

Intralesion transplantation of serotonergic precursors enhances locomotor recovery but has no effect on development of chronic central pain following hemisection injury in rats

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Abstract

The effects of intralesion grafts of serotonergic precursors on locomotor recovery and development of chronic pain were assessed after chronic spinal cord hemisection injury (SCI) in rats. Serotonin- and brain-derived neurotrophic factor-secreting (RN46A-B14) and RN46A-vector-only cells were transplanted into the site of T13 lateral hemisection 10 days following injury in immunosuppressed animals, and locomotor and pain related behaviors were assessed weekly for 28 days. There were significant improvements in the degree of spontaneous locomotor recovery, but no significant difference was found in the magnitude of development of mechanical allodynia or thermal hyperalgesia in any transplant group. From these results, we conclude that intraparenchymal engraftment of RN46A-B14 cells is largely ineffective in influencing somatosensory outcomes after SCI, in contrast with the efficacy of dorsal intrathecal placement. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

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In previous studies, our laboratory and others have demonstrated that cell-based therapies are effective treatment methods after spinal cord injury (SCI). Specifically, we have shown that intrathecal engraftment of serotonin (5-HT)- and brain-derived neurotrophic factor (BDNF)-secreting serotonergic precursor cells, RN46A-B14, are able to improve locomotor function and ameliorate chronic central pain-like syndromes after T13 lateral spinal hemisection injury, presumably by increasing cerebrospinal (CSF) and tissue levels of cell-secreted compounds near the site of transplantation that influence spinal circuitry [8–10].

Others have shown that intraparenchymal transplantation is effective. After thoracic transection and transplantation of embryonic raphe cells, alternating rhythmic locomotor-like activity was achieved through activation of central pattern generator (CPG) circuitry [6,19]. In another study, intraparenchymal transplantation of BDNF-secreting Schwann cells

improved the regenerative response across a transected site in the spinal cord, particularly in descending 5-HT and DBH fibers [15] which can influence locomotion [12]. In a contusion injury paradigm, BDNF-secreting fibroblasts injected directly into the injury site resulted in improved locomotor recovery [13].

As of yet, the effects of intraparenchymal grafts on somatosensory function after SCI has been largely unexamined, but evidence exists that intrathecal grafts are effective in correcting abnormal pain syndromes. For example, lumbar transplants of neurons engineered to secrete BDNF are able to reduce allodynia and hyperalgesia after sciatic nerve constriction [2], and intrathecal transplantation of 5-HT secreting cells after chronic constriction injury reduces chronic central pain [5]. Intrathecal adrenal medullary chromaffin cells also reduce allodynia and hyperalgesia after SCI [7].

In the current study, we tested the outcome of intralesion transplantation of RN46A-B14 cells on locomotor recovery and on the development of chronic central pain after SCI.

Male Sprague–Dawley rats ($n = 30$), 150–175 g, were

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deeply anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and the T13 spinal cord segment was unilaterally hemisectioned using a #11 scalpel blade. Postoperative treatments included saline (1.0 cc s.c.) and penicillin-G (0.35 ml/kg i.m.).

RN46A-B14 (secretes BDNF and 5-HT) and RN46A-V1 cell lines were proliferated at 33°C, in Dulbecco's modified minimum essential/F-12/10% fetal bovine serum/250 µg/ml G418/10 ml penicillin-streptomycin. At confluence, RN46A-B14 cells produce 54.2 ± 4.12 ng/ml of 5-HT and of 347 ± 26.8 pg/ml BDNF, per 3×10^6 cells in vitro and continue to secrete in vivo [8]. Following a partial laminectomy and small dural puncture, 10^6 cells were stereotaxically injected into the lesion cavity of animals ($n = 10$, RN46A-B14; $n = 10$, RN46A-V1; $n = 10$ sham transplant) with a 30G needle, 10 days following hemisection. Cyclosporine A (40 mg/kg sid) was given for 7 days postoperatively to all animals.

