



amphetamine, or deltorphin ( $\delta$  opioid receptor ligand) induced motor activity. Moreover, naloxone (5.0 mg/kg, sc) has recently been reported to decrease the basal motor activity in rats (16).

Opioids are implicated in both the nociception, and spontaneous motor activity which have been reported to be antagonised by naloxone in separate studies with different doses. The pattern and direction of change in nociceptive and motor activity status simultaneously by the same dose and route of administration of naloxone is not clear from the literature. These experiments were therefore, designed to explore the nociceptive, and motor behavioral status simultaneously in naloxone treated rats.

## METHODS

*Animals*: Experiments were conducted on 10 adult male wistar rats weighing 200-250 g. They were housed in separate cages in rat room maintained under 14:10 light-dark period  $28 \pm 2^\circ\text{C}$ . They received rat pellets and tap water *ad libitum*. Pain and behavioral testing were done in accordance with the ethical guidelines formulated by the International Association for the Study of Pain (17).

*Formalin test*: Tonic pain was induced by injecting the formalin and the related behavior was scored in 10 rats (18). Briefly each rat was conditioned for 30 min in the specially designed test chamber or the cage of videopath activity analyser system before formalin injection (19). Following conditioning, 5% formalin solution in 50  $\mu\text{l}$  volume was injected subcutaneously into the plantar region of either the right or the left forepaw. A 4-point scale was used to quantify the behavior in an one-hour session. The behavior was rated as category 0 when the whole body was resting or moving on all the four paws; as 1 when rat was grooming or the injected paw was partially resting; as 2 when the injected paw was elevated or tucked under the body; and as 3 when the treated paw was licked or shaken by the rat. Behavioral scores

were continuously entered into a pocket computer (SHARP-PC 1402) for 60 min and a weighted average pain score for a duration of 5 min each was calculated by multiplying the time spent in each category with the rating (20).

*Motor activity*: Videopath activity analyser system (Coulbourn Instruments, Inc., U.S.A.) was programmed to track the rat's path with a video camera and analyse the different types of behaviors such as total distance travelled, stereotyped movements, corner or wall-hugging behavior and the time spent in each quadrant of the metallic cage (50 x 50 x 35 cm). The behavioral data was obtained for 5 min epochs during one-hour session. The analyser establishes an X-Y coordinate with the camera picture and generates the cursor block to superimpose over the rat's image on the video monitor and logs the data for analysis. Motor behavioral testing was done between 10:00 and 18:00 h. Before starting the experiment each animal was habituated to the test cage for 30 min. Motor behavior and pain score were simultaneously recorded immediately after formalin injection into the forepaw.

*Experiment I*: To study the effect of low dose of naloxone (6  $\mu\text{g}/\text{kg}$ , sc) on nociception and spontaneous motor behavior:

Experiments were conducted in a group of 5 rats. Each rat served as its own control. Spontaneous motor activity was recorded in the video path activity analyser in response to subcutaneous injection of formalin and naloxone with formalin. The spontaneous motor activity was recorded 5 min after injection of naloxone. In case of formalin alone, activity was recorded immediately after injection. A rest period of 48 hours was given in between the interventions. Pain rating was simultaneously done in the formalin injected groups by the observer.

*Experiment II*: To study the effect of high dose of naloxone (3mg/kg, sc) on nociceptive and spontaneous motor behavior:

The experimental schedule was as described in experiment I except that the rat received a

higher dose of naloxone. This study was conducted on a separate group of five rats.

#### Analysis of data :

The data were analysed using the paired Student's t-test and Wilcoxon's matched paired signed rank sum tests (Wilcoxon test). Pain rating of the basal and the naloxone-treated groups were compared using Student's t-test. Motor activity and forepaw licking behavioral scoring data were analysed by using the Wilcoxon test and comparing the formalin treated condition with the naloxone or the naloxone and formalin treatment. Values are expressed as mean  $\pm$  standard deviation (SD) for formalin pain rating while as standard error of mean (SEM) for motor activity and paw licking behavior.

