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Le rôle des cellules dopaminergiques dans la locomotion induite par l'olfaction chez la lamproie

Par
Philippe-Antoine Beauséjour

Département de neurosciences, Faculté de médecine

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Présentée par

Philippe-Antoine Beauséjour

A été évaluée par un jury composé des personnes suivantes

Marina Martinez
Président-rapporteur

Réjean Dubuc
Directeur de recherche

Jean-François Pflieger
Membre du jury

Frédéric Brocard
Examinateur externe

Résumé

La détection de molécules chimiques par l'odorat est importante pour guider le comportement des animaux. Chez la lamproie marine, *Petromyzon marinus*, l'olfaction est vitale pour plusieurs fonctions telles que l'alimentation, l'évitement des prédateurs et la reproduction. Les différents comportements olfactifs de la lamproie sont les mieux caractérisés parmi tous les vertébrés aquatiques et ils font l'objet du premier chapitre de l'introduction.

Les circuits du cerveau responsables des mouvements produits lors de la détection de stimuli olfactifs ont été examinés chez la lamproie. Des études récentes révèlent qu'il existe deux organes olfactifs périphériques ayant des projections parallèles qui innervent des parties distinctes du bulbe olfactif (BO). Dans les deux cas, le signal olfactif atteint éventuellement les cellules réticulospinales (RS), qui activent les réseaux locomoteurs spinaux. La littérature portant sur ces circuits neuronaux est décrite dans le deuxième chapitre introductif. Le substrat neuronal par lequel le signal olfactif est transmis aux cellules RS n'est pas complètement caractérisé mais des données du laboratoire Dubuc suggèrent que le tubercule postérieur (TP) serait une cible importante des projections du BO. Puisque cette région contient des neurones dopaminergiques (DA) impliqués dans le contrôle moteur, l'objectif principal de cette thèse était de déterminer leur rôle dans le traitement du signal olfactif et la production de locomotion.

Nos résultats ont permis de caractériser l'innervation DA du BO de la lamproie et d'observer que les neurones DA du TP projettent à la partie médiane du BO chez les animaux de stade larvaire et adulte. De plus, l'activation de récepteurs D2 dans cette région diminue la transmission du signal olfactif aux cellules RS. Dans le reste du BO, des neurones DA apparaissent au stade adulte. Ces observations sont rapportées dans le premier chapitre des résultats. Puisque les neurones DA du TP peuvent moduler la transmission olfactomotrice au niveau du BO, ils pourraient aussi jouer un rôle via leurs projections connues vers le tronc cérébral. Le deuxième chapitre des résultats se penche donc sur l'implication du TP dans le relai de l'information olfactive au système moteur. L'étude des projections du BO montre que les neurones DA sont ciblés, incluant ceux qui projettent à la région locomotrice mésencéphalique (RLM), responsable de l'initiation et du contrôle de la locomotion. Aussi, la stimulation olfactive active des neurones du TP qui projettent à la RLM.

Dans une préparation dont la tête est fixée mais le corps peut se déplacer, la stimulation olfactive induit de la nage en recrutant simultanément le TP et les cellules RS. Nous montrons aussi que le TP est recruté durant la nage survenant spontanément, ce qui indique que cette région joue un rôle important dans le contrôle locomoteur.

Cette thèse révèle que les neurones DA du TP peuvent 1) être activés par la détection d'odeurs et ensuite 2) moduler la transmission au niveau du BO ainsi que 3) recruter la RLM pour produire un épisode de nage. Ces données suggèrent qu'ils occupent une position-clé dans l'intégration sensorimotrice des stimuli olfactifs puisqu'ils encodent à la fois de l'information sensorielle et motrice.

Mots-clefs: Olfaction, Intégration sensorimotrice, Locomotion, Dopamine, Modulation, Neuroanatomie, Neurophysiologie, Lamproie.

Abstract

The detection of chemical molecules by smell is important in guiding the behavior of animals. In the sea lamprey, *Petromyzon marinus*, olfaction is vital for several functions such as feeding, predator avoidance and reproduction. The various olfactory behaviors of the lamprey are the best characterized among all aquatic vertebrates and they were reviewed in the first chapter of the introduction.

The brain circuitry responsible for producing movement upon sensing olfactory stimuli has been examined in lamprey. Recent studies revealed that there are two peripheral olfactory epithelia with parallel projections that reach distinct parts of the olfactory bulb (OB). In both cases, the olfactory signal eventually reaches reticulospinal (RS) cells, which activate the locomotor networks of the spinal cord. The literature describing these neural circuits is thoroughly reviewed in the second chapter of the introduction. The neuronal substrate by which the olfactory signal is transmitted to RS cells is not fully characterized, but data from the Dubuc laboratory suggest that the posterior tubercle (PT) may be an important target for OB projections. Since this region contains dopaminergic (DA) neurons involved in motor control, the main objective of this thesis was to determine their role in olfactory signal processing and the production of locomotion.

Our results have allowed to characterize the DA innervation of the lamprey OB and show that DA neurons of the PT send projections to the medial part of the OB in larval and adult animals. In addition, the activation of D2 receptors in this region decreases the transmission of the olfactory signal to RS cells. In the rest of the OB, DA neurons appear in adult animals. These observations are reported in the first chapter of the Results. Since DA neurons of the PT can modulate olfactory-motor transmission at the level of the OB, they could also play a role through existing descending projections to the brainstem. Thus, in the second chapter of the Results, we studied the involvement of the PT in the relay of olfactory information to the motor system. The analysis of OB projections shows that DA neurons are targeted, including those that project to the mesencephalic locomotor region (MLR), which is responsible for initiating and controlling locomotion. Moreover, olfactory stimulation activates PT neurons that project to the MLR. In a head-fixed preparation in which the body moves, olfactory stimulation induces swimming simultaneously with PT and RS cell activity.

We also show that the PT is recruited during spontaneously occurring swimming, which indicates that this region plays an important role in locomotor control.

This thesis reveals that DA neurons in the PT can 1) be activated following odorant detection and then 2) modulate the transmission at the level of the OB as well as 3) recruit the MLR to produce a swimming episode. These data suggest that they occupy a key position in the sensorimotor integration of olfactory stimuli since they encode both sensory and motor information.

Keywords: Olfaction, Sensorimotor integration, Locomotion, Dopamine, Modulation, Neuroanatomy, Neurophysiology, Lamprey

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Français

ATV: Aire tegmentaire ventrale
BO: Bulbe olfactif
DA: Dopamine, Dopaminergique
ÉOP: Épithélium olfactif principal
GABA: Acide γ-aminobutyrique
MPTP: l-méthyl-4-phényl-l, 2,3,6-tétrahydropyridine
OOA: Organe olfactif accessoire
PL: Pallium latéral
PPSEs: Potentiels postsynaptiques excitateurs
RLM: Région locomotrice mésencéphalique
RS: Réticulospinal
SNc: Substance noire *pars compacta*
TP: Tubercule postérieur

Anglais

1-ene 3k-PZS: 3 keto-1-ene petromyzonol sulphate
3k-PZS: 3-keto petromyzonol sulfate
5-HT, 5HTergic: Serotonin, Serotoninergic
ΔF/F: Relative changes in fluorescence
AOO: Accessory olfactory organ
AP5: 2-amino-5-phosphonopentanoic acid
BSA: Bovine serum albumin
CNQX: 6-cyano-7-nitroquinoxaline-2,3-dione
CPG: Central pattern generator
DA, DAergic, DA+: Dopamine, Dopaminergic, Dopamine-immunopositive
DHN: Dorsal hypothalamic nucleus
DkPES: 3,12-diketo-4,6-petromyzonene-24-sulfate

EPSPs: Excitatory postsynaptic potentials

G: Glutaraldehyde

GABA: γ-aminobutyric acid

GAD: Glutamic acid decarboxylase

GSIB4: Griffonia simplicifolia isolectin β4

LPal: Lateral pallium

medOB: medial olfactory bulb

MOB: Main olfactory bulb

MOE: Main olfactory epithelium

MLR: Mesencephalic locomotor region

mRNA: Messenger ribonucleic acid

MRRN: Middle rhombencephalic reticular nucleus

OB: Olfactory bulb

ON: Olfactory nerve

PBS: Phosphate-buffered saline

PC: Pallium/cortex (Lateral pallium)

PT: Posterior tuberculum

PZS: Petromyzonol sulfate

RS: Reticulospinal

TBS: Tris-buffered saline

TBS-m: Tris-buffered saline with low sodium-metabisulfite

TBS-M: Tris-buffered saline with high sodium metabisulfite

TH, TH+: Tyrosine hydroxylase, Tyrosine hydroxylase immunopositive

TRDA: Texas Red-conjugated dextran amines, 3000 M.W.

vGluT: Vesicular glutamate transporter

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1. Introduction

1.1 Vue d'ensemble du sujet de la thèse

Les stimuli olfactifs génèrent des comportements appétitifs et aversifs chez les animaux. Dans plusieurs contextes tels que l'alimentation ou la reproduction, la détection de molécules chimiques dans l'environnement est essentielle pour la survie. De la réponse chimiotaxique d'un eucaryote unicellulaire à la perception des proies à plusieurs kilomètres de distance chez l'ours polaire (Owen et al., 2015), la détection chimiosensorielle influence directement le comportement moteur des animaux.