Locomotor function was tested using the Basso, Beattie and Bresnahan (BBB) Locomotor Rating Scale [1]. Mechanical allodynia was quantified by measuring paw withdrawal frequency in response to a 9.96 mN von Frey filament. A normally noxious pinprick stimulus was used to assess analgesic effects. Thermal hyperalgesia was measured by latency of paw withdrawal in response to radiant heat stimuli (4.7 amps) with constant glass temperature (30°C) for non-tested limbs [4]. Supraspinal responses accompanied noxious stimuli. In both mechanical and thermal tests, since no significant side-to-side differences were found, data were collapsed into combined forelimb or hindlimb scores for ease of comparison.

Statistical tests were evaluated at the alpha level of significance of 0.05, by two-tailed analyzes using analysis of variance and Friedman's rank test using one-way repeated measures, followed by tests of factors including pair-wise comparisons with either the paired or two sample Student's *t*-test. Statistical analyzes were performed using Jandel SigmaStat (v1.0), and graphed with Jandel SigmaPlot (v5.0).

Acutely hemisectioned animals displayed loss of ipsilateral hindlimb function as indicated by the BBB scores (mean \pm standard deviation, SD): 2.20 ± 0.95 , 3.15 ± 1.69 , and 1.94 ± 0.60 for RN46A-B14, RN46A-V1, and sham groups respectively (Fig. 1). At day 10 after injury, animals were transplanted. By day 28, rats gradually spontaneously regained use of their ipsilateral hindlimb and attained scores of 15.4 ± 1.28 , 12.28 ± 1.72 , and 11.80 ± 1.58 , for the same three groups respectively. RN46A-B14 animals demonstrated significantly ($P < 0.05$) improved locomotor function at three timepoints: 19, 24, and 28 days after injury.

Mean \pm SD number of paw withdrawals to 9.96 mN von Frey filaments are shown for forelimbs and hindlimbs (Fig. 2). Prior to injury, baseline forelimb withdrawal frequencies were 0.50 ± 0.23 , 0.31 ± 0.20 , and 0.44 ± 0.25 for RN46A-B14, RN46A-V1, and sham groups, respectively. By 28 days after hemisection, forelimb paw withdrawals

increased significantly ($P < 0.05$) in all treatment groups to 4.36 ± 0.32 , 4.78 ± 0.50 , and 4.05 ± 0.51 for RN46A-B14, RN46A-V1, and sham groups (Fig. 2A). Baseline hindlimb withdrawal frequencies were 0.72 ± 0.34 , 0.90 ± 0.40 , and 1.01 ± 0.46 for RN46A-B14, RN46A-V1, and sham groups, respectively. By 28 days after hemisection, hindlimb paw withdrawals increased significantly ($P < 0.05$) in all treatment groups to 4.65 ± 0.51 , 3.91 ± 0.47 , and $4.37.02 \pm 0.54$ for RN46A-B14, RN46A-V1, and sham groups (Fig. 2B). Between all transplant groups, no significant differences were seen in the degree of development of mechanical allodynia after injury in forelimbs ($P = 0.341$) or hindlimbs ($P = 0.421$).

For the pinprick test, responses were maximal at baseline (ten out of ten) for forelimbs and hindlimbs. Immediately following hemisection, there was a transient disruption in normal nociception but by 28 days normal responsiveness to noxious stimuli was restored. In forelimbs, animals response frequency was 9.98 ± 0.11 , 9.92 ± 0.10 , and 10.0 ± 0.00 for RN46A-B14, RN46A-V1, and sham groups, respectively (Fig. 3A). In hindlimbs, animals withdrew 9.90 ± 0.10 , 9.97 ± 0.11 , and 10.0 ± 0.00 times for RN46A-B14, RN46A-V1, and sham groups (Fig. 3B). No significant differences were observed between treatment groups.

Prior to injury, animals demonstrated thermal paw withdrawal latencies in forelimbs of 12.9 ± 0.82 , 12.8 ± 0.64 ,

