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Pain Chronification and Chronic Pain Impair a Defensive Behavior, But Not the Ability of Acute Pain to Facilitate It, Through the Activation of an Endogenous Analgesia Circuit

Monaliza Simone Lamana and Josiane Miranda
State University of Campinas

Gláucia Tobaldini and Luana Fischer
Federal University of Parana

Cláudia Herrera Tambeli
State University of Campinas

The endogenous ability to decrease pain perception during life-threatening situations is crucial to the prevention of recuperative behaviors and to leave the subject free to engage in appropriated defensive responses. We have previously shown that acute pain activates the ascending nociceptive control—an endogenous analgesia circuit dependent on opioid mechanisms within nucleus accumbens—to facilitate the tonic immobility response, an innate defensive behavior. Now we asked whether chronic pain and pain chronification impairs either the tonic immobility response or the ability of acute pain to facilitate it by activating the ascending nociceptive control. We found a significant decrease in the duration of the tonic immobility response in rats during the induction and maintenance phases of the persistent mechanical hyperalgesia. This finding suggests that chronic pain and its development impair defensive responses. However, during the induction and maintenance phases of persistent hyperalgesia, the ascending nociceptive control activation, by a forepaw capsaicin injection, increased the tonic immobility response, an effect prevented by the blockade of μ-opioid receptors within nucleus accumbens. This finding suggests that pain chronification and chronic pain do not prevent the ability of acute pain to facilitate the defensive behavior of tonic immobility by activating the ascending nociceptive control. Therefore, although chronic pain states decrease the ability to engage in a defensive behavior, they may not prevent the expression of defensive behaviors during life-threatening situations accompanied by acute pain. The biological purpose of such a mechanism may be to increase the chances of survival of a wounded subject exposed to acute pain in a novel life-threatening situation.

Keywords: defensive behavior, persistent mechanical hyperalgesia, nucleus accumbens, ascending nociceptive control, rat

Acute pain is essential to life, it signals that something is wrong and a change in behavior is needed. However, during life-threatening situations, the ability to suppress pain perception in response to noxious stimulation is also essential to life preservation (Porreca & Navratilova, 2017). The temporary suppression of acute pain perception prevents recuperative behaviors, leaving the subject free to engage in appropriated defensive responses, a set of species-specific reactions with the adaptive function of increasing survival probability (Harris, 1996). The tonic immobility response is an innate defensive response of profound inactivity and relative lack of responsiveness to the environment (Klemm, 2001) that occurs during prey–predator confrontations. The state of profound inactivity during the tonic immobility response has the adaptative function of increasing the chances of prey escaping, because it decreases the predator's interest in the attack (Thompson et al., 1981). A neural mechanism able to decrease pain perception and facilitate defensive behaviors would be of great value during life-threatening situations. The ascending nociceptive control may serve both purposes. It is a neural circuit physiologically activated by peripheral noxious stimuli that induce potent heterosegmental analgesia (Gear, Aley, & Levine, 1999; Gear & Levine, 2009) and facilitates the immobility response (Tambeli et al., 2012). The ascending nociceptive control includes spinal (Tambeli, Levine, & Gear, 2009; Tambeli, Parada, Levine, & Gear, 2002; Tambeli, Quang, et al., 2003; Tambeli, Young, et al., 2003) and nucleus accumbens mechanisms (Schmidt, Tambeli, Barletta, et al., 2002; Schmidt, Tambeli, Gear, & Levine, 2001; Schmidt, Tambeli, Levine, et al., 2002; Schmidt, Tambeli, Levine, & Gear, 2003). In

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Monaliza Simone Lamana and Josiane Miranda, Department of Physiology, Piracicaba Dental School, State University of Campinas; Gláucia Tobaldini and Luana Fischer, Department of Physiology, Division of Biological Sciences, Federal University of Parana; Cláudia Herrera Tambeli, Department of Structural and Functional Biology, Institute of Biology, State University of Campinas.

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Correspondence concerning this article should be addressed to Cláudia Herrera Tambeli, Department of Structural and Functional Biology, Institute of Biology, State University of Campinas, Campinas, SP 13083-862, Brazil. E-mail: tambeli@unicamp.br

the spinal cord, the inhibition of ongoing excitatory neural activity induces nucleus accumbens-mediated endogenous analgesia (Tambeli et al., 2002). To activate the ascending nociceptive control the peripheral noxious stimulation activates inhibitory spinal mechanisms that inhibits the ongoing spinal excitatory activity resulting in heterosegmental analgesia mediated by endogenous opioids in the nucleus accumbens (Tambeli, Quang, et al., 2003). For example, the administration of the selective μ-opioid receptor antagonist CTOP into nucleus accumbens prevents both the endogenous analgesia and the facilitation of the tonic immobility response induced by the activation of the ascending nociceptive control. Therefore, nucleus accumbens is a key element of the ascending nociceptive control and is involved in both the endogenous analgesia and the facilitation of a defensive response.

Unlike acute pain, chronic pain has neither protective nor warning function for life preservation. Chronic pain develops in response to complex neuroplastic mechanisms with poorly understood consequences in different aspects of behavior. It is now clear that chronic pain is associated with general decrease in motivation, reward-related responses, and endogenous pain modulation (Ossipov, Morimura, & Porreca, 2014; Simons, Elman, & Borsook, 2014), including the duration of ascending nociceptive control-mediated analgesia (Ferrari, Gear, & Levine, 2010; Miranda et al., 2015). However, whether chronic pain affects defensive behaviors remains unknown. Therefore, in this study we asked whether chronic pain and its development impair either the defensive behavior of tonic immobility or the ability of acute pain to recruit the ascending nociceptive control to facilitate it.

