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NMDA receptor-dependent long term hyperalgesia after tail amputation in mice

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Abstract

Amputation of the mouse tail tip (2.5 cm) caused long term thermal and mechanical hyperalgesia in the remaining part of the tail. Hyperalgesia of the hindpaw to noxious heat (55°C) and cold (0°C) stimuli were also observed. Hyperalgesia at both the tail and hindpaw had a rapid onset ($\leq$ 30 min) and long lasting ($\geq$ 7 days) effect. Skin temperature of the remaining tail or hindpaw was not significantly affected by the amputation. Heat injury of the tail in normal mice induced short but not long term hyperalgesia ($\leq$ 48 h). Intrathecal pretreatment with NMDA receptor antagonists significantly attenuated long term hyperalgesia caused by tail amputation. These results strongly suggest that spinal NMDA receptors are critical for the induction of hyperalgesia by tail amputation, and the current mouse model may prove useful for investigating mechanisms of persistent pain after amputation.

Keywords: Phantom pain; (Mouse); Tail amputation; Hyperalgesia; Cold pain

1. Introduction

Tissue or nerve injury often induces persistent pain which can last for several days or months (for reviews, see the works of Coderre et al. (1993), Dubner and Ruda (1992), Willis (1992), Meller and Gebhart (1993)). Hyperalgesia (enhanced response to noxious stimuli) and allodynia (pain induced by non-noxious stimuli) are two consequences associated with persistent pain. Hyperalgesia to thermal and mechanical stimuli are usually observed at the injury site, although hyperalgesia can also be demonstrated in uninjured tissue (called secondary hyperalgesia) (see the works of Woolf (1983) and Kayser and Guilbaud (1987); for a review, see also the work of Guilbaud et al. (1992)).

Amputation of a part of the body, for various medical reasons (see the works of Weinstein (1994) and Wesolowski and Lema (1993)), often leads to persistent pain which lasts for months to years, including stump and phantom pain. Mechanisms of persistent pain after the amputation are not well understood. During previous investigations of synaptic plasticity using genetic mutant mice (Qi et al., 1996; Brandon et al., 1995), where the tip of the tail was commonly amputated and used for genotyping mutant mice (see the work of Matthes et al. (1996); see also Methods section in the work of Sora et al. (1997)), I noted that tail amputated mice appeared hypersensitive. Considering the potential usefulness of mutant mice to explore molecular mechanisms of nociception and analgesia, it is important to systematically characterize potential plastic changes in nociceptive transmission after removal of the tail tip. In this study, I measured thermal (heat and cold) and mechanical nociceptive thresholds of the tail and hindpaw before and after amputation of the tail tip. A preliminary report of these data has been made (Zhuo, 1997).

2. Materials and methods

2.1. Animals and surgical procedure

The experimental procedures were approved by the Animal Studies Committee at Washington University and conform to the guidelines of the International Association

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for the Study of Pain (Zimmermann, 1983). Male, 4–6 week old C57BL/6j mice were used (Jackson). Some mice were ordered from another supplier (Harlan; C57BL/6NHsd), and similar results were obtained. The data were pooled. The room temperature was always maintained at 20°C. Tail amputation was performed in mice anesthetized with 2–3% halothane (Ohio Medical Products) delivered via a nose cone (with 30% O_2 balanced with nitrogen; Puritan Bennett, KS). Amputation did not cause any movement of the tail or body, indicating that the depth of anesthesia was sufficient. The normal length of the mouse tail is about 6.5 to 7.5 cm, and only a tip of the tail (2.5 cm) was removed using surgical scissors. A drop of crazy glue was used to stop bleeding, and the mice usually recovered from anesthesia within 5–10 min. In a control group, mice were anesthetized with halothane for the same period of time without any surgery. For inducing tail heat injury, the protocol was modified from a previous study (Woolf, 1983). Mice were similarly anesthetized as those in amputation experiments, and the tip of the tail (2.5 cm) was immersed in hot water (55°C) for 20 s. The tail skin would usually turn red after the heat injury.

2.2. Mechanical stimulation of the tail

Mechanical (pressure) stimuli were used to produce a reflexive withdrawal of the tail. Von–Frey-like stimulation with nylon monofilaments (Semmes-Weistein Anesthesiometer; Stoelting, Wood Dale, IL) was applied to one site on the dorsal surface of the tail between 2 to 3 cm from its root end (Fig. 1). Filaments of different thickness, requiring different pressures (2.0–85 g) to bow the filament, were applied sequentially until the tail was reflexively withdrawn: this was considered the nociceptive mechanical (pressure) withdrawal threshold. Each trial consisted of three to four measurements taken at a few second intervals, with the baseline mechanical threshold being the mean of two or three trials taken at 3 min intervals. Unlike rats, mice generally were active and difficult to hold steady, so a small cloth bag was made to keep a mouse inside during the test, the tail being exposed. Measurements were started when mice were relatively comfortable inside the bag. It was difficult to obtain reliable measurements for mechanical withdrawal thresholds of the hindpaw or forepaw. Thus, only the mechanical withdrawal of the tail was measured.

