# OLFACTORY-INDUCED LOCOMOTION IN LAMPREYS

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## Abstract

The olfactory system allows animals to navigate in their environment to feed, mate, and escape predators. It is well established that odorant exposure or electrical stimulation of the olfactory system induces stereotyped motor responses in fishes. However, the neural circuitry responsible for the olfactomotor transformations is only beginning to be unraveled. A neural substrate eliciting motor responses to olfactory inputs was identified in the lamprey, a basal vertebrate used extensively to examine the neural mechanisms underlying sensorimotor transformations. Two pathways were discovered from the olfactory organ in the periphery to the brainstem motor nuclei responsible for controlling swimming. The first pathway originates from sensory neurons located in the accessory olfactory organ and reaches a single population of projection neurons in the medial olfactory bulb, which, in turn, transmit the olfactory signals to the posterior tuberculum and then to downstream brainstem locomotor centers. A second pathway originates from the main olfactory epithelium and reaches the main olfactory bulb, the neurons of which project to the pallium/cortex. The olfactory signals are then conveyed to the posterior tuberculum and then to brainstem locomotor centers. Olfactomotor behavior can adapt, and studies were aimed at defining the underlying neural mechanisms. Modulation of bulbar neural activity by GABAergic, dopaminergic, and serotoninergic inputs is likely to provide strong control over the hardwired circuits to produce appropriate motor behavior in response to olfactory cues. This review summarizes current knowledge relative to the neural circuitry producing olfactomotor behavior in lampreys and their modulatory mechanisms.

**Keywords:** Olfaction; Locomotion; Sensorimotor integration; Neuromodulation; Lamprey

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## Introduction

Odorant detection is essential for animal behavior such as feeding, mating, and predator avoidance. For instance, the lamprey, a basal vertebrate that has diverged from the main vertebrate lineage some 560 million years ago (Kumar and Hedges 1998), migrates over long distances and locates suitable spawning grounds due to olfactory stimulating molecules that act as directional cues. These molecules are emitted by conspecific animals and induce vigorous and precise tracking responses in their natural habitat (Bjerselius et al. 2000; Johnson et al. 2009). In fish, it was shown that exposure to identified odorants elicits robust motor responses (von Frisch 1941). Moreover, movements indistinguishable from normally induced behavior are elicited by electrical stimulation of olfactory brain areas (Grimm 1960). The stereotyped nature of the motor responses suggests a strong neural link between olfactosensory areas and the centers in the central nervous system that initiate and control movements. In lampreys, early studies (Wickelgren 1977a, 1977b) have demonstrated that electrical stimulation of the olfactory nerve or bulb evoked sustained depolarizations in reticulospinal (RS) cells. However, the neural substrate (Fig. 1) linking olfactory input to motor output has not been characterized until recently (Derjean et al. 2010).

The lamprey brain has a general organization very similar to that of other vertebrates but contains fewer neurons and is also much simpler. It is considered as the mammalian brain blueprint (Stephenson-Jones et al. 2013; for reviews, see Robertson et al. 2014; Grillner and Robertson 2016; Ryczko and Dubuc 2017; Suryanarayana et al. 2021a). Moreover, lamprey whole-brain preparations can be isolated *in vitro*, maintaining the neural connections between the olfactory and motor system, thus allowing researchers to measure responses to olfactory stimulation in motor command cells (i.e., brainstem RS cells). Using the lamprey to bridge the gap between odor detection and motor behavior, a neural substrate responsible for olfactomotor transformations was described for the first time in any vertebrate species. The circuitry (Fig. 1) consists of two segregated neural pathways (Derjean et al. 2010; Daghfous et al. 2018) originating from distinct regions of the peripheral olfactory organ (Green et al. 2017). In both pathways, olfactory sensory neurons project from the periphery to the olfactory

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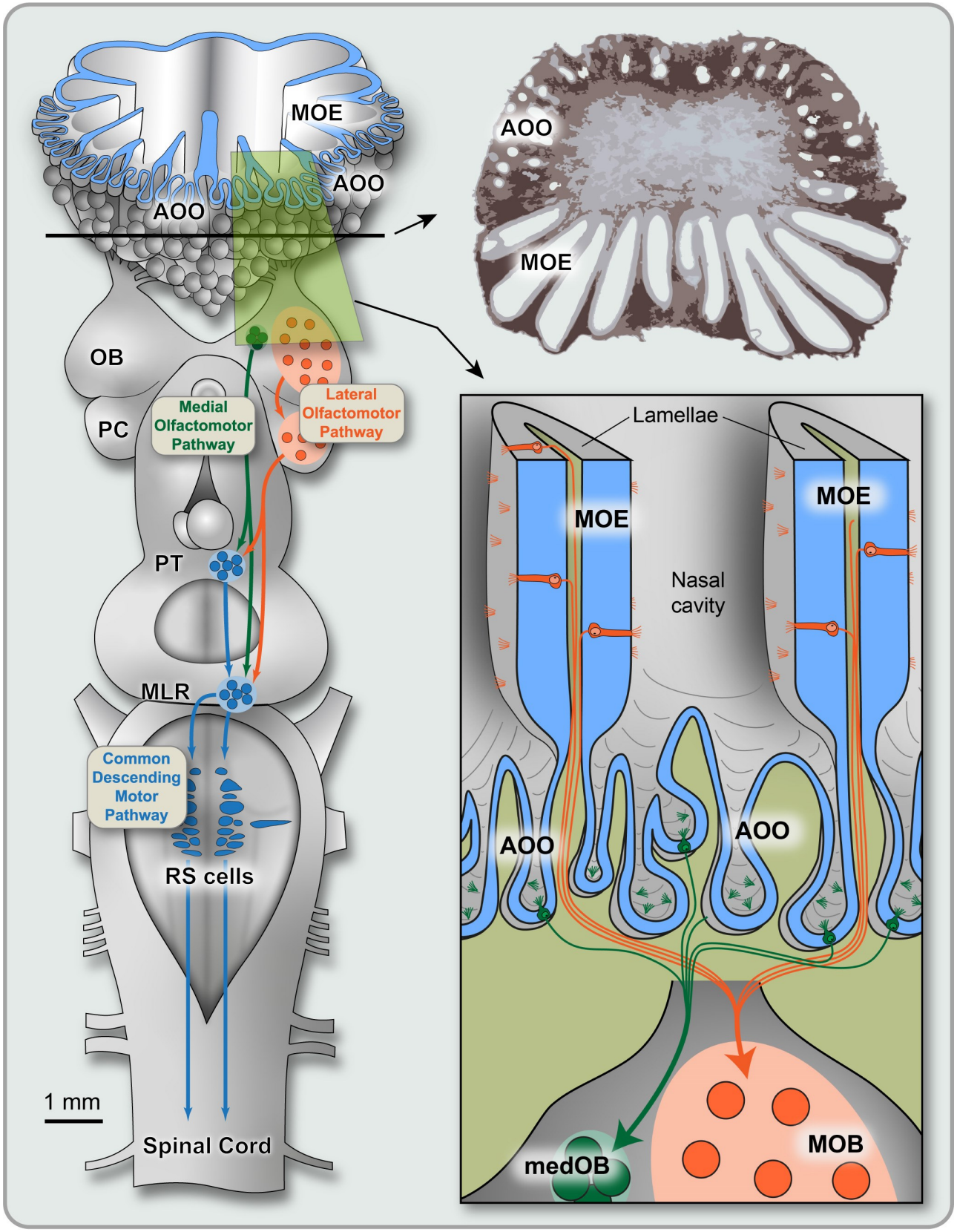


