AMYGDALAR INVOLVEMENT IN PAIN

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Abstract: The limbic system has been implicated in the modulation of pain. The aim of this study was to determine the role of amygdala in different types of pain, viz., phasic and tonic. Unilateral stimulation of central nucleus of amygdala (CeA), basolateral nucleus (BL) and medial amygdaloid (MeA) in conscious rats resulted in the reduction of the tonic formalin-induced pain. The thresholds for simple vocalization (SV) and vocalization after-discharge (VA) were elevated during amygdalar stimulation in the tail-flick (phasic pain) test. However, the threshold for tail-flick (TF) evoked by electric shock was not affected. Tail-flick latency (TFL) to noxious heat was accentuated during amygdalar stimulation. These results suggest that amygdala has a modulatory role in the descending endogenous pain control mechanisms.

Key words: amygdalar stimulation   formalin test
              phasic pain   tonic pain   analgesia
              simple vocalization   vocalization after-discharge

INTRODUCTION

Recent anatomical and electrophysiological evidence suggests that nociceptive impulses from the dorsal horn lamina I, pass through the spino (trigemino)-pontoamygdaloid pathway via the parabrachial area to the nucleus centralis amygdala (1-9). Single neuronal activity from the central nucleus of the amygdala has been shown to be inhibited as well as facilitated in response to peripheral noxious stimuli (10). A physiological role for these somesthetic noxious stimuli projecting to the amygdala has been postulated in the affective-emotional (fear, memory of aggression), behavioral (vocalization, flight, freezing, defense, offense), and autonomic, adrenocortical and micturition reactions (2, 10). Besides the opioids, a cholinergic mechanism has also been suggested in the modulation of tail-flick by the cortical and medial nuclei of the amygdala while the basolateral nucleus exerts a milder effect (11). The central and corticomedial nuclei of the amygdala have been implicated in the modulation of pain by microinfusion technique whereas the lateral nucleus is not effective in pain modulation. The nociceptive responses evoked by flinch and thermal stimuli were modulated by microinfusion of morphine (10-20 μg) and the enkephalinase inhibitor (SCH-32615) locally in the central and corticomedial nuclei respectively (12, 13). Recently, Kowada et al (14) have shown that stimulation of central nucleus of the amygdala produced inhibition of the nociceptive jaw opening reflex in cats. Other limbic structures have been implicated in the endogenous pain modulatory mechanisms. The amygdalar lesions (electrolytic) did not affect the reaction to subcutaneous injection of formalin but eliminated both the defensive freezing behavior and the hypoalgesia (15). Stimulation of other limbic structures viz., lateral septum and the dorsal hippocampus induced antinociception in association with epileptiform after-discharges, whereas in the amygdalar stimulated rats such an association was not found (16). The role of the amygdala in the behavioro-emotional component and the endogenous nociceptive

mechanisms has not been studied in detail in the unanesthetized animals. The present study was therefore addressed to the amygdalar influence on different types of pain viz., tonic, phasic and their affective components.

METHODS

Experimental animals :

Adult male albino rats (n=19) weighing 225-300 g were used in the present study. They were maintained under 14 h : 10 h light and dark conditions and were given food and water *ad libitum*. Each animal was housed individually in a polypropylene cage. Effects of amygdalar stimulation on pain were quantified using the formalin and tail-flick tests. Each animal served as its own control. In a few cases, both the tests were used on the same rat with an interval of one week.

Surgery :

Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Concentric bipolar steel electrodes of 22G (guide) and 26G stimulating electrodes were prepared from hypodermic needles and stainless steel wires respectively. The stimulating electrode was 1 mm below the guide. The tips (0.5 mm) of electrodes were uninsulated. The electrodes were stereotaxically implanted into the amygdalar nuclei (Central nucleus - posterior to the bregma (AP) = —1.8; lateral to the midline (ML) = 3.5; ventral to the dura (DV) = 8.0; Basolateral nucleus AP = —2.8; ML = 4.5; DV = 8.5) according to the rat brain atlas by Paxinos and Watson (17). The anchoring screws were driven into the skull and the electrode assembly was fixed to the skull with dental cement. After 4-5 postoperative days, different pain tests according to the ethical guidelines (18) were performed on these rats.