Experimental Procedures

Animals

Male adult Wistar rats (200 g to 300 g; $n = 148$, divided into 26 experimental groups) were used in this study. They were housed five per cage with free access to food and water and were maintained under a 12-hr light–12-hr dark cycle. All animal experimental procedures and protocols were approved by the Committee on Animal Research of the State University of Campinas, Brazil, and followed the guidelines of the Ethics Standards of the International Association for the Study of Pain in Animals (Zimmermann, 1983). Each animal was subjected to a single trial.

Drugs and Doses

Prostaglandin E_2 (PGE_2 100 ng/hindpaw), capsaicin (125 μg/forepaw), and the selective μ-opioid receptor antagonist CTOP (Cys2, Tyr3, Orn5, Pen7amide, 1.0 μg bilaterally injected into the nucleus accumbens) were obtained from Sigma-Aldrich (São Paulo, SP, Brazil) and their doses were based on previous studies (Ferreira, Lorenzetti, & De Campos, 1990; Gear et al., 1999; Schmidt, Tambeli, Barletta, et al., 2002).

Stock solutions of PGE_2 in 10% ethanol (1 μg/μl) were further diluted in phosphate buffered saline (PBS) to a final concentration of 3.33 ng/μl. The ethanol concentration of the final PGE_2 solution was 1%. Capsaicin was dissolved in ethanol (50%) and Tween 80 (50%) to an initial concentration of 50 μg/μl and further diluted in PBS to a final concentration of 4.16 μg/μl. CTOP was dissolved in PBS.

Paw Injections

Subcutaneous injections of drugs or their vehicle into the dorsum of the paw were performed using a 30-gauge needle attached to a polyethylene tubing and also to a Hamilton syringe. Paw injection volumes were 30 μl. PGE_2 (or its vehicle) was injected into the hindpaw to induce persistent inflammatory hyperalgesia (see the following text) and capsaicin or its vehicle into the forepaw.

Microinjections Into the Nucleus Accumbens

The rats were anesthetized with xylazine chloride (10 mg/kg) and ketamine hydrochloride (60 mg/kg). Two 23-gauge stainless steel guide cannulas were stereotaxically implanted bilaterally into the nucleus accumbens core (1.3 mm rostral, 5.2 mm ventral, and 1.8 mm from the bregma; Paxinos & Watson, 2007). The cannulas were then fixed to the skull with a screw and dental cement. Intraaccumbal injections were performed seven days later, via the insertion of a 30-gauge stainless steel injection cannula extending 2 mm beyond the tip of the outer guide cannula, attached to a polyethylene tubing and also to a 2-μl Hamilton syringe. Nucleus accumbens injection volumes was 0.25 μl and was delivered over a period of 120 seconds. The injection cannulas were kept in place for another 30 seconds after injection to avoid backflow. Injection sites were confirmed by injecting Evans blue dye (1%, 0.25 μl) and performing 50-μm postmortem coronal sections to determine the location of the dye (Tobaldini, Aisengart, Lima, Tambeli, & Fischer, 2014).

Chronic Pain Model: Persistent Inflammatory Hyperalgesia

The PGE_2-induced persistent mechanical hyperalgesia model has been previously described (Ferreira et al., 1990). Briefly, PGE_2 (100 ng/30 μl) was daily injected into the subcutaneous tissue of the dorsal surface of the rat's hindpaw for 14 days (induction phase of persistent hyperalgesia). After the discontinuation of the PGE_2 injection, the mechanical hyperalgesia persists for 30 days (maintenance phase of persistent hyperalgesia). The development of persistent inflammatory hyperalgesia and its intensity were assessed by measuring the mechanical nociceptive threshold on Days 1, 7, and 14 of the induction phase (measurements made before the PGE_2 injection) and on Days 1, 7, 14, and 21 of the maintenance phase of persistent mechanical hyperalgesia.

Nociceptive Testing

The nociceptive mechanical thresholds were determined using the paw withdrawal test (Randall & Selitto, 1957) and used as a measure of nociceptive activity. The mechanical paw-withdrawal test was performed in a blinded fashion, always before the PGE_2 injections. Briefly, an algesiometer (Ugo Basile, Varese, Italy) was used to apply a progressively increasing mechanical force (in grams) to the dorsal surface of the rat's hindpaw until the animal withdrew its paw. The nociceptive mechanical threshold is defined as the force (mean of three readings) in grams at which the rat withdrew its paw. Hyperalgesia is characterized by a significant decrease in mechanical paw withdrawal threshold.

Assessment of Tonic Immobility Response

Each rat was lifted off its feet by grasping its skin at the back of the neck. The tonic immobility response duration (in seconds) was recorded by a chronometer from the time the animal was suspended until it made escape-like movements (Meyer, Cottrell, & Van Hartesveldt, 1993).

Rotarod

The rotarod (Stoelting, Chicago, IL) was used to evaluate the effect of the intraaccumbal injection of CTOP or its vehicle on motor function. The latency to fall (in seconds) from the rotating apparatus (18 rpm) was recorded. The cut-off time was 200 s. The animals were trained three times a day for 2 days before testing.

Study Design

To investigate whether pain chronification and chronic pain impair defensive behaviors, we measured the duration of the tonic immobility response in rats with persistent mechanical hyperalgesia (see the preceding "Chronic Pain Model: Persistent Inflammatory Hyperalgesia" section). The 14 day-period of injections are the induction phase of persistent mechanical hyperalgesia and allows studying the mechanisms underlying chronic pain development, that is, pain chronification. After this 14 day-period, PGE_2 injections are discontinued but the nociceptive response remained exacerbated, that is, the mechanical nociceptive threshold remain decreased, for at least 30 days. This is the maintenance phase of persistent mechanical hyperalgesia and allows studying the mechanisms underlying chronic pain maintenance. Data from Figure 1 show the tonic immobility response and the nociceptive paw-withdrawal threshold evaluated in the same rats during the induction (Days 1, 7, and 14, behavioral tests performed before PGE_2 injection) and maintenance phases (Days 1, 7, 14, and 21 after discontinuing the PGE_2 injections) of persistent mechanical hyperalgesia. In these rats the nociceptive paw-withdrawal threshold was evaluated immediately after the tonic immobility response.

