Neural Correlates of Asymptotic Avoidance and Classical Conditioned Leg Flexion

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Brain correlates of conditioned leg flexion behavior were studied in dogs bearing chronically implanted electrodes. Animals were trained to asymptote under either classical aversive or avoidance contingencies, and then were retrained under the other contingency; the order of training was balanced across subjects. The training groups were further subdivided into 3- and 5-sec CS–US intervals. These procedures produced stable behavior and large differences in response latency such that CS onset controlled latency under the avoidance contingency and CS duration controlled latency under the classical contingency. The discharge rate of multiple unit activity recorded from motor, association, and auditory cortices during asymptotic performance was characterized by primary and secondary peaks. Primary peak latencies were invariant with respect to flexion behavior. The latencies of secondary peaks varied as a function of the behavioral response latency and generally preceded the onset of flexion behavior.

INTRODUCTION

In the study of neural mechanisms of learning it is essential to utilize paradigms permitting reliable behavioral control of quantifiable overt responses (7). The present experiments explore specific relations between neuronal activity in several areas of the cerebral cortex and conditioned leg flexion behavior in the dog. In order to insure significant behavioral

variations under conditions that would allow for control and evaluation of nonspecific factors such as level of activation, within-animal comparisons were made of flexion responding for two major classes of conditioning procedures, classical aversive and avoidance. It has been shown (8) that dogs trained under the classical contingency (where a shock is presented a fixed time period following onset of a tone, regardless of the animal's behavior) respond just prior to shock onset, while animals that can avoid the shock respond just after the onset of the tone.

In the current study a large difference in the latencies of classical and avoidance asymptotic responses is used as the behavioral baseline for an analysis of cortical activity. Multiple unit activity was chosen as the measure of brain activity because it is both sensitive enough to reflect ongoing changes in activity during the trial and is sufficiently robust to be collected in the chronic behavioral preparation (1, 2). It was thought that certain features of the multiple unit activity response would differ as a function of the latency of the behavioral responses produced, while others would not.

METHODS

Twenty mongrel dogs, five in each cell of the two-by-two design, completed both training procedures. Half of the subjects (ten) began training under a classical contingency (shock was always presented at the end of the CS–US interval). After reaching asymptote, these animals were switched to the avoidance procedure (shock was presented unless a criterion leg flexion occurred during the CS–US interval) and run to asymptote. The other half of the subjects were trained in the reverse order. These groups were further subdivided so that five dogs within each group were trained using a 5-sec CS–US interval, and the remainder a 3-sec CS–US interval.

A stock and nylon belts held the subject in place during experimentation. A leg bar was attached with a nylon cuff to the right foreleg of the subject which passed through a slit in the upper floor of the stock. When lifted, the leg bar rotated a potentiometer from which the analog leg response was recorded. In addition, two disk electrodes were taped to the back of the right foreleg for shock delivery. The shock (US) duration was 0.5 sec and had a selectable constant current of 0–6 ma. The apparatus was contained in a sound-attenuating chamber with white noise present throughout the session. A 3- or 5-sec 1000-Hz tone was presented against this background and served as the CS. The US was delivered at the end of the CS presentation (a) for all trials under classical conditions (b) for those trials on which a criterion response was not made during the CS–US interval under avoidance conditions. The intertrial

interval was varied over a range of 22–52 sec with a mean of 34 sec for both conditions. Two Schmitt triggers detected the height of the leg: one at leg lift onset and the other at the criterion height, the latter preventing the occurrence of the scheduled US in the avoidance contingency session. If the leg was lifted above the onset Schmitt trigger during the interval before the CS, the trial was postponed until the leg remained down for the required 2 sec.[2]

A typical trial's events were as follows. A trial programming tape halted, initiating an eight-channel tape recorder; 4 sec later, a 2 sec period before the CS was initiated. If the leg was raised above the lower leg lift criterion, the trial was continually postponed until the right forepaw was on or near the floor for the required 2 sec period before CS. Following this period, the CS was initiated and remained on for the duration of the CS–US interval, whether the subject responded or not. The tape recorder was turned off, the latency information based on the leg lift Schmitt triggers was punched out on IBM cards, and the programmed trial tape restarted.