**Fig. 1** Medial and lateral olfactomotor pathways transform olfactory inputs into locomotor output. Left: Schematic representation of a dorsal view of the brain of a young adult sea lamprey illustrating different regions involved in olfactomotor transmission. Right, above: Schematic coronal section in the lamprey peripheral olfactory apparatus depicts two distinct olfactory epithelia: the main olfactory epithelium (MOE) and accessory olfactory organ (AOO). Right, below: A schematic drawing of the nasal cavity shows the axonal projections of olfactory sensory neurons in the MOE (orange) and the AOO (green) that terminate into anatomically segregated olfactory bulb (OB) glomeruli. There are two pathways, the medial and the lateral olfactomotor pathways. In the medial olfactomotor pathway (green), olfactory signals from the AOO are relayed in the medial OB (medOB). They then reach the posterior tuberculum (PT) and the mesencephalic locomotor region (MLR). In the lateral olfactomotor pathway (orange), olfactory signals from the MOE are transmitted to the main OB (MOB) and then to the pallium/cortex (PC), which projects to both the PT and the MLR. The MLR controls locomotion through synaptic inputs to reticulospinal (RS) cells that in turn activate the spinal central pattern generators for locomotion

bulb (OB), which relays the input to the posterior tuberculum (PT). The olfactory signals are then transmitted to the mesencephalic locomotor region (MLR) that exerts a powerful control over RS cell activity. The MLR elicits a graded and coordinated activation of RS cells that act as command neurons in the brainstem and constitute the final common descending pathway for eliciting locomotion. The RS cells send excitatory projections to spinal neural networks generating the rhythmic motor activity that induces the coordinated muscle contractions necessary for propulsion during swimming. The spinal neural networks are referred to as central pattern generators (reviewed in Grillner 1981; Grillner et al. 2007). Reticulospinal cells play a crucial role in starting, maintaining, and stopping locomotion (Bouvier et al. 2015; Juvin et al. 2016; Capelli et al. 2017; Grätsch et al. 2019b). We presume that the neural circuitry responsible for the transformation of olfactory inputs into motor commands is highly sensitive to and recruited by chemical compounds that induce migratory (Bjerselius et al. 2000; Johnson et al. 2009) and reproductive behavior (Johnson et al. 2006, 2012), predator avoidance (Wagner et al. 2011), and foraging (Kleerekoper and Mogensen 1963). The section below further details the olfactomotor pathways from the periphery to the motor centers in the brainstem, involved in the transformation of olfactory signals into characteristic locomotor neural activity underlying swimming behavior of lampreys (Fig. 1).

## Olfactomotor circuitry in lampreys

Lampreys possess a single nostril containing a simple, but well-developed olfactory organ. There are two distinct olfactory subsystems, each with its own specialized sensory mucosa: the main olfactory epithelium and the accessory olfactory organ (Ren et al. 2009). Neurons from both these peripheral structures project to the OB (Green et al. 2013) where separate circuits eventually converge onto motor centers that elicit swimming movements in response to olfactory stimulation (Derjean et al. 2010; Daghfous et al. 2018; Beauséjour et al. 2020). According to the dual olfactory hypothesis (reviewed in Suárez et al. 2012), the two olfactory subsystems could act synergistically in the regulation of olfactory-guided behaviors (Salas et al. 2015). However, the specific contribution of each subsystem is still unknown.

First, olfactory sensory neurons in the main olfactory epithelium detect odorant molecules via receptors on their apical dendrites. Three distinct olfactory sensory neuron morphotypes (tall, intermediate, and short) were identified in the main olfactory epithelium of lampreys (Laframboise et al. 2007). The accessory olfactory organ represents a second, distinct olfactory organ (Scott 1896), which is composed of several small spherical cavities located caudal to the main olfactory epithelium and linked to it through tiny ducts (Hagelin and Johnels 1955). These vesicles are lined with an epithelium containing short, ciliated cuboidal neurons with apical projections to the luminal surface (Ren et al. 2009). The olfactory nature of these cells was never physiologically demonstrated due to their inaccessibility for electrophysiological recording of their chemosensory activity. Nonetheless, they are considered as putative olfactory sensory neurons based on their morphology and direct projections to the OB via the olfactory nerve (Ren et al. 2009; Green et al. 2017).

Odorants bind to selective chemosensory receptors to depolarize olfactory sensory neurons. Lampreys possess the most ancient, documented family of vertebrate olfactory receptors, which was evolutionarily conserved from lampreys to mammals (Freitag et al. 1999). However, the olfactory receptor gene family of lampreys (40 olfactory receptor genes; Zhang et al. 2020) is considerably smaller than that of mammals (over a thousand olfactory receptor genes in rodents; Buck and Axel 1991; Zhang and Firestein 2002). Chemosensory receptors are G protein-coupled transmembrane receptors that are highly variable in structure and can thus bind diverse ligands. Thus far, analysis of the lamprey genome has revealed 72 chemosensory receptor genes: 40 olfactory receptor genes, 28 trace amine-associated receptor genes, 4 vomeronasal type-1 receptor genes, and no vomeronasal type-2 receptor genes (Hashiguchi and Nishida 2007; Grus and Zhang 2009; Libants et al. 2009; Zhang et al. 2020). The expression of these three gene families was confirmed in the olfactory organ of lampreys (Chang et al. 2013). The small repertoire of chemosensory receptor genes may account for the limited range of odorants that activate olfactory sensory neurons in lampreys, i.e., basic amino acids, biogenic amines, few bile acids, and sex steroids (Li 1994; Li et al. 1995; Libants et al. 2009). When an odor ligand binds to a chemosensory receptor, the G protein subunits linked to the receptor dissociate, leading to olfactory sensory neuron depolarization. In mammals (Belluscio et al. 1998), amphibians (Mezler et al. 2001) and teleost fishes (Hansen et al. 2003), $G_{olf}$ is involved in olfactory transduction (Jones and Reed 1989). Since this G protein subunit was also detected in olfactory sensory neurons of the lamprey main olfactory epithelium, it may also be involved in olfactory transduction (Frontini et al. 2003).