To confirm our previous data that acute pain facilitates tonic immobility response by activating the ascending nociceptive control (Tambeli et al., 2012), we measured the duration of tonic immobility response in pain-free rats randomly assigned to receive a forepaw injection of either capsaicin (125 μg) or its vehicle 10 min after the intraaccumbal injection of either CTOP or its vehicle. The intraaccumbal injection of CTOP is a procedure classically used to prevent the endogenous analgesia elicited by the activation of the ascending nociceptive control (Gear et al., 1999).

The next step was to investigate whether pain chronification and chronic pain affect the ability of acute pain to facilitate defensive behaviors by activating the ascending nociceptive control. To this end, we measured the duration of tonic immobility response during the induction and maintenance phases of persistent mechanical hyperalgesia in rats assigned to receive a forepaw injection of capsaicin (125 μg) or its vehicle 10 min after the intraaccumbal injection of either CTOP or its vehicle.

To rule out the possibility that CTOP by itself might affect the immobility response we measured this response in rats randomly assigned to receive capsaicin's vehicle into the forepaw 10 min after the intraaccumbal injection of CTOP on Day 7 of the induction phase and on Day 7 of the maintenance phase of persistent mechanical hyperalgesia. CTOP or its vehicle was bilaterally injected into the nucleus accumbens only once, and capsaicin or its vehicle was injected into the left forepaw only once. Table 1 provides an overview of all the experimental groups and the numbers of rats in each.

Data Presentation and Analysis

Mechanical nociceptive threshold is presented as the force in grams necessary to evoke a paw-withdrawal response. The tonic immobility response is presented as the time in seconds until an escape-like response be evoked (Figure 1B) or as tonic immobility duration change, which is the variation (before–after) calculated by subtracting the basal tonic immobility duration (measured before any experimental intervention performed on that day) from that measured 5 min after the forepaw injection (subsequent figures). Data were normally distributed (Shapiro-Wilk test, $p > .05$) with equal variances ($p > .05$). Thus, they were analyzed by one-way analysis of variance (ANOVA) with repeated-measures (see Figure 1) or by one-way ANOVA (subsequent figures). The post hoc Student–Newman–Keuls tests were performed to determine the basis of the significant difference. Data are expressed as $M \pm SEM$. The Pearson correlation analysis was performed to evaluate the correlation between persistent mechanical hyperalgesia development and the tonic immobility response duration. A p value of $<.05$ was considered statistically significant in all analysis.

Results

Pain chronification and chronic pain impair the tonic immobility response. Daily PGE_2 injection (100 ng/30 μl) into the rat's hindpaw during the induction phase of persistent hyperalgesia significantly reduced the mechanical nociceptive threshold (Figure 1A, One Way Repeated Measures ANOVA and Student–Newman–Keuls Method, $F(6, 24) = 81.21$, $p < .001$, measures performed before PGE_2 injection) and the duration of the tonic immobility response (Figure 1B, One Way Repeated Measures ANOVA and Student–Newman–Keuls test, $F(6, 30) = 10.77$, $p < .001$, measures performed before PGE_2 injection). These findings suggest that pain chronification impairs defensive behaviors.

The mechanical nociceptive threshold (Figure 1A) and the duration of the tonic immobility response (Figure 1B) remained significantly reduced 1, 7, 14, and 21 days after discontinuing the PGE_2 injections (maintenance phase of persistent hyperalgesia). These findings suggest that chronic pain impairs defensive behaviors. Importantly, there was a strong and positive correlation between persistent hyperalgesia development and the decrease in tonic immobility response, as indicated by the Pearson correlation analysis (correlation coefficient = 0.802, $p < .001$). The duration of tonic immobility response was not affected on animals receiving PGE_2's vehicle into the hind paw, $F(2, 8) = 1.714$, $p = .240$, five rats/group data not shown).

Acute pain increases the tonic immobility response through the activation of the ascending nociceptive control. The acute noxious stimulation induced by the forepaw injection of capsaicin (125 μg) significantly increased the immobility response duration (Figure 2, one-way ANOVA and Student–Newman–Keuls test,

