EMOTIONAL REACTIVITY AFTER FRONTEMEDIAL CORTICAL, NEOSTRIATAL OR HIPPOCAMPAL LESIONS IN RATS

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Abstract. Emotional reactivity following lesions in structures corresponding to the main components of dorsal prefrontal system of the monkey was evaluated in a total of 165 rats by a six-categorial rating scale. Frontomedial cortical damage (FM) and lesions in anterodorsal (DH) or posteroventral (VH) hippocampal regions elicited marked increases in emotional reactivity, relative to sham operated controls. The most profound effect was observed in FM rats. VH rats were somewhat more emotional than DH ones. Anteromedial neostriatal lesions (NC) evoked increased emotionality only on the first day of testing. Damage to different structures differentially affected the various categories examined. The magnitude of overall reactivity and the pattern of changes indicate a similarity of effects from frontomedial and hippocampal lesions. This similarity was stronger between FM and VH than DH lesions. The negligible effect of NC damage on emotional reactivity suggests a functional dissociation between the enteromedial neostriatum and the anatomically related frontomedial cortex.

INTRODUCTION

Rosvold (29) has suggested that the frontal lobe system is organized into two subsystems: a dorsal system originating in the dorsolateral prefrontal cortex and an orbital system originating in the orbital prefrontal cortex. Each system was related to different subcortical structures: the anterodorsal portion of the head of the caudate nucleus and the hippo-
campus were considered the main structures of the dorsal system, while the ventrolateral part of the head of the caudate nucleus, the septum and the hypothalamus were the main structures implicated in the orbital system. This notion, based mainly on studies of monkeys, is consistent with data obtained from cats and dogs (1, 3, 4, 6, 8, 20, 27, 30, 31).

In the rat, consideration of a separate frontal subsystem was an impossibility because of the difficulty in establishing homologous relationships with other species, both for prefrontal cortex as a whole and for its subregions. However, recent anatomical studies (9, 23) provided evidence that the medial area of the pregenual medial cortex in the rat may be homologous with the dorsolateral cortex of the monkey, while the rat's sulcal cortex is probably homologous with the monkey lateral orbital cortex. Since these reports, several authors (5, 7, 10–12, 16–19, 26, 28, 32) have pointed to a functional dissociation between the two frontal cortical subfields and to different relations between the subfields and subcortical structures, similar to those observed in monkey. However, some differences between the rat and the monkey regarding the dissociation of the two frontal systems have also been noted, which have been concerned with emotional reactivity after the removal of the frontal subregions. Butters and Snyder (2) demonstrated a profound effect from orbitofrontal cortical lesion on emotional reactions in monkeys, whereas dorsal lesion had little or no effect. On the other hand, lesions in both frontal subregions of the rat produced an increase in emotionality, although the pattern of effects was different (16, 26). Increased emotionality following ablation of the frontomedial cortex in rats was observed also in our earlier studies (24, 25).

Nonneman et al. (26) using King's multiple-scale rating procedure found that large destruction of the hippocampus (from 50% to 85%) caused a pronounced increase in the rat's emotionality with a pattern more similar to that observed after lesions of the sulcal cortex than the frontomedial region. This finding points to stronger functional relations between the hippocampus and the "orbital" cortical rather than the "dorsal" cortical region in the rat. However, a number of studies (see Jarrard 13) have indicated that the hippocampus can be divided into anatomically and functionally distinct subregions. Accordingly, selective lesions in this structure seem to be more appropriate for relating specific hippocampal areas with specific areas of the prefrontal cortex.

Although several investigations in the rat (5, 7, 9, 10, 12, 28, 32), using different behavioral tasks, have confirmed the functional relation between the frontomedial cortex and anteromedial region of the neostriatum, little attention has been directed toward the comparison of emotional reactivity within this couple of structures. Kirkby (15) re-
ported increased defecations in an open field situation following antero-
dorsal neostriatal damage in the rat. However, he considered the eleva-
tion of emotionality as a secondary phenomenon resulting from the dis-
order in modulation of arousal processes. Apart from this interpretation,
the measure of defecations alone cannot be considered as an adequate
dimension of emotionality changes. It may be inferred from the above
considerations that the functional relations within the two frontal sub-
systems in the rat are still far from being clear. The experiment repor-
ted here attempted to assess the involvement of the main structures of
the dorsal frontal system i.e., the frontomedial cortex, the anteromedial
part of the neostriatum and the hippocampus — in the regulation of the
emotional state, evaluated by a modified use of King's scale (14). With
regard to functional heterogeneity of the hippocampus, two different
hippocampal regions, anterodorsal and posteroverentral, were damaged.