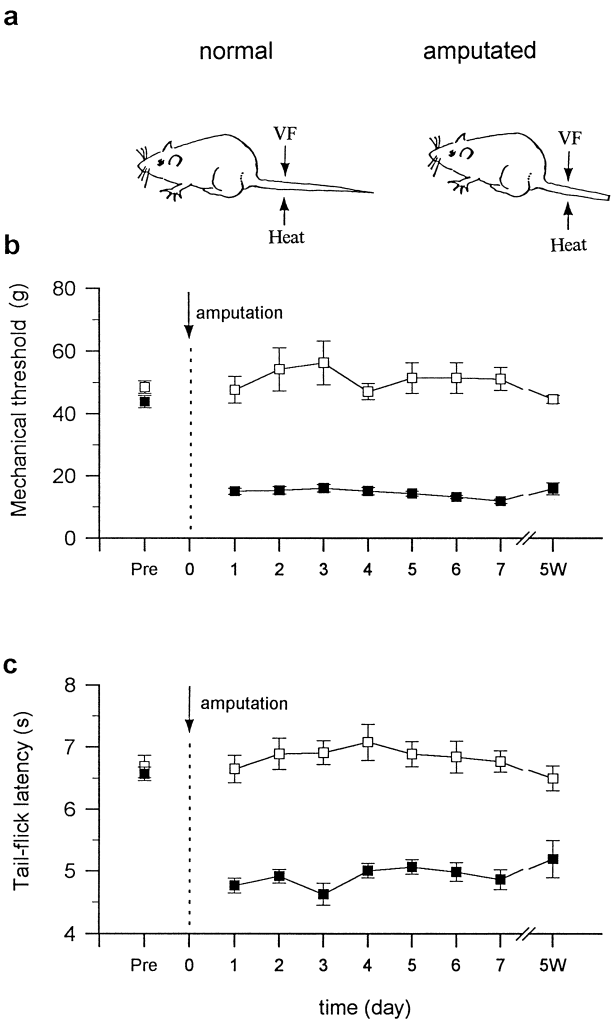

Fig. 1. Long term mechanical and thermal heat hyperalgesia induced by amputation of the tail tip. (a) A diagram eliciting the tail site for delivering noxious heat in tail-flick reflex (Heat: indicated by lower arrows) and mechanical pressure stimuli (VF: indicated by upper arrows) in normal and tail amputated mice. (b) Mechanical withdrawal thresholds before and after amputation (indicated by arrow) of the tail tip (filled squares). Data from control mice are also shown (open squares). The mean baseline mechanical withdrawal thresholds ('Pre' in figure) were 48.5 ± 2.0 g in control (open squares) and 43.8 ± 2.0 g in tail amputated (filled squares) mice. (c) Tail-flick latencies before and after amputation (indicated by arrow) of the tail tip (filled squares). Data from control mice are also shown (open squares). The average baseline tail-flick latencies ('Pre' in figure) were 6.7 ± 0.2 s in normal (open squares) and 6.6 ± 0.1 s in amputated (filled squares) mice. Data are presented as mean ± 1 S.E.M.

2.3. Tail-flick reflex

The spinal nociceptive tail-flick reflex was evoked by noxious radiant heat (8 mm in diameter) focused on the underside of the tail (Columbia Instruments; Columbus, OH) 2 to 3 cm from the tail's root end. The latency for reflexive removal of the tail from the heat was measured by a digital photocell timer to the nearest 0.1 s. The baseline tail-flick latency was set between 5–7 s by changing the intensity of the heat, which induced a sharp, burning pain sensation within 4 s of being applied to the author's finger. The baseline tail-flick latency was the mean of two or three measurements taken at 3 min. Similar to mechanical withdrawal tests, mice were kept inside a cloth bag during the tail-flick measurements. Since tail-flick response latency may be affected by skin temperature of the tail, skin temperature of the heated site was measured

using a digital thermometer with a small probe (~1.5 mm in diameter).

2.4. Hot-plate test

The hot-plate reflex was measured on a metal plate at 55°C (Columbia Instruments; Columbus, OH). Nociceptive responses were licking, lifting a hindpaw or jumping off the hot-plate, and the latency for response was recorded with a digital timer to the nearest 0.1 s. All mice tested showed behavioral responses within 25 s. Mice were removed from the chamber immediately after the first response. The baseline hot-plate latency was an average of three or four measurements taken at 10 min intervals. In some experiments, mice were tested on a 50°C hot-plate in order to test potential changes in nociceptive thresholds. Skin temperature of the hindpaw was measured as in the tail-flick test.

2.5. Cold-plate test

A cold-plate (frozen ice in a plastic container, 21 cm diameter × 21 cm height) was used to study behavioral responses to noxious cold stimuli. Temperature of the iced surface was monitored with a digital thermometer and maintained at 0°C by placing ice around the outside of the container. Response latencies on the cold-plate were similarly measured using the same criteria as in the hot-plate test (see above). Unlike the hot-plate test, mice showed significant individual variation within the first few measurements, with some mice developing a sensitized response to the cold-plate (see Section 3). Thus, all animals were used, including those that did not have a cold-plate response (≥ 60 s) on the first test. Cold stimuli (0°C) are believed to be noxious and to activate specific cold-nociceptors (LaMotte and Thalhammer, 1982; Yarnitsky and Ochoa, 1990), so changes in cold-plate response latency indicate cold hyperalgesia.