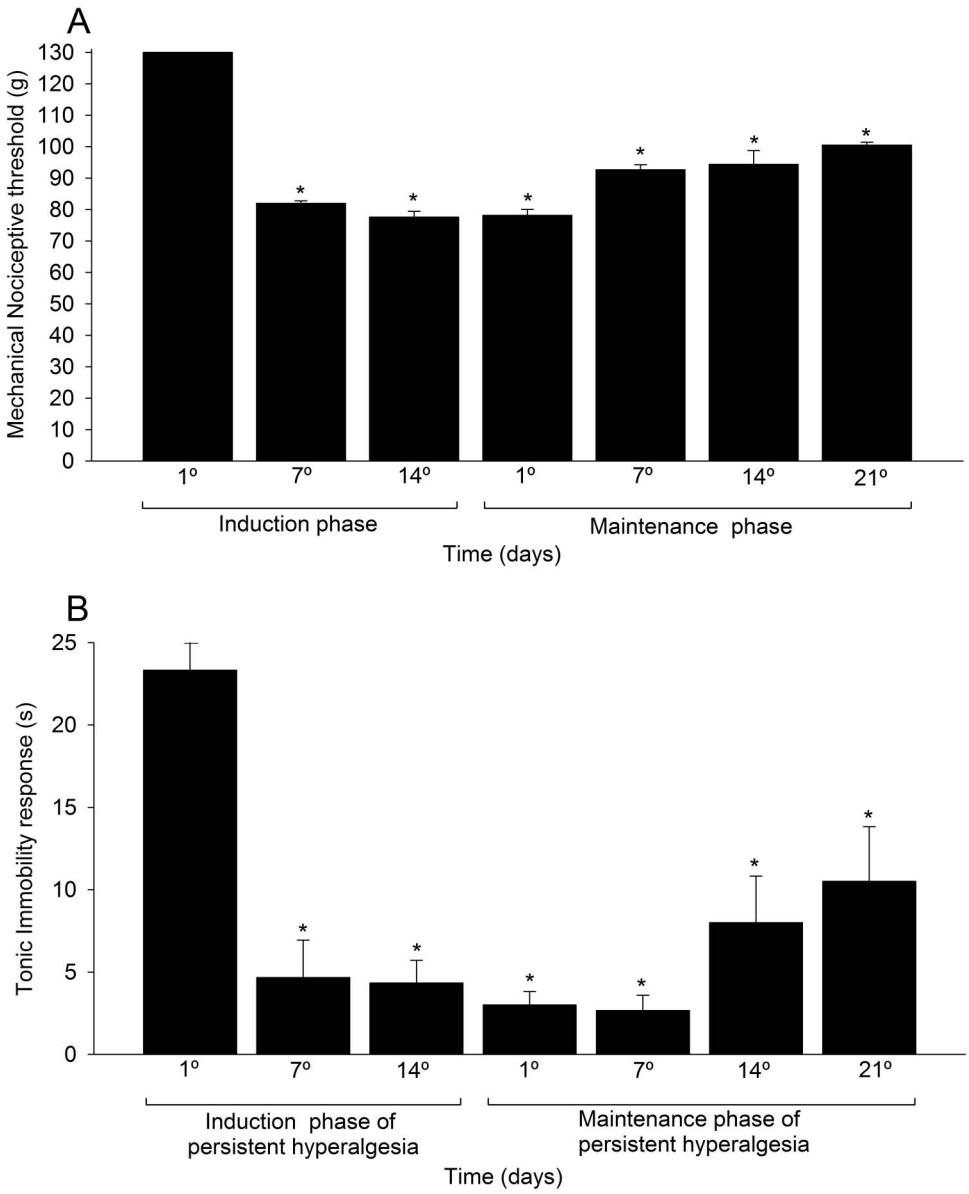

Figure 1. Effect of persistent hyperalgesia development on the tonic immobility response duration. Panel A: Chronic pain development. The mechanical nociceptive threshold measured by the Randall-Selitto paw-withdrawal test was significantly decreased on Days 7 and 14 of the induction phase of persistent hyperalgesia (defined as the 14-day period of daily subcutaneous injections of PGE_2 into the rat's hind paw; nociceptive threshold was measured before PGE_2 injection) and on Days 1, 7, 14 and 21 of the maintenance phase of persistent hyperalgesia (defined as the 21-day period of reduced mechanical nociceptive threshold following the discontinuation of the PGE_2 injections). Panel B: Defensive behavior**.** The duration of tonic immobility response was significantly decreased on Days 7 and 14 of the induction phase of persistent hyperalgesia (test performed before PGE_2 injection) and on Days 1, 7, 14 and 21 of the maintenance phase of persistent hyperalgesia. Asterisks indicate a response significantly lower than that of the 1° day of induction phase of persistent hyperalgesia (pain-free rats; $p < .05$, one-way repeated-measures analysis of variance and Student–Newman–Keuls post hoc test). Data are expressed as $M \pm SEM$ of the mechanical nociceptive threshold in grams (A) and of the tonic immobility duration in seconds (B) that were evaluated in the same five rats.

$F(2, 15) = 18.23$, $p < .001$). The intraaccumbal administration of the μ-opioid receptor antagonist CTOP (1.0 μg, 10 min before capsaicin) prevented this effect. The administration of CTOP (1.0 μg) into the nucleus accumbens of animals receiving capsaicin's vehicle into the forepaw had no effect (1.25 ± 0.39 s, $p = .896$, five rats/group data not shown in figures) indicating that CTOP by itself does not affect the immobility response. Together, these findings confirm our previous findings (Tambeli et al., 2012) that

Table 1
Study Design the Experimental Groups, Procedures, Numbers of Animals (n), and the Associated Figures

