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Transient tactile allodynia following intrathecal puncture in mouse: Contributions of Toll-like receptor signaling

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ABSTRACT

Studies of spinal drug action in mice often involve percutaneous intrathecal drug administration delivered in a lightly anesthetized animal. A successful lumbar intrathecal (IT) needle stick of a lightly anesthetized (isoflurane) mouse evokes a tail flick, which is an indication of local spinal nerve stimulation. Immediately upon arousal, a hind paw tactile allodynia, as measured with von Frey hairs (pre 1.55 ± 0.11 g vs. injected 0.66 ± 0.08 g) lasts 3–4 h. In a similarly anesthetized mouse without the needle stick, a 1-h allodynia was noted. In studies on spinal Toll-like receptor (TLR) signaling, we observed that following intrathecal puncture and mechanical stimulation of the nerve roots mice deficient in TLR down-stream signaling ($Myd88^{-/-}/Trif^{lps2}$), displayed only the transient (1-h) allodynia otherwise observed following isoflurane alone. These data suggest that the extended period of hyperalgesia observed with needle penetration of the dura and mechanical stimulation of the nerve roots requires signaling through the MyD88/TRIF pathways and supports the intrinsic role of Toll-like receptors in the allodynia secondary to the minor nerve activation occurring during the intradural puncture.

Intrathecal (IT) injections allow for the direct and anatomically limited introduction of agents to the spinal cord. This approach permits the assessment of the pharmacology of the physiological and behavioral effects mediated by spinal systems in the intact animal [27]. Such preclinical work has particularly led to an understanding of complex mechanisms involved in pain processing and the development of drugs for spinal use in humans. Benefits of IT injections include a decrease in the effective dose required, and the prevention of unwanted side-effects that result from systemic administration and drug accessibility to other organs [10]. IT injections are usually administered by catheter implantation in larger animals (rats, dogs), but in smaller species (mice) IT injections are performed with an acute lumbar puncture [12,22]. Here the needle is slowly inserted between the lumber vertebra L5 and L6 and then advanced, eliciting a tail flick when passing the dura, indicating contact with an intradural nerve root [12]. While the procedure can be undertaken in unanesthetized mice [12,13,22], the use of short lasting volatile anesthesia can aid in this procedure by allowing the investigator more time to locate the injection area and reduce the stress of restraint [7,8,22].

The procedure has several complicating variables. First, volatile anesthetics, introduce a drug variable into acute experiments. Several reports suggest that isoflurane has both analgesic and hyperalgesic affects depending on the concentration of isoflurane used [6,15,23,29]. The hyperalgesic effects are most reported following low concentrations of isoflurane [5,6,15,23]. Secondly, the needle insertion as noted involves the direct activation of the nerve root (the tail flick) signaling successful entry into the intradural space [12].

In the course of work examining the role of spinal Toll-like receptors (TLR) we made the unexpected observation that in the early period after anesthesia and sham injection there was a robust tactile allodynia that persisted for about 4 h. We sought to characterize this effect and found an involvement of spinal TLR signaling.

All animal experiments were carried out according to protocols approved by the Institutional Animal Care and Use Committee of the University of California, San Diego (under the Guide for Care and Use of Laboratory Animals, National Institutes of Health publication 85-23, Bethesda, MD, USA). Mice were housed up to four per standard cage at room temperature, maintained on a 12-h light/dark cycle (light on at 07:00 h). Testing was performed during the light cycle. Food and water were available *ad libitum*. C57BL/6 mice (male, 25–30 g) were purchased from Harlan (Indianapolis, IN). To study the role of the TLR receptors, we repeated these studies in mice deficient in MyD88 and TRIF. The MyD88 protein is involved in the pro-inflammatory activation pathway and is common to all

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TLRs except TLR3, while the TRIF pathway results in the production of type I Interferons and is shared by only TLR3 and TLR4. The Myd88$^{-/-}$ mice were a kind gift from Dr. S. Akira (Osaka University, Japan) and were backcrossed for over ten generations onto the C57Bl/6 background. Trif^{lps2} were a gift from Dr. B. Beutler (The Scripps Research Institute, La Jolla, CA) and were directly generated on the C57Bl/6 background. These two strains were intercrossed to generate Myd88/Trif^{lps2} mice.

