
A Segmental Chronic Pain Syndrome in Rats Associated with Intrathecal Infusion of NMDA: evidence for selective action in the dorsal horn

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Abstract: We explored the effects of chronic lumbar intrathecal NMDA infusion (mini-osmotic pumps) in Sprague-Dawley rats on motor and sensory axon integrity. Several different infusion protocols, each given over a 4 week period were examined: 0.15 M NMDA in phosphate buffered saline; phosphate buffered saline without NMDA; and 0.20 M magnesium sulfate plus 0.15 M NMDA; 0.35 M NMDA. In two additional protocols, 0.15 M NMDA or phosphate buffered saline were infused for a total of 8 weeks. Within 1-2 weeks of the onset of NMDA, but not phosphate buffered saline infusions, the rats exhibited irritability, circling, biting and excessive grooming resulting in loss of hair, and skin ulcerations from autotomy localized to lumbar and sacral innervated dermatomes. Co-infusion of NMDA with magnesium sulfate almost completely prevented these findings. The behavioural changes were not associated with abnormalities of sensory or motor conduction. Intrathecal infusion of NMDA induces a chronic "central" experimental pain disorder in rats, localized to the cord segment with the greatest exposure to the infusion, without involvement of peripheral sensory axons and sparing the axonal integrity of anterior horn cells.

Résumé: Un syndrome de douleur chronique segmentaire associé à l'infusion intrathécale de NMDA chez le rat: données indiquant une action sélective dans la corne supérieure de la moelle. Nous avons exploré les effets d'une infusion intrathécale chronique de NMDA au niveau lombaire sur l'intégrité des axones sensitifs (pompes mini-osmotiques) chez des rats Sprague-Dawley. Plusieurs protocoles d'infusion différents, tous administrés sur 4 semaines, ont été examinés: NMDA 0.15M dans un tampon salin phosphaté; un tampon salin phosphaté sans NMDA; NMDA 0.15M et sulfate de magnésium 0.20M; NMDA 0.35M. Dans deux protocoles supplémentaires, du NMDA 0.15M ou un tampon salin phosphaté ont été infusés pendant 8 semaines. Après 1 ou 2 semaines d'infusion du NMDA, les rats présentaient de l'irritabilité, des mouvements giratoires, ils mordaient et se toilettaient de façon excessive, causant une perte de poils et des ulcérations de la peau au niveau des dermatomes dont l'innervation est lombaire et sacrée. La co-infusion de NMDA avec du sulfate de magnésium prévenait presque complètement ces manifestations. Les changements du comportement n'étaient pas associés à des anomalies de conduction sensitive ou motrice. L'infusion intrathécale de NMDA induit un trouble chronique "central" expérimental de la douleur chez le rat, localisé aux segments de la moelle qui sont les plus exposés à l'infusion, sans implication des axones sensitifs périphériques et épargnant l'intégrité des cellules de la corne antérieure.

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"Central" pain disorders are known to arise in man from lesions at varying levels of the neuraxis including cortex, central white matter, thalamus, brainstem and spinal cord. The dorsal horn of the spinal cord is the initial central purveyor of nociceptive information and there is considerable evidence of plastic alterations in its structure and function following peripheral nociceptive activation.¹ There is less information about what mechanisms might allow it to become a primary generator of "pain" in the absence of peripheral nociceptive influences. Candidate neurotransmitter agents involved in the local generation of dorsal horn nociceptive volleys would undoubtedly include glutamate, aspartate, Substance P (SP) and calcitonin

gene-related peptide (CGRP). The participation of NMDA receptors in this local circuitry has been indicated by direct activation of nociceptive dorsal horn neurons by NMDA and the observation that NMDA receptor antagonists block nociceptive

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inputs.^{2,3} Dorsal horn neurons undergo polysynaptic excitation by both excitatory amino acids acting on NMDA receptors and by peptides, acting together in a synergistic fashion.⁴

In mice, single intrathecal injections of NMDA induced behavioural changes suggesting nociceptive activation.^{5,6} The longer term consequences of NMDA excitotoxin action on the dorsal horn have not been explored. In previous work, we (SN and RJR) examined histological consequences of chronic NMDA infusions in rats.⁷ Local changes at the site of infusion included segmental gliosis and necrosis, with higher doses, of both anterior and posterior horn neurons.

