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# Critical Role of the Rostral Ventromedial Medulla in Early Spinal Events Leading to Chronic Constriction Injury Neuropathy in Rats

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**Abstract:** **Neuropathic pain is a major clinical problem, and several animal models have been developed to investigate its mechanisms and its treatment. In this report, the role of the rostral ventromedial medulla (RVM) in the early events of the chronic constriction injury (CCI) model was investigated in behavioral and electrophysiological experiments. Placing the 4 CCI ligatures around the sciatic nerve induced large discharges and residual ongoing activity in spinal nociceptive neurons. Two weeks after CCI ligation, the rats showed behavioral hyperalgesia and allodynia as well as increased ongoing activity and responsiveness of spinal nociceptive neurons to innocuous and noxious stimuli. Blockade of excitatory synapses in the RVM by a kynurenate microinjection (2 nmol in 0.5 μL) 5 minutes before placement of the sciatic ligatures had no immediate effect on spinal neuronal activity but largely prevented the activation of spinal neurons. In kynurenate microinjected rats, behavioral hyperalgesia and allodynia developed slowly and incompletely, which corresponded with an incompletely developed hyperexcitability of spinal neurons. To the best of our knowledge, these results show for the first time that the initial response to nerve damage requires facilitation from the RVM.**

***Perspective:*** ***The present and previous findings indicate that descending facilitation from brainstem nuclei critically contributes to the spinal hyperexcitability that underlies neuropathic pain. The present results indicate that this contribution begins at the very moment the nerve is damaged and should be prevented and treated accordingly.***



***Key words:*** *Chronic constriction injury, descending control of pain, rostral ventromedial medulla, neuropathic pain, spinal dorsal horn, spinal neurons.*

Pain derived from damage to a peripheral nerve is one of the most complex forms of suffering, and, unfortunately, no invariably effective treatment for neuropathic pain is available, as some current therapeutic agents are successful in relieving pain but only in some patients. Peripheral nerve damage induces various degrees of hyperalgesia and allodynia, which may be accompanied by sensory and motor deficits.[22,33,49] Several phenomena, both at the periphery and at the spinal cord, are responsible for the increased excitability of spinal nociceptive neurons as well as for the abnormalities in axonal connectivity and in expression of neuromediators that underlie neuropathic pain.[3,22,31,42,46]

Another important player in neuropathic pain is the so-called "descending pain-control system."[25,30,36,43,45] To a great extent, the spinal hyperexcitability that characterizes neuropathic pain is due to facilitatory influences that "descend" from the nucleus raphe magnus and other structures of the rostral ventromedial medulla (RVM) on the spinal dorsal horn. Two classes of neuron in RVM, the on- and the off-cells, have been shown to project to the spinal cord[9,44] and to affect spinal nociceptive transmission in a wide variety of acute and sustained pain models and pharmacological manipulations.[8,15,17] On- and off-cell activity is in turn influenced by peripheral noxious events via the spinal cord. At the moment a nerve is damaged, the ensuing surge of spinal neuronal impulses will reach on- and off-cells, and their reactions should in turn influence spinal neurons, but this had not been investigated until now. From earlier studies it was known that blocking the ligature-triggered nerve dis-

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charges prevented the development of neuropathy. Indeed, in the chronic constriction injury (CCI) model of neuropathy,[1] 4 ligatures are loosely placed around the sciatic nerve, and these ligatures cause high-frequency discharges in spinal nociceptive neurons.[34] Placing the ligatures when the nerve is locally anesthetized causes a reduction in the subsequent development of hyperexcitability.[7,20,34,35] This established the importance of the early nerve discharges. In the present study, we investigated whether an influence from the RVM on the spinal events triggered by the nerve ligatures was critical for these events and the subsequent neuropathy. We used the CCI model to record activity of nociceptive spinal neurons before, during, and after placing the nerve ligatures, and our results extended those of Sotgiu et al.[34] We then hypothesized that if early events in RVM have a facilitatory influence in CCI neuropathy, pharmacological interference with synaptic excitation in RVM at the time of nerve ligation should attenuate the development of neuropathy. The RVM was thus microinjected with kynurenate 5 minutes before placing the CCI ligatures on the sciatic nerve. Kynurenate is a broad-spectrum antagonist to excitatory amino acid neurotransmitters, and its infusion into RVM has been reported to decrease on-cell firing.[12] The early role of on-cells in other models of neuropathy has been challenged,[2] but the present results indicate that activation of RVM neurons at the time of ligation is critical for the development of CCI neuropathy in the next 2 weeks. Preliminary findings have been presented at meetings.[40,41]

