POST-STRESS ANALGESIA AFTER LESIONS TO THE CENTRAL NUCLEUS OF THE AMYGDALA IN RATS

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Abstract. In two experiments the role of the central nucleus of the amygdala under two modes of foot-shock analgesia was studied in 39 male Möll-Wistar rats. In Experiment I a 4 min continuous foot-shock, dependent on neural mechanism was used as a stressor. Analgesia was produced by regularly intermitted 20 min of foot-shock action in Experiment II which evoke an opioid, humorally mediated mechanism. The results suggested that the central nucleus is involved only in the humorale regulation of the opioid form of analgesia. This finding fits well with the concept of limbic control of the anterior pituitary and the pituitary-adrenocortical axis, and points to the role of the amygdaloid complex in processing of stressful stimuli.

INTRODUCTION

The participation of the amygdala in the control of the pituitary-adrenocortical axis (1, 2) may indicate that this brain area is involved in the regulation of an organism's reactivity to stressful stimuli. Several data point to a major role of the central nucleus of the amygdala in this process. First, due to attenuation of pain- and fear-evoked responses seen after destruction of the central nucleus (9, 24), this structure is supposed to modulate an animal's responsiveness to environmental stressors. Next, the abundance of enkephalin-containing neurons (21) may predict the importance of the central nucleus for stress induced anal-
gesia. Finally, this last presumption is corroborated by a high concentration of neurotensin-containing cells and fibers within the central nucleus (10), in relation to the role of neurotensin in the antinociception (13), and in the control of the anterior pituitary (16).

Exposure to a variety of noxious stimuli evokes fear and stress states which produce an analgesic response evidenced by decreased responsiveness to pain. This stress induced analgesia is not a homogenous phenomenon, and its different forms may depend on various neural and/or humoral backgrounds (4, and 22 for a review). Even a single stressor can produce different forms of analgesia depending on its temporal properties and pattern of application (2, 14, 22).

The purpose of the present study was to determine whether the central nucleus of the amygdala plays a role in two forms of foot-shock analgesia: one depending on a nonopioid neural mechanism (14), and the other on an opioid, humorally mediated mechanism (4, 14, 15).

METHODS

Experiment I

Nineteen adult male Möll-Wistar rats weighing 300-350 g at the beginning of the experiment were used. They were housed 9 or 10 per cage with free access to food and water throughout the experiment. Before testing the rats were assigned randomly to two groups. In the lesioned group (10 rats) electrolytic bilateral lesions of the central nucleus of the amygdala were produced under nembutal anesthesia (50 mg/kg). The animal’s head was immobilized in a stereotaxic instrument with the incisor bar elevated 5 mm above the ear bars. A tungsten 0.6 mm diam. electrode insulated except for 0.5 mm at the well sharpened tip was lowered through a hole trephined in the skull according to the coordinates established earlier (24): 1 mm behind bregma, 4 mm lateral to the midline, and 8.1 mm down from the top of skull at bregma. A 1.5 mA anodal current was passed for 15 s, an indifferent electrode placed on cut head skin. In the sham-lesioned control group (9 rats) the skin was opened and a trepanization was performed, but no electrode was insert.

After 10 days of recovery period, pain sensitivity was measured by two tests: the hot-plate and the tail-flick. In the first one the rats were placed on a cooper plate heated by water of 56°C, and the latency to lick a hind paw was recorded. The maximum trial length permitted was 60 s. After a 5 min period of rest, the subjects were given to the second test. The radiant heat produced by a 100 W bulb placed in the focus of
Fig. 1. Reconstruction of lesions through areas of maximum tissue destruction in individual subjects of groups CA-4 and XA-4 (Experiment I), and groups CA-20 and XA-20 (Experiment II). Dark-shaded areas mark injuries in the right hemisphere, solid line encircled areas in the left hemisphere.

A parabolic mirror was concentrated on the dorsal surface of the rat's tail. The latencies to flick the tail in response to heating were measured in two trials with maximum permitted time 7 s, separated by a 3 min period of rest. The mean of the two measurements was taken as the
tail-flick latency. Prior to the experiment, the heat intensity was adjusted to produce a tail-flick response with a latency of about 3 s from three naive Möll-Wistar rats.

Immediately after measuring basal pain sensitivity, subjects were placed in a cage (15×18×29 cm) equipped with a grid-floor that was continuously electrified for 4 min period with scrambled 2.5 mA current. Following foot-shock exposure, post-shock pain sensitivity was retested in the hot-plate and tail-flick tests.

