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Research report

Sensorimotor cortex aspiration: A model for studying Wallerian degeneration-induced glial reactivity along the entire length of a single CNS axonal pathway

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ABSTRACT

Although there are some similarities in the molecular and cellular pattern of Wallerian degeneration in the PNS and CNS, in the CNS the removal of axonal and myelin debris by microglia and astrocytes is not very efficient and occurs over a much longer time frame than seen in a peripheral nerve. Several animal models have been used to study Wallerian degeneration-induced glial reactivity in the CNS and PNS. Although these models have clarified some aspects of the mechanisms underlying the differential glial cell responses in the PNS and CNS, they do not lend themselves easily to deciphering the mechanisms governing the location and extent of Wallerian degeneration-induced CNS glial reactivity. The present study develops a new animal model that entails destruction of the left sensorimotor cortex of adult rats to induce Wallerian degeneration within the total length of a fiber tract (i.e. the dorsal corticospinal tract) that extends all the way from the cerebral cortex to the sacral level of the spinal cord. Since the axonal degeneration in the ventral medulla and dorsal funiculus of the spinal cord would be confined to the corticospinal tract, it was predicted the glial reactivity would also be restricted to this fiber tract. Three distinct proximal–distal levels of this pathway were examined to determine the morphology, distribution and immunophenotype of microglia and astrocytes between 1 day and 16 weeks after sensorimotor cortex aspiration. As expected, there was a proximal to distal gradient in the appearance of glial reactivity along the length of the pathway, with the microglial reactivity being seen as early as 3 weeks in the left pyramid, and by 4 weeks (i.e. at C6) and 6 weeks (i.e. at T11) in the right dorsal corticospinal tract. Astrocytic reactivity lagged behind that of the microglial response at each level of the pathway. The microglial and astrocytic reactivity persisted up to 16 weeks after cortical injury, which was the longest survival time studied. The sensorimotor cortex aspiration model should prove extremely useful in deciphering the molecular mechanisms controlling Wallerian degeneration-induced CNS glial reactivity and in determining the relative role of astrocytes vs microglia in clearance of axonal and myelin debris.

1. Introduction

There are similarities in the molecular and cellular pattern of Wallerian degeneration in the peripheral (PNS) and central (CNS) nervous system, including degradation of the myelin sheaths [18,21]. An early step of Wallerian degeneration is granular disintegration of the cytoskeleton [57], which in rats begins in both central and peripheral nerve fiber tracts between 18 and 72 h after the lesion and progresses distally from the site of initial injury [22]. This cytoskeletal disintegration occurs mostly as a consequence of the activation of proteases such as calpains through an increase in the intra-axonal concentration of Ca^{2+} [10,20]. Axons undergoing Wallerian degeneration do not possess detectable activation of the caspase family of cysteine proteases [16], suggesting that Wallerian degeneration in axons and programmed cell death in neuronal perikarya may be two distinct self-destruct programs [49]. In rodents, axonal degeneration is complete within 3 days in both the PNS and CNS [22], but removal of the axonal and myelin debris is greatly delayed in the CNS as compared to the PNS [21,25,57]. This debris is cleared from the PNS within a matter of weeks [22,53], but persists in the CNS for several months [5,6].

The poor efficiency in removing axonal and myelin debris arising as a result of Wallerian degeneration in the CNS vs the PNS is

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likely due primarily to differences in the microglial and macrophage responses [57]. Vargas and Barres [57] have suggested that in the PNS the clearance of myelin debris occurs in two distinct phases, with the early phase being antibody-independent and mediated primarily by Schwann cells and the later phase being antibody-dependent and mediated primarily by macrophages. In contrast, the myelinating glia of the CNS, the oligodendrocytes, do not contribute much at all to myelin debris clearance from CNS fiber tracts nor is there much in the way of macrophage invasion except at the site of injury, which is also the only place where there is a disruption in the blood–brain barrier [1,48,51,54]. Removal of axonal and myelin debris from distal parts of CNS axonal pathways undergoing Wallerian degeneration, where the blood–brain barrier remains intact, is thus dependent mainly on the activation of resident microglia. Astrocytes do not appear to play more than a minor role in phagocytosis of the axonal and myelin debris [8,53]. Furthermore, the glial cell response in CNS pathways is slow to appear and is far less than that mounted by Schwann cells and macrophages in the PNS, such that clearance of axonal and myelin debris from CNS pathways also occurs over a much longer period of time (see [57] for a review).