Brain multiple unit activity was recorded during asymptotic sessions, i.e., after training had produced stable anticipatory responses. Multiple unit activity was recorded from motor, association, and auditory cortices, and from the mesencephalic reticular formation with stainless steel insect pins referenced to a screw affixed over the frontal sinus. Electrode implantation was done using sterile surgical technique with Nembutal anesthesia 4 weeks prior to the beginning of recording. Flexor electromyogram as well as analog leg response was also recorded. On playback, the recordings were filtered to eliminate slow waves and presented to Schmitt triggers which produced a standard pulse (-1.5 v) to a Lab 8 computer for each neural event greater than 10 μv. This technique excluded noise generated by the system (maximum 5 μv), and yielded a measure of multiple unit activity uncontaminated by spike amplitude. The number of spikes exceeding the 10-μv level were totaled for each 20-msec period (a bin) during the interval before the CS and the CS–US interval for each trial, yielding a multiple unit activity rate measure (spikes/20 msec). Activity at the brain sites, the EMG, and the leg position were collected and stored on computer tape for all 80 trials of each session. The taped neural activity was transformed into single trial z

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[2] This abort procedure introduces an instrumental contingency, i.e., Sidman avoidance, into the training paradigm. If this contingency had a differential effect, one might expect to find a preponderance of intertrial responses under the classical paradigm. However, in terms of absolute behavioral response levels there were significantly more responses during the CS and during the intertrial interval (excluding direct responses to the US) under the instrumental paradigm than under the classical paradigm in asymptotic performance.

scores and restored on computer tape. The resultant trial-by-trial z scores were summarized in histograms of response latency categories.[3]

RESULTS

Owing to the extremely large amount of time required to analyze even a single session of brain data, two dogs were selected from each group for complete analysis. Each of these dogs contributed four sessions of data, two classical and two avoidance. All data reported here are from the records of these eight sessions.

The effects of the experimental variables on the leg flexion behavior of the subjects are most clearly depicted by the composite probability distributions based on the anticipatory response latencies produced during the asymptotic sessions (Fig. 1). There were no reliable differences in the latencies attributable to order of training, so the probability distributions were contrasted for all four subjects/cell ignoring this factor. The proportions are calculated by dividing the number of anticipatory response latencies that occurred in each 200-msec interval by the total number of anticipations produced during the asymptotic sessions. The greatest probability of an anticipation during asymptotic classical training just precedes US onset (3-sec group = 2.6 sec; 5-sec group = 4.4 sec). The greatest probability of an anticipation during asymptotic avoidance training more closely follows CS onset, occurring at 1.2 sec regardless of the CS–US interval duration. These results replicate previous data (8) and extend the results in the predicted manner. They also provide the needed baseline for analyzing the neural multiple unit activity.

Patterns of brain activity and the position of the leg are shown in Fig. 2 for one subject[4] in the 5-sec interval group. Each column of Fig. 2 consists of the average single trial 20-msec z scores for two sessions during the 2-sec period before CS onset and the CS–US interval for auditory, association, and motor cortices and the analog leg response. The two columns represent data from trials falling within the interval most characteristic of the given contingency. The left column is from avoidance

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[3] The formula for the z score based on our data is $z = X - \bar{X}/\sigma$, where X = CS–US interval bin score; σ = standard deviation before CS; $\bar{X}$ = mean before CS. A more detailed treatment of the z score is given in the senior author's dissertation, University of California, Irvine, 1972 (also 6).

[4] The patterns of activity were generally quite stable from session to session for the various cortical placements, and histological verification of the placements in the respective loci were made. Because mesencephalic reticular formation placements were widely scattered and the patterns of activity were also varied, further analysis of information from this system is omitted from this report. EMG activity closely corresponded to the analog leg response, so only the latter measure is reported.
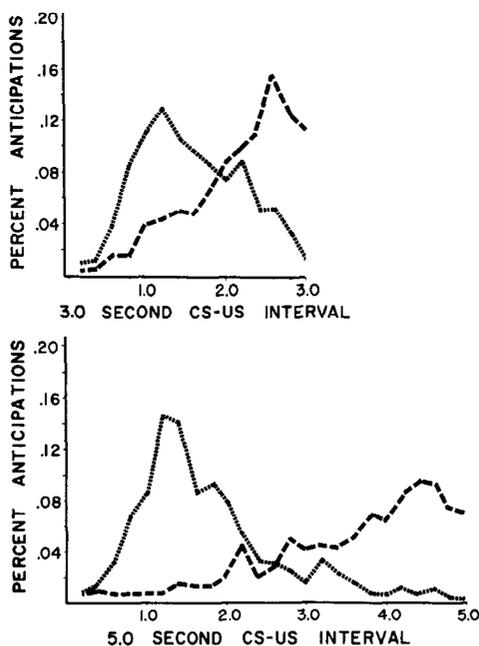
F IG. 1. The proportion of anticipatory responses in each 200-msec interval for asymptotic sessions under avoidance (dotted line) and classical (broken line) contingencies for the 3- and 5-sec CS–US interval groups. The proportions are based upon the total number of anticipatory responses for each condition: avoidance, 3 sec = 561, 5 sec = 607; classical, 3 sec = 362, 5 sec = 323.