Observation chamber :

For the formalin test an observation chamber, made of clear plexiglass (30 cm x 25 cm x 20 cm) with 10 small 0.5 cm diameter holes on top of the box for ventilation and passage of stimulating cables, was used. Under the floor, a large mirror was placed at a 45⁰ angle for observation of the animal (19).

Restrainer :

A rectangular clear plexiglass tube (22 cm x 6 cm x 5.5 cm) was used for restraining rats during brain stimulation in the tail-flick test. Ten small holes (dia. 4 mm) were made on the front wall for ventilation. The rear wall had a small semicircular slit for the protruding tail. A large oval shaped hole (dia. 2.5 cm) was made on the sliding top of the restrainer for cable connections to the stimulator.

Amygdalar stimulation :

Stimulation of the amygdala was carried out during the formalin and the tail-flick tests. Brain stimulation was delivered in 0.1 sec trains of 60 Hz, biphasic square wave pulses of 200 μsec by a Coulbourn Isolated Physiological Stimulator. Amplitude of the current was monitored on the oscilloscope (Tektronix-5223) by the voltage drop method through a 100 ohm resistor in series with the electrode. Current was given to the brain loci in the range of 50-750 μA. The current strength was increased gradually from 50 μA.

The amygdala was stimulated in a group of five different rats before subjecting them to the pain tests. Stimulation current was gradually increased from 50 μA to 1000 μA in steps of 50 μA and its effect on behavior, specifically freezing, aggressiveness, emotionality, urination, defecation and catalepsy were noted. Amygdalar stimulation at a high current strength of 1000 μA produced only aversive responses such as head and jaw movements.

Experiment 1 :

The effect of amygdala stimulation on tonic pain : Tonic pain was induced and the related behavior was rated (n=9) using the formalin test (19). Each rat was conditioned for 30 min in the observation chamber before formalin treatment. Following conditioning, 5% formalin solution in 50 μl volume was injected subcutaneously into the plantar region of either right or left forepaw. A 4-point scale was used to quantify the behavior in one hour session. The rating method followed was, briefly : Category 0-when the whole body was resting or moving on all the four paws; 1-grooming or the

injected paw was partially resting; 2-the injected paw was elevated or tucked under the body; 3-the treated paw was licked or shaken. Behavioral scores were continuously entered into a pocket computer (SHARP-PC 1402) for 60 min and a weighted average pain score for each 5 min interval was calculated by multiplying the time spent in each category with the rating (20, 21).

Basal pain scoring was continued till it attained control values in order to assess the duration and the rate of decrement of pain. It was observed that pain intensity remained stable for one hour and then began to decrease in the basal pain rating test. Therefore, the effect of amygdalar stimulation on the tonic pain was observed during the initial one hour. Unilateral amygdalar stimulation (100-600 μA) was started immediately after the formalin treatment into the forepaw and continued for 2 min. While the pain rating was being carried out, the amygdala was stimulated for 2 min duration again after 15, 35 and 50 min of formalin injection corresponding to the 3rd, 7th and 10th epochs in one hour of formalin pain scoring (22).

Experiment 2 :

The effect of amygdalar stimulation on phasic pain (tail-flick test) : Phasic pain was induced by noxious heat to the tail of the rat (n=10) and the tail-flick latency (TFL) was measured by tail-flick analgesia monitor (Omnitech, USA). Each rat was conditioned for 30 min in the restrainer before starting the experiment. The rat's tail was cleaned with spirit and placed on the heating coil which was 4 mm below the tail. Noxious heat was applied to the ventral surface of the caudal part of the tail. The cut-off time was set to 30 sec to avoid tissue damage. The response time was frozen on the display when the tail interrupted the infra-red beam. Heat was applied to the tail thrice at intervals of 5 min and the basal TFL was measured by taking mean of these 3 observations (23).

