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EEG CORRELATES OF BEHAVIOR IN THE CAT. I. PATTERN DISCRIMINATION AND ITS ALTERATION BY ATROPINE AND LSD-25[1]

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INTRODUCTION

It is difficult to correlate spontaneous variations of electrophysiological events in brain structures with specific behavioral states or with the myriad amount of information processed by the brain over any period of time. However, the presentation of an extrinsic conditioning stimulus limits the time interval wherein brain wave alterations should reflect specific behavioral states and may make it possible to identify the brain structures participating in their generation. Going one step further, the technique of using a "tagged conditional stimulus" developed by John and Killam (1959, 1960) permitted them to observe changes in the form and distribution of evoked responses from various areas of the brain during acquisition, performance, stabilization and, finally, degradation of approach and avoidance performance.

In the present experiments, their technique, which employs a light of a specified frequency as a conditional stimulus, has been utilized to locate areas of information processing within the brain during acquisition and performance of a series of conditional behaviors which represent visual discrimination tasks of increasing difficulty. Unlike John and Killam's studies, however, the stimulus field was restricted to geometric forms pulsed upon a translucent background. The influences of atropine and LSD-25 on the correlated EEG and behavioral responses to the stimulus have also been assessed.

METHODS

Recording electrodes were implanted in five cats under pentobarbital anesthesia. Stainless steel screws were placed over the cortical visual area of the lateral (marginal) gyrus (Lat Gy) and the anterior suprasylvian gyrus (ASS Gy), while bipolar electrodes were oriented stereotaxically into deeper structures as shown in Table I. All electrode placements were confirmed by histological examination. The bipolar electrodes which were used consisted of two, 32-gauge, insulated stainless steel wires laminated to an insulated strut. The electrodes extended 1 mm beyond the tip of the strut and were bared only at the cut tips.

Experimental procedures were carried out in a sound resistant, electrostatically shielded box with a one-way observation window. Three translucent panels were arranged at one end of the box so that light pressure on each of the panels could activate a micro-switch which under program control could deliver about 2 ml of milk to a dish immediately below the panel pressed. The conditional stimulus (CS) consisted of a geometric figure punched into a solid slide and projected. The slide projection path was interrupted 10/sec by a rotating disk. Slides were programmed in the projector tray so that the stimuli were presented in a random order on the three panels.

The animals were fluid deprived in their home environment but were allowed free access to a

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TABLE I

Electrode placements in five cats

Cortical and subcortical structures	BE 39	BE 57	BE 58	BE 66	BE 67
Visual cortex (Lat Gy)	+	+	+	+	+
Association cortex (Ass Gy)	+	+	+	+	+
Midbrain reticular formation (RF)	+*	+	+	+	+*
Lateral geniculate body (LG)	+**	+	+	+	+
Centre medianum (CM)		+	+		
Nucleus antero-ventralis (AV)		+			
Nucleus ventralis anterior (VA)	+			+	+
Superior colliculus (SC)				+	+
Ventro-medial hypothalamus (H Vm)	+	+	+		
Medial forebrain bundle (MFB)	+***		+		
Nucleus reuniens (RE)				+	+
Ventral hippocampus (Hipp)	+	+	+		
Amygdala (Amyg)				+	+

* Near or partly recording from central gray.
** Somewhat medial placement.
*** Electrode tip not precisely identifiable.

dry laboratory diet. Following a post-operative recovery period they were tested 5–7 days per week, and each daily training session consisted of 20 or more non-correction trials. Electrical recordings were taken at all daily sessions throughout behavioral training and drug studies, using a Grass Model IV electroencephalograph and a magnetic tape recorder (Ampex FR-100, 14 channels). The animals were first subjected daily for 2 weeks to a familiarization period which consisted of twenty unreinforced, 15 sec periods of the flickering stimulus. The animals were then trained to make the conditioned motor response, pressing a panel in order to receive a milk reinforcement. Then the behavior was brought under stimulus control by pairing the availability of the milk with the appearance of a lighted oval on one of the three panels. When the animals reached and maintained a criterion performance level of 85–100% correct, a period of drug testing was begun.

Subsequently, three animals were trained to select one of two geometric forms presented simultaneously on adjacent panels (BE 39, 57 and 58). As before, the forms were pulsed at 10 c/sec and the positive symbol continued to be the oval. The negative symbol was a diamond with approximately the same surface area and the same light intensity. The location of the positive pattern was randomized between the two panels but pre-programmed in the slide tray. When criterion performance was achieved the response to drugs was again evaluated.

Finally, using 30 trials per day, the schedule of presentation of the conditional stimuli was again changed. In 10 of the trials only the single geometric form was presented. In 20 other trials the two symbols were presented. The effects of drugs were also assessed after this schedule.

Two other animals of the series (BE 66 and 67) were subjected to a different training schedule. The 20 trial schedule using the positive CS alone was followed by 10 trials in which either the negative or positive pattern was presented; both order and location on the three panel board were randomized. The number of negative symbols presented was then gradually increased so that the animals received 10 single pattern trials with oval (positive CS) only and then 20 trials in which either oval or diamond appeared. When there was evidence that the response was withheld to the negative stimulus, the positive and negative were presented simultaneously on two panels, not necessarily adjacent, for 20 of the 30 trials. Since these animals were trained later to three pattern discrimination only a few drug studies to confirm other results were carried out on 2 pattern discrimination.

Two measures of performance were used: the percent correct performance (based on the

total number of correct responses out of total trials, including trials on which the animals failed to respond) and response latency (calculated to the nearest 100 msec for each trial and then averaging the latencies of all trials in a session omitting failures to respond). An animal was considered as having failed to respond only after he had stopped making responses towards the panel.

The effects of single doses of atropine sulfate or LSD-25 injected i.p. were studied only after the animal had performed at criterion levels for 1 week. Thereafter the animals were given further drug doses only when their performance had returned to criterion levels and had remained there for at least 2 or 3 days. Test sessions began 30–45 min after the drug injection, so as to include the period of peak action of the drug. In general the animals showed recovery to criterion on the first or second post-drug days.

EEG analysis was carried out by visual inspection of the records and measurements of evoked potential amplitude in each of daily records. Only certain records were digitized for computer analysis of various features of wave shape change which is still underway.

RESULTS

A. *Training period*

1. *Behavior.* The animals required 4–14 days to acquire the motor response necessary to activate the panel; 1–3 additional weeks were required to bring the motor response under stimulus control (criterion: 80–100% correct performance) using the single geometric form. In the first days of training, latencies of responses ranged from 6 to 14 sec; at criterion performance latencies stabilized for the different animals at 2–4 sec (Fig. 1). Following drug testing and a period of overtraining, the animals were trained to discrimination between two patterns by two methods. The three animals trained in the discrimination by presenting the two forms simultaneously required 2–3 months to reach criterion performance of 90% correct. Of the other two animals sequentially trained (see Methods), one of these acquired the task more rapidly than those of the first group and the other at about the same rate.

2. *EEG changes.* The changes in EEG activity described represent those during and after acquisition of the conditioned response; they are not related to familiarization or motor training.

Visual system: The lateral geniculate (LG) and the visual area of the marginal gyrus (Lat Gy) showed 10 c/sec evoked activity in response to the CS (5 animals). Lat Gy responses and sometimes the LG evoked potentials contained faster components at 2–4 times the stimulus frequency. The development of such faster components could not be correlated with any aspect of behavior. As the animals progressed from chance performance during the first days of training to criterion levels, the evoked activity from LG became more prominent: both sharper in form and increased in amplitude. This was true to a lesser extent in Lat Gy (Fig. 1). The evoked activity was of greatest amplitude and highest incidence at about the time the animals reached criterion and then declined gradually during the overtraining and drug testing period so that some 1–2 months after the animals reached criterion the evoked activity was definitely less often apparent and of smaller amplitude. The superior colliculus (2 animals) also showed rhythmic activity which was most pronounced at the criterion performance.