Primary olfactory afferents (i.e., the axons of olfactory sensory neurons) form the olfactory nerve and terminate in OB glomeruli where they activate projection neurons that act as second-order olfactory neurons. In lampreys, individual olfactory sensory neurons of the main olfactory epithelium project a single axon that arborizes within the limits of a single glomerular unit (Weiss et al. 2020). The glomeruli are organized in distinct territories (Frontini et al. 2003) and anatomical tracing experiments revealed that the projections from the main olfactory epithelium and the accessory olfactory organ innervate spatially segregated regions of the OB (Ren et al. 2009; Green et al. 2017). While the accessory olfactory organ projects exclusively to the medial OB, the main olfactory epithelium innervates the main OB. Moreover, $G_{olf}$ immunoreactivity is detected in olfactory sensory neuron axons throughout the main OB but not in the medial OB (Frontini et al. 2003). This suggests that in the accessory olfactory organ, another, yet unidentified G protein alpha subunit is responsible for intracellular signal transduction.

The medial and the main OB are composed of the same cell layers but contain anatomically distinct populations of projection neurons (Green et al. 2013), which occupy non-overlapping territories. In the medial OB, the somata of projection neurons are larger in size and are located within the glomerular layer, which may enable them to receive and process

olfactory inputs more efficiently. These observations support the presence of two different populations of bulbar projection neurons (Green et al. 2013).

In an isolated olfactory epithelium-brain preparation, synaptic activity in the OB was measured in response to the application of odorants directly in the olfactory organ. Extracellular recordings revealed distinct response profiles between medial and main OB to three different odorant categories: amino acids, lamprey-specific pheromones, and bile acids (Boyes 2014; Green et al. 2017). Surprisingly, the medial OB responded to all three categories of odorants, whereas the responses in different subregions of the main OB were selective to amino acids and others responded preferentially to bile acids and pheromones. These observations suggest that the medial OB integrates olfactory inputs induced by various odorant categories processed in the accessory olfactory organ, whereas sensory neurons in the main olfactory epithelium project to specific main OB subregions that integrate specific odorant categories. It was also found that the duration of odorant responses differed between medial and main OB (Green et al. 2017). The responses in the medial OB were generally shorter in duration than those induced in the main OB. This could reflect differences in olfactory signal transduction mechanisms in the medial OB vs. the main OB (Frontini et al. 2003; Green et al. 2017). Therefore, it appears that the accessory olfactory organ-medial OB and the main olfactory epithelium-main OB are two distinct olfactory subsystems, chemotopically organized into parallel odor-processing streams, which are likely to exert different functions (Derjean et al. 2010). The specific contribution of each pathway to the detection of individual compounds and resulting behavior remains to be characterized.

The presence of parallel olfactory subsystems is common across insects and vertebrates (Galizia and Rossler 2010). Indeed, in terrestrial mammals, no less than four structurally separate olfactory subsystems were identified, each thought to serve distinct functions (Munger et al. 2009). Among those, two major and well-characterized subsystems are the olfactory system and the vomeronasal system. The vomeronasal organ is an anatomically distinct chemosensory epithelium first observed in mammals (Jacobson 1811) that specializes in the detection of semiochemicals such as pheromones (Suárez et al. 2012). As in the lamprey main olfactory epithelium and accessory olfactory organ, sensory neurons in mammalian olfactory subsystems differ by their location in the nasal cavity, their repertoire of chemosensory receptors, their signal transduction mechanisms, and their projections to olfactory regions (Munger et al. 2009). These distinctions between parallel olfactory subsystems suggest that they serve different functions (Breer et al. 2006). Moreover, the existence of parallel olfactory processing streams in basal vertebrates suggest that this functional organization is a common ancestral feature of vertebrates, and that it has a highly adaptive value, being present in modern-day insects and mammals (Galizia and Rossler 2010). Interestingly, genes encoding specific proteins of the vomeronasal signaling pathway are expressed in the lamprey olfactory organ, which indicates that molecular components specific to the mammalian vomeronasal system existed in the common ancestor of all extant vertebrates (Grus and Zhang 2009). This finding suggests that the lamprey accessory olfactory organ constitutes a primitive form of the vomeronasal system in the vertebrate lineage (Grus and Zhang 2009; Suárez et al. 2012; Chang et al. 2013). However, this hypothesis is still speculative and requires further examination.

In the natural environment, lampreys are attracted to odorants such as amino acids and pheromones in feeding and reproductive contexts, respectively. Odorants were shown to increase and guide locomotor activity (Kleerekoper and Mogensen 1963; Bjerselius et al. 2000), and to stimulate sensory neurons in the main olfactory epithelium (Li et al. 1995; Li and Sorensen 1997). The brain regions responsible for generating motor activity in response to olfactory inputs were investigated in an isolated olfactory epithelium-brain preparation (Derjean et al. 2010), which allows experimental access to the whole brain, while maintaining intact connections from the olfactory epithelium to the brain. To monitor locomotor responses, RS cell activity was measured with calcium imaging and intracellular recordings. Activation of RS cells is a precondition for movement in vertebrates and serves as an indication of locomotor efferent activity in the isolated olfactory epithelium-brain preparation (Derjean et al. 2010; Ryczko et al. 2013). When amino acids or pheromones were individually applied onto the peripheral olfactory organ, large calcium responses were induced in RS cells (Derjean et al. 2010). These results suggested the presence of a strong neural link between the peripheral olfactory organ and motor command cells in the brainstem (Derjean et al. 2010).

The role of the OB in this circuitry was then determined by pharmacological activation/inactivation. Local microinjections of glutamatergic agonists in the OB induced RS cell activity and rhythmic bursts of discharge alternating in spinal ventral roots on both sides (Derjean et al. 2010) – an activity referred to as "fictive locomotion" (Viala and Buser 1971; Perret et al. 1972; Andersson et al. 1978). Conversely, microinjections of glutamatergic antagonists in the OB blocked RS cell responses to electrical stimulation of the olfactory nerve (Derjean et al. 2010). Thus, it is likely that OB neural activity may induce swimming movements in intact animals. The responses of RS cells to electrical stimulation of the olfactory nerve were also decreased by local microinjections of glutamatergic antagonists in the medial OB (Derjean et al. 2010). Moreover, electrical stimulation of OB subregions revealed that only the medial OB elicits RS responses, as stimulation of the main OB failed to induce RS cell activity, even at higher intensities (Derjean et al. 2010; Daghfous et al. 2018). These experiments demonstrate an important role of the medial OB as a relay of the olfactory signal to RS cells. However, following a local microinjection of GABA receptor antagonists, RS cell responses to main OB electrical stimulation were markedly increased (Daghfous et al. 2018), even after resecting the medial OB from the preparation. This suggests that the main OB is under a GABAergic inhibition that reduces the transmission of olfactory signals to motor centers (Daghfous et al. 2018). These observations further support the hypothesis of two distinct olfactory subsystems acting in parallel to regulate olfactory behavior in the lamprey.