sessions with responses occurring between 1.67–3.33 sec (78 trials). The right column shows data from the classical sessions of the same dog in which responses occurred between 3.34–4.99 sec (115 trials). The horizontal lines on each graph correspond to the 5% probability level based on the activity before the CS.[5] Note that in most cases the activity before CS remains within these boundaries, while the CS–US activity exceeds the boundaries. Several points are immediately obvious. Activity generally increases after CS onset. This increase precedes the onset of the leg response in all cortical areas, and is usually, but not always, depicted by an initial or primary peak immediately after onset, followed by a gradual rise to a secondary peak. There is little or no activity at the leg during the period before the CS and during the CS–US period up to the response.

[5] These critical area indicators (probability levels) differ among the response latency category's histograms because they are a function of the number of trials. The first 0.5 sec. of information of the period preceding the CS and the first 20-msec bin of the CS–US interval have been deleted from the analysis because of possible equipment onset artifacts.
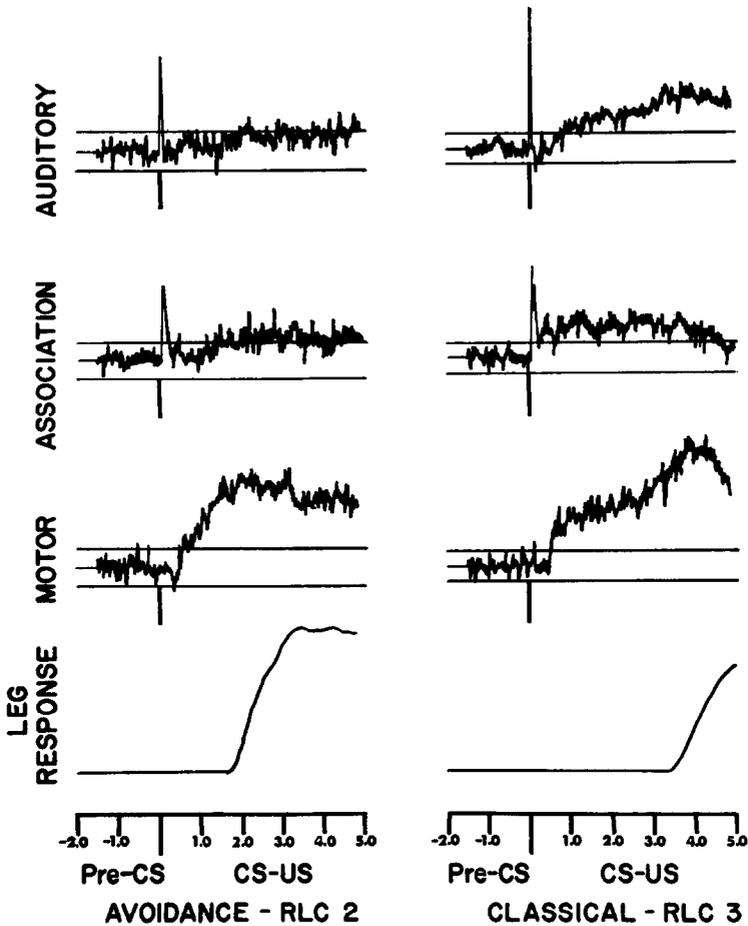
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FIG. 2. Average histograms of z scores from auditory, association, motor cortex and the analog leg response for one dog in the 5-sec CS–US interval group. Data depicted are averages of two sessions each under the avoidance and classical contingencies for the response latency category containing the greatest proportion of anticipatory responses for each contingency. RLC = response latency category.