In general the EEG changes seen during the two pattern training were similar to those seen during the single pattern training. Evoked activity in the Lat Gy and LG increased as the animal progressed from chance performance during the first day of training to criterion levels; evoked potentials were again sharper and increased in amplitude (compare the upper and lower traces in Fig. 2). On the first day of multiple training when the animal was at chance performance, 2/20 trials showed good evoked activity in LG. In contrast at the 90% level (Fig. 2) 16/20 trials showed well developed activity. Changes in evoked activity from Lat Gy in the session shown in the same figure were not as marked. While 5/20 trials had clear evoked potentials on the first day of training, 10/20 showed evoked activity at the 90% level. CS induced activity in both structures was most prominent at about the time the animals reached criterion and thereafter with overtraining decreased only slightly. Generally the lack of

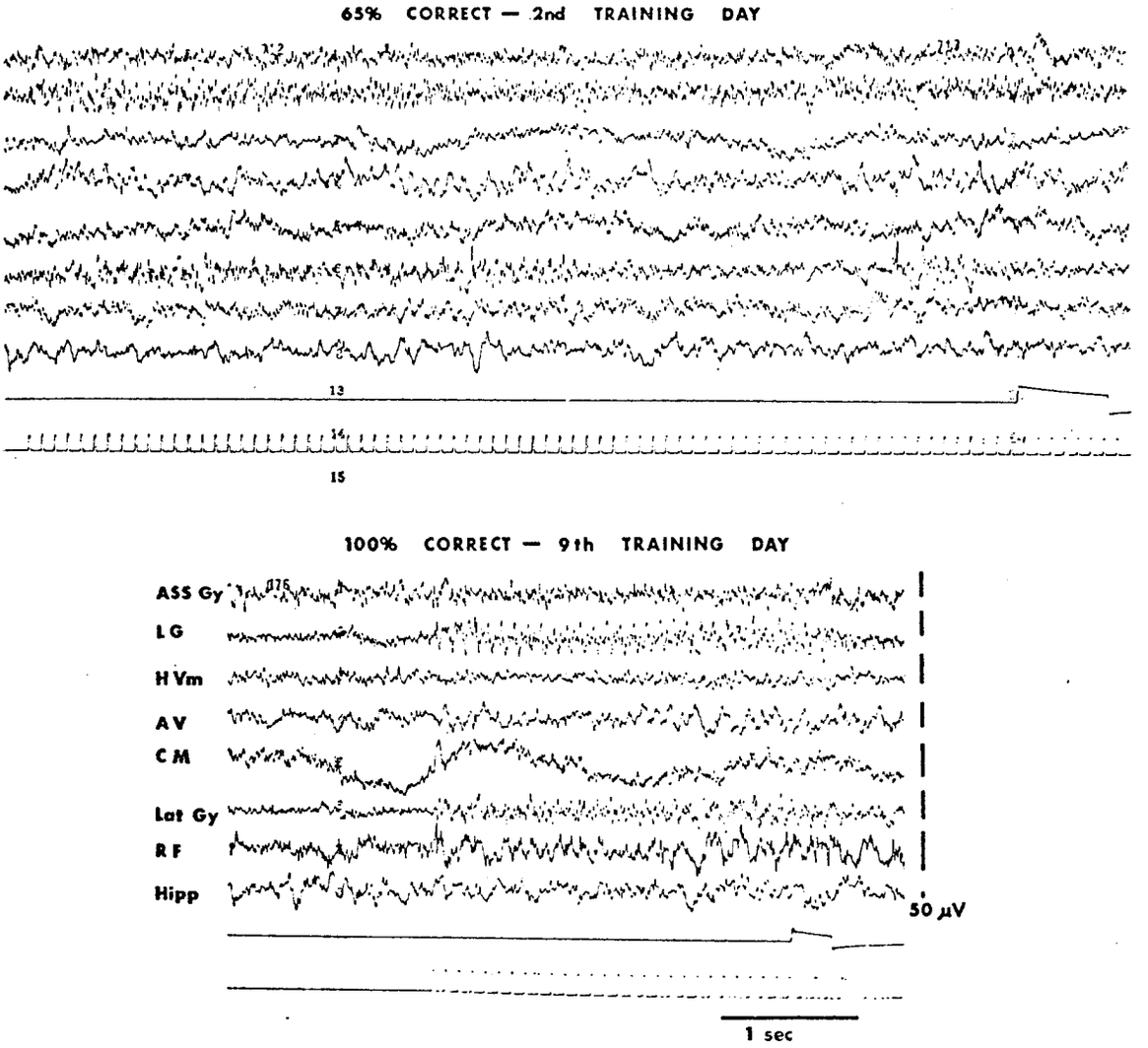

Fig. 1

EEG activity during acquisition of single pattern discrimination. ASS Gy = anterior suprasylvian gyrus; LG = dorsal nucleus of the lateral geniculate body; H Vm = ventro-medial hypothalamus; AV = antero-ventral nucleus of the thalamus; CM = centro-medial nucleus of the thalamus; Lat Gy = visual area of the marginal gyrus; RF = midbrain reticular formation; Hipp = ventral hippocampus. The EEG records at the top of the figure were taken from a correct trial on the second day of training when the animal's performance was 65% correct. The lower set of traces was obtained from a correct trial when the animal was at criterion. Below each set of EEG traces an upward deflection indicates a correct response. The 10 c sec conditioning stimulus is recorded on the bottom channel in each set. Note the decrease in the latency of responding at criterion and the change in the evoked activity in LG, Lat Gy, RF and AV which is sharper in form and increased in amplitude.

sharp decline during overtraining was in contrast to the results of overtraining to the single pattern.

Thalamic and tegmental areas: During the single pattern training, rhythmic activity related to the flickering stimulus also appeared in various thalamic areas and the midbrain reticular formation (RF). In the early training session illustrated in Fig. 1, 6/20 trials showed significant rhythmic activity in the RF whereas at 100%