Chez les vertébrés, la façon dont ces mécanismes sensorimoteurs sont traités est encore mal connue. La compréhension des circuits neuronaux générant des réponses motrices à la détection de molécules chimiques dans l'environnement est nécessaire pour mieux comprendre le comportement animal, qui est fortement influencé par l'olfaction. La lamproie marine, un vertébré aquatique, est un excellent modèle pour étudier les mécanismes responsables de la transformation olfactomotrice dans le système nerveux central. En effet, l'odorat a une importance capitale durant tout le cycle de vie de la lamproie bien mise en évidence par le volume substantiel occupé par les structures dédiées au traitement olfactif dans son cerveau. De plus, le système moteur de cet animal a été particulièrement bien caractérisé. Les connaissances antérieures sur le système nerveux de la lamproie en font donc un modèle idéal pour étudier les circuits neuronaux permettant la transformation d'informations olfactives en réponses motrices appropriées.

Des travaux antérieurs du laboratoire Dubuc ont démontré l'existence de circuits neuronaux pouvant générer une réponse motrice rapide à la détection d'odeurs (Derjean et al., 2010; Daghfous et al., 2018). Dans la périphérie olfactive, deux populations de neurones détectent les odeurs et projettent l'information au bulbe olfactif (BO) via deux voies anatomiquement distinctes. Des neurones de projection bulbaires de ces deux voies (médiale vs latérale) transmettent ensuite le signal vers une population neuronale du tronc cérébral impliquée dans la production d'une réponse locomotrice, soit les cellules réticulospinales (RS).

L'identification des circuits neuronaux responsables de la transformation olfactomotrice permet maintenant de poser plusieurs nouvelles questions. Notamment, les comportements olfactifs de la

lamproie varient au cours de son cycle de vie et les mécanismes régulant ces changements ne sont pas connus. Par exemple, avant la maturation sexuelle, la lamproie cesse ses comportements de prédation et devient plus attirée par l'odeur de partenaires sexuels potentiels que par l'odeur de ses proies. Nous suspectons que l'apparition de nouveaux comportements nécessite des modifications du système nerveux central et qu'il doit certainement exister des mécanismes de modulation des réseaux olfactomoteurs pour produire des comportements olfactifs appropriés à la survie de l'animal dans tous les contextes. Pour mieux caractériser ces circuits, il est important de comprendre comment ils sont modulés.

Chez les autres vertébrés, la transmission dopaminergique (DA) dans le BO a une importance capitale dans la modulation des comportements olfactifs (Kendrick et al., 1988; Keverne et al., 1993; Serguera et al., 2008). Des neurones DA locaux modulent le traitement de l'information olfactive et de plus, des projections DA provenant de la substance noire pars compacta (SNc) et de l'aire tegmentaire ventrale (ATV) et participant à la perception de stimuli olfactifs ont récemment été observées chez le rongeur (Höglinger et al., 2015; Zhang et al., 2019). Malgré les connaissances sur les systèmes DA et olfactif de la lamproie, la manière dont ils interagissent est encore méconnue. C'est pourquoi nous avons cherché à étudier les connexions anatomiques et les mécanismes physiologiques par lesquels la transmission DA peut influencer les comportements olfactifs chez la lamproie.

Dans un premier temps, nous avons examiné la façon dont les comportements olfactifs sont modulés par la transmission DA au niveau du BO chez la lamproie. Les mécanismes physiologiques de la transmission DA n'ont d'ailleurs jamais été étudiés dans le BO d'un vertébré basal tel que la lamproie. Ensuite, des projections du BO en provenance des voies olfactomotrices médiale et latérale ont été observées dans une région contenant une population importante de neurones DA, le tubercule postérieur (TP). Chez la lamproie et les autres anamniotes, cette région contient l'homologue de la SNc/ATV des mammifères. Toutefois, la fonction des neurones DA du TP dans le traitement et le relai de l'information olfactive pour produire une réponse de nage est encore inconnue. Ainsi, nous souhaitons mieux caractériser leur rôle dans l'intégration du signal olfactif en provenance des deux voies olfactomotrices: médiale et latérale. Nous avons examiné la possibilité que ces neurones DA soient directement contactés par des neurones de projection du BO et soient importants pour la production de comportements olfactifs.

La revue de littérature contenue dans l'introduction de cette thèse comporte deux sections principales. La première contient un manuscrit préparé pour une soumission dans le journal Animal Behaviour (Section 1.2). Il s'agit d'une revue de littérature portant sur les comportements olfactifs de la lamproie ainsi que les différentes molécules odorantes pouvant induire ou modifier ces comportements. Pour chaque stade comportemental, les odeurs et les comportements qu'elles induisent sont détaillés. La deuxième section contient un article de revue publié dans le journal Cell & Tissue Research (Section 1.3). Cette revue de littérature porte sur les circuits neuronaux qui induisent la transformation de signaux olfactifs en réponses motrices chez la lamproie marine. Elle traite principalement de leur organisation générale ainsi que des réseaux neuromodulateurs au niveau du BO qui peuvent influencer les comportements olfactifs en y modifiant l'activité synaptique. Suivant la revue de littérature, les différentes questions de recherche (Section 1.5) qui ont guidé les travaux de cette thèse sont détaillées et l'hypothèse générale (Section 1.6) est présentée. Les objectifs spécifiques de nos travaux sont énumérés à la section 1.7 et scindés en deux projets distincts. À la section 2 (Résultats), les deux projets qui constituent le travail de recherche de cette thèse sont présentés sous la forme d'articles (Sections 2.1 et 2.2). Ces résultats sont ensuite interprétés en lien avec les questions de recherche et la littérature du domaine dans la section 3 (Discussion).

1.2 Olfactory behavior in sea lampreys

Le manuscrit suivant est prêt depuis septembre 2020 pour une soumission à Animal Behaviour, un journal scientifique international de premier plan qui contient des articles portant sur tous les aspects du comportement animal. La lamproie marine étant une espèce parasitaire dans les Grands Lacs d'Amérique du Nord, son écologie et son comportement ont été abondamment documentés. Ce manuscrit tire profit de cette riche littérature pour exposer ce qui est actuellement connu sur les différentes odeurs de son environnement qui guident la lamproie marine pour lui permettre d'échapper aux prédateurs, de localiser des proies, ainsi que d'identifier des sites adéquats pour leur reproduction.

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Olfactory behavior in sea lampreys

by

Beauséjour, Philippe-Antoine[1]; Zielinski, Barbara[2]; Dubuc, Réjean[1,3]

1: Université de Montréal
Department of Neurosciences
C.P. 6128, Succ. Centre-Ville
Montreal (Quebec) Canada H3C 3J7

2: University of Windsor
Department of Integrative Biology
401 Sunset Avenue
Windsor (Ontario) Canada N9B 3P4

3: Université du Québec à Montréal
Department of Exercise Sciences and
Research Group in Adapted Physical Activity
C.P. 8888, Succ. Centre-Ville
Montreal (Quebec), Canada H3C 3P8

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1.2.1 Abstract

Sea lampreys (*P. marinus*) are aquatic animals that strongly depend on chemosensory signals to make vital choices in behavioral contexts such as feeding, predator avoidance, and reproduction. Throughout the lamprey life cycle, detection of naturally occurring odorant mixtures induces stereotyped motor responses in the wild and laboratory settings. Observational studies have led to the characterization of lamprey behavior in the past, but the study of olfactory behavior in the aquatic environment has been challenging because of a lack of knowledge about the exact structure of chemical stimuli that can induce such behavior. Recently, tremendous advances were made by isolating individual compounds from sea lampreys, which can replicate natural behavior when artificially applied in the wild. In no other aquatic vertebrate has the olfactory ecology been described with such extensive details.

We provide here a comprehensive review of specific odorants and the olfactory behaviors they may induce during every major developmental phase of the sea lamprey.