In this work, we studied long term (4 and 8 week) behavioural and electrophysiological consequences of intrathecal NMDA infusions. Chronic central nociceptive activation was assessed by daily examination of the rats for evidence of irritability and autotomy. Serial *in vivo* and endpoint *in vitro* measurements of multifibre myelinated motor and sensory conduction and unmyelinated sensory conduction were made. These studies assessed possible peripheral mechanisms of nociceptive pathway activation and the physiological integrity of motor axons. NMDA was delivered by mini-osmotic pumps alone or with magnesium sulfate and compared to control infusions of phosphate buffered saline. Infusions with magnesium indicated whether the action of NMDA could be prevented by blocking the NMDA receptor. We addressed the following questions: (i) what is the dermatomal distribution of autotomy and its time course following intrathecal NMDA? (ii) is the NMDA action blocked by magnesium? (iii) do abnormalities in peripheral conduction, especially sensory myelinated and unmyelinated fibres, account for the behavioural changes? (iv) is there excitotoxic damage to anterior horn cells or neurofilament synthesis as assessed by electrophysiological indices of motor axon integrity?

METHODS

The studies were conducted on male Sprague-Dawley rats of initial weight 400-500 g housed in wire or wood shaving floored cages and with free access to rat chow and water. Local approval for the work was given by the Animal Care Committee. Surgical procedures and nerve conduction studies were conducted with the animal anaesthetized with sodium amytal (80 mg/kg ip). Under sterile technique, a Silastic (Dow Corning) catheter (I.D. 0.3 mm; O.D. 0.64 mm) was introduced through the lumbar 5th and 6th space by a slit in the dura and was fed rostrally through the subarachnoid space such that the solutions from the catheter bathed the dorsal surface of the lumbar enlargement. The other end of the catheter was then connected to a infusate primed mini-osmotic pump (Alzet Alza Corporation, 14 day pump #2002) containing 200 µl and with a flow rate of 0.5 µl/hr. The pump and positioned catheter were sutured (Ethicon 4-0 silk) to the paraspinal muscles of the rat and then covered, along with the catheter by the approximated and sutured skin flaps. The pumps were replaced every 2 weeks through the protocol while the animal was under sodium amytal anaesthesia. Multifibre nerve conduction studies of caudal motor and sensory fibres were completed at onset (before pump placement) and 4 weeks or 8 weeks later. At endpoint, the sural and peroneal nerves were resected and placed in an *in vitro* chamber for measurements of myelinated and unmyelinated

conduction at 37°C. The techniques for *in vivo* and *in vitro* conduction have been previously described.⁸

The following infusion protocols in 8-12 rats were used, each given continuously over a 4 week period (Table 1): (i) 0.15 M NMDA in 0.1 M phosphate buffered saline (PBS); (ii) 0.1 M PBS alone; (iii) 0.2 M magnesium sulfate together with 0.15 M NMDA for 4 weeks; (iv) 0.35 M NMDA in PBS. Two further infusions were given over 8 weeks: (v) 0.15 M NMDA in PBS; (vi) PBS alone. Studies (i) through (iii) were completed together over a common time period. All rats in these groups were reared on wire cages whereas (iv), (v) and (vi) were reared in plastic shavings-covered shoeboxes. Study (iv) was conducted separately in smaller groups of rats (n = 3) since a higher dose of NMDA was employed and included its own control group that received intrathecal PBS alone. Rats were inspected daily (excepting weekends) for evidence of autotomy.

Statistical calculations included determination of means and standard errors. Endpoint studies were compared among the groups using one way analysis of variance at endpoint (ANOVA) for studies (i) through (iv) and by the Student's t test for study (v). The null hypothesis was rejected for $p < 0.05$.