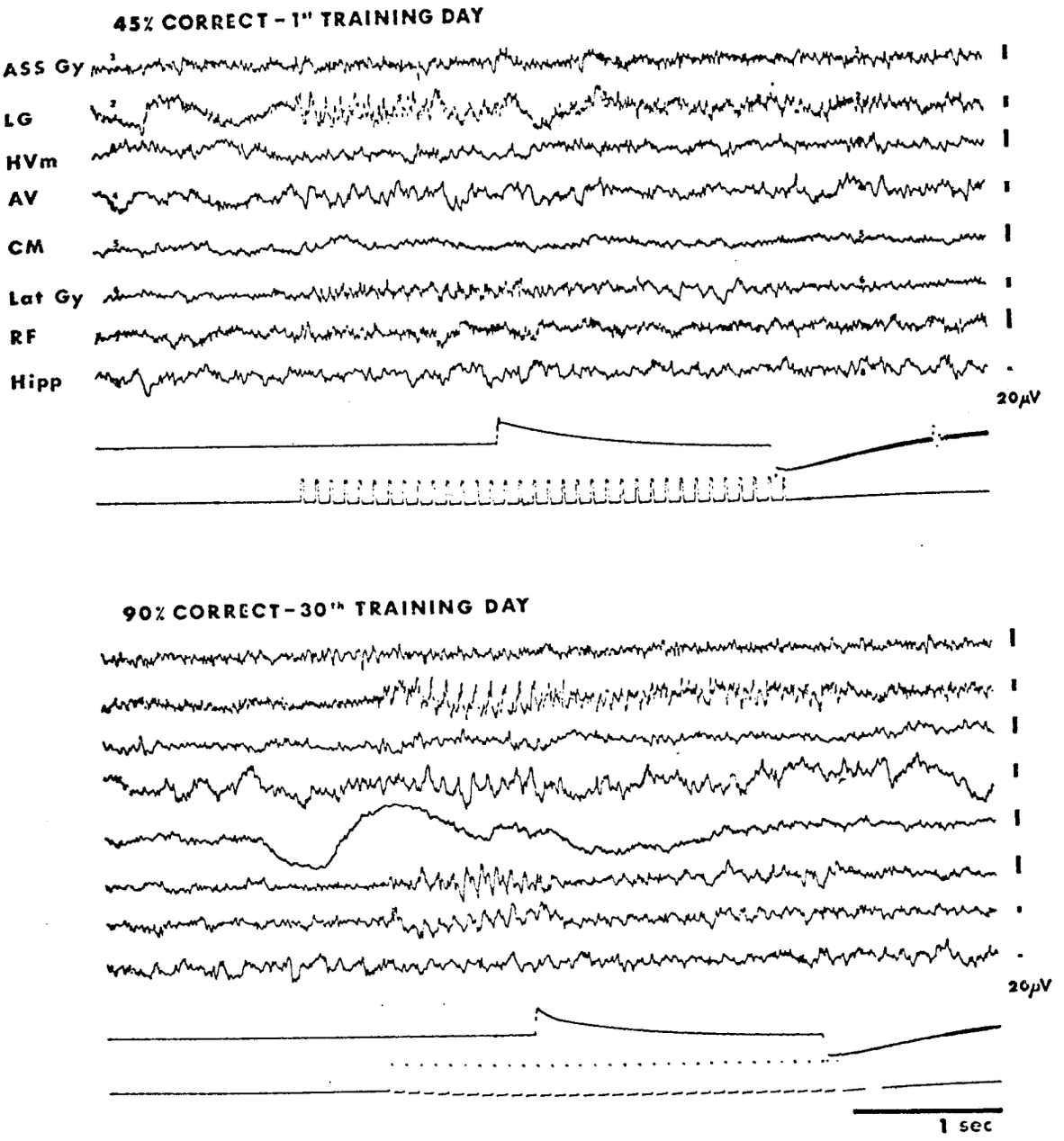

Fig. 2

EEG activity during acquisition of the two pattern discrimination. Legends as in Fig. 1. Upper set of traces is from a correct trial on the first day of training. Note that evoked activity in LG, AV, Lat Gy and RF during correct trials at criterion (lower set of traces) was well developed in contrast to that during early training.

correct it was present in 18/20 trials. This evoked activity had the CS frequency, its amplitude and incidence of occurrence increased as the percent correct performance increased. It was most evident about the time the animal first reached criterion and for about 2–3 weeks thereafter and became less pronounced in the course of the next month as drug testing and overtraining

continued. The evoked activity in the RF continued to decrease in amplitude, until some 2 months after the animal had reached criterion it had practically disappeared. Labeled activity in the anterior ventral thalamic nucleus (AV) behaved in a way comparable to that described for the RF. At 65% performance, 6/20 trials showed rhythmic AV activity and at the 100% level 15/20. Again with overtraining the labeled activity became less prominent.

With the beginning of two pattern discrimination training there was a resurgence of the frequency labeled activity in RF and in AV. For example, at the beginning of this training cycle, in 7 20 trials from AV and 1/20 from RF, labeled activity was evident. At 90% performance, in AV 16/20 and in RF 19/20 trials exhibited the labeled activity. The rate of disappearance of evoked activity during overtraining was less after two pattern training than after single pattern training. The appearance of labeled activity in centre medianum (CM) was intermittent and not correlated with correctness of performance or with the stage of training nor did it stabilize so as to allow it to be used to predict impending performance.

Other structures: Less striking labeled, evoked activity was also observed under both training schedules in ventral medial hypothalamus (H Vm) especially in the region of the medial forebrain bundle (MFB), but was not observed in anterior suprasylvian gyrus (ASS Gy), hippocampus (Hipp) or amygdala (Amyg) under either training condition. However, the activity from the Amyg showed 40 c/sec bursts of spindling. As the task became more difficult the waves lost their spindle characteristic and became continuous. This Amyg activity was not related specifically to the interval between the CS and motor response.

B. *Correlates of correct performance*

In general when the animals made incorrect responses, the evoked EEG was markedly different from that seen during correct responses. In single pattern trials in which the animals made errors, the evoked activity from the areas discussed above was either much less prominent or failed to develop altogether. An example of decrease in amplitude and sharpness of response in LG and Lat Gy with poorly developed potentials in AV and RF is shown in Fig. 3. The gradually increasing incidence of CS evoked activity during trials was correlated with increase in number of correct trials. Analysis of the evoked EEG during correct and incorrect responses during a 2 week period at criterion in this animal revealed that only 2/18 error trials showed evoked activity in LG, AV, Lat Gy and RF typical of that seen during almost all the 222 correct trials.

If the CS was not terminated simultaneously with the onset of the behavioral response, whether correct or incorrect, one noted a sharp decrement in the amplitude of evoked responses (Fig. 3), at the time the head was lowered to drink. Head position was not quantified but continuous observation during all trials showed orientation of gaze toward panels up to the point of response. In contrast to the great differences between correct and incorrect trials during single pattern discrimination, following two pattern training the EEG evoked responses during error trials were only slightly less well defined in form and slightly reduced in amplitude in comparison to those during correct trials.

C. *Drug studies*

1. *Atropine sulfate*

Behavior: The effect of atropine on the conditioned animals was examined at dose ranges from 0.125 to 2 mg/kg. The behavioral deficits after atropine were qualitatively and quantitatively different under the two paradigms. With the single pattern training the first sign of a behavioral deficit (at 0.125–0.25 mg/kg) was an increase in the latency of responding. Following higher doses of atropine, 0.5 mg/kg, the animals failed to respond (omission errors) in 20–100% of the trials (Fig. 4, *A*). In contrast, in the two pattern discrimination test after low doses (0.25 mg/kg) there were commission errors; *i.e.*, the animals pressed the panel illuminated by the unreinforced (negative) symbol (Fig. 4, *B*). With increasing doses of atropine there was increasing failure to respond (omission errors) just as in the simpler behavioral task. In general, the animals showed recovery to criterion on the first or second post-drug days.