Intrathecal needle placement procedure for the IT saline and IT sham treatment is performed as previously described [12] with modifications outlined in the following description. Mice were briefly induced with 3% isoflurane (with 2% oxygen and 2% room air) in a chamber until a loss of the righting reflex was observed (about 3 min). The mouse was then shaved on the lower back to help visualize the lumbar region, and placed in a nose cone for continued isoflurane administration during the procedure. At no time during the procedure did the animal show respiratory arrest. A 1″ 30-gauge needle attached to a 50 μL Hamilton syringe was inserted between the L5 and L6 vertebrae, evoking a flick of the tail. Tactile thresholds were measured using the up-down application of von Frey hairs along the following time course: 0 (baseline), 30-, 60-, 90-, 120-, 180-, and 240-min, and 24-h after treatment. Following recovery from anesthesia, as evidenced by a vigorous righting reflex and spontaneous ambulation, typically around 1–2 min, mice were evaluated for motor coordination and muscle tone.

Both mouse groups (C57Bl/6 and Myd88/Trif^{lps2}) received the following three treatments: (i) IT saline (vehicle). Following IT needle placement, 5 μL of sterile 0.09% saline slowly injected over 30 s. The animal was then withdrawn from the nose cone and allowed to ventilate with room air. (ii) IT Sham. For the IT sham injection groups, the same procedure was followed as above except the needle was simply held in place for 30 s to simulate the injection time. (iii) Anesthesia alone. The isoflurane control treated mice received 3 min of 3% isoflurane (with 2% oxygen and 2% room air) in a chamber, followed by transfer to the nose cone used for the IT sham procedure. The mouse received isoflurane via the nose cone for another minute to simulate the same amount of time under isoflurane for the IT sham procedure and then withdrawn form the nose cone and allowed to ventilate with room air.

Mechanical sensitivity was assessed using the von Frey up-down method. Filaments with values ranging from 2.44 to 4.31 (0.03–2.00 g) were applied to the paw as previously described [2]. The 50% probability withdrawal threshold (in principal, the calculated force to which an animal reacts to 50% of the presentations) was recorded. Mechanical values for both the paws were measured and averaged to produce a single data point per day of measurement.

Data are presented as group mean ± SEM. Tactile threshold time course curves (plotted as the mean ± SEM vs. time after treatment) were analyzed with a one-way analysis of variance (ANOVA) with repeated measures over time, followed by Dunnett's post hoc test to compare each time point to the same group's baseline. For Fig. 3, hyperalgesic indices were calculated for each mouse. The hyperalgesic index is the area under the time course curve after treatment, in which the percentage change from baseline threshold is plotted against time. This is calculated as follows: 100 × ((baseline threshold − treatment threshold)/(baseline threshold)) and presented as group mean ± SEM. Hyperalgesic index was first analyzed within each mouse strain via one-way ANOVA followed by Dunnett's post hoc test to the appropriate control (no treatment group). Second, to compare between the two mouse strains, a two-way ANOVA and Bonferroni post hoc test was used comparing mouse group and treatment. All analyses employed Prism statistical software, CA, USA.

To test the effect of the intrathecal (IT) injection procedure on baseline thresholds C57Bl/6 mice were anesthetized with isoflurane and underwent an IT injection receiving 5 μL of saline (Fig. 1A). A second group of mice received the IT needle placement but no injection (IT sham) followed by mechanical threshold testing with von Frey hairs (Fig. 1B). Tactile threshold testing of untreated C57Bl/6 mice for 4 h did not produce any significant changes in tactile thresholds. After anesthesia and IT saline, a significant drop in the tactile threshold (tactile allodynia) was observed at the earliest time point examined and lasted up to 4 h (Fig. 1A). This IT saline effect was not different from that observed in the IT sham group (Fig. 1B). No animals undergoing either the IT saline or sham displayed any detectable change in ambulation or behavioral signs such as failure to bear weight or paw cupping.

