

## PREDATOR INDUCED STRESS AND ITS ANALGESIC POTENTIAL ON ESTROUS AND ANESTROUS ALBINO RATS

H. H. SUDHAKAR AND D. VENKATESH\*

\*Department of Physiology,  
Dr B.R. Ambedkar Medical College,  
Bangalore - 560 045

and

\*Department of Physiology,  
Devaraj Urs Medical College,  
Tamaka, Kolar - 563 101

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**Abstract :** It has been established that physical stress induces antinociceptive effect. In the present study efforts were made to investigate the role of chronic intermittent psychological stress in the induction of the analgesic effect and the probable role of estrous cycle in modulating the antinociceptive response. Albino rats in regular estrous cycle (n = 15) and those in anestrus (n = 15) were exposed to psychological stress for a period of 20 min each day for 12 consecutive days. The predator (domestic cat) was used to induce the psychological stress. At the end of each session tail flick response time to heat was recorded as a measure of pain perception. It was observed that female estrous rats had a low pain threshold in the beginning but its tolerance increased gradually reaching a peak by 6th to 7th day and returned to control level by 11th day. The anestrus female had higher pain threshold at the beginning and showed a gradual decline to reach the control level as estrous females by 12th day. These results suggest that the ovarian hormones and the corticosterone may modulate the impact of stressor on endogenous pain inhibition and other stress responsive systems.

**Key words :** opioid analgesia  
anestrus

predators  
psychological stress

### INTRODUCTION

Stress is a threat or challenge to the integrity and survival of the organism (1). The animals are exposed to various types of stress. A repeated or a continuous exposure to stress factors are blamed to be the cause

of various diseases. Nevertheless different types of stress increase the pain threshold altering its behavioral response. Substantial evidence exists for the presence of multiple endogenous pain inhibitory systems that can be activated by environmental stressors. The opioid and non-opioid forms of stress

\*Corresponding Author



induced analgesia have been implicated which depends on the parameters of stressors (2). It has further become evident that a variety of biological factors like sex, reproductive status, species strain, behavioral and physical status can affect the expression of stress induced analgesia (3, 4). It is observed that female animals exhibit a greater anxiety reaction when exposed for stress of short duration and further they demonstrate a lower pain tolerance with respect to males having comparable characters (5, 6, 7). Currently available literature indicates the influence of physical stress and its analgesic effect. However very few references are available on the analgesic effect of psychological stress. Further many physiological systems that mediate the impact of stressors and the response of the animal thereon are strongly suggested to be influenced by estrous cycle and ovarian hormones. Several hypothalamic-pituitary, adrenal responses including plasma levels of corticosterone,  $\beta$  endorphins are believed to vary with various phases of estrous cycle (8, 9, 10).

Keeping in view the available references, an attempt has been made to determine the antinociceptive effect of chronic intermittent psychological stress on the female albino rats and the probable analgesic effect or analgesia modulating effect of the female sex hormones.

#### METHODS

The female albino rats used for the experiment were bred in the laboratory. The average weight of the animal was 175–200 g with their age between 150–160 days. The

rats were randomly assigned into two groups G1 and G2 with 15 animals in each group. The rats of G1 served as anestrus groups and were kept together in a spacious cage in an isolated room which was well ventilated and illuminated. A large group of female rats kept together totally isolated from males go into a phase of anestrus (11). The rats of G2 were kept in groups of 5 in separate cages along with the males and they served as estrous group. All the rats were allowed free access to food and water.

#### Procedure

Analgesiometer (INCO) was used to record the tail flick response time to heat as per the established procedures. The instrument was calibrated so as to attain a temperature of 42° C at the base of the rat's tail (12). The maximum duration of exposure to heat was restricted to 2 min to avoid heat necrosis of the tail. A modified rat restrainer (D. Venkatesh) was used to give a better front view and greater comfort to the rat during its stay. A control tail flick response was recorded after restraining the rat for 20 min. A predator (domestic cat) was used to induce psychological stress. The optimum time of exposure to obtain the best stress induced response was determined by using a different group of rats that were comparable to the test group. By a series of trials the optimum exposure time to predator was estimated to be 20 min. On the day of experiment the rats were kept in the restrainer for a duration of 15 min for them to get adapted to the restrainer and were later exposed to the cat which had freedom to move in one half of the cage. Care was taken to ensure a good view of



the cat by the rats throughout the time of exposure. In addition the rats received adequate olfactory and auditory cues from its predator. The tail flick response was recorded at the end of 20 min. For the next 11 days the experiments were conducted during the same time of the day and the tail flick response was recorded following exposure to the predator.

**Statistical analysis**

The results are presented as mean ± S.D. The data were analysed using student's 't' test. P values less than 0.05 were considered significant.

