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Tail-Flick Latency and Self-Mutilation Following Unilateral Deafferentation in Rats

N. KŘÍŽ, A. YAMAMOTOVÁ, J. TOBIÁŠ, R. ROKYTA

Department of Normal, Pathological and Clinical Physiology, Third Faculty of Medicine, Charles University, Prague, Czech Republic

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Summary

Unilateral deafferentation induced by transection of the C_4-C_8 dorsal roots of spinal cord, followed by a complex of abnormal self-mutilating behavior, is interpreted as an animal model of chronic nociception. The objective of our study was to test the differences in tail-flick latency between intact control and unilaterally deafferented animals and to assess the changes in their acute nociceptive sensation. The initial hypothesis was that deafferentation-induced painful sensation might cause stress-induced analgesia that should be manifested as prolonged tail-flick latency. The experiment was carried out on 11 male and 10 female adult Wistar rats. The tail-flick latency was repeatedly measured over a period of 10 consecutive weeks both in the preoperative baseline period and following multiple cervical dorsal rhizotomy. Contrary to our hypothesis, unilateral deafferentation was followed by a significant shortening of the tail-flick latency both in males and females. In deafferented animals, compared to the controls, variations of tail-flick latency were reduced. In individual animals after deafferentation, concurrent dynamic changes were observed in self-mutilating behavior, in a loss and regaining of body weight, and in tail-flick latency. Our data suggest that changes in tail-flick latency may be interpreted in terms of central sensitization and that tail-flick latency might be considered as a useful marker of chronic nociception.

Key words
Deafferentation • Nociception • Rats • Self-mutilation • Tail-flick

Introduction

Complete deafferentation of the brachial plexus or of the corresponding dorsal roots can provoke a syndrome of chronic pain in humans. In addition to a total loss of sensibility in the formerly innervated areas, these patients feel spontaneous pain in the corresponding segments and adjacent areas. Along with these symptoms, repeating bursts of severe pain occur (Nashold and Ovelmen-Levitt 1991).

In rats, after the section of dorsal roots corresponding to the brachial plexus in man, abnormal behavior consisting of scratching, nibbling, and depilation of ipsilateral forelimb areas was observed (Basbaum 1974, Lombard *et al.* 1979a, Albe-Fessard and Lombard 1980, Wynn-Parry 1980, Vaculín and Rokyta 2004). This self-mutilating behavior in animals might be consistent with the response to painful sensation in man (Albe-Fessard 1996).

There exist at least three possible physiological explanations for the self-mutilation process induced by unilateral deafferentation: 1) self-mutilation process

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

might reflect the animal's response to a painful sensation, 2) deafferentation from rhizotomy renders the forelimb anesthetized (in such a case, the animal does not recognize the limb as part of its body scheme and attacks it as a foreign object), and 3) it is also possible that the deafferentation induces abnormal paresthetic sensations that are not necessarily painful but elicit self-mutilation.

The animals bite the region in which they feel false sensation due to abnormal central activity induced by the deafferentation (Lombard *et al.* 1979a,b, Albe-Fessard and Rampin 1991, Kříž and Rokyta 2000), or due to lack of any afferent sensation (Gandevia and Phegan 1999). Unilateral deafferentation has been suggested as an animal model of chronic nociception (Basbaum 1974, Albe-Fessard and Lombard 1980). However, whether animals express autotomy in response to nociception has not yet been fully established.

Our hypothesis was that deafferentation induces a painful sensation, as manifested by self-mutilation, and this might cause stress-induced analgesia that should be manifested as increased tail-flick (TF) latency in deafferented animals. The objective of our study was to test the differences in TF latency between intact control and unilaterally deafferented animals and to assess the changes in their acute nociceptive sensation.

Methods

Animals

The experiments were carried out on both male (n = 11, six of them were tested after deafferentation only), and female (of mixed estrous phases, n = 10) Wistar rats. Adult animals (90-day-old) were housed individually in single cages with a free access to food and water. They were maintained in climate- and light-controlled rooms (temperature: 23±0.5 ^{o}C, 12/12 h light/dark cycle with lights on at 06:00 h). Control data were obtained in five males and ten females, and these animals were then deafferented. The body weight of all animals was monitored each week and their behavior was followed daily. In both control and deafferented animals, the condition of the skin and adjacent tissues in the deafferented area was scored on a scale quantifying abnormal behavior such as depilation and self-mutilation.