The neuronal circuitry through which olfactory signals are transmitted from the OB to RS cells was investigated with a combination of anatomical tracing experiments and physiologi-

cal experiments. Anterograde axonal tracer injection in the medial OB (Fig. 2a-b) labeled dense projections to a region in the ventral diencephalon: the PT (Derjean et al. 2010). Conversely, retrograde tracer injection in the PT (Fig. 2c-d) labeled a single population of OB projection neurons located in the medial OB (Derjean et al. 2010). This OB projection, previously identified in the lamprey as part of the olfacto-thalamic and hypothalamic tract (Heier 1948), consists of thick axons that course throughout the forebrain and terminate in close proximity with coarse dendrites of PT neurons. Moreover, OB projections terminating in the PT were also observed in two other species of lamprey (Northcutt and Puzdrowski 1988; Polenova and Vesselkin 1993) as well as in several fish species (Matz 1995; Rink and Wullimann 2001; von Bartheld 2004; Northcutt 2011; Northcutt and Rink 2012). Similarly, anterograde tracer injections in the main OB (excluding the medial OB) labeled distinct projection terminals in the PT (Suryanarayana et al. 2021b).

Physiological experiments were conducted to assess whether the PT recruits RS cell activity and elicits locomotion. In semi-intact preparations (*in vitro* isolated central nervous system with the tail of the animal kept intact), RS cell activity and swimming movements were induced in response to electrical stimulation or glutamate microinjections in the PT (Derjean et al. 2010; Ryczko et al. 2013, 2017, 2020). Moreover, RS cell responses to electrical stimulation of the olfactory nerve were blocked by microinjections of glutamate antagonists into the PT, suggesting that the latter region is an important relay for olfactory inputs and that the projections from the medial OB to the PT are glutamatergic (Derjean et al. 2010).

Located medially at the meso-diencephalic junction, the PT contains dopaminergic (DAergic) neurons that constitute the lamprey homolog of the mammalian substantia nigra *pars compacta* and ventral tegmental area (Baumgarten 1972). Most features of this DAergic system in lampreys are remarkably similar to those seen in mammals. It is connected to the basal ganglia nuclei (i.e., nigrostriatal loop; Baumgarten 1972; Pombal et al. 1997; Ericsson et al. 2013; Stephenson-Jones et al. 2013) and plays a role in the control of movements (reviewed in Grillner et al. 2013). Furthermore, this DAergic nucleus was shown to send efferent fibers in widely distributed brain regions, including ascending projections to the forebrain and descending projections to the brainstem (Baumgarten 1972; Nieuwenhuys 1977). These projections were more recently confirmed by Pérez-Fernández et al. (2014) using a combination of tracer injection and immunofluorescence. They also showed that PT projections to various motor centers were DAergic. In zebrafish, the PT also contains DAergic neurons with ascending projections to the striatum (Rink and Wullimann 2001). Interestingly, descending projections from the zebrafish OB terminate in close proximity with neurites of DAergic neurons in the PT (Miyasaka et al. 2014). However, the specific role of DAergic neurons in relaying olfactory inputs remains unknown. Further studies are required to identify and characterize functional aspects of the PT neurons receiving OB afferents, in lamprey as in zebrafish (Miyasaka et al. 2014).

The main OB also projects directly to the PT and axons from projection neurons of the main OB were observed terminating in close proximity with DAergic neurons (Suryanarayana et al. 2021b). Additionally, the OB has abundant projections to the pallium (see Fig. 2e-f; Northcutt and Puzdrowski 1988; Polenova and Vesselkin 1993; Derjean et al. 2010; Suryanarayana et al. 2017, 2021b), which was recently proposed to be the lamprey homolog of the mammalian neocortex (Suryanarayana 2019). In a recent publication, the lamprey pallium was referred to as the pallium/cortex (Suryanarayana et al. 2021a). This three-layered cortex integrates sensory inputs from several modalities: olfactory, somatosensory, and visual (Suryanarayana et al. 2017, 2020, 2021b). In turn, the pallium/cortex projects to several motor centers (Ménard et al. 2007; Ocaña et al. 2015). The ventral part of the pallium/cortex is a sensory area with olfactory functions (Suryanarayana et al. 2021b), whereas the dorsal part contains distinct visual, somatosensory, and motor areas and projects to brainstem motor centers (Ocaña et al. 2015; Suryanarayana et al. 2020). Interestingly, in both regions "pyramidal" projection neurons receive glutamatergic inputs from the OB (Suryanarayana et al. 2017, 2020, 2021b). Moreover, two subpopulations of main OB neurons that provide excitation to the pallium/cortex were identified and were shown to be markedly similar to the mammalian mitral and tufted cells with respect to their morphology, projection patterns, and membrane properties (Suryanarayana et al. 2021b). Mitral-like cells project directly to the ventral pallium/cortex, which has been proposed to be the piriform pallium (Heier 1948; Suryanarayana et al. 2021b). In parallel, tufted-like cell output is relayed in the dorsomedial telencephalic nucleus, which in turn also activates the ventral pallium/cortex. Interestingly, electrical stimulation of the pallium/cortex elicits various trunk, eye, oral, and locomotor movements (Ocaña et al. 2015). Because the PT receives abundant pallial/cortical projections, it was thus proposed that this region processes and relays olfactory information from the OB to the PT (Pérez-Fernández et al. 2014; Ocaña et al. 2015). Anatomical tracing revealed that descending fibers from the main OB are located close to dendrites of pallial/cortical neurons retrogradely-labeled after a PT injection (Daghfous et al. 2018). Furthermore, RS cell responses to electrical stimulation of the pallium/cortex are decreased following glutamate antagonist microinjections into the PT (Daghfous et al. 2018), suggesting that glutamatergic inputs from the main OB to the pallium/cortex (Suryanarayana et al. 2017, 2021b) are relayed to the PT before reaching RS cells. Altogether, the data reviewed above strongly suggests the existence of two parallel olfactomotor pathways (medial: accessory olfactory organ-medial OB-PT and lateral: main olfactory epithelium-main OB-pallium/cortex-PT; see Fig. 1) that converge upon the PT, which in turn relays the olfactory signal toward RS cells to induce locomotion. Hence, the PT may elicit swimming behavior in response to olfactory input originating from both the accessory olfactory organ and the main olfactory epithelium. However, the specific contribution of each of these two olfactory subsystems to the recruitment of PT neurons remains unclear and further investigations are needed.