After behavioral testing, subjects were sacrificed and the brains removed for histological verification of lesions (Fig. 1). In half of the subjects the central nucleus of the amygdala was bilaterally and symmetrically destroyed. No injuries in other structures were seen. However in the remaining five rats, the lesions were not quite symmetrical. The central nucleus of the amygdala was destroyed on only one side, and in the alternate hemisphere several areas of injury involved in different subjects the: globus pallidus, nucleus lateralis and baso-lateralis of the amygdala or small ventral part of the putamen. Therefore, the lesioned subjects were divided into two groups: CA-4 with correct bilateral lesions of the central nucleus, and XA-4 with asymmetrical and combined injuries. The results of Experiment I were finally analyzed in three groups: the control sham-lesioned, group N-4 (N = 9), and two lesioned CA-4 (N = 5) and XA-4 (N = 5).

Experiment II

Twenty adult male Möll-Wistar rats from the same colony and of the same weight as in Experiment I were used. The animals from the same experimental group were kept ten in a single home-cage containing food and water ad libitum. The rats were assigned to two groups: sham-lesioned, control (10 rats) and lesioned (10 rats). In both groups there were the same surgeries and experimental procedure as described in Experiment I. The type of stressor was the only difference between the Experiments I and II. The grid-shock of 2.5 mA was given in 1 s pulses every 4 s (regularly intermitted shock) for 20 min.

The histological verification of lesions (Fig. 1) also showed, exclusively bilateral and symmetrical injuries of the central nucleus of the amygdala in five of the lesioned subjects. However, in the other five subjects the lesions were asymmetrical. The central nucleus was unilaterally destroyed, but in the second hemisphere other injuries were found in different subjects in the: capsula externa, nucleus lateralis and baso-lateralis of the amygdala, the claustrum, or small fragment of putamen. Accordingly, the results of Experiment II were also analyzed in three groups: N-20 sham lesioned control (N = 10), CA-20 with strictly
bilateral and symmetrical lesions of the central nucleus ($N = 5$), and XA-20 with asymmetrical and combined injuries ($N = 5$).

In both experiments the hot-plate and the tail-flick latencies were converted into percentage values ($p$), assuming cut-off latencies of 60 s and 7 s respectively as 100%. For statistical analysis, latency scores were transformed to arcsin values according to the formula $-\arcsin\sqrt{p}$.

**RESULTS**

**Experiment 1**

The results of the hot-plate and the tail-flick tests, obtained before and after 4 min of continuous foot-shock action are presented in Fig. 2. In all groups interpolated the foot-shock exposure produced a prolongation of hind paw lick latencies during the hot-plate retest. The transformed data were subjected to a two-way analysis of variance (ANOVA) with one repeated factor (pre- vs. post-shock, time effect), and one between-group independent variable (symmetrical vs asymmetrical vs sham, lesion effect). Neither the lesion effect nor lesions-time interaction proved significant. Only a highly significant time effect was revealed ($F_{1/15} =$ $\ldots$
However, the post foot-shock analgesic effect seemed to be stronger in the sham-lesioned rats. Only one sham subject showed a very small post-shock effect, while latencies of the other eight animals were either just slightly shorter than the cut-off limit of 60 s or responses were not seen at all. Generally, post foot-shock analgesia was somewhat weaker in the lesioned groups. Two subjects, R-7 and R-10 from Group XA-4, reacted with almost the same latencies before and after foot-shock action. Both of these subjects had unilateral lesions involving lateral and baso-lateral nuclei of the amygdala, part of the globus pallidus and the nucleus caudatus, in addition to unilateral central nucleus injury.

Clear post-shock prolongation of the tail-flick response latencies was also seen in all groups. The ANOVA did not reveal any lesion effect or lesions-time interaction. The time effect alone reached high significance \((F_{1/8} = 68.94, P < 0.001)\). Moreover, a tendency similar to that observed in the hot-plate test was seen when analyzing individual data. The weakest post-stress analgesia appeared in the same two subjects: R-7 and R-10 from Group XA-4.

**Experiment II**

The major results of this experiment are shown in Fig. 3. Regularly intermittent 20 min foot-shock produced prolongation of hot-plate response latencies, only in the sham subjects, with was reflected in the ANOVA

![Fig. 3. Mean scores of the hot-plate and the tail-flick response latencies (percentage values) during pre- and post-shock periods in Group N-20 (broken line), Group CA-20 (solid line), and Group XA-20 (thin line).](image)
of transformed latencies of hind paw licking. A highly significant time effect \( F_{1/17} = 26.51, P < 0.001 \) and a lesion effect \( F_{2/17} = 6.81, P = 0.007 \) were shown. Duncan Tests revealed that post-shock response latencies in control group were significantly longer than those observed in Group CA-20 \( (P < 0.01) \) and in Group XA-20 \( (P < 0.05) \) but there was no difference between two lesioned groups. The interpretation of the sham lesioned subjects being the only group showing the analgesic effect of stress was confirmed by a significant time-lesions interaction effect \( F_{2/17} = 7.58, P = 0.005 \).