2.6. Intrathecal drug administration

Intrathecal drug injection was carried out as described by Hylden and Wilcox (1980) with some modifications. To avoid possible stress induced during intrathecal injection, mice were always anesthetized with halothane (2%) during injection. The injection tip was a steel tip of a 30-gauge needle connected with a PE-10 tube. The PE-10 tube then was connected to a 50-μl syringe. After the injection, it took 2–3 min for mice to recover. The volume of the injection was 5 μl and saline was used as a control. Drugs were always prepared freshly on the day of experiments and dissolved in saline. The doses of antagonists, (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[a,d] cyclohepten-5,10-imine hydrogen maleate (MK801) (7.4 nmol) or (±)-2-amino-5-phosphonopentanoic acid (AP-5) (25 nmol), were selected in part based on previous pharmacological reports from other investigators and on the effectiveness in abolishing NMDA-mediated postsynaptic currents of dorsal horn neurons evoked by electrical stimulation of primary afferent fibers in in vitro spinal cord slices using whole-cell patch recording techniques (Zhuo and Li, 1997). MK801 at higher doses was not used since it induced motor side-effects.

2.7. Experimental testing procedure

Different time frames were used in three sets of experiments. In the first set of experiments for measuring long term hyperalgesia induced by the amputation, behavioral responsive thresholds were measured before and 7 days after the amputation at 1 day interval. In some animals, in order to determine the early onset and duration of hyperalgesia, responses were also measured at 30 min or 5 weeks after the amputation (see Section 3). In the second set of experiments for comparing hyperalgesia induced by the amputation with tail heat injury, behavioral responsive thresholds were measured at 30 min or 24 h after heat

Table 1
Long-term hyperalgesia induced by amputation of the mouse tail tip (2.5 cm)

		n	Baseline	After treatment[a]	% of Baseline
Mechanical withdrawal	control	9	48.5 ± 2.0 g	51.3 ± 3.7 g	106.9 ± 2.5
	amputated	14	43.8 ± 2.0 g	19.0 ± 4.1 g*	34.2 ± 1.8
Tail-flick reflex	control	10	6.7 ± 0.2 s	6.9 ± 0.1 s	103.0 ± 0.9
	amputated	14	6.6 ± 0.1 s	5.1 ± 0.3 s*	75.1 ± 1.0
Hot-plate test	control	14	15.7 ± 1.2 s	12.9 ± 0.6 s	96.8 ± 2.1
	amputated	14	13.6 ± 1.0 s	10.2 ± 0.4 s*	81.6 ± 4.0
Cold-plate test	control	23	33.7 ± 3.0 s	23.5 ± 2.2 s	73.0 ± 5.0
	amputated	11	43.7 ± 5.4 s	6.2 ± 0.4 s*	16.9 ± 1.0
Tail temperature	control	10	21.2 ± 0.3°C	21.7 ± 0.1°C	102.5 ± 1.6
	amputated	7	22.7 ± 0.2°C	23.2 ± 0.2°C	102.1 ± 0.8
Hindpaw temperature	control	10	28.8 ± 0.4°C	28.7 ± 0.1°C	99.9 ± 1.1
	amputated	7	29.0 ± 0.4°C	28.3 ± 0.4°C	97.7 ± 1.7

Data are presented as mean ± 1 S.E.M.
[a] Data represent the mean of measurements during day 1 and day 7.
* $P < 0.05$ compared with baseline values before tail amputation or control animals.

injury of the tail tip. In the last set of experiments for investigating the effects of NMDA receptor antagonists, behavioral responsive thresholds were measured before and 6 days after the amputation at 1 day interval.

2.8. Data and analysis

Data are presented as the mean value ± 1 S.E.M. Hyperalgesia is also presented as a percentage of the control. Data for the tail-flick, hot-plate and cold-plate are presented as the latency (*s*), and data for the mechanical withdrawal of the tail as the force (in g) necessary to bow the filament. The effects of tail amputation on behavioral nociceptive thresholds were evaluated by one-way analyses of variance (ANOVAs) and post hoc comparison Dunn's method or Newman–Keuls tests. Comparisons of control treatment or antagonist pretreatment on long term hyperalgesia were analyzed by two-way ANOVAs (treatment × time) and post hoc comparison Tukey test. Student's test was applied for comparisons between paired groups. In all cases, $P < 0.05$ was considered significant.

3. Results

3.1. Hyperalgesia at the remaining part of the tail

Long term mechanical hyperalgesia of the remaining part of the tail was observed after amputation of the tail tip (Fig. 1 and Table 1; $n = 14$ mice, $F(10, 135) = 62.18$, $P < 0.001$). In order to determine the early onset of hyperalgesia, the mechanical withdrawal threshold was also measured at 30 min after amputation in 11 mice. The mechanical withdrawal threshold of the tail was significantly decreased as compared with baseline thresholds ($P < 0.05$). Mechanical withdrawal thresholds were not significantly affected in control mice ($n = 9$; $F(7,71) = 0.43$; see Fig. 1 and Table 1). The mechanical withdrawal threshold after tail amputation was significantly lower than control mice and that before amputation ($F(1,176) = 478.4$, $P < 0.001$; Table 1). In 10 mice, mechanical withdrawal thresholds were measured 5 weeks after tail amputation. Mechanical thresholds remained significantly decreased (mean 14.3 ± 1.4 g) as compared with thresholds before the amputation or thresholds of control animals ($P < 0.05$ in each case).

