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# ACQUISITION OF THE ESCAPE REFLEX IN CATS AFTER THE NUCLEUS CENTRALIS OF THE AMYGDALA LESIONS

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*Abstract.* Three groups of 6 cats were trained in bar-press escape responses from unsignalled shock. Lesions of dorsolateral part of the amygdaloid nucleus centralis were made either 10 days or 35 days before training in two groups of subjects. The remaining 6 cats served as intact control group. The lesions resulted in a lengthening of instrumental response latencies especially in the final period of training. This was not caused by any changes in animals sensitivity and reactivity to painful stimulation since no differences between control and lesioned groups were observed at earlier stages of training. Further, no group effects were observed in threshold reactivity to shock. However in contrast to the control cats, both lesioned groups performed escape responses to low intensity stimuli with very long latencies.

## INTRODUCTION

The results of our previous experiments (16) suggested a decrease of fear after lesions in the dorsolateral part of the amygdaloid nucleus centralis in cats. The lesions resulted in retarded active bar-press avoidance acquisition, which was especially pronounced for short-latency responses. It was shown that the destruction of the amygdaloid nucleus centralis affected not only the acquisition of the avoidance reflex, but also overtrained avoidance responses, which deteriorated. Moreover,

escape responses during the first postoperative period were performed with latencies longer than before surgery.

The method used in our previous experiments did not allow us to ascertain whether or not post-operative behavior was caused merely by changes in the sensitivity to painful stimuli. It is well know that aversive stimuli evoke a number of autonomic and motor responses some of which are necessarily incompatible with the bar-press response. Several investigators (2, 4, 7) have argued that amygdala lesions result in changes of reactivity and sensitivity of subjects to painful stimuli. However, those authors reported extensive lesions, often extending to beyond the amygdaloid complex. Therefore it was decided to investigate the effects of lesions restricted to the central nucleus on reactivity to painful stimuli. Similar to our previous work we decided to limit the extent of the lesions to dorsolateral part of the central nucleus, trying not to destroy the fibers of stria terminalis and ventral system.

## METHODS

Subjects in this experiment were 18 adult male cats. Training of instrumental escape responses was conducted in rectangular cage (54 × 54 × 40 cm), with a grid floor to apply electric shock to the paws of the animals. A bar (10 × 2 cm) was located in the center of an ablong wall of the cage, 8 cm above the floor. Alternating current of 50 cps and 0.05-1.95 mA from a shock generator with a scrambler (Grason–Stadler model E 1064 GS) was used as unconditioned stimulus (US).

Methods and the design of the experiment were similar to those used for study of the effects of prefrontal lesions on escape performance (17). An escape response, consisting of a bar-press reaction, automatically and immediatelly terminated the US. Tonic pressing of the bar during intertrial intervals did not preclude the start of the next trial and the cat was required to lift its paw and press again to terminate the shock. Each training session lasted for 10 trials. A trial started with the activation of the grid floor and was terminated by the bar-press response. The entire experiment consisted of 30 training sessions and 5 testing sessions. The first testing session was introduced after initial 10 training sessions, and the following after each 5 training sessions.

On the first training trial the threshold value of electric shock which elicited the unconditioned reaction in each cat was estimated to

obtain additional information about individual subjects reactivity to painful stimulation.

The shock intensities used during the first 10 training sessions were adjusted to the subject's behavior. During the first testing sessions the shortest latencies of escape responses were observed when the shock intensity was adjusted 0.42–0.49 mA higher than the threshold intensity. Accordingly, during all subsequent training sessions shock intensity for a given cat was constant and at least 0.42 mA higher than the lowest intensity which elicited the escape bar-press response during first testing session.

The testing sessions differed from the training sessions in three aspects: (i) each session had 12 trials, (ii) a testing trial was terminated by the bar-press reaction, or after 60 s, (iii) shocks of increasing intensities were used. In consecutive trials root-mean-square values of electric shock (R.M.S. current) were estimated and presented in Table I.

TABLE I

Shock intensities (in mA) in consecutive testing trials

| Number of trial | R.M.S values of electric shocks |
|---|---|
| I | 0.05 (±0.01) |
| II | 0.12 (±0.01) |
| III | 0.21 (±0.01) |
| IV | 0.29 (±0.01) |
| V | 0.42 (±0.01) |
| VI | 0.49 (±0.01) |
| VII | 0.56 (±0.02) |
| VIII | 0.77 (±0.02) |
| IX | 1.01 (±0.02) |
| X | 1.24 (±0.03) |
| XI | 1.52 (±0.03) |
| XII | 1.95 (±0.04) |

Subjects were assigned to one of three groups each with six cats: a nonoperated control group (Group 1), a group trained 35 days after surgery (Group 2), and a group trained 10 days after surgery (Group 3). Lesions were performed under aseptic conditions with Nembutal anesthesia (40 mg/kg). The dorsolateral part of the nucleus centralis of the amygdala was destroyed bilaterally. Lesions were done electrolitically in a stereotaxic apparatus. The tungstin electrodes (0.5 mm in diam) were insulated up to 0.5 mm of the tip. A cathodal current of 1.5 mA DC for 1 min was used, and the coordinates were AP — 13.0, H — 6.5, L — 9.2 according to the atlas of Jasper and Ajmone-Marsan (5), corrected by using the method of Kuciński (8). After the experiment the cats were sacrificed with overdoses of Nembutal and their brains were subjected to histological analysis using Klüver and Nissel's technique.

