Chronic pain produces hypervigilance to predator odor in mice

Kevin C. Lister[1], Sioui Maldonado Bouchard[1], Teodora Markova[1], Andrea Aternali[1], Pelin Denecli[1], Stephania Donayre Pimentel[1], Mariam Majeed[1], Jean-Sebastien Austin[1], Amanda C. de C. Williams[2], and Jeffrey S. Mogil[1,*]

1 Department of Psychology and Anesthesia, McGill University, Montreal, QC H3A 1B1, Canada.
2 Research Department of Clinical, Health and Educational Psychology, University College London, London WC1E 6BT, UK.
*E-mail: jeffrey.mogil@mcgill.ca

The adaptive significance of acute pain (to withdraw from tissue-damaging or potentially tissue-damaging external stimuli, and to enhance the salience of he stimulus resulting in escape and avoidance learning) and tonic pain (to enforce recuperation by punishing movement) are well-accepted [1]. Pain researchers, however, generally assert that chronic pain has no adaptive significance, representing instead a pathophysiological state. This belief was recently challenged by the observation [2] that nociceptive sensitization caused by a chronic pain-producing injury reduced predation risk in squid (*Doryteuthis pealeii*). In that study, injury to an arm (removal of the tip with a scalpel) 6 hours prior led to increased targeting by black sea bass, resulting in decreased survival of the squid in a 30-minute trial featuring free interaction between predator and prey. The surprising finding was that anesthesia during surgery, preventing the chronic nociceptor sensitization associated with such injuries, led to even lower probability of survival. That is, the likely presence of pain increased apparent fi tness, and the authors concluded that the chronic pain state and its associated nociceptive sensitization represented an adaptive function. Pain-induced defensive behaviors affecting fitness have also been reported in crustaceans (*Gammarus fossarum*) [3]. It is, however, currently unknown whether this may also be true in any other species, including in Mammalia.

We designed an octagonal O-maze (Figure 1A and Supplemental Experimental Procedures) in which food-deprived mice were placed in one octant. A food reward was placed ~30 cm away via the 'short route'; alternatively, mice could walk all the way around the maze (the 'long route'; ~200 cm) to retrieve the reward. Mice were trained for 6–8 days until they opted for the short route in >80% of 10 consecutive rewarded trials. The maze was designed with a fan and vacuum system such that volatilized odors could be introduced into the maze, but were restricted to the octant corresponding to the short route. On the single testing day immediately following training we found that fox urine caused mice to avoid the short route in favor of the long route in a concentration-dependent manner (n = 5–11; main effect of concentration: $F_{2,19} = 7.4$, p = 0.004; Figure 1B). This shift represents vigilance to potential predation, as the long route minimizes exposure to the fox odor. We avoided the use of yet higher volumes of fox urine to prevent spread of the odor beyond the short-route octant, and to prevent the possibility of floor effects.

Mice received sham surgery or spared nerve injury (SNI) 1–2 weeks prior to maze training and 2–3 weeks prior to maze testing by experimenters blinded to surgical status. SNI is a preclinical model of complex regional pain syndrome, type 2 (causalgia), involving transection of two of the three distal branches of the sciatic nerve innervating the hind paw. SNI is associated with nociceptive sensitization and robust and long-lasting neuropathic pain-related behaviors [4,5]. A two-way ANOVA revealed a significant odor x surgery interaction (n = 11–13; $F_{1,44} = 6.6$, p = 0.01). Mice given SNI displayed significantly more avoidance of the short route than those given sham (p = 0.037), and thus were hypervigilant to the fox odor (Figure 1C).

Most pain research is aimed at providing proximate explanations (regarding mechanism and ontogeny) for pain — especially the more pressing clinical concern of chronic pain — leaving two of Tinbergen's four questions [6] regarding adaptive signifi cance and phylogeny largely unaddressed. In fact, the leading hypothesis regarding the ultimate explanations for chronic pain is the 'smoke detector principle', which posits that excessive pain reporting and defensive responses to injury long past tissue healing are akin to a broken, or oversensitive, smoke detector, which continues to sound the alarm despite the lack of ongoing threat [7]. The observations of Crook and colleagues [2] in squid represent the fi rst empirical data to suggest that chronic pain may indeed have adaptive signifi cance. By continually reminding an injured organism of its increased risk of predation, chronic pain may increase vigilance, leading to increased Darwinian fitness. Ethical considerations precluded us from testing actual predation of mice, and thus fitness could not be evaluated. However, we believe that the hypervigilance to fox odor demonstrated here is a close, experimentally tractable proxy that mirrors in some respects the increased defensive behaviors displayed by injured squid [2]. We suggest, therefore, that the chronic-pain hypervigilance hypothesis of Crook *et al.* [2] may hold in mammals.

