

**Title:**

**Algesia after epileptic seizure**

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**Short title:**

**Postictal algesia.**

## Summary

The consequences of epileptic seizures related to postictal inhibition in early postictal period include postictal analgesia. We studied this phenomenon over 96 hours following (flurothyl gas) induced seizures in adult male Wistar rats. Nociception of control (no seizure) and seized groups were tested using the plantar and von Frey hair tests. We determined latency of forepaw and hind paw reactions using plantar tests and the number of von Frey hairs reactions. Shortly after seizures, longer plantar test latencies were seen relative to the control group. Plantar test reaction times of forepaws were significantly shorter than in hind paws before the seizures. The effect disappeared post-seizure and surprisingly, it also disappeared at the corresponding time in controls; it reappeared after 48 h in the seizure group and after 24 h in controls. Differences in the von Frey hairs test occurred at 5 and 60 minutes post-seizure, however, these differences could not be explained by limb anatomy; although, different thermal and mechanical nociception mechanisms could be significant. The unexpected reactions in controls could be related to brief social and physical interactions between the two groups.

**Key words:** epileptic seizure, nociception, flurothyl, rat, postictal analgesia

## Introduction

Perception in general, including pain perception, is strongly influenced by the functional status of the brain. Epileptic seizures rarely elicit central pain (Scholz, et al. 1999). They do however, lead to a variety of postictal phenomena, including changes in somatosensory perception. Decrease in nociception (algesia) is usually designated as antinociception. Postictal antinociception (PA) is accepted as an aftereffect of many types of experimentally induced epileptic seizures (Coimbra, et al. 2001a, Coimbra, et al. 2001b, de Freitas, et al. 2004, Freitas, et al. 2005, Homayoun and Dehpour 2004, Portugal-Santana, et al. 2004). The degree of experimentally induced PA is usually evaluated with thermic tests, e.g. tail flick, plantar and hotplate tests (Bocheva and Dzambazova-Maximova 2004, Coimbra, et al. 2001a, Coimbra, et al. 2001b, de Freitas, et al. 2004, Freitas, et al. 2005). The PA effects are usually tested between 30 and 120 minutes post-seizure. We were unable to find information describing longer-term PA effects resulting from single epileptic seizures. There is some information in the literature, which along with our previous experiments, suggest a possibility of differential pain sensations in forepaws and hind paws in rats (Yamamotoová, et al. 2004). There are many models of epileptic seizures. Seizures are commonly induced by the application of specific drugs or by rhythmic electrical stimulation of the brain. Interpretation of results based on chemically induced epilepsy is complicated by the persistence of the convulsant agent even after the seizure has ended. Therefore, the model of generalized seizures induced by flurothyl - 2,2,2-trifluoroethyl ether (Eger, et al. 2002) vapors is advantageous because flurothyl is rapidly exhaled. The exact mechanism underlying its pro-convulsive effect is still under discussion (Hashimoto, et al. 2006).

In the presented experiments, we tested short and long term changes in sensory functions using the plantar test and the von Frey hairs test. We were also interested to see if there were any differences between forepaw sensation and hind paw sensation and, if these differences existed, to further determine how these differences were affected during the postictal period.

## Methods

The study was carried out on freely moving animals. The animals were raised under a controlled light cycle (12 hours light, 12 hours dark, lights on at 6:00 a.m.) with free access to food and water. All procedures were performed in accordance with the 3<sup>rd</sup> Faculty of Medicine, Charles University Ethical guidelines and in agreement with the Guidelines of the Animal Protection Law of the Czech Republic, which correspond to the respective EU regulations. The experimental protocol was approved by the Faculty Ethics Committee, and special care was taken to minimize animal suffering.

Adult naive male Wistar rats, obtained from the Anlab breeding farm, (N = 20) were randomly assigned to an experimental (N = 10) and a control (N = 10) group. The mean body weight of the rats was  $254 \text{ g} \pm 15.6$  (SEM). The experimental protocol was performed simultaneously on both the control animals and the experimental animals. The control animals were exposed to the same procedures as the experimental (i.e. transportation, cages of the same volume, housing). The control animals were tested (plantar test, von Frey hairs test) in the same room, in alternating succession with the experimental animals. Control and experimental animals were housed together in the same room during the entire experiment and were allowed olfactory, auditory and visual contact. With the exception of exposure to Flurothyl, the protocol for the control animals was identical to that of the seized group. Additionally, the duration of procedures was the same for both groups of animals.

