CONDITIONED RESPONSES IN THE RETICULAR FORMATION

Thesis by

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ABSTRACT

Unit activity was recorded from the midbrain and pons of 40 freely moving rats in an appetitive classical conditioning situation. Responses to auditory stimuli were observed from 100 units before and during a conditioning procedure in which presentation of food occurred 1 sec after the onset of the auditory stimulus. Conditioned unit responses (i.e., spike rate accelerations or decelerations) were considered to be positive when 1) no similar responses appeared prior to conditioning, and 2) latencies were equal to or less than those of sensory responses derived from the inferior colliculus. Such short latency conditioned unit responses were recorded from 11 probes located in the mid-lateral part of the ventral region of the brain stem. This region was differentiated from paramedian, far lateral and dorsal parts of the brain stem reticular formation. Conditioned unit responses of considerably longer latencies were recorded from 76 probes located in these other regions. Among the longer latency responses interesting differences appeared in experiments conducted after the first conditioning series was completed. With additional training, units in the "reticular activating system" of midbrain and pons tended to yield stabilized responses in the early portion of the CS-US interval closely related in time to the orientation responses evoked by the CS. In contrast, the responses of units in the limbic midbrain tended to stabilize in the later part of the CS-US interval closely related in time to preparatory responses tied to the US.

During extinction when the auditory stimulus was no longer followed by presentation of food, many of the responses were reduced to their pre-conditioning levels. However, there was a tendency for units which had displayed short latency responses on the first conditioning day to be more resistant to extinction than units which had displayed longer latency conditioned responses. The data were interpreted as indicating a local correlate of learning in the reticular formation of midbrain and pons and a separation of the midbrain system into at least two areas: 1) the classical "reticular activating system" related to orienting reactions, and 2) the limbic midbrain areas related to drives and rewards. Because the ventral and mid-lateral area with very short latency conditioned responses was not clearly tied to either of these, it was considered as possibly representing a third division.

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INTRODUCTION

Herrick's classical study of the brain of the tiger salamander (1948) set the stage for the theory that the reticular neuropil at all levels between sensory and motor mechanisms constituted the predecessor of the vertebrate integrative mechanisms. At all anterior-posterior levels it was viewed as containing the basic requirements for decision making and conditioning. Its less organized parts from midbrain to medulla were seen as being ancestral to the more complex integrative mechanisms, including the cortex. Through a lengthy period following the discoveries of Magoun and his colleagues (Magoun and Rhines, 1946; Rhines and Magoun, 1946; Moruzzi and Magoun, 1949) special relations of the reticular formation to arousal tended to generate a view of it as a "waking mechanism." But even at this time there survived a belief that the reticular formation was to some degree a small and perhaps primitive brain, but nevertheless a more complete brain than would be suggested by the "waking mechanism" theory. The work of the Scheibels (1958) and of Brodal (1957), and more recent theorizing of Kilmer, McCulloch, and Blum (1968) carried forward the more total brain concept: a concept which received considerable sustenance from the centrencephalic views of Penfield (1952). The problem is that direct evidence from studies at the mammalian level did not offer substantial support to the view that decision processes and learning processes occurred in the reticular formation.

So far as learning is concerned, Sharpless and Jasper (1956) pointed to habituation, and Morrell and Jasper (1956) indicated

conditioning of the arousal reaction. The implication was that local changes occurred in the reticular formation. But these studies did not offer recordings from the reticular formation to substantiate the suggested source of the change. Studies by John and Killam (1959), Ellison, et al. (1968), and earlier studies from our laboratory (Olds and Hirano, 1969; Olds, Mink and Best, 1969; Phillips and Olds, 1969) all showed changes in the reticular formation. Particularly interesting were "learned responses" in the reticular formation that appeared to a stimulus depending on whether it was relevant to the currently active drive (Phillips and Olds, 1969), and responses in reticular formation that accompanied the animal's attentional interest rather than his motor responses (Ellison, et al., 1969). There were also interesting units that were accelerated just prior to the point in time when the animal anticipated the unconditioned stimulus and prepared for a consummatory response (Olds, Mink and Best, 1969). The difficulty in these cases was that no aspect of the experiment certified that the primary changes involved were local; alterations elsewhere could have caused the new response to be projected to the reticular formation.

With regard to decision making, the findings of Olds (1962) and others on self-stimulation and of Glickman and Schiff (1967) and others on changed drive behaviors caused by stimulating in specific parts of the tegmentum suggested a relationship between stimulation of fibers in this region and decision processes. At least the experiments suggested that fibers here were related to decisions about

major behavioral directions: and these were the decisions supposed to be made in this area according to the theory of Kilmer, McCulloch and Blum (1968). The difficulty in this case was that the stimulation experiments did not clarify the problem of whether these were only passing fibers or whether critical aspects of the drive and reward processes might be local.

The present experiment was designed to ask directly whether local integrative processes concerned with learning take place in the reticular formation. Learned changes in neuronal processes were studied with a special view to the question of whether they might involve local changes, and if these occurred the further aim was to determine whether they might be delimited in locus to particular sub-sections of the reticular formation. The data collected permitted other analyses of learned unit responses to be made to find whether some units were more involved with basic drive directions, and others with basic attentional mechanisms. These were considered to be tests for differentiations related to particular decision processes.

METHODS

Subjects and Probes

The subjects were 40 adult, male, albino, Holtzman rats. Probes for recording units were of fine nichrome wire (62.5μ diameter), factory insulated with enamel, and cut with scissors to form a blunt, uninsulated tip. Eight or nine of these probes were chronically implanted under stereotaxic and neurophysiological guidance in each animal. Probes were aimed at regions of the posterior diencephalon and brain stem extending from 3.5 to 9 mm posterior to bregma. All probes were approximately 1 mm lateral to the sagittal suture and 8 mm below the surface of the skull. Probes were lowered by stereotaxic methods to within .5 mm of the intended area and then slowly advanced until clear, unitary spikes were observed (4 to 1 signal-to-noise ratio). The background noise level was about 25 microvolts and acceptable unitary spikes were of 100 microvolts or more. In each animal one large uninsulated wire (250μ diameter, 5 mm in length) was implanted in the anterior lateral region of the cortex to serve as an indifferent probe. All probes were fixed in place with acrylic and brought out to a 10-contact plaque that was similarly fixed to the skull. At least 3 days were allowed for recovery before experiments were begun. During this period and during experiments animals were maintained on a 24 hr light schedule and on a limited diet so that weight was kept at 70-80 % of the preoperative level. Following completion of experiments animals were sacrificed with an overdose

of nembutal and perfused with physiological saline followed by a 10 % formalin solution. Brains were sectioned at 60μ and alternate sections were stained for fibers and cells with Weil and cresyl violet, respectively. The probes left fairly clear tracks in brain tissues, and the point of recording could be determined with relative ease. In some cases a small marking lesion (10μa for 15 sec) was made at the end of the experiment to facilitate localization of the tip of the probe (see Fig. 1). Histology was not available for 5 probes. The data from these probes are presented in the result section with that for probes implanted at the same coordinates and are indicated in Appendix II.

Cages and Stimuli

Experiments were carried out in a 13 inch diameter circular plastic cage (housed within a larger, sound-attenuating enclosure). Penetrating through the center of the top of the cage was an 11-wire cable which was fixed to the animal's plaque at the lower end and to a commutator and counterbalanced arm at the upper end. Ten of the wires were of low noise cable (Microdot) and were connected to the brain probes. The 11th wire was a length of noisy "hearing aid" lead which was open circuited at the lower end. Minimal movements by the animal caused relatively large voltages to be generated in this lead and its amplified signal served as an indicator of the animal's movement, thus providing a measuring system for the behavioral response of the animal. The output of the noisy wire was fed through an

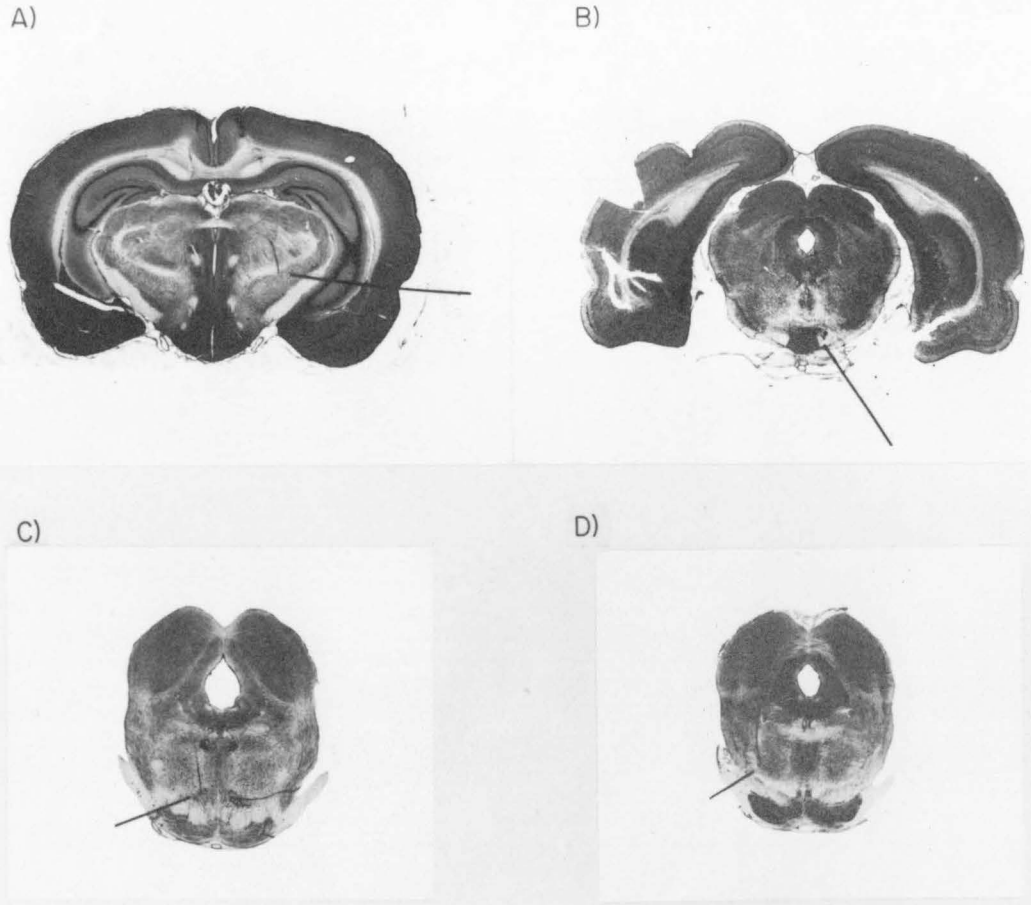


Fig.1 Histological material indicating probe locations in A) zona incerta, probe 8614-7; B) medial lemniscus, probe 8492-6; C) pontine tegmental nucleus, probe 8733-5; D) ventrolateral pontine reticular formation, probe 8216-6. B) and D) have small marking lesions. The code numbers identify the probes for comparison with Table 1.

amplifier with frequency range of 500-2000 Hertz and then into a Schmitt trigger. The trigger rate was then used as the measure of behavior.

The experimental cage was equipped with a loudspeaker for presentation of a tone CS, a mechanical pellet dispenser to deliver a food pellet US, and a continuously available water bottle. The pellet dispenser discharged with a loud auditory signal and dropped pellets into a localized part of a food chute. Animals were hungry prior to experiments and were hand trained prior to the beginning of the experiment to retrieve pellets rapidly after discharge by the dispenser. If pellets were not retrieved within 7 sec of magazine discharge they were withdrawn automatically. A count was kept of unretrieved pellets, and if this number amounted to more than 50 % of the total, the data were not accepted.

Recordings

Prior to the experiment all probes were screened for acceptable unitary activity. Electrical signals from the best of the fine wire probes were fed through amplifiers with a frequency range of 500-10,000 Hertz and then into waveform discriminators which utilized height and time-constant "window" discriminators to select single units for counting (Olds, 1965; Olds, 1967). Spikes from what appeared on a storage scope to be from several different neurons were often so similar in amplitude and wave-shape as to be indistinguishable by the automatic counting device. Nevertheless, each spike was in itself one action potential from one neuron, and all the neurons contributing

to the pool recorded as "one unit" were from a small family of similar neurons localized at the recording point. A computerized "quality control" system was used to plot out samples of the recorded units. These were plotted out in sets of 10 overlapped traces and about 30 sets (300 units) were sampled daily from each probe (see Fig. 2). These tracings were qualitatively evaluated by visual inspection and cases where units of widely different amplitude or wave-shape were counted as one, or cases where clearly non-unitary spikes were accepted were excluded from the data prior to further analysis. Careful comparison of the analog output from the fine probe amplifiers and the digital output from the discriminators indicated that not only were spikes of several similar shapes counted as though all were one single unit, but also sometimes two spikes that were identical in appearance on the storage tube of a CRO were discriminated so that one was counted and the other not. Nevertheless, all of the clearly observable correlations between behavioral events and changes in unitary spike rate were equally visible whether analog traces or computerized evaluation of digital output was used. Therefore, because the latter was faster and more objective, the present data are based on computerized counts derived from the automatic discriminator system.