Deafferentation

The laminectomy was performed in animals anesthetized with sodium pentobarbital (50 mg/kg i.p.) and a local anesthetic (Procain 1.5 % s.c.) injected into the neck region. A small unilateral incision in the dura mater was made just above the dorsal root entry into the spinal cord. Five dorsal roots at the cervical level C_4 to C_8 were cut on the left side proximally to the dorsal root ganglia. Care was taken to prevent trauma and bleeding of the spinal cord. An aseptic micro-surgical technique was used. All experiments adhered to the ethical guidelines for animal pain experimentation (Zimmermann 1983).

Somatic, physiological and behavioral observations

Body weight and TF latency were determined once a week and self-mutilation was evaluated using the following scores:

0 – intact,

1 – intermittent mutilation (mutilation of different quality and intensity, permanent licking, skin depilation and denuding, scratching, nail or skin nibbling with visible bleeding)

2 – severe mutilation, removal of the toe parts up to full forelimb autotomy.

The time from rhizotomy to the onset of abnormal behavior varied among different animals and also often exhibited fluctuation in the intensity of self-mutilation during the course of the experiment. Thereafter, all rats were followed for 10 weeks until stabilization of the process had occurred.

Tail-flick method

The tail-flick analgesia meter Model 33 (IITC Life Science Instruments, USA) was used for pain threshold testing with radiant heat. TF latency obtained in intact rats showed a high variation in individual animals (Ness *et al.* 1987, Kříž *et al.* 1997). To minimize the risk of spontaneous tail-flick reactions, the response to heat stimulation should be shorter than 5 s (Kříž *et al.* 1997). To obtain reproducible values, TF latency was measured once a week (session) with 6 trials per session over 10 weeks of both preoperative and postoperative periods.

It has been shown by Ness *et al.* (1987) that TF latencies were the shortest on the distal part of rat tail. Therefore, in one session, the TF latency was assessed at 60-s intervals starting with the skin of the distal part of the tail-tip and continuing proximally from the preceding spot. The short interstimulus interval between subsequent TF measurements was necessary for reducing the stress, caused by weak immobilization of animals during this procedure (Dubner and Bennet 1983).

Statistical analysis

The TF latencies were analyzed by four-way ANOVA (factors SEX x GROUP x SESSION x TRIAL) for repeated measures. A similar procedure was used for evaluating the effect of self-mutilation (factors SEX x GROUP x MUTILATION x SESSION x TRIAL). Differences between males and females were tested by factor SEX, the differences between control and deafferented animals by factor GROUP. Ten repeated sessions before and after deafferentation represented a within-subjects variable SESSION. Six trials of TF latencies in each session comprised the within-subjects variable TRIAL. Simple effects were computed in all significant interactions. The BMDP 386 Dynamic software (4V Program) performed all computations. $P<0.05$ value was considered significant. Data are presented as means ± SEM.

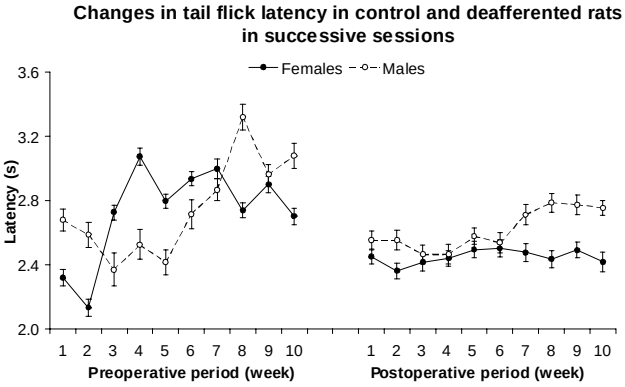

Fig. 1. Variations of the mean TF latency (± S.E.M.) in female (black circles) and male (white circles) rats during a 10-week preoperative (control animals) and a 10-week postoperative period (deafferented animals). Every point represents the average value from six consecutive measurements in each session.