Hyperalgesia was not limited to mechanical sensory transmission, as long term hyperalgesia of a spinal nociceptive tail-flick reflex was also observed (Fig. 1 and Table 1; $n = 14$, $F(10,138) = 10.59$, $P < 0.0001$). Tail-flick latencies were significantly decreased at 30 min after tail amputation compared with baseline or control animals ($P < 0.05$ for each case). In control normal mice ($n = 10$), tail-flick latencies showed no significant change over the same period of time ($F(7,79) = 0.38$; Table 1 and Fig. 1). The tail-flick latency after the amputation was significantly lower than control mice and that before amputation ($F(1,184) = 306.5$; $P < 0.001$, Table 1). In six mice, tail-flick latency was measured for 5 weeks, with tail-flick latency remaining significantly decreased at 5 weeks after the amputation (mean 5.0 ± 0.3 s; $P < 0.05$ compared

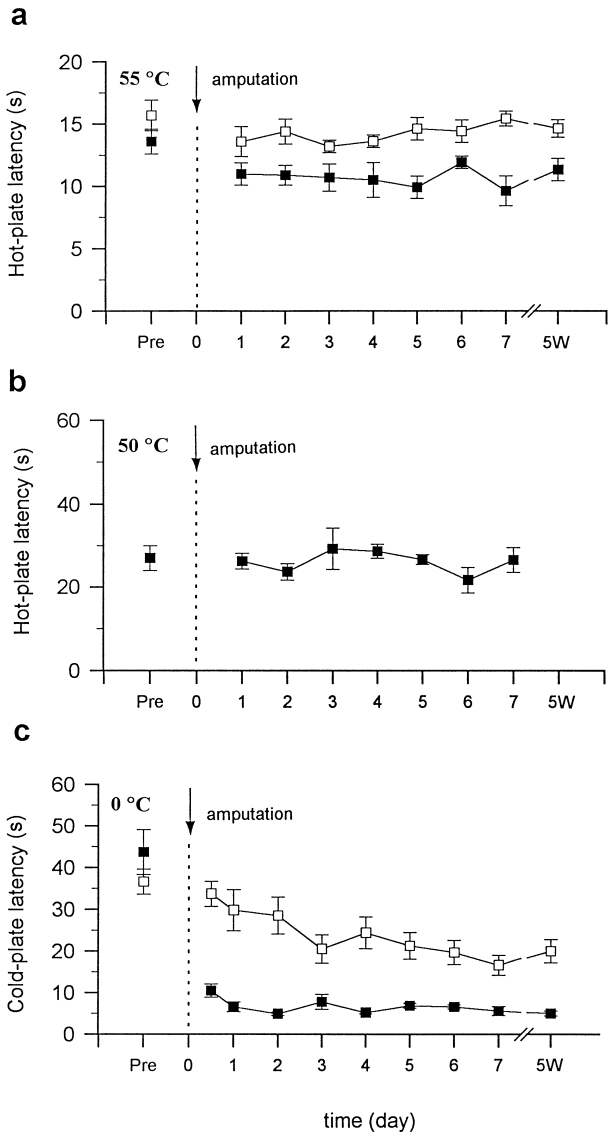

Fig. 2. Long term heat and cold hyperalgesia of the hindpaw induced by amputation of the tail tip. (a) The hot-plate latencies at 55°C before and after amputation (indicated by arrow) of the tail tip (filled squares). Hot-plate latencies after the amputation were significantly decreased except at 6 days after the amputation ($P < 0.05$). Data from control mice are also shown (open squares). The average baseline hot-plate latencies ('Pre' in figure) were 15.6 ± 1.6 s in normal (open squares) and 13.6 ± 1.0 s in amputated (filled squares) mice. (b) The hot-plate latencies at 50°C before and after amputation (indicated by arrow) of the tail tip (filled squares). The average baseline HP latency ('Pre' in figure) was 27.0 ± 3.0 s. (c) The cold-plate latencies at 0°C before and after amputation (indicated by arrow) of the tail tip (filled squares). Data from control mice are also shown (open squares). The average baseline cold-plate latencies ('Pre' in figure) were 36.6 ± 3.0 s in normal (open squares) and 43.7 ± 5.4 s in amputated (filled squares) mice. Data are presented as mean ± 1 S.E.M.

with tail-flick latency in control mice or that before the amputation).

3.2. Hyperalgesia in the hindpaw

Heat hyperalgesia of the hindpaw was evaluated using the hot-plate test. Surprisingly, hot-plate latencies were significantly decreased after tail amputation (Fig. 2a and Table 1; $n = 14$, $F(10,130) = 2.30$, $P < 0.05$). In seven mice tested, hot-plate latencies were significantly decreased at 30 min after tail amputation ($P < 0.05$). Hot-plate latencies were not significantly affected in control mice ($n = 14$, $F(7,111) = 0.41$; Table 1). The hot-plate latency after the amputation was significantly lower than control mice and that before the amputation ($F(1,210) = 36.36$; $P < 0.001$, Table 1). In five mice, hot-plate latencies were measured for 5 weeks after the amputation and was not significantly different from the pre-baseline level or control mice. In 10 experiments, mice were also tested at 50°C hot-plate. Hot-plate latencies were not significantly affected ($F(6,69) = 0.84$; Fig. 2b).

