Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chemical Neuroanatomy 29 (2005) 217–226
Journal of CHEMICAL NEUROANATOMY
www.elsevier.com/locate/jchemneu

Efferent connections of the ''olfactostriatum'': A specialized vomeronasal structure within the basal ganglia of snakes

Alino Martinez-Marcos [c,*], Isabel Ubeda-Bañon [c], Enrique Lanuza [b], Mimi Halpern [a]

[a] *Department of Anatomy and Cell Biology, Downstate Medical Center at Brooklyn, State University of New York, Brooklyn, NY 11203, USA*
[b] *Departament de Biologia Cel·lular, Facultat de Ciències Biològiques, Universitat de València, 46100 Burjassot, València, Spain*
[c] *Departamento de Ciencias Médicas, Facultad de Medicina, Centro Regional de Investigación Biomédica, 'Universidad de Castilla-La Mancha, 02006 Albacete, Spain*

Received 22 June 2004; received in revised form 22 November 2004; accepted 1 February 2005

Abstract

The olfactostriatum is a portion of the basal ganglia of snakes that receives substantial vomeronasal afferents through projections from the nucleus sphericus. In a preceding article, the olfactostriatum of garter snakes (*Thamnophis sirtalis*) was characterized on the basis of chemoarchitecture (distribution of serotonin, neuropeptide Y and tyrosine hydroxylase) and pattern of afferent connections [Martínez-Marcos, A., Úbeda-Bañón, I., Lanuza, E., Halpern, M., 2005. Chemoarchitecture and afferent connections of the ''olfactostriatum'': a specialized vomeronasal structure within the basal ganglia of snakes. J. Chem. Neuroanat. 29, 49–69]. In the present study, its efferent connections have been investigated. The olfactostriatum projects to the main and accessory olfactory bulbs, lateral cortex, septal complex, ventral pallidum, external, ventral anterior and dorsolateral amygdalae, bed nucleus of the stria terminalis, preoptic area, lateral posterior hypothalamic nucleus, ventral tegmental area, substantia nigra and raphe nuclei. Tracer injections in the nucleus accumbens proper, a structure closely associated with the olfactostriatum, result in a similar pattern of efferent connections with the exception of those reaching the main and accessory olfactory bulbs, lateral cortex, external, ventral anterior and dorsolateral amygdalae and bed nucleus of the stria terminalis. These data, therefore, help to characterize the olfactostriatum, an apparently specialized area of the nucleus accumbens. Double labeling experiments after tracer injections in the nucleus sphericus and the lateral posterior hypothalamic nucleus demonstrate a pathway between these two structures through the olfactostriatum. Injections in the olfactostriatum and in the medial amygdala show parallel projections to the lateral posterior hypothalamic nucleus. Since this hypothalamic nucleus has been previously described as projecting to the hypoglossal nucleus, both, the medial amygdala and the olfactostriatum may mediate vomeronasal influence on tongue-flick behavior.

Keywords: Nucleus accumbens; Nucleus hypoglossus; Nucleus sphericus; *Thamnophis sirtalis*; Ventral pallidum; Tongue-flick

Abbreviations: ac, anterior commissure; ACC, nucleus accumbens; AMB, nucleus ambiguus; AOB, accessory olfactory bulb; aot, accessory olfactory tract; BDA, biotinylated dextran-amine; BNST, bed nucleus of the stria terminalis; C, cerebellum; DBB, nucleus of the diagonal band of Broca; DC, dorsal cortex; DLA, dorsolateral amygdaloid nucleus; DM, dorsomedial thalamic nucleus; EXA, external amygdala; FDA, fluorescein-labeled dextran-amine; flm, fasciculus longitudinalis medialis; GLV, ventral lateral geniculate nucleus; ig, internal granular layer of the olfactory bulb; ip, internal plexiform layer of the olfactory bulb; IP, interpeduncular nucleus; LC, lateral cortex; LCDC, dorsocaudal lateral cortex; LCRV, rostroventral lateral cortex; lfb, lateral forebrain bundle; LHN, lateral posterior hypothalamic nucleus; m, mitral layer of the olfactory bulb; MA, medial amygdala; MC, medial cortex; mfb, medial forebrain bundle; MOB, main olfactory bulb; NAOT, nucleus of the accessory olfactory tract; NS, nucleus sphericus; OS, olfactostriatum; OT, optic tectum; POA, preoptic area; PV, periventricular hypothalamic nucleus; RA, raphe nuclei; RDA, tetramethylrhodamine-labeled dextran-amine; S, septal complex; sl, spinal lemniscus; SN, substantia nigra; st, stria terminalis; TS, torus semicircularis; VAA, ventral anterior amygdala; VE, vestibular complex; VMH, ventromedial hypothalamic nucleus; VP, ventral pallidum; VTA, ventral tegmental area; VIII, vestibulocochlear nerve; XIIN, hypoglossal nucleus

* Corresponding author. Tel.: +34 967599200x2962; fax: +34 967599340.
E-mail address: alino.martinez@uclm.es (A. Martinez-Marcos).