Most notably, predation risks are minimized during the larval stage by avoiding odorants emitted from potential predators or injured fishes. However, following metamorphosis, parasitic sea lampreys orient swimming towards specific amines released by their prey. Finally, sexually mature adults must gather into a suitable habitat for a single reproductive event, which occurs immediately before death. Among many compounds isolated from lampreys, such as bile acids and sulfated steroids, some guide lamprey migratory behavior over long distances, while others are hypothesized to be minor components that act as proximity cues. However, despite efforts to identify close-range pheromonal signals, characterization of the behavioral effects of individual compounds still requires more studies. This review summarizes new insight into lamprey olfactory behavior brought by recent advances in the field.

1.2.2 Introduction

The life cycle of the lamprey is peculiar and divided into larval, parasitic, and reproductive stages. After filter-feeding as larvae for many years, they metamorphose into parasitic adults and prey upon fishes to increase body mass. They finally migrate to spawning grounds to reproduce and die. Lampreys are nocturnal animals which live in aquatic, low-visibility environments and throughout the life cycle, depend strongly on chemosensory cues to make vital choices about survival and reproduction. Accordingly, lampreys have a well-developed olfactory apparatus that can perceive molecules in the picomolar range, which is enough to follow ‘’odor plumes’’ in their underwater environment. Lampreys can chemically detect fishes such as preys and predators, and of course other lampreys. Among the 41 species of lamprey, many live in the same geographic area and use common signalling mechanisms since they share predators and habitat preferences for rearing and spawning. Below is a review of the current knowledge regarding olfactory-induced behavior across the life cycle of the sea lamprey, Petromyzon marinus.

1.2.3 Larval phase

Lamprey eggs hatch in nests built within riverine gravel streambeds, where pro-larvae develop for 17-33 days, until they reach Piavis stage 17 (Piavis, 1971). The lamprey can then swim and leaves the nest before eyes even develop. However, the pro-larval lamprey is not completely blind to its environment, since its olfactory mucosa contains chemosensory neurons with microvilli and responds to application of various amino acids and bile acids, and water conditioned by conspecifics (Zielinski et al., 2005). This suggests the olfactory organ is very important early in life. Lampreys then reach in downstream silted areas where they burrow into fine sediments and consume organic matter for many years as filter-feeding larvae (Hardisty et Potter, 1971a). At this stage, they are very vulnerable and are readily eaten by predatory fish (Morman et al., 1980), but also amphibians, reptiles, birds and mammals (Dawson et al., 2015). Burrowing protects larvae from predation (Smith et al., 2012), so they are mostly sedentary but slowly disperse downstream (Derosier et al., 2007). They are reliant on chemosensory detection of soluble substances in the aquatic environment. Larvae respond to molecules emitted from injured of dead conspecific or heterospecific fishes, which represents a reliable predation risk indicator and evoke anti-predator behavior. In a laboratory setting (Perrault et al., 2014), conspecific lamprey extract and heterospecific fish extract evoked an increase in the rate of escape attempts and direction changes

(‘’zig-zag’’ movements), similar to antipredator behavior observed in other prey fishes (Kats et Dill, 1998). Furthermore, when sand is added to the bottom of the experimental area (Wagner et al., 2016), exposure to conspecific lamprey extract reduces downstream drift, suggesting that alarm cues could signal a predatory-event and result in larval retention within the protective burrow.

1.2.4 Metamorphosis and parasitic phase

Larvae then undergo a true metamorphosis to accommodate the considerable body plan changes that allow adult lampreys to be hematophagous ectoparasites feeding on large fishes. The adult morphology is so distinct that larvae were first described as a separate species: Ammocœte branchialis (de Filippi, 1844), and are still known as ‘’ ammocœtes ’’ to this day. Most important are the morphological modifications of the peripheral olfactory organ (Vandenbossche et al., 1995). Extensive olfactory receptor neuron neurogenesis and differentiation (Youson, 1980) accounts for the dramatic modification in shape and mass of the nasal sac, which doubles in relative weight (Vandenbossche et al., 1997). This important growth, in addition to the development of eyes and sucker mouth, have important behavioral consequences since it allows newly-transformed adult lampreys to immediately begin parasitic feeding in their natal stream (Davis, 1967; Silva et al., 2013).

At the start of the parasitic phase, sea lampreys migrate downstream to feed in lake or sea habitats. How do lampreys locate and attach to larger fishes? To locate prey within 200 mm, the lamprey produces a pulsating electrical field around the head region (Kleerekoper et Sibakin, 1956a; Kleerekoper et Sibakin, 1956b). However, long-distance orientation toward prey relies upon olfaction. Indeed, parasitic sea lamprey locomotor activity is strongly increased following exposure to water containing the composite ‘’body odor’’ of trout (trout water; Kleerekoper et Mogensen, 1963). This stimulus does not only increase locomotor activity, but it is also attractive to lampreys since they display strong preference for the areas perfused with trout water in a compartmentalized experimental tank. These responses are abolished if the nasal tube is blocked, suggesting olfactory involvement. A single amine isolated from trout water, arginine, could reproduce these results. Among other amino acids detected by lamprey, L- and D-arginine elicit the strongest olfactory responses at low concentrations (Li et Sorensen, 1992). Arginine is an essential amino-acid in fishes and is necessary for many metabolic reactions (Luo et al., 2004). Among the other essential amino acids, requirements are amongst the highest for arginine in many fishes (Wilson et Halver,

1986), including important prey species of the sea lamprey such as salmon (Halver et al., 1957) and trout (Kaushik et al., 1981; Ketola, 1983). Since the main olfactory epithelium of sea lamprey is a millionfold more sensitive to arginine than other amino acids (Li et Sorensen, 1992), arginine could be an important molecule to guide long-distance predatory behavior in sea lampreys. However, other amino acids that are detected at much higher concentrations may serve for close-range localization or identification of preys or for other, yet unknown purposes.

1.2.5 Male upstream migration

The growth rate is low in the larval filter-feeding phase, requiring many years to reach a few grams, but after only 12-18 months of parasitic feeding, adult lampreys can reach over two kilograms (Hardisty et Potter, 1971b). During this period, progressive gonadal development and atrophy of the gut leads to cessation of feeding and the start of spawning migration in adults (Larsen, 1980). Lampreys then face many challenges to reach a suitable habitat for their final act: reproduction. Since lampreys rely on a single reproductive opportunity in their lifetime, their ability to gather in appropriate spawning grounds is vital.

Although solitary animals, lampreys have evolved a complex communication system to congregate at the same time and place. The timing of reproduction is especially crucial since breeding events are seasonal and lampreys must rely on nutrients stored during their parasitic phase until death, which occurs shortly after spawning. The gathering location is also an issue: among tributaries to the Great Lakes, which contain a fifth of surface freshwater on Earth, only 7.5% have supported growth of larvae (Morman et al., 1980).

In this journey, migratory behavior rests heavily on chemosensory signals. In contrast with other migratory fishes, such as salmonids, which learn the chemical composition of their birthplace, lampreys do not home to their natal stream (Bergstedt et Seelye, 1995; Waldman et al., 2008). Instead, they display regional panmixia, using a ‘’suitable river’’ strategy, relying on olfactory assessment of spawning habitat based on contemporaneous chemical signaling (Nordeng, 1971; Waldman et al., 2008). First, males must navigate in open water and locate an appropriate stream. To do so, they perform an initial extensive search parallel to the shoreline (Vrieze et al., 2011) until they encounter river water, which they prefer over lake water (Vrieze et Sorensen, 2001). Then, they transition to an intensive stream-finding search and orient toward the river mouth, cued by chemical signals contained in the river plume (Wagner et al., 2009; Meckley et al., 2014). Indeed,

because river water flows unidirectionally and is constrained to the channel, upstream odor sources can ‘’activate’’ the entire stream discharge (Webster et Weissburg, 2009; Johnson et al., 2012a). Lampreys, when rendered artificially anosmic, are unable to even locate rivers (Vrieze et al., 2010).

What kind of olfactory cues would indicate the presence of a ‘’suitable river’’? They depend on two types of signals: 1) Attractive migratory pheromones and 2) repulsive alarm cues.