Cold hyperalgesia of the hindpaw was observed using a cold-plate test ($n = 11$, $F(9,98) = 2.83$, $P < 0.001$) (Fig. 2 and Table 1). In four mice tested, cold plate latencies were significantly decreased at 30 min after tail amputation ($P < 0.05$). In control mice ($n = 23$), cold-plate latency was measured for the same period of the time. The mean cold-plate latency measured on the second day was significantly decreased ($P < 0.05$, Fig. 2). The cold-plate latencies remained consistent for another 6 days of measurements (Fig. 2). The cold-plate latency after the amputation was significantly lower than control mice and that before the amputation ($F(1,267) = 44.33$, $P < 0.001$; Table 1).

Unlike the hot-plate test, responses of control mice in the cold-plate test were variable. For example, in nine of

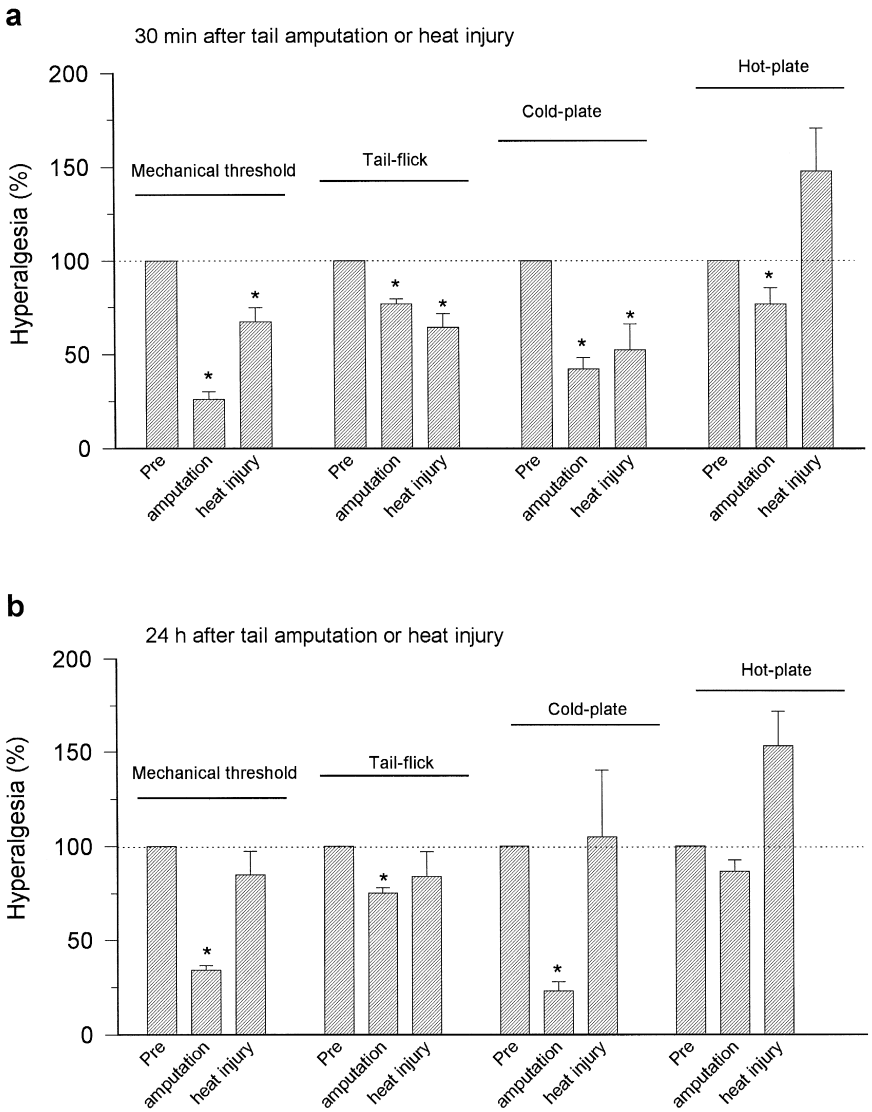

Fig. 3. Hyperalgesia induced by tail amputation and tail heat injury. (a) Hyperalgesia at 30 min after tail amputation and heat injury of the tail tip (55°C; 20 s). (b) Hyperalgesia at 24 h after the treatments. For the purpose of comparison between tail amputation and heat injury, data are presented as percentage change from the baseline. Data are presented as mean ± 1 S.E.M. $* P < 0.05$.

the 23 mice tested, cold-plate latency (baseline 29.0 ± 5.6 s) was not significantly changed over the 7 days of measurement. In the other 14 mice, cold-plate latencies were significantly decreased ($P < 0.001$). However, the cold-plate latency of the sensitive group ($n = 14$) at 7 days after the control treatment was still greater than that of tail amputated mice ($P < 0.05$), suggesting that cold hyperalgesia after amputation can not be simply due to sensitization caused by repetitive measurements.

Another six mice were tested on a cold-plate at 15°C 2 weeks after tail amputation. The mean cold-plate response latency at 0°C plate was 4.8 ± 2.0 s; the same mice did not show behavioral responses on a 15°C plate (> 60 s). These results suggest that the effect is temperature-dependent. In three mice, cold-plate latencies at 0°C were measured 5 weeks after tail amputation, and the mean cold-plate latency was 9.0 ± 1.6 s ($P < 0.05$ compared with cold-plate latency before amputation).