How does the PT recruit RS cells to induce swimming activity? Neurons in the PT project to several motor centers (Pérez-Fernández et al. 2014), including the MLR (Ménard et al. 2007;

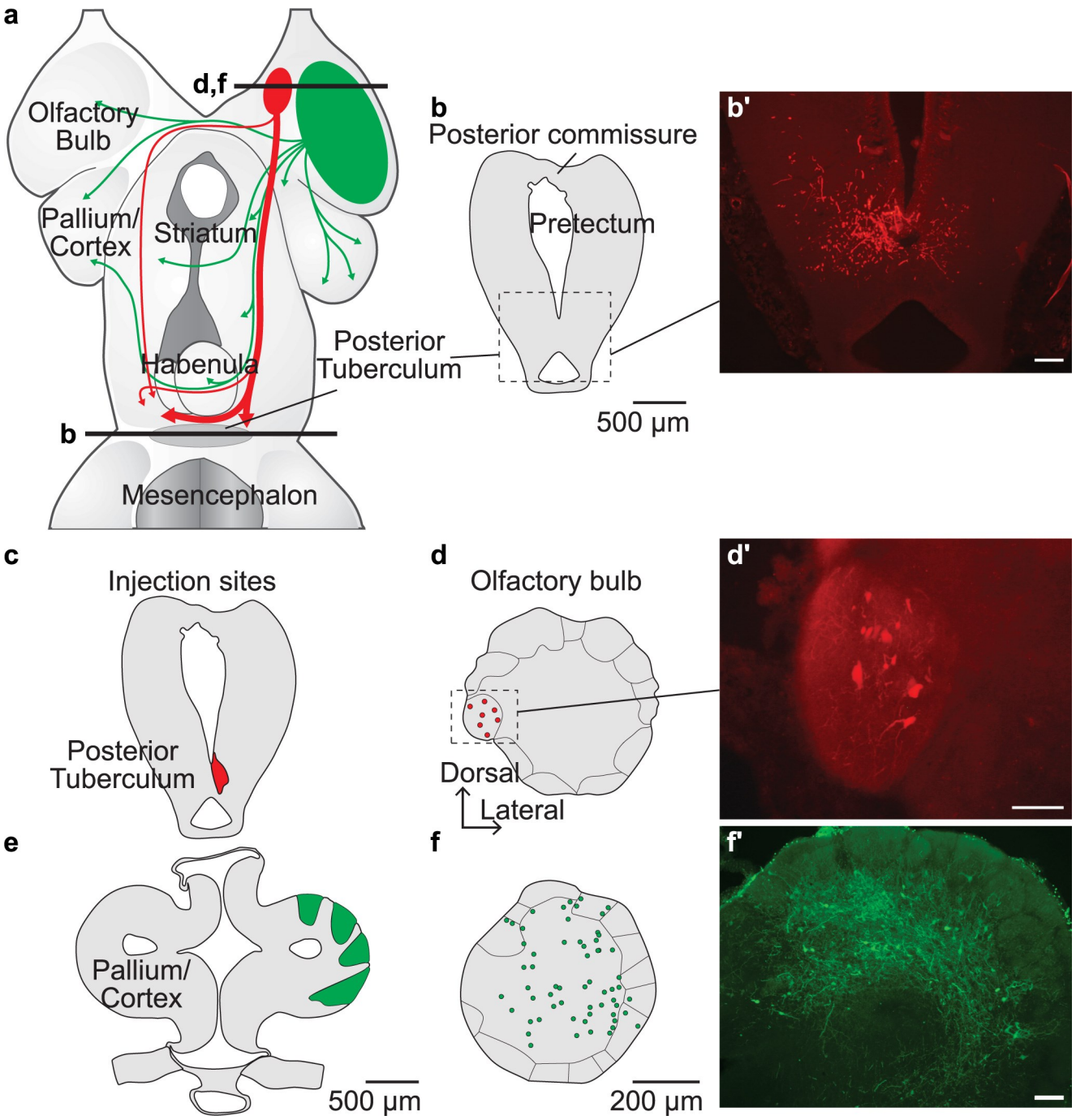


**Fig. 2** Efferent olfactory bulb projections involved in odor-induced locomotion. (a) Schematic representation of the lamprey forebrain illustrating secondary olfactory projections from the medial (red) and main (green) olfactory bulb. (b) Anterograde tracer injection in the medial olfactory bulb labeled fibers in the posterior tuberculum. (c-d) Conversely, retrograde tracer injection in the posterior tuberculum labeled neurons in the medial olfactory bulb. (e-f) Retrograde tracer injection in the pallium/cortex labeled neurons in the main olfactory bulb. Scale bars = 100 µm. Credits: Adapted with permission from (Derjean et al. 2010)

Ryczko et al. 2013, 2017), which is of particular interest considering the major impact that the MLR has on locomotor control in vertebrates (see Ryczko and Dubuc 2013). The MLR was discovered in cats nearly 60 years ago (Shik et al. 1966). It attracted considerable interest from the scientific community, because this physiologically defined region was shown at the time to be exclusively dedicated to the control of locomotion. The discovery of the MLR was a major step for understanding the neural control of locomotion. Since the original discovery of the MLR, it has been shown to be multi-functional, acting on many different aspects of motor behavior (for reviews, see Dubuc et al. 2008; Le Ray et al. 2011; Ryczko and Dubuc 2013; Grätsch et al. 2019a). The MLR is an important brainstem motor center that controls initiation, maintenance, and cessation of locomotion. Indeed, the lamprey MLR exerts a graded control over downstream RS cell activity to initiate swimming and regulate the intensity of the locomotor output (Sirota et al. 2000). The MLR projections to RS cells are monosynaptic and both glutamatergic (Brocard and Dubuc 2003) and cholinergic (Le Ray et al. 2003). Stimulation of the MLR elicits large depola-

rizations of RS cells on both sides of the brainstem (Le Ray et al. 2003; Brocard et al. 2010), in addition to alternating ventral root discharges or swimming in a semi-intact preparation. Cholinergic MLR projections activate muscarinoceptive cells lateral to the reticular formation. The latter cells amplify RS cell activity and boost the locomotor output (Smetana et al. 2007, 2010). These connections constitute an hyperdrive mechanism for locomotion (Smetana et al. 2010).

Olfactory stimulation induces sustained depolarizations of RS cells (Wickelgren 1977a, 1977b; Derjean et al. 2010; Daghfous et al. 2018; Beauséjour et al. 2020) and it is now well understood that these cells play a critical role in the behavioral responses to odorants. The descending projections of RS cells activate the spinal locomotor networks (Buchanan and Grillner 1987; Ohta and Grillner 1989) and control the frequency of rhythmic locomotor activity and thus locomotor speed.

Since direct PT projections to the MLR were observed in the lamprey (Ménard et al. 2007) and that injections of glutamatergic antagonists in the MLR block RS cell responses to stimulation of the olfactory nerve (Derjean et al. 2010), the mechanisms by which the PT recruits the MLR was examined in further details (Ryczko et al. 2013, 2017). The PT contains neurons immunopositive for glutamate, DA, or both that were retrogradely-labeled by a tracer injection into the MLR (Ryczko et al. 2013, 2017). In semi-intact preparations, where the brain is accessible and the body can produce locomotor movements, stimulation of the PT activated MLR neurons that evoked a graded increase in RS cell activity and, consequently, swimming speed (Ryczko et al. 2017). The descending glutamatergic projections from the PT to the MLR were shown to be essential to activate downstream locomotor circuits as glutamatergic antagonist microinjection into the MLR considerably decreased locomotor responses induced by PT stimulation. The parallel DAergic projections from the PT to the MLR were shown to provide additional excitation as activation of D1 receptors in the MLR increased swimming frequency (Ryczko et al. 2013, 2017). Interestingly, it was also shown that projections from the PT to the MLR are conserved from lamprey to mammals (Ryczko et al. 2016; Ryczko and Dubuc, 2017). Remarkably, the authors found that these projections were present in lampreys, salamanders, and rats, in addition to providing clear evidence that they could also exist in humans (Ryczko et al. 2016).