Results

Tail-flick latency over a 10-week period in control and deafferented animals

Unilateral deafferentation was accompanied by a general decrease in TF latency (2.74±0.02 vs 2.54±0.01 s; factor GROUP: $F_{1, 33} = 9.72$; $P = 0.0038$) equally in both sexes (GROUP x SEX: $F_{1, 33} = 1.23$; $P = 0.2746$). The values in control and deafferented animals differed in their time course (GROUP x SESSION: $F_{9, 297} = 4.67$; $P = 0.0001$).

When comparison was made between self-controls (the control rats later deafferented, $n = 5$) vs. experimentally naive groups of deafferented males ($n = 6$), no substantial differences were found either in the average TF latency (2.49±0.03 vs 2.71±0.03 s; $F_{1, 9} = 2.66$; $P = 0.1373$) or in the time course ($F_{9, 81} = 0.16$; $P = 0.9398$). Therefore, in the final statistical evaluation, both groups of deafferented males were pooled together.

The trends in TF latency before and after deafferentation differed in males and females (GROUP x SEX x SESSION: $F_{9, 297} = 3.93$; $P = 0.0007$). After deafferentation, the shorter TF latency was more stable in both sexes, without the oscillations typical for intact animals. The details are described in Figure 1.

Tail-flick latency during one session in control and deafferented animals

In each session, when the stimulation was repeated every 60 s, the TF latency continuously decreased to a constant level. The first value of TF latency was the longest (TRIAL: $F_{5, 165} = 260.06$; $P<0.0001$) (Fig. 2). The absolute decrease of TF latency was higher in deafferented animals than in the controls (GROUP x TRIAL: $F_{5, 165} = 2.6$; $P = 0.0501$). A basal level of TF latency was reached during the second or third stimulation in the controls, but after the fourth or fifth stimulation in deafferented animals.

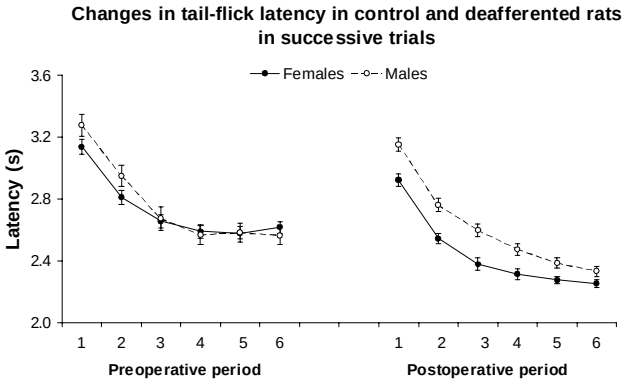

Fig. 2. Changes in the mean TF latency (± S.E.M.) in successive trials in female (black circles) and male (white circles) rats during preoperative (control animals) and postoperative periods (deafferented animals).

Similar effects of deafferentation on the differences in TF latency values were found in female and male rats. The decrease was greater in deafferented animals irrespective of their sex (GROUP x SEX x TRIAL: $F_{5, 165} = 0.49$; $P = 0.7065$). On the other hand, the decreasing trend was more prominent in female than in male animals (SEX x TRIAL: $F_{5, 165} = 3.48$; $P = 0.0152$).

Tail-flick latency: a comparison between the group of self-mutilating and non-mutilating animals

No significant effect of self-mutilation on the mean TF latency was found between the self-mutilating (n = 15) and non-mutilating groups (n = 6) (2.59±0.02 vs 2.66±0.01 s; $F_{1, 28} = 0.54$; $P = 0.4678$) neither the interaction of factors GROUP x MUTILATION was significant ($F_{1, 28} = 0.49$; $P = 0.4894$). In the non-mutilating group the mean TF latency decreased from 2.78±0.03 s to 2.59±0.02 s; in the mutilating group from 2.74±0.03 to 2.45±0.02 s. The interaction of factors GROUP x MUTILATION x SESSION was also not significant ($F_{9, 252} = 0.96$; $P = 0.4559$). The comparison between self-mutilating and non-mutilating animals is shown in Figure 3.