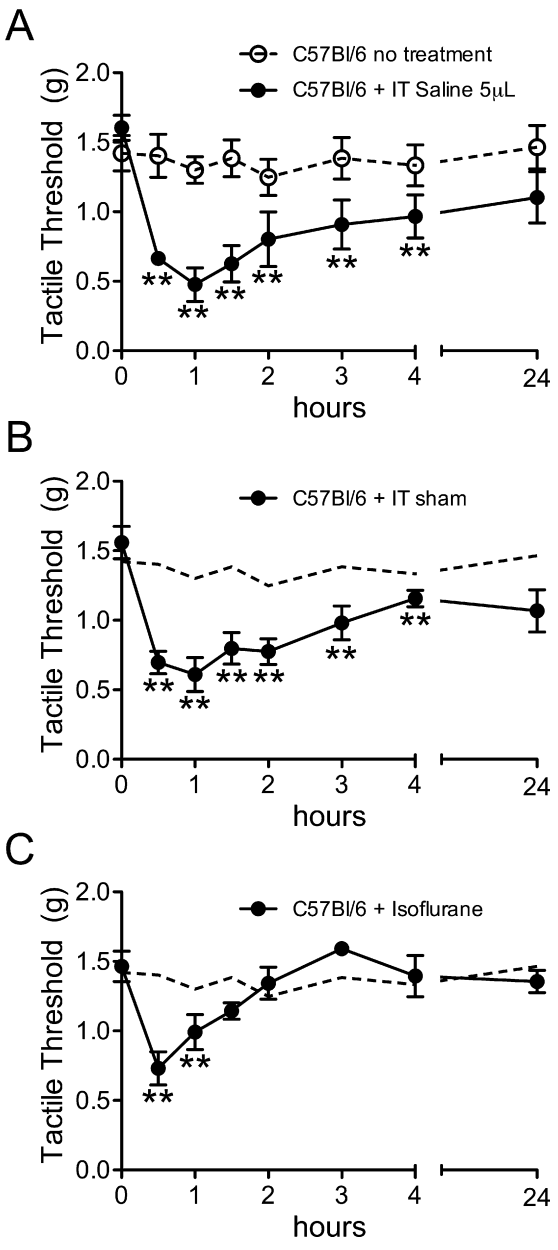

Fig. 1. Transient tactile allodynia observed in C57Bl/6 mice following IT saline and IT sham procedure. C57Bl/6 mice received IT saline (A) or underwent the IT sham procedure (B), followed by mechanical threshold testing with von Frey filaments. (C) C57Bl/6 mice were subjected to vaporized isoflurane anesthetic for the same treatment time as the IT sham group. The dashed line in B and C repeats the control no treatment group presented in A for comparison. Data expressed as mean ± SEM (n = 5–6 mice/group) $^{**}p < 0.01$ repeated measures one-way ANOVA, followed by Dunnett's post hoc test to compare each time point to the same group's baseline (t = 0).