In addition to the innervation of the MLR, PT neurons immunopositive for glutamate, DA, or both were also retrogradely-labeled by a tracer injection into reticular nuclei, suggesting that RS cells receive a direct DAergic innervation from the PT (Ryczko et al. 2020). Using fast-scan cyclic voltammetry, the authors showed that stimulation of the PT evokes DA release in the reticular nuclei. Local microinjection of D1 receptor antagonists into hindbrain reticular nuclei decreased the swimming frequency as well as the duration of locomotor bouts elicited by electrical stimulation of the PT. Moreover, the synaptic responses elicited in RS cells by stimulation of the PT were markedly reduced by local application of the D1 receptor antagonists. Therefore, it appears that while glutamatergic inputs from the PT provide strong excitation to the MLR, descending DAergic projections from the PT provide additional excitation to both the MLR (Ryczko et al. 2013, 2017) and RS cells (Ryczko et al. 2020).

Physiological experiments were performed to confirm the role of the MLR as a relay for olfactory signals. Pharmacological inactivation of the MLR with glutamatergic antagonists drastically decreased RS cell responses to electrical stimulation of the olfactory nerve (Derjean et al. 2010), or the pallium/cortex (Daghfous et al. 2018). These observations indicate that glutamatergic transmission in the MLR plays an important role in transmitting olfactory signals to RS cells in both the medial and lateral olfactomotor pathways. Furthermore, since medial OB neurons were retrogradely-labeled following a tracer injection in another locomotor center, the diencephalic locomotor region, it was previously suggested that odor-induced locomotor activity could be mediated by the diencephalic locomotor region (El Manira et al. 1997), well known to project directly to RS cells to evoke swimming (El Manira et al. 1997; Ménard and Grillner 2008; reviewed in Grillner and El Manira 2020). The lamprey diencephalic locomotor region is located in the ventral thalamus and was suggested to be homologous to the mammalian zona incerta (El Manira et al. 1997; Grillner and El Manira 2020). The zona incerta of mammals projects to the hindbrain (Schwanzel-Fukuda et al. 1984) and induces locomotor activity upon its stimulation (Parker and Sinnamon 1983; Milner and Mogenson 1988; Marciello and Sinnamon 1990; Sinnamon 1993). Pharmacological inactivation of the diencephalic locomotor region with glutamate antagonists failed to alter RS cell responses to electrical stimulation of the olfactory nerve, suggesting that it is not an essential relay of the olfactomotor circuitry (Derjean et al. 2010).

Local injections of retrograde axonal tracers were performed to investigate other potential sources of inputs to the MLR. Retrogradely-labeled cell bodies were observed in both the medial OB and the pallium/cortex (Ocaña et al. 2015; Daghfous et al. 2018), suggesting that the medial OB and pallium/cortex could also induce locomotion by recruiting the MLR directly. However, the specific contribution of each of these descending projections (i.e., medial OB-MLR vs. pallium/cortex-MLR vs. PT-MLR) to relay the olfactory signals has not been established yet and further experiments are required to ascertain their role in the olfactomotor circuitry.

## Neuromodulation in the olfactory bulb

The neural circuitry described above is hardwired to generate locomotion upon detection of numerous odorants (Derjean et al. 2010; Green et al. 2017). However, it is noteworthy that the olfactomotor circuitry is not simply turned on and off by odorants, but it must also be regulated to adapt olfactory responses to changing internal and external conditions (Beauséjour et al. 2020). In their natural environment, lampreys are exposed to a variety of chemical stimuli that are found in different ranges of concentration and are of variable relevance for survival and reproduction. The behavioral olfactory responses need to be modified according to the biological state and needs of the animal. Odor-driven behaviors can thus vary across life and will be adapted to internal cues, such as gender and developmental stage (Siefkes et al. 2005), or external factors, such as light/dark cycle and water temperature (Di

