Neuroscience Letters 584 (2015) 113–118

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Projections from the oral pontine reticular nucleus to the spinal cord of the mouse

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HIGHLIGHTS

- The possible boundary of PnO is proposed based on the tracing result.
- The fibers from the PnO terminate in the entire spinal cord.
- Fiber terminals are present mainly in laminae 7–10 of the spinal cord.
- The difference between fibers arising from PnC and SubC is described.

ARTICLE INFO

Article history:
Received 8 August 2014
Received in revised form 7 October 2014
Accepted 11 October 2014
Available online 23 October 2014

Keywords:
Oral pontine reticular nucleus
Spinal cord
Caudal pontine reticular nucleus
Reticulospinal tract
Muscle atonia
REM sleep

ABSTRACT

The present study investigated projections of the mouse oral pontine reticular nucleus (PnO) to the spinal cord by (a) injecting a retrograde tracer fluoro-gold (FG) to the lumbar cord and (b) an anterograde tracer biotinylated dextran amine (BDA) to PnO. We found that PnO projects to the entire spinal cord with an ipsilateral predominance. PnO fibers mainly travel in the ipsilateral ventral funiculus in the entire cord, terminating in laminae 7–10 with a lower density of fibers and boutons in lower segments. A small number of fibers travel in the contralateral ventral funiculus in the cervical cord with a similar terminating pattern to the ipsilateral counterpart. The present study is the first demonstration of PnO fiber terminals in the mouse spinal cord. This pathway might be responsible for muscle atonia during REM sleep, but needs physiological research to confirm this.

1. Introduction

The oral pontine reticular nucleus (PnO) has been well known for its involvement in wake-sleep cycle especially the rapid eye movement (REM) phase [1–4]. In pharmacological studies, REM and anti-gravity muscle atonia were induced after injecting an adenosine A1 receptor agonist to PnO [3,4]. Therefore, PnO must have a role in muscle relaxation during REM sleep. PnO has been shown to have spinal projections in many species [6–11] and its fiber termination in the spinal cord has also been reported in the rat [12,13] and cat [9,14–17]. However, controversies in the projecting pattern also exist.

Some studies reported that PnO does not project to the lumbar and lower segments of the spinal cord [18,19], whereas others support the notion that PnO projects to the entire spinal cord [7,8,11,20]. In the mouse, this nucleus has been reported to project to the cervical and thoracic cord [5,6]. But the termination pattern of PnO fibers in the mouse spinal cord has not been investigated. The present study examined the PnO projections to the spinal cord with retrograde and anterograde tracer injections. We found that PnO projected to all levels of the mouse spinal cord, with its fibers terminating predominantly in ipsilateral laminae 7 to 10. Collaterals were also observed in the contralateral lamina 7. The present results suggest an anatomic substrate for the involvement of PnO in modulating muscle tone during REM sleep.

2. Materials and methods

2.1. Animals

All animal procedures were reviewed and approved by the Animal Care and Ethics Committee of The University of New South Wales (11/75A). C57/BL6 mice of 12 weeks of age, weighing 25–30 g, obtained from the Animal Resource Center were used.

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http://dx.doi.org/10.1016/j.neulet.2014.10.025
0304-3940/

2.2. Retrograde tracing

Mice were anaesthetiszd with an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (5 mg/kg) before laminectomy was performed on the T12 or L1 vertebra. 40 nl fluoro-gold 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, 5 mice were injected with fluoro-gold to the upper lumbar segments. The control group either received normal saline injections into the spinal cord (2 mice) or fluoro-gold injections into the cisterna magna (2 mice).

2.3. Anterograde tracing

Mice were anesthetized as described above and placed in the stereotaxic head holder. After drilling the skull above PnO, 20–40 nl of biotinylated dextran amine (BDA) solution (10,000 M_W, Invitrogen) was injected into PnO (Bregma: −4.15 to −4.83 mm, midline: +0.50 to +1.30 mm, surface: −3.50 to −4.50 mm; 6 mice) with a 5 μl Hamilton syringe. Control animals received BDA injections either into the cisterna magna (2 mice) or into the adjacent caudal pontine reticular nucleus (PnC) and the subcoeruleus nucleus (4 mice). In each case, the syringe was left in place for 15 min after the injection. At the end of the procedure, the skin was sutured, buprenorphine was injected subcutaneously, and topical tetracycline was sprayed over the incision.