## RESULTS

*Escape reflex performance.* Changes in performance were analized in six 50-trial blocks. The groups mean total shock duration in each

block are presented in Fig. 1. An analysis of variance of this index did not show any significant group effect but did indicate a highly significant effect of session blocks ($F = 15.43$, $df = 5/75$, $P < 0.001$). The longest mean time of shock application was observed in Block I, when total time of shock administration in 50 trials was about 21 min in each group of subjects. In the course of training responding became more efficient reaching asymptote values in Blocks III or IV. The shortest mean total times of shock application were observed in the last three blocks of training sessions. The Duncan tests revealed significant differences between the Block I and all consecutive blocks

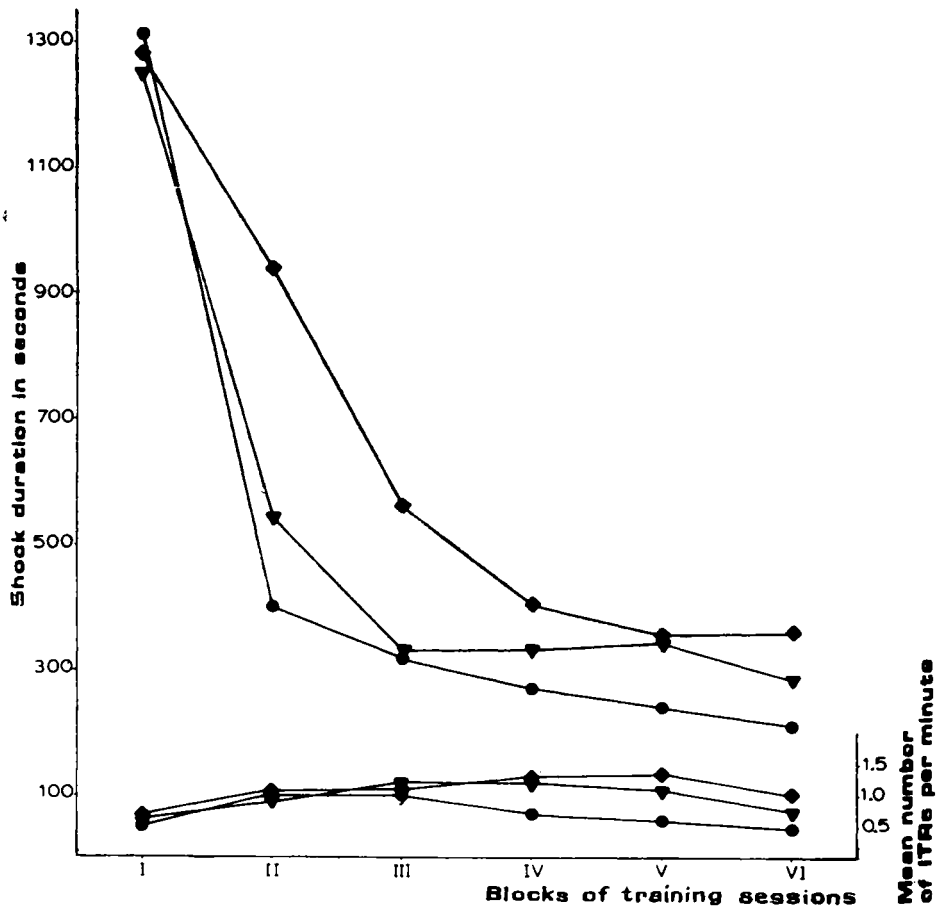


Fig. 1. The mean total time of shock application and the number of intertrial responses (ITR) per min. Circles denote Group 1, triangles denote Group 2, and diamonds denote Group 3.

of sessions ($P$'s $< 0.01$) and also between Block II and Blocks IV, V and VI ($P$'s $< 0.05$). No other comparisons reached significance.