Tail-flick was also elicited by applying electric shock to the tail. Rats were conditioned in the plexiglass restrainer for 30 min before starting the experiment. Two needle electrodes, each 1 cm long were prepared from size 00 insect pins. Electrodes were inserted intradermally, 2 mm in depth, into the middle portion of the tail and a 2 cm distance between the two electrodes was maintained. Both the needle electrodes were fastened with adhesive tape. Rats were conditioned for 30 min following the needle insertion. Noxious electrical stimulus was applied to tail with the following stimulus parameters. Biphasic square wave pulses of 40 Hz, 1.5 ms width and varying current strength (mA) for 200 msec. Stimulus was delivered by the voltage drop method through the Grass S4 stimulator and stimulus Isolation Unit B. Current strength was gradually increased in step-wise fashion at an interval of 5 min. The current at which TF occurred, was taken as the threshold value. Mean value of 3 current threshold values (mA) was determined as the baseline threshold.

To study the amygdalar influence on the phasic pain (TF), mediated at the spinal level, unilateral amygdalar stimulation was set up 30 sec prior to the noxious heat or the electric shock to tail and was continued for 15 sec (24).

Experiment 3 :

The effect of amygdalar stimulation on emotional component of pain : Simple vocalization (SV) and vocalization after-discharge (VA) manifest the emotional components of pain. Thresholds for SV and VA were determined (n=10) by applying electric shock to the tail as described for the tail flick test. Similarly, the thresholds for SV and VA were established (25, 26).

Histology :

At the end of amygdalar stimulation experiments, the site was lesioned electrolytically by passing 1 mA anodal current for 10 sec. Brains were perfused with saline and 10% formalin solution transcardially. Paraffin brain sections were cut to 35-40 μm thickness and stained with eosin-hematoxylin for determining the electrode site.

Statistical analysis :

Effect of amygdalar stimulation on pain rating was analysed using Student's t-test. To establish the effect of amygdalar laterality on

pain rating, one-way analysis of variance (ANOVA) was used. The significance was evaluated at 5% level. The data was pooled since the responses obtained from CeA and BL stimulation were not different except in the case of MeA.

RESULTS

Effect of amygdalar stimulation on tonic pain: Basal average pain rating (2.04±0.1) was scored after 5% formalin was injected subcutaneously in 9 rats. During and after amygdalar stimulation the formalin-induced pain rating was reduced (1.25±0.2) significantly (P<0.001) (Fig. 1 inset). During the first, third, seventh and tenth 5 min epochs when amygdala was stimulated pain scoring was significantly decreased to 1.78±0.7, 1.22±0.3, 1.09±0.2 and 1.21±0.4 respectively from the corresponding basal values of 2.29±0.3, 1.98±0.4, 2.09±0.2 and 2.04 ± 0.1. To study the role of the contralateral amygdala, stimulation was applied either ipsilaterally (n=3) or contralaterally (n=6) with respect to the formalin injection into the forepaw. The side of the stimulated amygdala had no significant differential effect on pain rating
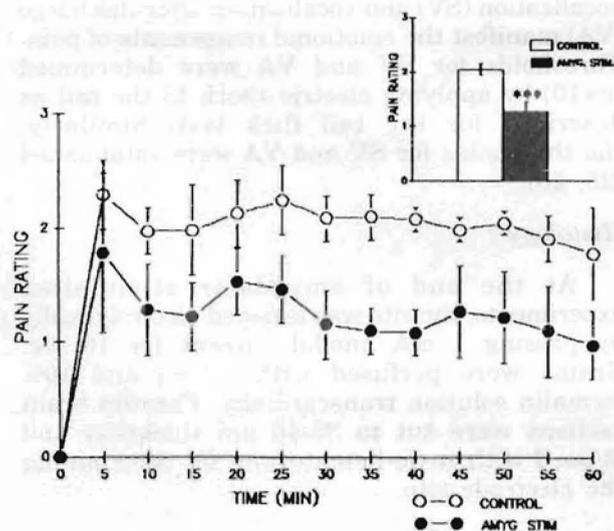
Fig. 1 : Pain rating curves after formalin test before and during stimulation of amygdala. Inset shows the average pain score of 60 min in rats before and during amygdala stimulation (n=9; ***P<0.001).