*Flurothyl seizures.* The experimental animals were placed in an airtight chamber (14 l) with an external air supply. Flurothyl (di-(2,2,2,-trifluoroethyl)ether -  $\text{C}_4\text{H}_4\text{F}_6\text{O}$ ) was administered at a constant rate (30  $\mu\text{l}/\text{min}$ ; via an infusion pump onto a filter pad suspended at the top of the chamber) until a tonic-clonic seizure was observed. Immediately after the onset of the generalized tonic-clonic seizure, the air inside the chamber was exchanged. Seizures ceased within  $100.2 \pm 9.17$  s of evacuation of flurothyl vapors. Seizure latencies (time needed to induce a tonic-clonic seizure) were measured. The mean latency ( $\pm$  SEM) was  $8 \text{ min } 20 \text{ s} \pm 29.76 \text{ s}$ , which is similar to the values obtained in previous experiments. The animals were then transferred to another room (distant approx. 18 m) where the plantar tests and somatosensory sensitivity (von Frey hairs) tests were conducted. Control animals were placed in similar chamber (without flurothyl vapor) for 10 minutes. The chamber was not located in the same room where the seizures were evoked.

Special note: The time at which seizures were induced in the experimental group is an important reference point in time. Other measurements, for both the control and experimental group are made relative to this point in time. Recognize that terms like pre-seizure and post-seizure are used to reference this point, however, only the animals in the experimental group actually experienced a seizure.

The first post-seizure measurements were taken 5 minutes after the seizure ended. The next measurement was taken 60 min post-seizure. Subsequent measurements were taken at 24 hour intervals, i.e. 24, 48, 72 and 96 hours post-seizure. The same tests were performed on the control animals at the same, above mentioned, times.

#### *Plantar test*

Pain threshold for thermal stimulation was determined using plantar test equipment (Ugo Basile, Comerio, Italy). The latency (in seconds) of withdrawal to noxious thermal stimulation was measured. We tested all four paws of all animals. Animals were placed individually in a clear plastic box with a clear glass floor. The cut-off value was set at 22 s to prevent animal injury. The testing box was cleaned between each use.

To minimize any influence the pain threshold test might have on seizures, we measured threshold values, in both, the control and experimental group, 72 hours prior to seizure induction in the experimental group. With regard to this first measurement, the animals were allowed 10 min to adjust to the test environment before testing began.

#### *von Frey hairs*

In our experiments, the mechanical sensitivity of forepaws and hind paws was measured using following von Frey hairs: 4.08, 4.56, 5.07 and 5.88. The presence of mechanical response was evaluated in a binary fashion (0 or 1) in each animal for all hair thicknesses. Testing of each paw was done five times with each filament (hair). The average number of all mechanical responses in both groups (control and experimental) for all latencies before and after the seizure was established and statistically compared. This approach expressed changes in sensitivity. This method was adapted according to the description by Chaplan (1994) and Coddere (2004) (Coderre, et al. 2004, Chaplan, et al. 1994).

#### *Statistics*

For evaluation of results, we used the two-way repeated-measures ANOVA and one-way repeated-measures ANOVA (with Tukey's post-tests) measures. The normality distribution was pretested for each measurement.

## **Results**

#### *Plantar test*

Control measurements were performed in all animals. We observed significantly different latencies in forepaw and hind paw movements, measured after nociceptive stimulation. After 3 days (72 hours) were animals divided in two groups and flurothyl seizures were elicited in experimental group. The significant difference between forepaw and hind paw latencies disappeared in this group following seizures, but reappeared 48 hours later (Tab. 1). Surprisingly, the difference between hind paw and forepaw sensitivity also disappeared in the control group (Tab. 1, Fig 1) when the control animals were tested together with seized animals – see methods. It has been observed at 5 min and 60 min after the seizures were elicited in experimental animals.

