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TERMINATION OF VESTIBULOSPINAL FIBERS ARISING FROM THE SPINAL VESTIBULAR NUCLEUS IN THE MOUSE SPINAL CORD

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Abstract—The present study investigated the vestibulospinal system which originates from the spinal vestibular nucleus (SpVe) with both retrograde and anterograde tracer injections. We found that fluoro-gold (FG) labeled neurons were found bilaterally with a contralateral predominance after FG injections into the upper lumbar cord. Anterogradely labeled fibers from the rostral SpVe traveled in the medial part of the ventral funiculus ipsilaterally and the dorsolateral funiculus bilaterally in the cervical cord. They mainly terminated in laminae 5–8, and 10 of the ipsilateral spinal cord. The contralateral side had fewer fibers and they were found in laminae 6–8, and 10. In the thoracic cord, fibers were also found to terminate in bilateral intermediolateral columns. In the lumbar and lower cord, fibers were mainly found in the dorsolateral funiculus bilaterally and they terminated predominantly in laminae 3–7 contralaterally. Anterogradely labeled fibers from the caudal SpVe did not travel in the medial part of the ventral funiculus but in the dorsolateral funiculus bilaterally. They mainly terminated in laminae 3–8 and 10 contralaterally. The present study is the first to describe the termination of vestibulospinal fibers arising from the SpVe in the spinal cord. It will lay the anatomical foundation for those who investigate the physiological role of vestibulospinal fibers and potentially target these fibers during rehabilitation after stroke, spinal cord injury, or vestibular organ injury.

Key words: vestibular system, spinal cord afferents, hindbrain, posture control, sense of balance.

INTRODUCTION

The vestibular nuclei play an important role in maintaining postural stability and gaze by integrating afferent signals from the labyrinth of the inner ear, the visual and proprioceptive sensations and issuing efferents to the spinal cord (in monkeys: Roy and Cullen, 1998, 2001; Gdowski and McCrea, 1999, 2000; in cats: Peterson and Coulter, 1977; Donevan et al., 1992a,b; in rats: Lannou et al., 1982; Fuller, 1985; Niklasson et al., 1988; Dieringer and Meier, 1993; Reber et al., 1996; Plotnik et al., 1999; Brettler et al., 2000; Bácskai et al., 2002; in frogs: Matesz et al., 1997, 2002a,b). Among these nuclei, the lateral (LVe) and spinal vestibular nuclei (SpVe) are the main source of spinal cord projections. Our previous study has shown the location of the vestibulospinal neurons in the LVe and their fiber termination pattern in the mouse spinal cord (Liang et al., 2014).

The SpVe contains a large number of neurons projecting to the spinal cord (in mice: VanderHorst and Ulfhake, 2006; Liang et al., 2011; in rats: Leong et al., 1984; Valla et al., 2003; in cats: Nudo and Masterton, 1988; in monkeys: Carlton et al., 1985; Nudo and Masterton, 1988). The majority of these neurons are seen in the caudal part of this nucleus (in rats: Zemlan et al., 1979; Leong et al., 1984). However, their fiber termination in the spinal cord has not been reported though retrograde studies have suggested the possible course of them after injecting a retrograde tracer into different parts of the spinal cord. In the rat, only a small number of labeled neurons were found after injections of HRP into the dorsolateral funiculus (Watkins et al., 1981), ventrolateral funiculus (Zemlan et al., 1979), or the ventromedial gray matter of the lumbar cord (Shen et al., 1990). In the cat, Hayes and Rustioni (1981) found a large number of labeled neurons in the SpVe after injecting HRP to the intermediate zone with encroachment into the dorsal horn. An anterograde study in the rat (injections involved LVe, medial vestibular nucleus (MVe), and SpVe) revealed that most of the vestibulospinal fibers were in the laminae 8 and 9, and to a lesser extent in laminae 2–7 (Matesz et al., 2002a,b). In the anterograde study on the cat, Donevan et al. found that fibers from the SpVe traveled in the dorsal, dorsolateral and lateral funiculi of the cervical cord and terminated in the ventral part of the dorsal horn or the intermediate zone (Donevan et al., 1992b). The present study investigated the vestibulospinal neurons in the SpVe and their fiber termination in the mouse spinal cord using both retrograde and anterograde tracing methods.

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Abbreviations: BDA, biotinylated dextran amine; CC, central canal; FG, fluoro-gold; IML, intermediolateral column; LVe, lateral vestibular nucleus; MVe, medial vestibular nucleus; Sol, solitary nucleus; SpVe, spinal vestibular nucleus.

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EXPERIMENTAL PROCEDURES

Animals

All animal procedures were reviewed and approved by the Animal Care and Ethics Committee of The University of New South Wales (14/94A – Mammalian Brain Structure in Health and Disease) in accordance with the Council Directive 2010/63EU of the European Parliament and the Council of 22 September 2010 on the protection of animals used for scientific purposes. Twenty-six C57/BL6 mice of 12–14 weeks of age, weighing 25–30 g were used. The mice were obtained from the Animal Resource Center in Western Australia.

