Reprinted from EXPERIMENTAL NEUROLOGY, Volume 20, Number 2, February 1968

EXPERIMENTAL NEUROLOGY 20, 175-187 (1968)

Multiple Measures of the Orienting Reaction and Their Dissociation after Amygdalectomy in Monkeys

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Received June 22; Revision Received September 25, 1967

In an earlier study we proposed that amygdalectomy results in the dissociation of the orienting reaction. One component of this reaction, the galvanic skin response (GSR) disappears after amygdalectomy and we made the hypothesis that failure to habituate was closely linked to this failure in the orienting GSR. We called this GSR component an indicator of "registration." The present study was undertaken to discover what other measures of orienting could be classified with the GSR as indicators of registration. Accordingly, six bilaterally amygdalectomized and four shamoperated rhesus monkeys were given 50 irregularly repeated presentations of a pure tone while GSR, heart rate, respiratory rate, EEG, and ear movements were recorded. Amygdalectomized monkeys failed to show the GSR, heart-rate, and respiratory-rate components of the orienting reaction while EEG activation and ear movement-orienting responses remained essentially intact.

Introduction

The amygdaloid complex has been shown to be involved in the orienting reaction by the finding of depression of the galvanic skin response (GSR) to novelty in monkey brain ablation studies (1, 5). This result came as a surprise in view of other reports that the locomotor behavior of such monkeys fails to habituate (3, 8). The apparent paradox suggested the hypothesis that the GSR component of the orienting reaction is in some way intrinsic to the registration of novelty without which habituation is retarded. Therefore, the present study was undertaken to survey simultaneously several indicators of the orienting reaction in amygdalectomized monkeys to determine whether a category related to "registration" could be discerned.

Method

Subjects. Nine preadolescent rhesus monkeys (*M. mulatta*) which had been subjected to bilateral amygdalectomy (group A, $N = 6$) or to a bilateral sham opeartion (group N, $N = 4$) were used. Nine months

[1] This research was supported by NIMH grant MH, 12970. Dr. Benzies is now at Illinois State Pediatric Institute, Chicago.

previously they had been trained in a visual-discrimination problem. Amygdalectomy was accomplished using subpial suction under direct vision in a single-stage bilateral operation. Details of the surgery have been published (1). The sham procedure was identical except that no brain tissue was suctioned. Reconstructions of the lesion are shown in Fig. 1.

Apparatus. Grass model-111D polygraph recordings were made simultaneously of EEG, EKG, respiratory excursions, chair movement, and of

