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Research report

Co-expression of GAD67 and choline acetyltransferase in neurons in the mouse spinal cord: A focus on lamina X

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ABSTRACT

Lamina X of the spinal cord is a functionally diverse area with roles in locomotion, autonomic control and processing of mechano and nociceptive information. It is also a neurochemically diverse region. However, the different populations of cells in lamina X remain to be fully characterised. To determine the co-localisation of the enzymes responsible for the production of GABA and acetylcholine (which play major roles in the spinal cord) in lamina X of the adult and juvenile mouse, we used a transgenic mouse expressing green fluorescent protein (GFP) in glutamate decarboxylase 67 (GAD67) neurons, combined with choline acetyltransferase (ChAT) immunohistochemistry. ChAT-immunoreactive (IR) and GAD67-GFP containing neurons were observed in lamina X of both adult and juvenile mice and in both age groups a population of cells containing both ChAT-IR and GAD67-GFP were observed in lumbar, thoracic and cervical spinal cord. Such dual labelled cells were predominantly located ventral to the central canal. Immunohistochemistry for vesicular acetylcholine transporter (VAChT) and GAD67 revealed a small number of double labelled terminals located lateral, dorsolateral and ventrolateral to the central canal. This study therefore describes in detail a population of ChAT-IR/GAD67-GFP neurons predominantly ventral to the central canal of the cervical, thoracic and lumbar spinal cord of adult and juvenile mice. These cells potentially correspond to a sub-population of the cholinergic central canal cluster cells which may play a unique role in controlling spinal cord circuitry.

1. Introduction

The neurotransmitters GABA and acetylcholine (ACh) play important functional roles in the spinal cord. GABAergic inhibitory interneurons are critical components of local circuits and are abundant in the dorsal horn and ventral horn where they modulate sensory processing and motor activity (Todd and Maxwell, 2000). Neurons containing immunoreactivity for GABA or the synthesising enzyme glutamate decarboxylase (GAD) are found in the dorsal horn of the rat and mouse (Kaduri et al., 1987; Polgar et al., 2013; Todd and McKenzie, 1989) with scattered somata in other layers, particularly lamina X and cerebrospinal fluid contacting neurons in the ependymal layer surrounding the central canal (Kaduri et al., 1987). The cholinergic system is also important in the spinal cord, since ACh is an important modulator of sensory processing and motor output at the spinal cord level. The ACh synthesising enzyme choline acetyltransferase (ChAT) is expressed by ventral horn motoneurons, small cells clustered around the central canal, partition neurons in the intermediate grey area, preganglionic sympathetic and parasympathetic neurons and neurons in the dorsal horn of the adult rat (Barber et al., 1984).

One study suggested the possibility of a role for cells expressing both ACh and GABA in lamina X in the cervical spinal cord of the rat (Kosaka et al., 1988), but did not examine other spinal levels that are also critical for locomotion and autonomic control. Lamina X is an important site for the convergence of somatic and visceral afferent inputs conveying mechanoreceptive and nociceptive information (Honda, 1985; Honda and Lee, 1985; Honda and Perl, 1985; Nahin et al., 1983; Ness and Gebhart, 1987). This area also contains neuronal networks responsible for the generation of locomotor behaviours (Bertrand and Cazalets, 2011), including cholinergic neurons (Zagoraiou et al., 2009). In addition, there are inhibitory neurons in lamina X that project to sympathetic preganglionic neurons of the intermediolateral cell column (Deuchars et al., 2005). Since lamina X also contains sympathetic preganglionic neurons (Deuchars and Lall, 2015), it is a neurochemically and functionally diverse region.

Understanding the neurochemical diversity of cells in lamina X may therefore contribute to unravelling the function of this region.

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Since each level of the spinal cord may contain unique circuitry underlying specific functions, lamina X may vary in cellular composition and structure throughout the cord. Further, potential species differences in cell phenotypes should be considered. Therefore, since mouse is a commonly utilised experimental model we have examined the distribution of double labelled cholinergic and GABAergic cells in lamina X of the spinal cord. Since previous studies have focussed on the cervical spinal levels, we extend this to lumbar and thoracic cord. To enhance detection of GAD expressing neurones, we utilised a transgenic mouse in which GAD67 is reported by expression of GFP in conjunction with immunohistochemistry for ChAT. Since understanding the localisation could be a precursor to electrophysiological studies and juvenile mice are frequently used for such studies *in vitro*, we examined the distribution in both juvenile and adult mice. To investigate where the double labelled cells could project, the localisation of vesicular acetylcholine transporter (VAChT) and GAD67 labelled terminals in the spinal cord were examined.

