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LONG-LASTING HYPERALGESIA AND SYMPATHETIC DYSREGULATION AFTER FORMALIN INJECTION INTO THE RAT HIND PAW

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Abstract—Subcutaneous formalin injection has been used extensively to evaluate acute effects (over several hours) of chemical nociceptive stimulation on nociceptive reflexes. Also, a persistent hyperreflexia for mechanical and thermal stimulation, lasting 3 weeks after formalin injection, has been revealed and related to microglial activation in the spinal dorsal horn. The present study demonstrates more prolonged effects of formalin injection, lasting 6 weeks, on operant escape from nociceptive thermal stimulation. Operant escape requires cerebral processing of nociceptive input and can detect effects that are not limited to spinal or spinal–brain stem–spinal reflex circuits.

Compared with rats injected with saline, escape responding to 44.5 °C and 47 °C stimulation was increased after bilateral s.c. injection of 5% formalin into the dorsal hind paws. The hyperalgesia outlasted visible signs of trauma (e.g. paw edema). Responses to 36 °C were not altered after formalin injection, providing a control for effects of the peripheral injury on activity levels or exploratory tendencies. Skin temperature recordings from the forepaws and contralateral hind paw during 44.5 °C stimulation of the left hind paw provided an indirect measure of cutaneous blood flow in formalin- and saline-injected animals. Normal reductions in skin temperature during thermal stimulation were attenuated (nearly eliminated) at 1 and 2 weeks after formalin injection and partially recovered by 10 weeks. Thus, formalin-induced tissue injury produced a long-term secondary hyperalgesia, accompanied by a reduced sympathetic responsivity. The similar time-course for these phenomena suggests that there are mechanistic linkages between focal injury, autonomic dysregulation and enhanced pain sensitivity. **© 2008 IBRO. Published by Elsevier Ltd. All rights reserved.**

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Abbreviations: ANOVA, analysis of variance; CCI, chronic constriction injury of the sciatic nerve; HPA, hypothalamic–pituitary–adrenal; L/G, licking and guarding reflexes of a hind limb to nociceptive stimulation.

Key words: formalin hyperalgesia, sympathetic dysregulation, inflammation, operant escape, tissue injury, pain.

Within several hours of formalin injection into the plantar hind paw of rats, two distinct periods of licking and guarding are associated with activation of nociceptors (Dubuisson and Dennis, 1977) and sensitization of spinal reflex circuits (Wiertelak et al., 1994). Subsequently, axonal endings in the injected region die back, and a period of inflammatory repair ensues. For several days after injection, primary hyporeflexia has been observed for plantar stimulation.

After formalin injection into the dorsal skin of a hind paw, licking and guarding responses to nociceptive heat stimulation of plantar skin of the paw are increased for 3 weeks (secondary hyperreflexia; Fu et al., 2001). Secondary hyperreflexia for limb withdrawal from mechanical stimulation is also observed. A somatotopically appropriate microglial proliferation is observed during this period (Wu et al., 2004). The proliferation of glia and secondary hyperreflexia for segmental spinal responses (limb withdrawal; Advokat and Duke, 1999) and spinal–brain stem–spinal reflexes (licking and guarding; Woolf, 1984) suggests that spinal sensitization results from influences of tissue injury on pain transmission systems. The present study evaluates this possibility with an operant test of escape that relies on processing of pain throughout the neuraxis (Vierck 2006a,b).

A previous examination of lick/guard (L/G) reflexes and operant escape after chronic constriction injury (CCI) of the sciatic nerve (Vierck et al., 2005) obtained temperature dependent and temporal differences with the effect on these two withdrawal reflexes. For this model of neuropathic pain, responding on L/G and operant escape tests was increased after CCI for cold but not for heat stimulation. These effects lasted as long as the animals were tested after CCI (60 days for L/G, 100 days for escape), corresponding to the persistence of neuropathic pain from nerve injury in humans. In contrast, numerous studies of CCI have shown that enhancement of limb withdrawal reflexes is temporary (recovering within 40 days) for both cold and heat (reviewed in Vierck et al., 2005). Thus, effects of peripheral injury on spinal reflex circuits should not be generalized to represent effects on neural substrates responsible for pain transmission and processing.

The time-course of behavioral changes following neuronal and/or tissue injury is a critical consideration for understanding mechanisms of chronic pain. Studies intended to reveal substrates of abnormal pain sensitivity relate the duration of changes in neuronal reorganization

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(e.g. alterations of receptor distribution or sensitivity) to the time-course of behavioral changes. If there is not a match, then the neuronal change(s) may have contributed to but are not entirely responsible for the behavioral consequences of injury. Also, the behavioral effects must represent a change in pain sensitivity and be appropriate for the clinical condition modeled. In the case of CCI, mechanistic observations have been related to a time-course of segmental reflex effects which does not match clinical experience or the duration of hyperalgesia as measured by operant escape (reviewed in Vierck et al., 2005). The present study compares the time-course for hyperalgesia and hyperreflexia following formalin tissue injury.

EXPERIMENTAL PROCEDURES

All experimental procedures were approved by the University of Florida Institutional Animal Care and Utilization Committee and conformed to National Institutes of Health guidelines for care and use of experimental animals. The experiment was designed to minimize the number of animals used and their suffering. Thirty-nine adult female Long-Evans hooded rats were housed communally, in groups of four, in large enclosures (32 in. high, 18 in. wide, 24 in. deep) containing a hammock, shelves, an exercise wheel, and cardboard and PVC compartments. Gnawing blocks of cypress and shredded paper for nesting were provided.