## RESULTS

### *Experiment I : Effect of low dose of naloxone (6 $\mu$ g/kg, sc) on nociceptive and motor behavior of rats :*

The results of the nociceptive and motor behavior are presented in Table I; Fig. 1a and b. The time spent in locomotor activity, gross movements and distance travelled were analysed for epoch of 5 min each during a one-hour session after formalin (sc) injection. The pattern of change in the various parameters of spontaneous

motor activity viz., locomotion, movements and distance travelled were similar and therefore only the data of locomotion are being presented.

There was a marked increase in the spontaneous motor activity throughout the one-hour session following formalin induction of pain. Locomotion increased to  $10.9 \pm 5.3$  sec during 15 min epoch in contrast to the basal ( $3.8 \pm 1.48$  sec) (Table I and Fig. 1b). The nociceptive rating was also high ( $2.34 \pm 0.4$  and  $2.00 \pm 0.7$ ) during the initial 5 and 15 min respectively. The pain rating continued to be in this range till the period of observation (60 min). The duration of category 3 (forepaw lick) indicating severe pain was  $94.00 \pm 31.90$  sec and  $192.40 \pm 75.75$  sec during 0-5 and 15-30 min respectively (Fig. 2). To correlate between the pain status and spontaneous locomotor activity, naloxone (6  $\mu$ g/kg, sc) was injected 5 min prior to formalin treatment. Low dose of Nx and formalin produced an increase in pain intensity ( $2.65 \pm 0.3$ ) during initial 5 min as compared to basal ( $2.08 \pm 0.4$ ), although the difference did not attain statistical significance. However, during later phase (20-45 min), there was a decrease in pain intensity. It significantly ( $P < 0.05$ ) reduced ( $1.72 \pm 0.3$ ) during 6th epoch as compared to its basal pain ( $2.25 \pm 0.4$ ) (Fig. 3a). The spontaneous motor activity decreased markedly throughout 1 h recording session. A significant reduction in

TABLE I: Effect of formalin(5%) on spontaneous motor activity in rats.

Epochs	Pain score	Locomotion (sec)		Movements (sec)		Distance (cm)	
		Basal	FOR	Basal	FOR	Basal	FOR
1	$2.34 \pm 0.4$	$10.7 \pm 2.4$	$11.7 \pm 2.2$	$53.5 \pm 11.9$	$58.5 \pm 11.0$	$188.5 \pm 41.1$	$206.3 \pm 37.6$
2	$1.87 \pm 0.2$	$8.7 \pm 2.8$	$9.1 \pm 3.1$	$43.5 \pm 13.9$	$45.5 \pm 15.3$	$155.6 \pm 48.7$	$163.5 \pm 54.2$
3	$2.00 \pm 0.7$	$3.8 \pm 1.4$	$10.9 \pm 5.3$	$12.6 \pm 4.8$	$54.5 \pm 26.5$	$100.8 \pm 32.5$	$195.4 \pm 92.4$
4	$2.14 \pm 0.3$	$4.7 \pm 1.9$	$9.3 \pm 3.1$	$23.5 \pm 10.0$	$46.5 \pm 15.3$	$84.0 \pm 32.7$	$162.9 \pm 53.0$
5	$2.19 \pm 0.2$	$1.4 \pm 0.6$	$9.3 \pm 4.1^*$	$7.0 \pm 3.4$	$46.0 \pm 20.5^*$	$30.2 \pm 11.7$	$167.5 \pm 72.1^*$
6	$2.13 \pm 0.3$	$1.8 \pm 0.8$	$8.1 \pm 2.6$	$9.0 \pm 4.2$	$41.5 \pm 12.6$	$38.4 \pm 15.7$	$141.7 \pm 42.9^*$
7	$1.95 \pm 0.2$	$2.4 \pm 1.2$	$9.2 \pm 3.1^*$	$12.0 \pm 6.0$	$46.0 \pm 15.5^*$	$46.0 \pm 20.9$	$162.2 \pm 53.8^*$
8	$2.05 \pm 0.3$	$5.8 \pm 2.6$	$8.4 \pm 3.8$	$28.5 \pm 13.2$	$42.0 \pm 18.8^*$	$105.6 \pm 46.6$	$151.5 \pm 68.9$
9	$2.05 \pm 0.3$	$6.0 \pm 2.8$	$5.8 \pm 1.5$	$30.0 \pm 14.3$	$45.0 \pm 21.4$	$107.2 \pm 49.8$	$161.9 \pm 76.3$
10	$1.96 \pm 0.3$	$4.1 \pm 2.2$	$8.6 \pm 3.9$	$19.0 \pm 11.3$	$43.0 \pm 19.3$	$72.7 \pm 37.1$	$160.3 \pm 71.5$
11	$1.95 \pm 0.1$	$7.2 \pm 4.1$	$5.2 \pm 1.7$	$37.5 \pm 20.5$	$26.3 \pm 8.6$	$128.3 \pm 71.6$	$94.1 \pm 29.3$
12	$1.81 \pm 0.4$	$7.1 \pm 4.9$	$8.1 \pm 2.9$	$35.5 \pm 24.7$	$43.0 \pm 15.4$	$129.8 \pm 87.6$	$151.8 \pm 52.1$