3.3. Other observations

Skin temperature of the tail stump or tail in normal ($n = 7$) mice was monitored during the same period of time. Neither the baseline skin temperature of the tail nor hindpaw was significantly affected by tail amputation (Table 1). After amputation of the tail tip, mice displayed normal appetites and motor behaviors and were indistinguishable from normal mice except for the shorter tail. Similar weight increases at the end of 7 days after the treatment (amputation or control) were observed in both mice with amputated tails ($n = 7$) and in normal mice ($n = 10$). Neither spontaneous pain nor vocalization was recorded during the observation period (from 1 day to 5 weeks), and the mice performed different types of behavioral functions without difficulty, such as walking, climbing and jumping.

3.4. Hyperalgesia induced by heat injury

In order to compare long term hyperalgesia induced by the amputation with hyperalgesia caused by tissue injury, nociceptive thresholds were studied in mice after heat injury of the tail tip (2.5 cm). Heat injury of the distal tail caused rapid mechanical and heat hyperalgesia of the remaining part of the tail and cold hyperalgesia of the hindpaw ($n = 6$) at 30 min after the injury (Fig. 3). Both mechanical withdrawal thresholds and tail-flick latencies were significantly decreased. Hot-plate latencies were not significantly affected, while cold-plate latencies were significantly decreased. Unlike hyperalgesia after the amputation, hyperalgesia induced by heat injury was short-lasting. All response latencies/mechanical thresholds returned to the pre-injury level within 24 h (Fig. 3).

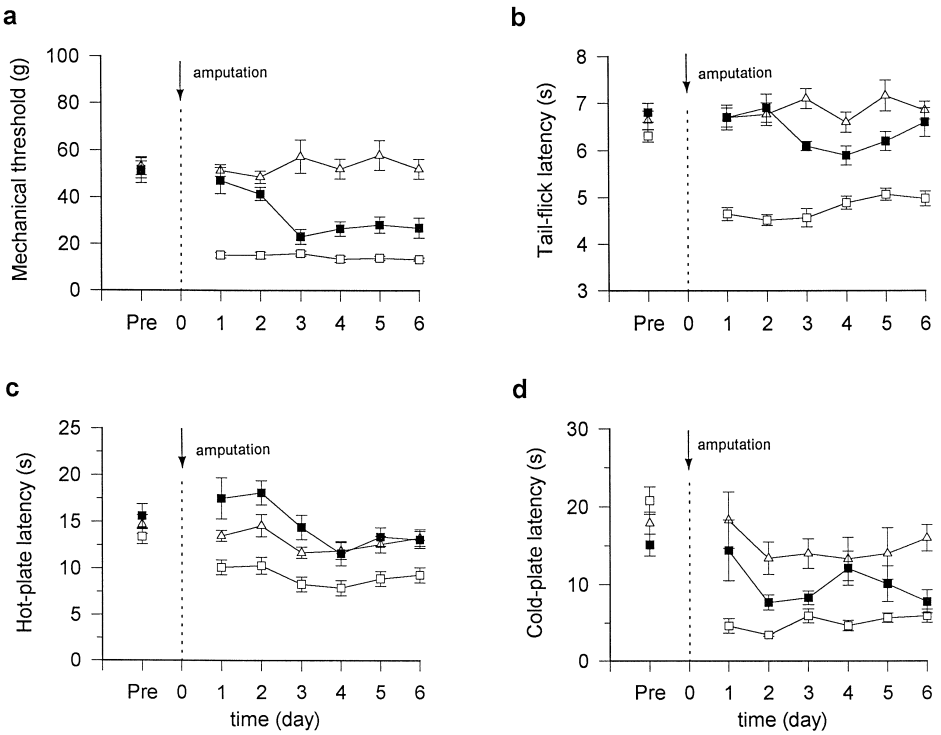

Fig. 4. Effects of intrathecal pretreatment with NMDA receptor antagonists MK801 or AP-5. Behavioral nociceptive thresholds, including mechanical withdrawal thresholds (a), tail-flick latencies (b), hot-plate latencies (c) and cold-plate latencies (d), before and after tail amputation (indicated by arrow) of the tail tip (filled squares). Data from MK801 or AP-5 were similar (see the text for analysis) and thus pooled together for the purpose of simplified illustration. Three different groups were shown: intrathecal injection of MK801/AP-5 (open triangles), intrathecal injection of MK801/AP-5 and tail amputation (filled squares), and intrathecal injection of vehicle (saline) solution and tail amputation (open squares). The mean baseline thresholds were given above 'Pre' in figures. Data are presented as mean ± 1 S.E.M.