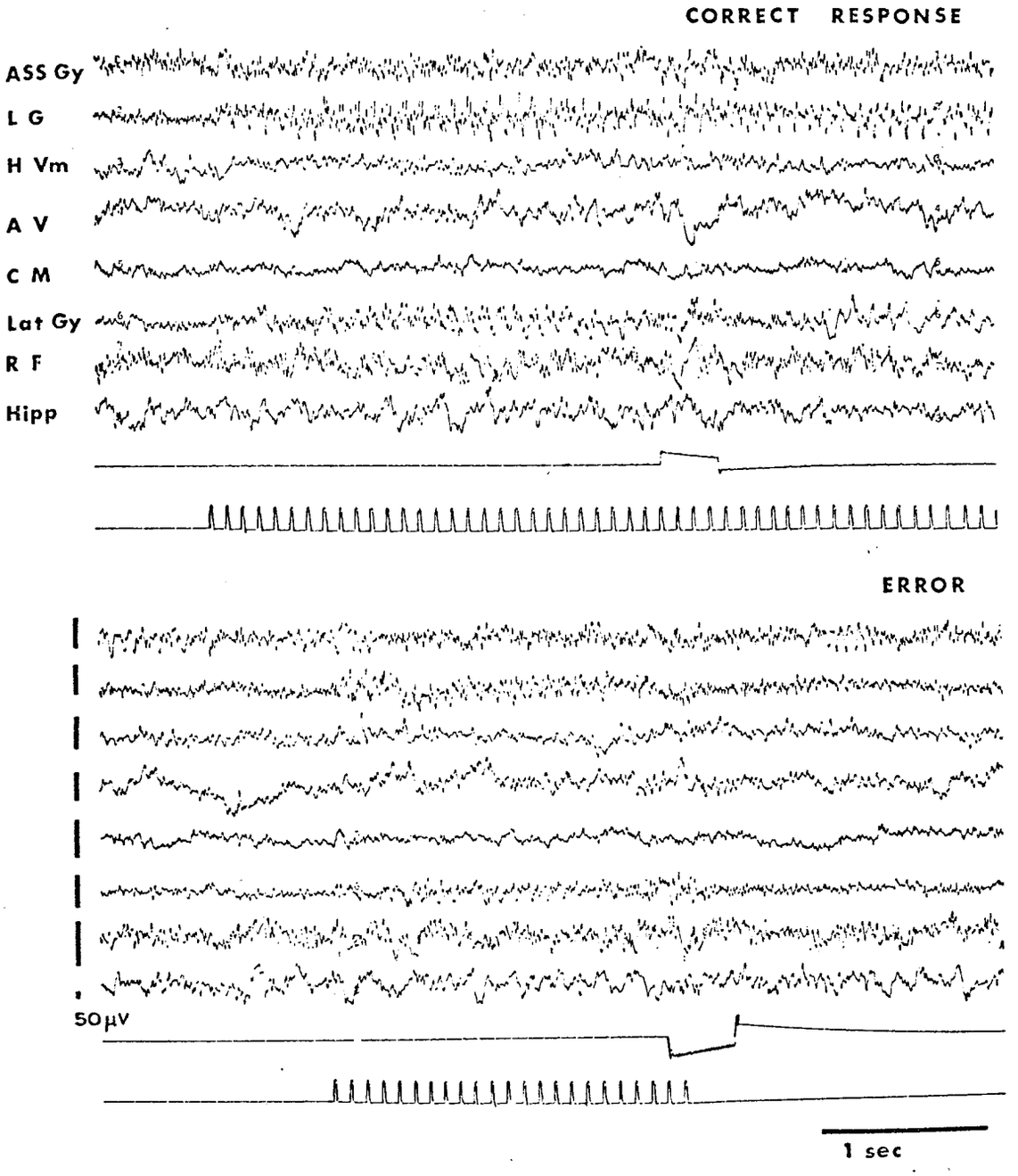

Fig. 3
Differences in EEG activity during correct and incorrect trials at criterion, single pattern discrimination behavior. Legends as in Fig. 1. Note that during the error trial (lower set of traces) the evoked activity was either much less prominent or failed to develop altogether.

Under both paradigms some increases in response latency following low doses of atropine occurred in almost all trials for a single day, but marked increases up to 4–5 times normal occurred in as many as 50% of the total number of trials. In these latter the animals exhibited a

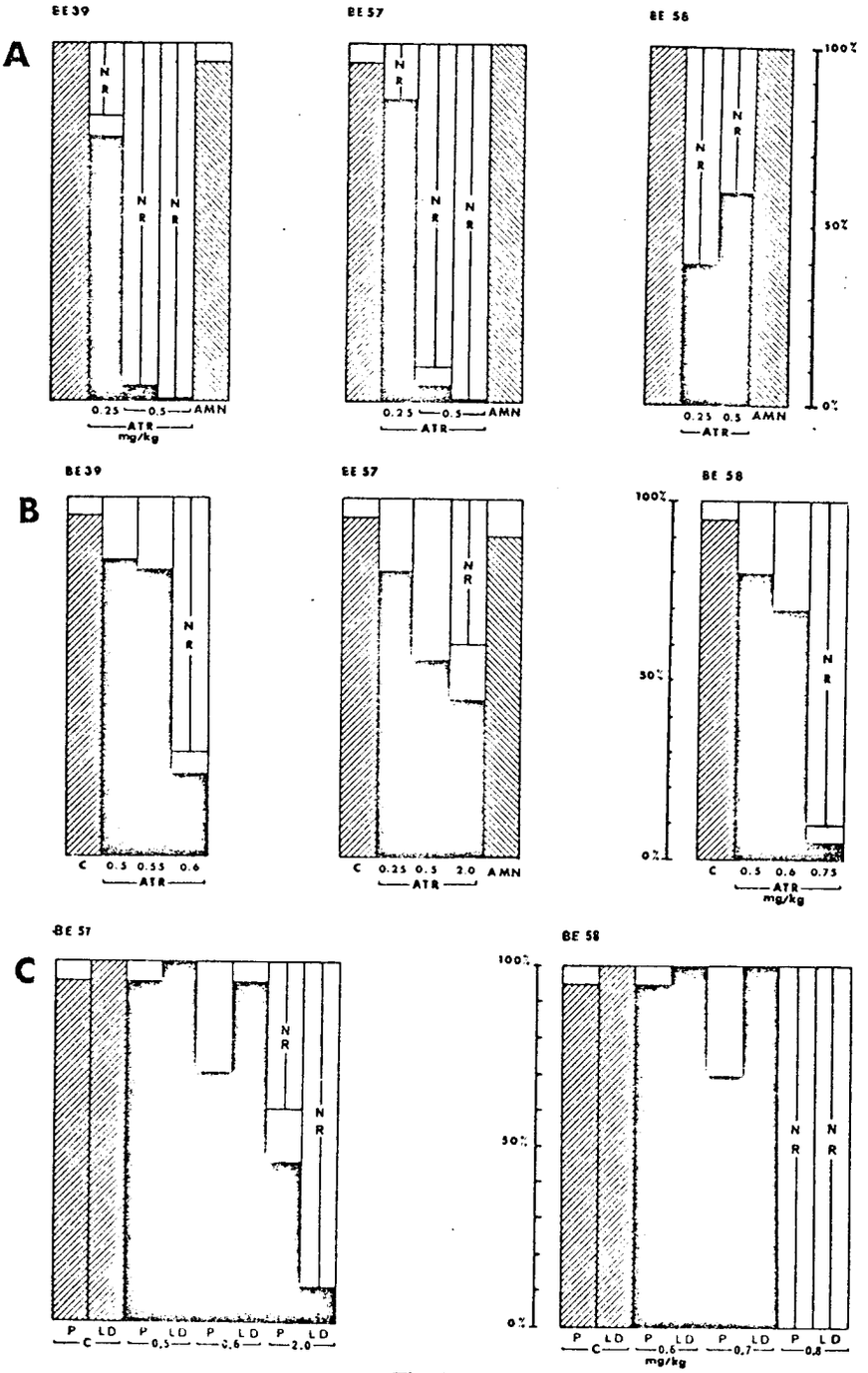

Fig. 4

Effect of atropine sulfate on single pattern (*A*), two pattern (*B*), and combined two pattern and single pattern (*C*) discrimination behavior. First crosshatched bar in each set = % correct response at criterion. ATR = performance after atropine sulfate at dose indicated. Black area in bar = % correct, clear area in bar = commission errors, NR = % omission errors (failures to respond). AMN = performance after atropine methylnitrate 1 mg kg. At *C*, paired bars show two pattern (P) and single pattern (LD) performance in individual sessions under control conditions (crosshatched) or under atropine sulfate at the dose indicated. Note the tendency to commission errors in the two pattern task (*B*) not seen in single pattern discrimination (*A*).

spectrum of ineffective responses that appeared to be a fragmenting of the appropriate sequence of behavior.

To make sure no shift in sensitivity over time could explain the differences in effects of atropine sulfate on single pattern (omission errors) and on two pattern discrimination (commission errors) two of the animals were tested in a situation in which the first 20 trials involved two pattern discrimination and the last 10 trials single pattern testing. Fig. 4, *C* represents the results of these studies. At the lowest doses animals made errors of commission on two pattern discrimination without any impairment on the single pattern task. At 0.8 mg/kg atropine or more, when performance was impaired on the single pattern task in this series, only failures to respond occurred (omission errors); at that dose and above the animal also made omission errors on the two pattern trials, with few if any commission errors. No single dose of atropine was found at which there were only commission errors on the two pattern trials and omission errors on the single pattern task.