RESULTS

Behavioural Alterations

Behavioural changes were noted within 1-2 weeks of the onset of infusions. These consisted of irritability, especially when the hindlimbs were handled, circling, excessive grooming of the hindquarters, and chewing or biting of the hindquarters. Later effects were hair loss over portions of the hindquarter and skin lesions from self biting. The autotomy lesions, noted between 7 and 26 days were confined to the lumbar and sacral segmental sensory supply and generally did not involve the upper body, or upper limbs except when higher doses of NMDA (0.35 M) were used. The most common lesion involved the skin over the junction between the hindtrunk and tail. The rats were observed to chew and bite this area within a few days of the start of infusion. Hair loss over the area preceded skin ulceration. The behavioural changes occurred earlier in rats given 0.35 M NMDA than those that received 0.15 M NMDA. In the first 3 groups of animals studied simultaneously (see above) the incidence of autotomy, defined as animals with open skin ulcerations, was 6 out of 12 rats receiving 0.15 M NMDA for 4 weeks, none out of 11 rats receiving PBS alone for 4 weeks, and 1 out of 7 rats who were co-infused with NMDA and magnesium sulfate.

Table 1. Final Weights.

Group	n	Weight (grams)
Control PBS (4 weeks)	11	537 ± 17
0.15 M NMDA (4 weeks)	10	502 ± 15
0.15 M NMDA + 0.2 M MgSO ₄ (4 weeks)	7	530 ± 9
Control PBS (8 weeks)	8	577 ± 18
0.15 M NMDA (8 weeks)	9	574 ± 9
Control PBS (4 weeks)	2	462 ± 22
0.35 M NMDA (4 weeks)	3	468 ± 35

Values are means ± SEM

In Vivo and In Vitro Multifibre Conduction

The results of serial multifibre *in vivo* measurements of caudal nerve action potential amplitudes are given in Figure 1 for control animals (0.1 M PBS infusate) and those given NMDA. At all time points, caudal sensory conduction velocities and nerve action potential amplitudes were similar between controls and those given NMDA. In both groups there were expected maturational increases in these indices with time. Rats given a combination of 0.15 M NMDA with 0.2 M magnesium sulfate also had caudal sensory studies that were similar to controls.

The results of *in vitro* measurements of sural myelinated and unmyelinated fibres are given in Table 2. As in the *in vivo* measurements, NMDA infusion was not associated with loss of sural sensory fibres or changes in conduction velocity.

The results of *in vivo* measurements of caudal and sciatic-tibial motor conduction are given in Figures 2 and 3. Motor conduction velocities in both caudal and sciatic tibial territories were maintained in NMDA treated animals. There was no evidence of motor unit loss as gauged by the amplitudes of the compound muscle action potentials in dorsal foot and caudal muscles. Results of *in vitro* measurements of peroneal motor conduction are given in Table 3: as in the *in vivo* studies, peroneal myelinated and unmyelinated fibres were not altered by NMDA infusion. Results were again comparable to those of control animals.

High dose (0.35 M) NMDA infusion over 4 weeks did not alter caudal sensory, caudal motor, sciatic-tibial motor, sural myelinated, sural unmyelinated, or peroneal myelinated conduction when compared to control PBS infused rats.

DISCUSSION

The major findings of this work were: (i) rats infused with intrathecal NMDA developed adverse behavioural reactions followed over a few days by self-injury or autotomy in the hindquarter skin appropriate to the spinal cord level exposed to the agent; (ii) autotomy was not associated with carrier infusion alone; (iii) autotomy was largely prevented by co-infusion of NMDA with magnesium sulfate; (iv) NMDA infusion was not associated with defects in myelinated and unmyelinated sensory conduction or motor conduction in the hindlimb or tail.

