Tissue and Cell 46 (2014) 103–111

Contents lists available at ScienceDirect

Tissue and Cell

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

Morphological properties of tectal neurons that project to the nucleus geniculatus lateralis, pars ventralis (GLv) and the surrounding ventral thalamus in chicks

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ARTICLE INFO

Article history:
Received 18 April 2013
Received in revised form
12 December 2013
Accepted 13 December 2013
Available online 21 December 2013

Keywords:
Tectum
Efferent neurons
GLv
Calcium-binding proteins
Chick

ABSTRACT

Layer 10 neurons of the chick tectum were morphologically investigated. The layer 10 neurons displayed heterogeneous immunoreactivities to calcium-binding proteins (CaBPs). Calbindin (CB)-immunoreactive (ir) neurons had pyramidal or round somata, primarily found in layers 5, 9, and 13. Parvalbumin (PV)-ir neurons were of various shapes with small to large somata ($109.7 \pm 48.6\ \mu m^2$) that were located mainly in layers 4 and 10. Calretinin (CR)-ir neurons had small to middle-sized somata ($79.3 \pm 9.7\ \mu m^2$) located primarily in layers 10 and 13, and most of them were similar to typical radial cells in size and shape. Two distinct types of neurons that projected to the nucleus geniculatus lateralis, pars ventralis (GLv) and ventral thalamus were demonstrated in layer 10. Type 1 cells had small to middle-sized somata ($74.3 \pm 33\ \mu m^2$), and each cell had a single apical dendrite that ramified into bush-like branches in layer 7. These cells corresponded to CR-ir neurons and radial cells in size and shape. Type 2 cells had larger somata ($124.7 \pm 52.6\ \mu m^2$), and their shapes were pyramidal, polygonal, or oval. They had multiple obliquely ascending dendrites that ramified into bush-like branches in layer 7. These cells often appeared similar to PV-ir neurons.

1. Introduction

The avian optic tectum, corresponding to the mammalian superior colliculus, is composed of 15 cellular and fibrous layers that process visual information (Cajal, 1911) and send ascending and descending efferents to various regions of the brain. Concerning ascending efferents of the stratum griseum et fibrosum superficiale (SGFS), layers 6–9 send many efferents to the nucleus opticus principalis thalami (OPT) (Wild, 1989). The OPT consists of small nuclei of the dorsolateral thalamus and is considered to be homologous to the mammalian lateral geniculate nucleus (Karten, 1979). Most layer 10 neurons project to the GLv and the ventral thalamus (Hunt and Künzle, 1976; Crossland and Uchwat, 1979). The SGFS and the stratum griseum centrale (SGC, layer 13) give off a large number of ascending efferents to the nucleus rotundus (ROT) (Benowitz and Karten, 1976; Karten et al., 1997; Hellman and Güntürkün, 2001; Luksch and Poll, 2002) as well as some efferents to the nucleus dorsolateralis posterior thalami, pars caudalis (DLPc) (Gamlin and Cohen, 1986). Some neurons of layer 10 (Hunt and Künzle, 1976), and the deep tectum (Gamlin and Cohen, 1986) also project to the pretectum.

On the other hand, many neurons in the subretinorecepient layers (layers 8–15) send descending efferents to various regions of the brain stem; specifically, layers 8 and 9 send efferents to the nucleus isthmo-opticus (IO) (Reiner and Karten, 1982; Woodson et al., 1991; Uchiyama et al., 1996), and layer 10 sends efferents mainly to the nucleus isthmi, pars parvocellularis and pars semilunaris (Woodson et al., 1991; Tömböl and Németh, 1999; Hellman et al., 2001; Sebestény et al., 2002; Wang et al., 2004, 2006), IO, and ipsilateral tectopontine pathway (ITP) (Reiner and Karten, 1982). Layers 13–15 send descending efferents to the ITP and contralateral tectobulbar pathways (CTB) (Reiner and Karten, 1982). Reiner and Karten (1982) proposed that the efferent system of the avian tectum is so complicated that ascending and descending efferent neurons are not grouped into superficial and deeper layers as found in mammals, but intermingle with each other in several layers.