\* $P < 0.05$  (formalin treated group is compared with basal group;  $n = 10$ ; Wilcoxon matched pairs signed ranks test; Pain rating and motor activity values are expressed as Mean  $\pm$  SD and Mean  $\pm$  SEM respectively). FOR-formalin (5%) in 50  $\mu$ l.

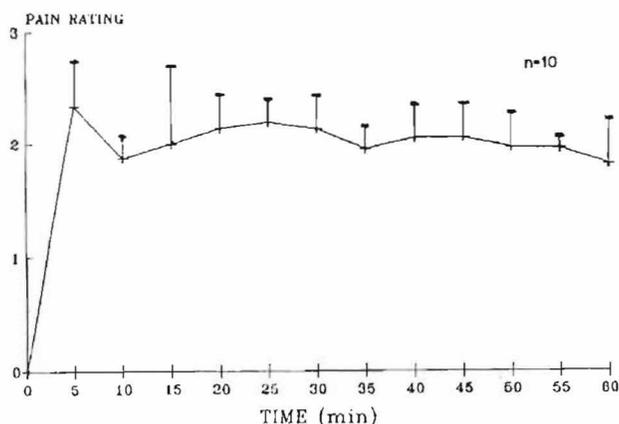


Fig. 1a : The basal formalin pain rating curve.

locomotor activity ( $2.2 \pm 0.89$  and  $1.0 \pm 0.89$  sec) was observed during first and second epochs, as compared to its basal ( $9.4 \pm 1.78$  and  $11.6 \pm 2.68$  sec) respectively (Fig. 3b). The decrease in pain intensity was also reflected in the during of category 3 (paw lick). Following low dose of Nx and formalin injection the paw lick duration was  $216.60 \pm 82.20$  sec and  $89.00 \pm 74.68$  sec during 0-5 min and 15-30 min period (Fig. 2).

*Experiment II: Effect of high dose of naloxone (3 mg/kg, sc) on nociceptive and spontaneous motor activity:*

In a separate group of five rats, the pain intensity was  $2.83 \pm 0.2$  during the first five

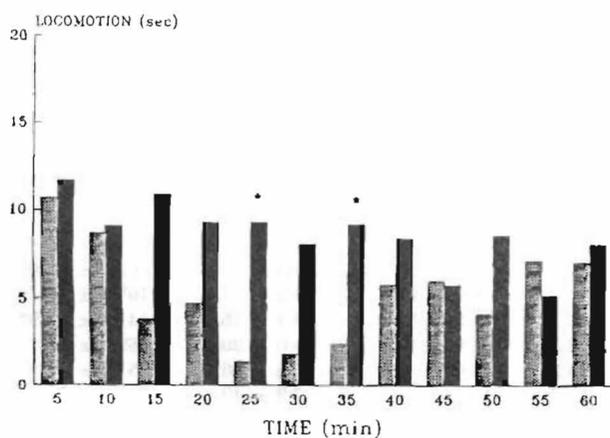


Fig. 1b : Effects of formalin (5% in 50 µl) on locomotor activity. Basal (stippled) and formalin injected (solid) rats during an one-hour recording session (\* $P < 0.05$ ).