Retrograde tracing

Mice were anaesthetised with an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (5 mg/kg) before being positioned into a mouse stereotaxic head holder (Kopf Instruments, Tujunga, CA, USA). Laminectomy was performed on the T12 or L1 vertebra and 40 nl fluoro-gold (FG) was injected into the right half of the lumbar cord with a 5 μl Hamilton syringe (Hamilton Company, Reno, NV, USA). The syringe was left in place for 10 min following the injection. Altogether, five mice were injected with FG to the upper lumbar segments. The control group either received normal saline injections into the spinal cord (two mice) or FG injections into the cisterna magna (two mice). At the end of the procedure, the skin was sutured, buprenorphine was injected subcutaneously, and topical tetracycline was sprayed over the incision.

Anterograde tracing

Mice were anaesthetised as stated above before being positioned into the same mouse stereotaxic head holder as above. After drilling the skull over the SpVe, 20–40 nl of biotinylated dextran amine (BDA) solution (10,000 MW, Life Technologies, Melbourne, VIC, Australia) was injected with a 5 μl Hamilton syringe (10 mice) (rostral SpVe coordinates: bregma: −6.23 to −6.59 mm, midline: +1.00 to +1.60 mm, surface: −2.90 to −3.50 mm, four mice were used; caudal SpVe coordinates: bregma: −6.59 to −7.07 mm, midline: +1.00 to +1.60 mm, surface: −2.90 to −3.50 mm, six mice were used). Control animals received the same tracer BDA injections either into the cisterna magna (two mice), the solitary nucleus (Sol) which is ventral to the SpVe (three mice), or the cerebellum (two mice). In each case, the syringe was left in place for 15 min after the injection. The following procedures were the same as in the retrograde tracing step.

Tissue preparation

After a survival time of 1 week (FG experiments) or 3 weeks (BDA experiments), mice were anesthetised with a lethal dose of pentobarbital solution (0.1 ml, 200 mg/ml) and perfused through the left ventricle with 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Brains and spinal cords were removed and put into 30% sucrose for two days before being sectioned at 40 μm in the coronal plane using a Leica CM 1950 cryostat. Every second section from FG injections and every 4th from BDA injections were collected for immunohistochemical staining.

Immunohistochemistry

Sections from FG injected mice were washed in 1 × PBS (phosphate buffered saline) and incubated in 1% H_2O_2 in 50% ethanol for 30 min, followed by incubation in 5% goat serum in 1 × PBS to block the non-specific sites. The sections were subsequently incubated in the primary anti-FG antibody (Millipore, VIC, Australia, 1:4000; raised in rabbit) and the secondary antibody (biotin conjugated goat anti-rabbit IgG; Sigma, Sydney, NSW, Australia, 1:200) before they were transferred to an extravidin peroxidase solution (Sigma, 1:1000). Sections were then visualized with 3,3′-diaminobenzidine (DAB) reaction complex (Vector lab, Burlingame, CA, USA) until optimal color developed. Sections from the dextran injected mice were treated with 1% H_2O_2 in 50% ethanol before they were incubated in the extravidin peroxidase solution (Sigma, 1:1000). Subsequent procedures were the same as for the FG immunostaining. At the end of the procedure, the sections were rinsed, mounted onto gelatinized slides, dehydrated in gradient ethanol, cleared in xylene, and coverslipped.

Data analysis

Sections were scanned with an Aperio scanner (ScanScope XT) under 20× magnification. Scanned images were opened with a software Imagescope and images of different magnifications were extracted and organized in Adobe Illustrator CS6. Brain and spinal cord sections were then compared with the diagrams of the mouse brain (Paxinos and Franklin, 2013) and spinal cord (Sengul et al., 2012) atlases, respectively. The final images were then processed in Adobe Photoshop 6. Cell counting was conducted on every second section. The total number of labeled neurons was corrected with the Abercrombie formula (1946). In this formula, the corrected count (A) is calculated by multiplying the total number of neurons counted (P) by a factor in which the section thickness is divided by the section thickness plus the diameter of the neuronal nucleus. To estimate the size of labeled neurons, the long and short diameters of a neuron with the visible nucleus were measured.

RESULTS

Retrogradely labeled neurons in the SpVe

After FG injections into the upper lumbar cord, labeled neurons were found bilaterally with a contralateral predominance (Fig. 1a–f). Interestingly, there were no labeled neurons in the rostral pole of the SpVe (Fig. 1a). This resulted in an area void of labeled neurons between labeled neurons in the LVe and the SpVe. Following this gap, there were labeled neurons predominantly on the contralateral side (Fig. 1b, c). More caudally, there were similar amounts of labeled neurons on both sides (Fig. 1d). Occasionally, there