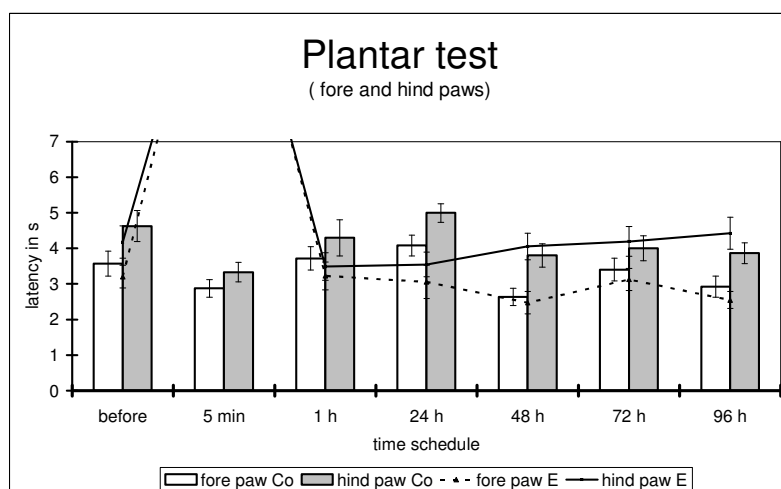
**Table 1.** Comparison of mean latencies (plantar test) between forepaws and hind paws and differences between the mean numbers of reactions to von Frey hairs

PLANTAR TEST							
Fore- vs hind paws	before seizure	after seizure in experimental group					
		5 min	1 h	24 h	48 h	72 h	96 h
Experiment							
Forepaws	3.21±0,32	14.41±2,61	3.23±0.39	3.06±0,47	2.47±0.31	3.13±0.31	2.55±0.24
	*	NS	NS	NS	*	*	*
Hind paws	4.18±0,45	14.07±2,59	3.49±0.39	3.55±0.35	4.05±0.37	4.20±0.42	4.43±0.45
Control							
Forepaws	3.57±0,35	2.88±0,25	3.72±0,33	4.08±0.29	2.64±0.24	3.40±0.32	2.93±0.3
	*	NS	NS	*	*	*	*
Hind paws	4.63±0,44	3.33±0,28	4.30±0,51	5.00±0.26	3.8±0.33	4.00±0.35	3.87±0.29
VON FREY HAIRS							
	before seizure	after seizure in experimental group					
		5 min	1 h	24 h	48 h	72 h	96 h
Experiment	7.9±1.88	0	2,4±0.85	5,7±1.8	9.1±2.28	8.1±2.27	7.1±1.75
	NS	*	*	NS	NS	NS	NS
Control	8.8±2.176	10.4±2.51	8.9±2.51	7.0±2.08	7.5±2.52	9.6±2.21	9.5±2.00
Experimental group - significant differences							
Before		*	*	NS	NS	NS	NS
5 min	*		*	*	*	*	*
1 h	*	*		NS	*	*	NS

*Plantar test: numbers represent mean latencies ±SEM in seconds; von Frey Hairs: mean numbers of reactions ±SEM; \* p < 0.05; NS non significant*

In both forepaws and hind paws, significantly longer latencies were found 5 minutes post-seizure in the experimental group relative to the control group; at 24 h post-seizure, the latencies were shorter in experimental group relative to the control group.

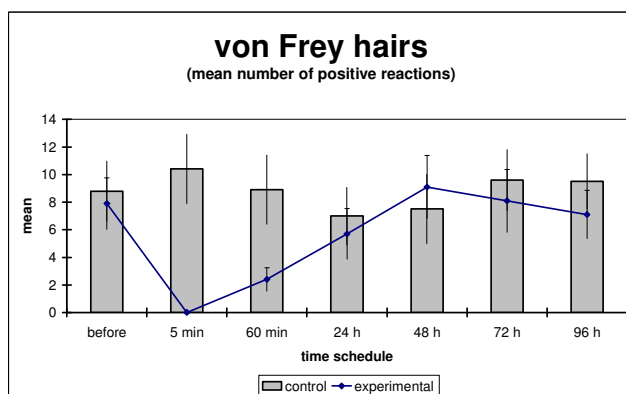
We also compared the latencies with repeated measures inside each group for the both fore and hind paws. We used one-way repeated-measures ANOVA. Significant differences in control group: hind paws  $P < 0.0005$ ;  $F = 4.801$ ;  $r^2 = 0.2017$ ;



**Fig. 1.** Comparison of nociception of forepaws and hind paws in plantar tests. Mean latencies in  $s \pm SEM$ , Co - control and E – experimental animals.

experimental animals. These prolongations in the control group were even more conspicuous when comparing plantar test latencies measured at 5 minutes post-seizure to the latencies at later scheduled test times.