Group	Hindpaw injection	NAc injection	Forepaw injection	Behavioral test	*n*	Figures
1	PGE2	—	—	Tonic immobility and Randall Selito	5	1A and 1B
2	Vehicle	Vehicle	Vehicle	Tonic immobility	6	2
3	Vehicle	Vehicle	Capsaicin	Tonic immobility	6	2
4	Vehicle	CTOP	Capsaicin	Tonic immobility	6	2
5	PGE2	Vehicle	Vehicle	Tonic immobility–Day 7 induction	6	3A
6	PGE2	Vehicle	Capsaicin	Tonic immobility–Day 7 induction	6	3A
7	PGE2	CTOP	Capsaicin	Tonic immobility–Day 7 induction	6	3A
8	PGE2	Vehicle	Vehicle	Tonic immobility–Day 14 induction	6	3B
9	PGE2	Vehicle	Capsaicin	Tonic immobility–Day 14 induction	6	3B
10	PGE2	CTOP	Capsaicin	Tonic immobility–Day 14 induction	6	3B
11	PGE2	Vehicle	Vehicle	Tonic immobility–Day 1 maintenance	6	4A
12	PGE2	Vehicle	Capsaicin	Tonic immobility–Day 1 maintenance	6	4A
13	PGE2	CTOP	Capsaicin	Tonic immobility–Day 1 maintenance	6	4A
14	PGE2	Vehicle	Vehicle	Tonic immobility–Day 7 maintenance	5	4B
15	PGE2	Vehicle	Capsaicin	Tonic immobility–Day 7 maintenance	5	4B
16	PGE2	CTOP	Capsaicin	Tonic immobility–Day 7 maintenance	5	4B
17	PGE2	Vehicle	Vehicle	Tonic immobility–Day 14 maintenance	6	4C
18	PGE2	Vehicle	Capsaicin	Tonic immobility–Day 14 maintenance	6	4C
19	PGE2	CTOP	Capsaicin	Tonic immobility–Day 14 maintenance	6	4C
20	PGE2	Vehicle	Vehicle	Tonic immobility–Day 21 maintenance	6	4D
21	PGE2	Vehicle	Capsaicin	Tonic immobility–Day 21 maintenance	6	4D
22	PGE2	CTOP	Capsaicin	Tonic immobility–Day 21 maintenance	6	4D
				Total A	128	
23	Vehicle	—	—	Tonic immobility	5	Data not shown
24	Vehicle	CTOP	Vehicle	Tonic immobility	5	Data not shown
25	PGE2	CTOP	Vehicle	Tonic immobility–Day 7 induction	5	Data not shown
26	PGE2	CTOP	Vehicle	Tonic immobility–Day 7 maintenance	5	Data not shown
				Total B	20	
				Total A + B	148	

Note. Total A represents the total number of animals used in figures; Total B represents the total number of animals used in the groups not shown in figures; Total A + B is the sum of the amount of animals used; PGE2 = Prostaglandin E2; NAc = Nucleus Accumbens.

acute pain facilitates defensive behaviors through the activation of the ascending nociceptive control.

Pain chronification and chronic pain do not alter the ability of acute pain to facilitate the tonic immobility response through the activation of the ascending nociceptive control. The acute noxious stimulation induced by the forepaw injection of capsaicin significantly increased the tonic immobility response duration assessed on Days 7 (Figure 3A; one-way ANOVA and Student–Newman–Keuls test, $F[2, 15] = 11.34$, $p < .001$) and 14 (Figure 3B; one-way ANOVA and Student–Newman–Keuls test, $F[2, 15] = 20.07$, $p < .001$) of the induction phase of persistent mechanical hyperalgesia. This effect was prevented by the administration of CTOP into the nucleus (1.0 μg, 10 min before capsaicin). The intraaccumbal administration of CTOP had no effect on animals receiving capsaicin's vehicle into the forepaw (-1.20 ± 3.99 s, $p = .884$, five rats/group data not shown in figures, experiment performed on Day 7 of the induction phase) indicating that CTOP by itself does not affect the immobility response during the induction phase of persistent mechanical hyperalgesia.

The duration of the tonic immobility response was significantly increased by the acute noxious stimulation induced by the forepaw injection of capsaicin on Days 1, 7, 14, and 21 of the maintenance phase of persistent mechanical hyperalgesia (Figure 4A, B, C, and D, respectively, one-way ANOVA and Student–Newman–Keuls test, $F[2, 15] = 4.99$, $p = .022$; $F[2, 12] = 16.22$, $p < .001$; $F[2, 15] = 15.07$, $p < .001$; $F([, 15] = 5.95$, $p = .012$, respectively). This effect was prevented by the intraaccumbal administration of CTOP, that had no effect on animals receiving capsaicin's vehicle into the forepaw (-4.00 ± 1.09 s, $p = .455$, five rats/group experiment performed on Day 7 of the maintenance phase, data not shown in figures). Together, these results suggest that chronic pain and its development do not alter the ability of acute pain to increase defensive behaviors by activating the ascending nociceptive control. Stereotaxic surgery or intraaccumbal CTOP did not affect rat's motor function assessed by the Rota-rod test since all animals included in the experiments stayed on the rotating apparatus (18 rpm) for 200 s. The injection sites were located within the nucleus accumbens in all animals included in the experiments (see Figure 5).

Discussion

The present study shows that chronic pain and its development impair defensive behaviors. The evidence is that the duration of the tonic immobility response was significantly reduced during the induction and maintenance phases of the persistent mechanical hyperalgesia (see Figure 1). However, pain chronification and chronic pain do not impair the ability of acute pain to facilitate defensive behaviors by activating an ascending pain modulation pathway. Specifically, acute pain induced by a forepaw injection of capsaicin significantly increased the tonic immobility response duration in animals with persistent inflammatory hyperalgesia, via

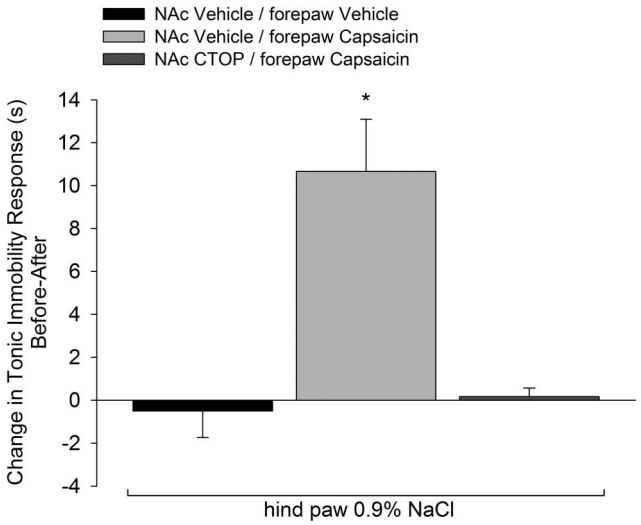

Figure 2. Effect of acute pain on tonic immobility response. The subcutaneous administration of capsaicin (125 μg) into the rat's forepaw significantly increased the duration of the tonic immobility response in previously pain free animals. This effect was prevented by intraaccumbal administration of the μ-opioid receptor antagonist CTOP. Asterisks indicate a response significantly greater than that of other groups ($p < .05$; one-way repeated-measures analysis of variance and Student–Newman–Keuls post hoc test; six rats/group). Data are expressed as $M \pm SEM$ of the change in tonic immobility duration in s, which was calculated by subtracting the tonic immobility duration measured before any experimental intervention performed on that day from that measured 5 min after the forepaw injection (before–after). NAc = Nucleus Accumbens; CTOP = Cys2, Tyr3, Orn5, Pen7amide.

the endogenous opioid release in the nucleus accumbens (see Figures 3 and 4).