0891-0618/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchemneu.2005.02.001

1. Introduction

Most vertebrates sense chemical cues through the olfactory and vomeronasal systems. A myriad of volatile odorants are detected by the olfactory system, whereas high-molecular-weight molecules such as prey chemicals and pheromones are predominantly sensed through the vomeronasal system (Brennan, 2001; Dulac and Torello, 2003; Halpern and Martinez-Marcos, 2003).

Among squamate reptiles (lizards, snakes and amphisbaenians), snakes posses a highly developed vomeronasal system (Schwenk, 1993; Eisthen, 1997). Anatomically, the vomeronasal epithelium relays chemosensory information to the accessory olfactory bulb (AOB), which in turn projects to secondary vomeronasal-recipient areas such as the medial amygdala (MA) and, especially, to the nucleus sphericus (NS) (Lanuza and Halpern, 1998). In turn, the main output of the NS is a bilateral projection to a structure located in the basal telencephalon (Lanuza and Halpern, 1997) historically named ''olfactostriatum'' (OS) (Johnston, 1923; Herrick, 1926; Durward, 1930; Warner, 1946), although its striatal or pallidal designation is debated (Martínez-Marcos et al., 2005; see below). Accordingly, the OS is the main tertiary vomeronasal structure of snakes and it is localized in the basal ganglia. The chemoarchitectonic organization, as well as the afferent connections of the OS, have been described recently (Martínez-Marcos et al., 2005).

The aim of the present work has been, therefore, to investigate the efferent connections of the OS, as well as the efferents from the nucleus accumbens (ACC) for comparison, to better understand the neuroanatomical circuits of the vomeronasal system as well as its relationships to other systems. For instance, a pathway from the MA to the lateral posterior hypothalamic nucleus (LHN) and from there to the hypoglossal nucleus (XIIN) was described as a possible pathway for vomeronasal influence on tongue-flick behavior (Martínez-Marcos et al., 2001). Herein, we have investigated the possibility of a comparable pathway through the OS. We have included double labeling experiments in order to study in parallel descending projections to the hypothalamus arising from the MA and from the OS.

2. Materials and methods

Male and female adult garter snakes, *Thamnophis sirtalis*, weighing between 18 and 75 g, were used in this study. Animals were purchased from a licensed dealer and maintained in terraria under a regulated 12:12 h light/dark cycle at 22–24 °C. They were given water ad libitum and fed earthworms weekly.

Twenty-four animals were injected intramuscularly with 1% sodium brevital (methohexital sodium, Eli Lilly and Company, Indianapolis, IN) at a dose of 1.4 μl per gram of body weight. Animals received iontophoretic injections of dextran-amine conjugated to biotin, tetramethylrhodamine or fluorescein (BDA, RDA and FDA, respectively; 10,000 molecular weight, lysine fixable; Molecular Probes, Eugene, OR) (Schmued et al., 1990; Veenman et al., 1992; Köbbert et al., 2000; Reiner et al., 2000). Tracers, diluted to 10% in 0.01 M phosphate buffer, pH 7.4, were delivered from micropipettes with an outer tip diameter of 15–40 μm by means of positive current pulses (2–5 μA; 7/7 s on/off) using a current generator (World Precision Instruments, Sarasota, FL). Injection times ranged from 5 to 15 min. Negative current was applied as the pipette was withdrawn from the brain. Animals received one, two or three injections. A total of 33 injections were placed in 24 animals (see Table 1).

After survival of 8–12 days, animals were perfused transcardially under an overdose of sodium brevital anesthesia (2 μl per gram of body weight) with saline solution followed by 4% formaldehyde (in 0.1 M phosphate buffer, pH 7.4). The brains were removed, postfixed overnight and cryoprotected with 30% buffered sucrose. Frozen parasagittal (olfactory bulbs) or frontal sections (35 μm thick) were collected into three matching series that

Table 1
Animals used in the present study, including tracers delivered and injection sites

Case	Verified injection site						
	OS	ACC	BNST/MA	LC DLA	LHN+NS	OS + BNST/MA	Total
	BDA0118	BDA0148	BDA9845	BDA9638	RDA0153	RDA0155	
	BDA0135[a]	BDA0152	BDA9846	BDA9639	FDA0153	FDA0155	
	BDA0137	RDA0136	BDA9852	BDA9829			
	BDA0156	FDA0135[a]	FDA9845				
	RDA0118	FDA0136	FDA9846	BDA9602			
	RDA0119		FDA9852	BDA9842			
	RDA0121		FDA9853	RDA9835			
	RDA0138			RDA9851			
	RDA0156			FDA9829			
Animals	7[a]	4[a]	4	8	1	1	24[a]
Injections	9	5	7	8	2	2	33

For abbreviations, see the list.

[a] Note that animal 0135 is included in both, injections in the OS as well as injections in the ACC. In the column LC DLA, the three first cases corresponded to injections in the LC and the next five cases to injections in the DLA.