Procedure

The first day of the experiment was devoted to a pseudo-conditioning and habituation procedure. At intervals of about one

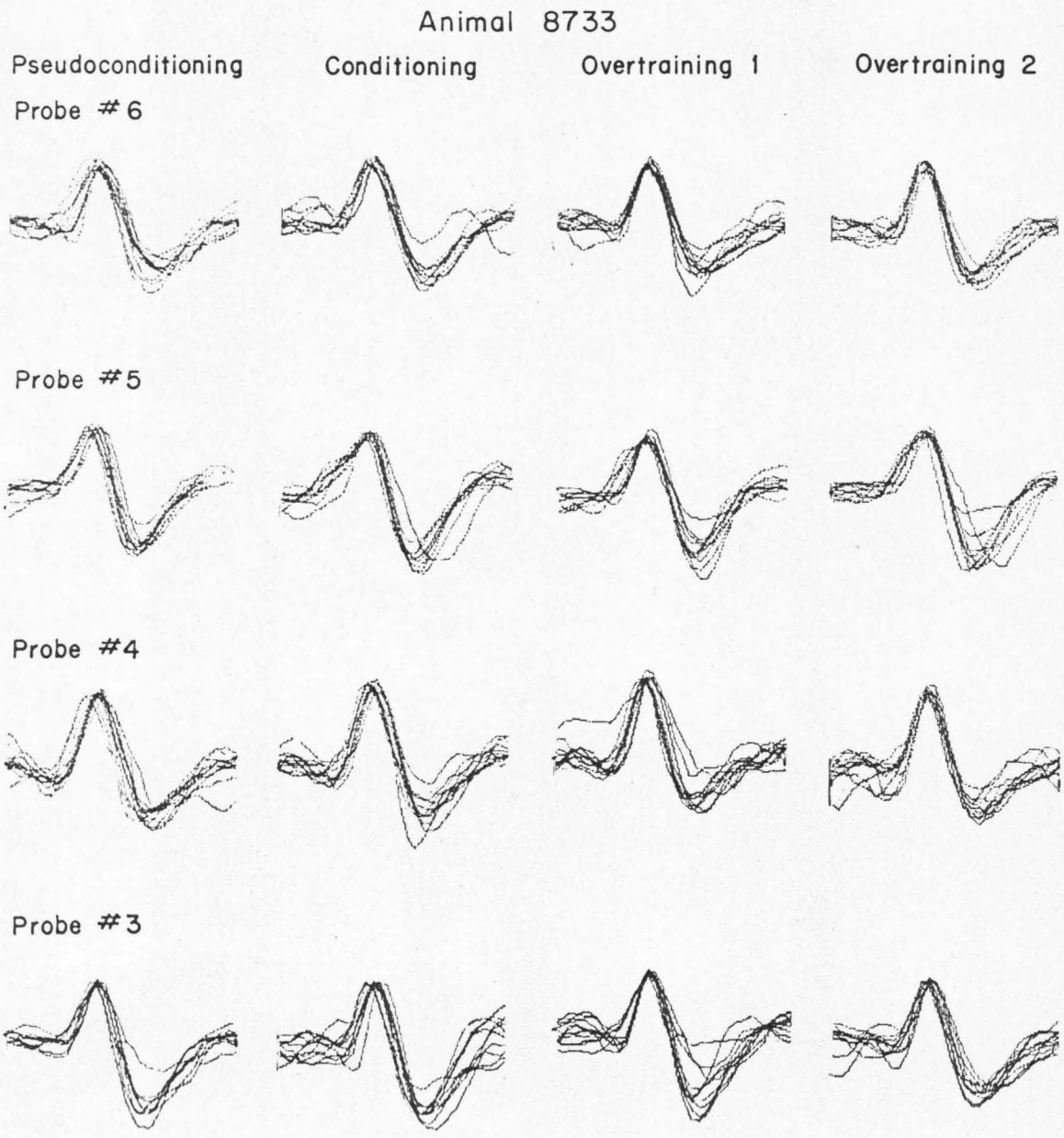


Fig. 2 Quality control pictures for 4 probes in animal 8733 over 4 days. Each sample consists of 10 overlapped traces. Probe #3, dorsal midbrain reticular formation; probe #4, lateral pontine reticular formation; probe #5, pontine tegmental nucleus; probe #6, superior colliculus.

per minute, 3 sec trials were presented. The first second involved only recording of background unit and behavioral activity (with no sign to the animal that a trial had started). At the beginning of the second second, one of three stimuli was presented (an auditory signal of 1,000 cps, square pulses; a different auditory signal of 10,000 cps; or the pellet dispenser which yielded a noise and a 45 mg pellet). If one of the two tones was applied, it was continued for the remaining two seconds of the trial. If the food magazine was discharged this was discrete, but the animal usually retrieved the pellet and had begun to eat prior to the end of the trial. Unit and behavior recording was continuous for the whole 3 sec period. On each trial one of the three stimuli was selected on a pseudo-random basis so that the incidence of the three was about equal over the 16 hr course of the day's experiment. There were about 320 trials of each of the three types; 960 trials in all. The experiment was run automatically between 4 pm one day and 8 am the following day. Then there was an 8 hr pause before the second day's experiment was begun.

The second day was primarily devoted to a conditioning experiment. However, for the first 150 trials (i.e., about 50 of each of the three kinds) the schedule of pseudo-conditioning was retained. The switch was then made to a conditioning series without any other break in the procedure. Time intervals between trials remained the same. In this case the three kinds of trials were: 1) tone 1 (called CS+) presented at the end of the first second and continued as before, but with the pellet dispenser (US) presented at the end of the second second;

2) tone 2 (called CS-) presented at the end of the first second without any correlated US, and 3) no stimulus presented at all. For half of the animals the high tone was the CS+ and for the others the low tone was so used. There was roughly the same number of presentations of tone 1, tone 2, and the food magazine as on day 1, but now the magazine was correlated with one of the two tones so that the tone preceded and overlapped the presentation of the food magazine (with a 1 sec CS-US interval). The third (blank) time period was inserted so that the total distribution of magazine and stimulus presentations over time would be equal for the two procedures (pseudo- and real conditioning).

Some of the animals received additional days of conditioning in order to assess the effects of overtraining. In these cases the procedure was identical to that of the first real conditioning day, except that no pseudo-conditioning trials were presented at the beginning of these days.

After either 1 or 2 days of overtraining, several animals were placed on an extinction schedule to determine if, and in what manner, the conditioned responses would extinguish. The procedure was identical to that of pseudo-conditioning.

Time Intervals

For each probe averages were computed, i.e., pre-stimulus and post-stimulus histograms. The averages included all of the trials for a given day. There were about 320 trials for each stimulus.

Within each trial the minimum time division (bin) was 10 msec; thus there were 100 divisions per second. Finer grain analysis of latencies therefore could not be made. On each trial (for each probe) a 1 or a 0 was placed in each minimum time bin depending on whether there had been any unit identifications made during that time interval. While it would have been more accurate to place the actual counts in these bins, critical evaluation of preliminary data showed that the same latencies and the same curve shapes were generated by either method. This was partly due to the low probability of firing in the units studied. Therefore, because binary entries were less expensive, this method was chosen.

In computing averages, the contents of all the first bins were added, similarly the contents of all the second bins, and so forth. In each case, the sum was divided by the number of trials; the result was a fraction denoting the proportion of times that a unit detection had been made in the indicated time interval (see Fig. 3). Each of the first 100 intervals (which were prior to stimulus application) provided separate estimates of the proportions to be expected from a random selection of a number of time bins equal to the number of trials. The time bins immediately following stimulus application provided an estimate of the change in background firing rate caused by application of the auditory stimulus. Similar analyses were made of data derived from the movement detector. In this case the "trigger level crossings" were substituted for unit detections. Movements occurred in response to the auditory stimulus at about 80-100 msec

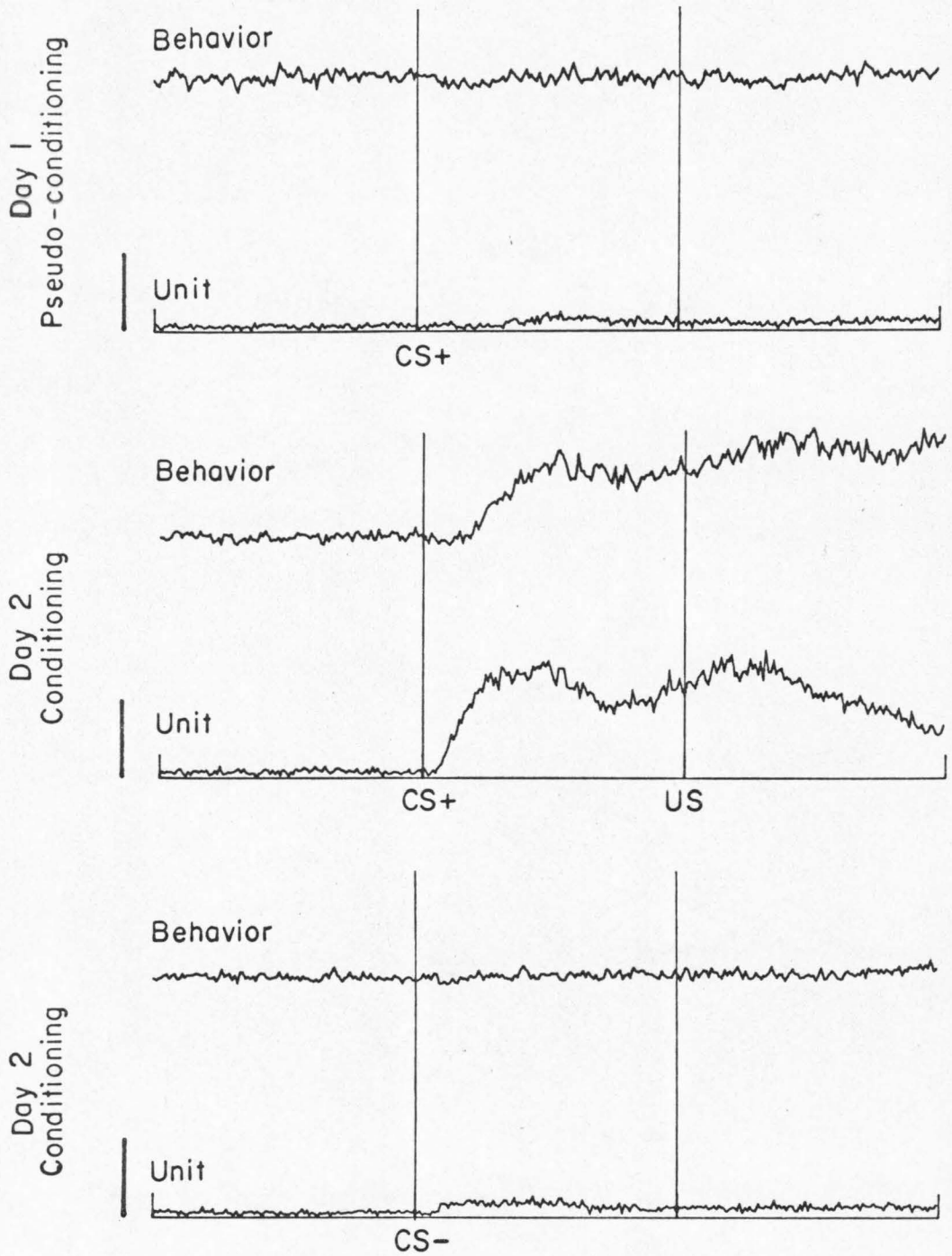


Fig. 3 Computer average curves (pre- and post-stimulus histograms) for probe 8680-2 located in the dorsomedial midbrain reticular formation. The vertical line at the left represents a probability of unit firing of .20 for each 10 msec bin.

after its onset. The analysis of the unit data was conducted in two ways. In one case, only the first 8 bins following stimulus onset were considered in order to mitigate unit effects fed back from behavior (called early response analysis). In the second case, the entire second following stimulus onset was considered (called late response analysis).

Responses and Latencies

A "response" was an acceleration or deceleration of unit spike rate caused by presentation of the auditory signal. The method of analysis was to establish a mean and standard deviation on the basis of the first 100 (pre-stimulus) bins. In the early response analysis the post-stimulus bins were then grouped in twos: 1 and 2 = the period from 0-20 msec after stimulation; 3 and 4 = the period from 20-40 and so forth. The average rate over all 320 trials for each of these pairs was computed separately and this rate was converted into a standardized deviation by subtracting the background mean over all 320 trials and dividing by the standard deviation of the background mean. A response was considered to characterize a time interval (0-20, 20-40, 40-60, or 60-80 msec after stimulus onset) if the average score for the pair of bins involved was at least 1.55 standard deviations ($p < .03$, two-tailed) from the mean background rate (see Appendix I). The end of the first time interval to show such a deviation was counted as the latency of the response. Latencies are stated as 10, 20, 40 msec and so forth from the onset of auditory

stimulation. Because of the interest in very short latency conditioned responses a single bin computation for the first bin was made. In this case the requirement of a score of at least 2 standard deviations ($p < .046$, two-tailed) from the mean background rate was made (see Appendix I). The first bin was most likely to contain electrical and mechanical artifacts from the tone presentation and 10 msec responses were still considered somewhat questionable.

