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Research Report

Cytoarchitecture of the accessory olfactory bulb in the salamander *Plethodon shermani*

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ABSTRACT

Plethodontid terrestrial salamanders are emerging models in the study of the evolution of chemical communication in vertebrates. Their vomeronasal system is well defined. It comprises sensory neurons in the epithelium of the vomeronasal organ, whose axons form the vomeronasal nerve projecting to the accessory olfactory bulb (AOB), which in turn projects to the vomeronasal amygdala through the accessory olfactory tract. A detailed description of the cellular elements of the urodele AOB is lacking. Neuronal morphology in the AOB was studied by means of biocytin intracellular injections and retrograde tract tracing in the salamander *Plethodon shermani*. The AOB exhibits the characteristic lamination of olfactory bulbs, except that it displays a mixed periglomerular and mitral somata layer superficially. Mitral cells are the only AOB neurons projecting to the vomeronasal amygdala. Each mitral cell sends multiple axonal branches, generally through both dorsal and ventral portions of the accessory olfactory tract. Some mitral cells additionally send axon collaterals in the white matter immediately ventral to the AOB. AOB interneurons are divided into superficial periglomerular and deep granule cells, each category exhibiting morphological variety. Some neurons in the granule cell layer of the AOB or the region ventral to the AOB have dendritic trees that cover both regions. The present study is the first to highlight the full anatomical extent of single AOB neurons and surprisingly suggests that the ventrolateral telencephalon found below the AOB is part of the salamander vomeronasal system.

1. Introduction

Studies of the vomeronasal system (VNS, or accessory olfactory system) are mostly conducted in rodents in the context of chemical communication in mating and social behaviors. Involvement of the VNS in other aspects of olfactory behavior has rarely been investigated in mammals. However, at least one predator cue (cat odor) activates the accessory olfactory bulb (AOB) preferentially in rats suggesting that additional functions of the mammalian VNS might still await discovery (McGregor et al., 2004). Many studies suggest that the VNS mediates detection of diverse biologically relevant chemical cues in reptiles and amphibians. For example, the snake vomeronasal organ is involved in the detection of reproductive, prey and predator cues (Noble, 1937; Wilde, 1938; Kubie et al., 1978; Halpern and Frumin, 1979; Kubie and Halpern, 1979; Kirschenbaum et al., 1986; Burghardt, 1993; Alving and Kardong, 1996; Miller and Gutzke, 1999). In terrestrial salamanders, a role of the VNS in the detection of reproductive pheromones, prey cues and home areas was experimentally demonstrated (Graves, 1994; Placyk and Graves, 2002; Wirsig-Wiechmann et al., 2002; Laberge et al., 2008), and these animals can also detect predator cues on the

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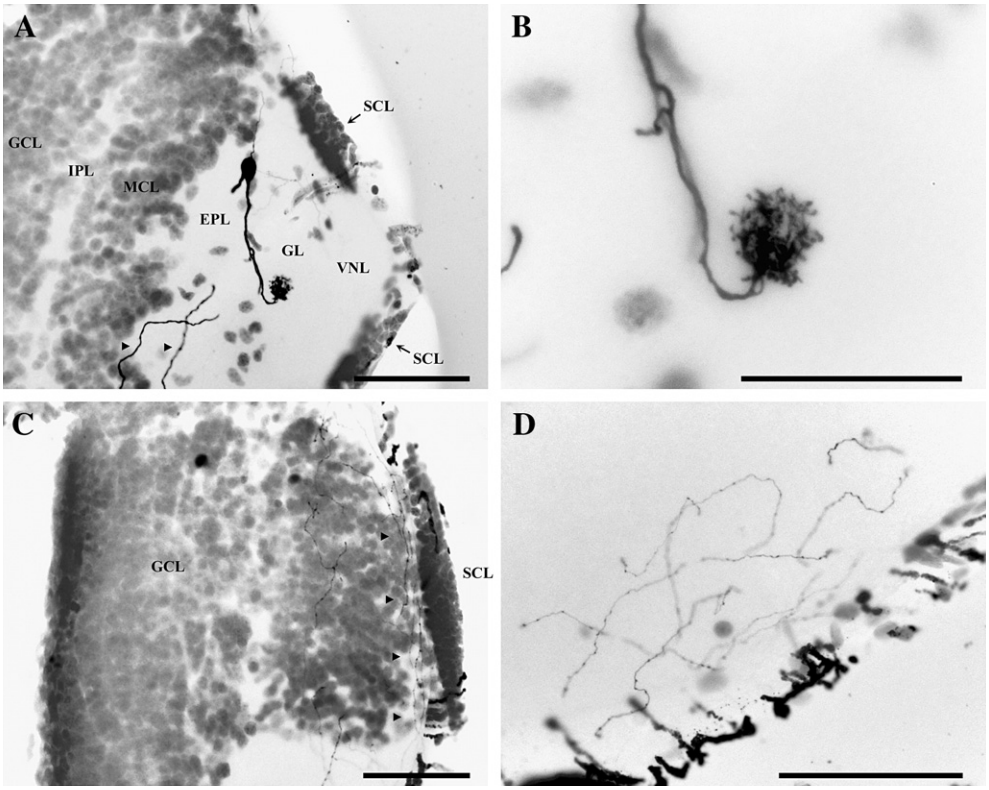