**RESULTS**

The control tail flick response latency in anestrus rats was 25.06 ± 4.4 sec and that for the estrous rats was 21.5 ± 3.5sec. Thus the estrous and anestrus female rats showed a comparable control tail flick response time. Exposure to the domestic cat induced psychological stress which significantly increased the tail flick response time in anestrus rats (66 ± 8.02sec) and

estrous rats (38 ± 6.56 sec) on the first day. Thus the tail flick latency after the exposure of animal to psychological stress was lower in estrous rats when compared to anestrus rats and the difference was statistically significant. As shown in Fig. 1 and Table I, the anestrus rats showed a gradual decrease in the tail flick response time over the subsequent days reaching the control values by the 12th day. However, estrous females showed a steady and a significant increase in the response time reaching a maximum by the 6th day, maintained at the same level for the 7th day and then showed a decline on the 8th day to reach the control values by the 11th day.

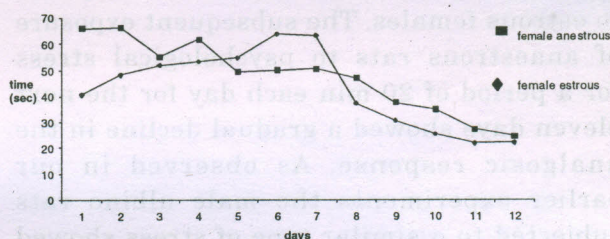


Fig. 1: Comparison of tail flick response time in female estrous and anestrus rats exposed to chronic intermittent psychological stress.

TABLE I: Tail flick response in estrous and anestrus rats on exposure to chronic intermittent psychological stress as compared to their control values.

	Control	Stress		
		Day 1	Day 6	Day 12
Estrous	21.5±3.5	38±6.56 P<0.001	63.67±5.6 P<0.001	21.7±3.1 P>0.05
Anestrus	25.06±4.4	66±8.02 P<0.001	50.07±4.6 P<0.001	24.6±4.6 P>0.05

Values are mean ± SD of 15 animals in each experimental group.



## DISCUSSION

The earlier studies have demonstrated the analgesic effect produced by various types of physical stress. They have suggested the opioid and non-opioid mechanisms for this analgesic effect depending on the duration of exposure. The estrous cycle is suggested to modulate the expression of opioid and non-opioid pain inhibition. Short-term exposure to various stressors are shown to activate the non-opioid pain inhibition and a prolonged exposure would trigger the endogenous opioid analgesic system (10). The present data revealed that the anestrous females showed a better pain tolerance with an initial increased threshold for pain in response to psychological stress as compared to estrous females. The subsequent exposure of anaestrous rats to psychological stress for a period of 20 min each day for the next eleven days showed a gradual decline in the analgesic response. As observed in our earlier experiments the male albino rats subjected to a similar type of stress showed a comparable trend though the pain threshold was much higher than anestrous females (13). The ovariectomized females exposed to physical stress also showed a greater analgesic response (4). A better pain tolerance in anestrous female rats and the ovariectomized rats could be due to the low circulating levels of oestrogen which increases the number of opioid receptors and maintains them in a highly sensitive state. A much higher pain threshold in male rats could be due to the potentiating effect of testosterone on the endogenous opioid pain inhibiting system. The estrous females exhibited tail flick latency in response to psychological stress on the first day, which

was much less when compared to their anestrous counterparts. Exposure of rats in regular estrous cycle to a state of stress can exhibit estrogen induced modulation of analgesic system. Estrogen is demonstrated to reduce the receptor number and binding in the opioid system as well as electro physiological actions of  $\mu$  opioids (14, 15, 16). The estrous females subjected to chronic intermittent psychological stress demonstrated a gradual increase in pain tolerance upto the seventh day. Subsequently there was a decline in the analgesic effect returning to the control level by about 11th day. The presence of estrogen or its metabolites at opiate receptors could desensitize and down regulate it whereas disappearance of these would upregulate the receptors or leave it in a very sensitive state. This might explain the enhancement of analgesia following decline in estrogen activity. These types of interaction at receptors have now been commonly recognised in a number of central analgesic systems (17). Better pain tolerance observed in estrous females with chronic stress could also be attributed to the increasing corticosterone levels, which is suggested to play a permissive role in long term analgesia and to be a critical factor in mediating this phenomenon (18). The observation of estrous females exhibiting a gradually increasing pain tolerance can be attributed to the decreased responsiveness of the opioid receptors to the circulating sex steroids. These observations are contrary to the suggestion that estrogen potentiates the analgesic effects by activating the opioid system (10). Our observations indicating absence of cyclical variations in the antinociceptive effect corresponding to the cyclical hormonal changes in the estrous



female rats are contrary to the views of Ryan and Maier (10), who have reported a demonstrable correlation between estrous cycle and antinociceptive effect. Further it substantiates the earlier inferences of Romero and Bodner (3) who found no definite relationship between the analgesic response over the different phases of estrous cycle for physical stress. A comparative study of analgesic response of anestrus females, estrous females and males in the later part of exposure to chronic intermittent stress showed a similar decreasing trend. It could probably be due to a mechanism of adaptation or habituation (11), which is contrary to the possibility of minimal habituation of the rats defensive response to the predator-cat over the

exposure schedule as observed by Blanchard et al (19).

#### In conclusion

- 1) Psychological stress induced greater analgesic effect in nonestrous females when compared to their counterparts in regular estrous cycle.
- 2) The various phases of estrous cycle did not appear to influence the analgesic effect to psychological stress.
- 3) The physical and psychological stress induced similar anti nociceptive effect in experimental animals.

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