The late response analysis was conducted in a similar manner. The post-stimulus second was divided into $\frac{1}{4}$ sec periods: bins 1-25 = the period from 0-250 msec after stimulation; 26-50 = the period from 250-500 msec, and so forth. The average rate for each of these groups was computed separately and this rate was also converted into a standardized deviation. A response was considered to characterize a time interval if the average score for the group of bins was at least 1.00 standard deviations ($p < .001$, two-tailed) from the mean background rate (see Appendix I). Such responses were highly visible.

The latency of an unconditioned response was considered to be the end of the first time interval which showed a significant deviation in the same direction on both the first day (pseudo-conditioning) and the second day (conditioning). The latency of a conditioned response was counted as the end of the first time interval which after conditioning showed a significant deviation from the background rate and was also twice any deviation in the same direction on the pseudo-conditioning day. During overtraining a response was considered to be altered if it differed from the previous day's response by $\pm$ 0.5

standard deviations or more. This degree of change was apparent on visual inspection.

The criteria of conditioning were chosen to assure 1) that there was a marked change caused by conditioning, and 2) that the change did not represent merely the disappearance of a prior response. Conditioning seemed to cause some responses to disappear or to become smaller, but it was not clear whether this was so. Some of these changes must have been due to habituation, the change being complete at the end of the first day. Others were likely due to conditioning, the whole change occurring on the second day. There were only 14 cases with latencies of 80 msec or less that met the criterion which was the inverse of that for conditioning: namely, a significant response on day 1 and a response of less than half of that on day 2. Because there were few cases and the source of the change was unclear, it was deemed wiser to put these aside for further experimental analysis. The criteria used were limited to identifying changes caused by conditioning if these consisted in the appearance of responses where there were none before, or in doubling of pre-existing responses (whether these were in the excitatory or inhibitory direction). The criteria described above were not considered to be statistical procedures, but merely tools used as objective criteria for selection of new responses.

Learning Curves

The development of conditioned responses was analyzed in the

trial-by-trial sequence of training. For each probe which showed a learned response for which data was available the post-stimulus histogram from the conditioning day was examined to determine the period of the response. This consisted of the portion of the CS-US interval which showed a continuous change in the same direction outside the range of variability observed in the background activity of the pre-stimulus interval. Learning curves were obtained by subtracting the average probability of firing during the background period from that during the portion of the CS-US interval of interest. The difference in firing rate from background was plotted for the CS+ in 10 trial groups. The average pre-stimulus background rate was plotted in 20 trial groups (10 each for CS+ and CS-). Average curves are presented for the first 250 trials of pseudo-conditioning and for 20 pre-pairing trials and the first 250 trials after pairing began during conditioning for the various brain regions and behavior.

An analysis was made to determine the initial point of changed responsiveness to the CS+ for each unit and for the behavior of each rat. The largest pre-pairing point was compared with successive points on the learning curve. The first of three successive points on the learning curve which were greater than the largest pre-pairing point was considered as the initial point of changed responsiveness to the CS+.

RESULTS

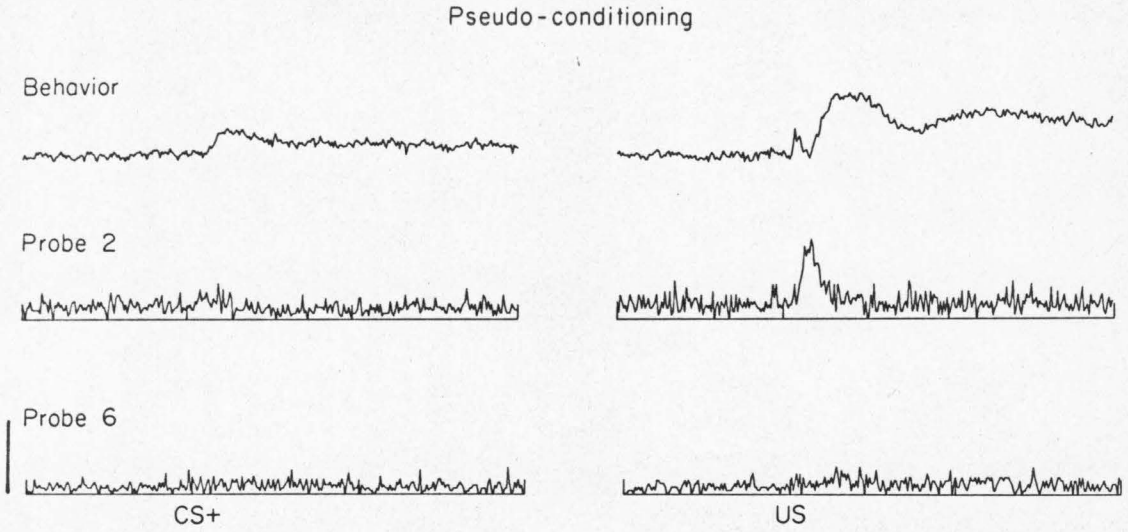
One hundred units in 40 animals were observed. Unit responses were thus generally recorded from more than one probe in each animal. The unit responses observed were a function of the location of the recording probes. Fig. 4 is an example of different responses from two probes in the same animal.

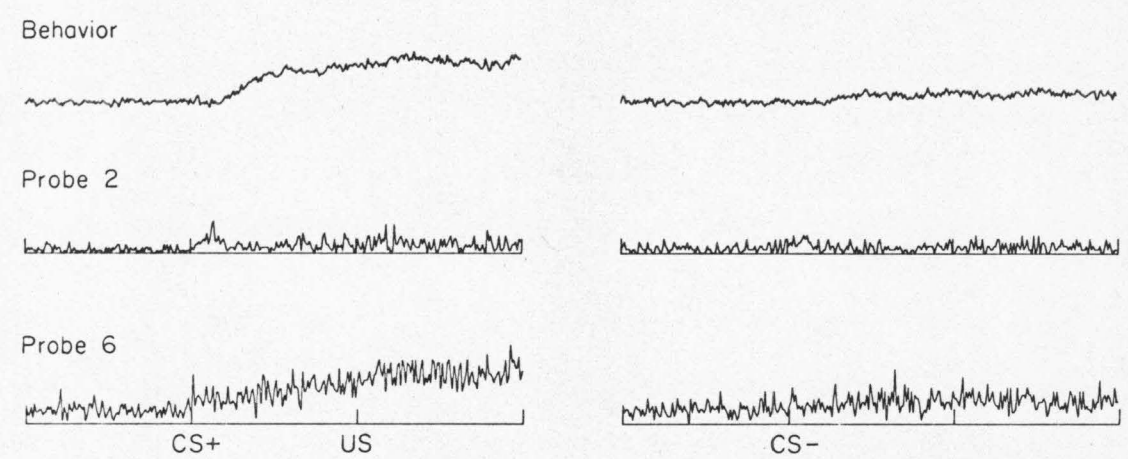
Unconditioned Responses

Unconditioned responses were recorded from a total of 52 units (see Appendix II for the latencies and distribution of these responses). These responses were most frequent in the posterior midbrain and central pontine reticular formation. Most of these responses occurred within 80 msec after stimulus onset.

Seventeen units displayed unconditioned responses with latencies of 20 msec or less. These were mainly in the posterior midbrain and the central pontine reticular formation where they appeared in 6 of 14 cases (43%) and 4 of 14 cases (29%), respectively. The other 7 short latency unconditioned responses occurred in the central region of the posterior pontine reticular formation, the dorsolateral region of the anterior pontine reticular formation, the central lateral region of the anterior midbrain reticular formation, the region of the medial lemniscus, dorsomedial red nucleus, dorsomedial central gray and zona incerta. Only 2 units had unconditioned responses with latencies in the 10 msec interval which was considered questionable because of the possibility of artifacts. These units were located in

Fig. 4. Example of different responses from two probes in animal 8437. Each line represents a 3 sec period. The first sec of each histogram shows the pre-stimulus background activity. Each histogram is an average over a 16 hour period of all trials in a particular class. The average was obtained as explained in the text. During pseudo-conditioning responses are shown to the tone that will become the CS+ and to the pellet dispenser (US). During conditioning responses are shown to the paired CS+ and US, and to the CS-. The vertical line at the left represents a probability of unit firing of .05 for each 10 msec bin. Probe 2 is located in the ventral tegmental area of Tsai and probe 6 is located in the region of the rubrospinal tract in the posterior midbrain. The response recorded from probe 2 to the CS+ on the conditioning day is similar to the response of that probe to the US on the pseudo-conditioning day. Probe 6 showed little response to either the CS+ or US on the pseudo-conditioning day, but displayed a large, sustained response to the CS+ during conditioning, with some generalization of response to the CS-.


Pseudo-conditioning
Behavior
Probe 2
Probe 6
CS+
US


Conditioning
Behavior
Probe 2
Probe 6
CS+
US
CS-

the substantia nigra and dorsomedial region of the posterior midbrain reticular formation.

Thirty-nine units displayed unconditioned responses which began within 80 msec after stimulus onset. The largest percentage of these responses occurred in the posterior midbrain where 9 of 14 units (64%) showed such responses. The proportion of these responses in other regions was: central pons, 8 of 14 units (57%); posterior pons, 4 of 9 units (44%); anterior pons, 6 of 14 units (43%); posterior diencephalon, 4 of 13 units (31%); central midbrain, 6 of 26 units (23%); and anterior midbrain, 2 of 10 units (20%). Figure 5 gives an example of an unconditioned response from a unit in the region of the medial lemniscus.

In 13 cases unconditioned unit responses displayed latencies greater than 80 msec.

There were only 5 units which displayed inhibitory unconditioned responses. These units were located in 1) superior colliculus, 2) central midbrain reticular formation, 3) medial midbrain reticular formation, 4) ventromedial midbrain reticular formation, and 5) central pontine reticular formation.

Conditioned Responses

A significant conditioned response characterized 87 of the 100 units studied (see Fig. 6 and Appendix II for the latencies and distribution of these responses). These responses were most frequent in the posterior midbrain and the anterior and posterior pons. Half

Fig. 5. Example of an unconditioned response from probe 8744-5 located in the region of the medial lemniscus. There is an unconditioned response to both of the tones on the pseudo-conditioning day and the conditioning day which began about 40 msec after stimulus onset. In addition, there is a new conditioned component to the response to the CS+ on the conditioning day which began 10 msec after stimulus onset. The vertical line at the left represents a probability of unit firing of .10 for each 10 msec bin.