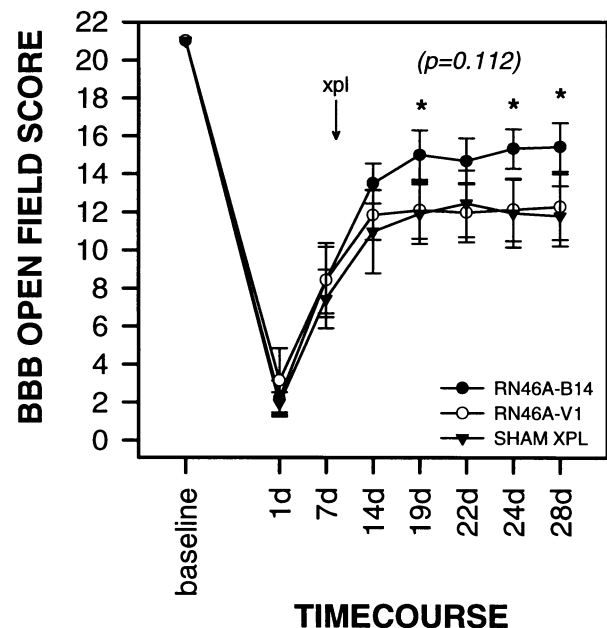


Fig. 1. BBB analysis of ipsilateral locomotor function following T13 hemisection and intralésion transplantation (arrow) of RN46A-B14, RN46A-V1, or no cells displayed as mean \pm SD score. Twenty-one represents normal locomotion, while zero represents no observable limb movement or weight support. By 28 days after hemisection animals demonstrate spontaneous recovery of function, and significant ($*P < 0.05$) differences at days 19, 24, and 28. All other limbs scored 21 at all time points.

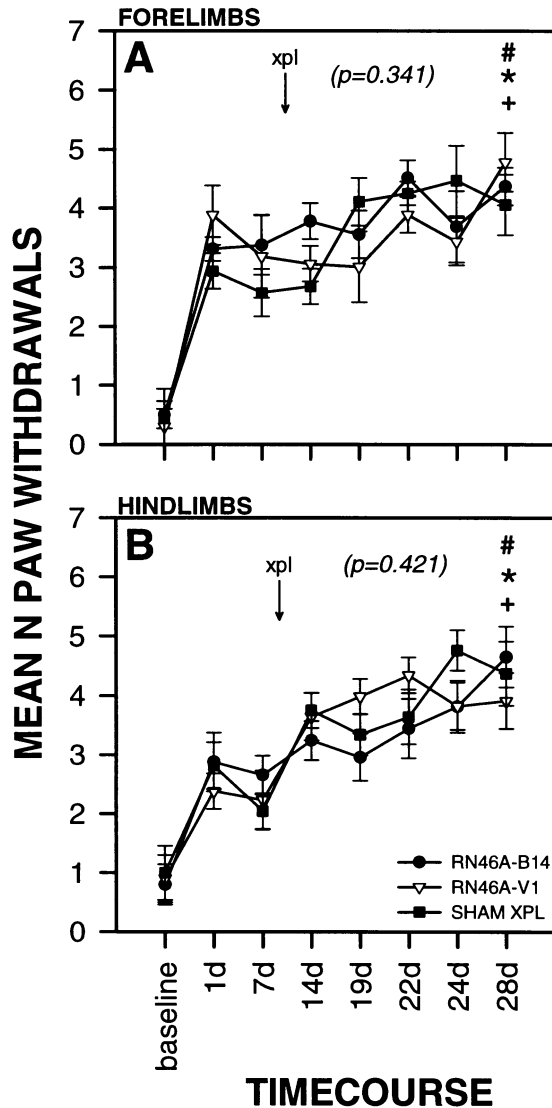


Fig. 2. Change in mean number of paw withdrawals (mean \pm SD) of combined forelimbs (A); and hindlimbs (B) plotted in days after T13 hemisection and intraslesion transplantation (arrow) of RN46A-B14, RN46A-V1 or no cells, to 9.96 mN von Frey mechanical stimuli compared to baseline, and various injury conditions. All groups demonstrate significant (#, *, + $P < 0.05$) development of mechanical allodynia by 28 days after hemisection injury, however no significant differences are observed between treatment groups in forelimbs ($P = 0.341$), or hindlimbs ($P = 0.421$).

and 13.5 ± 0.72 s for RN46A-B14, RN46A-V1, and sham groups, respectively (Fig. 4A). Spinal hemisection resulted in forelimb withdrawal latencies that were significantly ($P < 0.05$) decreased when compared to pre-surgical values, 7.65 ± 0.99 , 8.27 ± 0.84 , and 8.50 ± 0.92 s for RN46A-B14, RN46A-V1, and sham groups. In hindlimbs, baseline withdrawal latencies were 21.2 ± 0.95 , 19.8 ± 1.21 , and 20.0 ± 0.85 s in animals to receive RN46A-B14, RN46A-V1, or no transplants, respectively (Fig. 4B). Twenty-eight days after injury, paw withdrawal latencies decreased significantly ($P < 0.05$) to 12.8 ± 1.35 ,