The mean number of intertrial responses (ITRs) per min for six blocks of sessions were compared in the three groups of cats (Fig. 1). A significant block of sessions effect was obtained ($F = 6.62$, $df = 5/75$, $P < 0.001$). However, each group emitted maximal ITRs rates in different blocks, which was confirmed by Fergusson Tests (3) of trends (Table II). Each subject's ITRs rates in consecutive blocks of sessions

TABLE II

Comparisons (z scores) of gradient slopes with hypothesized coefficients for ITRs/min frequency in three groups of cats in consecutive six blocks of training sessions

| Coefficients | | | | | | Group 1 | Group 2 | Group 3 |
|---|---|---|---|---|---|---|---|---|
| 1,5 | 3,5 | 5,5 | 5,5 | 3,5 | 1,5 | 3.04* | 3.75* | 1.38 |
| 1 | 2 | 3 | 4 | 5 | 6 | 0.70 | 0.54 | 2.53* |
| 1 | 6 | 5 | 4 | 3 | 2 | 2.72* | 2.08* | 0.08 |
| 1 | 2 | 6 | 5 | 4 | 3 | 1.78* | 3.32* | 1.61* |
| 1 | 2 | 4 | 6 | 5 | 3 | 1.01 | 2.85* | 2.22* |
| 1 | 2 | 3 | 4 | 6 | 5 | 0.08 | 1.46 | 2.22* |

* $P < 0.05$

were ranked and independently compared with the hypothetical monotonic and bitonic polynomial coefficients listed in Table II. The selection of six presented coefficients (distributions of ranks) was based upon evidence from our observations and data (14) which concerned the most typical distributions of ITRs frequency observed during defensive instrumental reactions training.

Histograms showing distributions of instrumental response latencies for each group and blocks of sessions are presented in Fig. 2, and the same data are shown in Fig. 3 in the form of cumulative response latencies distributions. These data indicate that during initial periods of training (Blocks I and II) the escape latencies of the three groups did not differ. Lesioned cats similar to controls performed about 66% of escape reactions in Block I and about 80% of responses in Block II with latencies shorter than 10 s. The most frequent escape reaction latencies during these training periods were of 2 and 3 s duration. However starting with Block III the control group differed from the two operated groups especially in the proportion of short escape reaction latencies, and this difference increased with prolonged training.

In spite of lack of any between-groups differences in mean total

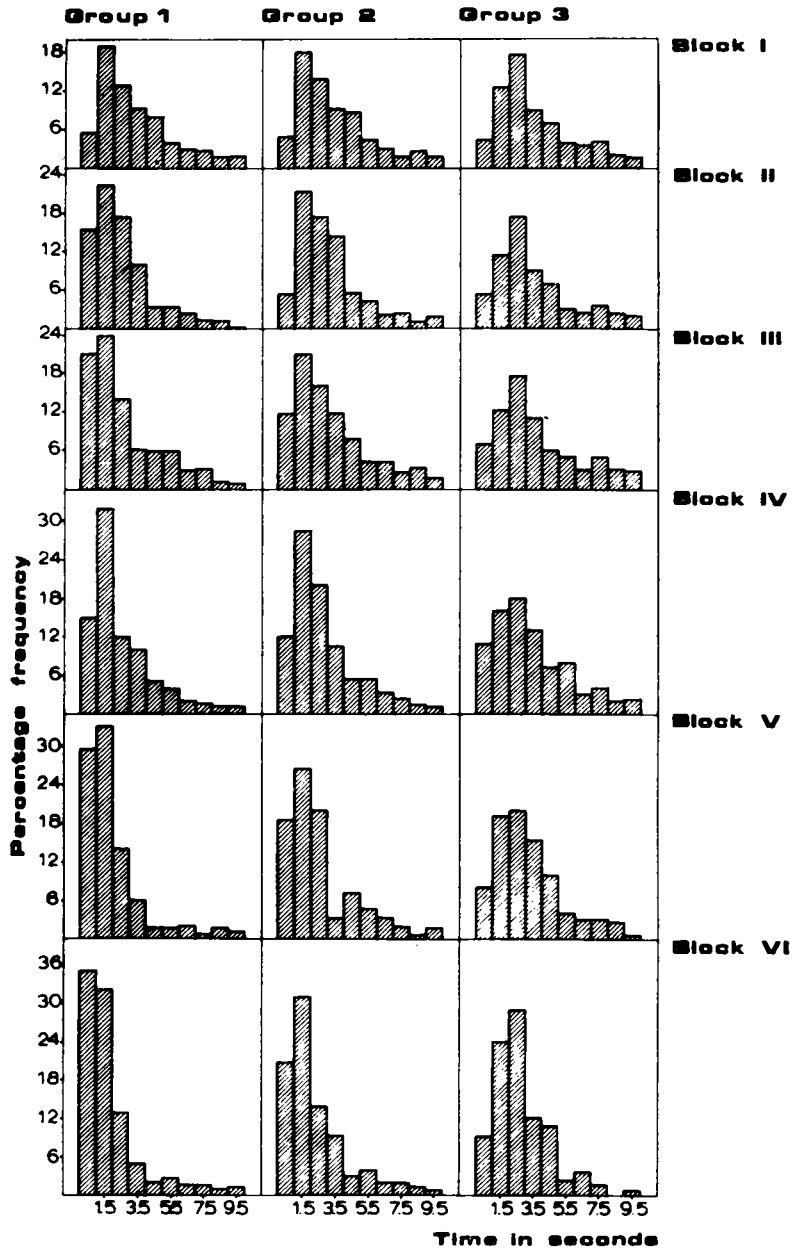


Fig. 2. Frequency histograms showing distributions of latencies of escape responses during consecutive blocks of training sessions.