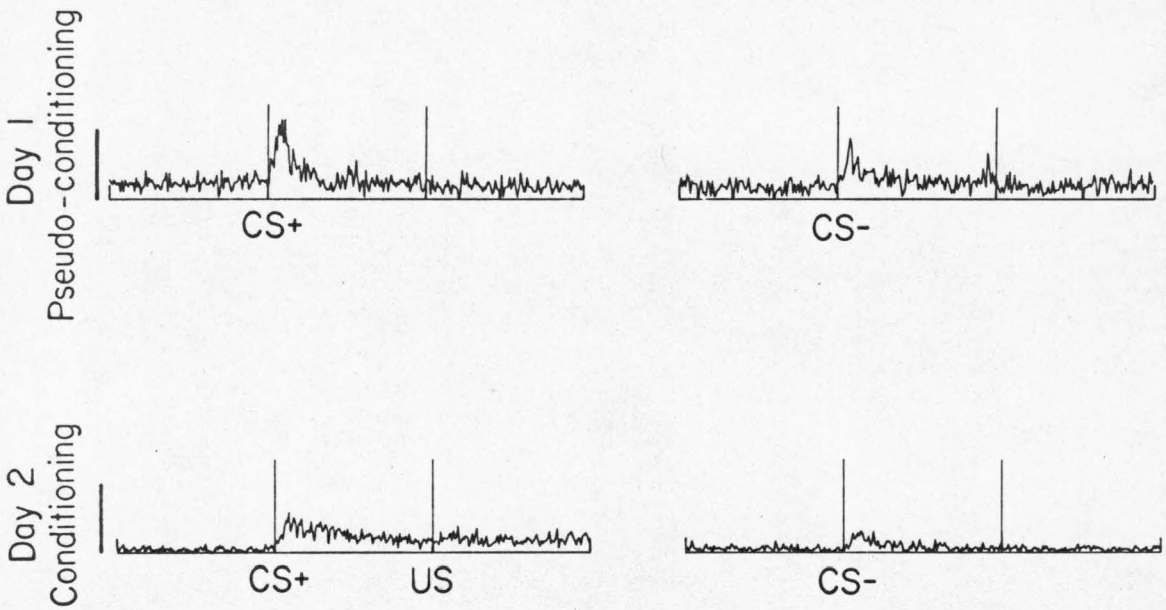
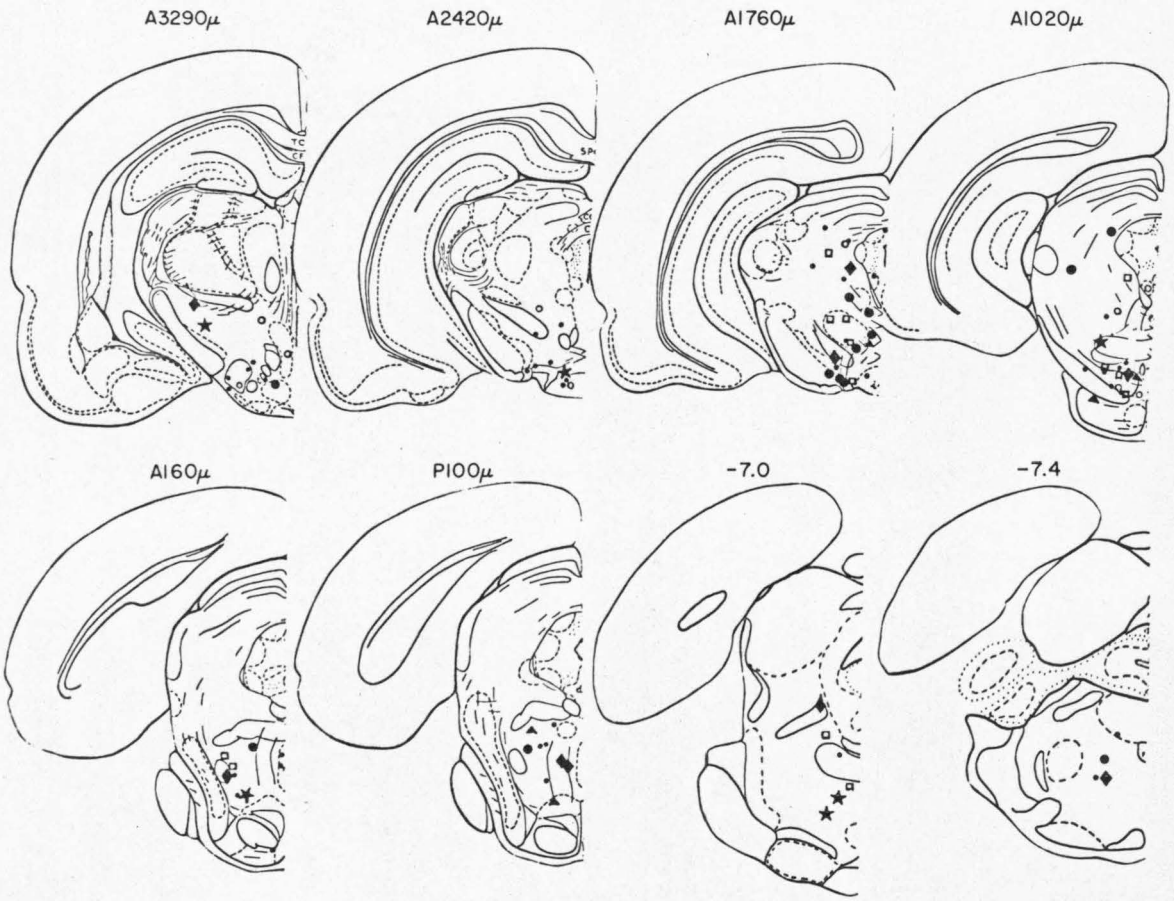

Day 1
Pseudo-conditioning
CS+
CS-
Day 2
Conditioning
CS+
US
CS-

Fig. 6. Map indicating latencies and distribution of conditioned responses. Sections A3290-P100 from atlas of Koenig and Klippel (1963). Sections -7.0 and -7.4 from atlas of Pelligrino and Cushman (1967).

LATENCY OF CONDITIONED RESPONSE



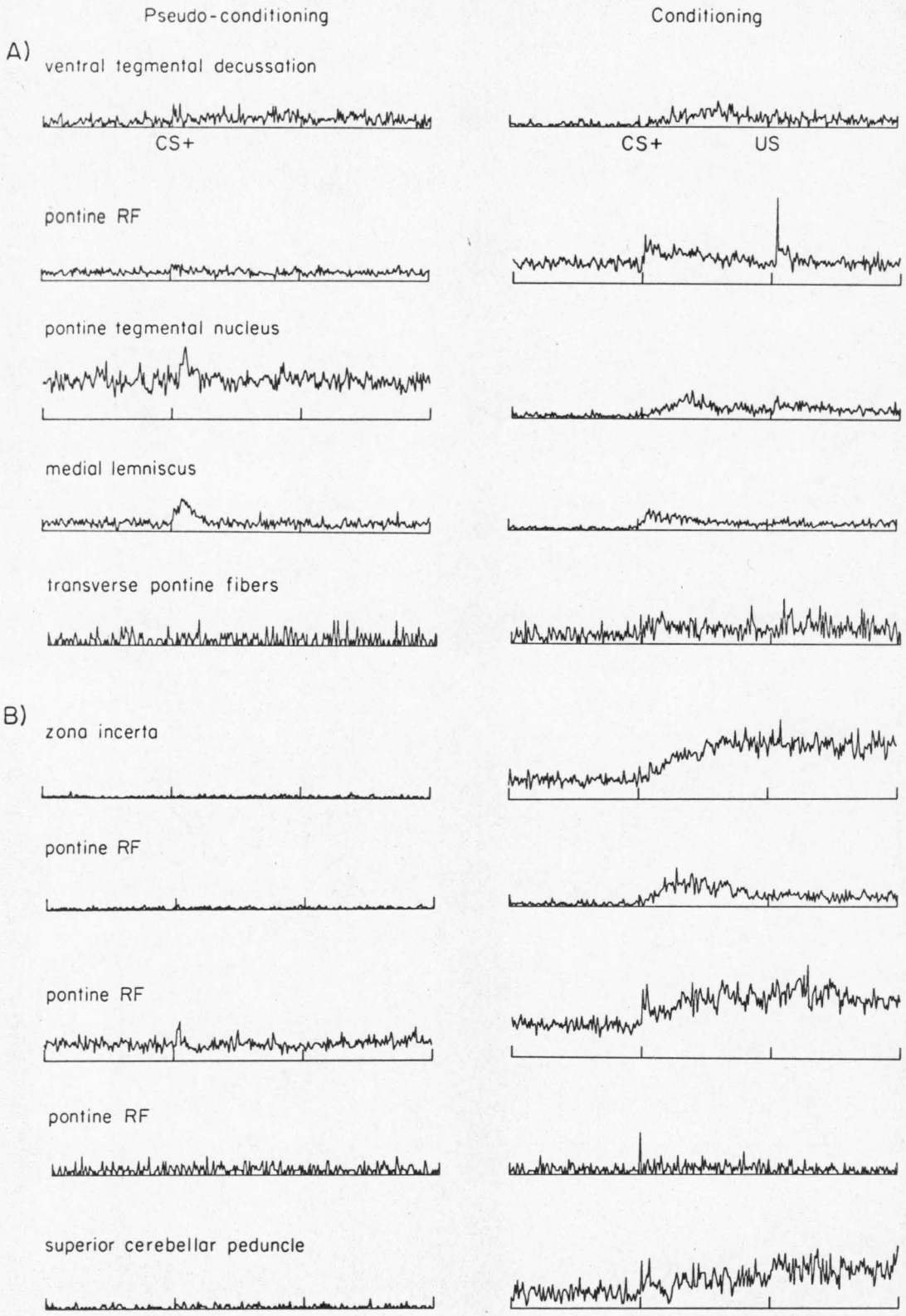
▲ 10 msec; ★ 20 msec; ◆ 40 msec; ▫ 60 msec; ○ 80 msec; • >80 msec;
● No conditioned response.

of the conditioned unit responses began before the behavioral response (prior to 80 msec). Forty-six of the units also displayed unconditioned responses.

Very short latency conditioned responses in a questionable 10 msec category) characterized 5 units located in 1) region of the ventral tegmental decussation, 2) dorsal portion of the pontine reticular formation, 3) pontine tegmental nucleus, 4) region of the medial lemniscus, and 5) region of the transverse pontine fibers (see Fig 7A). Only the response of the unit localized in the region of the medial lemniscus was followed by a significant conditioned response in the 20 msec interval.

Short latency conditioned responses with 20 msec latencies (appearing 0-20 msec after stimulus onset) characterized 6 units located in 1) zona incerta, 2) central region of the posterior pontine reticular formation, 3) ventrolateral region of the anterior pontine reticular formation, 4) dorsal region of the posterior pontine reticular formation, 5) region of the superior cerebellar peduncle, and 6) ventral tegmental area of Tsai (see Fig. 7B). Most of these responses were of short duration. The unit in the ventral tegmental area of Tsai was the only one to show a sustained conditioned response in every interval of the early response analysis. These short latency conditioned responses were not augmentations of unconditioned responses since there was no indication of response in this interval on the pseudo-conditioning day. There was no generalization of these responses to the CS- during this interval.

Fig. 7. Short latency conditioned responses. The traces on the left are the average pre- and post-stimulus histograms for the pseudo-conditioning day and the traces on the right are for the conditioning day. The responses in A) have latencies of 10 msec and in B) of 20 msec. The responses in A) were considered questionable because of the possibility of artifacts. Traces were not available for the unit in the ventral tegmental area of Tsai which had a response latency of 20 msec.


Pseudo-conditioning
Conditioning
A)
ventral tegmental decussation
CS+
CS+
US
pontine RF
pontine tegmental nucleus
medial lemniscus
transverse pontine fibers
B)
zona incerta
pontine RF
pontine RF
pontine RF
superior cerebellar peduncle

There were 44 units in all with conditioned responses displaying latencies of less than 80 msec (prior to the overt behavior). These were present throughout the extent of the brain stem, occurring most frequently in the posterior midbrain and anterior pons where 8 of 14 units (57%) showed such latencies in each group. The proportion of these responses in other areas was: posterior pons, 5 of 9 units (56%); central pons, 6 of 14 units (43%); posterior diencephalon, 5 of 13 units (38%); central midbrain, 10 of 26 units (38%); and anterior midbrain, 2 of 10 units (20%). Nineteen of 41 units in the non-specific reticular formation of the midbrain and pons were in this group. These were not localized to a particular region of the reticular formation. In 6 cases the response in the first significant interval was an augmentation of an unconditioned response.

There were 43 units with conditioned responses displaying latencies greater than 80 msec.

Five of the 87 units displayed inhibitory conditioned responses. Three of these were derived from that part of the midbrain "reticular activating system" surrounding the widest part of the central gray matter.

Thirteen of the units studied did not significantly change their response to the CS+ as a result of the conditioning procedure. These units were distributed in the following manner: 5 units in the central midbrain; 2 units in the anterior midbrain; 2 units in the posterior midbrain; 2 units in the central pons; 1 unit in the posterior diencephalon; and 1 unit in the posterior pons. Six of these units

displayed significant unconditioned responses.

Comparison of Unconditioned and Conditioned Responses

A comparison was made of the response magnitude of those units which showed only an unconditioned response with those that showed only a conditioned response during the first 80 msec after onset of the CS+ over 4 days of the experiment. For each unit the interval of peak response prior to 80 msec on the first conditioning day was chosen for comparison. The median standardized score for each group is presented (see Table 1). There was no significant difference in the magnitude of unconditioned and conditioned responses on the conditioning day or on either of the overtraining days by the median test (Hays, 1966).

Background (Spontaneous) Firing Rates

Within a day there was little change in the unit background firing rates. There was some change in the background rate of firing from one day to the next. Such changes were most likely due to changes in the "setting" of the unit discriminators between days. However, it is also possible that the firing rates of these units are not constant over long periods of time or that a shift occurred in recording to a different neuron from the small family of similar neurons localized at the recording point.

Learning Curves

The average learning curves for behavior and for the units in

Table 1. Response magnitude of units showing either unconditioned or conditioned responses during the first 80 msec after onset of the CS+.

	Median Standardized Score	
	Unconditioned Response Only	Conditioned Response Only
Pseudo-conditioning	5.70 (N=14)	0.55 (N=21)
Conditioning	3.57 (N=14)	2.46 (N=21)
Overtraining 1	2.80 (N=2)	2.75 (N=10)
Overtraining 2	3.01 (N=2)	1.82 (N=10)

Units were classified as showing either unconditioned or conditioned responses during the first 80 msec after onset of the CS+. For each unit the interval of peak response prior to 80 msec on the conditioning day was chosen for comparison across days. The median standardized score for each group is presented.