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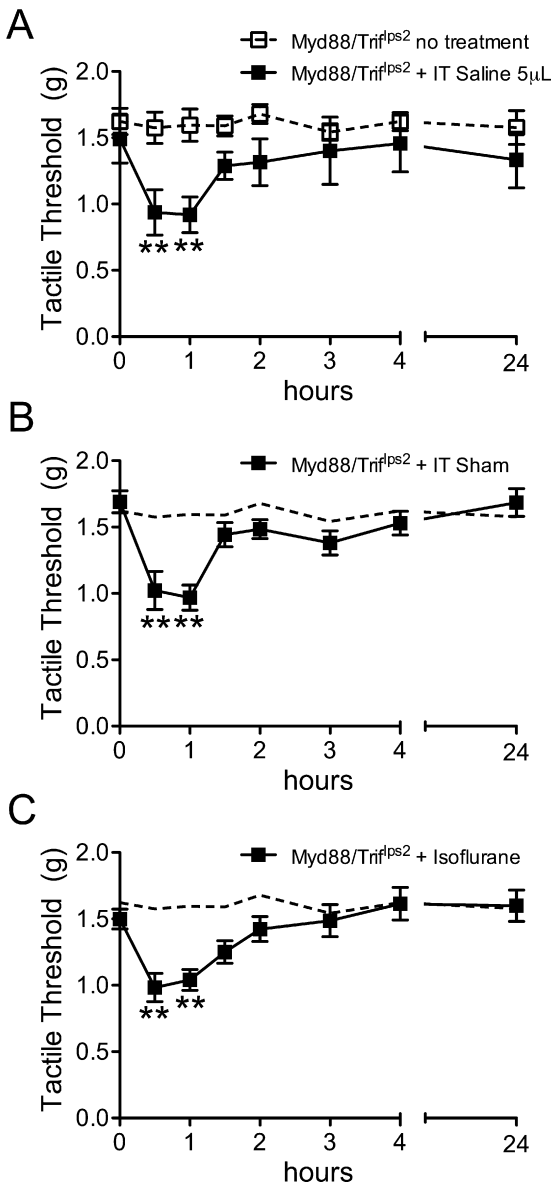

Fig. 2. Lack of transient tactile allodynia observed in Myd88/Trif^{lps2} mice following IT sham. Myd88/Trif^{lps2} mice received IT saline (A) or underwent the IT sham procedure (B), followed by mechanical threshold testing with von Frey filaments. (C) Myd88/Trif^{lps2} mice were subjected to vaporized isoflurane anesthetic for the same treatment time as the IT sham group. The dashed line in B and C repeats the control no treatment group presented in A for comparison. Data expressed as mean ± SEM (n = 5–6 mice/group) $^{**}p < 0.01$ repeated measures one-way ANOVA, followed by Dunnett's post hoc test to compare each time point to the same group's baseline (t = 0).

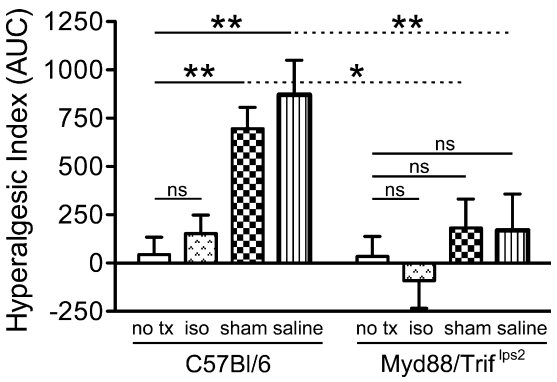

Fig. 3. Hyperalgesic index analysis of the treatment groups. Hyperalgesic indices were calculated for each mouse using their individual baseline threshold and calculating the area under the curve. Data expressed as mean ± SEM (n = 5–6 mice/group). Hyperalgesic index was first analyzed within each mouse strain via one-way ANOVA followed by Dunnett's post hoc test to the appropriate control (no treatment group), represented by the solid black lines. A two-way ANOVA followed by Bonferroni post hoc test was used comparing mouse group and treatment, represented by the dashed lines ($^{*}p < 0.05$; $^{**}p < 0.01$; ns = not significant).

To assess the effect of transient exposure to isoflurane alone in the tactile thresholds, C57Bl/6 mice were exposed to isoflurane for the same amount of time and at the same concentrations as the mice that underwent the IT sham procedure (Fig. 1C). Here, we observed a transient decrease in tactile thresholds up to 1 h post isoflurane exposure.

