Available online at www.sciencedirect.com
SCIENCE DIRECT
Zoology 108 (2005) 297–306
ZOOLOGY
www.elsevier.de/zool

The development of the hindbrain afferent projections in the axolotl: Evidence for timing as a specific mechanism of afferent fiber sorting

Bernd Fritzsch[a,*], Darin Gregory[a], Eduardo Rosa-Molinar[b]

[a]Department of Biomedical Sciences, Creighton University, Omaha, NE 68178, USA
[b]Julio Garcia Center for Investigations in Biology, University of Puerto Rico-Rio Piedras, San Juan, PR, 00931-1809, Puerto Rico, USA

Received 18 July 2005; accepted 29 August 2005

Abstract

The aim of this study is to reveal the timing and growth pattern of central octavolateral projection development in the Mexican axolotl, *Ambystoma mexicanum*. In this amphibian species the development of the inner ear occurs first, followed by mechanosensory lateral line organs, and finally by ampullary electroreceptors. Several hypotheses have been proposed about how the development of peripheral organs, including differential projections of the ear, might relate to the development of central projections. Our data suggest that the sequence of maturation of the ear, mechanosensory lateral line, and ampullary electroreceptive organs is closely accompanied by the timed development of the trigeminal, inner ear, mechanosensory lateral line organs, and the ampullary electroreceptor afferent projections in the axolotl. Our data suggest that segregation of central termination within the alar plate is a function of time and space: later forming organs are likely innervated by later forming ganglia that project centrally later and to more dorsal areas of the alar plate that have not yet received any other afferents. Later forming ganglia of the same type may grow along existing pathways of earlier formed neurons.

Keywords: Electroreception; *Ambystoma mexicanum*; Afferent; Ampullary organ; Central projection

Introduction

Among tetrapods, many salamanders and some caecilians (Fig. 1) have the full complement of octavolateral organs (inner ear, mechanosensory lateral line, ampullary electroreceptors; Fritzsch, 1981; Fritzsch and Wahnschaffe, 1983). Such organs are also found in other aquatic vertebrates such as lampreys, elasmobranchs and other non-teleosts (Bullock and Heiligenberg, 1986; Northcutt, 1997). In contrast, frogs (Fig. 1) never develop ampullary electroreceptors (Fritzsch, 1988a; Schlosser, 2002a) and many frogs lose the lateral line system completely during metamorphosis (Fritzsch, 1990; Schlosser, 2002b). Amniotes develop only an inner ear (Fritzsch et al., 1998) and the tympanic organ in birds (Neeser and von Bartheld, 2002; von Bartheld, 1990). The central projections of these different organs are well stratified in adults and show no overlap (Fritzsch, 1988a, b; Northcutt, 1980) despite an earlier contention that they do so (Larsell, 1967). In all vertebrates possessing those organs, the ampullary electroreceptors project to the most dorsal part, and the inner ear to the most ventral part of the

*Corresponding author
E-mail address: Fritzsch@Creighton.edu (B. Fritzsch).

0944-2006/$ - see front matter © 2005 Elsevier GmbH. All rights reserved.
doi:10.1016/j.zool.2005.08.003

octavolateral area of the alar plate of the hindbrain (Fritzsch, 1981, 1988b). More ventral areas of the alar plate appear to be molecularly specified to receive trigeminal and taste bud fibers (Qian et al., 2001). Among urodele amphibians, a developmental sequence has been described that shows that the inner ear develops first followed by the mechanosensory lateral line organs and the ampullary electroreceptors (Fritzsch and Bolz, 1986; Northcutt and Brandle, 1995; Northcutt et al., 1994, 1995). However, except for generalized nerve staining of salamanders (Northcutt and Brandle, 1995) and experimental tracing studies on zebrafish, a teleost without electroreception (Gompel et al., 2001a), there are no data as yet on the timing of arrival of vestibular and cochlear afferents or on their mode of segregation within the alar plate (Rubel and Fritzsch, 2002). For the ear, experimental data in mammals suggest that specific projections of the vestibular epithelia as well as differential projections of the cochlea develop early (Fig. 1) and may be specific from the onset (Maklad and Fritzsch, 2003a). Differences in central and peripheral development have been noted for chicken (von Bartheld et al., 1991), but later more extensive work showed this to be otherwise (Fritzsch et al., 1993).