There were no significant sex differences in the changes of TF latency over the postoperative period between self-mutilating (eight female and seven male rats) and non-mutilating animals (two female and four male rats) (MUTILATION x SEX x SESSION: $F_{9, 252} = 1.46$; $P = 0.1922$).

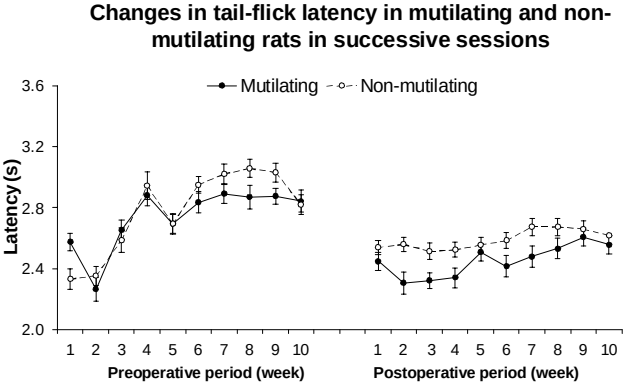

Fig. 3. Variations of the mean TF latency (± S.E.M.) in mutilating (black circles, n = 15) and non-mutilating (white circles, n = 6) rats during 10-week preoperative (control animals) and 10-week postoperative periods (deafferented animals). Every point represents the average value from six consecutive measurements in each session.

Self-mutilating behavior, body weight and tail-flick latency

Changes in body weight were monitored weekly both in control and deafferented animals (Fig. 4)**.** The body weights of females were relatively stable during the whole experimental period while the body weights of males reached the highest level only from the eighth week of the preoperative period (GROUP x SEX x SESSION, $F_{9, 117)} = 36.88$, $p<0.0001$).

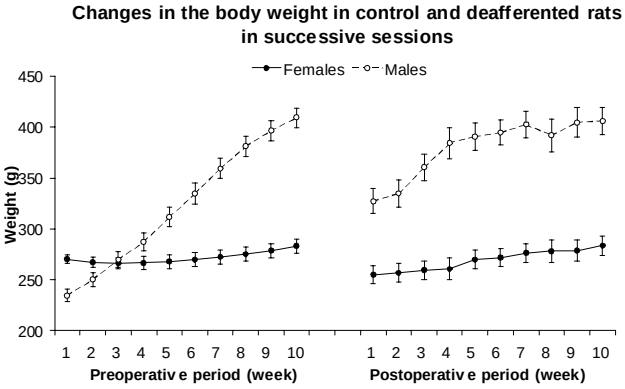

Fig. 4. Changes in the mean body weight (± S.E.M.) in female (black circles) and male (white circles) rats during 10-week preoperative (control animals) and 10-week postoperative periods (deafferented animals).

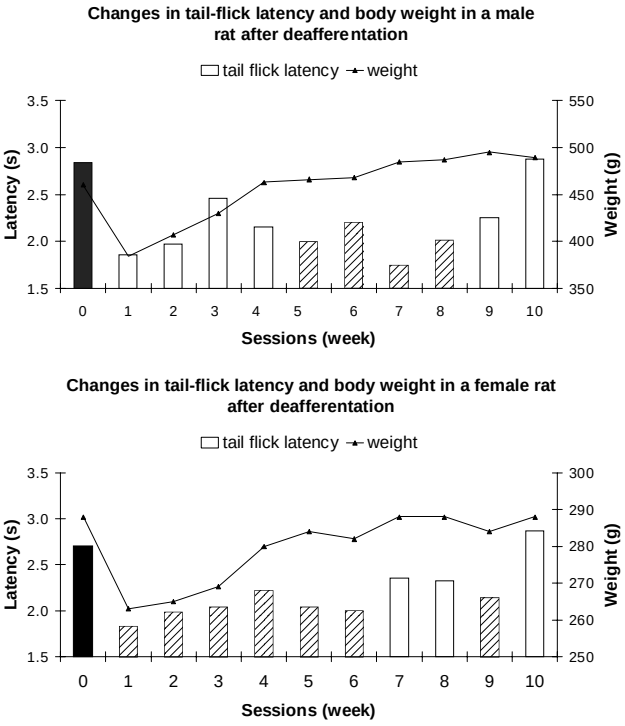

Fig. 5. The column graphs show changes in the mean TF latency during 10-week postoperative period in male and female rats, zero represents the last TF latency before rhizotomy. An empty column represents the weeks of non-mutilating behavior, hatched column self-mutilating behavior, and black column corresponds to preoperative period. The line graph follows changes in body weight.