The most prominent peripheral effects of these doses of atropine were mydriasis and failure of accommodation of the pupil to changes in light intensity, other autonomic changes were not measured. There was no generalized depression and minimal, if any, changes occurred in the normal motor activity associated with the performance of behavior: the cats generally did not fail to drink milk offered before or after the test session.

Under similar conditions atropine methylnitrate at doses up to 1 mg/kg induced the same side effects as those observed for atropine sulfate. This drug failed to alter either of the paradigms (Fig. 4, *A* and *B*).

EEG: The conditional behavioral deficits induced by atropine sulfate were associated with alterations in spontaneous and evoked EEG. Following single doses of 0.125-0.25 mg/kg, the background EEG showed dose related slow waves and bursts of high voltage activity from neocortical and subcortical areas and slow waves of increased amplitude from the ventral hippocampus. At 0.5 mg/kg the record was one of almost continuous slow wave, high voltage activity. In general, bursts of slow waves of higher voltage at a frequency of about 1/5 or 10 sec occurred in ASS Gy, H Vm, AV, RF and CM, with smaller bursts in LG and Lat Gy and CM. The EEG from the Hipp showed a large increase in background slow wave activity without any obvious burst-like character. There were no slow wave bursts from the SC, Reuniens or Amyg, and amygdaloid spindling was decreased significantly.

In single pattern conditioning, atropine sulfate also altered the EEG activity associated with presentation of the CS. Following doses of 0.125 and 0.25 mg/kg when the latency of the behavioral response had increased 2–3 times, rhythmic labeled activity in LG and Lat Gy developed much more slowly after onset of the CS than under control conditions. Although visual observations of the animals indicated immediate orienting of the animals to the stimulus panels, the evoked activity took approximately twice as long or longer to develop following atropine. However, when developed the evoked potentials in the visual system were increased in amplitude and simplified in form in four of the five cats, and slightly decreased in amplitude in one cat. For example, in the cat whose records are shown in Fig. 5, following atropine, 95% of the trials showed better evoked activity in LG at 0.5 mg/kg than in the control day while 90% of the trials showed better Lat Gy evoked activity than in controls.

During performance of the two pattern discrimination task atropine increased labeled activity in the visual system as in the single pattern situation. In addition, there was an increase in the rhythmic activity from H Vm and an increase in evoked activity from the MFB during the presentation of the CS in almost all the trials. In neither behavioral situation did doses of atropine methylnitrate up to 1 mg/kg alter spontaneous or evoked EEG.

2. *LSD-25*

Behavior: In contrast to the effect of atropine sulfate, LSD-25 administration was generally followed by commission rather than omission errors. In this linear study in which each animal served as its own control and successive doses at week or longer intervals were used, it was not possible to make a quantitative dose–effect

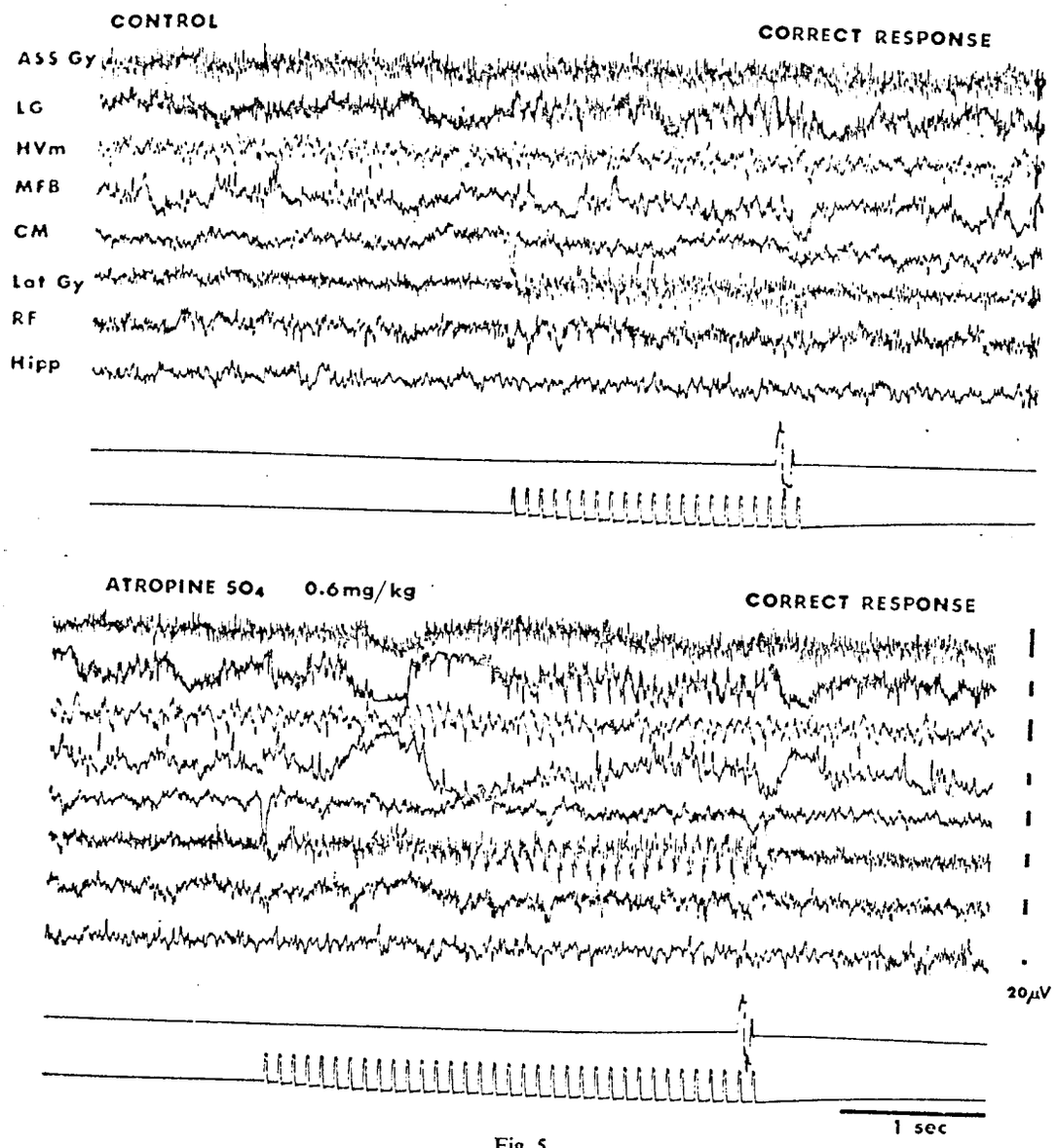

Fig. 5

Effect of atropine sulfate on two pattern discrimination at criterion. Legends as in Fig. 1. Latency of correct response increased as in single pattern task. Evoked EEG activity in LG, Lat Gy and H Vm was increased in amplitude by 0.6 mg/kg (lower set of traces).

comparison with LSD-25. The dose range of LSD-25 explored was from 25–200 μg/kg. Much greater variability in the response to a given dose between animals and in each animal was obtained with LSD-25 than had been found with atropine sulfate. It was our impression that following a dose of LSD-25 all subsequent doses were relatively less effective irrespective of time interval between doses.