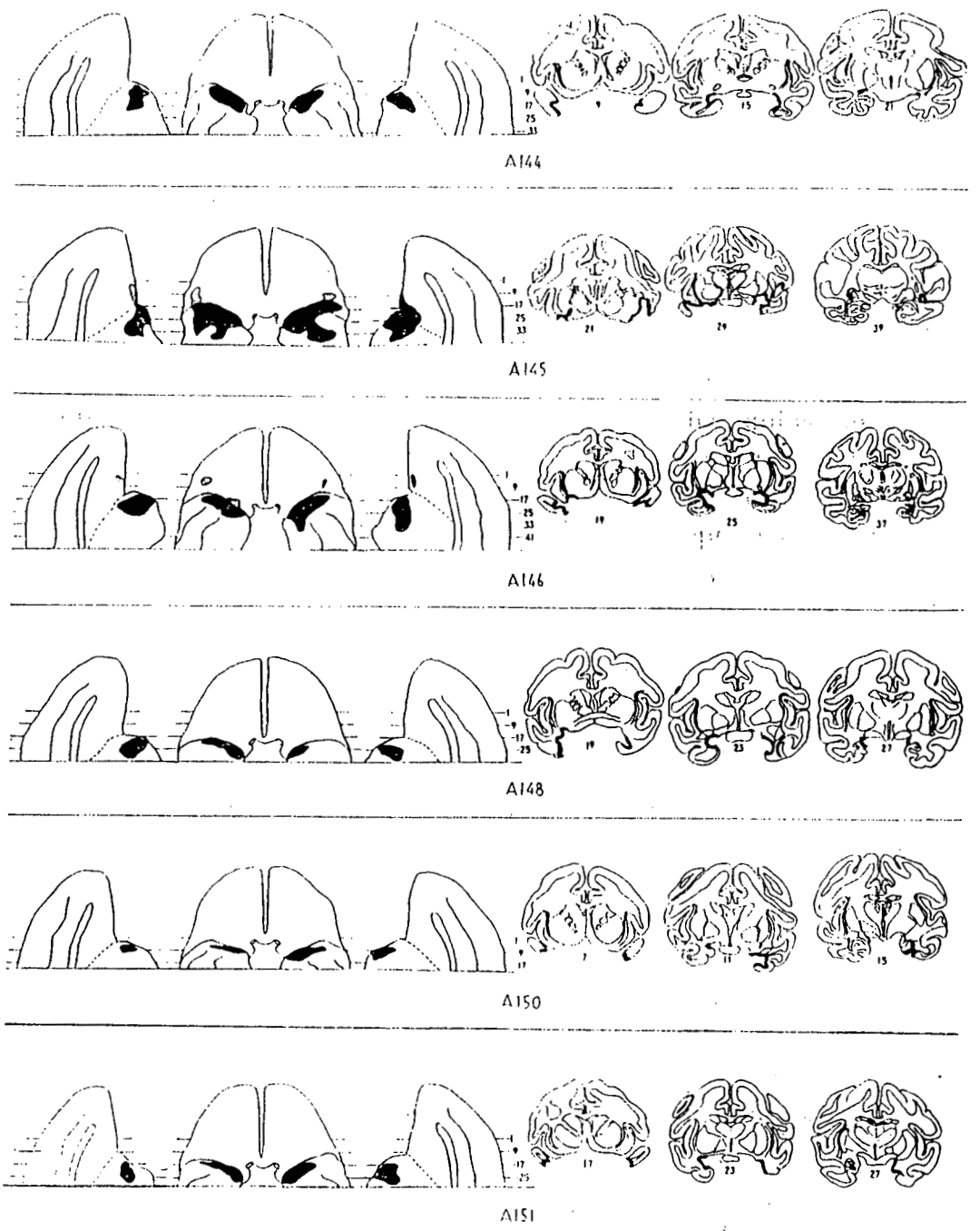

FIG. 1. Ventral and medial reconstructions of the brain lesions and representative cross sections of the brains of six monkeys in group A.

a finger-button switch for the recording of ear movements. Skin resistance was measured with a Fels Dermohmeter and simultaneously recorded on an Esterline-Angus graphic ammeter (model AW). Pure tones and background white noise was generated by a Grayson-Stadler (905D) twin oscillator and presented through a Jensen coaxial speaker mounted 60 cm above the vertex of the animal's head. A modified phonograph cartridge was mounted on the animal chair as a gross-movement pickup.

Procedure. Each animal was tested in one session for responses to a 2-sec pure tone of either 1500 or 1000 cycle/sec at 30-db above a 53-db background white noise as measured by a General Radio 1551-B sound-level meter. Restraint was in a Foringer primate chair with the wrists and ankles comfortably secured to the chair to limit twisting body movements. After the head hair was clipped and depilated, EEG electrodes were placed subcutaneously. This was accomplished by threading No. 36 enameled-copper wire through a 23-gauge hypodermic needle, scraping the enamel from the tip for 2 mm, and bending it back over the needle shaft to form a small hook. This allowed the wire to be slipped through the skin and remain subcutaneously while the needle was withdrawn over the wire. Then the wire was soldered to a shielded pickup lead. Two parietal (R + L) and two occipital (R + L) leads were placed, all approximately 2 cm apart. The lead wires were then firmly taped onto the scalp. Silver EKG electrodes (13 × 51 mm) were strapped on the chest on either side of the heart and contact made by rubbing Sanborn electrode jelly into the skin which had been exposed with hair clippers. Laboratory-made GSR electrodes were then applied to the right palmar surface of the right foot and the right calf, after the skin had been cleansed with Phisohex and water. These electrodes consisted of a 7-mm end portion of a Penlite battery cell (containing $ZnMnO_2$) with a saline-dampened cellulose sponge secured over the open battery end. Elastic webbing cuffs and Elastoplast wrappings secured them in place. These electrodes produced less than 1000-ohms resistance when tested before application to each animal. A light mask made of eye patches was taped in place to prevent GSR responses due to sudden eye opening.