Table 2
Effects of intrathecal pretreatment with NMDA receptor antagonists on long term hyperalgesia induced by tail amputation

		n	Baseline	After treatment[a]	% of Baseline
Mechanical withdrawal	MK801/AP-5	11	53.3 ± 3.9 g	53.3 ± 1.5 g	103.7 ± 2.9
	saline + amputated	11	51.7 ± 3.8 g	14.5 ± 0.4 g	29.0 ± 0.8
	MK801/AP-5 + amputation	10	51.4 ± 5.3 g	32.2 ± 3.9 g*	69.3 ± 8.5
Tail-flick reflex	MK801/AP-5	11	6.5 ± 0.2 s	6.9 ± 0.1 s	103.8 ± 1.4
	saline + amputated	11	6.3 ± 0.1 s	4.8 ± 0.1 s	76.1 ± 1.5
	MK801/AP-5 + amputation	10	6.8 ± 0.2 s	6.4 ± 0.2 s*	94.5 ± 2.3
Hot-plate test	MK801/AP-5	11	14.7 ± 1.0 s	13.0 ± 0.4 s	90.4 ± 2.9
	saline + amputation	11	13.4 ± 0.8 s	9.1 ± 0.4 s	70.8 ± 3.2
	MK801/AP-5 + amputation	10	15.6 ± 1.3 s	14.7 ± 1.1 s*	101.4 ± 7.5
Cold-plate test	MK801/AP-5	11	17.8 ± 1.4 s	14.9 ± 0.8 s	83.8 ± 6.7
	saline + amputation	11	20.8 ± 1.8 s	5.1 ± 0.4 s	25.6 ± 2.2
	MK801/AP-5 + amputation	10	15.1 ± 1.4 s	10.1 ± 1.1 s*	71.4 ± 8.6

Data are presented as mean ± 1 S.E.M.
[a]Data represent the mean of measurements during day 1 and day 6.
* $P < 0.05$ compared with animals which received intrathecal saline treatment.
Except for the hot-plate test, nociceptive thresholds in MK801/AP-5 pretreated, amputated mice are still significantly lower than those receiving MK801/AP-5 but without tail amputation ($P < 0.05$).

3.5. Intrathecal treatment with glutamate NMDA receptor antagonists

To test if the induction of long term hyperalgesia by tail amputation requires activation of spinal NMDA receptors, two different types of NMDA receptor antagonists were injected intrathecally into the lumber spinal cord at 10 min before tail amputation. Nociceptive behavioral responses were measured for another 6 days. Intrathecal administration of MK801 ($n = 6$; 7.4 nmol) 10 min before tail amputation significantly attenuated or blocked hyperalgesia. Hyperalgesia in MK801 treated mice was significantly less than that in saline treated mice (mechanical withdrawal threshold: $F(1,83) = 89.10$, $P < 0.001$; tail-flick reflex: $F(1,83) = 160.6$, $P < 0.001$; hot-plate test: $F(1,83) = 33.87$, $P < 0.001$; cold-plate test: $F(1,83) = 13.5$, $P < 0.001$) (see Fig. 4 and Table 2). In control animals which received intrathecal administration of MK801 alone, behavioral nociceptive thresholds were not significantly affected. Similar results were obtained with an another NMDA receptor antagonist AP-5 ($n = 4$; 25 nmol). Hyperalgesia in AP-5 treated mice was significantly less than that in saline treated mice ($P < 0.05$ in each case). Intrathecal injection of AP-5 ($n = 5$) alone did not significantly affect nociceptive thresholds over the same period of time (Table 2). Since results with MK801 or AP-5 experiments were similar, data were pooled and summarized in Fig. 4.

4. Discussion

The present study characterizes long term hyperalgesia in mice with amputation of the tail tip (2.5 cm). Hyperalgesia was observed at the remaining tail as well as the hindpaw. Hyperalgesia was independent of the skin temperature of the remaining tail or hindpaw and lasted for at least 5 weeks. Unlike nerve injury, amputation of the tail tip did not cause obvious spontaneous pain or allodynia, and the hyperalgesia produced tail amputation was not mimicked by heat injury of the same part of the tail.

4.1. Comparison with tissue and nerve injury

Persistent pain is induced by different types of tissue injury, such as inflammation (Hargreaves et al., 1988; Kayser and Guilbaud, 1987), heat injury (Woolf, 1983) and a surgical incision (Brennan et al., 1996). Mechanical and/or thermal hyperalgesia after tissue injury are detectable within a few hours (from 1 to 3 h). The magnitude of mechanical hyperalgesia of the remaining tail in the present study is similar to that after tissue injury (Hargreaves et al., 1988; Kayser and Guilbaud, 1987; Woolf, 1983; Brennan et al., 1996). However, the duration of mechanical hyperalgesia is different. In both inflammation and heat injury models, the mechanical thresholds return to normal at 24 h after the inflammation or injury (Hargreaves et al., 1988; Woolf, 1983). In the case of incision pain, mechanical hyperalgesia lasts for 3 days (Brennan et al., 1996). The duration of mechanical hyperalgesia in the present study was much longer and lasted for at least 7 days. The onset of thermal hyperalgesia is similar to that of mechanical hyperalgesia (heat injury), or faster than that of mechanical hyperalgesia (inflammation) (Hargreaves et al., 1988; Woolf, 1983). The duration of thermal hyperalgesia is short for both inflammation and heat injury (≤ 24 h), but thermal hyperalgesia of the remaining tail in the present study was much longer (≥ 5 weeks).

Both mechanical and heat hyperalgesia were observed with different animal models of nerve injury (Bennett and Xie, 1988; Kim and Chung, 1992). The onset of hyperalge-

sia (≥ 1–3 days) is slower than that after tail amputation. The magnitude of mechanical hyperalgesia is similar or greater than that after tail amputation. Furthermore, mechanical allodynia is observed after nerve injury. No mechanical allodynia (e.g., response to gentle touch or brush) was observed in tail amputated mice.