1.2.5.1 Larval attractive migratory pheromones

Bile acids specific to the sea lamprey (Haslewood et Tökés, 1969) are excreted by stream-resident larvae (Li et al., 2002) and attract adults to migrate upstream to the same breeding grounds (Teeter, 1980). These bile acids are produced in the liver, stored in the gall bladder, and excreted through the intestine along with feces (Haslewood, 1980). As a by-product of larval feeding, these odorants are released at higher rates from well-fed larvae (Fine et Sorensen, 2010) and indicate the presence of high-quality reproductive habitat (Wagner et al., 2009), adequate for spawning and rearing of offspring. These products diffuse through large volumes of turbid water but are released at rates sufficient to produce biologically relevant concentrations in river water (Polkinghorne et al., 2001) and degrade sufficiently slowly for the entire pheromone to persist in river mouths (Fine et Sorensen, 2010). At nanomolar concentrations, these ligands induce strong electrophysiological responses in the main olfactory epithelium (Li et al., 1995), enhance swimming activity (Bjerselius et al., 2000) and guide male adults in upstream (Bjerselius et al., 2000). Hence, the smell of well-fed larvae attracts upstream-migrating adults in streams.

Identification of pheromonal compounds in the larval odor led to the discovery of novel bile acids and sulfated steroids (Sorensen et al., 2005) which are detected and attractive to adult sea lamprey in the sub-picomolar range (10^{-13} M; Hoye et al., 2007). However, since these compounds failed to induce the behavioral effect of the full larval odor in the natural habitat (Meckley et al., 2012; Meckley et al., 2014), efforts were continued to discover previously unknown molecules that could act as larval migratory pheromones. Notably, petromyzonin (a sulphated hexahydrophenanthrene; Li et al., 2013a), petromyroxols (fatty acids; Li et al., 2015a; Li et al., 2015b), and petromyric acid A (a fatty acid; Li et al., 2018a) stimulate the main olfactory epithelium of adult sea lamprey and could putatively induce behavioral bias in river selection. However, the full extent of bioactive molecules contained in the larval pheromone mixture is not completely identified to this day.

Interestingly, since land-locked sea lampreys share habitat preferences with many different species of lampreys (Johnson et al., 2015) that overlap spatially in their geographic distribution (Renaud, 2011), heterospecific lampreys use common signalling molecules to guide upstream-migration. Indeed, adult sea lampreys also respond to migratory pheromones emitted from heterospecific larval odors (Fine et al., 2004), allowing for a more detailed assessment of habitat quality and guiding them to syntopic rearing habitats which can support populations of multiple lamprey species (Dawson et al., 2015).

1.2.5.2 Repulsive alarm cues

Alarm cues constitute a second type of chemical signals guiding upstream migration. For the purpose of this review, alarm cues are divided into two main categories: chemicals released from dead or injured animals (Necromones) and chemicals released from potential predators (Predator cues). Alarm cues, released in the aquatic environment, are publicly available and lead to antipredator behaviors (for an extensive review, see Ferrari et al., 2010). They are critical in stream selection since they allow for safe, indirect predation risk assessment of a stream before entering the river (Siefkes, 2017). Indeed, upstream migrants are very vulnerable since they are confined by the river channel and driven to swim in increasingly narrow and shallow streams. They are thus persistently exposed and killed by many predators such as mammals, birds, water snakes, and fishes (Surface, 1899; Morman et al., 1980; Hume et Wagner, 2018). However, chemosensory detection of alarm cues allows lamprey to avoid areas with potential predators during their spawning migration.

The presence of lamprey necromones reduce the probability of adult lampreys to enter a stream (Di Rocco et al., 2016). Within a river, upstream migrants also reduce risk exposure by avoiding or accelerating upstream movement through areas activated with necromones (Bals et Wagner, 2012; Hume et al., 2015; Luhring et al., 2016). During daytime, immobile sea lampreys did not show responses to chemosensory alarm cues (Di Rocco et al., 2014).

Since sea lampreys share common predators with other species of lamprey (Cochran, 2009), avoidance and flight responses are also induced by damage-released necromones from heterospecific lampreys (Bals et Wagner, 2012; Byford et al., 2016; Hume et Wagner, 2018). Moreover, strong avoidance was also observed in response to necromones from fish species sympatric with sea lamprey populations, C. commersonii (Imre et al., 2014). Different predator

cues, such as human saliva (mammalian predator cue), N. sipedon washings (reptilian predator cue) and 2-phenylethylamine (chemical found in the urine of many mammalian carnivores) also induce anti-predator behavior in lamprey (Di Rocco et al., 2014; Imre et al., 2014). Behavioral responses to both necromones and predator cues were found to increase with stimulus concentration (Bals et Wagner, 2012; Di Rocco et al., 2016). Interestingly, responses were stronger when both stimuli were applied in combination (Imre et al., 2014; Di Rocco et al., 2016). Hence, the chemosensory detection of various alarm cues in the environment allows the adult lamprey to be highly responsive to predatory threats (Wagner et al., 2011). Identification of the chemical constituents that compose the lamprey alarm cue responsible for anti-predator behavior is underway (Dissanayake et al., 2019) and suggests that they are water-soluble nitrogenous compounds, such as amino acids.

1.2.5.3 Male attractive migratory pheromones

Many weeks following stream entrance, the now sexually mature male sea lamprey reaches the spawning area and soon builds and aggressively defends a nest (Applegate, 1950; Manion et Hanson, 1980). The spermiated male then releases a multicomponent pheromone to attract females over long distances to their nest (Li et al., 2002; Johnson et al., 2009). The major component is 3-keto petromyzonol sulfate (3k-PZS), a lamprey-specific bile alcohol that acts as a migratory pheromone. Interestingly, this molecule is also the major component of the larval pheromone and is secreted again by lampreys as sexually mature adults to attract potential mates upstream. In adults however, 3k-PZS is released at a much higher rate and via a different mechanism. Indeed, since the gall bladder and bile ducts degenerate during metamorphosis and are thus absent in adults (Yamamoto et al., 1986), 3k-PZS is now released from the gills (Siefkes et al., 2003) through a complex mechanism of bile salt biosynthesis and excretion that takes place upon sexual maturation (Brant et al., 2013) under the influence of progestin (Bryan et al., 2015). Secreted first during the larval stage and then after sexual maturation, 3k-PZS is detected by downstream migratory adults and induces long-distance upstream navigation into habitats that can sustain larval rearing (Brant et al., 2015; Brant et al., 2016b).

Since lampreys share syntopic reproductive habitats where multiple species can be sustained (Dawson et al., 2015), heterospecific populations of lamprey are present in these habitats and also emit odors that are added to the downstream blend. Chemical profiling of odors emitted from different species of adult male lamprey allowed the observation of substantial overlap in male-

released compounds that guide reproduction, including the presence of 3k-PZS (Buchinger et al., 2017). Importantly, electrophysiological and behavioral tests confirmed the existence of interspecific responses to lamprey pheromones. Notably, female sea lampreys detect, and respond to odors of adult males from every lamprey species tested (Buchinger et al., 2017), despite interspecific variation amongst male odors (Buchinger et al., 2019). Hence, at the river mouth, the sexually immature female lamprey is exposed and responsive to an overlap of heterospecific larval (Fine et al., 2004) and adult male (Buchinger et al., 2017) compounds that are shared among different sympatric lamprey species. This chemical information represents honest signals of successful spawning and rearing (Wagner et al., 2009; Hume et Wagner, 2018) and could thus enable the female sea lamprey to have a more accurate olfactory assessment of habitat quality and presence of spermiated males. A female lamprey must be thorough when choosing the river where it will reproduce and die.

1.2.6 Female upstream migration

Females follow males in the same streams to find reproductive partners, avoiding predation by exploiting the above-mentioned alarm cues, although responses attenuate following sexual maturation (Bals et Wagner, 2012). Arriving close to nesting habitat, the now sexually mature females encounter an overlap of many generations of larval lampreys that drifted downstream from previous reproductive events (Dawson et al., 2015). They now face another olfactory challenge: exposed and attracted to 3k-PZS from both larvae and potential breeding partners, they must distinguish between the two to complete their migration to spawning grounds.