METHOD

Subjects. The subjects were 165 male Wistar albino rats, 90–100 days
old and weighing 190–240 g at the beginning of the experiment. They
were housed in groups of 5–7 with food and water available ad lib. The
rats were maintained in the laboratory for two weeks after their arrival
from an animal breeding farm. During this time they were handled for
5 min each day for 6 days prior to surgery in order to diminish individu-
al differences in emotionality. About 40 rats were assigned to each
of four surgical groups: frontomedial cortical (FM), anteromedial neo-
striatal (NC), anterodorsal hippocampal (DH) and posteroverental hippo-
campal (VH). Due to a large number of subjects in the groups, the ex-
periment was conducted successively, so that only one group was tested
in a given time period. Since differential seasonal influences were ex-
pected, as well as those of other uncontrolled factors, within each group
the subjects were randomly divided into two subgroups of operated
and controls, which were tested simultaneously. This allowed com-
parisons of experimental data of operated rats with those of respective
controls.

Surgery. The operations were performed under clean but not aseptic
conditions. The subjects were anesthetized with Nembutal (50 mg/kg)
and positioned in a Kopf stereotaxic instrument, so that bregma and
the point just behind lambda were in the same horizontal plane. All
brain lesions were performed bilaterally by passing anodal current
through a tungsten electrode, insulated with enamel except for 1 mm
at the tip. The orientation points for coordinates were: bregma — for
AP and for L, dura — for H. For FM surgery a series of 10 closely spaced lesions were made using an electrode of 0.5 mm diameter and 2 mA of current for 10 s. The intended area was the medial cortical projection field of the n. medialis dorsalis thalami as defined by Leonard (23). Coordinates were: AP, from +5.2 to +2.2, L, 0.5, H, from 1.1 to 3.0 down from the dura.

The NC lesions were made by placing an electrode of 0.4 mm diameter at three points using Divac's (5) coordinates: AP, +0.5 and +1.5, L, 2.2 and H, 5.5 and 5.0 for the anterior placement and H, 5.0 for the posterior placement. Anodal current of 3 mA was passed for 15 s. The DH lesions were produced by passing a 2.5 mA current for 15 s through an electrode of 0.4 mm diameter which was placed at two points: AP, -1.0, L, 2.2, H, 2.9 and AP, -2.0, L, 2.2, H, 2.9. VH lesions were made with the same parameters of electrode and current as DH lesions but using different coordinates: AP, -3, L, 5, H, 5.5 and AP, -4, L, 5, H, 5. The subjects of the control subgroups received a skin incision and trephine holes were drilled, but no lesions were made.

Histology. The lesioned rats were deeply anesthetized and perfused intracardially with isotonic formalin. Frontal 50 pm sections were subsequently made on a cryostat and stained, with the Klüver-Barrera method. The extent of the damaged tissue was identified following the atlas of König and Klippel (21).

Procedure. Emotionality testing was conducted for 5 days starting on the 5th postsurgical day. Each rat was removed from the home cage and placed in the middle of a 30 × 30 cm table. Two observers independently rated the animals reactions using a modification of King's emotionality scale (14), consisting of the following categories: (i) resistance to capture in the home cage, (ii) muscular tension and resistance to handling, (iii) vocalization during testing, (iv) the reaction to a pencil presented close to the rat's snout, (v) the reaction to a light tapping on the back with a pencil, and (vi) urination and defecation during the testing session. The reactions were scored from 0 to 5 points in each category. The sum of scores from all categories represented the daily emotionality rating for a given subject.

RESULTS

Anatomy. The largest and smallest lesions in the four brain damaged groups are presented in Fig. 1. In the FM group the cortex was ablated from the frontal pole to the genu of corpus callosum. In the nomenclature of Krieg (22), the damage included the medial portion of field 10 and
major parts of fields 24 and 32, sparing the most ventral pregenual part of the medial surface. The neostriatal lesions were similar to those reported by Divac (5). A few rats sustained slight damage of corpus callosum. The DH lesions were confined to the anterodorsal part of this

structure. In the majority of rats, the destruction comprised partly the fimbria of the hippocampus and the dorsal section of gyrus dentatus. Sporadically, the ventral part of corpus callosum was also touched. One rat showed slight damage of n. lateralis thalami. The VH lesions destroyed the posterior and ventral parts of the hippocampus including also the ventral aspect of the gyrus dentatus and the medial fragment of the subiculum. The most ventral part of the hippocampus was left intact.