Fig. 1 – Mitral cell processes. A: Micrograph showing a mitral cell labeled by intracellular injection of biocytin in the external plexiform layer of the accessory olfactory bulb. The cell displays a glomerular arbor and two lateral dendrites (arrowheads) within this 50 μm-thick coronal section. The glomerular arbor is shown at a higher magnification in panel B. The arrowheads in panel C show the descending course of several axon branches belonging to this mitral cell in the most caudal part of the accessory olfactory bulb. D: Axon meshwork of the mitral cell in the white matter of the vomeronasal amygdala, where the accessory olfactory tract terminates. Note the abundant axonal varicosities. Scale bars are 100 μm in panels A, C and D, and 50 μm in panel B. Abbreviations: EPL: external plexiform layer; GCL: granule cell layer; GL: glomerular layer; IPL: internal plexiform layer; MCL: mitral cell layer; SCL: superficial cell layer; VNL: vomeronasal nerve layer.

substrate suggesting that the VNS could be involved in that case too (Sullivan et al., 2002).

Eisthen (1997) remarked that the presence and features of the VNS are highly variable in tetrapods. This presents an opportunity to study the selection pressures leading to elaboration or reduction of the vomeronasal organ. However, the precise role of the VNS remains unknown, and the suggestion that it is an adaptation to terrestrial life was rejected on the ground that it is present throughout the lifecycle of salamanders (aquatic and terrestrial), caecilians and frogs (Schmidt and Wake, 1990; Eisthen, 1997; Eisthen, 2000). Herrick (1921, 1924, 1931) described the urodele AOB, when present, as a very rudimentary structure compared to its counterpart in anurans. However, recent studies have shown that the VNS of some salamanders is particularly well developed (Schmidt et al., 1988; Schmidt and Roth, 1990; Laberge and Roth, 2005). This makes them good model species to elucidate VNS function. In addition, North American plethodontid salamanders are used as models for the evolution of chemical communication because they are diverse, display conserved mating sequences, and use pheromones during courtship following discrete delivery modes that are well characterized phylogenetically (see Watts et al. (2004), Palmer et al. (2005), Palmer et al. (2007)).

The objective of the present study is to complete the description of the cellular substrate for olfactory processing in the vomeronasal pathway of the salamander *Plethodon shermani*. The olfactory pathways and amygdala complex of this species (previously referred to as *P. jordani*) were previously described by Schmidt et al. (1988) and Laberge and Roth (2005). The present article concerns itself with the AOB. In amphibians, the AOB forms a bulge on the lateral telencephalon caudal to the main olfactory bulb (MOB). It is found in a dorsolateral position in urodeles, as opposed to a ventrolateral position in anurans. Olfactory bulbs are crucial structures in all vertebrate olfactory pathways. They are involved in shaping olfactory signals before transmission to central regions and receive centrifugal modulation from the latter. Olfactory bulbs are laminated structures comprising projection neurons, typically mitral cells, and interneurons, mostly periglomerular and granule cells although additional neuron types are sometimes observed depending on the species (reviewed in Allison (1953), Nieuwenhuys (1967), Meisami and Bhatnagar (1998)).