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Colocalization of serotonin and vesicular glutamate transporter 3-like immunoreactivity in the midbrain raphe of Syrian hamsters (*Mesocricetus auratus*)

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Abstract

Vesicular glutamate transporter 3 (VGLUT3) expression has been specifically localized to brain regions rich in serotonergic cells. It has been suggested that this transporter may contribute to the regulation of extracellular glutamate concentrations via a nonsynaptic mechanism. In this study, we examine the colocalization of vesicular glutamate transporter 3 immunoreactivity with serotonin immunoreactivity in the dorsal and median raphe nuclei of Syrian hamsters. Brain sections from adult hamsters were fluorescently labeled for serotonin-ir and VGLUT3-ir and examined using confocal microscopy. The results indicate that most serotonergic cells of the midbrain raphe also expressed vesicular glutamate transporter 3. In addition, nonserotonergic cells in these brain regions also show immunoreactivity for the transporter. These data confirm previous findings of vesicular glutamate transporter 3 expression in serotonergic and nonserotonergic neurons in rats. These findings suggest that the location of vesicular glutamate transporter 3 may be as much a function of neuroanatomical location as of the neurochemical identity of the expressing neurons.

Keywords: 5HT; VGLUT3; Dorsal raphe; Median raphe

Glutamate is the primary excitatory neurotransmitter in the mammalian brain. Recently, two vesicular glutamate transporters (VGLUT1 and VGLUT2) were identified as the molecules responsible for concentrating glutamate in synaptic vesicles [1,15]. Between them, these two transporters appeared to account for the known distribution of glutamatergic neurons in the brain [8,9]. Subsequently, however, a third vesicular glutamate transporter was identified [12,14,5]. This molecule, named VGLUT3, has a much more restricted distribution and has been colocalized with cholinergic, serotonergic, and GABAergic cells [6,3]. The function of VGLUT3 is unknown, but the evidence strongly suggests that it is involved in packing vesicles with glutamate for exocytotic release [3].

In addition to its more confined distribution, VGLUT3 also differs from the other two vesicular glutamate transporters in its intracellular location. In cholinergic neurons of the striatum, VGLUT3 is detected in cell bodies, not just in terminals [5,3]. This suggest the possibility of an unconventional mode of glutamate release, perhaps from the cell soma, by these neurons [4]. VGLUT3 has also been localized within astrocytes as well as neurons, and in cells outside the nervous system [3]. It has also been identified in cultured serotonin neurons [12,2] and in midbrain raphe neurons in rats [7]. The specificity of VGLUT3's localization within specific brain nuclei would suggest that it is involved in a type of glutamatergic function specific to those brain regions. However, localization of the VGLUT3 protein has not been examined in the midbrain raphe nuclei other than in rats. In the present study, we sought to determine whether VGLUT3 protein was expressed in the midbrain raphe nuclei of the Syrian hamster, and whether VGLUT3 colocalized with serotonin neurons within these nuclei.

Adult male and female Syrian hamsters (ages 10–20 weeks) were group-housed (separated by sex) in a 14 h light/10 h dark cycle with food and water available ad libitum until the start of the experiment. Throughout the experiment, care was taken to minimize pain and discomfort to the animals. All procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Kent State University Institutional Animal Care and Use Committee. Animals were deeply

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anesthetized with an overdose of sodium pentobarbital (Nembutal, 200 mg/kg) and transcardially perfused with approximately 100 ml of phosphate-buffered saline (PBS) and 200 ml of 4% paraformaldehyde in phosphate buffer. Whole brains were removed and postfixed overnight in 4% paraformaldehyde at 4 °C. Brains were then transferred to PBS and kept at 4 °C until ready for slicing.

Coronal sections through the midbrain were cut at 40 μm on a vibratome. Every fourth section was collected and stored in PBS. In all subsequent steps, sections were rinsed three times for 5 min in PBS between incubations. Sections were incubated in blocking solution (5% normal donkey serum, 0.3% Triton-X in PBS) for 30 min, followed by overnight incubation at room temperature in guinea pig anti-VGLUT3 (Chemicon, 1:4000) and rabbit anti-serotonin (Immunostar, 1:2000). Sections were then incubated in Cy3-conjugated donkey anti-guinea pig and Cy2-conjugated donkey anti-rabbit (Jackson Immunoresearch, 1:200) for 1 h at room temperature. After rinsing in PBS, sections were mounted on gel-coated slides and allowed to air dry before coverslipping with DPX. Slides were stored at 4 °C.

The extent of colocalization was qualitatively evaluated using an Olympus FV500 confocal microscope with Fluoview software. Confocal image stacks of the dorsal and median raphe were taken at multiple resolutions, and examined for the distribution of both labels. NIH ImageJ software was used to create composite images from confocal stacks and to extract single layer images. Adobe Illustrator was used to convert images to CMYK format for publication. Image contrast was adjusted for maximum clarity of printed images.