The animal was then placed in a lighted Acoustic Industries sound-insulated chamber (120 × 120 × 215 cm) to reduce ambient noise and allow observation via a one-way window.

A 5-min control period of recording was allowed. The pure tone was then presented at random intervals determined by the appearance of slow waves on the EEG record (criterion of at least three waves of 50 μV/2 sec) and absence of observed or recorded movement. This time was in the range of 30–180 sec. Half of the animals in each group were presented with a 1000-cycle/sec tone and half with one of the 1500 cycle/sec. The novel

(alternate) tone was presented after the completion of 50 trials plus a habituation criterion of four consecutive presentations with no GSR. The original stimulus was again repeated until four consecutive no responses occurred and the novel stimulus was again presented twice. One experimenter observed for any forward ear motion and depressed a finger button for the duration of each movement.

Scoring. Galvanic skin responses which occurred between 0.8 and 5 sec after tone onset were scored as decrease in resistance from onset to peak of the response in kohms resistance. Any response which was 0.5 kohms or greater was accepted. Trials on which movement occurred were discarded.

The disruption in regular respiratory rhythm for 6 sec after tone onset was rated along a 0–3 scale: 0 = none observable, 1 = just noticeable, 2 = moderate, and 3 = marked. Trials 1–5, 46–50, and novel 1 and novel 2 were selected. Deceleration, acceleration, and irregularity were the acceptable criteria for disruption. Reader reliability was measured on similar records and found to correlate at +.78.

Heart-rate changes were measured in two ways. Comparison of heart rate in beats per minute before, during and after the stimulus was made by measuring the distance for five beats on the EKG record in these periods. Percentage response scores were also determined. A criterion of at least 3 beat/min increase poststimulus onset was chosen as a response. Secondly, maximum beat-by-beat changes were scored by measuring the RR intervals in millimeters and comparing the mean of the last five beats before the stimulus with the maximum (peak of deceleration) and minimum (peak of acceleration) length of RR interval in the 20 beats immediately following onset of the stimulus. These values were then converted to beats per minute and compared quantitatively.

For ear movements the percentage of trials with responses and the cumulative duration of the responses (total mean seconds per trial that the ears remained forward) were taken for the 0–6 sec poststimulus period and the intertrial intervals. In the ITI-periods values were corrected with a factor $x/\mathrm{ITI}\ (100)$ to obtain measures per 100 sec of intertrial time of the number of ear responses and proportional time for the duration of these responses (x = mean rate or cumulative duration of response per ten-trial block).

In scoring EEG low-voltage fast-wave onset on a criterion of at least three waves of 50-μv amplitude in 2 sec was used to estimate the existence of slow-wave activity at stimulus onset. All trials were scored and any trials on which movement occurred or where slow waves were absent were discarded. The onset of low-voltage fast activity (LVF) was scored if there [illegible] than three waves of 50 μv in 2 sec in the 6-sec poststimulus-

onset period. Duration of LVF in seconds and latency of onset were also measured. Because this was determined by each animal's slow-wave EEG activity, an analysis for group differences in intertrial interval was done on all trials. Table 1 shows that both groups had decreasing intervals with time but only block 3 was significantly different bewteen groups. This block of trials was excluded for any statistical purposes.

TABLE 1

MEAN INTERTRIAL INTERVAL (SEC)

Ten-trial block	1	2	3	4	5
Group N	144.7	95.6	73.1	58.5	58.4
Group A	94.1	69.4	48.8	40.6	52.6
t	1.21	1.14	2.41*	0.51	0.36

* $p<.05$.