The engagement of a threatened organism in the appropriate defensive behaviors may be the difference between death and survival. When flight or fight responses are no longer effective, the immobility response, that is "playing dead," becomes the last chance of surviving because the predator gives up attacking a prey that does not react (Thompson et al., 1981). In humans, this response occurs in life-threatening situations associated to strong fear (Rocha-Rego et al., 2009). Threat events are frequently accompanied by noxious stimuli (Harris, 1996). However, pain is incompatible with defensive behaviors, because to engage in defensive responses, a threatened organism needs to be free from conflicting motivation related to an injury. The ascending nociceptive control is a neurobiological mechanism underlying noxious stimuli, analgesia, and defensive behaviors, since it is activated by acute nociceptive stimulation, induces analgesia (Gear et al., 1999; Gear & Levine, 2009) and increases tonic immobility response (Tambeli et al., 2012). Therefore, during a threat situation, acute pain activates a neuronal mechanism to decrease nociception (see Figure 6 [1]) and facilitate (see Figure 6 [2]) defensive responses.

Here we showed that in contrast to acute pain, chronic pain and its development impairs the defensive behavior of tonic immobility (see Figure 6 [3]). Seven days after the initiation of daily hindpaw PGE_2 injections, the duration of the immobility response significantly decreased. This decrease persisted throughout the induction and maintenance phases of the persistent inflammatory hyperalgesia (see Figure 1B). In fact, there is a strong and positive correlation between the decrease in the nociceptive threshold and in the immobility duration. The inability to engage in defensive behaviors can be interpreted as a decrease in the ability to fight for survival during chronic pain. It is in agreement with the general changes in behavior observed in chronic pain states such as impaired motivation, reward-related responses and endogenous pain modulation (Ossipov et al., 2014; Simons et al., 2014).

Despite impairing defensive behaviors, chronic pain does not affect the ability of acute pain to increase the immobility response (see Figure 6 [4]). An acute noxious stimulus induced by the capsaicin injection into the forepaw significantly increased the immobility response duration in animals with per-

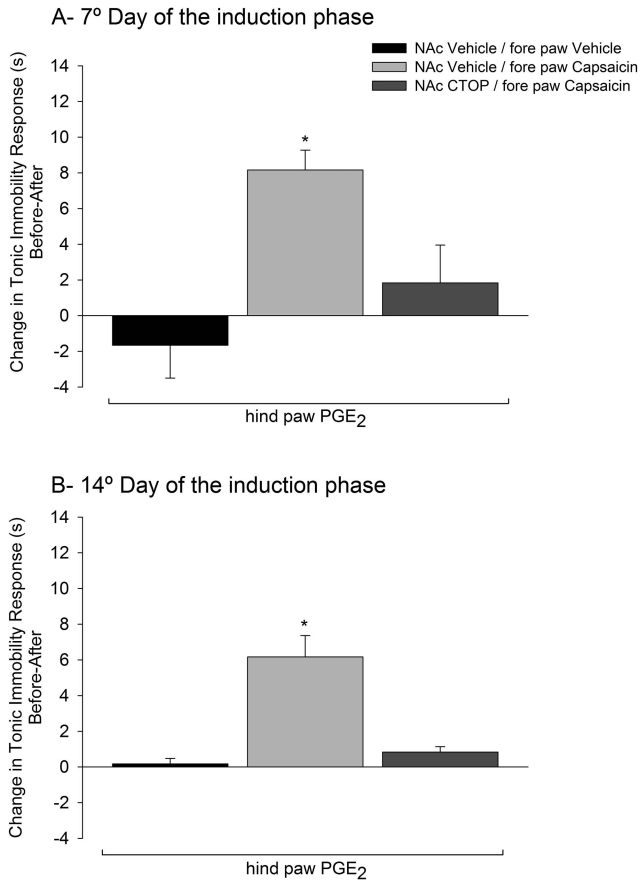

Figure 3. Effect of pain chronification on the ability of acute pain to recruit the ascending nociceptive control to facilitate the tonic immobility response. The subcutaneous administration of capsaicin (125 μg) into the rat's forepaw significantly increased the duration of the tonic immobility response on Days 7 (Panel A) and 14 (Panel B) of the induction phase of the persistent hyperalgesia. This effect was prevented by intraaccumbal administration of the μ-opioid receptor antagonist CTOP. Asterisks indicate a response significantly greater than that of other groups, one-way repeated-measures analysis of variance and Student–Newman–Keuls post hoc test, six rats/group. NAc = Nucleus Accumbens; CTOP = Cys2, Tyr3, Orn5, Pen7amide, 1.0 g bilaterally injected into the nucleus accumbens.