Supplemental information

Supplemental Information includes the experimental procedures and supplemental reference, and can be found online at https://doi.org/10.1016/j.cub.2020.06.025.

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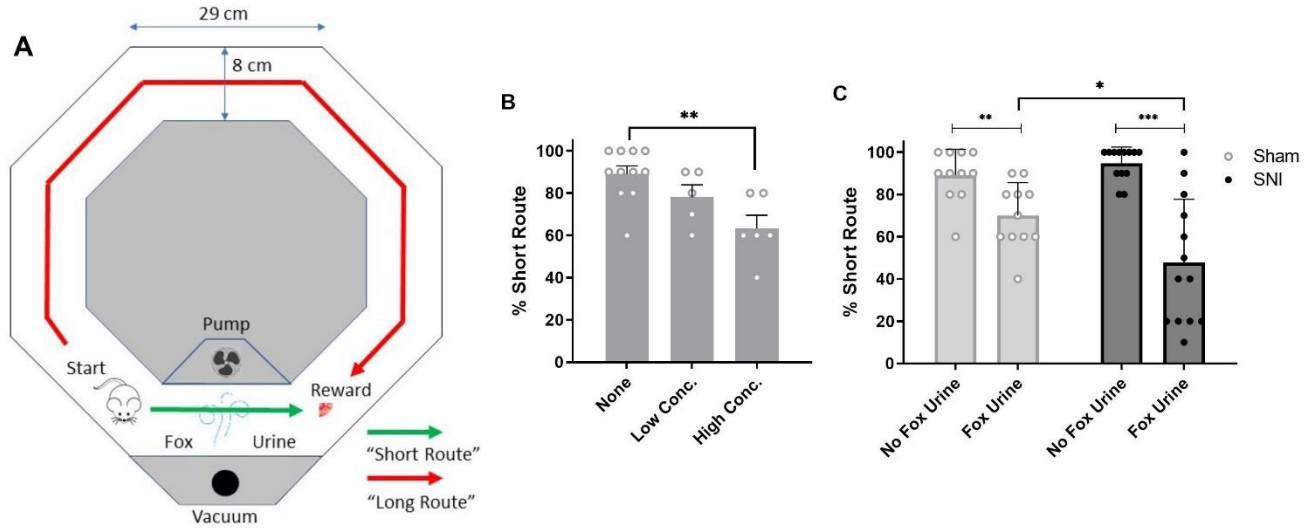
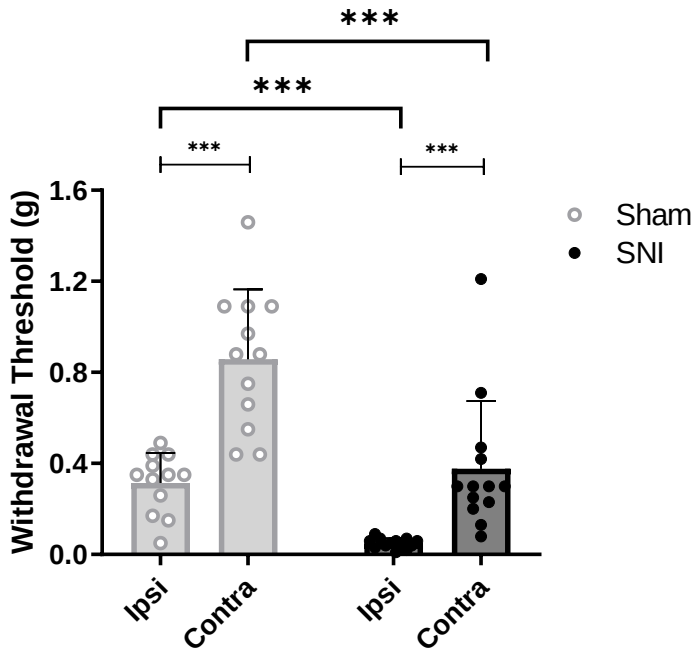
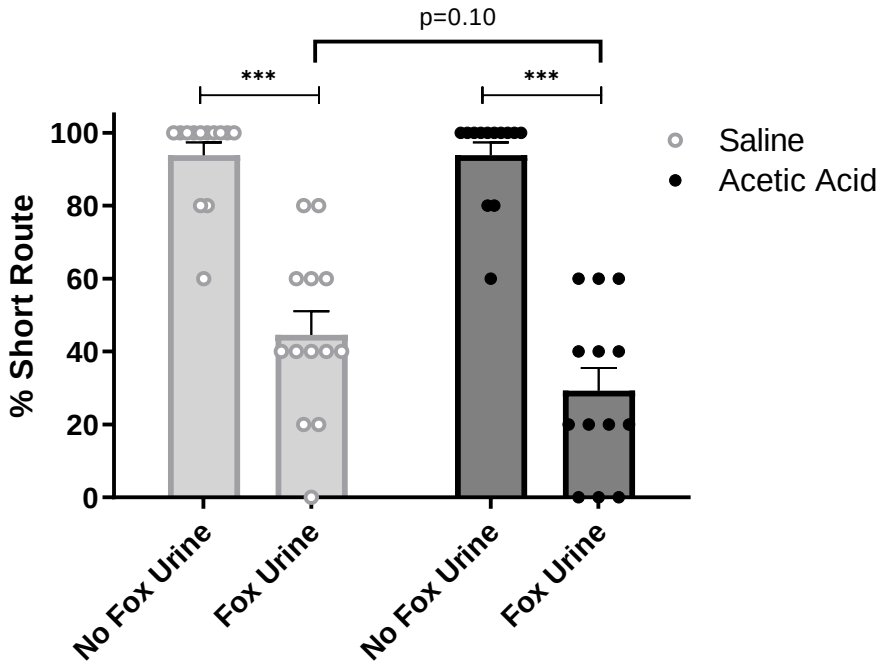
Figure 1. Hypervigilance to predator odor in mice produced by spared nerve injury (SNI).
(A) Cartoon description of the octagonal O-maze used for training and testing. Mice were placed in the start octant and could choose to retrieve a reward via short or long routes. During testing, fox urine was pumped into and vacuumed out of the octant representing the short route; avoidance of this route would thus represent vigilance towards possible predation.
(B) Concentration-dependent avoidance of the maze short route infused with fox urine. Bars represent mean ± SEM percentage of ten trials in which the mouse took the short route (that is, through the odor) to the reward. Trials in separate groups of mice featured no fox urine (room air pumped through; n = 11), or fox urine at low concentration (Low Conc.; 1 ml of 100% fox urine over □10 min; n = 5) or at high concentration (High Conc.; 2 ml; n = 7). **p < 0.01 by Tukey's post-hoc test.
(C) Mice experiencing chronic pain from SNI display hypervigilance to fox urine. Bars as in graph (B) (n = 11–13 mice/condition); mice received SNI or sham surgery 2–3 weeks prior to testing in a maze featuring room air or 2 ml fox urine. *p < 0.05, **p < 0.01, ***p < 0.001 by Student's *t*-tests as indicated.