#### *von Frey hairs*



**Fig. 2.** Mean number ( $\pm SEM$ ) of reactions to von Frey hairs elicited 3 days before, and 5 min, 1, 24, 48, 72 and 96 hours after flurothyl induced seizures.

$< 0.0005$ ;  $F = 4.801$ ;  $r^2 = 0.2017$ ;

forepaws  $P < 0.0001$ ;  $F = 6.979$ ;  $r^2 =$

0.2686. In experimental group: hind

paws  $P < 0.0001$ ;  $F = 38.27$ ;  $r^2 =$

0.6683; forepaws  $P < 0.0001$ ;  $F =$

39.09;  $r^2 = 0.6729$ . In a post hoc test in

the experimental group, we observed a significant prolongation of latencies, in

both forepaws and hind paws, but only at 5 minutes post-seizure. However, in

the control group, significant

prolongations of latencies were seen at

1 hour and 24 hours after the seizure in

We did not observe any statistically significant differences between reactions of forepaws and hind paws in control or in experimental animals.

However, we did observe significant differences between the experimental group and the control group in the mechanical responses to pressure elicited by all previously mentioned diameters of von Frey hairs ( $p < 0.005$ ,  $F = 8.787$ ; two-way repeated-measures ANOVA). This

difference was mostly apparent at 5 and 60 minutes post-seizure (Fig. 2).

In the group of control animals we did not find any significant differences related to repetition of measurements ( $F = 1.118$ ;  $r^2 = 0.1105$ ; one-way repeated-measures ANOVA). In the experimental group of animals we observed significant differences in means ( $P < 0.0001$ ;  $F = 6.669$ ;  $r^2 = 0.4256$ ; one-way repeated-measures ANOVA). Differences between individual measurements (times relative to the start of experiment) - see Fig. 2 and Table 1.

## Discussion

Antinociception occurs following epileptic tonic-clonic seizures in both experimental animals and humans. Other sensory inputs are also altered. Mechanisms involved in postictal antinociception remain to be explained in detail. Duration of sensory changes may correlate with postictal changes in transmitter systems.

The postictal period is characterized by many short and long-lasting functional changes in the brain. Postictal inhibition is a prominent phenomena directly linked to the end of the seizure. It is difficult to induce another epileptic seizure during this period (Mares, et al. 1982). Some functional changes during the postictal phase are usually explained by the persistence of the increased inhibition that occurs at the end of a seizure. Changes in extracellular concentrations of various substances may also play an important role. Many of these substances could be related to antinociception; for example increases in the extracellular concentration of adenosine (During and Spencer 1992, Kulkarni, et al. 1994, Kulkarni, et al. 1997), opioid peptides or amino acids (Engel and Rocha 1992, Halonen, et al. 1992, Rocha, et al. 1991, Velisek and Mares 1992). Adenosine decreases nociceptive perception in both animals and in humans. It acts both centrally and peripherally (Hayashida, et al. 2005, Chizh, et al. 2004). GABA has been shown to have anticonvulsant as well as antinociceptive effects (McGaraughty, et al. 2005). Several compounds formerly considered as GABAergic are specifically used for the treatment of neuropathic pain, e.g. gabapentin and pregabalin with different mechanism for each of them (Backonja 2002, McGaraughty, et al. 2005). These compounds bind to specific subunit of voltage gated calcium channels and modulate release of transmitters (Maneuf, et al. 2006, Taylor, et al. 2007). Therefore, there is little doubt that opioid peptides are involved in postictal antinociception (Portugal-Santana, et al. 2004).