2. Results

2.1. Verification of GAD67-GFP knock-in mice

To verify that the GFP labelled cells in this model contain GAD67 we performed immunohistochemistry for GAD67 on spinal cord sections from GAD67-GFP mice. Co-localisation of GAD67-GFP and GAD67-IR was observed in cells throughout the spinal

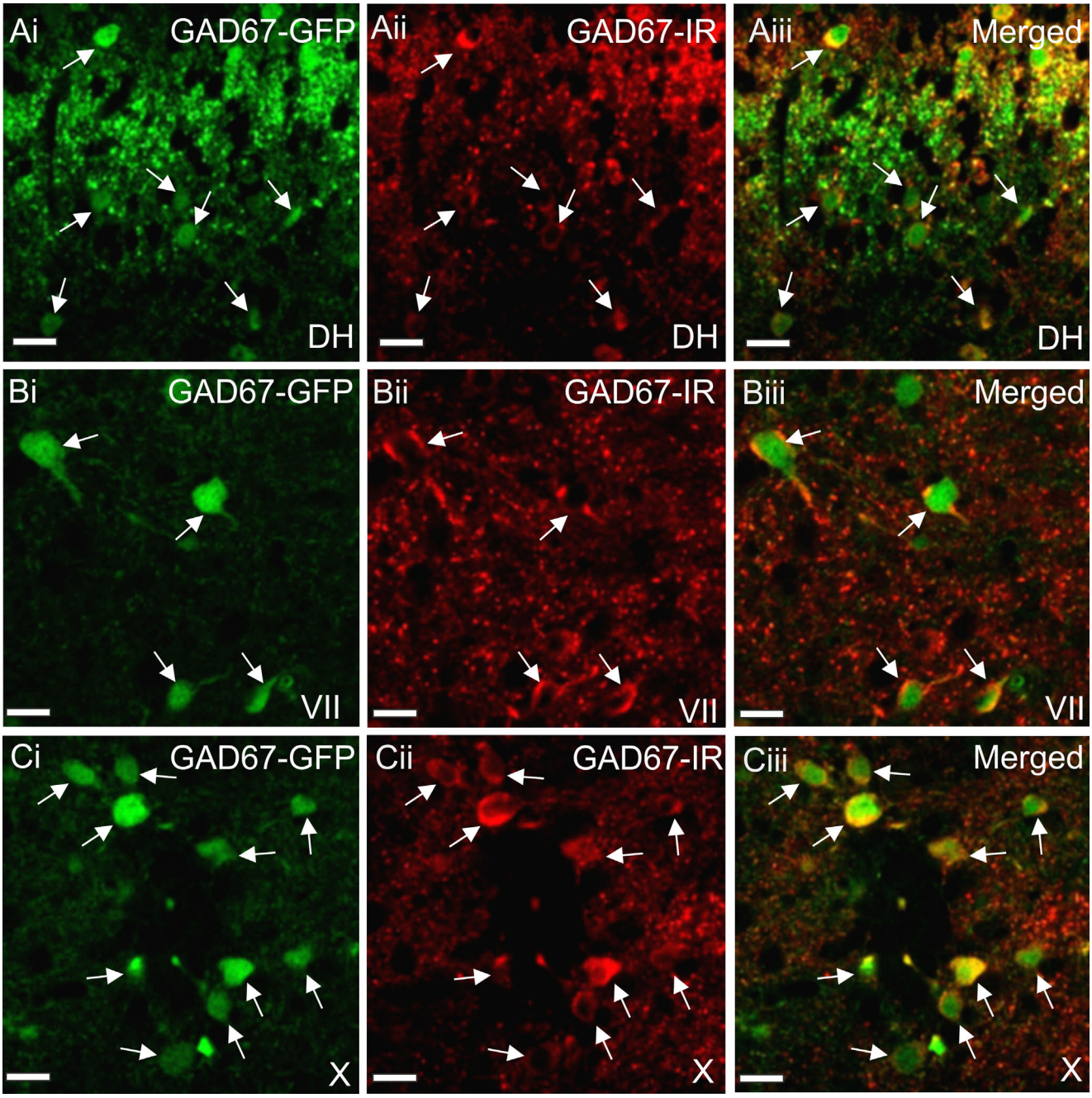

Fig. 1. *Distribution of GAD67-GFP and GAD67-IR cells in the adult mouse spinal cord.* Confocal images showing GAD67-GFP containing cells in the dorsal horn (Ai), lamina VII (Bi) and Lamina X (Ci). GAD67-IR cells are also observed in these areas (Aii, Bii, Cii) and merging of the images reveal that GAD67-GFP and GAD67-IR is observed in the same cells, arrows indicate double labelled cells. Scale bars = 20 μm.