Several animal models have been used to study Wallerian degeneration-induced glial activity in the CNS and use of these models have clarified some aspects of the mechanisms underlying the differential responses in the PNS and CNS. For example, Fujiki et al. [19] used an extradural crush injury of the T8 dorsal columns of the spinal cord in adult wild-type and Wallerian degeneration slow (WLD^s) mice; this lesion induces Wallerian degeneration of ascending fibers in the fasciculus gracilis. The WLD^s mutation delayed the appearance of the morphological signs of axonal degeneration and use of the dorsal column crush injury model revealed a corresponding delay in the appearance of microglial and astrocytic reactivity. Wang et al. [60] used a dorsal funiculotomy (T8 injury) model in adult rats to study Wallerian degeneration-induced glial reactivity in the dorsal corticospinal tract (dCST) and dorsal columns during the first 30 days after injury. However, due to the complexity of the tissue response following a compression or contusion injury to the spinal cord it is difficult to make mechanistic interpretations that pertain specifically to the appearance of axonal and myelin debris.

Thus, more suitable models with which to study the CNS glial cell response to axonal degeneration and the consequent build-up of axonal and myelin debris are ones that induce Wallerian degeneration along the total length of a single axonal pathway that travels separately from other descending or ascending pathways. Optic nerve transection [47] or crush [31] accomplishes this nicely, as does transection of dorsal roots of the spinal cord [63]. Use of the optic nerve as a model system provides a CNS axonal pathway that can be predictably and reliably damaged, but does not provide for the concurrent study of glial cell responses (or lack thereof) in adjacent undamaged tissue. Wallerian degeneration of the primary sensory afferents in the dorsal columns as a result of lower thoracic or lumbar dorsal root injury provides a long length of a CNS axonal pathway for study. However, the multiple root contribution to the fasciculus gracilis and the contribution of new fibers by more superior roots means that the proportion of degenerating axons decreases as one proceeds towards the terminal territory in the nucleus gracilis in the medulla. This complicates the interpretation of mechanisms governing the location and extent of Wallerian degeneration-induced CNS glial reactivity. Although the study of glial reactivity along the perforant pathway following entorhinal cortex injury has proved useful for identifying the potential contribution of circulating monocytic cells to the glial cell response [4], the pathway itself is relatively short and does not provide an opportunity to examine the temporospatial aspects of CNS glial reactivity in response to axonal degeneration.

In the present study, left sensorimotor cortex aspiration was used to induce Wallerian degeneration along the full extent of the dCST, including the internal capsule, basis pedunculus, basis pontis, medullary pyramid, and in the base of the dorsal column of the contralateral side of the spinal cord. Any Wallerian degeneration-induced glial reactivity in this animal model should be limited to the right dCST, with the left dCST functioning as an internal control. The goal of this study was to determine the time frame over which Wallerian degeneration-induced microglial and astrocytic reactivity appeared in the left (ipsilateral to injury) pyramid and in the right (decussated) dCST at the level of C6 and T11.