Our results supports the hypothesis that antinociception is closely related to the above-mentioned substances. It has been demonstrated that changes in their concentrations occur after epileptic seizures and these changes have been shown to last for tens of minutes (de Freitas, et al. 2004, Freitas, et al. 2005, Portugal-Santana, et al. 2004). Consistent with the above, we observed that the period of decreased sensitivity following a seizure was short. On the other hand, comparison with control animals revealed an interesting increase in sensitivity 24 hours post-seizure. These results suggest the existence of long lasting the existence of long lasting changes that cannot be explained by changes in concentrations of the above

mentioned substances. In our other experiments with flurothyl induced seizures (Mareš, et al. 2004), we observed changes in learning, associated with a water maze, 24 hours post-seizure. Because of the oxidative stress related to seizures (Patel 2002) these longer-lasting changes could be related to changes in free radical concentrations that occur during a seizure. Oxidative stress, as measured by free radicals and singlet oxygen, is one possible explanation for increased nociception following either acute or chronic painful stimulation (Rokyta, et al. 2003, Rokyta, et al. 2004). Additionally, it has been shown that the increased nociception can be reduced with antioxidants. The anti-nociceptive mechanism in our model of postictal changes is dominant during the early postictal period. The question is whether the increase of reactive oxygen species (ROS) levels during a seizure may cause increased sensitivity 24 h post-seizure relative to controls. It is known that ROS act as mediators for activation of processes connected to apoptosis and also as growth signals (Min, et al. 2006). It may elucidate some delayed functional changes. Whether ROS have some immediate direct functional effect is not clear.

The difference in sensitivity of forepaws and hind paws in intact rats is rarely mentioned in the literature (Morgan and Whitney 1996, Yamamotová, et al. 2004). The simplest explanation of this phenomenon is that rats are bearing more weight on the hindpaws. In our experiments rats were tested in the same position but after the seizure the latencies were not different (tab. 1). Our results support the suggestion of a different mechanism of nociception for forepaws and hind paws (Yamamotová, et al. 2004). This has been explained by the involvement of different receptor-transmitter systems. Nociception in forepaws is preferentially influenced by the opioid antinociceptive system, while nociception in hind paws is more influenced by the non steroidal anti-inflammatory drugs (NSAIDs) system (Hirate, et al. 2006, Lewis 1986). That the forepaw / hind paw difference was still absent 24 hours post-seizure, in our experimental group, indicates the fragility of these systems of sensation. The loss of this difference in nociceptive sensitivity, in the control group, at the same time that the experimental animals underwent induced seizures and entered the postictal period was surprising. As was already mentioned, in the method section, the experimental and control animals were in contact during the postictal period. We hypothesize that olfaction was, most probably, the sensory modality responsible for the coincidental and analogous change in nociceptive sensitivity seen in the controls (Kavaliers, et al. 1998). However, it does not explain the nature of the phenomenon. The control group also exhibited changes in latencies of reactions of both forepaws and hind paws in the plantar test when compared with subsequent measurements. The explanation of this phenomenon could be similar, i.e. olfactory, visual and auditory contact with the experimental group.

The cranial - caudal difference between forepaws and hind paws revealed by the plantar test was not observed when the animals were tested using von Frey hairs. Anatomical differences alone are not sufficient to explain the results seen in the von Frey test. Changes in latencies post-seizures lasted longer than in plantar test. Additionally, there was no increased sensitivity in experimental animals 24 hours post-seizures nor was there decreased sensitivity in control animals as was seen in the plantar test at the corresponding



time. This supports the idea that both modalities (somatosensory and thermal) are governed by different systems.

Our results demonstrate that nociception, as measured by the plantar test, can be influenced, by a simple short epileptic seizure, for up to 24 hours post-seizure. Hypoesthesia, relative to controls, occurred shortly after seizures (5 min. post-seizure) in experimental animals and had transitioned to hyperesthesia, relative to controls, 24 hours post-seizure. This result must be confirmed by an independent experiment. We used plantar test to estimate the difference in pain perception between forepaws and hind paws. This difference disappears 24 hours post-seizure. Differences between forepaws and hind paws were not observed during testing with von Frey hairs. This shows that the difference between the forepaws and hind paws can not be explained by differences in anatomy between paws. Instead it suggests that different mechanisms of thermal and mechanical nociception might play an important role. The reaction of the control group to the induced seizures in the experimental group illustrates a sensitivity of perception along other parameters. Further research into this intriguing phenomenon may result in some very interesting and surprising findings.