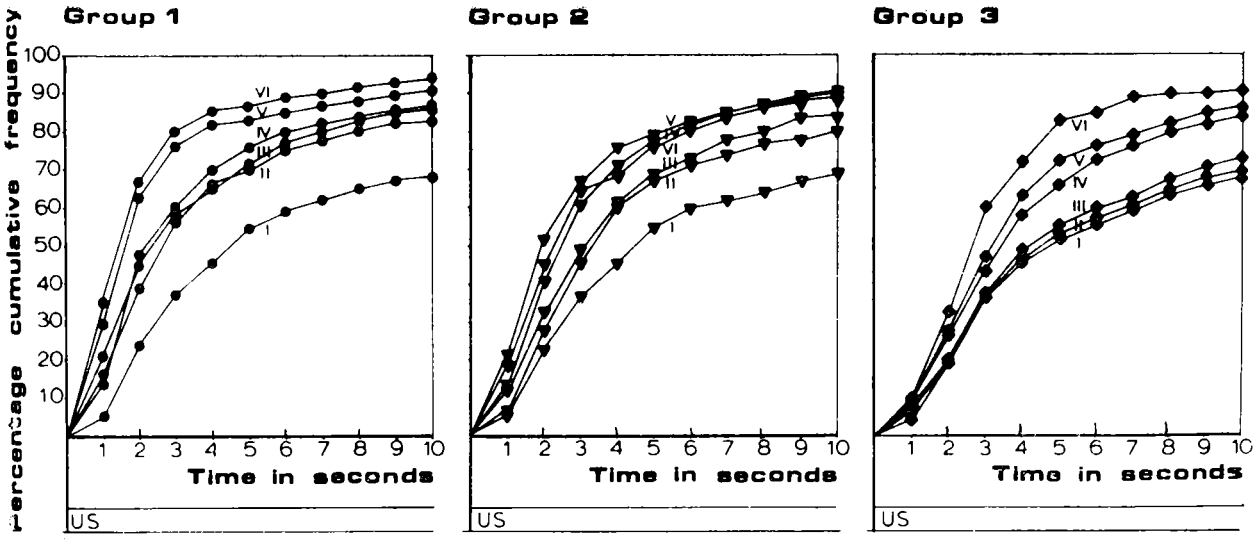


Fig. 3. Cumulative frequency distributions of bar-press escape latencies during consecutive blocks of training sessions. Group denotation as in Fig. 1.

TABLE III

The group mean of latencies of escape reactions (in s) in consecutive periods of training

| Groups | | Blocks of sessions | | | | | |
|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | VI |
| Group 1 | | 4.63 | 2.60 | 2.72 | 2.70 | 1.74 | 1.61 |
| Group 2 | | 4.85 | 4.20 | 3.00 | 2.57 | 2.43 | 2.05 |
| Group 3 | | 5.37 | 5.35 | 5.07 | 3.45 | 3.37 | 2.86 |
| Source of variation | *df* | Values of the *F* statistics | | | | | |
| Groups (A) | 2/15 | 4.26* | | | | | |
| Blocks (B) | 5/75 | 11.53*** | | | | | |
| Interaction (AB) | 10/75 | 0.79 | | | | | |

* $P < 0.05$; *** $P < 0.001$

time of shock application, an analysis did revealed significant group effects on latencies of escape reactions (Table III). The longest latencies were observed in Group 3 while the shortest in control subjects (Group 1). When the block of sessions effect was collapsed the Duncan tests showed significant differences between Group I and III ($P < 0.05$) and no differences in the other comparisons were found.

The blocks of sessions effect was also significant. The longest mean median latencies were observed in Block I, and they shortened in the course of training. The Duncan tests showed significant differences between Blocks I and II ($P$'s $< 0.05$), and between Block I and all remaining blocks of training sessions ($P$'s $< 0.01$). Significant shortening of mean median escape latencies was observed in Blocks: IV ($P < 0.05$), V and VI ($P < 0.01$) in comparison with Block II, and also in Blocks V ($P < 0.05$) and VI ($P < 0.01$) in comparison with Block III. No differences were seen in the remaining comparisons.