11.2 ± 0.56 , and 10.6 ± 1.28 s for RN46A-B14, RN46A-V1, and sham groups. All animals developed thermal hyperalgesia. No significant differences were achieved between treatment groups, for either forelimbs ($P = 0.619$) or hindlimbs ($P = 0.499$).

Previous experiments in our laboratories show that intrathecally-implanted neural precursors can survive for long periods of time, and produce and secrete both 5-HT and BDNF into the CSF that diffuses into the spinal parenchyma without evidence of a host-immune response or active rejection [8]. We have also shown that intrathecal RN46A-B14 cells promote improvements in open-field

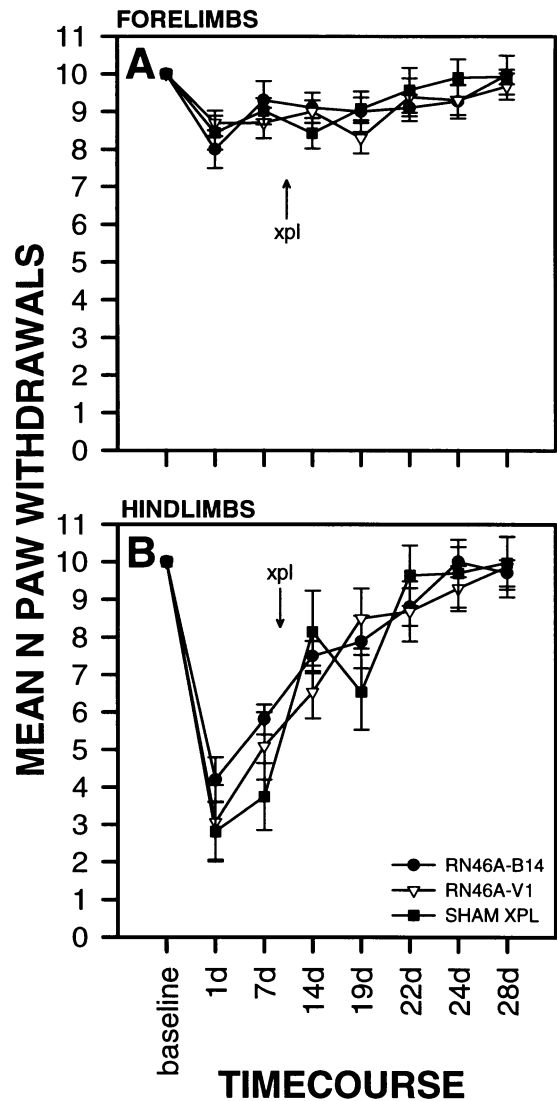


Fig. 3. Number of paw withdrawals (mean \pm SD) to noxious pin stimulus in forelimbs (A); and ipsilateral hindlimbs (B) following T13 hemisection injury and intraslesion transplantation (arrow) of RN46A-B14, RN46A-V1 or no cells. Immediately after injury, animals demonstrate an impairment in processing of normally noxious stimuli that returns by 28 days after injury. No groups demonstrate any differences in restoration of pain-related behaviors, before or after transplantation. Contralateral hindlimb values were 10 at all time points.