To obtain information about the relation between leg response latency and multiple unit activity latency, the CS–US interval was divided into four response latency categories. Each response latency category consists of all trials for which the leg response was initiated within the prescribed duration of the CS–US interval. For the 3-sec CS–US interval, the response latency categories were 0.00–0.99, 1.00–1.99, 2.00–2.99, and 3.0+ sec. For the 5-sec CS–US interval the corresponding response latency categories were 0.00–1.66, 1.67–3.33, 3.34–4.99, and 5.0+ sec. Within each of these behavioral response intervals two aspects of latency
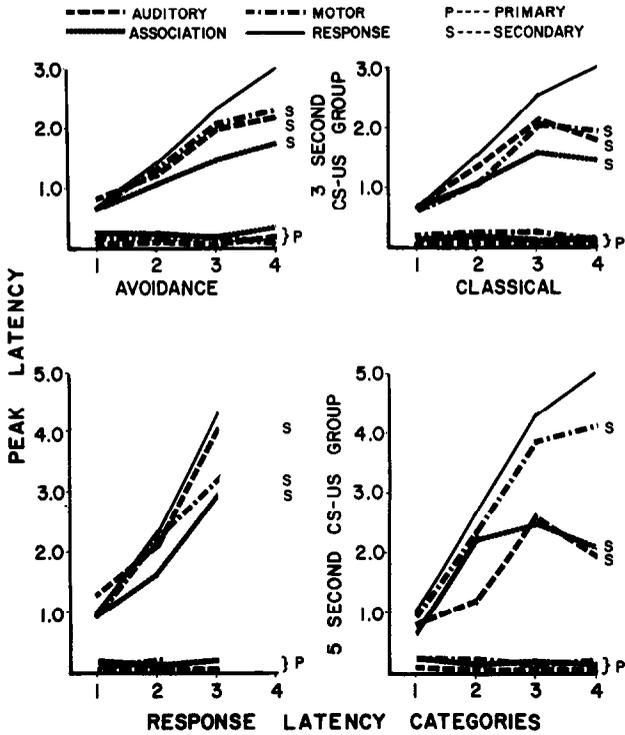
FIG. 3. Average latencies of the primary (P) and secondary (S) multiple unit activity peaks, and the latency of leg flexion as a function of response latency category. Each graph depicts the averages for four dogs. (No trials occurred for RLC 4 in the 5-sec CS–US interval avoidance condition.)

were analyzed. First, "primary peak" was defined as the largest z score following the first occurrence of multiple unit activity greater than a $z = 1.96$ (5% critical area) in the first 500 msec following CS onset. Second, "secondary peak" was defined as the point where the multiple unit activity reaches a maximum following the first 500 msec of the trial.

Figure 3 summarizes the average latencies of primary and secondary peaks for each group (3 sec/5 sec—classical/avoidance) and for response latency categories. It is evident from Fig. 3 that primary peak latencies are quite stable, showing no correlation with a shift in experimental conditions or behavioral response latency. In marked contrast, secondary peaks exhibit systematic variation. The most striking effect is the consistent high positive correlation between behavioral response latency and latency to the secondary peak. For the average data of Fig. 3, the secondary peak consistently precedes the behavioral response onset in all cortical regions studied for all response latency categories except #1. There is also a consistent tendency for the secondary peak in

association cortex to precede the peaks in auditory and motor cortical areas. Another way of stating this is that the first brain event predictive of behavioral response (among those that we measured) is the secondary peak in association cortex.

DISCUSSION

These patterns of neutral events taken in conjunction with the large differences within and between subjects in leg flexion responding to the two contingencies suggest that highly specific relations exist between conditioned responses and cortical events associated with them. The most consistent effect is the strong positive relationship between latency of the secondary peak of multiple unit activity and latency of the behavioral response. These results cannot be explained in terms of generalized effects like nonspecific activation because of the differing latencies of the secondary peak at various cortical loci.

The facts that secondary peaks occurred and their latencies exhibited the same general ordering with latency of response onset in all three cortical areas, including the auditory cortex, was somewhat unexpected. It is, however, generally consistent with recent data of Olds and colleagues (5) showing changes in unit activity in many brain regions following conditioning, and it is in close correspondence with the studies of John and his associates (3, 4), where characteristic secondary evoked field potentials were found to occur in widespread areas of the brain in highly trained animals. These secondary brain potentials are, in general, predictive of learned behavioral responses (7). Whether they represent "memory readout," as John suggested, or some initial set of brain events leading to the generation of behavioral response, remains to be determined.

The present results indicate that the multiple unit activity of the three cortical areas examined constitute predictive correlates of behavior in so far as all three regions exhibited secondary peaks prior to the flexion response. However, they do not behave in an identical manner even though their general patterns of multiple unit activity are similar, i.e., a short-latency primary peak followed by a long-latency secondary peak in discharge rate. Of particular interest, the association cortical field generally exhibited a shorter latency for the secondary peak than the motor and auditory regions. The fact that the secondary increase in the association cortex preceded that of the motor and auditory areas suggests that this region might bear a causal relationship to the other two. This position must be tempered by the fact that this temporal sequence was equivocal for the 5-sec classical condition. Additional analyses and studies should help clarify the temporal sequencing of cortical multiple unit activity with respect to conditioned behavior.

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