*Threshold reactivity in testing sessions.* The threshold of unconditioned reactions evoked by shock was estimated in the first trial of training. The shock intensity was slowly increased until the cat oriented itself to the grid floor and displayed the flight response as defined by Kaada (6). Mean values of this index were similar in all groups: 0.38 mA in Groups 1 and 2 and 0.41 mA in Group 3. As mentioned before, the shock intensities used during the first 10 training sessions were adjusted to the cat's behavior. However, the lowest shock intensity which evoked an instrumental reaction in the first testing session determined the shock intensity used during all further training sessions. The mean threshold values found in the first testing session were: 0.60 mA (range 0.21–1.01 mA) in Group 1, 0.55 mA (range 0.12–1.01 mA) in Group 2, and 0.42 (range 0.21–0.77 mA) in Group 3.

Changes of the mean threshold value for each group of cats in consecutive testing sessions presented in Fig. 4 did not show any group effect, whereas a significant testing sessions effect was revealed ($F = 3.69$, $df = 4/60$, $P < 0.01$). The Duncan tests indicated that a significant decrease of escape threshold values appeared in the third and fourth testing sessions compared to the first session ($P < 0.01$). It is worth mentioning that the threshold value had a tendency to a systematically increase in the final testing sessions, reaching a level similar to that which was observed in the first testing session. Nevertheless, this increase was not significant.

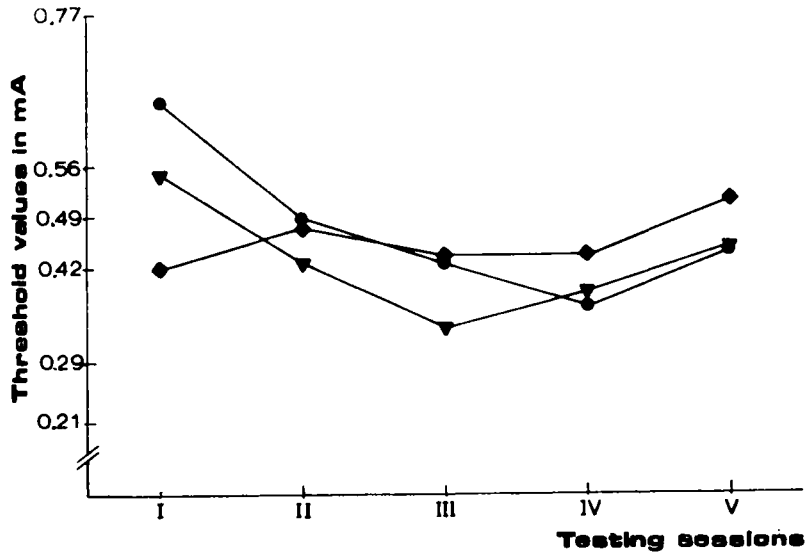


Fig. 4. Threshold values of escape reactions in consecutive test sessions. Group denotations as in Fig. 1.

Sporadicly elicited escape reactions by 0.12 mA shock intensity were found in the control group and in the group of cats trained 35 days after surgery. The lowest threshold value in the group of cats trained 10 days after operation was 0.21 mA. No differences were noticed between control and lesioned cats in performance of escape reactions to shock applied in consecutive testing trials. The mean percent of reactions increased in all groups as the shock intensity increased, reaching the 100% level of performance in response to 1.01 mA shock intensity.

Latencies of escape reactions changed also on consecutive testing trials, reflected by the mean median latencies of instrumental responses on the three groups shown in Fig. 5. As seen from this figure

both lesioned groups responded to low intensity stimuli with longer latencies than the control group. Latencies of response shortened in all groups of cats as the shock intensity increased but this tendency was far more marked in lesioned cats than in the control group.