Fig. 1. Composite representation of the largest (dotted area) and the smallest (darkened area) lesions in the four brain damaged groups. FM, frontomedial cortex; NC, anteromedial neostriatum; DH, anterodorsal hippocampus; VH, posteroventral hippocampus.
Behavior. The concordance of ratings of the two experimenters was evaluated by means of linear correlation. A highly significant positive correlation ($P < 0.001$) was found between the ratings in each category.

Total emotionality scores. Figure 2 presents the emotionality ratings (sum of scores from all categories) for all groups across the 5 days of testing. An overall analysis of variance (lesion groups $\times$ surgical subgroups $\times$ testing days) disclosed reliable differences among groups with various lesions ($F = 11.43$, $df = 3/157$, $P < 0.001$). In all groups scores of lesioned rats were higher than those of controls (subgroup effects, $F = 60.47$, $df = 1/157$, $P < 0.001$). The differential effect of various lesions is shown by significant interaction between groups and subgroups ($F = 3.57$, $df = 3/157$, $P < 0.025$). The groups and subgroups displayed a decrease of emotionality scores over testing days as indicated by a reliable days effect ($F = 128.09$, $df = 4/628$, $P < 0.001$). This decrease differed in various groups as indicated by a significant interaction between groups and days ($F = 3.72$, $df = 12/628$, $P < 0.001$). On the other hand, a nonsignificant subgroups $\times$ days interaction ($F < 1$) pointed to similar decrease in operated and control subgroups. However, the effects of lesions on the decreasing in emotionality should be quali-
fied by the interpretation of higher order interaction. It is apparent from Fig. 2 that, in contrast to other subgroups, the decrease in emotionality of the NC operated subgroup was greatest between the first and second day of testing, which was reflected in a significant groups × subgroups × days interaction ($F = 2.75$, $df = 12/628$, $P < 0.001$). To further allow for more precise comparisons, it seemed reasonable to take advantage of the simultaneous testing of each operated subgroup with the respective control. Accordingly, separate two-way analyses of variance between subgroups and testing days were performed for each group, and Duncan tests were done for post hoc analyses, when appropriate. This procedure seemed additionally justified by the significant interactions between groups and subgroups obtained from overall ANOVA.

In the FM group, rats with cortical lesions were more emotional than sham operated controls ($F = 72.47$, $df = 1/40$, $P < 0.001$) while overall emotionality declined over successive days in both subgroups ($F = 18.41$, $df = 4/160$, $P < 0.001$). The lack of a significant interaction between subgroups and testing days ($F < 1$) indicates a similar course of decreasing emotionality in the lesioned and control rats, suggesting that the difference in emotionality between subgroups persisted until the end of test sessions.

For the NC group the ANOVA showed a nonsignificant subgroup effect, however, the interaction between subgroups and testing days was significant ($F = 4.34$, $df = 4/168$, $P < 0.005$). Duncan tests revealed that the rats lesioned in the neostriatum differed reliably from the controls only on Day 1 ($P < 0.01$). On further days the emotionality scores decreased in both subgroups ($F = 77.69$, $df = 4/168$, $P < 0.001$).

The hippocampally lesioned rats of the DH and VH groups showed higher emotionality scores than their respective controls. The subgroup effect was significant, both for the DH group ($F = 7.49$, $df = 1/38$, $P < 0.01$) and the VH group ($F = 16.20$, $df = 1/37$, $P < 0.001$). During successive testing days emotionality diminished in the DH group ($F = 30.63$, $df = 4/152$, $P < 0.001$), as well as in VH group ($F = 19.75$, $df = 4/148$, $P < 0.001$). Since the rate of decrease was similar in the hippocampally lesioned and control subgroups, as indicated by the nonsignificant interaction ($P > 0.2$), it may be inferred that differences in emotionality scores of the rats damaged in either hippocampal region and respective controls were maintained throughout the entire testing period.