Results

The percentage of trials with galvanic skin responses of at least 500 ohms in the first 20 vs. the last 20 trials showed a replication of an earlier report. Amygdalectomized animals failed to generate orienting reactions to the tone (Fig. 2). The control group gave 50.7% R in the first 20 trials which fell to 20% in the last 20. The amygdalectomized group scored only 20.3% in trials 1–20 which was at approximately the same level, 25.8% in trials 31–50. The change scores (block 5—block 1) averaged only —4.5% for this group while the controls showed —21.8% ($U = 2$, $p < .02$).

Increased amplitude of GSR as an indicator of orienting was difficult to analyze because of differing response rates between the groups, i.e., amplitude scores which included zero-response trials caused biasing of amplitude measures in the direction of the frequency of response. If zero-response trials are disregarded, the median response amplitude in early trials vs. later trials was not significantly different for either group.

Respiratory responses in group-A animals failed to show normal high respiratory-rhythm disruption scores (Fig. 3). In trials 1–5 group N scored 2.4 (along a 3.0 scale) while group A scored only 0.6 (median test, $p < .01$). By trials 46–50 group N was responding less than in trials 1–5 (Walsh test, $p < .062$) and group A at about the same level as in the earlier trials (.7) ($p > .062$). On the first two presentations of the novel tone, group N increased response from 1.75 in trials 49 and 50 to 2.5 (Walsh test $p < .062$). Group A changed from 0 to 1.0 ($p > .062$).

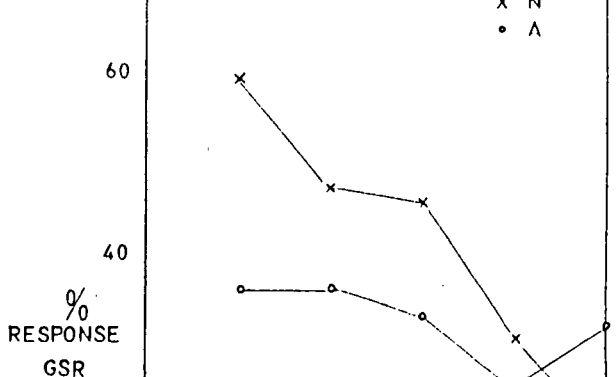

FIG. 2. Mean percentage GSR response to 50 trials of the initial tone for control (N) and amygdalectomized (A) groups. (The two groups are similarly denoted in all subsequent figures.)

FIG. 3. Group mean respiratory-disruption scores for representative trials of the original tone and two trials of the novel tone.

A more exact measure, respirations per minute, was applied and showed the same general effect but because of the small N and the variability of the prestimulus rates, both among and within individuals, it was not possible to obtain sufficient matched pairs for statistical tests.

Group A also failed to display the cardiac-orienting pattern observed in group N. This was acceleration during the first six beats after stimulus onset (Fig. 4). In trials 1–10, normals showed 77% response which fell to 38% by trials 41–50 and increased to 88% for the two novel trials. The group A mean percentage response was 41% in trials 1–10, 46% in trials 41–50 and 20% in the two novel trials. The high scores of the control group in the first ten trials and the two novel trials were significantly different from group A ($p = .01$, U test). Differences in heart rate between groups *before* stimulus onset for these trials was not significant ($p > .2$, U test). As a further check that a trend in faster group prestimulus heart rates was not the cause of the amygdalectomized group failure to show acceleratory responses, three pairs of subjects were matched for the mean prestimulus heart rates and the percentage response values were found comparable to those of the larger groups.

Figure 4 also shows that heart-rate response measured both in change in beats per minute and percentage response parallel each other very