In the wild, and in laboratory settings, sexually mature females are not attracted to the larval odor but are strongly attracted to the spermiated male odor (Buchinger et al., 2020). The mechanism is that in addition to 3k-PZS, larvae also secrete petromyzonol sulfate (PZS) in high concentration, which is repulsive for female adults to prevent misguided orientation toward larval odor and focus on mate search (Buchinger et al., 2020). The main olfactory epithelium of sea lampreys possesses distinct and sensitive mechanisms to detect PZS and 3k-PZS (Li et al., 1995; Li et Sorensen, 1997; Siefkes et Li, 2004). Interestingly, through an unknown mechanism, PZS reduces responses to 3k-PZS in the olfactory epithelium of female sea lamprey (Siefkes et Li, 2004; Johnson et al., 2006; Buchinger et al., 2020). Accordingly, it also abates the behavioral preference of ovulated females for 3k-PZS in the laboratory and the natural habitat (Buchinger et al., 2020). Even if they both

secrete 3k-PZS and PZS, larvae and males release them at opposite proportions (male-typical ratio = 100:1 3k-PZS/PZS; larva-typical ratio = 1:10 3k-PZS/PZS) and the relative abundance of each component allows females to robustly discriminate between male and larval odor. In fact, Buchinger et al. (2020) observed that mixtures of 3k-PZS and PZS at typical ratios were sufficient to replicate the response of the natural odorants in behavioral assays, in which females chose streams and nests treated with male odor (or male 3k-PZS/PZS ratio) over larval odor (or larval 3k-PZS/PZS ratio). Hence, female sea lampreys respond to a ratio of compounds that act as a reliable cue for male location.

1.2.6.1 Different responses of females to migratory cues after migration

Why is upstream migration maintained in females despite exposure to larval and male-released PZS? First, downstream females are exposed to a much more diluted concentration of PZS, being at a great distance away from larvae and sexually mature males. Furthermore, the female adult olfactory epithelium is one hundred times more sensitive to 3k-PZS than PZS (Siefkes et Li, 2004) and a ratio of 1:1 3k-PZS/PZS must be achieved to neuter EOG responses to 3k-PZS (Buchinger et al., 2020). Second, in downstream females that still are sexually immature, PZS does not suppress the locomotor response to 3k-PZS (Brant et al., 2016b) which allows the spawning migration to take place. Indeed, sexual maturation of female sea lampreys induces new behavioral responses to male pheromones, which may contribute to the reproductive synchrony (Walaszczyk et al., 2016). In sexually immature females, 3k-PZS does not increase odor-source location (nest entry) but increase swimming speed and general search behavior (Hume et al., 2015; Johnson et al., 2020), which differs greatly from the response of sexually mature females who display attraction to the source at close range (Johnson et al., 2006; Johnson et al., 2009; Johnson et al., 2013; Johnson et al., 2020). Notably, 3k-PZS induces more daytime movement only in ovulating females (Walaszczyk et al., 2013), which is consistent with the fact that in the natural habitat, ovulated females stop limiting activity to nighttime (Manion et McLain, 1971) and are now also active during the day (Manion et Hanson, 1980). Thus, females respond differently to 3k-PZS and PZS downstream vs upstream because they are at different concentrations and also because sexual maturation induces new behavioral responses in females. Akin to females, males also display repulsive behavioral responses to PZS after sexual maturation, which could prevent orienting toward larvae during spawning (Buchinger et al., 2020). As such, they can also benefit from this

pheromonal cue and avoid tracking larval 3k-PZS into fine sediment habitats which are unsuitable for their nest-building needs.

Hence, 3k-PZS acts as an attractive migratory pheromone (Go signal) that guides immature adults upstream to proper spawning grounds. However, since nursery and spawning habitat are interspersed in streams, 3k-PZS from a larval source may interfere with reproductive behavior. PZS then acts as a repulsive cue (No-Go signal) to maintain sexually mature adults away from nurseries. Both of these lamprey-specific cues act as olfactory traffic lights to guide individuals efficiently through this riverine journey and allow for timely gathering of sexually mature adults in one of the rare tributaries of the Great Lakes sustaining productive spawning sites.

1.2.7 Olfactory spawning behavior

During a reproductive event, hundreds of lampreys, synchronously go up a particular river to breed (Moore et Braem, 1965). In the spawning lek, males establish and defend their own nesting territory while females visit various nests for approximately one week before adults rapidly die of senescence (Johnson et al., 2015). Adults form actively breeding pairs and spawn intermittently (every five minutes). Although monogamous pairs were observed (Manion et Hanson, 1980), lampreys are primarily polygynandrous (Johnson et al., 2015), and sex ratios in the spawning population determine the number of females in each nest (Hanson et Manion, 1978; Hanson et Manion, 1980). Moreover, the spawning season and territory of the sea lamprey overlap with those of the other four upper Great Lakes lamprey species (Johnson et al., 2015). While other lampreys often share common nests and can engage in mass spawning (Case, 1970) without antagonistic behavior between species (Morman, 1979), neighboring male sea lamprey are competitive, especially when joined by a female, and viciously remove male invaders from their nesting area with their sucker mouth (Manion et Hanson, 1980). In this chaotic breeding event, olfaction is essential to ensure identification of adequate sexual partners.

Olfaction is essential at this stage where sexually mature females must select suitable mates among competing spermiated males. If they are artificially anosmiated, ovulatory females are unattracted to male odor and unable to even locate spermiating males in a spawning stream (Johnson et al., 2006). After being guided upstream by a collective, stream odor and now in a much closer range, the smell of individual lampreys is now accessible. There is high variation of odorant compounds between males (Buchinger et al., 2019). In addition to 3k-PZS, they also release various rates of

molecules that are hypothesized to be minor components of the pheromones and to act as proximity cues (short-range-individual pheromones, as opposed to long-range-collective migratory pheromone 3k-PZS). These putative proximity cues are detected by the main olfactory epithelium of ovulating females, which show preference to certain ratios of pheromonal blends. However, the full spectrum of molecules present in the full male odor is still under extensive investigation (Li et al., 2018b).

1.2.7.1 (3k-PZS) 3-keto petromyzonol sulfate

Among the many putative pheromone compounds discovered in spermiated male lamprey washings, most are steroids. The most well-known is 3k-PZS, which elicits migratory behavior and is considered the major component of the male pheromone. In addition to driving long-range migration and congregation of multiple lamprey species in spawning grounds, 3k-PZS also is the main component for near-source courtship actions and has profound behavioral effects on sexually mature females. Notably, 3k-PZS contributes to nest localization (Johnson et al., 2012b) and selection, and elicits nest construction and pair maintenance behaviors (Siefkes et al., 2005; Johnson et al., 2012b).

1.2.7.2 (DkPES) 3,12-diketo-4,6-petromyzonene-24-sulfate

However, 3k-PZS alone does not induce the full extent of sexual behaviors as the complete male odor. Other minor components of the male odor include additional lamprey-specific, biologically active steroids that are released in lesser quantities by spermiated males. Among those, 3,12-diketo-4,6-petromyzonene-24-sulfate (DkPES) is the most well-characterized. This bile alcohol was shown to enhance the attractiveness of 3k-PZS when the two compounds are mixed, which assists closeby females in finding and choosing mates (Li et al., 2013b). Interestingly, the male-typical ratio of 30:1 3k-PZS/DkPES is more effective than 3k-PZS alone for female attraction and retention in artificial nests (Li et al., 2013b; Brant et al., 2016a). Each is independently detected by distinct receptors in the main olfactory epithelium of adult sea lamprey, although the threshold of detection is a thousandfold greater for DkPES (Brant et al., 2016a). This difference in sensitivity suggests that DkPES may not be detectable until the receiver-female is within proximity of the signaler-male and thus, the authors hypothesized that DkPES allows females to gauge distance to males.

1.2.7.3 Other steroids

Compounds of the male pheromone necessary to maintain females in the nest for near-source courtship and spawning behavior also remain unidentified but may include other recently discovered steroids that elicit EOG and behavioral responses. These novel, lamprey-specific steroids extracted from sexually mature male washings are petromyzestrosterol (Li et al., 2012), petromyzones A, B and C (Li et al., 2017b), petromyzenes A and B (Li et al., 2017a), and petromylidenes A, B and C (Li et al., 2018c). All of them are detected in the picomolar or subpicomolar range by the female olfactory epithelium, except for petromylidene A (10^{-9} M) and petromyzestrosterol (10^{-6} M). Moreover, these steroids induce behavioral responses in laboratory settings: all are attractive to females except for petromyzone B and C that are repulsive, and petromyzestrosterol that is untested yet. Another noteworthy steroid is 3 keto-1-ene petromyzonol sulphate (1-ene 3k-PZS), a lamprey-specific unsaturated sulfated bile alcohol which has the same potency than 3k-PZS in attracting ovulated females to nests (Johnson et al., 2014). However, a mixture of 3k-PZS and 1-ene 3k-PZS shows no additive effects compared with 3k-PZS alone, which suggests both molecules may bind the same olfactory receptor and are therefore perceived as the same stimulus.