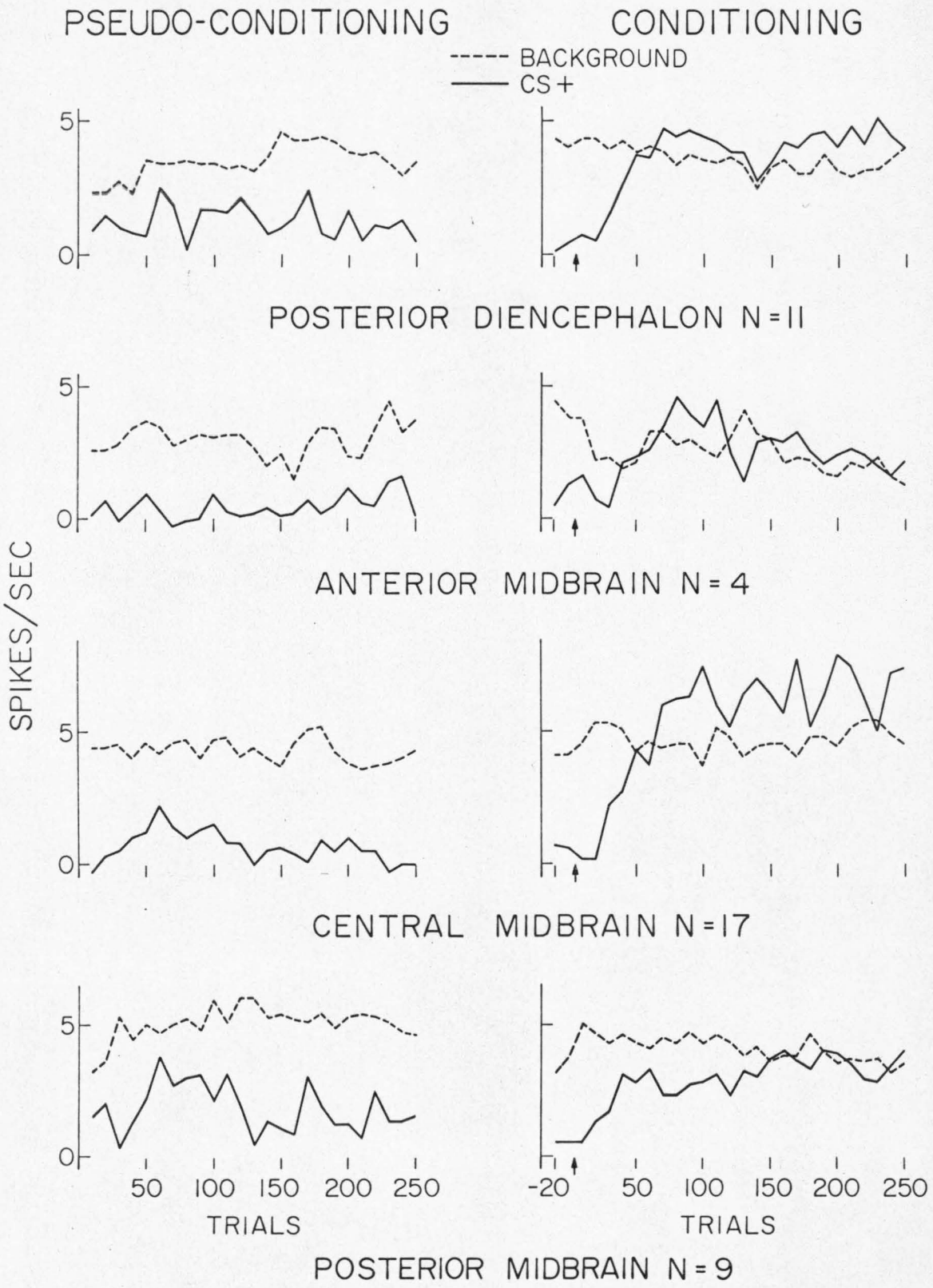
each brain area studied are presented in Fig. 8. Comparison of the first and second halves of each individual curve by a Mann-Whitney test (Hays, 1966) indicated that the trends seen in the average curves were representative of the members of the group making up that curve. Each average curve was analyzed for a consistent increase or decrease using a one-way analysis of variance with repeated measures over trials (Winer, 1962).

During pseudo-conditioning none of the brain areas showed significant changes in firing rate to the CS+ over trials. Almost all of the brain areas showed no significant changes in background firing rate. The only exception was the central pons which showed a significant increase ($p < .05$) in background firing rate during the course of pseudo-conditioning. There was a significant decrease ($p < .01$) in the behavioral curve in response to the CS+ during pseudo-conditioning.

All of the brain areas and behavior showed significant increases ($p < .01$) in response to the CS+ during conditioning. None of the brain areas showed significant changes in background activity during conditioning. Behavior showed a significant decrease ($p < .01$) in background rate during both pseudo-conditioning and conditioning.

The results of the analysis of the initial point of changed responsiveness to the CS+ are presented in Table 2. The mean number of trials after pairing began for a learned trend to emerge ranged from 28 to 75 for the various brain areas. The mean for the behavioral learning curves was 42. The anterior pons ($\overline{X} = 75$) and the posterior pons ($\overline{X} = 28$) were the only areas to show a significant

Fig. 8. Average curves for behavior and for the units in each brain area studied during the first 250 trials of pseudo-conditioning and the 20 trials before pairing and the 250 trials immediately after pairing of the CS+ and US during conditioning. The background curves represent 20 trial periods for each unit and behavior member of the respective groups. The CS+ curves represent 10 trial periods and are expressed as response minus background.


PSEUDO-CONDITIONING
CONDITIONING
----- BACKGROUND
—— CS+
5
0
POSTERIOR DIENCEPHALON N=11
ANTERIOR MIDBRAIN N=4
SPIKES/SEC
CENTRAL MIDBRAIN N=17
50 100 150 200 250
-20 50 100 150 200 250
TRIALS
TRIALS
POSTERIOR MIDBRAIN N=9

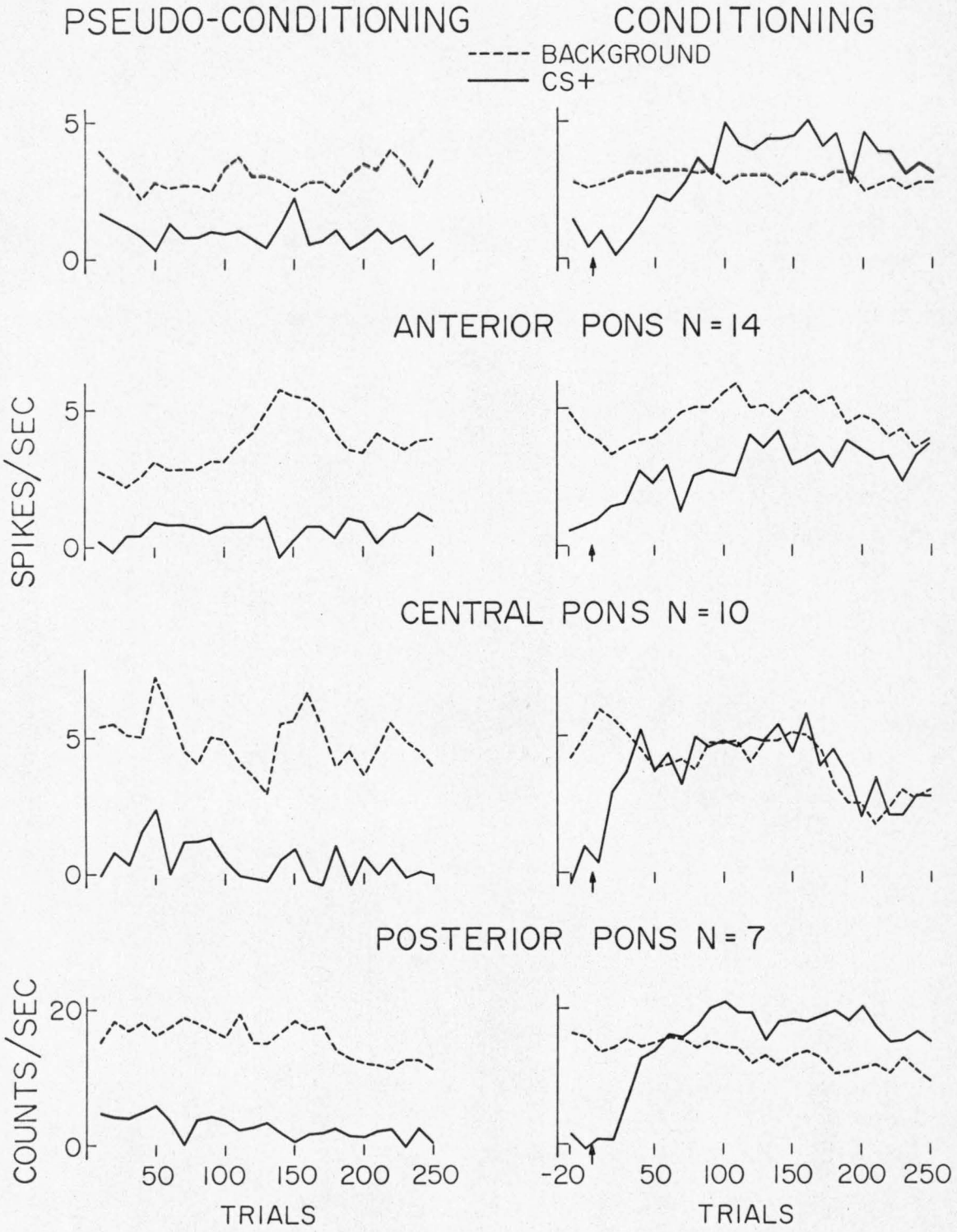

PSEUDO-CONDITIONING
CONDITIONING
----- BACKGROUND
—— CS+
5
0
ANTERIOR PONS N = 14
SPIKES/SEC
5
0
CENTRAL PONS N = 10
5
0
POSTERIOR PONS N = 7
COUNTS/SEC
20
0
50 100 150 200 250
TRIALS
-20 50 100 150 200 250
TRIALS
BEHAVIOR N = 72

Table 2. Initial point of changed responsiveness to the CS+.

	$\bar{X}$ number of trials	S.D.
Posterior diencephalon	63	54.7
Anterior midbrain	43	4.7
Central midbrain	50	37.4
Posterior midbrain	63	39.3
Anterior pons	75	54.3
Central pons	53	32.6
Posterior pons	28	21.1
Behavior	42	30.5

Anterior pons vs. Posterior pons	$t = 2.13$, p .05
Anterior pons vs. Behavior	$t = 3.23$, p .002

Individual unit and behavior learning curves were examined for the first point of a series which was out of the pre-pairing range. The values in the table are the means and standard deviations of the initial point of changed responsiveness to the CS+ for each group.

difference ($p < .05$, two-tailed) in the mean number of trials to show a learned trend by a t test (Hays, 1966). The anterior pons was the only brain area which differed significantly ($p < .002$, two-tailed) from behavior in the mean number of trials to show a learned trend.

Overtraining

There were 41 units with data available for 2 days of overtraining. Responses during the first and last quarter of the 1 sec CS-US interval were analyzed separately in order to obtain a general picture of these changes. A response was considered to be altered by further training if it differed from the previous day's response by $\pm$ 0.5 standard deviations or more. Eight units were eliminated from the first quarter second data and 4 units from the last quarter second data because there was no significant conditioned response in those intervals on any of the three conditioning days.

