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CHOLINERGIC CELLS IN THE TEGMENTUM SEND BRANCHING PROJECTIONS TO THE INFERIOR COLLICULUS AND THE MEDIAL GENICULATE BODY

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Abstract—The pontomesencephalic tegmentum (PMT) provides cholinergic input to the inferior colliculus (IC) and the medial geniculate body (MG). PMT cells are often characterized as projecting to more than one target. The purpose of this study was to determine whether individual PMT cholinergic cells, (1) innervate the auditory pathways bilaterally via collateral projections to left and right auditory thalamus; or, (2) innervate multiple levels of the auditory pathways via collateral projections to the auditory thalamus and inferior colliculus. We used multiple retrograde tracers to identify individual PMT cells that project to more than one target. We combined the retrograde tracer studies with immunohistochemistry for choline acetyltransferase to determine whether the projecting cells were cholinergic. We found that individual PMT cells send branching axonal projections to two or more auditory targets in the midbrain and thalamus. The collateral projection pattern that we observed most frequently was to the ipsilateral IC and ipsilateral MG. Cells projecting to both MGs were somewhat less common, followed by cells projecting to the contralateral IC and ipsilateral MG. Both cholinergic and non-cholinergic cells contribute to each of these projection patterns. Less often, we found cells that project to one IC and both MGs; there was no evidence for non-cholinergic cells in this projection pattern. It is likely that collateral projections from PMT cells could have coordinated effects bilaterally and at multiple levels of the ascending auditory pathways.

Key words: acetylcholine, pedunculopontine tegmental nucleus, laterodorsal tegmental nucleus, sensorimotor gating, acoustic startle, prepulse inhibition.

Cholinergic cells of the midbrain tegmentum are involved in a wide range of functions, including arousal and the sleep-wake cycle, sensory gating, attention, novelty detection, motor control, and reward prediction (Koyama et al., 1994; Reese et al., 1995b; Rye, 1997; Swerdlow et al., 2001; Kozak et al., 2005; Pan and Hyland, 2005; Chen et al., 2006; Jones, 2008; Rostron et al., 2008; Jenkinson et al., 2009). The cholinergic cells occupy two nuclei—the laterodorsal tegmental nucleus (LDT) and the pedunculopontine tegmental nucleus (PPT)—that can be referred to collectively as the pontomesencephalic tegmentum (PMT). The cholinergic cells of the PMT accomplish their wide-ranging functions through broad projections that extend to the spinal cord and to nuclei throughout the brainstem and into the diencephalon (Woolf and Butcher, 1986, 1989; Rye et al., 1988; Cornwall et al., 1990; Losier and Semba, 1993).

The widespread projections of the cholinergic nuclei arise from a relatively modest number of cholinergic cells (there are approximately 20,000 cholinergic cells/side in humans and 3000/side in rats; Jones, 1990; Manaye et al., 1999), leading to the expectation that many of the cholinergic axons must branch to innervate multiple targets. Evidence for such branching has come from a variety of approaches, including juxtacellular labeling of individual PMT cells (Mena-Segovia et al., 2008), antidromic activation from electrical stimulation of multiple brain areas (Kayama and Ogawa, 1987), and from studies with dual retrograde tracers. The retrograde tracer studies have identified projections to two thalamic nuclei (lateral geniculate nucleus and central-lateral nucleus, Shiromani et al., 1990; ventroposteromedial nucleus and ventrolateral nucleus, Beak et al., 2010; left and right dorsal lateral geniculate nuclei, Turlejski et al., 1994), or to nuclei in different areas but with shared functions (visual thalamus and superior colliculus, Billet et al., 1999; principle sensory trigeminal nucleus and facial motor nucleus, Beak et al., 2010).

There is growing evidence for a close and extensive relationship between the PMT and the auditory system. First, approximately half of the neurons in PMT respond to auditory stimuli (Reese et al., 1995a). Second, the cholinergic cells of the PMT are essential for prepulse inhibition of the acoustic startle response (Koch et al., 1993). This

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Abbreviations: AF 488, AlexaFluor 488; AF 647, AlexaFluor 647; APT, anterior pretectal nucleus; Aq, aqueduct; ChAT, choline acetyltransferase; cIC, contralateral inferior colliculus; cMG, contralateral medial geniculate body; CO, cytochrome oxidase; dl, dorsolateral subdivision of the medial geniculate body; FB, Fast Blue; FG, FluoroGold; GB, green beads; IC, inferior colliculus; ICc, central nucleus of the inferior colliculus; ICd, dorsal cortex of the inferior colliculus; ICx, external cortex of the inferior colliculus; iIC, ipsilateral inferior colliculus; iMG, ipsilateral medial geniculate body; LDT, laterodorsal tegmental nucleus; m, medial subdivision of the medial geniculate body; MG, medial geniculate body; PMT, pontomesencephalic tegmentum; PPT, pedunculopontine tegmental nucleus; RB, red beads; s, shell; SC, superior colliculus; sg, supragenicular subdivision of the medial geniculate body; SN, substantia nigra; v, ventral subdivision of the medial geniculate body; 3, oculomotor nucleus.