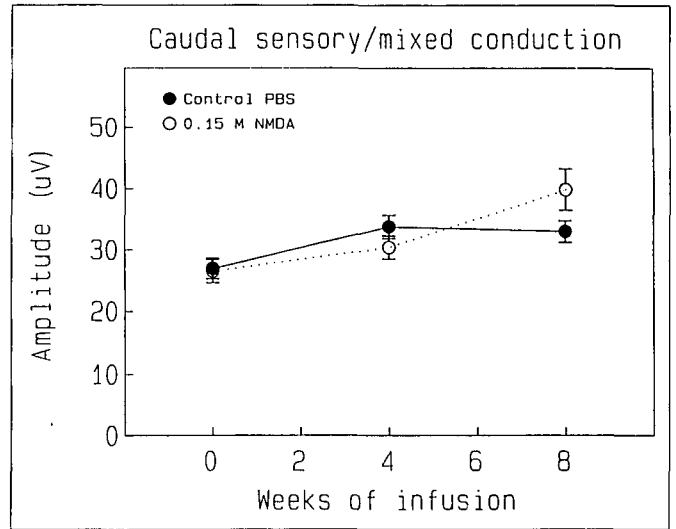


Figure 1: Serial *in vivo* compound nerve action potential amplitudes from caudal fibres.

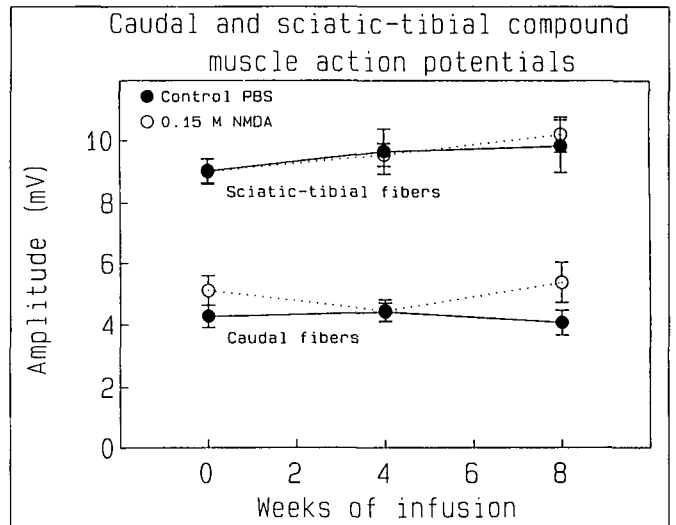


Figure 2: Serial *in vivo* compound muscle action potential amplitudes from caudal and sciatic - tibial fibres.

Table 2. Sural *In Vitro* Conduction.

Group	Myelinated Fibres		Unmyelinated Fibres	
	CV(m/s)	Amp(mV)	CV(m/s)	Amp(µV)
Control PBS (4 weeks)	62.8 ± 4.8 (9)	2.67 ± 1.20 (9)	1.05 ± 0.09 (7)	57 ± 13 (7)
0.15 M NMDA (4 weeks)	57.1 ± 2.9 (8)	2.01 ± 0.40 (8)	1.14 ± 0.09 (8)	62 ± 16 (7)
0.15 M NMDA + 0.2 MgSO ₄ (4 weeks)	50.6 ± 4.3 (5)	0.89 ± 0.20 (5)	0.85 ± 0.58 (5)	29 ± 9 (5)
Control PBS (8 weeks)	50.6 ± 3.2 (7)	1.54 ± 0.16 (7)	1.09 ± 0.05 (6)	39 ± 5 (6)
0.15 M NMDA (8 weeks)	52.2 ± 3.5 (9)	1.45 ± 0.43 (9)	1.07 ± 0.12 (4)	43 ± 21 (4)

Values are means ± SEM
() bracketed numbers indicate n

Table 3. Peroneal *In Vitro* Conduction.