1.2.7.4 Spermine

In the natural habitat, breeding pairs can engage in three days long sequences of nest maintenance and spawning (Manion et Hanson, 1980), and yet, no odorant mixture can induce this behavior except for the complete male odor. Indeed, the above-mentioned steroids are insufficient to retain females on nests for such extended durations, which suggests that other factors promote maintenance of spawning pairs (Johnson et al., 2015). In a riverine lamprey spawning congregation, each male defends its own nest while females visit different nests and can form pairs to spawn intermittently (Johnson et al., 2015). Remarkably, a molecule was found in sea lamprey semen that could act as a reliable and localized signal of closeby spawning males. Originally discovered in human seminal plasma in the 17th century (Tabor et Tabor, 1984), spermine is an odorous polyamine found in a wide diversity of organisms and tissues. Sea lamprey milt contains high levels of spermine, which promotes attraction specifically in sexually mature females (Scott et al., 2019). Spermine stimulates the main olfactory epithelium at concentrations as low as 10^{-14} M and much interestingly, Scott et al. (2019) have identified an olfactory receptor involved in its detection after screening most receptors expressed in the lamprey olfactory epithelium. This

constitutes a new male chemical signal which is detected by females with subpicomolar sensitivity and induces mating behaviors. This cue is a reliable indicator of sperm availability and could also contribute to synchronized gamete-release, which is crucial for productive external fertilization.

1.2.8 Conclusion

Despite efforts to identify close-range pheromonal signals, the odor of mature males is still significantly more attractive to mature females than any mixture of compounds tested so far. Individual compounds activate the main olfactory epithelium of females in various concentrations and could act at different spatial ranges. Moreover, the extraction of these novel, lamprey-specific steroids in quantities sufficient for behavioral testing in natural streams is a challenge (Li et al., 2018b). Characterization of their behavioral effects alone and in different mixtures will be a difficult task, especially in the natural habitat (Johnson et Li, 2010).

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1.3 Olfactory-induced locomotion in lampreys

Le manuscrit suivant est un article de revue publié chez Cell & Tissue Research, un journal scientifique international de premier plan qui contient des articles portant sur la biologie cellulaire. Plusieurs travaux du laboratoire Dubuc, de proches collaborateurs et d'autres laboratoires ont permis d'identifier chez la lamproie plusieurs régions du cerveau possiblement impliquées dans la production d'une réponse locomotrice lors de la détection d'odeurs. Ce manuscrit contient une description exhaustive de ce qui est actuellement connu sur ces réseaux neuronaux, leur modulation, ainsi que les manques à combler pour compléter notre compréhension de ce système.

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Olfactory-induced locomotion in lampreys

by

Beauséjour, Philippe-Antoine[1]; Zielinski, Barbara[2]; Dubuc, Réjean[1,3]

1: Université de Montréal
Department of Neurosciences
C.P. 6128, Succ. Centre-Ville
Montreal (Quebec) Canada H3C 3J7

2: University of Windsor
Department of Integrative Biology
401 Sunset Avenue
Windsor (Ontario) Canada N9B 3P4

3: Université du Québec à Montréal
Department of Exercise Sciences and
Research Group in Adapted Physical Activity
C.P. 8888, Succ. Centre-Ville
Montreal (Quebec), Canada H3C 3P8

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1.3.1 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. However, olfactory cues induce different behavioral responses adapted to the lamprey situation, which demands modulation of the hardwired neural circuits by modulatory mechanisms such as GABAergic and serotoninergic inputs. 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

1.3.2 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 et 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; Wickelgren, 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 et Robertson, 2016; Ryczko et 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 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., 2019a). 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; Johnson et al., 2012), predator avoidance (Wagner et al., 2011), and foraging (Kleerekoper et 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).

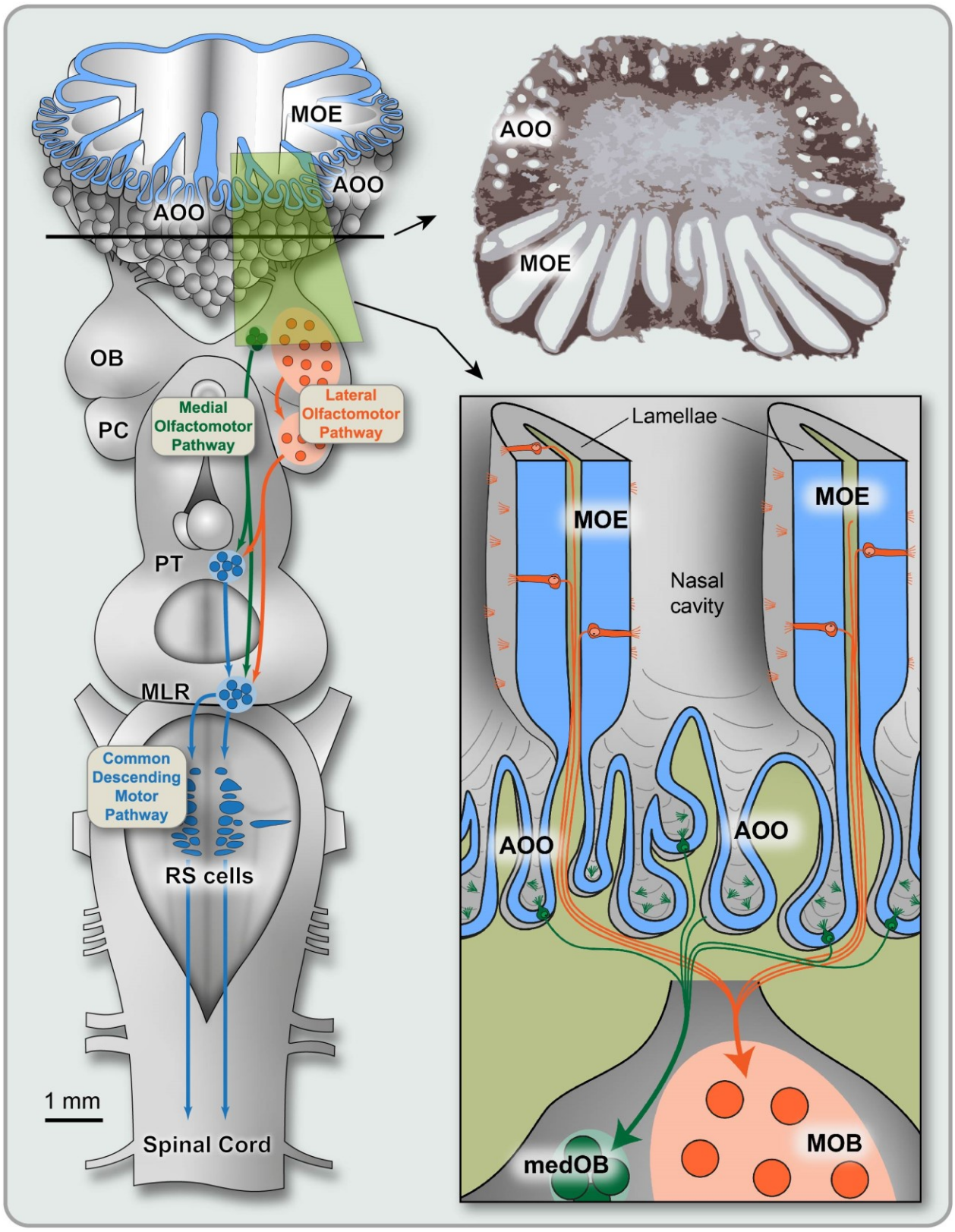
MOE
AOO
AOO
OB
PC
Medial Olfactomotor Pathway
Lateral Olfactomotor Pathway
PT
MLR
Common Descending Motor Pathway
RS cells
1 mm
Spinal Cord
AOO
MOE
Lamellae
MOE
MOE
Nasal cavity
AOO
AOO
MOB
medOB

Figure 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.

1.3.3 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.

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 et 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 is not 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 et Axel, 1991; Zhang et 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 et Nishida, 2007; Grus et 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 et 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 in these animals (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. 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 et 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 et 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 et Zhang, 2009). This finding suggests that the lamprey accessory olfactory organ constitutes a primitive form of the vomeronasal system in the vertebrate lineage (Grus et 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 et Mogensen, 1963; Bjerselius et al., 2000), and to stimulate sensory neurons in the main olfactory epithelium (Li et al., 1995; Li et 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 apparatus 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 et 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 physiological 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 et Puzdrowski, 1988; Polenova et Vesselkin, 1993) as well as in several fish species (von Bartheld, 1984; Matz, 1995; Rink et Wullimann, 2001; Northcutt, 2011; Northcutt et Rink, 2012).

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; Ryczko et al., 2017; Ryczko et al., 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 et 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).