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Table 1. Summary of injection sites and immunolabel. List of tracers injected in each case and the fluorescent label used to visualize the ChAT immunoreactivity. Values in parentheses indicate the total volume of tracer injected into the indicated structure. "x" indicates that no injections were made at that location in that animal. * denotes a Nanoliter injection; all other injections were made with a microsyringe

Case	Tracer in left IC (total volume)	Tracer in left MG (total volume)	Tracer in right MG (total volume)	Immunolabel
GP 481	RB (0.6 μl)	FG (0.05 μl)	GB (0.4 μl)	AF 647
GP 482	RB (0.6 μl)	FG (0.05 μl)	GB (0.4 μl)	AF 647
GP 484	RB (0.6 μl)	FG (0.05 μl)	GB (0.2 μl)	AF 647
GP 585	FB (0.6 μl)	GB* (69 nl)	RB* (69 nl)	AF 647
GP 586	FB (0.6 μl)	GB* (69 nl)	RB* (69 nl)	AF 647
GP 587	FB (0.6 μl)	GB* (69 nl)	RB* (69 nl)	AF 647
GP 595	FG (0.3 μl)	GB* (69 nl)	RB* (69 nl)	AF 647
GP 604	GB (0.6 μl)	FG (0.05 μl)	x	AF 647
GP 633	x	RB (0.4 μl)	FG (0.05 μl)	AF 488

AF 488, AlexaFluor 488 [green]; AF 647, AlexaFluor 647 [near-infrared]; FB, Fast Blue (EMS-Chemi GmbH, Gross Umstadt, Germany); FG, FluoroGold (FluoroChrome, Inc., Englewood, CO, USA); GB, green beads (Luma-Fluor, Inc., Naples, FL, USA); RB, red beads (Luma-Fluor, Inc.).

function relates to sensory gating and has been tied to PMT connections with the inferior and superior colliculi (Yeomans et al., 2006). Third, the PMT projects to several auditory nuclei, including the cochlear nucleus (Motts and Schofield, 2005), the inferior colliculus (IC; Motts and Schofield, 2009), and the medial geniculate body (MG; Hallanger et al., 1987; Steriade et al., 1988; Motts and Schofield, 2010). Such projections could affect the auditory pathway throughout its subcortical extent. In an earlier study, we identified collateral projections from PMT cholinergic cells to the left and right inferior colliculi (Motts and Schofield, 2009). These results were reminiscent of the earlier studies (cited above) reporting cholinergic collaterals in other systems, and suggested further that individual PMT cells exert effects bilaterally on the auditory pathways. The purpose of the present study was to determine whether PMT cholinergic cells innervate the auditory thalamus bilaterally (as described for the visual thalamus, Turlejski et al., 1994) and/or innervate multiple levels of the auditory pathways via collateral projections to the auditory thalamus and inferior colliculus.

EXPERIMENTAL PROCEDURES

All procedures were performed in accordance with the Institutional Animal Care and Use Committee and the National Institutes of

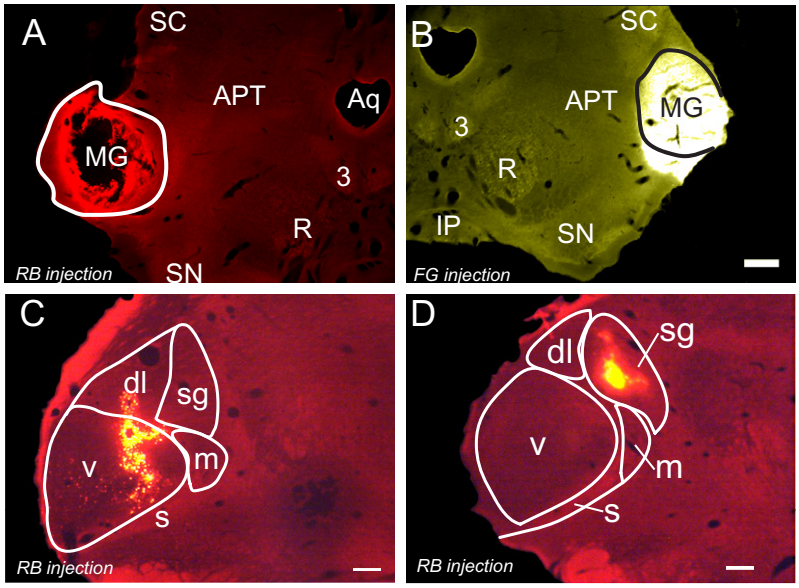

Fig. 1. Photomicrographs of representative large and small tracer injections into the MG. (A) Large red bead (RB) injection that was confined to the left MG (solid outline). GP 633. The black area in the center of the injection represents an area in which the tissue fell loose during processing. (B) A large FluoroGold injection that spread ventrally beyond the borders of the right MG. GP 633. Solid line—approximate borders of the MG. Scale bar applies to (A) and (B) (500 μm). (C) Smaller injection of red beads that is centered in the ventral MG (v) and extends into the dorsolateral subdivision (dl). GP 595R. (D) A small injection of red beads within the suprageniculate subdivision (sg). GP 586R. Transverse sections; dorsal is up; lateral is to the left in (A, C, D) and to the right in (B). Scale bars=500 μm. APT, anterior pretectal nucleus; Aq, aqueduct; dl, dorsolateral; m, medial; s, shell; SC, superior colliculus; sg, suprageniculate; SN, substantia nigra; v, ventral; 3, oculomotor nucleus. (C) and (D) adapted from Motts and Schofield, 2010, with permission. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.