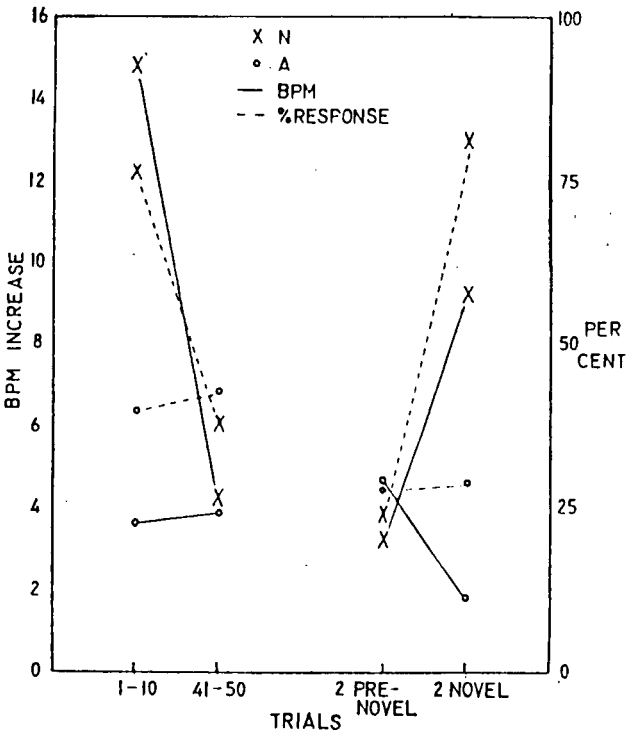

FIG. 4. Group mean percentage response (more than 3 beat/min increase in cardiac rate) as dotted lines, and actual cardiac rate change (solid lines) in the first six beats after stimulus onset. (Representative trials of the original tone and two trials of the novel tone.)

closely. Amygdalectomized animals showed consistently low beat per minute changes in the first six poststimulus beats in block 1 (3.7), block 5 (4.9), the two trials just preceding the novel stimulus (4.6) and in the two novel stimulus trials (1.8). Control values were significantly higher than those for group A in block 1 (14.7 beat/min) ($p = .002$, U test) and in the two novel trials (9.2) ($p = .001$, U test), but not different for block 5 (3.6) and the two prenovel trials (3.2).

Maximum cardiac acceleration in the first 20 beats after stimulus onset (approximately 10 sec) was also found to be higher in normal than amygdalectomized animals in trials 1–6 and the two novel trials (Fig. 5; $p = <.05$, U test). The exact beat-by-beat histogram curve of the acceleration response was not determined, but the same treatment of the R-R measurements were tested for maximum decrease in rate and no group differences were found. Decrease in rate for both groups was approximately 2 beat/min and did not change either across trials or with novelty.

Percentage ear movement response in the first 6 sec after tone onset was highest in trials 1–5 for both groups and habituated rapidly, by the tenth trial (Fig. 6). Intertrial responses, however, were generally lower in number for group A. Net change in intertrial responses for both groups from block 1 to block 5 was not significantly different. Habituation of intertrial

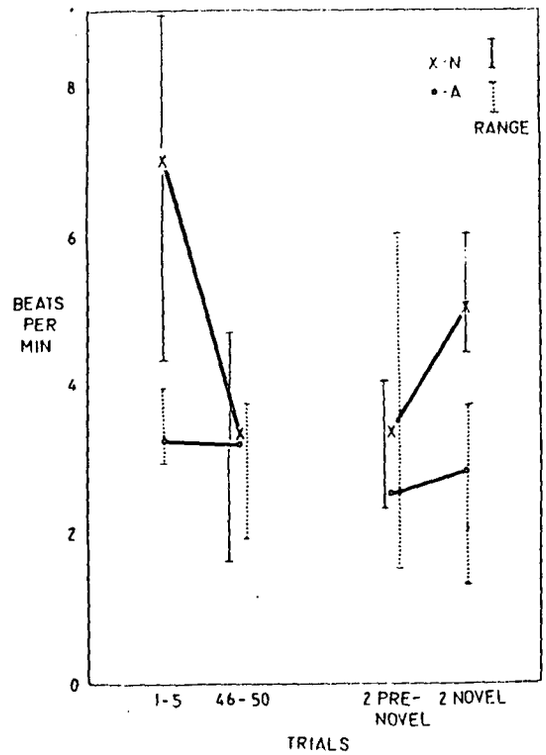

FIG. 5. Group mean maximum single-heart-beat rate increase in the first 20 beats after stimulus onset. (Representative trials of the original tone and two trials of the novel tone.)