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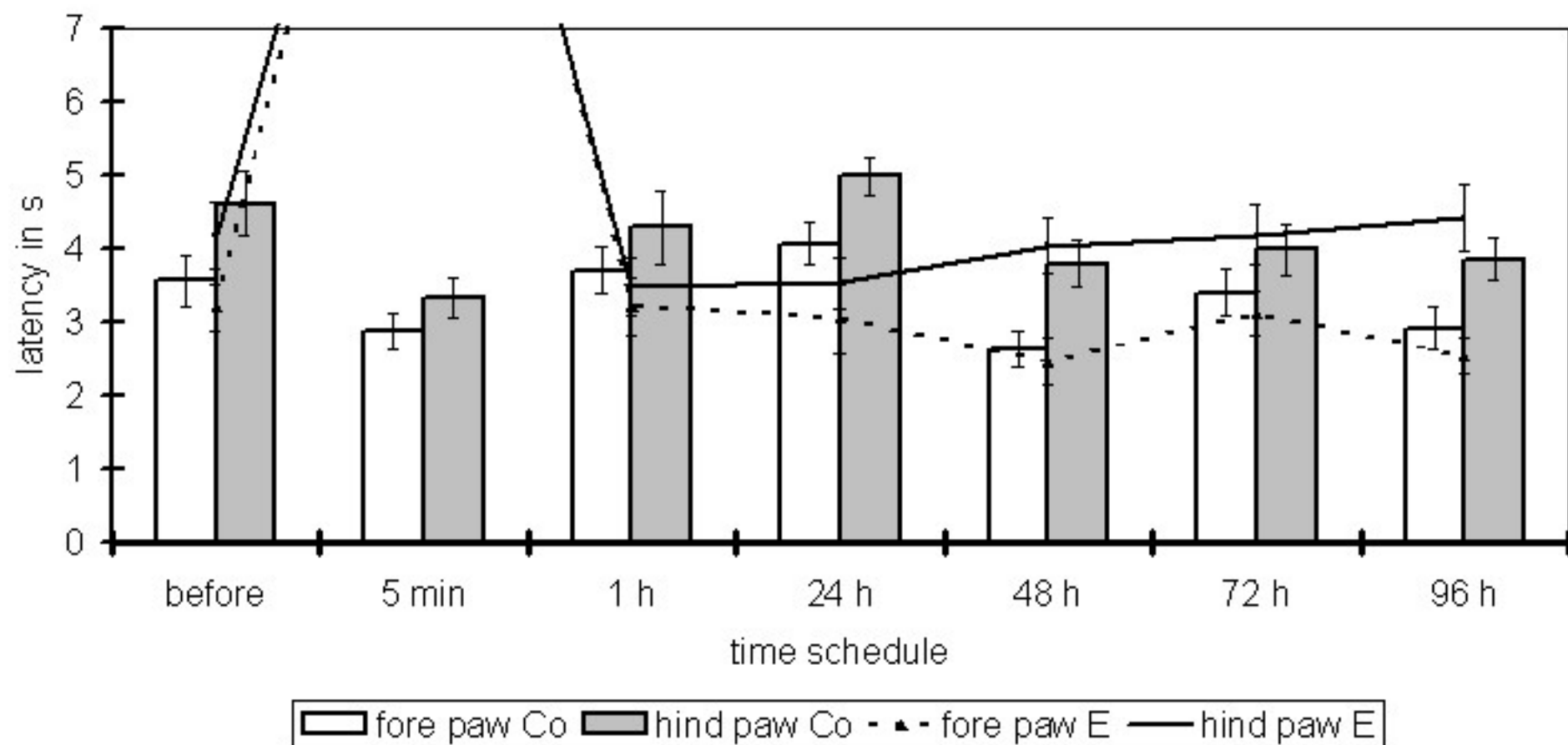
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# Plantar test

( fore and hind paws)



# von Frey hairs

(mean number of positive reactions)