Cell somata stained for 5HT and/or VGLUT3 are easily detectable in images captured from both the dorsal and median raphe nuclei. In both the dorsal raphe and the median raphe, VGLUT3-ir is detected in both 5HT-ir neurons as well as in non-5HT-ir cells (Fig. 1A and B). While 5HT-immunoreactive

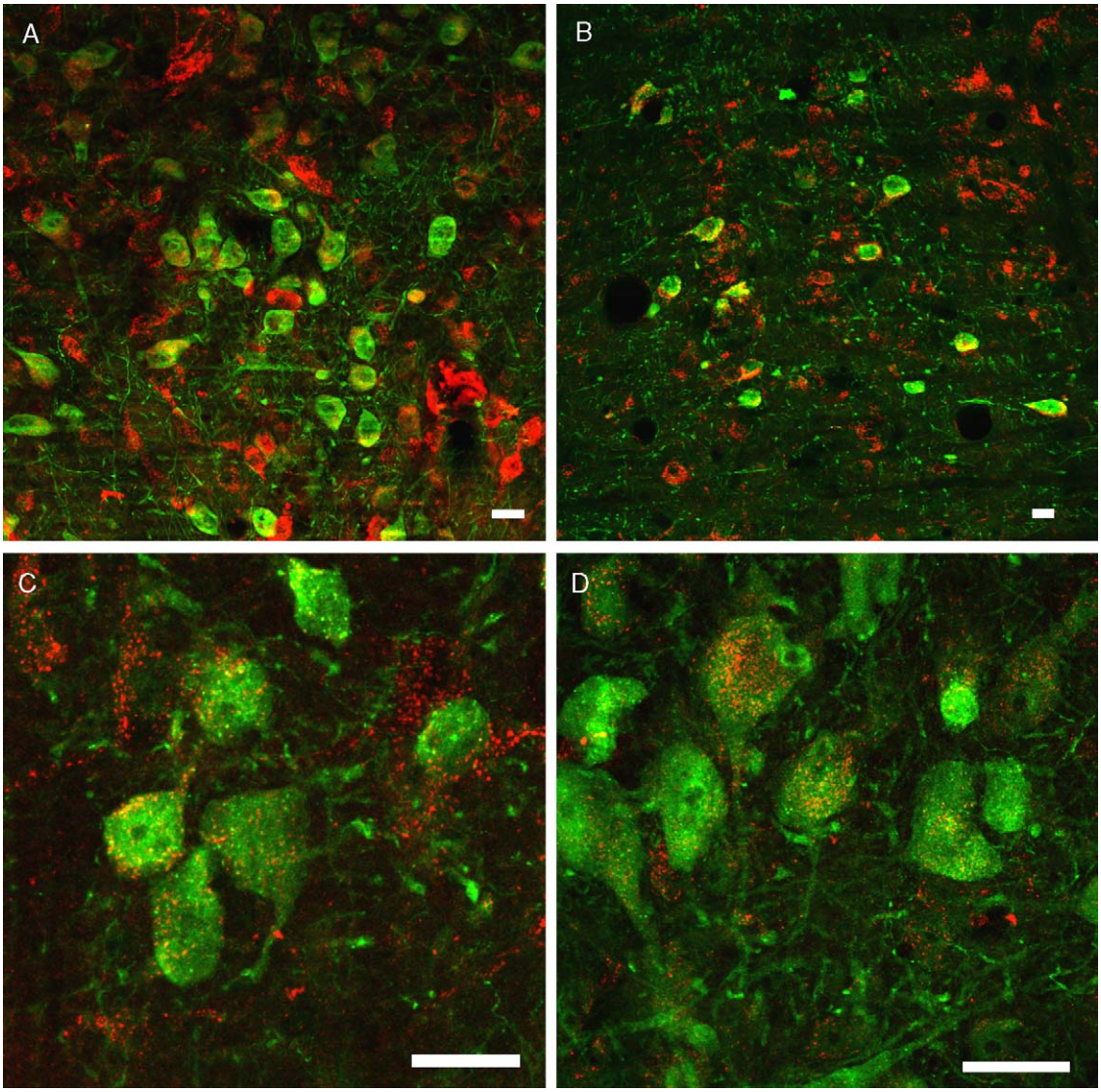

Fig. 1. Confocal fluorescent images of the dorsal (A and C) and median (B and D) raphe nuclei. 5HT-ir is shown in green, and VGLUT3-like-ir is shown in red. VGLUT3 is present in both 5HT-ir and cells and in cells that do not show 5HT-ir. In particular, note the VGLUT3 labeling in (C), with label highlighting the plasma membrane and out along processes extending from the cells. Scale bars = 25 μm.