Group	Myelinated Fibres		Unmyelinated Fibres	
	CV(m/s)	Amp(mV)	CV(m/s)	Amp(μ V)
Control PBS (4 weeks)	62.9 \pm 3.9 (10)	2.01 \pm 0.34 (10)		
0.15 M NMDA (4 weeks)	68.2 \pm 4.8 (9)	3.31 \pm 1.07 (9)		
0.15 M NMDA + 0.2 MgSO ₄ (4 weeks)	45.0 \pm 2.1 (4)	1.39 \pm 0.45 (4)		
Control PBS (8 weeks)	54.6 \pm 1.7 (7)	2.43 \pm 0.32 (7)	1.06 \pm 0.11 (5)	14 \pm 4 (5)
0.15 M NMDA (8 weeks)	54.3 \pm 3.2 (9)	1.72 \pm 0.51 (9)	1.06 \pm 0.14 (2)	19 \pm 13 (2)

Values are means \pm SEM
() bracketed numbers indicate n

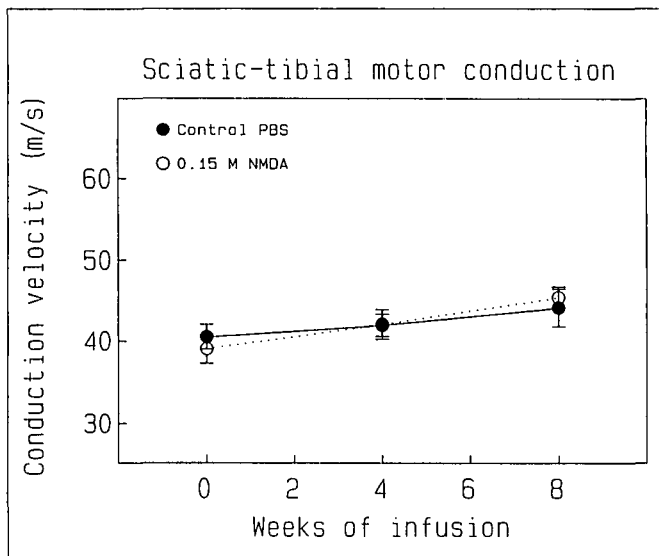


Figure 3: Serial *in vivo* motor nerve conduction velocities from sciatic-tibial fibres.

Several features of the behavioural response to NMDA suggest that its infusion induces an experimental "central" pain disorder, arising from alterations of nociceptive pathways in the lumbar cord. Firstly, the behavioural syndrome appeared to be selectively related to the NMDA receptor because coinfusion with magnesium sulfate largely prevented it. Second, the autotomy lesions and observed biting and chewing reactions were largely confined to the lower hindquarters, i.e., at dermatomes appropriate to the level over which NMDA was infused. Although it is likely that NMDA would have bathed higher cord levels and perhaps cortex, it may have been sufficiently diluted by CSF to prevent action at higher levels. We observed that the onset of autotomy was earlier with the higher dose infusate, arguing in favour of a concentration-dependent mechanism of the local response pattern. Third, the behavioural reaction could not be attributed to peripheral mechanisms because NMDA did not alter peripheral sensory myelinated or unmyelinated conduction. We did not exclude the presence of ectopic afferent activity from sensory nerves or dorsal root ganglia as initiators of a pain

syndrome^{9,10} but think this would be unlikely in the absence of actual damage to these peripheral structures. Models of pain that have evaluated autotomy have previously involved the study of peripheral nerve injury.¹¹ We have not excluded sensory root damage as contributing to the pain disorder in this study but are unaware of reports that there could be NMDA receptors on dorsal roots. To fully exclude this mechanism of pain in our model would require appropriate recordings from dorsal roots or their histological examination.

Two central mechanisms could have accounted for this behavioural syndrome in rats: i) chronic activation of nociceptive pathways by an excitotoxic action of NMDA. ii) elimination of antinociceptive interneurons by excitotoxin-mediated cellular necrosis. Both mechanisms have been postulated by Dubner and Ruda.¹² In favour of the first mechanism was the early onset. In our work the behavioural reaction developed within 1-2 weeks and previous work using mice noted an immediate onset of the aversive behavioural reaction. Aanonsen and Wilcox⁵ and Sakurada et al.⁶ noted that single intrathecal doses of NMDA induced hyperalgesia, and caudally directed scratching and biting behaviour in mice. This behaviour closely resembled that we observed in rats. In mice, an NMDA receptor antagonist (APV), mu opiate agonists, sigma receptor agonists, and phencyclidine all inhibited the behavioural and hyperalgesic effects.

