Post-stress analgesia in rats with partial amygdala lesions

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Abstract. . The role of the dorsal basal and the central nuclei of the
amygdala under two modes of foot-shock analgesia was studied in 45
Möll-Wistar rats. Four minutes of continuous foot-shock produced
post-stress analgesia in control and in all lesioned rats, but 20 min of
regularly intermittent foot-shock did not evoke analgesia in rats with
central nucleus lesion. This result indicates different involvement of
dorsal basal and central nuclei in processing of stressful stimuli.

Key words: amygdala, analgesia, stress, rats

INTRODUCTION

We have recently shown (Werka and Marek 1990) that the central nucleus of the amygdala is involved in the hormonal regulation of the opioid form of analgesia. This finding fits well with our hypothesis (Werka and Zieliński 1992) that the dorsomedial group of nuclei evaluates the emotional value of unconditioned stimuli, and also other cues that occur in temporal proximity to the reinforcers, by augmenting pituitary-adrenocortical responses to their occurrence. The basolateral part of the amygdala seems to be significant in the analysis and discrimination of stimuli that acquire only conditioned properties. Some authors showed that this complex is a main sensory interface of the amygdala (LeDoux et al. 1990), but its involvement in the regulation of an organism's reactivity to painful and stressful stimuli is still unknown. Indeed, there are some data suggesting that the dorsal basal nucleus, which belongs to the basolateral part of the amygdala, plays a role in pain reactivity depending on humoral (Coover et al. 1973), or neural mechanisms (Werka and Marek 1990). However, the lack of precise locations of lesions in those studies did not allow a definitive conclusion.

There were two purposes of the present study. The first was to determine the significance of dorsal basal nucleus in two forms of foot-shock analgesia. It has been demonstrated that the behavior elicited by short-lasting continuous foot-shock is dependent on nonopioid neural mechanisms (Lewis et al. 1980), but the behaviour evoked by long-lasting and/or repeated shock action is related to opioid mechanisms, and modulated by the pituitary-adrenal system (Lewis et al.1980, Mclennan et al. 1982). The second purpose was to reexamine the effect of central nucleus lesions and to compare them with the effect of dorsal basal injury. We supposed that such a comparison is important to understand the functional organization within the amygdala complex and to reconsider relevant hypotheses.

METHOD

Subjects and group treatment

Forty five 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 rats were assigned to three groups, a control group (Group N, 16 rats) and two experimental groups with different amygdalar lesions. In Group BL (18 rats) the dorsal basal nucleus, and in Group CE (11 rats) the central nucleus were bilaterally destroyed.

Surgery

Surgical procedures were done under chlorohydrate (360 mg/kg) anaesthesia. Electrolytic lesions were produced by passing 2.0 mA of anodal current for 15 s in Group BL, and 1.5 mA for 15 s in Group CE. The electrode was 0.4 mm diam. tungsten wire insulated except for 0.4 mm of its well-sharpened tip. The stereotaxic coordinates for Group BL were: 1 mm behind bregma, 4.8 mm lateral to the midline, and 8.8 mm down from the top of the skull at bregma. For Group CE the electrode was inserted 1 mm behind bregma, 4.4 mm lateral to the midline and 8.1 mm down from the top of the skull. In Group N the skin was opened and a trepanization was performed but no electrode inserted.

Apparatus and procedure

After a 10 day recovery period, pain sensitivity was measured by two tests: the hot-plate and the tail-flick. In former, 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 5 min rest period, the rats were subjected to the tail-flick test performed with use of the Letica S.A LI 7106 light beam analgesimeter. The heat produced by a halogen light (12 V DC, 100 W) placed in a moveable lamp was concentrated on the dorsal surface of the

rat's tail. The animal was put in a restrainer that left its tail completely free, but in such a way that it remained lying between the two adjustable sections of the experimental unit base. The latencies to flick the tail in response to heat were measured in two trials with maximum permitted time of 7 s, separated by a 3 min period of rest. The mean of the two measurements was used in further analysis. Preliminary study using an additional seven naive Möll-Wistar rats allowed adjustment of the heat intensity to produce a tail-flick response with a latency of about 3 s.

Immediately after measuring basal pain sensitivity, subjects were placed in a cage (15 x 18 x 29 cm) equipped with a grid-floor that was electrified by two types of foot-shock stressors. A continuous 4 min foot-shock with scrambled 2.5 mA current was the stressor in 8 rats from Group N, 9 from Group BL and 6 rats from Group CE. For other subjects the stressor was the foot-shock of 2.5 mA given in 1 s pulses every 4 s (intermittent shock) for 20 min in 8 rats for Group N, 9 rats from Group BL and 5 rats from Group CE. Immediately after foot-shock exposure the post-shock pain sensitivity was retested in the hot-plate and tail-flick tests.