The comparison of the extent of emotionality changes following different lesions was made on the basis of the "$F$" distribution of Snedecor (33). The exact probability of the incidental difference between
control and operated subgroups was $2 \times 10^{-10}$ for the FM group, $2.8 \times 10^{-4}$ for the VH group and $9.4 \times 10^{-3}$ for the DH group. Thus, it might be supposed that the difference between control and operated subgroups was higher in the FM group than in the VH group. A still smaller difference was observed in DH group.

**Scores in various categories.** Similar to the total emotionality scores, the overall ANOVA of the different category scales (groups × subgroups × categories) showed reliable differences among groups ($F = 11.47$, $df = 3/157$, $P < 0.001$). Scores of lesioned rats differed from those of control ones (subgroup effect, $F = 61.32$, $df = 1/157$, $P < 0.001$). These differences depended on the type of lesion as shown by significant interaction between groups and subgroups ($F = 3.44$, $df = 3/157$, $P < 0.025$). The category effect ($F = 825.95$, $df = 5/785$, $P < 0.001$) points to different magnitude of reactions in the various emotional scales examined. A highly significant groups × categories interaction ($F = 9.27$, $df = 15/785$, $P < 0.001$) indicates that scores of a particular category were different in various groups. The differential effect of various types of operations on scores in a particular category is shown by a highly significant subgroups × categories interaction ($F = 10.04$, $df = 5/785$, $P < 0.001$). The highly significant higher order interaction (groups × subgroups × categories; $F = 6.93$, $df = 15/785$, $P < 0.001$) may be interpreted as focussing on the differential effects of all three factors, i.e., categories examined, types of brain lesions and control operation. As in the analysis of total emotionality scores separate two-way ANOVAs (subgroups × categories) and Duncan tests were performed for each group, in order to obtain the more detailed comparisons between operated and control rats' scores in particular categories.

The FM group. An ANOVA indicated significant subgroup ($F = 72.50$, $df = 1/40$, $P < 0.001$) and category effects ($F = 274.54$, $df = 5/200$, $P < 0.001$), as well as their interaction effect ($F = 14.85$, $df = 5/200$, $P < 0.001$). Duncan tests showed, that the lesioned rats had reliably higher scores than the control rats on 4 categories: removal from home cage ($P < 0.01$), handling ($P < 0.01$), tapping the back ($P < 0.01$) and defecation/urination ($P < 0.01$). Vocalization and pencil reaction failed to differentiate the subgroups.

The NC group. The differences among categories ($F = 185.65$, $df = 5/210$, $P < 0.001$) and the interaction of subgroups × categories ($F = 8.61$, $df = 5/210$, $P < 0.001$) were both significant, but the subgroup main effect was not significant ($P > 0.2$). Duncan tests revealed that rats with neostriatal lesion differed from controls only in vocal reaction
(P < 0.01). Differences in all remaining categories were nonsignificant (P > 0.2).

The DH and VH groups. The ANOVAs indicated significant subgroup effects in both the dorsal hippocampal group (F = 7.79, df = 1/38, P < 0.01) and the ventral hippocampal group (F = 16.21, df = 1/37, P < 0.001). The differences among categories were significant in both dorsal and ventral group (F = 259.59, df = 5/190, P < 0.001 and F = 145.44, df = 5/185, P < 0.001, respectively); and significant interactions emerged in both groups as well (F = 4.56, df = 5/190, P < 0.001 and F = 2.94, df = 5/185, P < 0.025, respectively). Duncan tests showed that the rats with lesion in the dorsal hippocampus were more reactive than controls in 3 categories: removal from the home cage (P < 0.01), handling (P < 0.05) and back tapping (P < 0.05). The ventral hippocampal lesion, similar to the dorsal damage, increased emotionality scores for handling (P < 0.05) and back tapping (P < 0.01), and also evoked higher defecation and urination (P < 0.01) with no effect on the reaction to removal from the home cage.

Fig. 3. Changes in various rating categories of emotional measurement. Along the ordinate are indicated the percent differences of operated subjects with respect to control subgroups (100%). Dots represent significant differences revealed by Duncan tests for raw scores of operated and control subgroups in particular categories.
The above results are summarized in Fig. 3 which presents the percent differences of various operated subgroups with respect to control ones in particular categories.