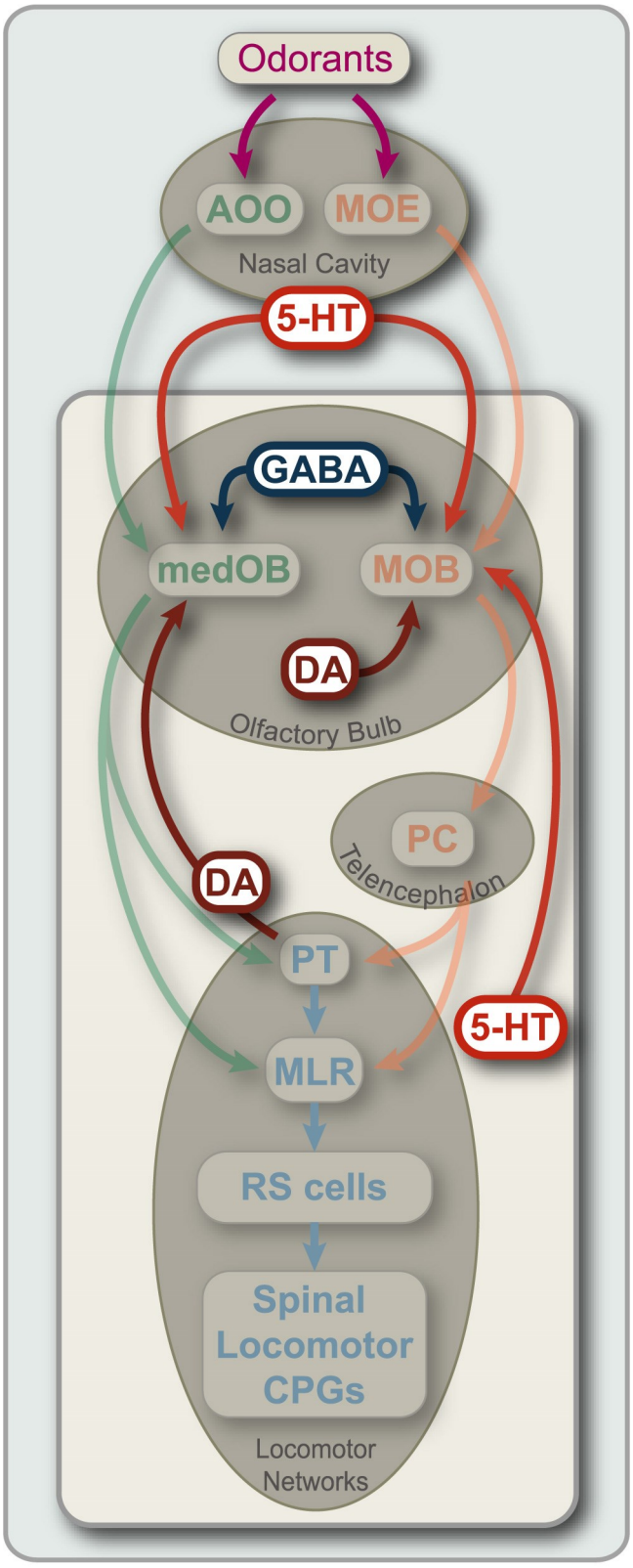


**Fig. 3** Modulation of transmission in olfactomotor pathways at the level of the olfactory bulb. Schematic representation of the medial (green arrows) and lateral (orange arrows) olfactomotor pathways illustrating brain regions involved in the transformation of olfactory inputs into motor output. Sources of innervation at the medial olfactory bulb (medOB) level include serotoninergic (5-HT, red) cells from the olfactory organ, local GABAergic interneurons (blue), and extrinsic dopaminergic (DA, dark red) cells from the posterior tuberculum (PT). Sources of modulation in the main olfactory bulb (MOB) involve 5-HTergic cells from the olfactory organ, local GABAergic interneurons, local DAergic interneurons, and mesencephalic 5-HTergic cells. This neuromodulatory circuitry provides means to adapt motor responses elicited by olfactory input to different external and internal states of the animal. AOO accessory olfactory organ, CPGs central pattern generators, MLR mesencephalic locomotor region, MOE main olfactory epithelium, PC pallium/cortex, RS reticulospinal

Rocco et al. 2014). Therefore, modulatory mechanisms must be present in the olfactomotor circuitry.

The OB is the primary processing region for the olfactory signal entering the brain and, as such, it represents an ideal region for the modulation of olfactory inputs before they are transmitted to downstream regions in the central nervous system. Neurons in the OB must stay tuned to relevant stimuli and filter other signals; the OB is thus well suited for the central gating of olfactory inputs. Local interneurons and extrinsic fibers modulate neural transmission in the OB circuitry, ultimately regulating the activity of projection neurons, which carry the olfactory signal to secondary brain regions. Hence, the OB is not a simple olfactory relay, but it is a dynamic network of neurons where sensory processing is adapted to generate appropriate olfactory behavior. Neuromodulatory mechanisms in the OB could efficiently regulate motor responses to odorants and would accordingly ensure appropriate context-sensitive olfactory behavior and allow for a wide array of behavioral responses. Because fibers containing GABA, DA, and serotonin (5-HT) are present in the lamprey OB, these neurotransmitter systems could modulate olfactory inputs to the bulbar circuitry (Fig. 3).

**GABAergic modulation in the lamprey OB**

The lamprey OB possesses a well-developed GABAergic system, which constitutes an ideal candidate for the regulation of neural activity in the OB. Immunocytochemical studies revealed up to five different GABAergic cell populations located in every OB layer except for the glomerular layer (Meléndez-Ferro et al. 2001). GABAergic fibers are localized in every layer of the OB, suggesting that GABA plays an important role in the modulation of bulbar circuits (Meléndez-Ferro et al. 2001). Moreover, recent anatomical studies (Daghfous et al. 2018) revealed GABAergic fibers overlapping with retrogradely-labeled projection neurons in the medial OB and main OB (PT or pallium/cortex tracer injection, respectively). Microinjection of $GABA_A$ receptor antagonists induced a dramatic increase of OB responses to electrical olfactory nerve stimulation (Daghfous et al. 2018), suggesting that glomerular activity is under GABAergic inhibitory control. To investigate whether this modulation may also affect olfactomotor transmission and thus behavioral output, synaptic responses were measured in RS cells. Localized microinjections of a $GABA_A$ receptor antagonist into the medial or main OB revealed that gating of GABAergic transmission occurs in both regions. Indeed, removal of GABAergic inhibition drastically amplified RS cell responses to electrical stimulation of the olfactory nerve, so that a minimal stimulation intensity induced sustained locomotor activity (Daghfous et al. 2018; Beauséjour et al. 2020). Thus, GABAergic modulation in the OB networks presumably leads to inhibition of olfactory-induced behavior. However, the cellular mechanisms by which the GABAergic inhibition is removed are not yet understood.

**Dopaminergic modulation of the lamprey OB**

In the lamprey OB, DAergic somata are observed within the granular layer and DAergic fibers are localized in both the granular and glomerular layers, suggesting that DA could modulate the neural activity in the OB (Pierre et al. 1997; Pombal et al. 1997). Moreover, two distinct types of DAergic fibers were identified in the lamprey OB (Beauséjour et al. 2020). First, extrinsic DAergic axons localized exclusively in the medial OB terminate in close proximity to medial OB projection neurons and olfactory nerve terminals. Tracer injections in the medial OB revealed that these DAergic axons arise from DAergic neurons in the PT (Beauséjour et al. 2020). Interestingly, these observations indicate the presence of reciprocal PT-medial OB connections that allow PT neurons to regulate the olfactory inputs they receive from medial OB projection neurons. To test whether DA may modulate olfactomotor transmission, DA receptor agonists or antagonists were locally microinjected in the medial OB of whole-brain preparations isolated *in vitro*. Microinjections of DA, D1 receptor agonists, or D2 receptor agonists in the medial OB decreased the RS cell responses to electrical stimulation of the olfactory nerve (Beauséjour et al. 2020). Conversely, microinjection of D2 receptor antagonists induced an increase of RS cell responses, suggesting that D2 receptors mediate inhibitory effects on olfactomotor transmission in the medial OB. Furthermore, *in situ* hybridization of DA receptor mRNA in the lamprey OB revealed widespread expression of D1 and D2 receptors in the granular layer (Pérez-Fernández 2013; Pérez-Fernández et al. 2014). Interestingly, somata expressing D2 receptors were also found in the glomerular layer, but exclusively in the medial OB (Pérez-Fernández et al. 2014). Hence, D2 receptors might be expressed by medial OB projection neurons and directly modulate their output.

As mentioned above, pharmacological inactivation of local GABAergic transmission in the OB increases dramatically the size of RS cell responses to electrical stimulation of the olfactory nerve (Daghfous et al. 2018). In comparison with GABAergic modulation of the OB, pharmacological inactivation of D2 receptors did not drastically increase RS cell responses to olfactory nerve stimulation. However, following microinjection of $GABA_A$ receptor antagonists, subsequent microinjection of DA in the medial OB reduced olfactomotor responses in RS cells to the point of eliminating spiking activity (Beauséjour et al. 2020). There is therefore a DAergic modulation in the medial OB that can lead to reduced motor responses to olfactory inputs. This DAergic innervation could thus enable flexibility in olfactory-induced motor responses.