(1.29±0.2 and 1.24±0.3, respectively with ipsilateral and contralateral sides) (ANOVA, F=0.16, P=0.69) (Fig. 2).
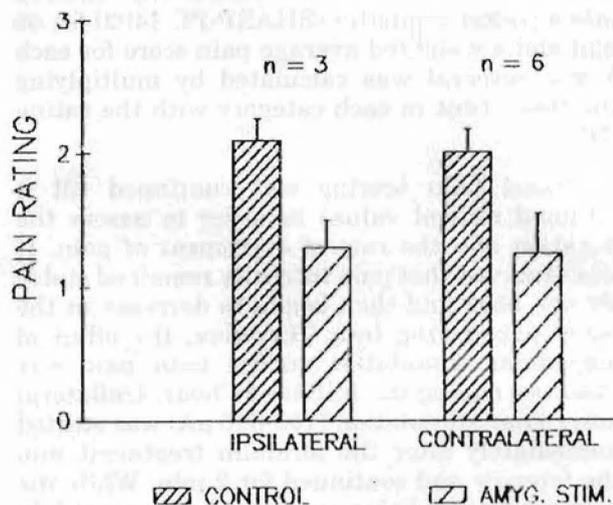
Fig. 2 : Contralateral and ipsilateral amygdalar stimulation have similar modulatory effects on the mean pain rating in the formalin test.

Effect of amygdalar stimulation on phasic pain (tail-flick test) : Tail-flick latency to noxious heat was measured in fifteen rats. Basal TFL (10.61±1.3 sec) enhanced to 17.99±1.9 sec during unilateral amygdalar stimulation. The increase in TFL was statistically highly significant (P<0.001; Table I). The duration of antinociceptive effect of amygdalar stimulation on tail-flick was studied in five rats. The effect lasted for 6-7 min after bilateral amygdalar stimulation (Table II).

Basal threshold level (0.22 ± 0.04 mA) for eliciting TF was not affected (0.25 ± 0.05) during amygdalar stimulation (ANOVA, F=1.97, P=0.17) (Table I).

Effect of amygdalar stimulation on emotional component of pain : Before the amygdala was stimulated, threshold for simple vocalization was 0.46±0.2 mA while for vocalization afterdischarge it was 1.03±0.5 mA (n=10) (Table I). Threshold levels for SV and VA were elevated to 0.94±0.3 and 1.89±0.5 mA respectively during amygdalar stimulation. The increase in threshold

was highly significant statistically (P<0.001) (Table I).

Effects of extra-amygdaloid (internal capsule and globus pallidus) stimulation was studied on thresholds (mA) for TF, SV and VA in rats (n=2). The basal thresholds for eliciting TF, SV and VA (0.57±0.01; 0.74 ± 0.1; 1.1±0.1 mA) were slightly higher than the amygdalar sites (0.22±0.04; 0.46±0.2; 1.03±0.5 mA respectively) (Table I).