Supplementary files



Supplementary Fig. 1. Mechanical allodynia after SNI surgery in mice. Bars represent mean ± SEM 50% withdrawal thresholds (n=12–13 mice/surgical condition) to von Frey fibers applied to the sural territory of the plantar hind paw ipsilateral (Ipsi) and contralateral (Contra) to the injury, using the up-down method of Dixon [1]. Baseline (pre-surgery) thresholds were not measured; shown are von Frey thresholds measured ≈1 week post-sham or post-SNI surgery, and ≈1–2 weeks prior to maze testing. Note that SNI-induced allodynia is extremely stable for long periods of time once developed [2]. Highly significant main effects of surgery ($F_{1,23}$ = 27.7, p<0.001) and side ($F_{1,23}$ = 65.4, p<0.001) were observed. ***p<0.001 as indicated. Although SNI produced more robust mechanical allodynia than sham surgery (defined via comparison to the contralateral hind paw), we also observed mechanical allodynia after sham surgery (compare Ipsi versus Contra for sham group) and likely "mirror" allodynia after SNI (compare Contra of sham versus SNI). The former phenomenon may represent long-lasting postoperative pain, but is more likely related to the social transfer of pain phenomenon as previously reported [3], since sham and SNI mice were housed together in the same cage. Mirror allodynia after SNI is a well-known (albeit strain-dependent) phenomenon [4].



Supplementary Fig. 2. A trend towards predator hypervigilance produced by 0.6% acetic acid. This study was conducted similarly to that reported in the main text except that training and testing sessions employed 5 trials instead of 10. Mice were pain-naïve during training. On the testing day mice were injected intraperitoneally with 0.6% acetic acid, and 30 min later placed in the maze. Bars represent mean ± SEM percentage of 5 trials in which the mouse took the short route (i.e., through the odor) to the reward (n=13 mice/condition). A two-way ANOVA revealed a highly significant main effect of fox urine ($F_{1,48}$ = 123.0, p<0.001), and a trend towards a pain x fox urine interaction ($F_{1,48}$ = 2.2, p=0.14). ***p<0.001 as indicated by Student's t-test. The reduced effect (of pain) here compared to SNI might be related to the shorter duration of pain before maze testing, the lack of sensitization produced by the noxious stimulus, and/or the shorter number of trials employed.