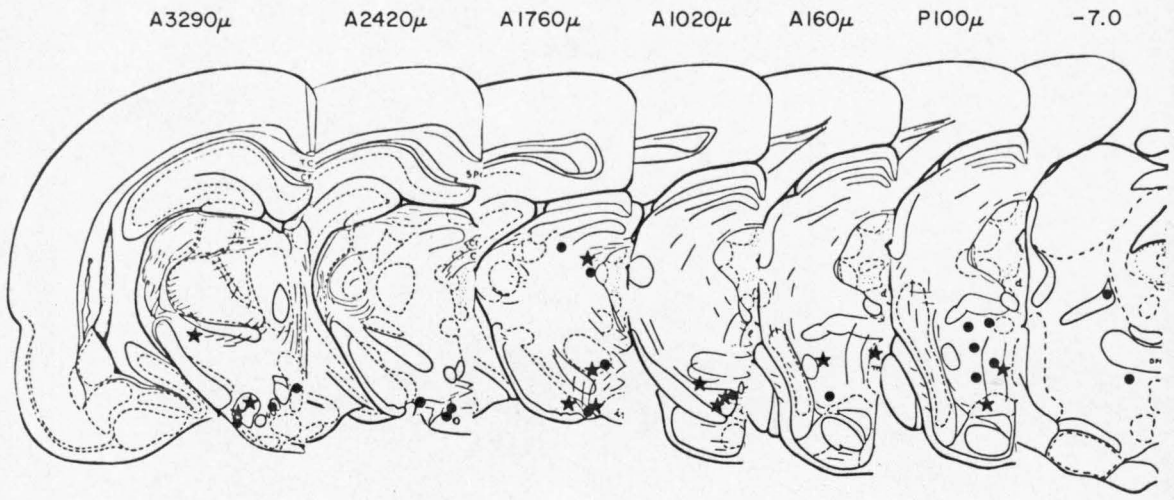
The response in the first quarter of the second was increased or stayed the same in 14 units and showed some decrease in 19 units during the course of overtraining. Nine of the unit decreases reached the pseudo-conditioning level by the end of overtraining. These units were located in the 1) posterior hypothalamic nucleus, 2) posterior lateral hypothalamic area, 3) medial mamillary nucleus, 4) region of the mamillary peduncle, 5) region of the mamillothalamic tract, 6) ventromedial red nucleus, 7) region of the medial lemniscus, 8) ventral tegmental area of Tsai, and 9) dorsal pontine reticular formation. All but one were adjacent to the limbic areas. There were

8 units which first showed a significant conditioned response during overtraining. These units were located in the 1) posterior lateral hypothalamic area, 2) region of the mamillary peduncle, 3) superior colliculus, 4) red nucleus, 5) region of the medial lemniscus, 6) substantia nigra, 7) dorsomedial pontine reticular formation, and 8) medial raphe. In the last quarter of the second the response was increased or stayed the same in 7 units and showed some decrease in 30 units during overtraining. Ten of the unit response decreases reached the pseudo-conditioning level by the end of overtraining. These units were located in the 1) zona incerta, 2) medial forebrain bundle region, 3) region of the basal optic tract, 4) ventromedial red nucleus, 5) region of the crus cerebri, 6) dorsal midbrain reticular formation, 7) ventral midbrain reticular formation, 8) dorsomedial pontine reticular formation, 9) medial pontine reticular formation, and 10) ventrolateral pontine reticular formation. Only 2 of these were near the limbic midbrain areas. There were 6 units which first showed a significant conditioned response during overtraining. These units were located in the 1) region of the mamillary peduncle, 2) basal optic tract and nucleus, 3) ventral midbrain reticular formation, 4) substantia nigra, 5) dorsal pontine reticular formation, and 6) medial pontine reticular formation. Figure 9 indicates the direction of response change during overtraining in the brain areas studied. Figure 10 gives an example of a response which stabilized in the early portion and a different response which stabilized in the later portion of the CS-US interval during overtraining. Although the behavioral latency of the former is less than

Fig. 9. Map indicating the pattern of response in overtraining. A) shows responses from the first quarter of the CS-US interval. B) shows responses from the last quarter of the CS-US interval. Representative brain sections as in Fig. 6.

PATTERN OF RESPONSE IN OVERTRAINING

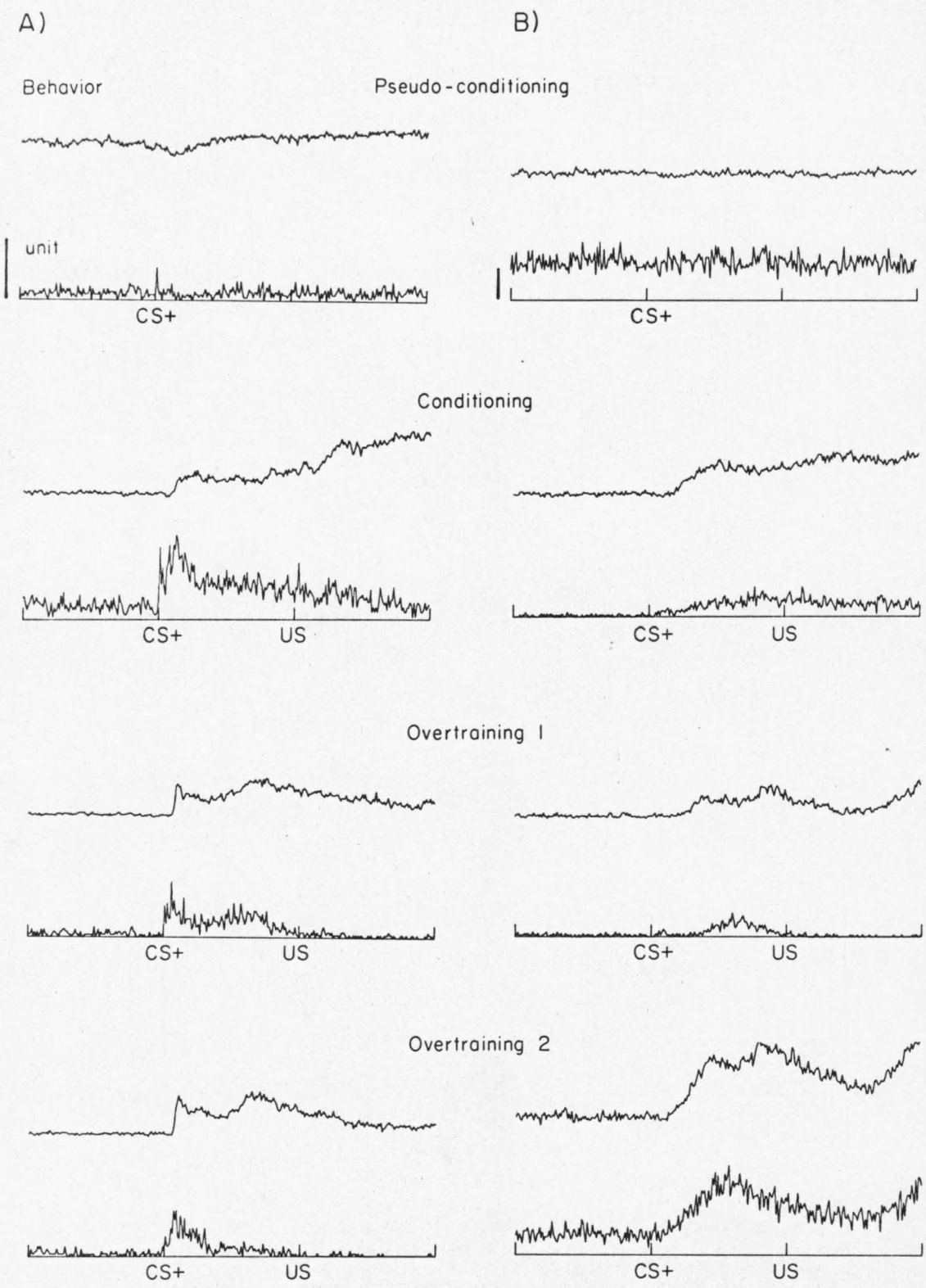
A)



B)

★ Response which increases or stays the same in overtraining. ● Response which shows some decrease in overtraining.

Fig. 10. Example of responses from A) probe 8614-7 located in the zona incerta in which the response stabilized in the early portion of the CS-US interval during overtraining, and B) probe 8680-7 located in the red nucleus in which the response stabilized in the late portion of the CS-US interval during overtraining. The upper trace represents the behavior and the lower trace represents the unit response. The vertical line at the left represents a probability of unit firing of .05 for each 10 msec bin.


A)
B)
Behavior
Pseudo-conditioning
unit
CS+
CS+
Conditioning
CS+
US
CS+
US
Overtraining 1
CS+
US
CS+
US
Overtraining 2
CS+
US
CS+
US

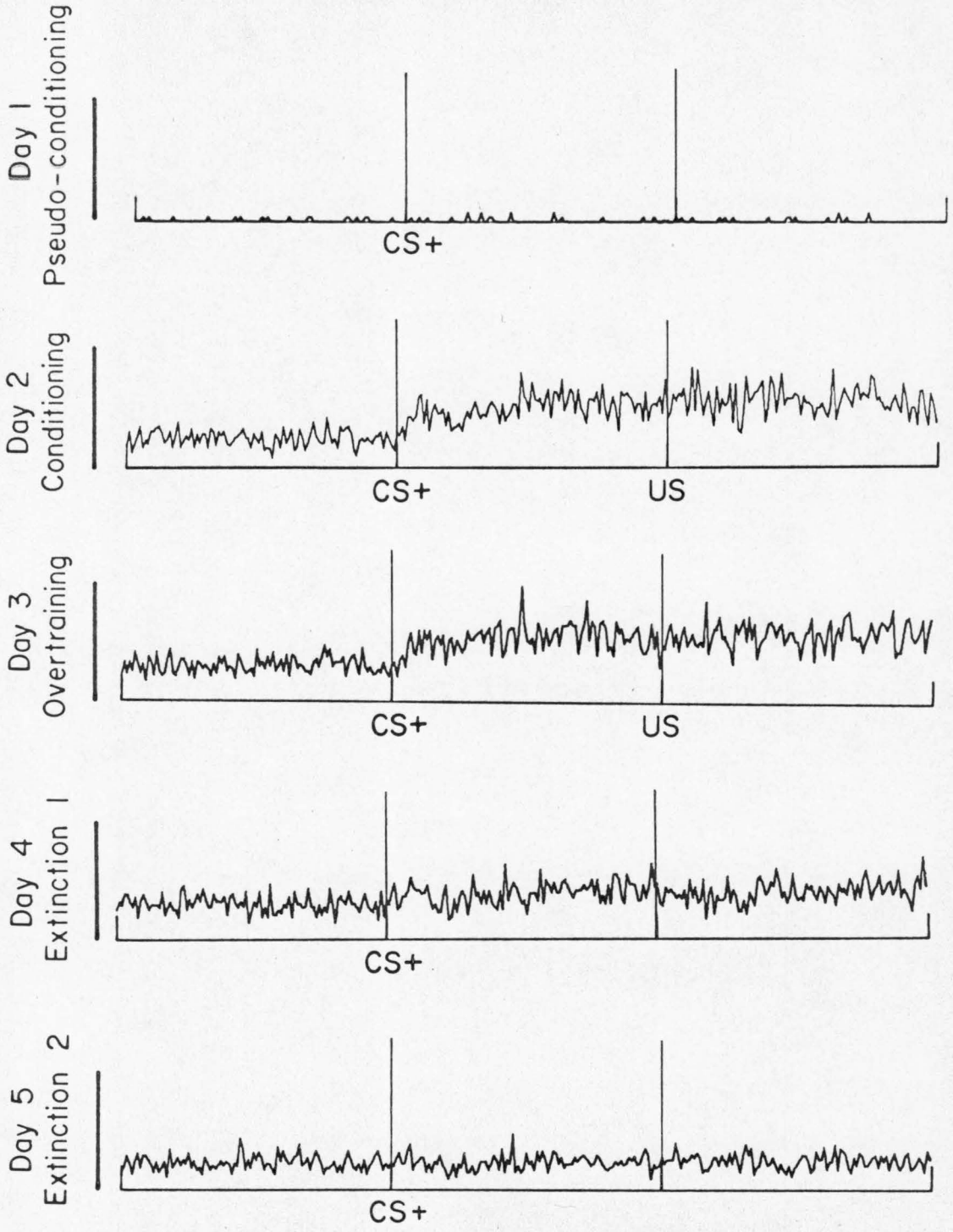
that of the latter, the behavioral latency for a particular subject does not appear to shift over days, implying that the change in the unit response over days is not directly related to the behavior.

Extinction

There were 16 units with data available for 2 days of extinction. The quarter second interval data were analyzed to determine if the responses would return to their pseudo-conditioning level when the CS+ was no longer followed by presentation of food.

In 2 cases with units located in the posterior mamillary nucleus and substantia nigra the response was reduced to the pseudo-conditioning level on the first extinction day. In 8 cases the unit response was reduced to the pseudo-conditioning level on the second extinction day. These units were located in the posterior hypothalamus, posterior lateral hypothalamic area, region of the mamillary peduncle, substantia nigra, ventral midbrain reticular formation, and 3 units in the region of the medial lemniscus. Only 2 of the 10 responses which did extinguish had displayed conditioned responses with latencies of 80 msec or less on the first conditioning day. Both of these units were in the region of the medial lemniscus. Figure 11 gives an example of a response which did extinguish from a unit in the region of the medial lemniscus. The shift in background firing rate from day 1 to day 2 may reflect a release from inhibition as a consequence of conditioning or a shift in recording to a different neuron from the small family of similar neurons localized at the

Fig. 11. Example of a response that was extinguished from probe 8817-5 located in the region of the medial lemniscus. The vertical line at the left represents a probability of unit firing of .10 for each 10 msec bin.


Day 1
Pseudo-conditioning
CS+
Day 2
Conditioning
CS+
US
Day 3
Overtraining
CS+
US
Day 4
Extinction 1
CS+
Day 5
Extinction 2
CS+

recording point.

There were 6 units which still displayed a significant conditioned response in at least 1 of the quarter second intervals after 2 days of extinction. These responses were not confined to any particular portion of the CS-US interval. The units were located in the 1) H_1 fields of Forel, 2) region of the mamillary peduncle, 3) region of the medial lemniscus, 4) ventral midbrain reticular formation, 5) ventral tegmental area of Tsai, and 6) ventral pontine reticular formation. Four of these 6 units in which the conditioned response did not extinguish had conditioned responses with latencies of 80 msec or less on the first conditioning day.