TABLE I : Effects of amygdalar and extra-amygdaloid stimulation on the thresholds (mA) for tail-flick (TF), simple vocalization (SV) and vocalization after-discharge (VA).

a) Amygdalar stimulation

Test	Before stimulation	During stimulation
TF (Noxious heat)	10.61±1.3 (sec)	17.99±1.9 (sec)***
TF (Nox. electrical)	0.22±0.04 (mA)	0.25 ± 0.05 (NS)
SV	0.46±0.2	0.94 ± 0.3***
VA	1.03±0.5	1.89 ± 0.5***

b) Extra-amygdaloid stimulation

TF (Nox. electrical)	0.57±0.01 (mA)	0.60±0.1 (NS)
SV	0.74±0.1	0.79±0.07 (NS)
VA	1.10±0.1	1.17±0.03 (NS)

Significant increase in thresholds as compared to control rats (***P<0.001); NS: statistically not significant as compared to basal (before stimulation).

TABLE II : Duration of amygdalar stimulation effect on tail-flick latency (Mean±SD sec).

	Tail-flick latency (mean±SD sec)
Basal	10.88 ± 0.8
During amyg. stim.	19.36 ± 1.9***
4 min after stim.	17.38 ± 3.5**
7 min after stim.	9.70 ± 0.5

n=5; **(P<0.01); ***(P<0.001)

Histological verification of stimulation sites in the brain sections revealed electrode position in the amygdala (central, basolateral and medial nuclei), internal capsule and globus pallidus (Fig. 3).
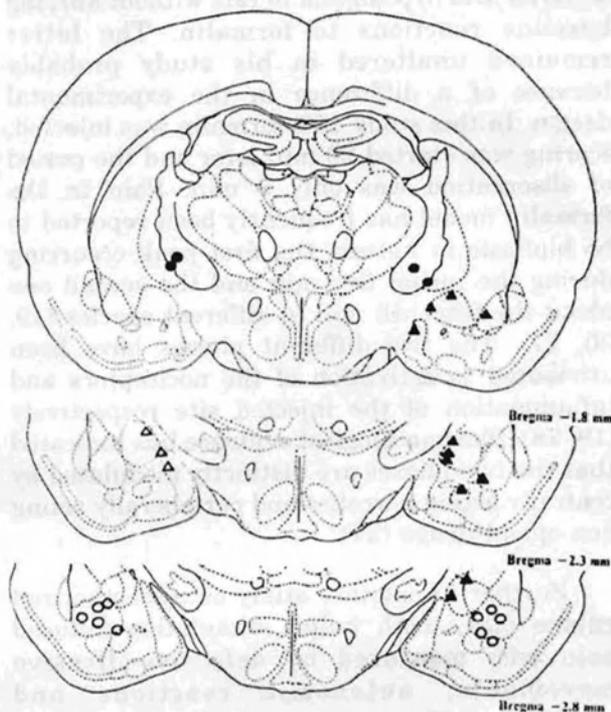

Fig. 3 : A schematic diagram showing electrode sites in coronal sections of the brain. Unilateral (right ▲; left △; n=14) and bilateral (O; n=5) amygdalar, internal capsule and globus pallidus (●; n=2) stimulation.

DISCUSSION

The results suggest that stimulation of the central, basolateral and medial amygdalar nuclei leads to a significant decrement in pain intensity. Pain, tonic or phasic, induced in the present study by formalin injection or noxious heat application respectively was alleviated by amygdalar stimulation. Hypoalgesic effect induced either by ispilateral or contralateral amygdalar stimulation was not significantly different. Amygdalar stimulation also increased the threshold of stimulus for eliciting vocalization and vocalization after-discharge which reflect emotional components of pain.

Stimulation of the amygdala led to hypoalgesia in our rats. Helmstetter (15) produced a lesion of the central or the basolateral nucleus which eliminated both defensive freezing behavior and hypoalgesia in rats without altering baseline reactions to formalin. The latter remained unaltered in his study probably because of a difference in the experimental design. In this study 15% formalin was injected; scoring was started 20 min later and the period of observation was only 8 min. Pain in the formalin model has frequently been reported to be biphasic in nature, the first peak occurring during the initial five min and the second one about the fifteenth min in different species (19, 20, 27). The two different phases have been attributed to activation of the nociceptors and inflammation of the injected site respectively (19, 28). Pharmacological evidence has indicated that the two phases are distinctly modulated by centrally acting narcotics and peripherally acting non-opioid drugs (27).