In favour of a mechanism resulting in loss of antinociception is our (SN and RJR) previous histological evidence of localized cord gliosis, and neuronal death in both anterior and posterior horns of the spinal cord⁷ but this may be a later event, after considerable nociceptor activation has already occurred. Glutamate receptors are particularly distributed in substantia gelatinosa¹³ and glutamate is colocalized with SP (Substance P) in dorsal root ganglion cells.¹⁴ There seems little reason to believe that glutamate terminals project exclusively to antinociceptive interneurons. Indeed, in experiments in which NMDA was iontophoresed into spinal cord gray matter, not only was there increased firing of a number of wide dynamic range (WDR) lumbar gray matter neurons, but subthreshold doses also increased their firing to peripheral stimulation.² There is also evidence that NMDA antagonists attenuate aversive reactions from peripheral stimuli and block "wind up" activation

of dorsal horn nociceptive neurons.^{3,15} The release and actions of excitatory amino acids in the dorsal horn are enhanced by peptidergic primary afferent neurotransmitters CGRP and SP: there appears to be close cooperativity between amino acid and peptidergic nociceptive cord circuitry¹⁶⁻¹⁸ (for review see 4). Both SP and serotonin antagonists interrupt NMDA adverse effects.⁶

Peroneal nerve action potential amplitudes (having a major contribution from motor axons), and sciatic-tibial or caudal compound motor action potential amplitudes were preserved despite the use of high doses of NMDA. This is good evidence that the axon processes of anterior horn cells did not atrophy (as would be reflected in reductions of motor conduction velocity) or drop out (as would be reflected in declines in the compound nerve or muscle action potential amplitude) in numbers detectable by our techniques. Since proximal section of a motor nerve is only associated with failure of conduction of distal fibres at an interval of several days,¹⁹ single exposures of anterior horn cells to excitotoxins, with eventual drop out of these cells, would require up to 2 weeks to be identified. These considerations justified our approach in studying electrophysiological consequences of the infusions at 2 and 4 weeks. In human disorders with gradual loss of anterior horn cells, serial measurements of compound muscle action potentials are sensitive indices of motor unit loss.

How do we correlate the apparent loss of anterior horn neurons in our previous work with these findings? It appears that the dorsal horn consequences of NMDA infusion (nociceptive pathway enhancement) are earlier and more sensitive indices of excitatory amino acid toxicity in the rat. The loss of anterior horn cells may be a relatively late event, could be a retrograde event from loss of dorsal horn interneurons, or might be gradual and mild such that distal sprouting from intact motor units compensates for loss of motor units and preserves the compound muscle action potential. There may have been insufficient injury to the anterior horn cell population to permit detection of decline in the compound muscle action potential. By extending our infusion protocol for 8 weeks, and increasing the NMDA dose we deliberately looked for evidence of a more gradual loss of motor axons from anterior horn cell destruction and tried to accelerate such a process. We did, however, not observe loss of motor axons. Also, the peroneal nerve amplitudes were preserved, arguing against axonal loss. Thus, our findings suggest that anterior horn neurons and their axonal processes are relatively resistant to excitatory toxicity. The absence of electrophysiological evidence for axonal atrophy also suggests that excitotoxin damage does not target somadirected synthesis of neurofilaments, important constituents of cell calibre. The results in this work also clearly differ from electrophysiological features of domoic acid intoxication in humans, a disorder linked to inappropriate activation of glutamate receptors from systemic ingestion. Systemic domoic acid intoxication was associated with evidence of axonopathy, not detected by our intrathecal model.²⁰

Of course, to better characterize the motor consequences of amino acid excitotoxicity would require detailed time course studies of anterior horn cell morphology and expression of the appropriate marker molecules on these cells.

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