DISCUSSION

Short latency conditioned unit responses (within 20 msec after CS onset) were recorded from 11 of the 100 probes. This was a sufficiently large set of very early conditioned unit responses so that the brain stem reticular formation might be considered as a site of local changes involved in some of the aspects of classical conditioning. These very short latency conditioned responses were not present in large enough numbers to permit precise localization of the specific parts of the brain stem which might have been particularly involved. The fact that our statistical criterion for an early conditioned response would be met sometimes by chance during a large number of tests makes the data difficult to interpret. Fortunately, the points discovered were not evenly distributed among the points tested. They were not observed in the far lateral third of the midbrain tegmentum and they were not found in the medial third either; thus they took a mid-lateral position. At the midbrain level they were present only in the lower half of the region studied. These 20 msec conditioned responses were not only absent in the tectum, but they were also absent in the upper half of the midbrain reticular formation. They were present in the lower half of the midbrain reticular formation in a mid-lateral position. In the pontine reticular formation they also had a mid-lateral position. But the pontine reticular formation in its most posterior part seems to have only a "lower half," being limited above by colliculi and fibers to the cerebellum. Thus it was no longer appropriate to speak of the

responses as being mainly in the lower half of the pontine reticular formation. In general, these responses were characteristic for the "integrative" region of the reticular formation described by Brodal (1957) for the cat.

By one criterion these short latency conditioned responses could be considered to be fairly specific since only a small proportion of these units showed such responses (about 1 in every 14 cases). In a similar recent study (Olds, et al., 1972) responses in the posterior thalamus were found to be more frequent (there was more than 1 short latency response for every 2 cases), while in the cortex and hippocampus short latency responses amounted to only about 1 in 30, and 1 in 21 cases,respectively. When responses appeared in an area in large proportion they were considered to be non-specific. By this analysis, the reticular examples were more specific than those in posterior thalamus but less so than those in cortex.

In order to study the long-run characteristics of learned responses observed in the brain stem the group was increased in size by adding to those elements yielding very early conditioned responses the others that showed conditioned changes during the course of the CS-US interval. These were studied with two main questions in mind: 1) what were the eventual stable levels of these responses after overtraining (as the behavior became more automatic), and 2) what were the characteristic changes during extinction when the behavior was "unlearned."

The most interesting outcome of these tests was that units broke

up into two families during overtraining. One group eventually yielded stable responses in the latter portion of the CS-US interval (at the time when the animal was making preparatory responses related to the US). The other group eventually yielded stable responses only in the early portion of the CS-US interval (at the time when the animal was making orienting responses related to the CS). These two kinds of stabilization patterns which appeared during overtraining characterized two meaningfully different anatomical areas. These can be indicated by the following description. At the midbrain level the non-specific parts of the tegmentum are divided into at least two relatively clearly separated systems. One is the "reticular system" described by Magoun and his colleagues (Magoun and Rhines, 1946; Rhines and Magoun, 1946; Moruzzi and Magoun, 1949). This system is identified with that part of the brain usually called the reticular formation by anatomists (Brodal, 1957; Scheibel and Scheibel, 1958). The other system in the ventral tegmental area has strong bi-directional connections and close functional similarity with the hypothalamic medial forebrain bundle area described as a reward or motivational system (Olds, 1962). This limbic midbrain system extends from the supramamillary region through the area of Tsai, including an area proximal to the substantia nigra and interpeduncular nucleus. These two non-specific systems yield respectively "arousal" on the one hand, and "motivational" effects on the other when stimulated electrically (Olds and Peretz, 1960). While there are relations between them, they are clearly differentiated. In the present study the responses

that stabilized in the early portion of the CS-US interval were derived from probes in the reticular arousal system. The responses that stabilized in the latter portion of the CS-US interval were derived from probes in the limbic midbrain motivational system.

During extinction tests the most interesting differences followed similar, but not identical lines. In this case early responses (closer in time to the CS) were more resistant to extinction and later responses (closer in time to the conditioned behavior itself) were less resistant, usually being completely extinguished when the behavior was also gone.

Several objections may be raised to the interpretation of this data as indicating parts of the brain stem where local changes occurred during learning. It might be argued that alterations in other parts of the brain could have been projected to the reticular formation. A tonically active element (a "dynamic engram") might result in the message taking a path which was functionally closed prior to conditioning. In this case the point of the observed new conditioned response would be the effective site of the dynamic engram. If such a mechanism were involved it should be observable as a change in background firing rate of the tonically active element. The somata of these elements might be in the recording area or elsewhere, but their fibers would necessarily project to the recording region. It is on this basis that the local region might be considered to be the effective site of this "dynamic engram." The time course of the change in background firing rate for these elements

would exhibit a "learning curve" that would have a trial-to-trial character similar to that of the conditioned response. While there were no such changes in the background activity of the brain stem units observed here, it is possible that such changes occurred in other structures which project to the brain stem. In future experiments attempts will be made to determine whether this in fact occurs using the present methods. Trial-to-trial changes in background firing rates are already measured in these tests. In further studies it will be necessary to map the brain with care, seeking the background rate changes that correlate with these response changes. Then tests will be made for projection from areas with background changes to areas with response changes. While the mapping approach may seem time consuming, a finite number of samples can give a fair representation even if the population approaches infinity as sampling theory clearly indicates.

It might be suggested that the animals were more aroused after conditioning and that this added arousal made the units more responsive. Since the animals were equally aroused and in an essentially identical situation on the first day during pseudo-conditioning, background arousal could not account for the observed conditioned responses. Moreover, if background arousal was a factor then the conditioned responses should have been equal for the CS+ and the CS-, and they were not.

One might question the validity of generalizing the anatomical work of Brodal (1957) done on the cat (indicating a lateral input

region, a middle integrating region, and a medial output region) to the present study of the brain stem of the rat. Herrick (1948) described a very similar picture for the brain stem of the salamander. Since the reticular formation is so similar in species as diverse as the cat and the salamander, it seems reasonable to assume that these divisions apply also to the rat.

Historically, the reticular formation was viewed as an undifferentiable mass of diffusely interconnected short-axoned cells in the central portion of the brain stem extending from the lower border of the medulla to the diencephalon. The assumed lack of orderly arrangement within the reticular formation and the accompanying difficulty in investigating such an area may account for the relative paucity of analysis of the reticular formation in the first few decades of this century. This attitude was expressed by W. F. Allen writing in 1932 that "It is well known from embryology that most of the left-over cells of the brain stem and spinal cord which are not concerned in the formation of motor root nuclei and purely sensory relay nuclei are utilized in the production of the formatio reticularis."

It is now generally agreed that the reticular formation receives input from all of the ascending afferent systems (Amassian and Devito, 1954; Scheibel, et al., 1955) and cortical areas. In turn, it projects primarily to the spinal cord, cerebellum, thalamus, and cortex (Brodal, 1957). Investigations of the fine structure of the reticular formation have shown it to possess a fair amount of intrinsic

organization (Scheibel and Scheibel, 1958). Each portion of the stem receives input primarily from collaterals of fibers passing by that region. This separation of input in layers or "discs" along the anterior-posterior axis may be represented by the different rates of learning in the various regions along the brain stem. Afferents enter the reticular formation in a plane perpendicular to the long axis of the brain stem, while reticular cell dendrites show maximal arborization along this same plane. Therefore, synapses are formed between axons and dendrites running parallel to each other. Cells in the lateral region tend to project medially, whereas most medially situated reticular cells have bifurcating axons that project long distances rostrally and caudally, emitting frequent collaterals along their course. There is also some separation in the medial region of cells that project primarily in a rostral or caudal direction. In the medulla and pons, the former tend to be more caudally located than the latter, implying a considerable potential for integration between ascending and descending projections. In the present study recordings were probably made from the large elements observed by the Scheibels. The data are consistent with the view of the Scheibels that these are integrative mechanisms involved in higher processes. Other investigators have postulated that the reticular formation is importantly involved in learning processes (Penfield, 1952; Gastaut, 1958; Kilmer, et al., 1968; Bloch, 1970).

Some studies of conditioned unit responses and evoked potentials have focused on the latency of the conditioned response (Woody, et al.,

1970); others have focused on mapping the conditioned responses in the brain (John and Killam, 1959). The maps by John and Killam were interpreted by them as indicating that the reticular formation may be involved in some phase of the learning process. Thompson (1969), using lesion techniques, found the ventral tegmental area and the posterior nucleus of the thalamus were apparently necessary for visual discrimination learning. Sparks and Travis (1968) observed definite response patterns from reticular neurons during performance of a discriminative task which the authors considered to be related either to the sensory stimulus or to the motor response. They did not have a pre-conditioning baseline with which to compare their conditioned responses, and their "bin size" was so large as to rule out analysis of conditioned unit responses occurring before the conditioned overt behaviors. Ellison, et al. (1968) described differences in reticular and pyramidal multiple-unit activity in a conditioning situation designed to separate instrumental and classically conditioned responses. Pyramidal neurons responded during the instrumental response, while reticular neurons responded during both the instrumental and classically conditioned behavioral responses. The authors concluded that reticular formation responses were related to the motivational and arousal aspects of the situation. Buchwald, et al. (1966) observed conditioned multiple-unit responses in the reticular formation prior to the appearance of a leg flexion response. While these results are similar to those found in the present study, these authors did not localize the responses within the reticular formation, nor did they

present latencies to permit evaluation of the relative "primacy" of these conditioned responses.

If one assumes that learning "involves the rerouting of nerve impulses within the central nervous system...new pathways become available to incoming excitation;" (Burns, 1957), then after training the excitation would take an old pathway up to a point and then be routed into a new functional one which might consist of several steps. Synaptic connections between the old pathway and the first step of the new one might be altered in a variety of ways by the training procedure, while further connections from step one of the new pathway to successive steps need not be affected. "Conditioned brain responses" would appear however in the neuronal activity at all the successive steps because the excitation of elements at step one would initiate a progression of excitation along the chain. The present study attempted to differentiate between the neuronal activity at step one and that at later steps. Temporally, the new responses at step one would precede the later ones in the chain of events from stimulus to response. The shortest latency conditioned brain responses might be considered to be "at more primary sites" of conditioning, while other later conditioned responses would be considered to be more likely (but not necessarily) secondary to them. It is possible that some of the longer latency conditioned responses might also be at primary sites of change, but the present method does not allow distinctions to be made between primary and secondary conditioned responses when these both occur late in the CS-US interval. It might be possible to separate primary from

secondary responses in this pool of elements on the basis of when in the trial sequence (the "learning curve") the conditioned response develops. A long latency response that developed early in conditioning before any shorter latency ones had appeared might be considered as a primary change necessary for conditioning because its latency was the shortest at the time when it first appeared.

In the present study there was little difference between brain regions in the number of trials necessary for development of the conditioned response, and therefore a detailed analysis could not be made. However, the "earliest learning" in the brain stem did characterize a narrow region in the anterior pons. When the present data are compared with that from other studies (Disterhoft and Olds, in preparation; Segal and Olds, in preparation) a sequence of development of learned responses emerges. Conditioned unit responses appeared first in the pons, second in the thalamus and dentate gyrus, third in sensory cortex and CA3 of hippocampus, and fourth in motor cortex and CA1 of hippocampus.

The present study combined latency and mapping approaches in an attempt to map the latencies of the conditioned responses within the brain stem. This method indicated certain regions of the ventral brain stem as "significant sites" where specific changes might occur during conditioning. The results however, provide only a first step toward elucidating the regions actually involved in the learning process. A finer grain time analysis would permit a more definite identification of the earliest conditioned responses. A more complete

map of the brain stem with particular emphasis on the ventral regions would be most helpful in clarifying the areas involved. The next step must be to find whether some critical change elsewhere is projected to the recording point and could account for the short latency conditioned responses. First, the latency refinements (smaller time bins) will permit an answer to the question of whether other learned events triggered by the stimulus precede the responses recorded in the ventral brain stem and are projected to these points. Second, analysis of changes in tonic unit activity during learning must be mapped to determine whether relevant activity could be projected to these points by units whose changes in background rates actually constitute the "memory trace."

It is worth noting that if changes in background firing rates actually account for the short latency conditioned responses, then these tonically active elements must have very specialized characteristics. They will not be "turned on" by the auditory stimulus, by arousal, by the behavior, or by the reinforcement. All of these aspects of the experiment were identical during pseudo-conditioning and conditioning. They must therefore be "turned on" by the specific association of the auditory stimulus with reinforcement. In addition, these elements will not be acting in a general manner. This is because they would not act equally on the response to the CS-. In almost every case the response to the CS+ was larger than the response to the CS-. Thus they would be elements triggered or accelerated by the association of a particular auditory stimulus with reinforcement.