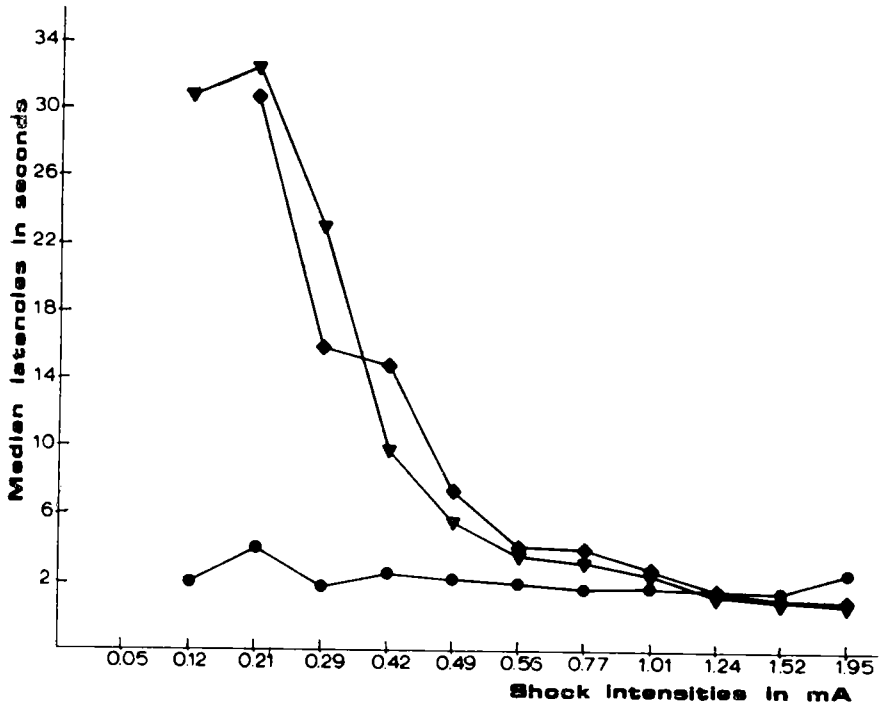


Fig. 5. Median latencies of escape reactions in consecutive test trials. Group denotations as in Fig. 1.

An analysis of variance compared mean latencies of escape reactions emitted by the three groups of cats to the four highest intensities (1.01–1.95 mA) in consecutive testing sessions (Table IV). In general, the higher the shock intensity, the shorter the response latencies were revealed. However, significant differences in the mean latencies of responses were also caused by the systematic acceleration of responding in succesive testing sessions (Fig. 6).

Three cats from the control group behaved in a different way. Their escape latencies were longer as shock intensity increased, probably because of the deleterious influence of strong shock on instrumental responding. However, the interaction between groups and shock intensity was not significant. A significant interaction between shock intensity and consecutive testing sessions was observed, which was caused by different distribution of escape latencies emitted to increasing shock

TABLE IV

Analysis of variance of the mean escape latencies emitted by three groups of cats to the four highest intensities (1.01–1.95 mA) in consecutive test sessions

| Source of variation | *df* | Values of the *F* statistics |
|---|---|---|
| Groups (A) | 2/15 | < 1 |
| Testing sessions (B) | 4/60 | 3.88** |
| Shock intensities (C) | 3/45 | 10.67*** |
| Interaction (AB) | 8/60 | 1.12 |
| (AC) | 6/45 | 1.19 |
| (BC) | 12/180 | 2.17* |
| (ABC) | 24/180 | 1.04 |

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

intensities (Fig. 6). During the first testing session, response latencies became systematically shorter as the shock intensity increased. It is evident, however, that in the remaining testing sessions, escape responses tended to be faster only to the 1.01 mA shock intensity. More-

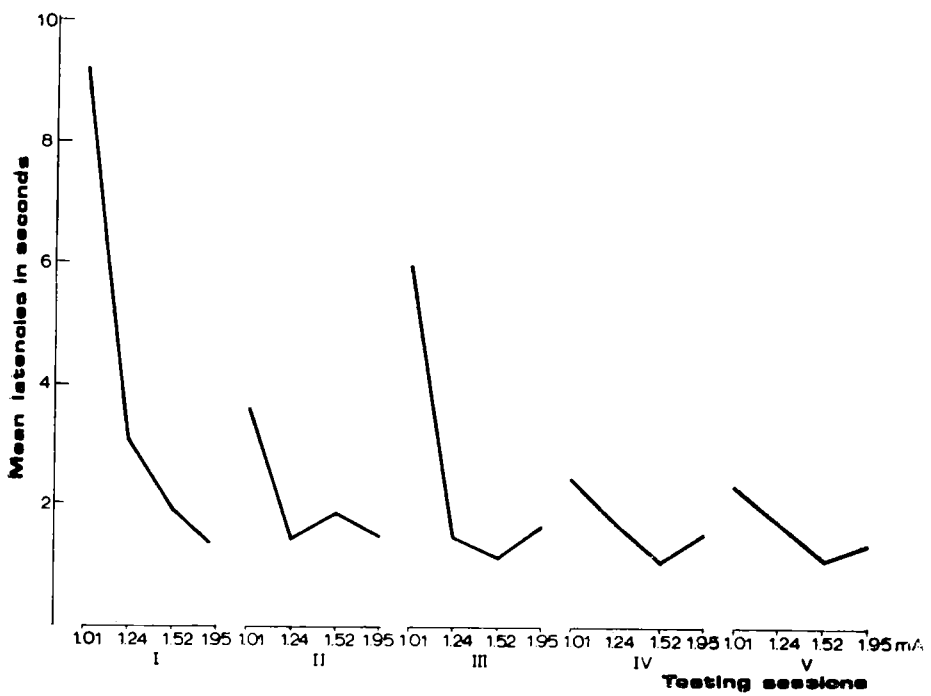


Fig. 6. Mean latencies of escape reactions for all groups of cats in consecutive test sessions.

over, faster responses appearing in both initial testing sessions were evoked by the strongest shock. Nevertheless, the shortest escape latencies, observed in the third and all remaining testing sessions, were evoked by 1.52 mA shock intensity, and were loger in last trial.