As described above, varied pathways originate from many tectal layers, particularly layers 10 and 13, which are the principal efferent layers. Although the efferent system of layer 13 neurons has been studied extensively, the same is not true for the efferents of

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http://dx.doi.org/10.1016/j.tice.2013.12.005

layer 10 neurons. More generally, the existence of various kinds of neurons in the efferent layers may be meaningful in their reflection of the diversity of the efferent system. In fact, the subretinorecepient layers (layers 8–15) are composed of several morphologically different neuronal types. These neurons have been assigned various names based on their appearance, such as radial, stellate, pyramidal, bipolar, and multiform. The most common and characteristic neurons found in layer 10 are radial cells with vertically-oriented fusiform somata and shepherd's crooked axons that emanate from the radial dendritic shaft. Radial cells were originally described by Cajal (1911), and have been reported to be piriform cells in chicks (LaVail and Cowan, 1971), bipolar cells in ducks (O'Flaherty, 1970), ovoid cells in sparrows (Cajal, 1911), and radial cells in quail (Hilbig et al., 1998). According to Hilbig et al. (1998), quail radial cells with pear- or spindle-shaped somata, and one or two primary dendrites with numerous fine processes are found in layer *i* (layer 10). Most secondary dendrites run dorsally in a fashion parallel to a primary dendrite and terminate in the uppermost layers. The axons of these radial cells emanate from the soma or a proximal dendrite, and while some of the axons extend in the dorsal direction, other axons run in a ventral direction.

Furthermore, Stone and Freeman (1971) divided radial cells into three subgroups (r1, r2, and r3) in pigeons. Type r1 cells are the largest group and probably include most SGFS cells. They have globular cell bodies from which a prominent apical dendrite emanates in a radial fashion toward the tectal surface. These dendrites pass through afferent plexus layers and terminate in a layer of superficial stellate cells. Type r2 cells are bipolar and fusiform and have both an apical and a basal dendrite. The two dendrites are oriented strictly in the radial direction and usually display equal prominence and length. Both of these dendrites have numerous short and laterally spreading branches. The apical dendrites always reach the plexus layers, and in some cases terminate in the superficial stellate cell layer. Type r3 cells are pyramidal in shape and have cell bodies that are situated in sublayer *j* (layer 12). In addition to the radial cell group, stellate cells, or non-radial cells have been observed in deeper levels (Stone and Freeman, 1971). Marginal cells, which correspond to stellate cells, are oriented vertically and upside down relative to radial cells (Hilbig et al., 1998). Interestingly, O'Flaherty (1970) described small pyramidal cells that are scattered throughout layers 8–10 and appear to be interneurons. The axons of these interneurons emanate from the soma and ramify horizontally.

As described above, although various neuronal types have been studied in the tectum, the morphological properties of tectal neurons that relay retinal afferents to the GLv and the ventral thalamus are not yet clear. We sought to elucidate the morphological properties of the tectal neurons that project to the GLv and its surrounding regions in relation to the immunoreactivity of CaBPs which are used as dependable neuronal markers of specific neuronal populations (Celio and Heizmann, 1981; Braun, 1990; Celio, 1990; Baimbridge et al., 1992; Andressen et al., 1993).

2. Materials and methods

2.1. Animals

Chicks employed in this study were hatched from fertilized white-leghorn eggs (pathogen-free) in our laboratory using an egg incubator (Showa Furanki, Tokyo, Japan) at 38 °C with 70% humidity. Chicks remained in the incubator for 1-half-day after hatching and were then reared into a brooder at ~30 °C with conventional fluorescent lighting (30 1x, 12 h of light per day). Eighteen chicks (from 1 to 14 days old) and 38 chicks (from 1 to 2 days old) were used for WGA-HRP labeling and DiI labeling, respectively. Nine chicks (3–8 days old) were used for immunostaining and two chicks (5 days old) were employed for Nissl staining.