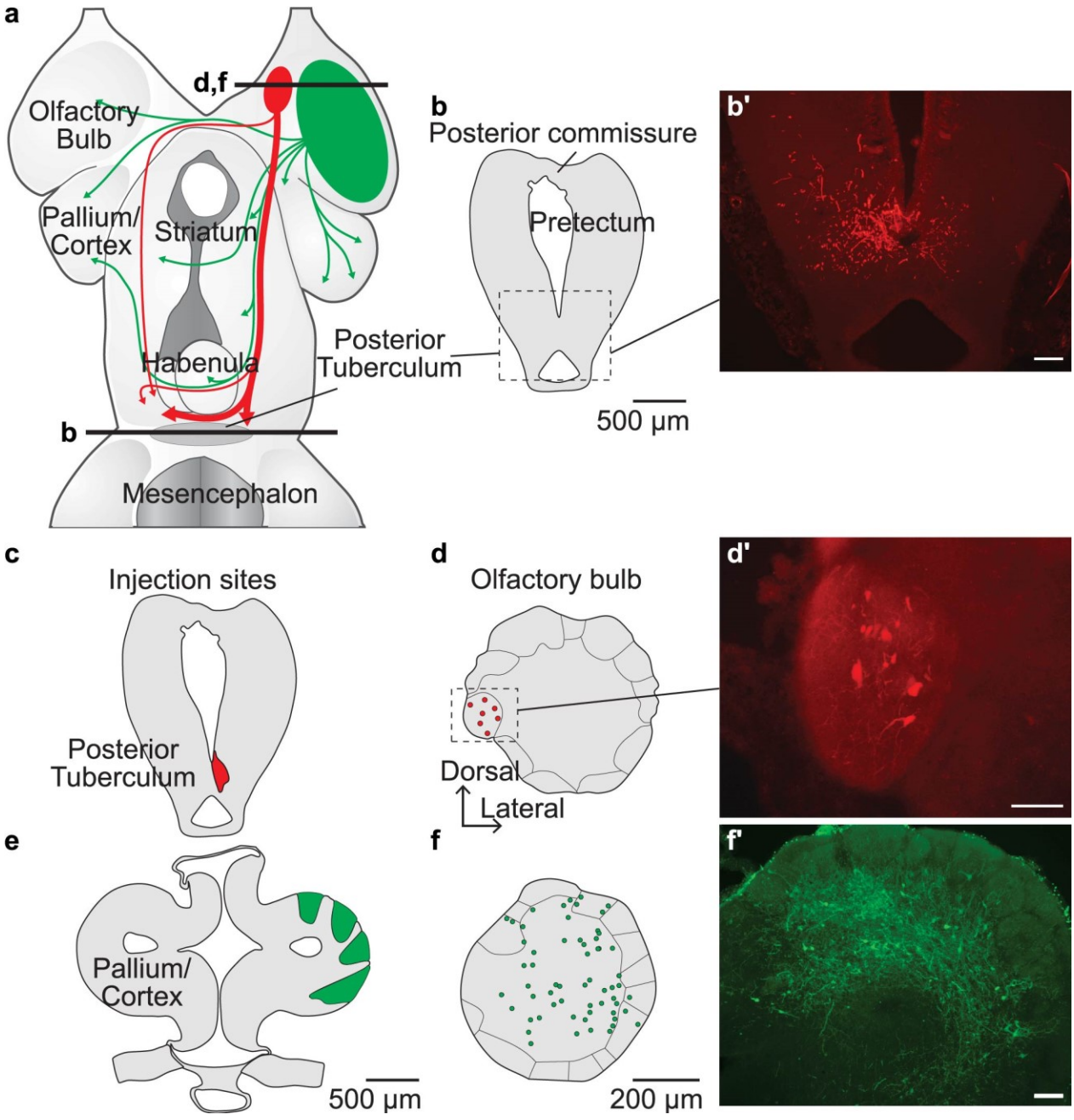

Figure 2. Efferent olfactory bulb projections involved in odor-induced locomotion.

(a) Schematic representation of the lamprey forebrain illustrating secondary olfactory projections from the medial 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 in b', d' and f' = 100 μm. Credits: Adapted with permission from (Derjean et al., 2010).

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 et Puzdrowski, 1988; Polenova et Vesselkin, 1993; Derjean et al., 2010; Suryanarayana et al., 2017; Suryanarayana et al., 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; Suryanarayana et al., 2020; Suryanarayana et al., 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; Suryanarayana et al., 2020; Suryanarayana et al., 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; Suryanarayana et al., 2021b) are relayed to the PT before reaching RS cells. Altogether, the data reviewed above strongly suggest 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; Ryczko et al., 2013; Ryczko et al., 2017), which is of particular interest considering the major impact that the MLR has on locomotor control in vertebrates (see Ryczko et 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 behaviors (for reviews, see Dubuc et al., 2008; Le Ray et al., 2011; Ryczko et Dubuc, 2013; Grätsch et al., 2019b). 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 et Dubuc, 2003) and cholinergic (Le Ray et al., 2003). Stimulation of the MLR elicits large depolarizations 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; Smetana et al., 2010). These connections constitute a hyperdrive mechanism for locomotion (Smetana et al., 2010).

Olfactory stimulation induces sustained depolarizations of RS cells (Wickelgren, 1977a; Wickelgren, 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 et Grillner, 1987; Ohta et 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; Ryczko et al., 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; Ryczko et al., 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; Ryczko et al., 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 et 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; Ryczko et al., 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 et Grillner, 2008; reviewed in Grillner et 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 et 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 et Sinnamon, 1983; Milner et Mogenson, 1988; Marciello et 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.

1.3.4 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 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 and serotonin (5-HT) are present in the

lamprey OB, these neurotransmitter systems could modulate olfactory inputs to the bulbar circuitry (Fig. 3).

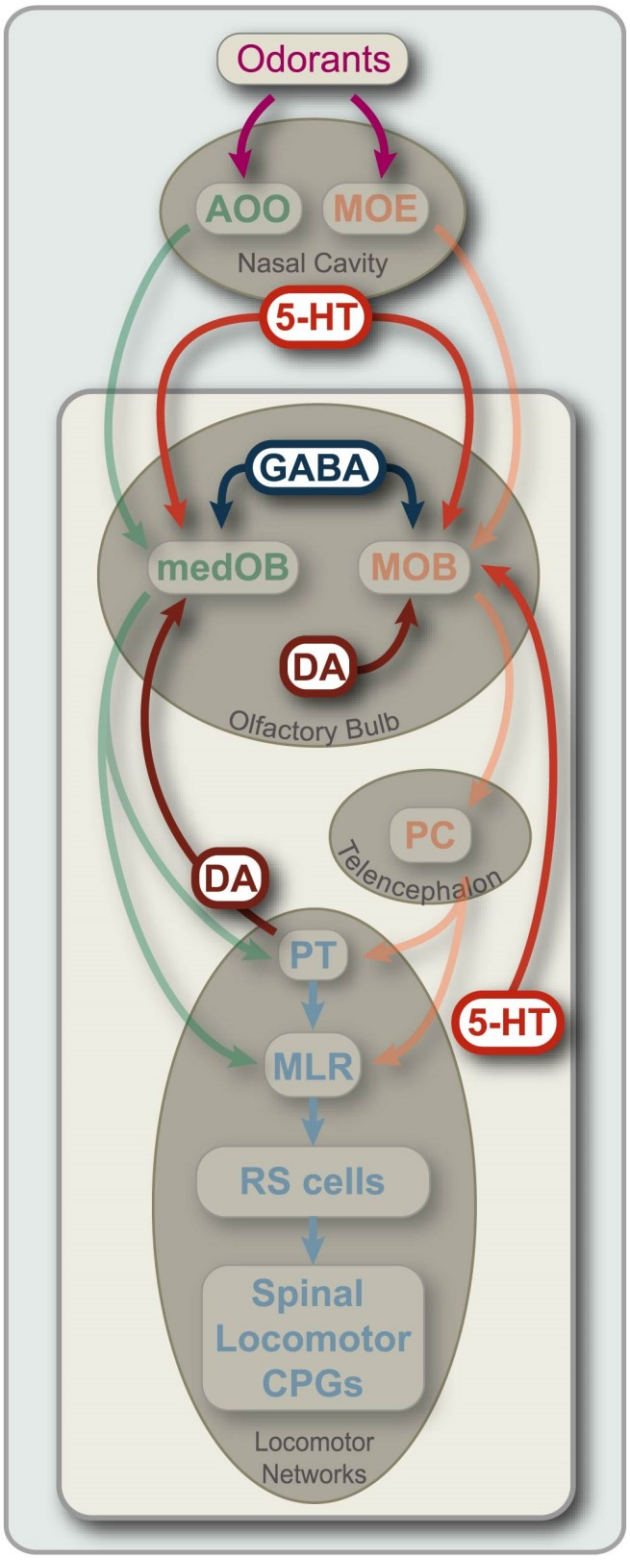

Figure 3. Modulation of transmission in olfactomotor pathways at the level of the olfactory bulb.