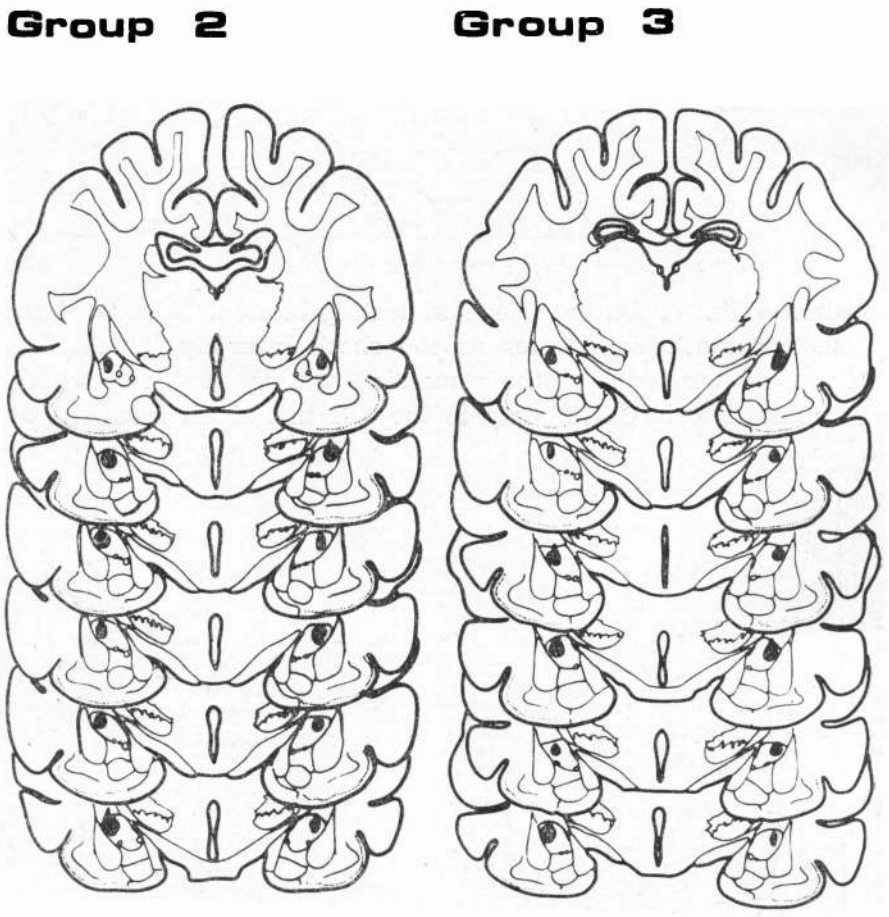


Fig. 7. Reconstruction of lesion sections through areas of maximum tissue destruction in individual cats of Groups 2 and 3.

*General behavior of cats and results of neurological tests.* All cats showed many symptoms of fear during the initial four or five training sessions. They attempted to flee from the chamber, sometimes urinated, defecated, showed piloerections and vocalizations. Two cats from the control group had a tendency to react aggressively, especially when

being taken from their home-cages. After several training sessions, the cats became quiet and fear reactions occurred only sporadically. No clear effects of surgery on locomotor activity, the intensity and patterns of reactions to new external stimuli (including a white mouse shown from the door of the home cages) were observed.

Neurological observations were made before and after surgery of the visual placing response, righting reflex and hopping reactions. Orientation of the head toward the source of such sounds as the rustling of paper or clapping hands, and head shaking to a puff subjects' ears were also noted. Additionally subjects were allowed to explore a room and mount several obstacles like chairs and stools. No post-operative changes in these tests over pre-operative behavior were noticed.

*Anatomical verification of the lesions.* In all subjects bilateral lesions were symmetrical and included a fragment of the dorsolateral part of central nucleus without injury of the stria terminalis. Most likely the

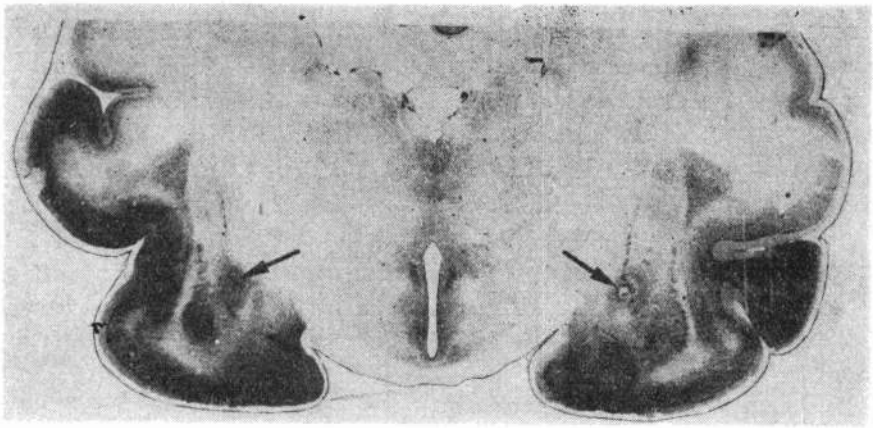

Fig. 8. Frontal section of the brain of representative cat with typical lesions in dorsolateral part of central nucleus of the amygdala.

longitudinal association fibers were not damaged. Small unilateral damage to the region of the putamen adjacent to the central nucleus was found in three cats. In one cat, a fragment of the anterior part of the amygdaloid nucleus centralis was also unilaterally destroyed. There were no correlations between lesion extent and the magnitude of post-operative changes in any subject's behavior. The maximal extent of the lesions for both lesioned groups are presented in Fig. 7, and example of histological preparate is given in Fig. 8.