They would reflect back selectively to facilitate or withdraw inhibition from particular synaptic channels specialized for carrying one auditory message to the exclusion of others. Finally, they would be elements that had discharge rate as a "settable parameter," at least for a given context or environment.

Further corroboration of my results could come from studies involving discrete lesions. If lesions between reticular areas and their cortical afferents did not abolish the phenomena, then it would be less likely that they resulted from dynamic changes occurring elsewhere. Studies employing presentation of stimuli in different modalities, each serving as the CS for the same response (or presentation of the same stimulus modality as the CS for a different response), are required to indicate the extent to which the conditioned unit responses were related to the stimulus and response components of the learning situation.

Theories of learning which assume specific physiological or anatomical changes have generally suffered from a lack of evidence for clear neuronal changes or neuronal growth in the central nervous system of the adult animal. Such changes have usually been observed in very young animals. Some recent investigations have further indicated the capacity for change in the central nervous system of young animals as a result of previous experience, and some similar events have been observed in older animals. Globus and Scheibel (1967) and Valverde (1967) observed changes in the spines of the apical dendrites of visual cortex pyramidal cells as a consequence of early

visual deprivation. Rosenzweig, et al. (1969) noted increases in both the weight and depth of visual cortex as a result of living in an enriched environment. Raisman (1969) reported heterotypical re-innervation of septal nuclei from persisting intact fibers in the de-afferented area, demonstrating the existence of "new" synaptic connections formed in the adult central nervous system. Fifkova (1970) has more recently reported changes in the volume of the lateral geniculate and the thickness of the visual cortex caused by visual deprivation even when this was instituted after considerable visual experience during maturation. Although these studies did not prove that specific physiological or anatomical changes occurred as a result of a particular learned association, they did indicate that changes could occur in the adult central nervous system as a result of experience.

While the present method identified regions where changes in the unit activity occurred as a function of conditioning experience, it did not indicate the mechanism underlying the change or the way in which this mechanism was controlled. Such information will hopefully be developed by further studies of the electrophysiology, pharmacology, and fine anatomy of the critical regions implicated by the present kind of mapping experiments. However, as Horridge (1968) has pointed out, "The ultimate validation of the claim that any observed change is the cause of learning is to impose it artificially and produce a behaviour change."

We are at the beginning of a new phase in the study of the mechanisms underlying the learning process. It is hoped that a com-

bination of approaches will enable us to understand these pervasive, yet elusive processes.

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APPENDIX I

Early Response Analysis

1) The mean of the background:

$$\bar{X}_b = \frac{\sum_{i=1}^{100} X_i}{100}$$

X_i = the average probability of firing in a bin over 320 trials

b = background

2) The standard deviation of the background:

$$\sigma_b = \sqrt{\frac{\sum_{i=1}^{100} (X_i - \bar{X}_b)^2}{100}}$$

3) The standardized score (Z score) for a bin:

$$Z_i = \frac{X_i - \bar{X}_b}{\sigma_b}$$

4) The standardized score for a pair of bins:

$$Z_{i+(i+1)} = \frac{X_{i+(i+1)} - X_b}{\frac{\sigma_b}{\sqrt{2}}}$$

$$Z_{i+(i+1)} = \frac{Z_i + Z_{i+1}}{2} \cdot \sqrt{2}$$

$$X_{i+(i+1)} = \frac{X_i + X_{i+1}}{2}$$

$Z_{i+(i+1)}$ is compared to a sampling distribution made of pairs of bins in the background. In order to convert the single Z scores to Z scores for pairs of bins, the standard deviation has to be divided by the square root of the sample size (2).

5) The real values of Z were compared to the expected Z score yielding $P < .03$, two-tailed. Z values were considered significant when

$$\frac{Z_i + Z_{i+1}}{2} \cdot \sqrt{2} \geq Z_{(P \leq .03)} \; ; \; Z_{(P \leq .03)} = 1.55 \; (\sqrt{2}) = 2.19$$

6) For the first 10 msec bin:

$p(Z \geq 2) \leq .046$, two-tailed

Late Response Analysis

1) The standardized score for a quarter of a second (25 bins):

$$Z_{i+(i+1)+\ldots+(i+24)} = \frac{X_{i+(i+1)+\ldots+(i+24)} - \bar{X}_b}{\frac{\sigma_b}{\sqrt{25}}}$$

$$X_{i+(i+1)+\ldots+(i+24)} = \frac{X_i + X_{i+1} + \ldots + X_{i+24}}{25}$$

$$Z_{i+(i+1)+\ldots+(i+24)} = \frac{Z_i + Z_{i+1} + \ldots + Z_{i+24}}{25} \cdot \sqrt{25}$$

$Z_{i+(i+1)+\ldots+(i+24)}$ is compared to a sampling distribution made of groups of 25 bins in the background. In order to convert the single Z scores to Z scores for groups of 25 bins, the standard deviation has to be divided by the square root of the sample size (25).

2) The real values of Z were compared to the expected Z score yielding $P < .001$, two-tailed. Z values were considered significant when

$$\frac{Z_{i+} + Z_{i+1} + \ldots + Z_{i+24}}{25} \cdot \sqrt{25} \geq Z_{(P < .001)}$$

$$Z_{(P < .001)} = 1.00 \; (\sqrt{25}) = 5.00$$

APPENDIX II

Summary of Unconditioned (o) and Conditioned (x) Responses

In Each Interval for Each Probe for the First Two Days of the Experiment

SUBJECT ELECTRODE	STRUCTURE	10 msec	20 msec	40 msec	60 msec	80 msec	250 msec	500 msec	750 msec	1000 msec
	POSTERIOR DIENCEPHALON									
8216-1	zona incerta		x				x	x	x	x
8614-7	zona incerta		o	x	x	x	x	x	x	x
9195-7	H_1			o	o	x	xo	xo	xo	xo
9207-1	posterior LHA							x	x	x
8801-1	MFB					x				x
9084-8	MFB						x	x	x	x
8492-1	posterior MFB						x	x	x	x
8921-2	posterior HTH nucleus					xo	x			
8817-7	posterior HTH						x	x	x	x
8801-2	mamillothalamic tract			o	o	o	xo	xo	x	x
8817-2	dorsal premamillary nucleus									
8801-7	medial mamillary nucleus						x			
8817-3	posterior mamillary nucleus							x	x	x
	ANTERIOR MIDBRAIN									
8174-6	central midbrain RF					o-	o-	x	x	x
8105-3	central lateral midbrain RF		o	o	o	xo	o	xo	x	x
8542-7	ventral midbrain RF									
8984-7	ventral midbrain RF							x	x	x
8343-1	ventromedial midbrain RF						o	x	xo	
8216-7	medial lemniscus							x	xo	x
9243-3	ventral tegmental area Tsai or supramamillary decussation		x	x	x	x	x	x	x	x
9084-7	mamillary peduncle						x	x	xo	x

(continued)

SUBJECT-ELECTRODE	STRUCTURE	10 msec	20 msec	40 msec	60 msec	80 msec	250 msec	500 msec	750 msec	1000 msec
	ANTERIOR MIDBRAIN									
8801-3	basal optic tract and nucleus									
8921-7	basal optic tract								x	x
	CENTRAL MIDBRAIN									
8733-6	superior colliculus				o–		o	o	xo	x
8293-4	dorsal midbrain RF				xo	x	x	x	x	x
8733-3	dorsal midbrain RF							o	xo	xo
8294-5	dorsomedial midbrain RF			x		x	x	x	x	x
8680-2	dorsomedial midbrain RF					x	x	xo	xo	xo
8736-2	dorsomedial midbrain RF						x–	x–	x–	x–
8184-6	medial midbrain RF							o–	o–	
8105-4	medial midbrain RF									
8277-6	dorsomedial central gray		o	o			o	x	x	x
8293-5	nucleus darkschewitsch						x	x	x	x
8136-3	dorsomedial red nucleus		o	o	x	x	x	x	x	x
8176-1	dorsolateral red nucleus				x	x	x			
8680-7	ventromedial red nucleus				x	x	x	x	x	x
8736-7	ventromedial red nucleus							x	x	x
8431-2	ventral red nucleus									
8612-8	medial lemniscus			x	x	xo	x	x	x	x
8437-2	ventral tegmental area Tsai				x	x	x		x	x
8343-2	ventral tegmental decussation	x					o	xo	xo	o
9243-5	substantia nigra, zona reticulata	o								
9195-3	substantia nigra, zona compacta							x		x
9243-6	mamillary peduncle									
9207-3	interpeduncular nucleus						x	x	x	x
8183-7	no histology				x			x	x	
8200-6	no histology							x	x	
8340-5	no histology						x	x	x	x
8340-4	no histology							x	xo	xo

(continued)

SUBJECT-ELECTRODE	STRUCTURE	10 msec	20 msec	40 msec	60 msec	80 msec	250 msec	500 msec	750 msec	1000 msec
	POSTERIOR MIDBRAIN									
8277-5	dorsolateral midbrain RF		o	o	o		o	o	o	o
8277-4	dorsomedial midbrain RF	o	o	o	o	o	o	o	o	
8460-3	dorsomedial midbrain RF				x–			xo	xo	x
8105-6	central midbrain RF		o	o	o	o	xo	xo	xo	xo
7967-7	ventromedial midbrain RF		o	o	xo	xo	xo	xo	xo	xo
7967-5	ventromedial midbrain RF		o	o	o	x	xo	xo	xo	xo
8492-5	ventromedial midbrain RF				o–	o–	o–			x–o–
8921-5	ventral midbrain RF				x		xo	x		
9195-4	ventral midbrain RF							o	xo	xo
8426-7	principal oculomotor nucleus					x	x	x	x	x
8437-6	rubrospinal tract		o		o		x	x	x	x
8492-6	medial lemniscus		o	x	x		x	x	x	xo
8510-6	interpeduncular nucleus			o		x	x	x	x	
8174-4	no histology					x	x	x	x	x
	ANTERIOR PONS									
8437-3	ventrolateral pontine RF		x	x	o	x	x	x	x	x
8491-4	dorsolateral pontine RF		o	o	xo	xo	xo	x	x	x
8431-6	medial raphe							x	x	x
8736-6	medial raphe							x	x	x
8612-7	superior cerebellar peduncle		x		x	xo		x	x	x
8510-3	medial lemniscus		o	o	x		x	x	x	x
8744-6	medial lemniscus					x	x	x	x	x
8744-5	medial lemniscus	x	x	o	o	o	o	x	x	x
8817-5	medial lemniscus				x	x	x	x	x	x
9070-5	medial lemniscus								x	x
9084-5	medial lemniscus or crus cerebri							x	x	x
8402-6	medial lemniscus or crus cerebri			o						x
9207-5	crus cerebri								x	x
8984-4	transverse pontine fibers	x				x	x	x	x	x

(continued)

SUBJECT-ELECTRODE	STRUCTURE	10 msec	20 msec	40 msec	60 msec	80 msec	250 msec	500 msec	750 msec	1000 msec
	CENTRAL PONS									
8431-5	dorsal pontine RF							x	x	o
8460-5	dorsal pontine RF		o	o			o	o	o	
8575-3	dorsal pontine RF	x		xo	xo	o	xo	x	x	
8575-6	dorsomedial pontine RF								x	x
8680-4	medial pontine RF		o	x	x	o	x	x	x	x
8492-4	medial pontine RF		o	x	x	x	x	x	x	x
8176-6	lateral pontine RF			o						
8554-4	lateral pontine RF		o	o	o	o	o	x	x	xo
8733-4	lateral pontine RF				x-		x	x		
8402-5	ventrolateral pontine RF			o	o				x	x
8216-6	ventrolateral pontine RF or rubrospinal tract			x	xo	x	x	x	x	x
8744-4	ventral pontine RF						x	x	x	x
8733-5	pontine tegmental nucleus	x					xo	x	x	x
8542-5	medial pontine transverse fibers							x		x
	POSTERIOR PONS									
8216-4	dorsolateral pontine RF									
8437-5	dorsal pontine RF		x			x				
8343-4	central pontine RF		x			x	x	x	x	x
8612-5	central pontine RF		o	x	x	x	xo	x	x	x
8612-4	central pontine RF				o-	o-	x-	xo	x	x
8575-5	medial pontine RF				x	xo	xo	x		
8491-6	locus coeruleus							x		
8575-4	decussation of superior cerebellar peduncle							x	x	x
8614-5	decussation of superior cerebellar peduncle			x	x	xo	x	x	x	x
Total number of electrodes with first significant response in each interval		5	6	9	14	10	14	19	8	2

(continued)

o = significant conditioned response on first conditioning day ($p < .03$, two-tailed)

x = significant unconditioned response on pseudo-conditioning day and first conditioning day ($p < .03$, two-tailed)

- = inhibitory response

Abbreviations: H_1 = Forel's field H_1; LHA = lateral hypothalamic area; MFB = medial forebrain bundle; HTH = hypothalamic; RF = reticular formation