleus occurs as the criterion of acquisition is reached, rather than in the session of the first significant behavioral discrimination[9,28]. These results are consistent with the hypothesis that the anterior cingulate cortex, MD thalamic nucleus, and BL nucleus participate in a unified learning circuit.

Additional evidence of the functional unity of this circuit is provided by the effects of experimental lesions induced before training, which in the MD nucleus or in the anterior cingulate cortex yield a moderate retardation of behavioral acquisition, and no impairment of asymptotic avoidance performance[10,13]. Preliminary data indicate that BL nucleus lesions yield the same pattern of results (Poremba and Gabriel, unpublished observations). Thus, the circuit formed by the anterior cingulate,

MD thalamic and BL amygdaloid neurons would seem to be involved in mediating original behavioral acquisition in the avoidance task. The development in the first training session of discriminative activity in these areas suggests that the contribution made by this circuit begins to occur in the very earliest stages of acquisition. In contrast, lesions in the posterior cingulate cortex or anterior thalamic nuclei do not impair behavioral acquisition at all, but yield a moderate impairment of post-asymptotic performance[11,12], suggesting that this circuit is preferentially involved in maintenance of the well-learned behavior.

Behavioral acquisition is abolished by combined lesions of the anterior and posterior cingulate cortex, or by combined lesions of the anterior and MD thalamic nuclei[10,13]. The devastating effects of combined circuit damage coupled with the moderate effects of single-circuit damage suggests functional redundancy. That is, each circuit contributes in the ablated and possibly in the intact animal to the aspect of task performance (original acquisition or conditioned response maintenance) that is not its specialty.

Neurons in the central amygdaloid nucleus exhibit rapid development of training-induced plasticity and lesions in this nucleus, the anterior cingulate cortex and in the MD thalamus significantly impair the acquisition of conditioned bradycardiac responses in rabbits[1,3,4,20]. Along with the present data, these results indicate a rapidly-developing, early involvement of the amygdala and related cortical and thalamic circuitry in learning. In addition, these data raise a question as to the precise functional relevance of this early learning circuit. Several findings of past work with the present model[10] and work with other models[17] suggest that the cortical and thalamic components of this circuit carry out a rather general mnemonic function, recency encoding or working memory, i.e. the rapid and relatively short-lived encoding of novel environmental contingencies. We would suggest tentatively that the input to the central amygdala relayed from anterior cingulate and/or MD thalamus through the BL nucleus is involved in bringing mnemonic recency data to bear upon cardiovascular and other autonomic response systems. A compatible view of the role of central nucleus neurons has been presented recently by Kapp and his co-workers[21]. Studies now ongoing, of the interactions among all 3 circuit components, are intended to provide information about the specific contribution of BL amygdaloid neurons to the recency encoding process.

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