**DISCUSSION**

Lesions in the structures belonging to the dorsal prefrontal system differentially affected emotional reactivity of the rats. The smallest effect was observed in the subgroup with NC damage that differed from the controls only on the first day of testing, in contrast to other lesioned subgroups in which changes were greatly prolonged. The most profound effect was observed in the FM operated subgroup, while those observed in both hippocampal subgroups appeared to be considerably smaller. Our result differs from the findings of Nonneman et al. (26) who reported higher emotional reactivity in hippocampal rats than in rats with anteromedial cortical lesions. This discrepancy is probably due to the different extent of hippocampal damage. Nonneman et al. (26) applying an aspiration technique, destroyed large area of the hippocampus (from 50% to 85%), encroaching also on neighbouring tissue, while our lesions, made by electrocoagulation, were small and localized strictly in the hippocampal region.

Changes in particular categories of testing depended on the type of lesions, indicating differences in the character of emotional disturbances following damage of various structures. Comparing the two hippocampal groups, the main difference concerned defecation/urination scores which were much higher in VH operated rats than in the control rats, while these measures did not differ from the controls in the DH group. On the other hand, the operated DH group but not the VH group, showed stronger resistance to removal from the home cage than did control subjects. Operated rats from the FM group showed a pattern of changes similar to the DH group, regarding the reactions to removal from the home cage, handling and startle reactions to back tapping, and to the VH group regarding handling, defecation/urination scores and startle reactions. Again, our results are not consistent with those of Nonneman et al. (26). According to these authors, rats with frontomedial cortical lesions differed significantly from controls only in vocalization and defecation/urination. In our experiment almost all categories were affected, while changes in vocalization were not observed. Several factors might be responsible for this discrepancy. One concerns procedural differences. For instance, Nonneman et al. (26) removed rats from the home cage by the tail, while our rats were taken in hand by the experimenter. It seems likely, that the degree of stressfulness of manipula-
tions may influence the differences in reactivity between the normal and operated rats. It can be inferred from the data of Nonneman et al. that their hippocampal rats differed from the controls on responses to pencil presentation. Our experiment performed on a larger number of subjects showed that hippocampal rats, as well as the rats with other lesions and the controls, ignored the pencil unless they were incidentally touched on the nose. Accordingly, due to possible divergencies in procedure, King's emotionality test provided data which are hardly comparable between experiments from various laboratories. Furthermore, the type of statistical analysis employed is also important in comparing results. Nonneman et al. (26) and Kolb (16) applied Mann-Whitney U and Wilcoxon tests, which are not sensitive enough for evaluation of this kind of data, so that some information could be lost. The analyses of variance used in the present experiment seem to be a more appropriate tool.

The most striking result of the present study is that ablation of the anteromedial part of the neostriatum do not replicate the effects of frontomedial cortical ablation with respect to emotional reactivity. It cannot be said that the NC rats were indifferent to various manipulations during testing. Rather, they manifested the emotional reactivity in a different way than did the FM rats. The NC rats exclusively demonstrated vocal reactions and this was the only category (apart from pencil presentation) in which the FM rats did not differ from controls. Since the present neostriatal lesions almost certainly interrupted the fibers of the corticospinal tract it might be supposed that NC rats cannot express their emotionality in the way involving motor elements like struggling and resisting to handling. However, if this was the case the NC rats should show in some categories significantly lower scores than controls, but they did not. Furthermore, the supposition of limited motor expression of emotional state by NC rats seems to be untenable in view of our experiment on open field activity (in preparation) revealing higher motility in the NC group than in other operated groups. On the other hand, the concept of distinct effects of the FM and NC lesions on emotional reactivity is supported by finding that in the NC group the difference between operated and control rats disappeared by the second day of testing while in the FM group the subgroup differences lasted throughout the entire testing period. Functional affinity between the frontomedial region of the prefrontal cortex and the anatomically related anteromedial part of the neostriatum in rats was described in several papers (5, 7, 10, 12, 28, 32). According to our knowledge, emotional reactivity is up to this time the only measure dissociating functions of these structures.
Summing up, the comparisons of the magnitude of overall emotional reactivity and the pattern of changes suggest a functional affinity of the frontomedial cortex to the hippocampus. This affinity appears to be stronger in the posteroventral than in the anterodorsal region of the hippocampal formation. The negligible effect of lesions in the antero-medial neostriatum points to a dissociation between this structure and the frontomedial cortex, as well as the hippocampus, in the function of emotional state regulation.

We are greatly indebted to Dr. P. Jastreboff for suggestions on the statistical evaluation of experimental data. This investigation was supported by Project 10.4.1.01.2 of the Polish Academy of Sciences.

REFERENCES


Accepted 25 April 1980

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