To assess the role of TLR signaling in the allodynia initiated by the anesthesia and the sham injection, Myd88/Trif^{lps2} mice were subject to the same IT saline, IT sham, or isoflurane alone treatment followed by the same time course of tactile threshold testing (Fig. 2). Importantly, the tactile thresholds of untreated Myd88/Trif^{lps2} animals did not differ from the C57Bl/6 mice. The brief allodynia, lasting around 1 h observed following isoflurane anesthesia alone was unaltered in the Myd88/Trif^{lps2} (Fig. 2C). In contrast, the longer lasting allodynia, observed after the IT saline and IT sham in C57Bl/6 mice, was reduced to that observed after anesthesia alone in the Myd88/Trif^{lps2} mice (Fig. 2A and B). Analyzing these data using the hyperalgesic index showed identical results (Fig. 3).

In the present work, we show that during the hour following recovery of the mouse from a brief period of light isoflurane anesthesia a moderate, but significant, tactile allodynia was observed. Following the same protocol, the IT injection of saline through a 30-gauge needle resulted in a 4-h tactile allodynia. The magnitude of the effect was identical to that observed after the intradural placement of the needle without injection. This observation suggests that the critical variable regulating the extended allodynia was neither the anesthetic nor the injection of the saline vehicle, but the intradural insertion of the needle. The intradural placement of the needle is commonly accompanied by a brisk flicking of the tail, which is considered to be a positive marker for intradural penetration of the needle [12,22]. This motor response elicited by placement of the needle at the L5–L6 vertebral interspace reflects the contact with nerve roots and not the spinal cord. As the dorsal and ventral roots lie in close proximity, it is likely that both types are impacted by the needle insertion. Given those circumstances, allodynia is not surprising as the acute activation of high-threshold sensory afferents will initiate spinal facilitatory processes, which likely account for the observed tactile allodynia [16]. Though the animal is anesthetized, afferent-evoked spinal sensitization has been routinely demonstrated in animals anesthetized with volatile anesthetics [4,28]. Previous work, with intentional needle sticks of the peripheral nerve, has shown the development of evident and persistent tactile allodynia in animals [17,26]. As the effects observed here were transient, and there were no signs of motor impairment, significant nerve injury by the point of the 30-gauge needle appears unlikely. In humans, acute intradural needle sticks are known to produce evident and often transient painful sensations mediated by contact with the spinal root, though persistent pain states in humans has also been reported [1,11,20].

It is important to note that the volatile anesthetic itself had a clear but transient effect. The origin of this transient allodynia is not clear. Several reports suggest that isoflurane has both analgesic and hyperalgesic affects depending on the concentration of isoflurane used [6,15,23,29]. The hyperalgesic effects are most reported following low concentrations of isoflurane [5,6,15,23]. Excitatory events are commonly observed with emergence from volatile anesthetic, which may have parallels in the present observations.

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What ever the mechanism, the prominent finding in the present work was the unexpected observation that the block of TLR signaling had no effect upon the very transient allodynia observed immediately after anesthetic recovery in a normal animal, but prevented the delayed allodynia that occurred after the intradural needle stick. The extended tactile hyperalgesia observed in the C57Bl/6 mice but not the Myd88/Trif^{lps2} mice indicates that innate inflammatory cell signaling occurred following a needle stick into the intrathecal space that elicited a tail flick, but not following the anesthetic recovery, since Myd88/Trif^{lps2} mice are unable to signal via IL-1, IL-18 and the Toll-like receptors.

As the IT procedure evokes a reliable tail flick upon puncture of the dura, we suspect that a minor nerve activation or injury plays a role. Interestingly the TLR pathway has been shown to play a role in post injury hyperpathia [9,18,21,24]. Specifically, TLRs present on spinal microglia and astrocytes cells mediate this neuroinflammatory response especially in the case of neuropathic injury possibly through the production of proinflammatory cytokines [3,9,14,19,25]. While the IT injection with spinal nerve root stimulation is not considered a model of neuropathic pain, the transient lowering of the tactile thresholds do suggest an acute injury.

These presented data support the idea that the TLR pathway contributes to the extended period of hyperalgesia observed after needle penetration of the dura combined with mechanical nerve root stimulation, required in a mouse intrathecal injection. This may provide a simple model to identify the specific TLRs involved and perhaps the endogenous products released by this modest nerve impalement.

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