Statistical analysis did not exhibit significant differences in TF latency in mutilating and non-mutilating animals, we found a parallel decrease in TF latency with increasing intensity of self-mutilation in individual cases. Figure 5 shows two examples of simultaneous fluctuations in self-mutilating behavior, body weight loss and a decrease of TF latency in individual deafferented female and male rats.

The effects of deafferentation on self-mutilating behavior are summarized in Table 1. The incidence of different degrees of self-mutilation has not been proved to be sex dependent ($\chi^2 = 1.7138$, df = 2, P = 0.4244).

Table 1. Behavior during the postoperative period - the occurrence of different stages of self-mutilation after deafferentation.

Sex	Intact (0)	Mutilation (1)	Autotomy (2)
Female (n = 10)	2	5	3
Male (n = 11)	4	6	1

Numbers in parentheses indicate the score according to the self-mutilated behavioral scale. $\chi^2 = 1.7138$, df = 2, P = 0.4244

Discussion

Looking for suspected changes in pain perception induced by rhizotomy, we tested the differences in TF latency between intact controls and unilaterally deafferented animals. Our working hypothesis was that deafferentation, manifested behaviorally by self-mutilation and probably followed by long-lasting increased painful sensation, might influence the pattern of nociception in deafferented animals.

The present study shows that unilateral forepaw deafferentation generally decreases the TF latency in rats. The tail-flick is probably a spinal reflex (Irwin *et al.* 1951) that is also modified by supraspinal structures, which modulate the activity of dorsal horn interneurons (Danneman *et al.* 1994). Because of the lack of any other published data concerning the TF measurements in deafferented animals, it is only possible to speculate about the origin of underlying mechanisms. Nociception is regulated by a number of control mechanisms, including spinal (segmental or heterosegmental), subcortical, cortical and hormonal.

The decrease in TF latency induced by deafferentation could result from primary and/or secondary hyperalgesia in neighboring regions, in the close vicinity of inputs of sectioned dorsal roots (Wiesenfeld-Hallin *et al.* 1993).

Neuronal hyperactivity following deafferentation has been recorded in the lateral cuneate nucleus (Kjerulf and Loeser 1973, Albe-Fessard and Rampin 1991) and demonstrates an increased central sensitization mechanism. In rats with the chronic pain syndrome, induced by dorsal rhizotomy, Lombard *et al.* (1979b) found spontaneous 10 Hz bursting activity mainly in the contralateral VPL thalamic nucleus. Increased neuronal activity is also accompanied by an increase in extracellular potassium concentrations. After unilateral deafferentation, we found increased resting levels of extracellular potassium concentration in specific thalamic nuclei of the ventrobasal complex in rats (Kříž and Rokyta 2000). These results support the hypothesis of central hypersensitization due to deafferentation (Hník *et al.* 1981, Kříž and Rokyta 2000).

Altered nociception after deafferentation can also result from changes of the descending antinociceptive system. On-cells of the rostral ventromedial medulla in rats are thought to exert facilitatory effects on spinal nociceptive transmission, whereas off-cells in the rostral ventromedial medulla seem to exert an inhibitory effect on nociceptive transmission (Fields *et al.* 1983, Bederson *et al.* 1990, Hernández and Vanegas 1993). In another study, Hernández *et al.* (1994) showed that noxious stimulation elicited potent activation of on-cells followed by depression even when stimulation continued and the TF response was retarded or abolished. These results show that the TF response can be modulated by brainstem structures.

Our results could also be interpreted as changes of the endogenous opioidergic system in highly autotomized rats (Hájek *et al.* 1992). It has been shown that the expression of abnormal pain-related behavior in some spinally injured rats was tonically suppressed by the spinal opioidergic system (Hao *et al.* 1998). The absence or dysfunction of opioid inhibition can be the underlying mechanism responsible for the interindividual differences in neuropathic pain-like behavior in injured rats (Franěk *et al.* 2004).

