Pain threshold variations in female rats as a function of the estrus Cycle

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Summary: In this study, the response of female rats in different phases of the estrus cycle to nociceptive stimulation was evaluated using thermal (hot plate and tail immersion) and chemical (formalin) tests. In the hot plate test, the paw licking latency fell significantly ($p < 0.05$) in the metestrus and diestrus phases compared with the proestrus and estrus phases. The observations in the tail immersion test also followed the same pattern. The significant reductions in the paw licking and tail withdrawal latencies due to a lowered threshold denote an increase in pain sensitivity in the metestrus and diestrus phases. In the formalin test, the licking time fell significantly from the metestrus to the diestrus phase compared with the proestrus and estrus phases, the reduction in this test which was due to an increased threshold connotes a decrease in pain sensitivity. The results therefore seem test dependent. In conclusion, pain threshold in female rats depends on the estrus state.

Keywords: Pain threshold, Variation, Estrus cycle

INTRODUCTION

Most female rats have a four day estrus cycle consisting of four sequential stages called respectively, proestrus (P), estrus (E), metestrus (M) and diestrus (D) (Freeman, 1994). This cycle affects many of the rat's behaviour in response to social and environmental stimuli (Erskine, 1989; Pfatt, et al., 1994) with the most obvious changes occurring as the rats move into and through its fertile period (P and early morning of E). Thus, it is not surprising that many environmental studies in rats have shown that estrus cyclicity exists for pain behaviour in response to stimulation of somatic structures (Berkley, 1997).

The observations made in different studies were however inconsistent, for example some studies found out that behavioural response thresholds to noxious pressure or electrical stimulation of the hind foot or tail base were lower in P and E (i.e rats were more sensitive in those stages) than during M and D(Drury and Gold, 1978; Kayser et al.,1996). Others found out that whereas hind foot withdrawal responses to thermal stimulation did not vary with the estrus stage, the amount of thermal hyperalgesia produced by inflammation of the foot was significantly increased in P (Ruda, et al., 1998). Still others found out that tail flick thresholds were lowest during P, greater during E and highest during M (Frye et al., 1992; Sapsed –Byrne and Holdcroft, 1996) or were lower during E and M than during P and D(Martinez –Gomez et al., 1994). Similarly in rats, pain behaviours that occur in response to an experimentally implanted calculosis are significantly greater when the rats are in M and D phases than in P and E (Giamberardino et al, 1977a). Based on these inconsistencies, further research is deemed warranted, accordingly the present study looked at the dependence of pain threshold on the estrus cycle.

MATERIALS AND METHODS

Female cyclying Wistar rats (180-250g) were used for this study. They were bred and housed in the pre-clinical animal house of the College of Medicine, University of Ibadan, Nigeria. They were fed with rat cubes (Ladokun feeds, Ibadan, Nigeria) and had water ad libitum. The animals were divided into four groups: proestrus, estrus, metestrus and diestrus of six rats each depending on their cycling phases. They were subjected to two thermal tests (hot plate and tail immersion tests) and a chemical test using formalin.

The hot plate test
The test was carried out using the original method of Eddy and Leimbach (1953) as modified by Ibironke et al. (2004). The animals in the various groups were

placed in turn on a hot plate whose temperature was maintained at 52 ± 2.0 °C. A cut off time of 60sec was imposed to avoid significant tissue damage. Pain sensitivity was evaluated by the response latency to paw licking on the hot plate.

The tail immersion test

The details of the tail immersion procedure were essentially similar to those published earlier (Statile et al, 1998). Using a circulating immersion heater (Catalogue No 13-874-170, Fischer Scientific, Pittsburg, PA) a constant temperature of 50 ± 0.2 °C was maintained in the water bath in which the terminal 3cm of the animal´s tail in the various groups were immersed. The nociceptive end point was characterized by a violent jerk of the tail. The time taken for the animal to withdraw or flick its tail out of the water was taken as the tail withdrawal latency.

Formalin induced paw licking in rats

The details of the procedure were essentially similar to that of Hunskaar and Hole (1987). Briefly, 0.2 ml of 3 percent formalin was injected into the dorsal surface of the left hind paw of the rats in the various groups and the rats placed in a chamber with a mirror mounted on three sides to allow an unobstructed view of the paws. The time spent licking the injected paws (licking time) was recorded. The animals were observed for the first 5 min post formalin (early phase) and for 10 min starting at the 20th min post formalin (late phase).

Statistical Analysis

This was carried out using the students't-test. A value of $p < 0.05$ was regarded as significant.

RESULTS

The effects of estrus cyclicity on pain threshold are shown in tables 1, 2 and 3.

The hot plate and tail immersion tests

Table 1 (hot plate) and table 2 (tail immersion) tests showed that as the cycle progressed from proestrus to the estrus phase, there was an insignificant ($p>0.05$) decrease in paw licking and tail withdrawal latencies. There after the paw licking and tail withdrawal latencies fell significantly ($p<0.05$) as the cycle moves through the metestrus to the diestrus phase. Pain sensitivity was highest in the diestrus phase in both tests.