4.2. Secondary hyperalgesia and central sensitization

Secondary hyperalgesia is reported from tissue/nerve injury (Woolf, 1983; Kayser and Guilbaud, 1987; Hargreaves et al., 1988; Brennan et al., 1996). In the case of tissue inflammation, both mechanical and heat hyperalgesia are limited to the injected side of the hindpaw. The thresholds of the other side of the hindpaw are typically like those in normal animals (Hargreaves et al., 1988). However, different results were reported when vocalization was used to measure nociceptive responses. Decreases in vocalization threshold were observed not only at the injured area, but also at the remote parts of the body (i.e., forelimbs) (Kayser and Guilbaud, 1987). Mechanical and thermal thresholds of the other side of hindpaw were also decreased after heat injury of one side of the hindpaw (Woolf, 1983). After nerve injury, mechanical hyperalgesia can be observed at the adjacent area of the affected hindpaw and/or normal side of the hindpaw (see the work of Bennett and Xie (1988); see also the work of Guilbaud et al. (1992) for a review).

In the present study, thermal (heat and cold) hyperalgesia was observed at the hindpaw after the tail amputation. Although it is possible that heating of the tail during the hot-plate test may have also contributed to animals' behavioral response, hyperalgesia measured in the present study was likely due to thermal stimuli on the hindpaw. The magnitude of hyperalgesia of the hot-plate test was significantly less than that in the tail-flick reflex. Furthermore, hyperalgesia was not detected in the hot-plate test at 50°C. Since no change in skin temperature of the hindpaw was detected, hyperalgesia is likely due to central sensitization in the spinal cord or supraspinal structures (see the works of Urban et al. (1996) and Wiertelak et al. (1994); see also the works of Gebhart (1992), Fields (1992) and Maier et al. (1992) for reviews).

4.3. Distinguish temperature effect from hyperalgesia

Several previous studies indicate that nociceptive tail-flick latencies are affected by the tail skin temperature (Eide et al., 1988). No significant change in the tail temperature was found during the 7 day period, and hyperalgesia was observed throughout the same period of time. These results suggest that changes in tail-flick latency are not simply caused by changes in the skin temperature. Mechanical hyperalgesia, which is likely independent of skin temperature, supports this conclusion. Skin temperature of the hindpaw was also not affected after the tail amputation. These data indicate that thermal/mechanical hyperalgesia reported here are not secondary effects of changes in skin temperature after tail amputation.

4.4. Clinical relevance

In humans, amputation of an extremity often causes phantom pain, stump pain, or phantom limb sensation (see the works of Melzack (1990), Jensen and Rasmussen (1994) and Sherman et al. (1997) for reviews). They all could have a rapid onset (the first week) after surgery and last for months or even years. In addition to phantom pain, hyperalgesia and/or allodynia have been reported in the stump as well as adjacent areas (Baron and Maier, 1995; Jensen and Rasmussen, 1994; Sherman et al., 1997). In animals, large scale functional reorganization in the cortex happens after nerve injury or sensory deafferentation (Pons et al., 1991; Garraghty and Kaas, 1991). Ramachandran et al. (1992) demonstrated that sensory stimuli applied to the lower face evoked referred sensations of missing digits, providing a direct perceptual correlate of the physiological observations in animals (Pons et al., 1991; Wall et al., 1982; Garraghty and Kaas, 1991). Furthermore, the amount of cortical reorganization in the somatosensory cortex was found to correlate with the magnitude of phantom pain in humans (Flor et al., 1995), indicating that plastic changes in the cortex after amputation may contribute to phantom pain. The present study may provide an animal model for studying plastic changes in nociceptive transmission and modulation after amputation.

4.5. Contribution of spinal NMDA receptors

NMDA receptors are important for synaptic plasticity in various regions of the central nervous system (CNS), including long-term enhancement of synaptic transmission in dorsal horn neurons of the spinal cord (Bliss and Collingridge, 1993; Randic et al., 1993). Behavioral studies indicate that activation of NMDA receptors in the spinal cord is important for the induction of hyperalgesia/allodynia by tissue or nerve damage (see the works of Coderre et al. (1993), Meller and Gebhart (1993) and Dubner and Ruda (1992) for reviews). In the present study, intrathecal pretreatment with NMDA receptor antagonists could prevent or significantly decrease long term hyperalgesia caused by tail amputation. These results suggest that NMDA receptor-dependent plastic changes in the spinal cord during tail amputation is critical for the induction of hyperalgesia in both the remaining part of the tail and hindpaw. It is likely that different types of hyperalgesia induced by tissue/nerve injury, or amputation may share some common cellular mechanisms in the spinal cord.

Recently, new molecular mechanisms about pain and opioid analgesia have been obtained using mice with selective genetic deletion of a target protein (Konig et al., 1996;

Matthes et al., 1996; Sora et al., 1997; Ledent et al., 1997; Murata et al., 1997). Systematic comparisons of nociception between normal mice and tail amputated mice have not been carried out. The present results show that hyperalgesia can be induced by such procedures and hyperalgesia likely lasts for days to weeks. Although phantom pain can not be investigated in mice, the current mouse model of tail amputation may be useful for understanding mechanisms of long term plastic changes in the CNS after amputations like those in humans (Flor et al., 1995). Considering the rapid progress in mouse genetics, studies utilizing a combination of tail amputation and genetics in the mouse should facilitate our understanding of molecular mechanisms of pain after amputation such as hyperalgesia, stump pain, and phantom pain.

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