The latencies of responses in both tests were converted into percentage values, assuming cut-off latencies of 60 s and 7 s respectively as 100%. For statistical analysis, percentage latency scores were transformed to arcsin values.

Histology

After behavioural testing subjects were sacrificed and the brains removed for histological verification of lesions (Fig. 1). The lesions in Group BL symmetrically affected mainly the dorsal basal nucleus, a ventral fragment of the lateral nucleus and/or a lateral fragment of the ventral basal nucleus of the amygdala. In 8 rats there was some invasion to the capsula extrema region at least unilaterally. Group CE had bilateral symmetrical lesions of two third of the central nucleus. In some rats injury unilaterally involved a small ventral part of the putamen, the globus pallidus, stria terminalis or a small fragment of the lateral nucleus of the amygdala.
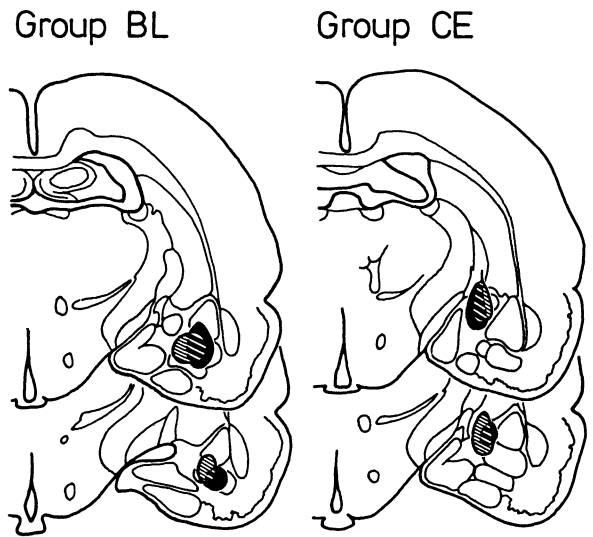
Group BL   Group CE

Fig. 1. Examples of typical lesions through areas of maximum tissue destruction in Group BL and CE. Hatched areas, mark injuries in the right hemisphere; dark areas, mark injuries in the left hemisphere.

RESULTS

The results of the hot-plate and the tail-flick tests, obtained before and after 4 min of continuous, or 20 min of intermitted shocks are presented in Fig. 2.

In all groups, the continuous 4 min foot-shock exposure produced marked prolongation of response latencies in both tests (see Fig. 2, upper part). More than half of the rats neither licked a hind paw nor flicked the tail in response to painful stimuli during retests, indicating strong post-stress analgesia. In spite of the fact that there were no systematic post-shock observations of emotional behavior beside those related to analgesimetric tests, animals in the hot-plate test showed some symptoms of increased activity. In all groups, general motility increased after continuous foot-shock stimulation.

Marked group differences of reactions were observed in both tests after 20 min of regularly intermitted shock exposition (Fig. 2, lower part). Two 2 x 2 x 3 ANOVA's, independently performed for each test, were used. The latencies expressed in percentages and submitted to arcsin transformation
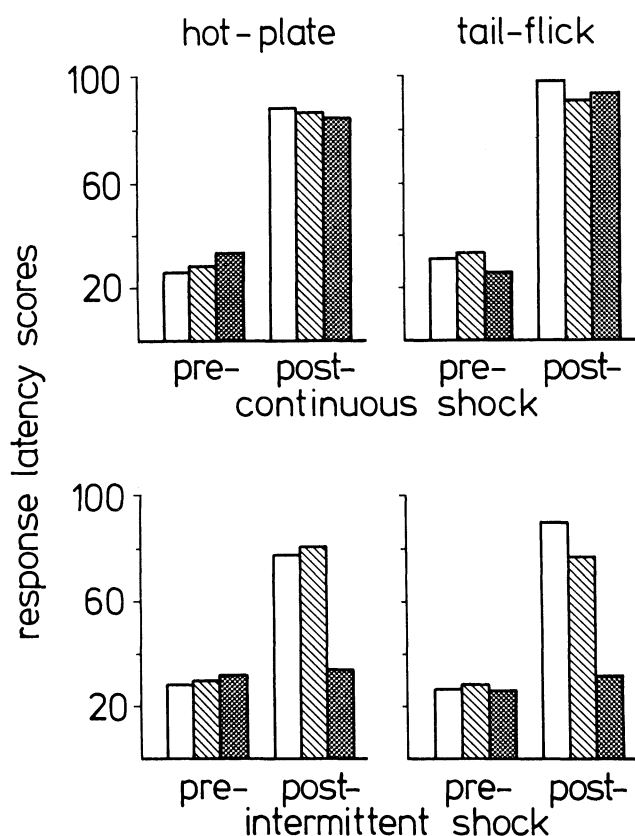
Fig. 2. Mean percentage values of the hot-plate and the tail-flick response latencies during pre- and post-continuous foot-shock expose, or during pre- and post-intermittent foot-shock period in Groups: N (open bars), BL (hatched bars), and CE (dark bars).