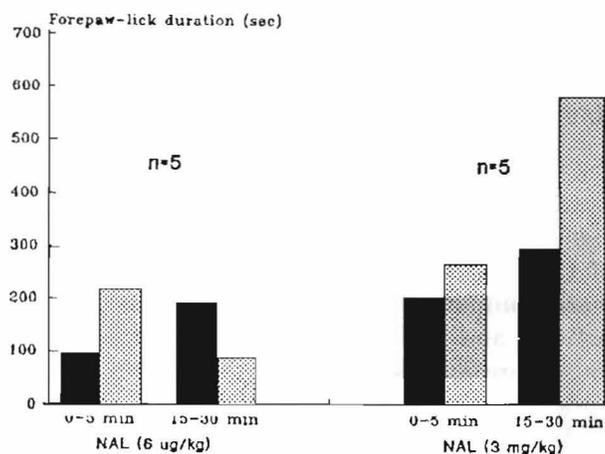


Fig. 2 : Effects of formalin (■) and low dose (6 µg/kg, sc) and high dose (3 mg/kg, sc) of naloxone (NAL-▨) on forepaw licking behavior of rats during early phase (0-5 min) and late phase (15-30 min) of formalin pain.

min after 3 mg/kg, sc Nx injection as compared to basal ( $2.60 \pm 0.2$ ). During 25-60 min period the pain intensity was more. A significant ( $P < 0.05$ ) increase in pain rating ( $2.47 \pm 0.3$ ) was observed during 7th epoch (Fig. 4a). The paw lick behavior categorised as 3 during 0-5 min was  $250.40 \pm 37.31$  sec after Nx injection, as compared to the basal  $183.60 \pm 29.75$  sec suggesting increase in pain level. However, during the late phase the paw-lick duration ( $538.60 \pm 80.62$  sec) increased markedly as compared to basal ( $290.20 \pm 92.34$  sec) (Fig. 2). A simultaneous record of the spontaneous motor activity showed a decrease during 5-35 min and 45, 50, 60 min (Fig. 4b).

## DISCUSSION

The results of our experiments suggest that the increase in spontaneous motor activity was parallel with the increase in the nociceptive rating during the tonic (formalin) pain. Secondly, the lower dose of naloxone (6 µg/kg) reduced the spontaneous motor activity simultaneously with the decrease in pain rating. Whereas, the higher dose of naloxone (3 mg/kg) decreased the spontaneous motor activity, while increasing the pain rating during the later half (after 20 min) of the formalin injection.

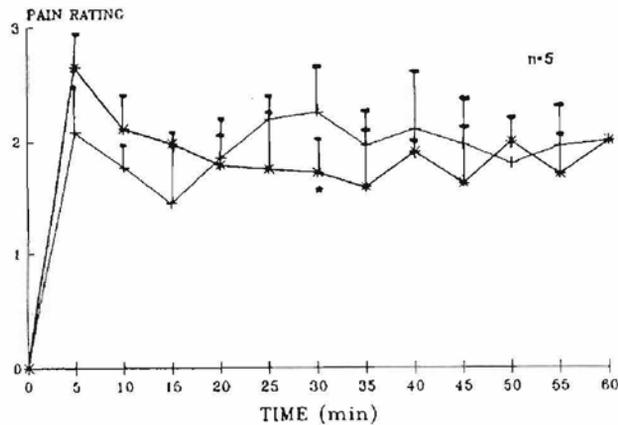


Fig. 3a : Effects of low dose of naloxone (6  $\mu$ g/kg, sc) on the formalin pain rating (—\*) of rats as compared to the basal pain rating (—+). \*P < 0.05.

There is a great deal of interest in the mechanism of naloxone action mostly because of its widely reported biphasic paradoxical or a simultaneous antinociceptive-pronociceptive effects in the same rat with the same dose. In the present study, we also report a dose dependent effect on pain viz., hypoalgesia with lower dose (6  $\mu$ g/kg) and hyperalgesia with higher dose (3 mg/kg) during the later phase of formalin pain. These differences may be based on the site of naloxone action viz., centrally or peripherally or both. However, for opioids a dual modulatory peripheral mechanism has been suggested. The low doses of opioids evoke a direct receptor-mediated excitatory, whereas the high doses evoke an inhibitory action on sensory neurons (21). Probably, naloxone also in low doses blocks a putative opioid system which antagonises antinociception (22) or an endogenous dynorphin pronociceptive system or specifically block the central kappa opioid-pronociceptive system (23). It is unlikely that the complex effects on nociception are merely based on peripheral or central sites of action.