Unilateral deafferentation was followed by a highly variable manifestation of self-mutilating behavior, lasting many weeks after the rhizotomy, which might also be accompanied by hyperalgesia. These behavioral findings are concordant with alternating changes of TF latency in deafferented rats. Our behavioral results in rats are in agreement with the clinical observations that only some patients suffer from neuropathic pain after nerve injury. It has been shown by many authors that the occurrence and variability of neuropathic pain-induced behavior is influenced by genetic factors and it has also been noted frequently after experimental nerve injury

within one strain of animals (Inbal *et al.* 1980, Wiesenfeld and Hallin 1981, Devor and Raber 1990, Wiesenfeld-Hallin *et al.* 1993). Such variability is usually explained by technical, environmental, or even dietary factors (Zeltser and Seltzer 1994). Since all these factors were kept constant in our experiments, we suppose that there may be additional mechanisms underlying such variability.

The onset, quality and the degree of self-mutilation may be explained by comparing them with other models of chronic pain-related behavior. Denervated limbs in animals are often scratched and bitten resulting in self-injury (autotomy) of the denervated limbs (Wall *et al.* 1979). The development of autotomy by transection and ligation of sciatic and saphenus nerves was monitored by Devor and Raber (1990), who demonstrated the genetic aspects of autotomy in rats. Deafferented animals, according to these authors, expressed relatively high or low levels of autotomy. Their data suggested that the greater probability of self-mutilating behavior could be inherited as a single-gene autosomal recessive trait. No indication of sex linkage was found. The rats with high autotomy proved to be more sensitive than the low-autotomy rats when the methods of hot plate, tail immersion, and mechanical stimulation were used (Devor and Raber 1990, Devor and Seltzer 1999).

In the present study, unilateral deafferentation decreased the TF latency in both sexes; however, there was a substantial sex difference during a 10-week period, both in control and deafferented animals. Variations in the TF latency over time showed larger alterations within subsequent sessions in female controls if compared with the males and generally in the controls as compared to deafferented animals. One of the possible explanations could be the influence of the body weight increase in males during the long-lasting experiment. From the obtained data a close relation between body weight and TF latency is evident, and both are dependent on general somatic and metabolic states, which are highly individual. The developmental growth curves of rats showed sex differences with faster growth rate in females than in males (Lát 1969). The earlier termination of the phase of growth acceleration is probably responsible for earlier stabilization of the TF latency in females. These conclusions are valid only for the control animals. After deafferentation the decrease in the TF latency is not sex dependent.

In each session, the last values of TF latency were the shortest in both control and deafferented animals. In each session, the TF measurement was assessed from the most distal part of the tail-tip before continuing more proximally. The distal part of the tail is generally known to be the most sensitive part compared to the more proximal ones (Ness *et al.* 1987, Carstens and Wilson 1993). Nevertheless, we obtained the longest TF latencies on the tip. Moreover, the difference between the first and subsequent values was greater in deafferented animals than in the controls; especially in deafferented females with a higher degree of self-mutilation. This trend may be explained by at least two different processes: by reduced stress-induced analgesia during one session and/or by increased sensitivity to a painful stimulus.

In the present study, deafferented rats exhibited a shorter TF latency. This decrease appeared almost simultaneously with self-mutilation, sometimes preceding the self-mutilation by one week. In intact animals, the supraspinal structures control spinal cord activity, while their influence is changed after rhizotomy. This apparently represents a mutual type of regulation. In phantom limb pain patients, the neural processing is changed (Larbig *et al.* 1996). A lack of sensory afferentation from the deafferented forelimb may change the original pattern of neural processing in its central projection – brain neuromatrix (Melzack 1990, 1999), and in this way it may evoke "deafferentation pain behavior". From our data we suggest that the deafferentation produces a heterosegmental nociceptive effect, which is partly mediated by a supraspinal mechanism and/or a local spinal mechanism.

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Reprint requests

R. Rokyta, Department of Normal, Pathological and Clinical Physiology, Third Faculty of Medicine, Charles University, Ke Karlovu 4, 120 00 Prague 2, Czech Republic. Fax: +420 222492 3827. E-mail: Richard.Rokyta@lf3.cuni.cz