## DISCUSSION

Data presented in this paper indicate that lesions in the central nucleus of the amygdala affected bar-press escape responses in cats. Inspite of not finding significant group effects in the total time of shock administration, the precise analysis of the distributions of escape median latencies revealed that both lesioned groups of cats responded with longer latencies than the control animals.

Some authors (2, 4, 7) have tended to explain the effects of amygdala lesions on defensive conditioned reflexes by significant changes in the animal's sensitivity and reactivity to shock. This suggestion was partially supported by the finding of anatomical connections between amygdala and sensory-motor cortex S-II in cats (12). Some investigators have also emphasized fibers which conduct sensory impulses from skin receptors to the amygdala (1, 13).

Deterioration in escape performance demonstrated in the present study, and marked in the final periods of training, can hardly be related to changes in sensitivity and reactivity to shock, since no differences between groups were observed at the early stages of learning. The results of our previous experiments on cats (16) and rats (15) as well as the results of neurological tests and observations of general behavior support the same conclusion. More precise information concerning this problem let us to an estimation of pain threshold values. Contrary to the results of other investigators (2, 4), we did not observe any post-operative changes in the threshold values of unconditioned reactions evoked by different shock intensities. Similarly, no differences between control and lesioned animals were seen in the threshold of instrumental reactions and the level of escape performance estimated on consecutive testing trials.

It is possible that these contradictions are mainly caused by different localizations and extents of lesions made by various authors. Kemble and Beckman (7) have described significant shortening of escape latencies evoked by strong as well as weak shock after lesions including "inhibitory centers" in basolateral part of the amygdala. However, our findings show that in contrast to control cats, both lesioned groups responded to low intensity stimuli with longer latencies. High intensity shock however evoked much shorter latencies of escape reactions in all groups of animals. It may be suggested that the reception of shock was impaired after the surgery, but this deficit was compensated by strong intensities of electric shock.

Is important to note that experiences of pain do not have positive relationship to the intensity of external stimulation. That is the in-

strumental responses evoked by painful stimuli are not determined only by perception of pain and its various aspects such as location and magnitude. Equally important are the animal's past experience with similar situation and the affective as well as the motivational concomitants of pain (10, 11). Our data give understanding the effects of pain, since the threshold values of escape responses and their latencies were changed during consecutive testing sessions.

The shock stimulus possesses at least two biological functions. First, it evokes unconditional motor and emotional reactions. Secondly, in the conditions of the present experiment, it acquires signalling properties and denotes that pain with fear will continue untill the performance of an instrumental response. As a rule the unconditioned reactions are not subjected to learning since they arise from innate and philogenetically-formed reflex arcs. The intensity and velocity of these reactions depend on the integrative action of all elements of the reflex arc — receptors, effectors, afferent and efferent pathways. Therefore, it is possible to reveal eventual impairment of reception by estimating the performance of unconditioned reactions evoked by various shock intensities.

Quite different problems appear when instrumental escape reflexes are investigated. It is well known that these reactions are strongly energized by fear which is normally elicited by noxius stimulation (9). Accordingly, escape reflexes are probably caused by functional association of the pain signaling center and instrumental reaction center. Thus, it is understandable that formation of such associations can be influenced not only by impairment of pain reception but also by too low and too high motivation level. One of the consequences of such impairment is the incorect evaluation of the biological value or signaling property of unconditioned stimuli, which inform that pain with fear will continue until the performance of the instrumental reaction. As far as the measurement of escape threshold reactivity allows us to estimate the magnitude of changes and impairments in pain reception, the escape reaction latencies provide information about the motivation level and the evaluation of the signalling properties of pain stimuli.

One may conclude that the signalling properties or biological values of even weak painful stimuli were sufficient for control animals to evoke efficient and rapid reactions. On the contrary, the lengthening of escape latencies observed in both lesioned groups during training and testing sessions were probably caused by a postoperative decrease of both the motivation level and signalling properties of shock. It is possible that the merked post-operative decrease in the performance of avoidance and escape responding shown in our previous experiment

(16) can not be related to a decrease in sensitivity and reactivity to unconditioned stimuli, but rather to the decrement in animals' evaluation of biological value of external pain evoking stimuli.

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