The superficial and deep sensory neurons in the medulla, ganglion, as well as the dorsal root of spinal cord respond differently to the noxious stimuli, naloxone, and the non-noxious

stimuli as well. Moreover, these neurons also respond differently to the initial or late phase of the formalin pain as evidenced by [14 C]2-deoxyglucose uptake and the appearance of fos-like immunoreactivity (24). Therefore it is likely that the naloxone affects the populations of neurons that respond specifically to the late phase of noxious stimulus of formalin. Besides, the site of naloxone action, the differential effect of naloxone are suggested to be mediated by the opioid receptor on the medullary dorsal horn sensory neurons (25). The affinity varies with the receptor subtypes. Highest for mu receptors, lowest for kappa and intermediate for delta (26). The excitatory effects of low doses of Naloxone are probably mediated by competitively inhibiting the actions of endogenous opioid effects via mu receptors (27). Whereas, the hyperalgesic action of naloxone has been attributed to its excitatory action on the multi receptive, deeper dorsal horn neurons of medulla.

Not only are the differential effects of naloxone determined by the neuronal site in medulla, but also on the site of projection of the activated neurons. Recently, Mokha (8) has reported that the pro or anti-nociceptive effects of naloxone may be determined by the activation of projection site by the selective nociceptive

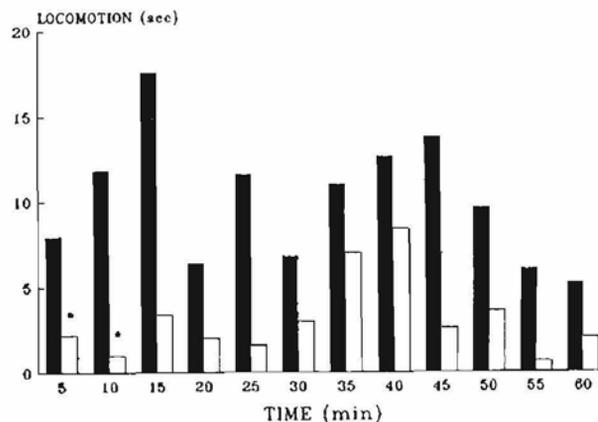


Fig. 3b : Shows the spontaneous locomotor activity in rats preinjected with formalin alone (■) and formalin in addition to low dose of naloxone (□) \*P < 0.05.

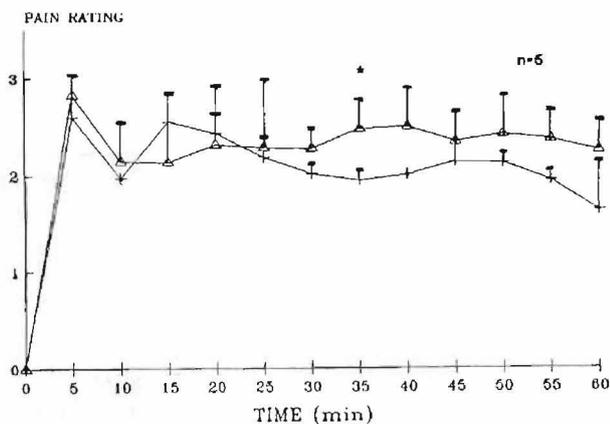


Fig. 4a: Effects of high dose of naloxone (3 mg/kg,sc) on the formalin pain rating (—△—) of rats as compared to the basal pain rating (—+—). \* $P < 0.05$ .

neurons. Naloxone reduces the responses of selectively nociceptive neurons in the superficial dorsal horn which is profusely connected with lateral thalamus (28), the nucleus submedialis thalamus (28, 29) or PAG (30) and the parabrachial areas. These neural sites are implicated in both the nociception and antinociception and the affective component of it. Depending on the projection in the brain the selectively nociceptive neurons mediate pronociception or antinociception.