In the one pattern task LSD-25 significantly increased errors in discrimination in two of five animals, had slight or no effect in two animals and caused one animal to fail to respond altogether the first time it received LSD-25 (Fig. 6, *A*). An important source of this variability was previous experience with the drug even after an interval of weeks or months. For example, in animal BE-66 in which several previous lower

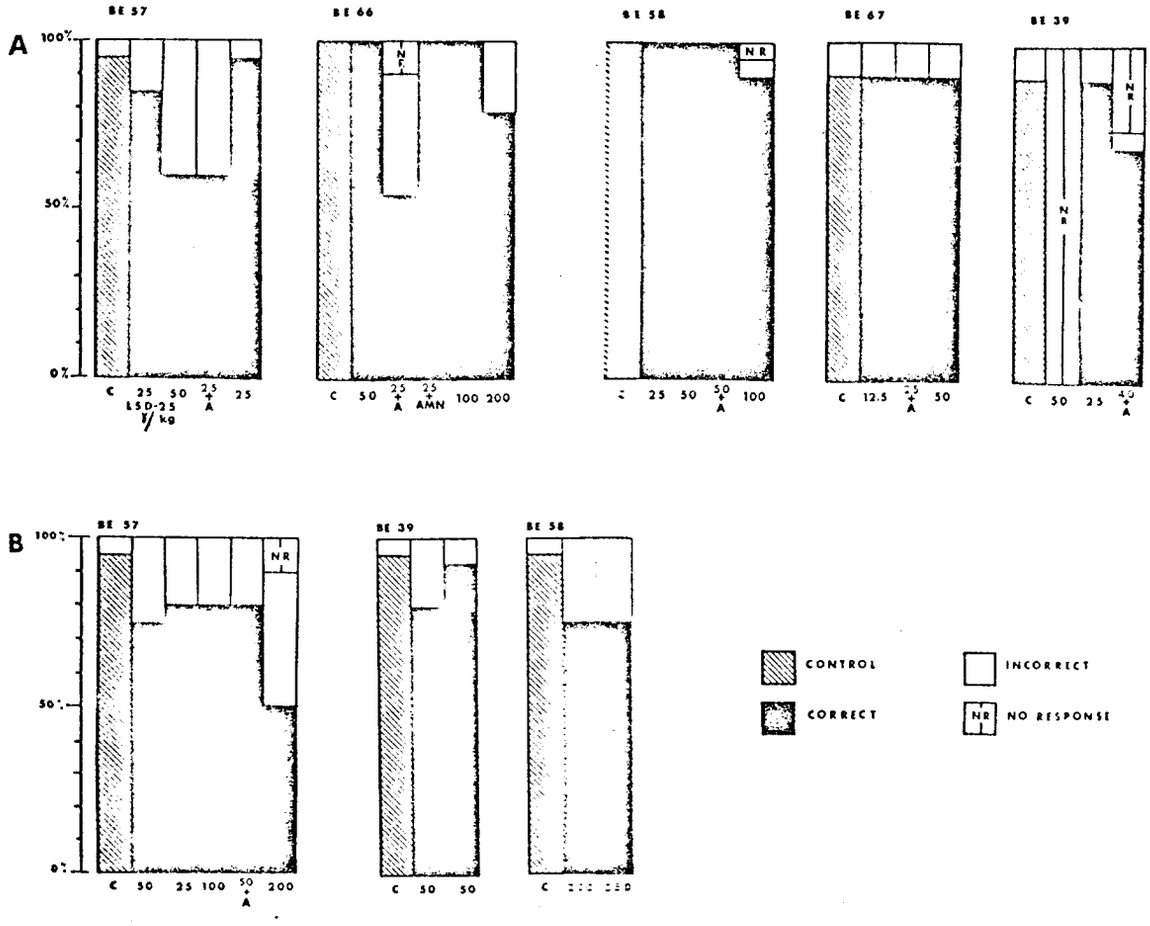

Fig. 5

Effect of LSD-25 alone and with atropine sulfate on single pattern (*A*) and two pattern (*B*) discrimination. Bar graphs similar to Fig. 4. A = 0.5 mg kg atropine sulfate and AMN = 0.5 mg kg of atropine methylnitrate given in addition to the LSD-25 dose shown in mg kg. Note preponderance of commission errors and the variability in the behavioral response to LSD-25.

ineffective doses were given, 200 μg/kg was required before behavior was affected. In contrast, in animal BE-57 to which only one previous lower dose was given, 50 μg/kg was able to reduce correct response to little above chance.

Following training in the two pattern paradigm, the three animals tested showed an increase in number of errors in panel selection (commission) after LSD-25 (Fig. 6, *B*). Animal BE-57 at doses of 25–100 μg/kg showed performance levels at 80% correct, and at 200 μg/kg his performance was 50% correct with 10% failure to respond. In two other animals (BE-58 and 39) 50 and 200 μg/kg were required to reduce performance to the 75 and 80% level.

In general the animals displayed few other behavioral alterations under LSD-25. They seemed to be more restless and agitated. The animal which had the most marked response to LSD-25 showed an increase in intertrial responding over from few, if any, responses on control days to a total of 20/session after 200 μg kg of LSD-25. The other cats showed no intertrial responding after LSD-25. The latency of responding in both paradigms was either unchanged or shorter. This slight alteration was in marked contrast to the increased latency after atropine.

EEG: In both paradigms at dosage levels in which behavioral deficits were observed, there

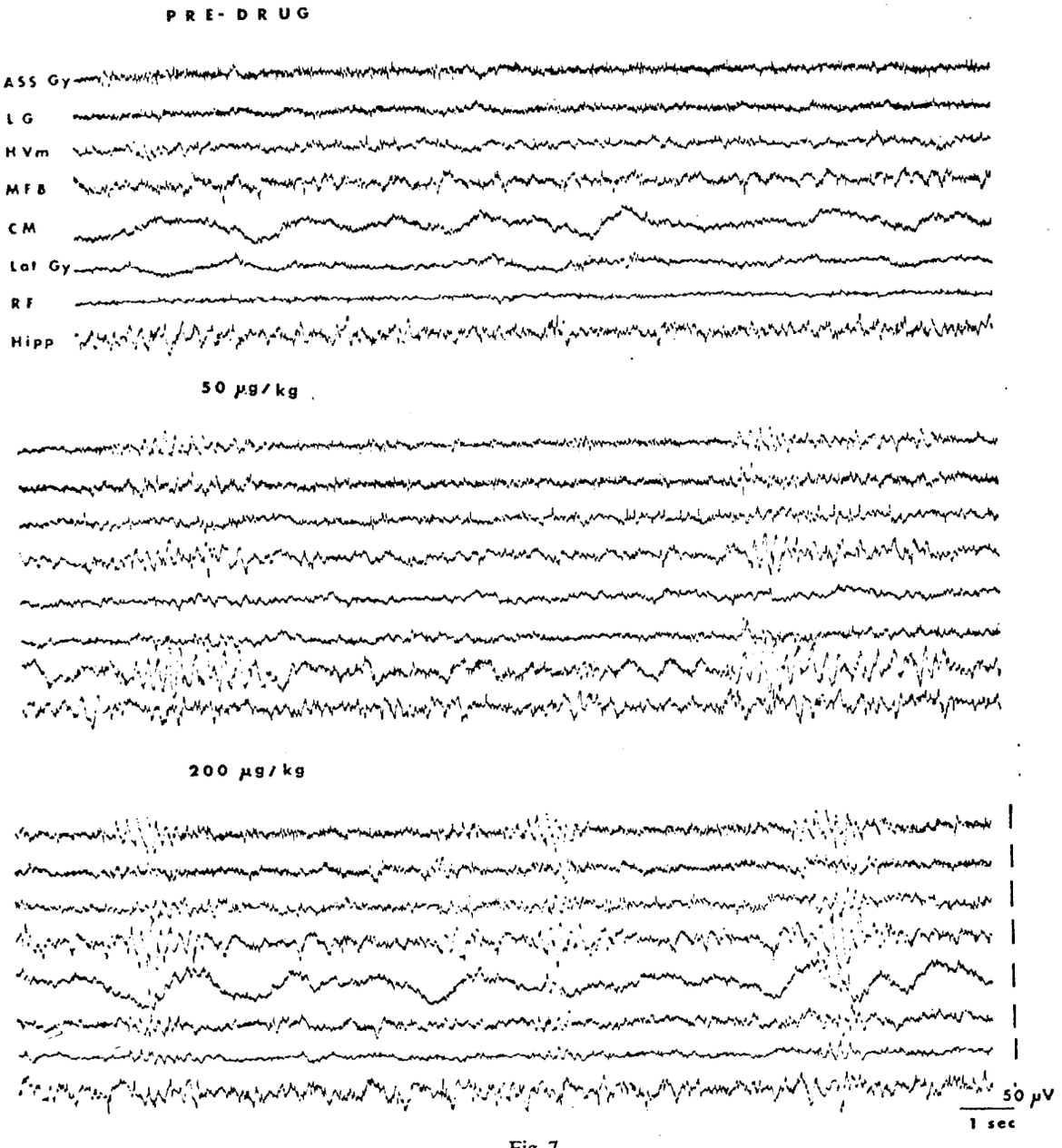

Fig. 7

Effect of LSD-25 on EEG. Legends as in Fig. 1 and 2. Note the development of rhythmic slow wave bursts particularly in ASS Gy, MFB and RF with smaller amplitude bursts in LG, Lat Gy, H Vm and CM following 50 mg/kg (middle traces) which increase in frequency of occurrence from 1/10 sec to 1/5 sec at 200 mg/kg (lower traces).