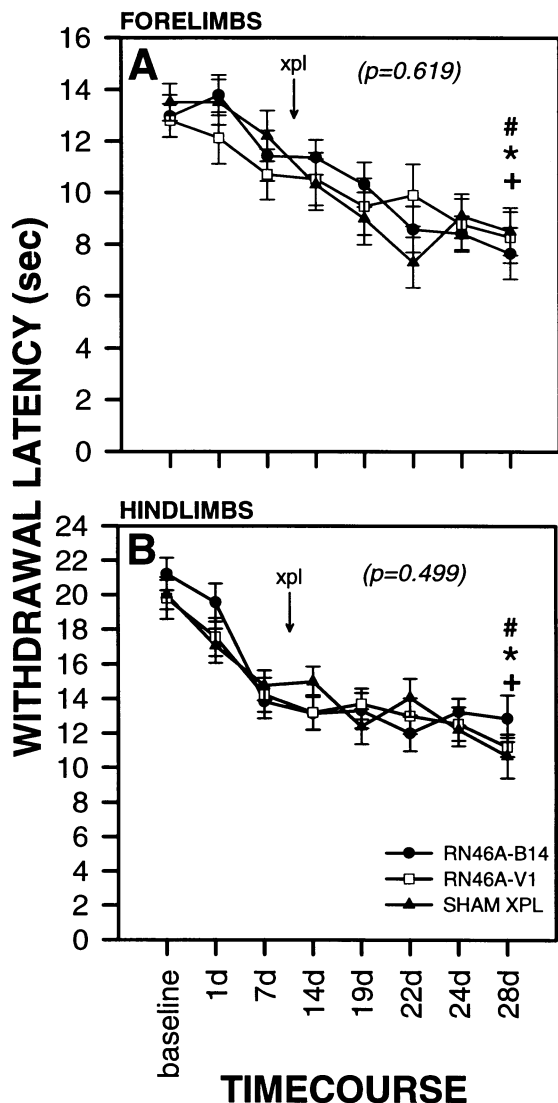


Fig. 4. Changes in mean \pm SD paw withdrawal latency (in seconds) to radiant thermal stimulus for forelimbs (A); and hindlimbs (B) following T13 hemisection injury and intrasession transplantation (arrow) of either RN46A-B14, RN46A-V1, or no cells. All groups demonstrate significant ($\#$, $*$, $+P < 0.05$) development of thermal hyperalgesia by 28 days after hemisection injury. Following group-group comparison, it is apparent that no significant differences in degree of hyperalgesia are evident in either forelimbs ($P = 0.619$) or hindlimbs ($P = 0.499$).

locomotor function as well as reductions in mechanical allodynia and thermal hyperalgesia at the behavioral level [9], and reduction of hyperexcitability of dorsal horn neurons involved in nociceptive processing [10]. Here, following intraparenchymal transplantation, we observe similar gains in locomotor function, but no differences in pain-related behaviors between treatment groups.

Like others [6,19], we were successful in augmenting locomotor function through intraspinal transplantation of raphe cells near CPG circuitry at low thoracic levels. Fibroblasts engineered to secrete BDNF show no growth responses after being transplanted into the central gray

matter of intact animals [16], however, BDNF-secreting cell grafts placed into the lesion site of thoracic spinal cord injury enhance growth of sensory, motor, and coeruleospinal axons but fail to elicit corticospinal fiber growth [14].

One possible reason for not observing emergence of fibers from the graft site in intraspinal-placement paradigms could be that high concentrations of neurotropic substances draw surviving fibers into graft material but do not permit their continued extension outwards. Functional bridging or connection with targets would thus be prevented, and this is perhaps the major caveat of this transplant method.

In order to maximally modulate nociceptive circuitry, it follows that cell grafts must secrete antinociceptive compounds that accumulate and exert their effects discretely upon nociceptive circuits in the dorsal horn. This is best achieved by engrafting secretory cells directly onto the surface of the spinal cord can influence spinal locations that mediate forelimb nociception (see ref. [3]) [7,8]. Intraparenchymal grafting limits the diffusion of transplant-secreted substances from a focal source to locations devoid of compounds. This process is retarded by factors such as extracellular geometry, cellular obstacles, and viscous components of tortuosity which exert a drag effect. Molecules can also experience varying degrees of uptake or clearance [17]. In the intraspinal paradigm, these compounds experience difficulty in reaching their targets which often reside many segments rostral or caudal to the site of transplantation. For example, to activate CPG circuitry after SCI, embryonic raphe cells [20] or intrathecally-delivered BDNF [11] are only effective at particular spinal segments, despite their ability to induce progressive reinnervation for long distances [18].

In sum, transplantation of cells which are known to secrete compounds that modulate locomotor function and nociception into a T13 hemisection lesion are less effective than intrathecal placement, and fail to provide a source of neuroactive compounds to distant segments where they can meaningfully effect function. These results suggest that time and place of transplantation is critical in influencing outcome measures.

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