Thus the differential influence of naloxone depends primarily on the central mechanisms residing in the deeper horn of the medulla. The location of nociceptive neurons activated there and the stimulus selectivity (multireceptive vs selectively nociceptive neurons) are the determinants of the response.

Naloxone in either dosage given by us did not affect the initial pain. It only affected the later pain from 20-50 min. The initial pain is attributed to the peripheral nociceptor stimulation by the noxious chemicals and the tonic late phase to the local inflammatory response. But the neural processes initiated during the initial phase sensitize and influence the neural processing vis-a-vis the behavior during the later phase of formalin pain. The

happenings in these neural substrates namely the hippocampus initiated through cingulum bundle or fornix are prolonged and continue in the absence of stoppage of stimulation by the noxious agent. The processing has been suggested to occur in structures that subserve both sensory-discriminative and motivational-affective function (reviewed in 31). Naloxone in addition to its central effects probably also modulates the neural signals initiated during the early phase of tonic pain.

There is a greater difficulty in interpreting the results of naloxone on the spontaneous motor activity in the literature available because besides the relationship with the dose of naloxone the 'data' varies with the recording process. It also varies with the time of the test, pretest, exposure to the equipment, conditioning prior to recording, physical, environmental factors, time and quality of previous meals and recording duration. To further add to this list are the parameters of locomotor activity recorded vis-a-vis the equipment used and the size of the observation chamber (32). Therefore, we compared the effects of different doses of naloxone on the spontaneous motor activity under the standardised conditions using the same equipment. Moreover, to eliminate these and other unspecified factors, the effect of

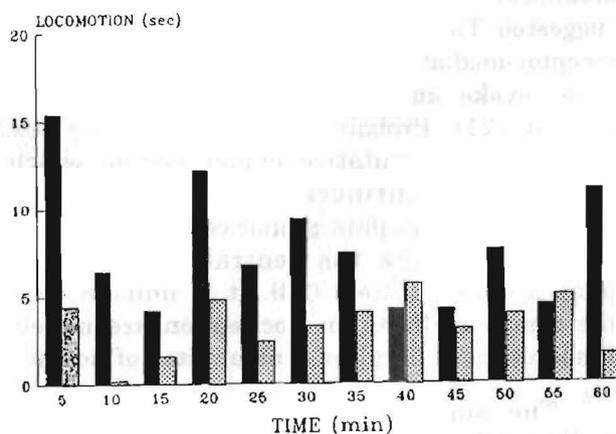


Fig. 4b: Shows the spontaneous locomotor activity in rats preinjected with formalin alone (■) and formalin in addition to high dose of naloxone (▨).

naloxone in two different doses was studied on the pain status and spontaneous locomotor activity simultaneously in the same rat, at the same hour of the day, using the same equipment. Besides, the correlation amongst the spontaneous locomotor activity and nociception level has not been reported in the literature.

The lower dose of naloxone (6 µg/kg) suppressed the spontaneous locomotor activity during the initial phase (5 min) and also during the later phase (35-50 min). The rats were conditioned to the equipment for 30 min prior to the recording. Our results are in agreement with the other reports in the habituated rats (reviewed in 32).

Naloxone (1,4 mg/kg) decreases the locomotor activity in the paired rats habituated to the experimental set up (32). In our rats also the motor activity remained suppressed despite the differences in the mode of injection and the study design. Our rats received naloxone subcutaneously, only 5 min prior to the recording session of 60 min after a 30 min conditioning period on every experiment day. Whereas, Dokla (32) injected naloxone intraperitoneally, 30 min prior to the recording session, lasting for only

5 min, which was repeated for five days. When the interval between injection and decrease in the spontaneous locomotor activity is compared in these two studies, it is nearly the same, that is 30-35 min.

The effect of naloxone on the spontaneous locomotor activity and nociception simultaneously has not been reported so far. Naloxone (6 µg/kg) while relieving some pain, reduced the basal spontaneous motor activity. Higher dose of naloxone on the contrary produced higher pain scores and still reduced the locomotor activity. From this observation, it emerges that naloxone affects locomotor activity and pain not by a totally common mechanism. Although the effect on motor activity are not non-specific, it may antagonize opioid release in relation to the social environmental situations (32).

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