were alterations in the spontaneous (Fig. 7) and evoked EEG patterns. Rhythmic slow wave bursts appeared in neocortical and subcortical areas, except in Hipp. As the dose of LSD-25 was increased the amplitude and frequency of appearance of bursts increased from about 1/10 sec to 1/5 sec but never became continuous within this range of doses. Evoked activity in the visual system during correct trials was increased (Fig. 8) in both behavioral paradigms. During in-

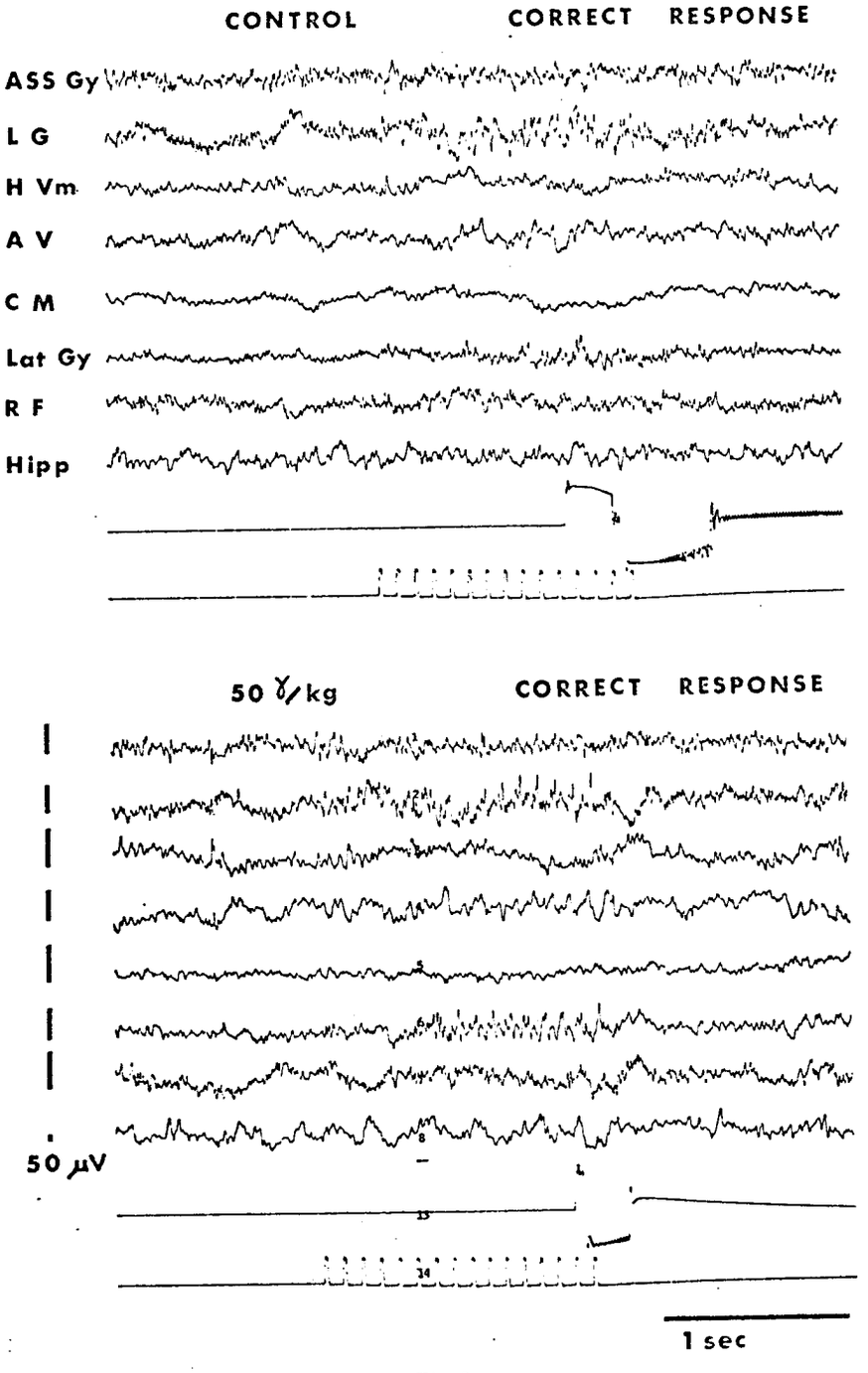

Fig. 8

Effect of LSD-25 on evoked EEG activity during correct single pattern trials at criterion. Legends as in Fig. 1. Note the increase in the amplitude of evoked potentials in LG and Lat Gy following 50 mg/kg (lower traces).

correct trials in the single pattern task, evoked potentials were markedly attenuated but not noticeably in the two pattern task.

3. *LSD-25 plus atropine sulfate*

Behavior: Following the single pattern training when there was a clear distinction between the deficits observed with atropine sulfate and LSD-25, a limited number of studies were done administering the two drugs simultaneously. An increase in the behavioral deficits characteristic of LSD-25 alone was seen in two cats (BE-57 and BE-66) no change over those observed with LSD-25 alone in one (BE-39) but results were equivocal in the other three (Fig. 6). As with LSD-25 alone, investigation of the possible potentiating effects of the two drugs together was complicated by the influence of previous LSD-25 experience.

EEG: The EEG effect of the LSD-25-atropine combination resembled that of LSD-25 alone and not of atropine alone. The spontaneous EEG in cats receiving both drugs showed bursts of rhythmic activity which were similar to the bursts under LSD-25 alone and appeared in the same brain areas. There was no evidence of the continuous background slow wave activity that was noted under atropine sulfate. Also the potentials in LG and Lat Gy evoked by the conditioning stimulus during the trials were somewhat enhanced, though not as much as under LSD-25 alone.

DISCUSSION

EEG changes with training seen in this study were not exactly comparable with those from previous reports (Grastyán *et al.* 1959; John and Killam 1959, 1960; Adey *et al.* 1960 and Liberson *et al.* 1960) because of differences in brain areas examined and differences in behavioral situations. However, some similarities should be pointed out. As was true for most of the studies mentioned above, CS related rhythmic activity in various areas of the brain became more prominent as the conditioned stimulus became more significant for the animal. Regarding the disappearance of evoked activity at criterion reported by Liberson *et al.* (1960), the present study like that of John and Killam (1960) indicated that, although some diminution occurred, the evoked activity disappeared only after some 1 or 2 months of overtraining in the simpler task and was persistent in the more complex two pattern discrimination task.

The possibility that the head position may be an important factor, as in the studies of Worden *et al.* (1964) with auditory stimuli, cannot be eliminated but the following facts mitigate its importance: (1) *constant* observation of the animals did not permit beginning of a trial unless the animal was oriented to the panel board; (2) attentive gaze close to the panel was more characteristic of early training during longer latencies to response than of the time of maximal evoked potentials; (3) diminution of amplitude during overtraining to the single pattern task was much more marked than in the two pattern task although response latencies and visible motor patterns were similar; (4) somewhat similar changes in evoked potentials occurred in John and Killam's studies (1959, 1960) in which the entire environment displayed the CS.