The figure also presents percentage values of the tail-flick response latencies. Due to generally high variability of the scores in this test, neither the lesion effect nor the lesion-time interaction effect emerged. The only significant was the time effect \( F_{1/17} = 23.38, P < 0.001 \), indicating post-stress analgesia in the tail-flick test in all groups. However, four of the ten sham control subjects showed weak or even no post-shock analgesic effect. On the other hand, only in symmetrically lesioned subject R-22 from Group CA-20, and asymmetrically lesioned subject R-27 from Group XA-20, were no post-shock reactions observed. This result have occured because of exceptionally small central nucleus injuries in these subjects. Inspite of the fact that there were no systematic post-shock observations of emotional behavior beside those related to analgesimetric tests, there was a general impression of smaller fear responsiveness of the animals in this experiment in comparison to the first one.

**DISCUSSION**

Our results show that bilateral lesion in the central nucleus of the amygdala does not influence basal pain sensitivity, but appears to block the post-stress analgesia, when long-lasting, 20 min of intermittent shock was used as a stressor (Experiment II). In spite of nonsignificant lesion effect when short-lasting, 4 min of continous foot-shock was used (Experiment I), two rats from the group with asymmetrical lesions (Group XA-4) did show a relatively weak analgesic effect. This finding suggests that the lateral part of the amygdala plays some role in the processing of short-lasting stressor stimuli, since unilateral lesions were located mostly in the nucleus lateralis of these subjects. The other lesioned structures, including the nucleus centralis, are not involved in that mechanism. In the hot-plate test of Experiment II the analgesic effect was clearly observed only in sham-lesioned rats. In the tail-flick test, the majority of subjects with unilateral and bilateral central nucleus injuries were not analgetized by long, regularly intermitted shock action. Probably, the lack of group differences was mainly due to within-group
variability of the scores in that test. Relatively high within-group variability is rather a common phenomenon in both the tail-flick, and the hot-plate tests. Nevertheless, it seemed that the larger the destruction of the central nucleus, the weaker was the post-stress analgesia.

Behavioral changes produced by any lesion depend not only on the nuclear damage but also on interruption of many fiber systems passing through the damaged area. The central nucleus is not homogenous in terms of its neurochemical organization and projections to and from other brain structures. However, there is evidence (8, 9, 23) that behavioral changes caused by lesions of this structure differ from the effects of injuries to the surrounding area. The results obtained in the groups with asymmetrical lesions are definitively insufficient to evaluate the role of other amygdalar nuclei in the post-stress analgesia.

There are two possibilities to explain why nucleus centralis injury blocked analgesia only after prolonged and intermitted shock. It is well known, that lesions of this structure decrease fear level (24) and the motivational value of stressful stimuli (25). Moreover, it has been demonstrated that the behavior elicited by long-lasting and/or repeated exposure of such stimuli is modulated by the pituitary-adrenal system (11, 19). Humoral factors of this system are involved in the analgesic effects provoked by prolonged and intermittent shock action (14). There is no direct evidence that the central nucleus lesions disturb or attenuate activity of pituitary-adrenal system, however, the lack of analgesic effect in lesioned subjects from Experiment II, might be a result of decreased activity of humoral factors. Since these factors do not play an important role in post-stress analgesia produced by short-lasting shock (14, 22), the effect of the central nucleus lesions could not be observed when the 4 min continuous shock was employed in Experiment I.

The second possible explanation of our results may be related to differences in behavioral consequences of exposure to continuous and/or regularly intermitted shocks. We know that inescapable (7, 20, 23) and unpredictable stimuli (3, 6) produce more fear than do escapable and predictable stimuli. Moreover, inescapable and unpredictable pain result in “learned helplessness” (17, 18) and analgesia (12). In the situation when several shocks are repetitively and regularly applied, the amount of fear produced by such a procedure might be reduced, since the next shock pulse is able to be predicted from the previous one (3). Such a situation existed in Experiment II in which poststress observations indicated weaker defensive reactions and relatively weaker analgesic effect in comparison to Experiment I. Thus, the situation in the first experiment seemed to be — more, and in the second — less “learned helplessness” provoking. Moreover, since nucleus centralis injury decreased
the level of fear and animal's capacity to evaluate the emotional significance of stimuli (25), they might reduce the emotional properties of regularly repeated action to a level such that blocking of post-stress analgesia appeared. This interpretation fits well with Bolles and Fanselow (5) concept of an activating role of fear in analgesic mechanisms.

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