2.4. Tissue preparation

After survival times of 1 week (fluoro-gold experiments) or 6 weeks (BDA experiments), mice were anesthetized with a lethal dose of pentobarbital solution (0.1 ml, 200 mg/ml) and perfused

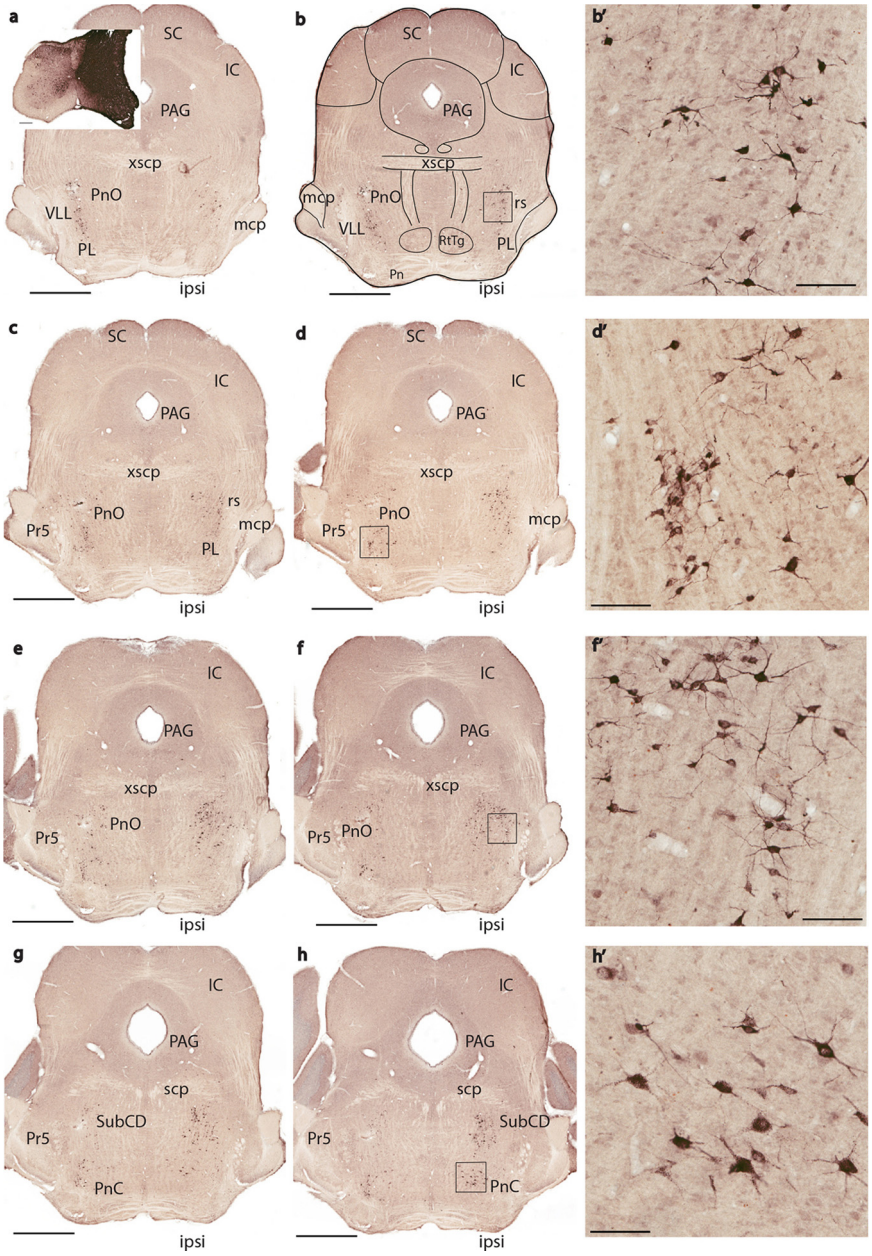

Fig. 1. Retrogradely labeled neurons in PnO and its adjacent nuclei after FG injections into the lumbar cord. (a)–(f) FG labeled neurons in PnO. The photomicrograph in (a) shows the injection site in the lumbar cord. (b′) and (f′) show the rectangular area of the ipsilateral PnO in (b) and (f) and (d′) shows the rectangular area of the contralateral PnO in (d). (g) And (h) shows FG labeled neurons in SubCD and PnC. (h′) Shows FG labeled neurons in the ipsilateral PnC. The scale bar = 200 μm for the photomicrograph in (a) = 1 mm for (a)–(h) = 100 μm for (b′)–(h′).