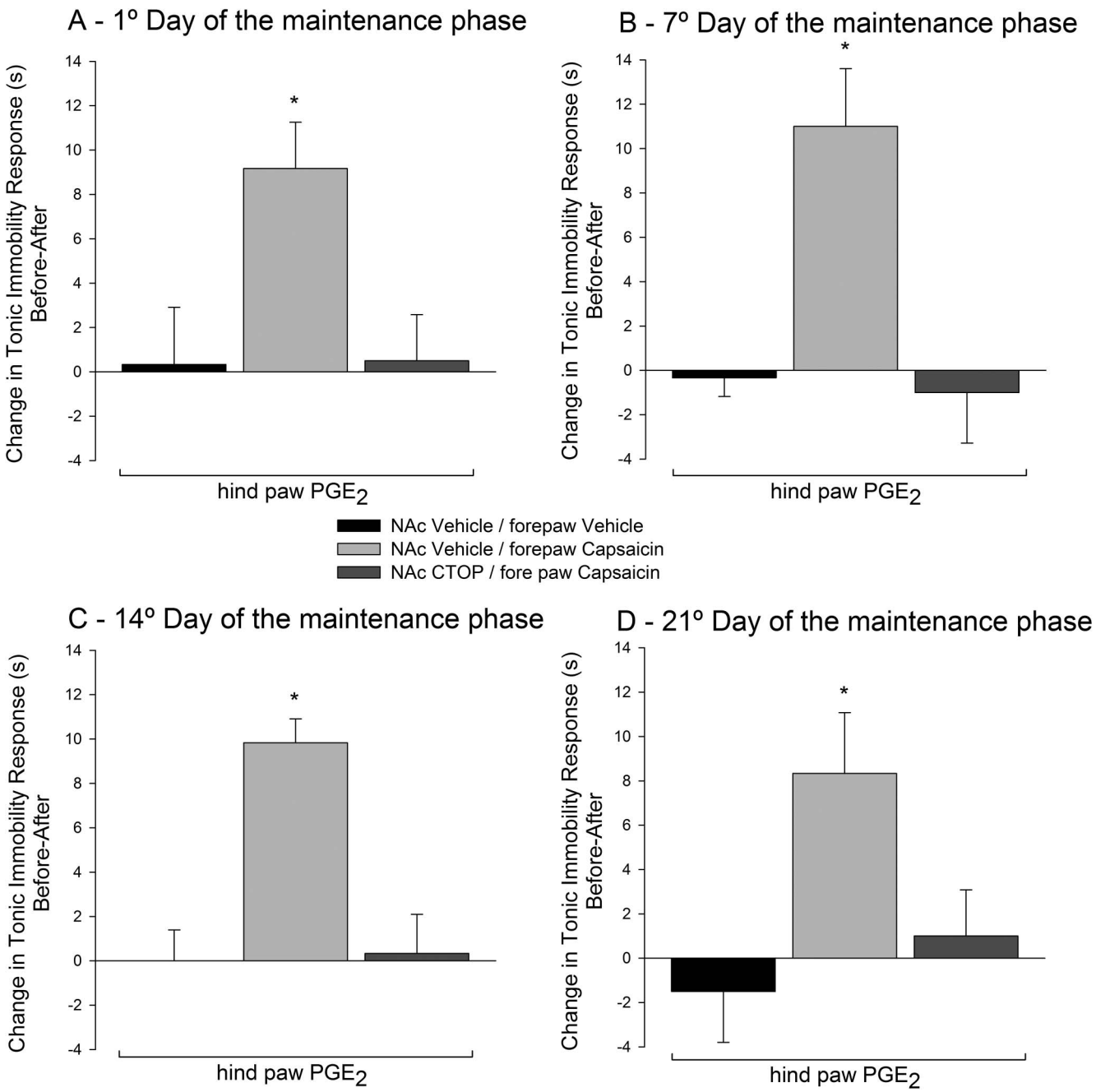

Figure 4. Effect of chronic pain on the ability of acute pain to recruit the ascending nociceptive control to induce the tonic immobility response. The subcutaneous administration of capsaicin (125 μg) into the rat's forepaw significantly increased the duration of the tonic immobility response on Days 1 (Panel A; six rats/group); 7 (Panel B; five rats/group); 14 (Panel C; six rats/group), and 21 (Panel D; six rats/group) of the maintenance phase of the persistent inflammatory hyperalgesia. This effect was prevented by intraaccumbal administration of the μ-opioid receptor antagonist CTOP. Asterisks indicate a response significantly greater than that of other groups ($p < .05$, one-way repeated-measures analysis of variance and Student–Newman–Keuls post hoc test). NAc = Nucleus Accumbens; CTOP = Cys2, Tyr3, Orn5, Pen7amide, 1.0 g bilaterally injected into the nucleus accumbens.

sistent hyperalgesia. This effect was observed with similar magnitude throughout the induction (see Figure 3) and the maintenance (see Figure 4) phases of the persistent hyperalgesia. Such preservation in the ability of acute pain to facilitate defensive behaviors even in the presence of chronic pain may have the purpose of increasing the chances of survival of a wounded subject exposed to acute pain in a novel threatening situation.