2.2. Application of axonal tracers

2.2.1. WGA-HRP injections

All animals were anesthetized with 0.03–0.06 ml of pentobarbital sodium solution (16 mg/kg BW) injected intraperitoneally. The anesthetized animals were placed on a stereotaxic instrument (Narishige, SR-60, Japan) where the beak was fixed at a 45° angle relative to the horizontal line with a specially prepared plastic holder and a protractor that were attached to the ear bar. A glass micropipette was lowered into the GLv (anterior/posterior, 6.4 mm; lateral, 3 mm; horizontal, 1 mm) based on coordinates acquired from Kuenzel and Masson's atlas (1988). A minimal amount of 3% wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) (Tbyobo, Japan) in distilled water was iontophoretically injected into the GLv through a glass micropipette (tip inner diameter 1 μm) with a 3–6 μA square wave (500 ms positive, 500 ms negative, electrode plus) for 3–6 min. The electrode was kept in place for 10 min to minimize diffusion of the tracer along the needle track (Hu et al., 2004). Chicks were allowed a survival period of 30 h in the brooder and were then deeply anesthetized again with 0.06–0.12 ml pentobarbital sodium solution (32 mg/kg BW) for perfusions. The chicks were perfused through the left ventricle first with a physiological saline for birds (0.75%, 40 °C) and then with 1% paraformaldehyde-l.25% glutaraldehyde in 0.1 M phosphate buffer (PB) (pH 7.4, room temperature RT), and last 10% sucrose in 0.1 M PB (pH 7.4, 4 °C). Following the perfusions, brains were immediately removed from the skulls and then brain blocks, which included both the mesencephalon and telencephalon, were trimmed and cut transversely on a cold microtome at a thickness of 50 μm. One out of every three sections was incubated in tetramethylbenzidine (TMB) media according to Mesulam (1982). A second set of sections was counterstained with neutral red to examine the cytoarchitecture of the brain. The cytoarchitectural classification of the chick brain was performed according to Kuenzel and Masson's atlas (1988).

2.2.2. DiI application

For injection of DiI (1,1′-dioctadecy1, 3,3,3′,3′-tetramethylindocarbocyanine perchlorate) (Molecular Probes, Eugene, OR), all animals were deeply anesthetized and perfused as described above except that a solution of 1% paraformaldehyde in 0.1 M PB followed the physiological saline. The animals were then placed on a stereotaxic instrument (SR 60, Narishige, Japan), and a l-μm-syringe was lowered into the GLv using the same coordinates described above. The syringe was driven at a speed of 0.06 μl/min, and 0.1–0.5 μl of a 0.5% DiI solution (0.8 mg DiI in a solution consisting of 8 μl dimethyl sulfoxide solution, 150 μl physio1ogical saline, and 0.1% Triton X-100) was injected into the GLv. The needles were kept in place for 10 min and then the brains were immediately removed from the skulls. For implantation of DiI crystals after perfusion of the fixative, the brain was transversely cut through a region containing the GLv at a beak angle of 45° on the stereotaxic instrument. A minimal amount of DiI crystals were then implanted into the GLv in the brain block that contained the thalamus and the tectum under a dissection microscope. All brain blocks were then immersed in l% paraformaldehyde in 0.1 M PB (pH 7.4) in the dark at 37 °C for 9 weeks. Following this incubation, the brain block was then embedded with 5% agar in 0.1 M PB (pH 7.4) and were transversely or parasagittally cut at a thickness of 200 μm in 0.1 M PB on a microslicer (Vibratome l500, Technical International Inc., USA). The slices were collected into 4% paraformaldehyde in 0.1 M PB in a brown glass container. The slices were then counterstained with two to three drops of 0.04% DAPI (4′,6-diamidino-2-phenylindole) (Molecular Probes, Eugene, USA) for 1–2 min to reveal the laminar