Further, in another study on anaesthetized guinea pigs, tooth pulp stimulation induced pain was measured by defensive-offensive movements, autonomic reactions and vocalization. These responses suggestive of pain intensity depended on the parameter of stimulation itself beside the electrode site in amygdala (29). With varying frequency of current from 10-100 Hz, painful, sedative or analgesic responses were recorded. High frequency of medial amygdalar stimulation elicited pain sensations and low frequency stimulation of the lateral amygdala evoked analgesic effects (29). Pre-stimulation control observation of pain response to 5% formalin in our rats showed heightened pain response which began soon after formalin injection into the forepaw and lasted for 5 min. Pain score then decreased until the 15th min. It again increased at 20 min (although less than the first peak) and remained so till the end of the first hour. This has also been reported in other species by several other workers (19, 20, 27).

Unilateral amygdalar stimulation produced a discernible inhibition of pain intensity in our rats and the initial peak and the later plateau phase of pain were both attenuated. The stimulation effect lasted for 15 min. As has already been discussed, the initial peak in the pain intensity is attributed to activation of nociceptors and the later plateau phase of it to inflammation of the tissue. They are mediated by two separate neuronal substrates and mediators (19, 27, 30). Our study suggests that amygdalar stimulation alleviates pain of both origins. Ipsilateral amygdalar stimulation has shown more effect than contralateral stimulation although these differences in effects were not statistically significant. Similar effects on pain response to formalin injection were also observed during lateral hypothalamic stimulation in rats (22). Unilateral amygdalar (CeA, BL and MeA) stimulation elevated the thresholds for the elicitation of SV as well as VA. Comparable observations are not available in the literature. However, lesions of bilateral amygdaloid complex in rats have been reported to increase basal thresholds for VA (31). Similarly, Charpentier (32) reported a decrease in nociceptive reactions involving emotions, following bilateral amygdalar lesions. Our results are not in agreement with them presumably because of differences in the technique. In other studies lesions of whole amygdaloid complex were made whereas we stimulated focal areas in the amygdala. It is quite possible that amygdala has a dual role in modulating pain information; it may both inhibit and facilitate through different neuronal groups. Our study supports this view proposed earlier. The medial regions were primarily involved in pain whereas the lateral amygdalar regions in analgesia (29). This division may not physiologically be strictly compartmentalized into medial and lateral parts. Recently, Bernard et al, (10) have reported that neurons in the rostral portion of the lateral capsular subdivision of the central nucleus, the peripheral edge of the lateral subdivision of the central nucleus and the ventral portion of the globus pallidus are activated by peripheral noxious stimuli, whereas neurons in the nucleus centralis are inhibited. Our electrodes led to excitation of areas involved in endogenous analgesic mechanisms.

Stimulation of extra-amygdaloid areas such as internal capsule and globus pallidus (lateral) did not alter the electrical nociceptive thresholds for tail-flick and affective pain components viz., simple vocalization and vocalization after-discharge in our rats. On the contrary the amygdalar stimulation increased the thresholds for elicitation of simple vocalization and vocalization after-discharge but not that of tail-flick response. No comparable studies are available in literature regarding the influence of globus pallidus on these electrical nociceptive responses in conscious rats. However, in anaesthetized rats neuronal responses to heat noxious stimulus has been reported by Bernard et al (10).

These results suggest that the amygdala plays an important role in the modulation of tonic and phasic pain. It has also an effect on different components of pain. In this study, the stimulation of amygdala has no effect on the tail-flick evoked by electric shock, probably, the amygdala may not influence the reflex behavior which is mediated predominantly at the spinal level. It is necessary to reveal the analgesia mechanisms not only with electrical activation of the amygdala neurons but with exogenous neurochemical modulation.

ACKNOWLEDGEMENTS

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