Neural circuits underlying endogenous pain modulation (Ossipov et al., 2014; Simons et al., 2014), including the ascending nociceptive control (Ferrari et al., 2010; Miranda et al., 2015) have impaired functioning during chronic pain states. However, although the antinociceptive efficacy of the ascending nociceptive control is decreased during pain chronification and chronic pain (Miranda et al., 2015), its ability to facilitate the immobility response is preserved. This is evidenced by findings showing that the capsaicin-mediated increase in the immobility response duration throughout persistent hyperalgesia is prevented by a previous CTOP injection into the nucleus accumbens (see Figure 3 and 4). Nucleus accumbens is a fundamental component of the ascending nociceptive control (Gear et al., 1999; Gear & Levine, 2009) and of the mesolimbic dopaminergic system, which is known for its involvement in reward and motivation (Baliki & Apkarian, 2015; Bromberg-Martin, Matsumoto, & Hikosaka, 2010; Volman et al., 2013). Nucleus accumbens dopamine mechanisms are involved in both fear (Albrechet-Souza, Carvalho, & Brandão, 2013) and motivation (Bergamini et al., 2016). Recently, the role of nucleus accumbens in plastic changes associated with chronic pain has emerged. Functional changes within nucleus accumbens circuits appears to be critical for pain chronification (Vachon-Presseau et al., 2016) and for the decreased motivation during chronic pain states (Schwartz et al., 2014). In fact, it was suggested that nucleus accumbens have opposite roles in nociceptive processing during chronic and acute pain states (Dias et al., 2015). Therefore, although the ability of the pain triggered μ-opioid mechanism to facilitate the immobility response is preserved, plastic changes in other mechanisms within the nucleus accumbens may contribute to the impairment in defensive behaviors during chronic pain states.

There is a well-known reciprocal interaction (see Figure 6, [2] and [5]) between defensive behaviors and endogenous pain controls (Harris, 1996). In fact, several brain regions, such as periaqueductal gray matter (Monassi et al., 1997), rostral ventromedial medulla (da Silva & Menescal-de-Oliveira, 2006), and nucleus accumbens (Meyer et al., 1993) mediate defensive responses and endogenous analgesia. Functionally, fear and the perception of life threat activate pain modulation systems in a preventive way. However, in the presence of real noxious stimulation, the recruitment of the ascending nociceptive control may function as a final resource to reinforce the expression of defensive behaviors when they are most necessary. The biological value of this mechanism may be so high that its functioning is preserved, as shown in the current study, even in the presence of chronic pain, a condition that, by itself, disrupts defensive behaviors (Harris, 1996) and endogenous pain modulation (Ossipov et al., 2014; Simons et al., 2014).

In summary, the present study demonstrated that pain chronification and chronic pain impair the defensive behavior of tonic immobility in rats (see Figure 6 [3]) but do not affect the ability of acute pain (see Figure 6 [4]) to facilitate it through the activation of the ascending nociceptive control. Therefore, while impairing the defensive behavior of tonic immobility, chronic pain does not affect the ability of acute pain to facilitate it. This is ensured by the activation of ascending nociceptive control that keeps its ability to facilitate a defensive response, even during chronic pain states.

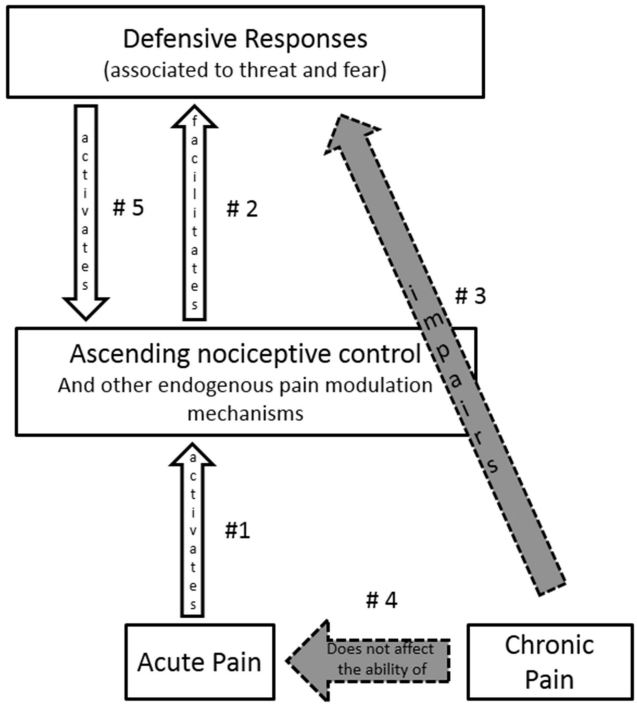

Figure 6. Proposed model—Interaction between chronic pain, endogenous pain modulation and defensive behaviors. Chronic pain impairs defensive behaviors (3), but does not affect the ability of acute pain (4) to facilitate them by activating the ascending nociceptive control (1 and 2). Life threat and defensive behaviors (5) also activate endogenous pain modulation systems in a preventive way. Mechanisms indicated by gray dashed arrows are suggested based on the findings of the present study.

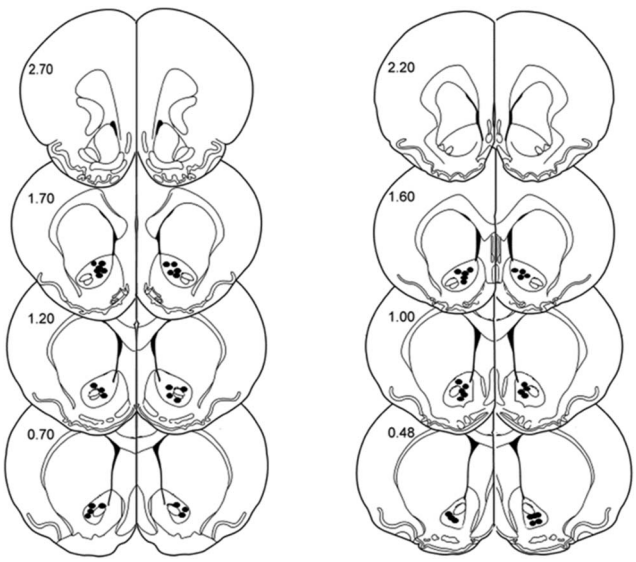

Figure 5. Injection sites in the nucleus accumbens. Nucleus accumbens injection sites (solid circles) plotted on drawings adapted from the atlas of Paxinos and Watson (Paxinos & Watson, 2007). Numbers represent distance caudal from bregma. Some symbols overlap others.

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