were analyzed with one repeated factor, pre- vs. post-shock period (trial effect), and two independent variables: 4 min of continuous vs. 20 min of intermittent shock (stressor effect) and Group N vs. Group BL vs. Group CE (group effect). Both ANOVA tests revealed highly significant effects of all factors and their interactions. Only the triple interaction in hot-plate response latencies did not reach significance (Table I). Obviously, only in Groups N and BL did the 20 min of intermittent foot-shock produce prolongation of response latencies in both tests. The results of the ANOVAs indicated that only in rats with nucleus centralis lesions of the amygdala did the post-stress response latency prolongation not appear. The effect was caused by discontinuous shock exclusively. In Group CE two rats in hot-plate and the three others in tail-flick re-

tests revealed the same or even shorter response latencies, indicating a total decrease of post-stress analgesia.

However, post-shock analgesic effect observed after intermittent shock seemed to be weaker in comparison to the 4 min continuous foot-shock exposition. Only one third of rats did not react in both retests. There was also a general impression of less fear responsiveness in animals subjected to the regularly intermittent foot-shock.

DISCUSSION

The results showed that bilateral lesion in the central and/or dorsal basal nuclei of the amygdala did not influence rats' basal pain sensitivity. In all subjects, there was also no change of post-stress analgesic reactivity, when continuous 4 min foot-shock was used as a stressor. Since the behaviour elicited by this type of stressor depends on nonopioid neuronal circuits (Lewis et al. 1980), it seems obvious that neither the basolateral nor central parts of the structure are involved in the regulation of these mechanisms. In our earlier study (Werka and Marek 1990), a weak analgesic effect was seen in a few rats with injury to the baso-lateral part of the amygdala. However, these animals had unilateral and asymmetrical lesions located mostly in the lateral nucleus and ventral putamen.

We find that the results of post-stress analgesia in rats with the central nucleus injury, observed after intermittent shock action in this experiment, are very similar to findings obtained earlier (Werka and Marek 1990). Both experiments used almost the same procedures, and the same lesion were made. The effect of the central nucleus injury has already been extensively discussed (Werka and Marek 1990). Much the same as before, we suppose that the dorsomedial group of nuclei analyzes and evaluates the emotional value of unconditioned stressful stimuli and other cues that occur in temporal proximity to unconditioned stimuli. The mechanism involves activation of the pituitary-adrenocortical system that modulates the level of fear (Coover et al. 1973, Smith 1973, Hennesy and King 1977) and pain sensitivity (Bolles and Fanselow 1980).

TABLE I

ANOVA results of transformed percentage latency scores in all groups and tests

Source of variation	df	Hot-plate test		Tail-flick test	
		F	P	F	P
Trial effect (A)	1/39	286.31	<0.001	487.25	<0.001
Stressor effect (B)	1/39	10.65	<0.005	24.82	<0.001
Group effect (C)	2/39	4.30	<0.02	10.52	<0.001
Interaction (AB)	1/39	11.76	<0.002	27.88	<0.001
Interaction (AC)	2/39	5.45	<0.01	8.92	<0.001
Interaction (BC)	2/39	6.82	<0.005	7.20	<0.005
Interaction (ABC)	2/39	2.92	NS	9.57	<0.001

However, in control rats and in subjects with dorsal basal nucleus lesion, intermittent shock action evoked post-stress analgesia. It is well known that pain sensitivity elicited by such a stressor is dependent on the pituitary-adrenal system (Lewis et al. 1980, McLennan et al. 1982). There is no clear and direct evidence that the dorsal basal nucleus lesions affect any humoral activity. Allen and Allen (1975) showed that the ventral amygdalofugal pathway (VAF) between the amygdala and the hypothalamus was critically involved in the ACTH response to stressful stimuli. However, most of VAF fibres which originates in central nucleus of the amygdala pass through the basolateral complex (de Olmos 1972), so that observed changes of humoral activity might not depend on fiber interruption but rather on nucleus centralis disfunction. Our results suggests no direct involvement of dorsal basal nucleus in the adreno-pituitary system.

All the amygdala nuclei contribute to stimulus processing mechanisms (Aggleton and Mishkin 1986, LeDoux et al. 1990). Specifically, the central nucleus and the baso-lateral part of the amygdala are involved in processing two aspects of the stimuli. One is related to the emotional and motivational function of a signal, the other is acquired in the course of learning and related to discrimination processes of different modalities. However, since the first aspect of stimuli is mostly processed by nucleus centralis, or even the entire dorsomedial part of the amygdala, the second is analyzed by the basolateral group of nuclei (Werka and Zieliński 1992). In other words, dorsal basal nucleus belonging to the basolateral complex processes all cues that acquire conditional properties, but no unconditioned stimuli. Therefore it seems to be not surprising, that lesions of the structure neither change nonopioid neural nor opioid humoral form of analgesia.

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