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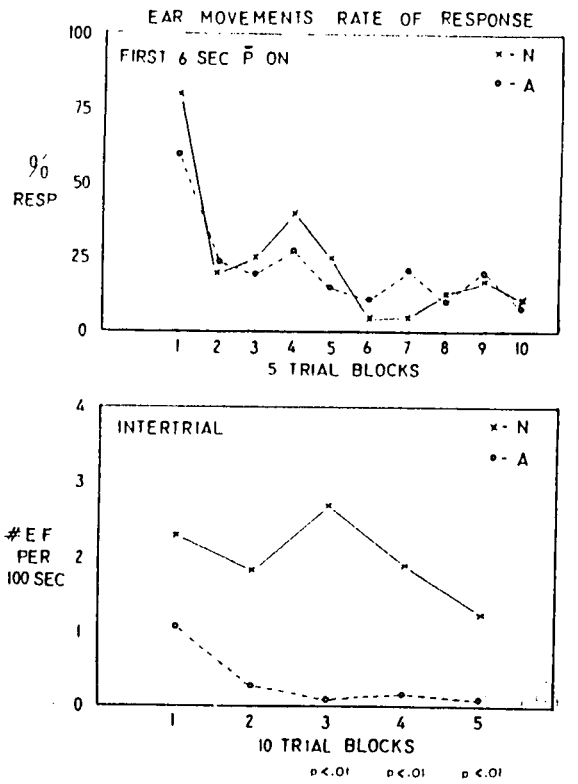

FIG. 6. Above: group mean percentage ear movement response to 50 trials of the initial tone during the first 6 sec after stimulus onset. Below: rate of ear movement responses in the intertrial intervals as number of ear movements per 100 sec.

responses was more gradual then those in the first 6 sec. Response to the novel stimulus was infrequent in both groups.

Duration of ear movement responses (cumulative seconds of response) were also found to be high in early trials for both groups and habituated at a rapid rate (Fig. 7). There were no group differences found. In the intertrial period the control group accumulated much more ear forward time than group A and this time decreased gradually across the 50 trials. Group A had virtually no intertrial EM time.

Percentage response curves for slow wave activation of the EEG showed no habituation of the occurrence of the response in either group (Fig. 8). The groups did differ in that amygdalectomized monkeys showed two patterns of response. Three animals responded on nearly every trial (total = 94–100%) compared to a normal range of 63–98%. Three other group-A subjects showed lower total response rates of 49–60%.

On the other hand, both latency and duration of the LVF response did change with stimulus repetition. Latency increased in the control group across trials in a gradual manner (Fig. 9, upper graph). By the last two trials there was a significant increase from first 2R (MD = 0.6 sec) to last 2R (MD = 2.0 sec) ($p < 0.62$, Walsh test). Group A did not follow

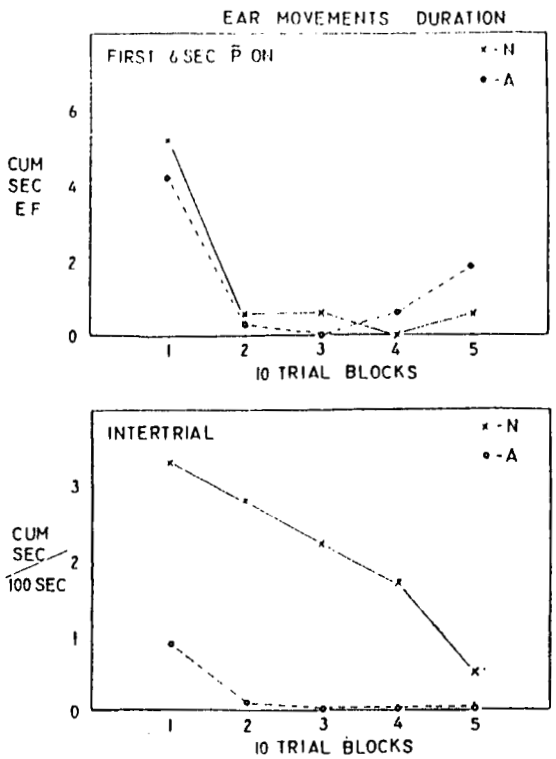

FIG. 7. Above: group mean cumulative duration of ear movement per trial in the first 6 sec after stimulus onset. Below: cumulative duration of ear movement responses per 100 sec in the intertrial intervals.