2. Material and methods

2.1. Sensorimotor cortex aspiration

Thirty-two female Wistar rats (3–4 months old) weighing 220–260 g (U of S Laboratory Animal Service Unit) were used in this study. The University of Saskatchewan Animal Protocol Review Committee approved the study design; food and water were available to all rats *ad libitum*. Each rat was anesthetized by inhalation of Halothane (MTC Pharmaceuticals, ON, Canada). A mixture of 3% Halothane in 100% oxygen was applied by using a Boyle anesthetic machine (Ohmeda, Mississauga, ON) to induce anesthesia. After approximately 5 min the animal became immobile and unresponsive to a pinch test on their hind paws (pedal reflex). To minimize animal pain and distress, a single intramuscular injection of Buprenex (0.05 mg/kg) was administered prior to making a skin incision along the midline of the scalp. The concentration of 3% Halothane was maintained during the initial parts of the surgery. The Halothane concentration was reduced, however, to 2–2.5% during sensorimotor cortex aspiration. When suturing the wound, the concentration of Halothane used was 1–1.5%. Throughout the surgery, each animal was carefully monitored (every 3–5 min) for both physiological parameters and analgesia, including their respiration, heart rate, pedal reflexes, and the color of their paws (pink in color).

Once the Halothane inhalation had induced a sufficient level of anesthesia such that the rat was immobile and unresponsive using the pedal reflex, the scalp was shaved with an electric razor, the rat positioned in a stereotactic apparatus, and the incision site was swabbed with Betadine antiseptic solution (10% provide-iodine topical solution UPS and 70% ethanol). A single intramuscular injection of Buprenex (0.05 mg/kg body weight) was then administrated prior to making a midline incision in the scalp, with the incision beginning just caudal to the eyes and continuing caudally to approximately 10 mm behind the ears. The periostium was deflected and a rectangular craniotomy was done using a surgical drill; this craniotomy extended rostrocaudally from approximately 4 mm rostral to the coronal suture to approximately 2 mm rostral to the lambdoid suture, and mediolaterally from approximately 1 mm lateral to the sagittal suture all the way to the temporal ridge. Upon completion of the craniotomy, the dura was grasped with a pair of very fine forceps, cut with a pair of iridectomy scissors, and carefully reflected out of the way. A Pasteur pipette was connected to a rubber tube, which in turn was connected to vacuum pressure created from the tap running. With the aid of a surgical microscope (Nikon-SMZ-1B) the cortical brain tissue (predominantly sensorimotor cortex) was aspirated by suction, with the brain tissue being removed from the area exposed by the craniotomy as deep as the cortical white matter.

For antiseptic purposes, the surgical area was washed with a 3% solution of hydrogen peroxide (diluted in dH_2O). Then the skin wound was sutured with 3-4 Ethicon 4-0 surgical silk sutures and Betadine solution diluted 1:1 with 70% ethanol was applied to the wound to prevent infection. A 2% Xylocaine local anesthetic gel was applied on top of the sutured skin wound (Lidocaine Hydrochloride Jelly, USP Sterile Topical Anesthetic, Astra Pharma Inc., ON, Canada).

During the first hour postoperatively the animal was monitored every 5 min for their respiration rate and for the color of their paws. Each rat was kept on a heated blanket for the first 15–30 min and was transferred to their own cage once they started to move and once their respiration and paw color were normal. The rats were then checked every hour post-surgically for the next 3 h and also the following morning to ensure they were drinking water and eating food. Subsequently, each rat was checked daily with particular attention being paid to the sutures and to the healing of the wound. None of the rats had any problem with the sutures and there were no complications with postoperative healing of the wounds, which typically occurred within 2–3 weeks. The postoperative scar was covered with new hairs by 4 weeks after the surgery.

The rats were also checked for their general behavioral status including their mobility, alertness, and their food and water consumption. No noticeable mobility problems were observed during the daily checkings in any of the rats. Although there have been several reports of motor impairment following damage to the sensorimotor cortex, each of these reports had to use skilled motor or behavioral tests to detect the functional impairment. These tests included tasks that tested skilled forelimb use [12,23,58], skilled ladder walking [12], and tests to detect limb use asymmetry [42,62].