## Materials and Methods

Male Sprague-Dawley rats (weight, 250–330 g), bred at the Instituto Venezolano de Investigaciones Cientificas (IVIC), were used for this study. All the experiments were approved by IVIC's Bioethical Committee for Animal Research and were carried out in accordance with the guidelines of the International Association of the Study of Pain and the Society for Neuroscience. First, the behavioral consequences of sciatic nerve surgery were verified. Some rats were then subjected to sciatic nerve surgery accompanied by neuronal recordings in the spinal cord. Finally, other rats were first subjected to sciatic nerve surgery, and behavioral testing followed by neuronal recordings was carried out 14 days later.

### CCI of the Sciatic Nerve

The CCI model of sciatic nerve damage was used throughout. Under thiopental anesthesia (60 mg/kg i.p.; Abbott, Caracas, Miranda, Venezuela) the left sciatic nerve was exposed and freed for 20 mm proximal to its trifurcation. Four segments of 4-0 sterile silk (Johnson & Johnson, Caracas, Miranda, Venezuela) were placed under the nerve separated by 1 to 1.5 mm and then loosely tied in a distal-proximal sequence. Blood circulation in the perineurium was preserved, as judged by microscopic examination. In sham-operated animals the sciatic nerve was only exposed and freed, but no threads were placed. The skin was sutured and the rats were kept on a 37°C thermostatic blanket in an oxygen-rich chamber until full recovery from anesthesia. Surgery was always carried out by the same person.

### Behavioral Testing

The mechanical threshold for paw withdrawal in awake rats was determined by means of von Frey–type nylon monofilaments (Stoelting, Wood Dale, IL) with bending forces of 1, 2, 4, 8, 10, 15, 26, and 60 g. The rat was placed on a metallic wire mesh through which the filaments were applied from below onto the plantar surface of the hind paw. Filaments were applied in ascending force order until a paw withdrawal occurred (threshold value).

Latency of paw withdrawal to noxious heat was determined by the method of Hargreaves et al.[11] The rat was placed in a chamber (Plantar Test Glass Stands, Series 8; IITC Life Science, Inc., Woodland Hills, CA) with a glass floor preheated to 30°C. Light was beamed from below onto the ventral surface of a hind paw to reach 50°C and was held until the paw was withdrawn or 30 seconds had elapsed.

The hot plate test was carried out by placing the rat on a metal plate heated to 49°C and measuring the latency to licking of the left hind paw. Cutoff was 60 seconds.

Testing took place on days 2 and 1 before nerve surgery, the results being averaged as baseline, and then on days 3, 4, 5, 6, 7, 10, 11, 12, 13, and 14 after surgery. Left and right paws were alternated.

Exploratory behavior was evaluated by the open field test. The rat was placed on a 1-$m^2$ checkerboard floor divided into 100 squares, 100 $cm^2$ each. The number of squares entered by the rat's forepaws within 30 seconds was counted. Exploratory behavior was determined on days 2 and 1 before nerve surgery and averaged as baseline and then on days 7 and 14 after surgery.

### Recording of Neuronal Activity

Recordings from spinal wide dynamic range (WDR) neurons were carried out at the time of CCI surgery to determine their responses to innocuous and noxious stimulation applied at their receptive fields as well as their responses to the sciatic ligatures. In further rat groups, spinal recordings were carried out 2 weeks after manipulating the sciatic nerve and the RVM.

Under thiopental anesthesia (60 mg/kg i.p.), a catheter was placed into the external jugular vein to maintain the rat deeply anesthetized and areflexic with a diluted solution of thiopental (8–10 mg/kg per hour). Rectal temperature was kept around 37°C by means of a thermostatic blanket, and moist oxygen was blown toward the rat's snout.

A laminectomy was performed in spinal segments L1-L5, and the rat was affixed to a stereotaxic frame with spinal clamps. Unitary action potentials were recorded with tungsten microelectrodes (9–12 MΩ; FHC, Bowdoinham, ME) and discriminated, amplified, displayed, and stored with the aid of the BrainWave software (Datawave Technologies, Berthoud, CO) and conventional means. Dorsal horn neurons were chosen for study if they fired in an incremental manner on the application of me-