The formalin test

The results of this test are as shown in table 3. In both phases, the licking time fell insignificantly from the proestrus to the estrus phase. As the cycle moves from the estrus through the metestrus to the diestrus phase, the decrease in the duration of paw licking

became significant ($p <0.05$). Least sensitivity to pain was obtained in the diestrus phase.

Table 1
Effects Of Estrus Cycle Variation On Paw Licking Latencies to Noxious Heat In Rats

Phases of Estrus Cycle	Paw Licking Latencies (s)	p-value
Proestrus	6.9 ± 0.29	-
Estrus	6.8 ± 0.24	> 0.05
Metestrus	5.8 ± 0.20	< 0.05
Diestrus	5.3 ± 0.30	< 0.05

Values are means $\pm$ SEM , n = 6

Table 2
Effects of Estrus Cycle Variation on Tail Withdrawal Latencies to Noxious Heat in Rats

Phases Estrus Cycle	Tail Withdrawal Latencies (s)	p- value
Proestrus	4.7 ± 0.26	-
Estrus	4.5 ± 0.34	> 0.05
Metestrus	3.5 ± 0.27	< 0.05
Diestrus	3.1 ± 0.18	< 0.05

Values are means $\pm$ SEM, n= 6

Table 3
Effects of Estrus Cycle Variation on Formalin Induced Paw Licking in Rats

	Duration of paw Licking (s)	Duration of Paw Licking (s)
Phases of Estrus Cycle	Phase 1	Phase 2
Proestrus	88.6 ± 2.08	99.5 ± 1.85
Estrus	82.9 ± 2.08 [ns]	99.3 ± 1.46 ns
Metestrus	$57.3 \pm 1.59*$	$69.9 \pm 1.34*$
Diestrus	$52.5 \pm 1.94*$	$58.9 \pm 1.24*$

Values are means $\pm$ SEM, n= 6, ns Not significant vs proestrus group, * $P < 0.05$ vs proestrus group

DISCUSSION

The results herein presented showed that pain threshold in female rats vary with the estrus cycle. We observed that as the cycle changes from the proestrus to the diestrus there was a gradual decrease in paw licking and tail withdrawal latencies due to increased sensitivity in agreement with the reports of Giamberardino et al. (1977a) whose study showed that tail flick thresholds were lowest (increased sensitivity) in the M and D phases of the estrus cycle but contrasted those of Martinez-Gomez et al. (1994) and Frye et al. (1992) both of which showed that tail flick thresholds were lowest in the P and E phases. The differences between our study and the two contrasting studies cited above could easily be explained considering the fact that Martinez-Gomez et al. (1994) and Frye et al. (1992) made use of electrical stimulation and noxious pressure while our

study made use the thermal method, this fact further buttressed our earlier suggestion that the results might be test dependent. Another contrasting study by Sapsed- Byrne and Holdcroft (1996) made use of anaesthesized rats while our rats in this study were not, the anaesthesia might be responsible for the difference.

While a number of other differences between these studies might account in part for these variables (e.g. diet, estrus assessment, time of day of the experimented, etc), results from a study by Giamberardino et al. (1977a) suggested that another important factor may be the bodily depth of the stimulus. These authors found out that the menstrual pattern of pain thresholds to electrical skin stimulation differed from the pattern of stimulation of the subcutaneous tissue and muscle, these inconsistencies in the rat studies might have been due in part to variations in the inclusion of deeper somatic tissues (e.g. muscle) in the somatic stimuli that were used during the study.

Our observations in the chemical (formalin) test ran counter to the results obtained in the thermal test as the rats were found to be more sensitive in the P and E phases compared to the M and D phases in the thermal test. This again underlines the fact that the results obtained might to a large extent depend on the test used. Unlike the thermal test where a lot of studies had been carried out, studies on chemical test were difficult to come by and in fact we did not come across a single study that made use of the chemical test. This made it difficult for us to directly compare our work with previous studies in this field, this study may therefore serve as a reference point for other workers in this field.

It appears from the discussion earlier on presented that most of the estrus changes that do occur in the rats behavioural responses to noxious stimulation of visceral and somatic structures take place as the rats move from diestrus through proestrus into estrus phase. This situation raises the question of hormonal involvement in pain sensitivity as we have previously reported (Ibironke and Olopade, 2004), because P is the stage during which estradiol and then progesterone levels rise to their highest point (giving rise to ovulation during the early morning of estrus, immediately after which the hormonal levels fall precipitously (Freeman,1994). In conclusion, we have shown that pain thresholds and therefore sensitivity in rats are hormonal dependent and that these changes may in part explain the alterations in behaviour that occurs during the M and D phases when thresholds

are lowest as our results have shown. The possibility of other factors being responsible for the increased sensitivity cannot be ruled out at the moment and is in fact the subject of an ongoing study in our laboratory.

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