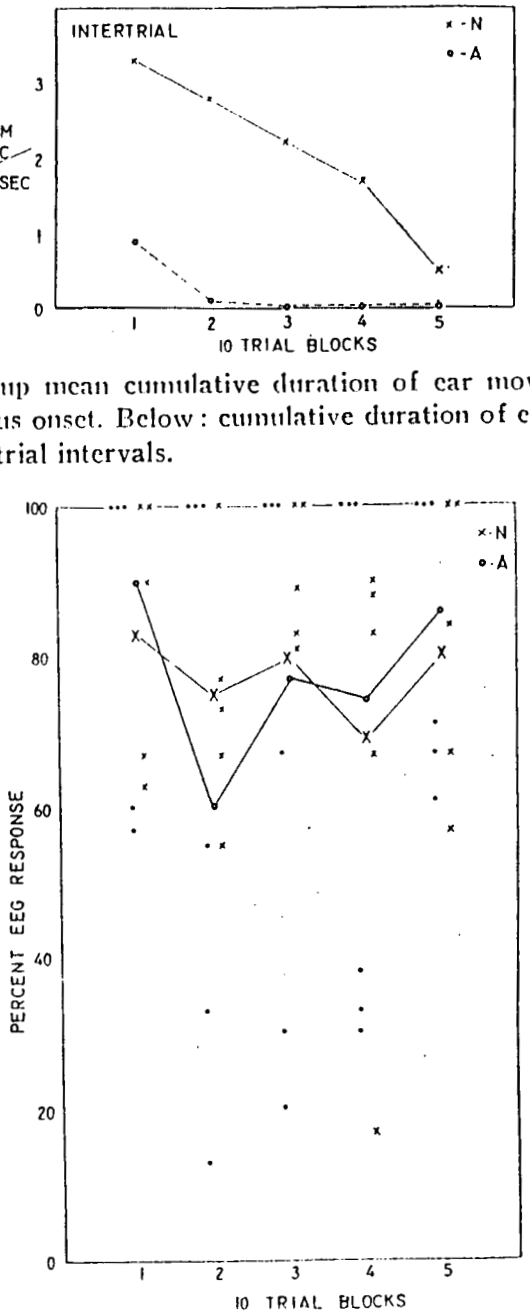

FIG. 8. Group mean percentage EEG low-voltage fast-wave onset response to 50 trials of the initial tone. Individual mean values are represented by smaller symbols.

this pattern and the latency remained short after fifty trials. The group-A subjects attain so short a latency in the first two trials thereby demonstrating no deficit in speed of the onset of the EEG reaction. Durational changes (Fig. 9, lower graph) in group A were much like those of the controls (shorter in the late vs. early trials) but there was a tendency toward shorter durations in block 1. This was not a marked difference, however, and tested at $p < .05$ level only in block 2 trials. However, duration of R in group A remained short on the two novel trials, showing failure of reorienting.

Thus, in the operated group latency followed the failure to habituate prediction and duration tended towards the other extreme—failure to orient. Percentage response reflected both extremes—hyper- and hyporeactivity.

Discussion

The results show that under the conditions of the experiment, the GSR, heart rate, and respiratory rate components of the orienting reaction were uniformly abolished by amygdalectomy. On the other hand, ear movements and EEG activation were for the most part undisturbed except for the *duration* of the EEG low-voltage fast response which did not change on dishabituation and the latency of LVF which failed to habituate.