In order to determine whether the sequential development of afferents of the three sensory systems of a salamander reflects the sequence of maturation of the ear, the mechanosensory lateral line organs, and the ampullary electroreceptors and whether the projections initially segregate or overlap, we examined the projection of the ear, mechanosensory lateral line organs, and the ampullary electroreceptors in the developing axolotl. Our study investigates the temporal and spatial organization of the octavolateral and trigeminal afferents with three hypotheses in mind:

(1) Afferents from placodal ganglion cells may grow randomly into the brain and become assigned to specific pathways only after they are connected with a specific peripheral organ, i.e. trigeminal, inner ear, lateral line, and ampullary organs. This will be referred to here as the retrograde determination hypothesis and would suggest that central projection specification occurs as a consequence of a naïve sensory neuron coming into contact with a specific,

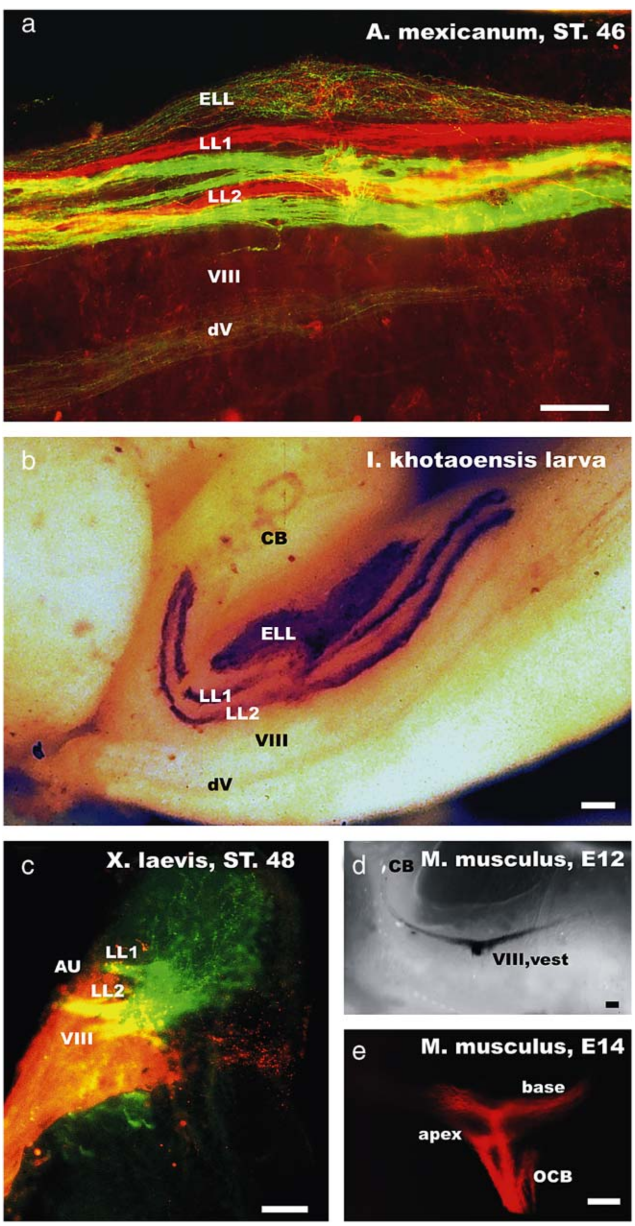

Fig. 1. The organization of the lateral line and inner ear afferent projections are shown for the axolotl (a), a gymnophionan (b), a frog (c) and the vestibular and cochlear projections of a mouse embryo (d, e). Note that older salamander and gymnophionan larvae show a clear segregation of mechanosensory afferents of a given peripheral lateral line nerve into two distinct fascicles (LL1, LL2). Labeling two peripheral nerve branches (in this case the anterodorsal and anteroventral lateral line nerve) with differently colored dextran amines results in labeling of two discrete fascicles for each nerve (a). In contrast, no such fasciculation is apparent in the electrosensory afferent projections (ELL; a, b). Frog lateral line projections show two entering fascicles, but widespread ramification throughout the entire lateral line nucleus (c). The frog inner ear projection shows the vestibular component (VIII) ventral to the lateral line projection, but also the formation of the auditory projection (AU) lateral to the lateral line projection. In mice, the vestibular (d) and cochlear (e) fibers are forming distinct projections from the earliest time they can be labeled and do so even for subdivisions derived from different parts of the cochlea (e). Labeling of all cochlear afferent fibers would result in a continuous projection with no distinct fascicles being apparent. CB, cerebellum; dV, descending tract of the trigeminal nerve; LL1, LL2, lateral line afferent fascicles; ELL, electroreceptive ampullary organ projection; VIII, vest, vestibular component; OCB, olivocochlear bundle. Bars indicate 100 μm in all images.