The second type of DAergic fibers in the OB stems from local interneurons, which are scattered homogeneously across the granular layer and innervate main OB glomeruli. Intriguingly, these DAergic interneurons only exist in adult lamprey, as they are not observed during the larval stage (Abalo et al. 2005; Beauséjour et al. 2020). The role of DAergic interneurons in the main OB remains unknown, but they may provide adult lampreys with additional processing mechanisms to modulate olfactory activity and odor-induced locomotion. Physiological experiments assessing the effects of DA microinjection into the main OB on RS cell responses to electrical stimulation of the olfactory nerve should be conducted. Altogether, the data reviewed here indicate that DAergic innervation modulates neural activity in the OB of lampreys and could regulate odor-driven behavior via distinct mechanisms in the medial and lateral olfactomotor pathways.

**Serotoninergic modulation of the lamprey OB**

As indicated above, DAergic innervation was shown to influence olfactory transmission in the lamprey OB. Other monoamine neurotransmitters, such as 5-HT, may also be involved in odor processing. Serotoninergic innervation of the lamprey OB was first observed as chains of yellow fluorescent varicosities coursing in the olfactory nerve and glomerular layers (Baumgarten 1972) following staining of monoamines with the Falck-Hillarp technique (Falck 1962). In the adult lamprey, the OB is devoid of 5-HTergic somata and is innervated exclusively by extrinsic 5-HTergic fibers that are distributed in the olfactory nerve layer, the glomerular layer, the mitral cell layer, and the internal granular layer (Pierre et al. 1992; Zielinski et al. 2000; Frontini et al. 2003; Abalo et al. 2007). It is believed that cell bodies immunoreactive for 5-HT in the lamina propria of the peripheral olfactory organ provide part of the 5-HTergic inputs to the OB via axons travelling in the olfactory nerve (Zielinski et al. 2000; Frontini et al. 2003). Furthermore, 5-HTergic fibers originating from the olfactory organ have a particularly rich innervation in the medial OB, where they terminate in close proximity to olfactory nerve fibers (Frontini et al. 2003), and presumably, projection neurons. It is noteworthy that occasional 5-HTergic neurons were seen in the OB by Abalo et al. (2007) and could also contribute to the 5-HTergic innervation of the OB. Olfactory nerve transection confirmed that the peripheral source of 5-HT innervates glomerular units, whereas a second, central source of 5-HTergic innervation terminates in the granular layer (Zielinski et al. 2000). Another source of 5-HTergic input could originate from the midbrain tegmentum, which contains 5-HTergic cell bodies (Baumgarten 1972; Pierre et al. 1992; Antri et al. 2006) and has direct projections to the OB (Northcutt and Puzdrowski 1988). The presence of 5-HTergic fibers in every OB layer (Abalo et al. 2007) is matched by the widespread bulbar distribution of 5-HT1a receptors (Cornide-Petronio et al. 2013). This 5-HT receptor is expressed in most cells of the mitral and granular cell layers and appears during early developmental stages (Cornide-Petronio et al. 2013).

The anatomical observations described above are suggestive of 5-HTergic modulation in the OB circuitry. Moreover, Boyes (2014) showed that 5-HT attenuates neural responses in the main OB to stimulation of the main olfactory epithelium with amino acids, lamprey-specific pheromones, or bile acids – all of which induce RS cell activity (Derjean et al. 2010). This inhibitory effect in the OB circuitry is consistent with the documented 5-HT1a receptor hyperpolarizing effects (Hoyer et al. 1994). Moreover, 5-HT1a receptor antagonists reversed the 5-HTergic inhibitory effect and increased main OB neuronal responses to all odorants (Boyes 2014). The most robust modulatory effects of 5-HT1a antagonists were observed during amino acid-induced responses in the lateral OB and interestingly,

the latter region was shown to respond exclusively to amino acids (Green et al. 2017). Because of the association between amino acid detection and predation in lampreys (Kleerekoper and Mogensen 1963), 5-HTergic transmission at the OB level may contribute to the modulation of odor-induced feeding behavior. In vertebrates, the role of 5-HT in the regulation of satiety and feeding behavior is well established (Blundell 1977; Fletcher 1988) and could be conserved from lamprey to mammals. Altogether, these observations demonstrate that 5-HTergic modulation occurs in the main OB, which presumably affects the activity of projection neurons. Whether this 5-HTergic innervation could also shape OB output has not been ascertained yet, and its functional impact on RS cell responses to olfactory stimulation should be assessed. Similarly, the physiological effect of 5-HTergic innervation specifically in the medial OB should also be tested.

## Conclusion

Sensorimotor transformations allow animals to integrate sensory signals from their environment to generate appropriate motor responses. These transformations are of key importance and are likely to have been amongst the first central nervous system functions to appear in evolution because they are crucial for animal survival. Sensory-evoked locomotion is often seen as a rather simple behavior, such as during escape. External stimuli are first detected, then processed as sensory information within brain neuronal networks before a locomotor command is sent to the muscles. For goal-directed behavior, internal cues play an additional role in starting, maintaining, and stopping locomotion. Despite major knowledge advances made on sensory and motor systems over the last decades, an important challenge remains: to elucidate the detailed neural connections that link stimulus detection and motor output.

This review focused on two parallel pathways involved in the transformation of olfactory inputs into a locomotor output, which were identified for the first time in the lamprey. We described a medial pathway from the accessory olfactory organ to the medial OB and a lateral pathway from the main olfactory epithelium to the main OB and then to the pallium/cortex. Both pathways converge onto the PT and the MLR to induce locomotor responses via RS cell activation. This neural substrate is recruited following peripheral detection of odorants and was proposed to be important to odor-induced behavior in lamprey (Derjean et al. 2010). Since feeding and reproductive requirements may vary throughout the life cycle, activity within this network must be regulated and modulatory mechanisms control this pathway at the OB level. The olfactomotor circuitry discussed here may provide a neural substrate that allows lampreys to detect, process and act accordingly following chemoreception of odorant molecules.

Many gaps need to be filled in order to gain a better understanding of the neural circuits that mediate odor-induced motor responses. For instance, a medial and a lateral pathway were characterized in lampreys, but their specific contribution to olfactory behavior is still incomplete. Many types of behavior are induced by odorants, such as escaping predators or finding mates, but we ignore which behavior is induced by the medial or the lateral pathway, or whether both of them are necessary to specific tasks. Additional studies are required to complete our comprehension of the olfactory subsystems described here. For example, lesion experiments in semi-intact animals would be helpful in determining the role of the medial vs. the lateral pathway in odor-driven behaviors. Moreover, both pathways converge onto the PT, a brain region that exerts control over brainstem motor centers through descending DAergic and glutamatergic projections and more details are needed on the integration mechanisms in this region. Besides, since the PT has been proposed as a homolog of the ventral tegmental area in other vertebrate species, the DAergic neurons located in this nucleus could then be involved in rewarding specific behaviors. The simpler organisation of the lamprey central nervous system provides an advantage to examine the cellular mechanisms of reward. Future experiments should aim at understanding the role of the PT in behavior, especially the contribution of DAergic and glutamatergic neurons to the transformation of olfactory inputs into motor output.

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**Author contribution**

The first draft of the manuscript was written by PAB and all authors commented on the manuscript. The authors read and approved the final manuscript.

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