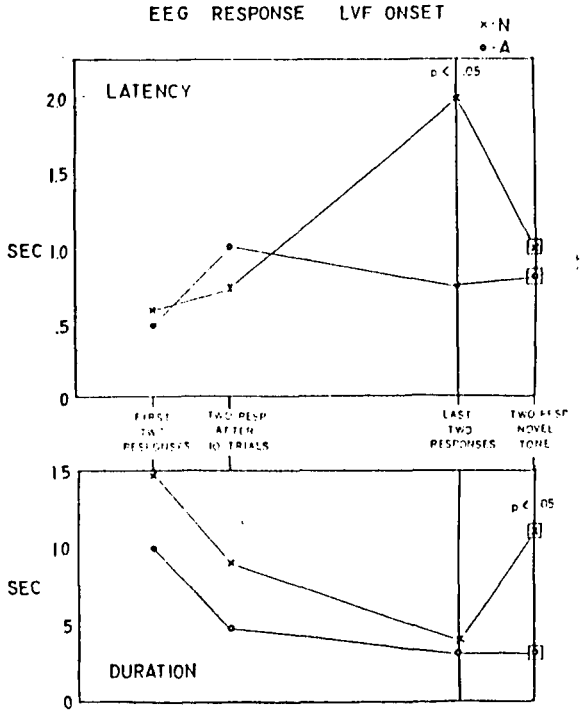

FIG. 9. Group mean latency (above) and duration (below) of EEG low-voltage fast-wave onset responses for two trial samples from the first ten, second ten, and last ten trials of the initial tone and (in squares) for the first two trials of the novel tone.

It is possible to interpret most of these results as straightforward change in the orienting responses. However, the heart-rate data pose a problem. In a recent review of American human and animal experiments, Graham (4) suggested that a heart-rate increase, as was seen in our control group, represents a "defense," or "startle" response, rather than an orienting reaction. She concluded that deceleration occurs most frequently when simple stimuli are presented and that when acceleration is observed it usually fails to habituate. On the other hand, Russian workers, including Siminoff, Latash, and Sokolov, routinely observed acceleration in the human orienting reaction (personal communications). Graham discounted these data on account of the lack of sufficient published details in Sokolov's review of these experiments (9). In addition, she compared the heart-rate-response characteristics observed in the orienting reaction with those observed in tests of readiness to respond where measurements are made after the stimulus during the response foreperiod. In such situations the Laceys (6) and others have reported deceleratory responses which are nonhabituating. However, to call such a signalling situation "simple attention" comparable to that observed in the orienting reaction experiments may well be misleading. Further, the Laceys' deceleratory response is obtained only with a fixed foreperiod. In addition, acceleration appears to follow immediately *both* the "ready" signal and "response" stimulus, with deceleration sandwiched between. In any case, our data, the first to be reported for the monkey, clearly support the Russian view: acceleration occurs and it habituates.

An additional problem in interpretation concerns the EEG response. The LVF response rate was not habituated in group N within fifty trials so the rate of occurrence of this response cannot be considered simply as an indicator of orienting. The normal pattern was one of variation of rates between 60–100%. In human beings, habituation of alpha blocking is reported to be the last component of the OR to habituate. We are struck by the two extremes of response in group A. Three showed very high reactivity with no variability; three others varied like normals but at a lower level of response. It is as if the amygdalectomized animals were not gauging the subtleties of the situation—their reaction tended to be either all or none. This characteristic has been observed in other experiments (2, 7).

Finally, intertrial ear movements persisted through forty trials in the group N and should not be viewed as a good measure of orienting. These responses were very rare in group A. This finding is in apparent contradiction to Bateson's (3) who found notably increased ear responses in amygdalectomized monkeys during short (6-sec) intertrial visual-discrimination training waiting periods. One explanation for this difference is that the intervals over which measurements were taken in Bateson's experi-

ment were comparatively much shorter and were more comparable to our first 6-sec measure. Also Bateson's monkeys were occupied in solving problems.

In summary, the current experiment has shown that the orienting reaction can be fractionated into two major components by amygdalectomy in monkeys. The suggestion arises that autonomic nervous system indicators are involved in one component and that orienting movements and EEG indicators, for the most part, form another. According to the hypothesis proposed earlier, the autonomic indicators signify some sort of registrational process; the significance of the other component of the orienting reaction remains to be explored.

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