Behavioral testing

After a period of acclimation to the testing apparatus, 12 female Long-Evans rats (300–350 g) were trained to escape from nociceptive thermal stimulation according to methods previously reported (Vierck et al., 2002, 2004, 2005). The testing apparatus consisted of a dark (0.5 foot-candles) compartment (6 in. wide, 8 in. long) with a thermally regulated floor (plate compartment) and a brightly lit (3200 foot-candles) escape compartment (6 in. wide, 6 in. long) with a thermally neutral floor (at room temperature). The animals could ambulate freely between the compartments, choosing between thermal stimulation in the dark plate compartment and an aversive level of bright light in the escape compartment. The apparatus was ventilated with room air to minimize differences in ambient temperature between compartments and between sessions with different floor temperatures. The average air temperatures recorded in the plate and escape compartments were 25.5 °C and 26.6 °C for test trials of 36 °C. For trials of 44.5 °C the temperatures were 26.7 °C and 26.5 °C, and for test trials of 47 °C the temperatures were 28.0 °C and 27.7 °C.

Behaviorally trained animals were tested 5 days per week and received two 15-min trials per day in adjacent apparatuses. The sequence of temperatures presented in the two daily trials was as follows: Monday: 36–36 °C, Tuesday: 36–47 °C, Wednesday: 44.5–36 °C, Thursday, 36–44.5 °C, Friday: 47–36 °C. Animals tested on such a schedule that varies temperatures between trials and days learn to sample the stimulus condition on each trial and respond according to its intensity. After stable pre-injection data were obtained (eight sessions for each combination of stimulus intensities), each animal received a s.c. injection of saline (50 μL) in one dorsal hind paw (Monday) followed by injection in the other hind paw on Tuesday of week 1 (no behavioral testing on injection days). The data presented in Figs. 1–3 are from the first trials on Wednesday through Friday (36 °C, 44.5 °C and 47 °C), which allowed data for each stimulus intensity to be obtained during the first and subsequent weeks after saline injection. Behavioral testing continued for 8 weeks after saline injection. Then, each animal received s.c. 50 μL injections of 5% formalin (5 mL of 37% formaldehyde in 95 mL saline) in one dorsal hind paw (Monday) and then the other on Tuesday. Behavioral testing continued for

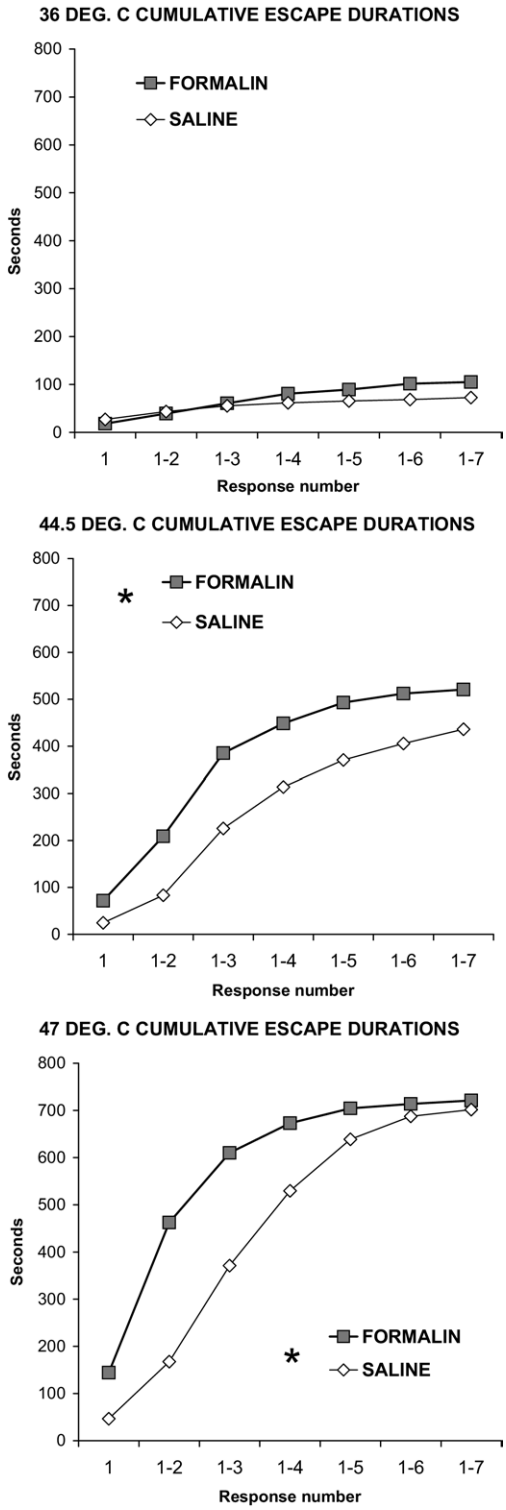

Fig. 1. Escape response durations, averaged over 6 weeks following injection of saline (open circles) or 5% formalin (closed squares) into the dorsal skin of the hind paws. Escape duration is summed from response to response; the last point in each panel shows the total duration of escape compartment occupancy over seven responses (1–7). Escape duration was positively related to stimulus intensity. Formalin injection did not affect responding to 36 °C (top panel) but significantly increased responding to 44.5 °C (middle panel) and 47 °C (bottom panel) (* $P<0.05$).

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