The presence of theta activity observed by Grastyán *et al.* (1959) and Adey *et al.* (1960) in the hippocampus during various stages of training was never seen in the present study. This discrepancy may be due either to the use of different behavioral situations and/or to the fact that the electrodes were in different hippocampal locations. The more restricted distribution of evoked activity seen in our study compared to that of John and Killam (1959, 1960) may be due to the limited stimulus field and the use of positive reinforcement exclusively in our experiments.

As in John and Killam's study (1960), evoked activity was observed to be better developed during correct responses than during errors in our single pattern task. The absence of a significant difference in labeled activity in correct *versus* incorrect trials in the two pattern task may be related to the contingencies of the test situation. When discrimination of the panel carrying an illuminated form from 2 dark panels was required, during errors the animal approached a dark panel. In the two pattern task, the discrimination required was between two geometric forms of equal light intensity. Thus, during errors the animal approached an illuminated

panel of a different form. However, since this problem did not exist in John and Killam's paradigm, task difficulty may be involved more directly. Evidence that under intermittent illumination form discrimination is a difficult task for the cat is found in the increase in 40 c/sec activity from the amygdaloid derivations. Lesse (1957) has related the appearance of 40 c/sec activity in the amygdala to emotional stress in the cat. Comparison of results in a variety of test situations are needed to resolve this question.

The alterations of performance elicited by the drugs were found to be different in their dependence upon the behavioral situations. Atropine sulfate caused a progressive increase in the latency of performance in the single pattern task and finally total abolition of the conditioned behavior with no dose inducing incorrect performance, whereas in the two pattern task under low doses an increased latency of responding was frequently accompanied by errors of commission. Only at higher doses of atropine did the animals fail to respond. This difference in the impairment in the two tasks appears genuine and not simply an artifact due to overtraining or change in drug sensitivity because similar findings were obtained when both single and two pattern problems were given during the same session. LSD-25, on the other hand, at effective doses, caused the animals to make incorrect responses in both behavioral paradigms. To rule out the possibility that the effects of atropine sulfate were related to its peripheral actions specifically on the pupil, salivary glands and gastrointestinal tract, similar experiments were undertaken using atropine methylnitrate. This quaternary compound is 3–4 times more potent peripherally than atropine sulfate (Herz *et al.* 1965) but does not readily penetrate the blood-brain barrier. In doses up to 1 mg/kg atropine methylnitrate failed to alter correct performance or the latency of responding.

From this study and others (Whitehouse 1964 and Rougel *et al.* 1965) it is clear that atropine sulfate has central actions quite distinct from peripherally mediated effects. Stein (1963) from studies comparing atropine and its quaternary congener proposed that atropine sulfate depressed drinking behavior centrally in the rat. However, in our experiments, when the animals failed to respond, they drank milk given to them before and after the experimental sessions, and were seen to lick the empty milk dishes during trials. Thus, failure of motivation for drinking cannot explain the effect of atropine sulfate on the conditional behavior.

The behavioral deficit could, perhaps, best be described as a fragmenting of the appropriate sequence of behavior; this fragmenting led to long latency responses and finally failure to respond.

Because atropine methylnitrate produced no change in behavioral performance or in EEG activity, and because the EEG and behavioral changes were dose related in parallel following atropine sulfate, the altered EEG picture must be presumed to be associated with the behavioral deficit. The altered activity somewhat resembled the EEG during sleep in normal animals but, as in other studies (Wikler 1952), was not accompanied by behavioral sleep or by generalized depression. It is to be emphasized that the high voltage, slow wave bursts in fact only bear superficial resemblance to the usual patterns seen in sleep. However, the background of continuous, high voltage, slow waves invariably preceded long latency responses or failure to respond in a substantial number of trials, and its appearance in a variety of neocortical, subcortical and limbic areas indicates that numerous brain mechanisms are involved.

Potentials evoked during trials were not only altered in hypothalamic areas which are considered to be involved in drinking behavior, but evoked activity in brain areas handling afferent input (visual system) and those involved in integrative function (AV, RF and Hipp) also showed changes following atropine. These data confirm the findings of Rougel *et al.* (1965) following ditran and the work of Sadowski and Longo (1962) after scopolamine in rabbits trained in an instrumental conditioning paradigm.

The effects of LSD-25 were variable in the two behavioral situations. After training in the single pattern task, two of five animals were unaffected, two others committed errors and one failed to respond. Dose–response relationships could not be determined both because of the limited range of doses and an apparent effect of previous exposure to the drug. An apparent

tolerance to repeated doses of LSD-25 has also been noted by Balistrieri and Fontinari (1959), Jarvik and Chorover (1960) and Adey *et al.* (1962). Our long term experiments suggest this effect of previous experience may last weeks or months. In the two pattern task all animals exhibited errors of commission.

There did not appear to be changes in the animals' behavioral repertoire after LSD-25 which would account for the incorrect performances. The latencies of responding were only slightly decreased, and only one animal exhibited increased intertrial responding. However, the demands on motor performance were much less severe than those imposed by Adey *et al.* (1962).

The background EEG activity after LSD-25 was characterized by bursts of high voltage, slow waves. With increased dosage and more profound behavioral effects, these bursts occurred more frequently but never became continuous as after atropine. These bursts are similar, in general, to those described by Bradley and Hance (1956) and Adey *et al.* (1962). Their absence in ventral hippocampus is at variance with the latters' data but may reflect the differences between the behavioral tasks and/or electrode location.

The evoked or labeled activity during correct trials became more prominent in the visual system after LSD-25 in both tasks. During error trials in the single pattern task the labeled evoked activity was poorly organized just as in the controls. In contrast, in the two pattern task there were no significant differences between EEG responses during correct and incorrect trials with or without drug. Possible explanations for these differences in control trials discussed above, apply equally to error trials after drugs.

When atropine and LSD-25 were given together, ineffective doses of atropine increased the LSD-25 effect in two of five cats. This is of interest because hallucinatory effects of anticholinergic agents have been reported clinically (Pfeiffer *et al.* 1959), and it has been suggested that atropine should potentiate actions of drugs in the LSD series (Pfeiffer and Jenney 1957). In the animals showing an increased behavioral deficit with the two drugs together, the spontaneous and evoked EEG activity more closely resembled that after LSD-25 alone than after atropine and the behavioral impairment was similar to that induced by a larger dose of LSD-25.

SUMMARY

1. Electrophysiological changes associated with the acquisition and retention of two visual pattern tasks in cats were found principally in the visual system (lateral geniculate and the visual area of the lateral gyrus), the midbrain reticular formation and the anterior ventral nucleus of the thalamus.

2. A comparison of EEG correlates of acquisition of the two behaviors failed to reveal consistent differences. Instead, a parallel, gradual development of evoked responses during initial training occurred. During overtraining, in the single pattern there was a diminution of evoked activity; however, this was not marked in the two pattern situation.

3. Atropine sulfate was found to increase latency or abolish response in a single pattern discrimination task. In a two pattern discrimination task, atropine increased response latency, caused errors of commission, and finally blocked responding in a dose related sequence. The drug effects were related to central rather than peripheral action as demonstrated by the lack of effect of atropine methylnitrate under the same conditions. The behavioral deficits were accompanied by slow waves and high voltage bursts in the spontaneous EEG from neocortical, hippocampal and subcortical areas, while the evoked responses to the conditioning stimulus during the trials were delayed in onset and increased in amplitude in the visual system as compared to the pre-drug trials.

4. The effects of LSD-25 were characterized by errors of commission in both behavioral tasks. The behavioral alterations were associated with rhythmic slow wave bursts in the spontaneous EEG from neocortical and subcortical areas, and the evoked responses to the conditioning stimulus during correct trials were increased in the visual system.

5. Differences in evoked responses between correct and incorrect trials were striking in the single but not in the two pattern task, but error

trials were not different under either drug from controls.

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