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 5HTergic cells from the olfactory organ, local GABAergic interneurons, local DAergic interneurons, and mesencephalic 5HTergic 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.

1.3.4.1 GABAergic modulation in the lamprey OB

The lamprey OB possesses a well-developed GABAergic system, which constitutes an ideal candidate for the regulation of local neural activity. 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.

1.3.4.2 Serotoninergic modulation of the lamprey OB

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 5HTergic somata and is innervated exclusively by extrinsic 5HTergic 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 5HTergic inputs to the OB via axons travelling in the

olfactory nerve (Zielinski et al., 2000; Frontini et al., 2003). Furthermore, 5HTergic 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 5HTergic neurons were seen in the OB by Abalo et al. (2007) and could also contribute to the 5HTergic innervation. Olfactory nerve transection confirmed that the peripheral source of 5-HT innervates glomerular units, whereas a second, central source of 5HTergic innervation terminates in the granular layer (Zielinski et al., 2000). Another source of 5HTergic input could originate from the midbrain tegmentum, which contains 5HTergic cell bodies (Baumgarten, 1972; Pierre et al., 1992; Antri et al., 2006) and has direct projections to the OB (Northcutt et Puzdrowski, 1988). The presence of 5HTergic 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 5HTergic 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 5HTergic 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 et Mogensen, 1963), 5HTergic 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 5HTergic modulation occurs in the main OB, which presumably affects the activity of projection neurons. Whether this 5HTergic 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 5HTergic innervation specifically in the medial OB should also be tested.

1.3.5 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 in the lamprey. 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 are present. 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, the respective contribution of the medial and the lateral pathways 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 help 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. Future experiments should aim at understanding the role of the PT in odor-induced behavior, especially the contribution of DAergic and glutamatergic neurons to the transformation of olfactory inputs into motor output. Because the PT has been proposed as a homolog of the ventral tegmental area in other vertebrate species including mammals, 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.

1.3.6 Declarations

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1.4 Contrôle locomoteur chez les vertébrés

La locomotion est une fonction motrice de base qui a pour but de déplacer efficacement le corps dans différents environnements et qui est apparue très tôt dans l'évolution des animaux. Chez les vertébrés, bien qu'il existe des modes de locomotion variés, l'organisation générale du système nerveux au contrôle de la locomotion est demeurée identique pour la nage du poisson, le vol de l'oiseau ainsi que la marche des humains. Cette section de l'introduction se veut un aperçu des régions et des mécanismes nerveux responsables de la locomotion chez l'ensemble des vertébrés, mais en particulier chez la lamproie et les mammifères. Nous discutons d'abord de l'organisation générale du contrôle locomoteur chez l'ensemble des vertébrés, puis nous abordons les différents centres supraspinaux impliqués. Ceux qui sont présents chez la lamproie (conservés évolutivement chez les vertébrés) et ceux qui sont absents chez la lamproie (apparus après la séparation de la lamproie de la lignée des vertébrés) seront discutés séparément.

1.4.1 Organisation générale du contrôle locomoteur

L'organisation générale de la locomotion (Fig. 4) est la même pour tous les vertébrés. La lamproie est un vertébré aquatique qui ne possède pas de membres et qui se propulse dans son environnement grâce à une nage ondulatoire. Pour produire ce mouvement, les muscles se contractent en alternance de chaque côté du corps et comme chez tous les vertébrés, la contraction musculaire est induite par les projections de motoneurones dont le corps cellulaire est localisé au niveau de la moelle épinière. Les motoneurones constituent la voie de sortie commune pour tous les programmes moteurs et, dans le contexte de la locomotion, leur activité est contrôlée directement au niveau de la moelle épinière (Sherrington, 1910; Graham Brown, 1914). Des réseaux de neurones spinaux nommés les générateurs centraux de patrons (GCP) permettent de produire des séquences précises de contractions musculaires qui permettent la locomotion et ce, indépendamment des entrées sensorielles et des voies supraspinales (Grillner et Zangger, 1974; Grillner et Zangger, 1975). En effet, le patron d'activité généré dans la moelle épinière isolée est aussi complexe dans l'animal spinal que dans l'animal intact (Dubuc et al., 1985).

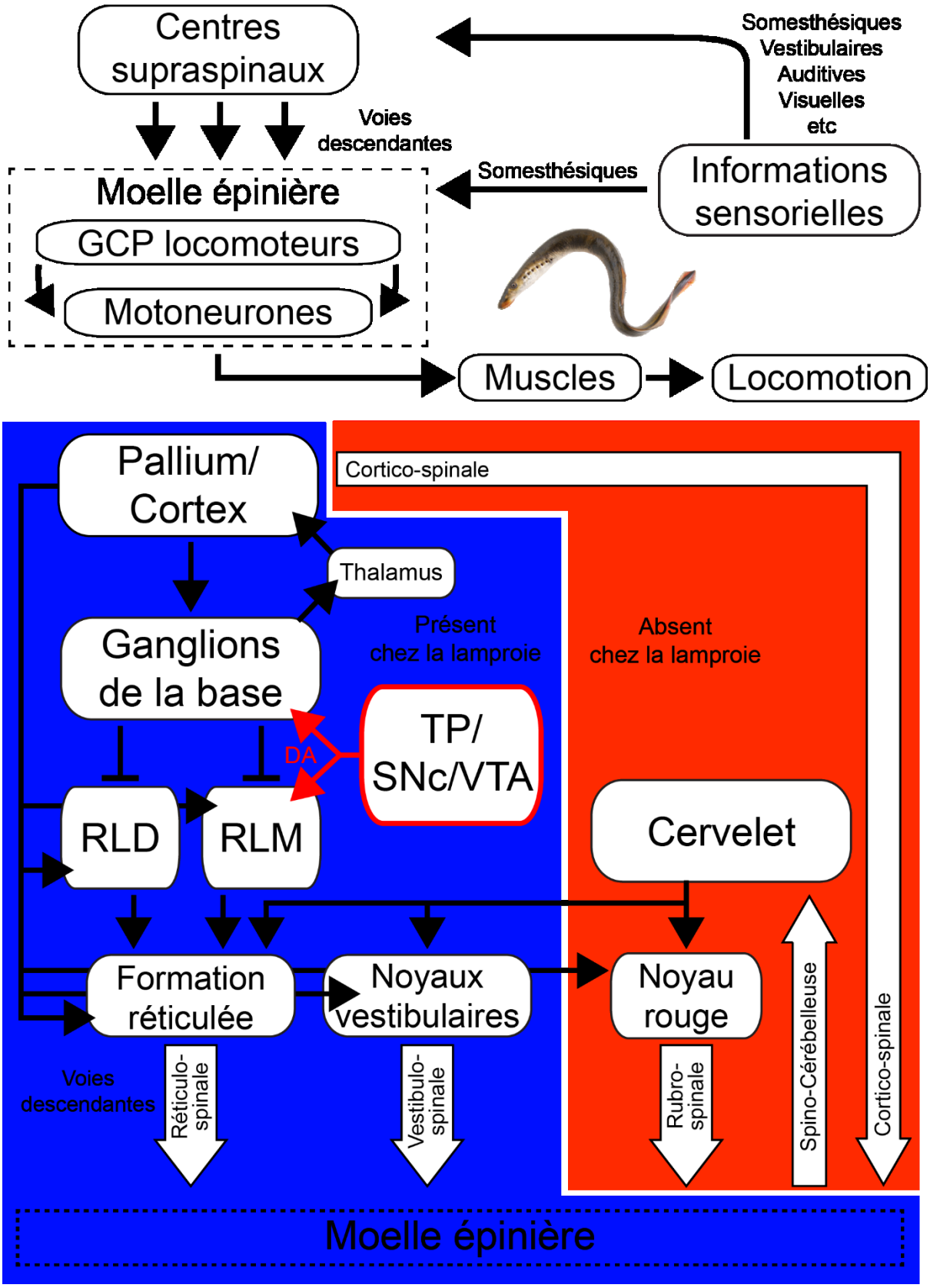
Centres supraspinaux
Somesthésiques
Vestibulaires
Auditives
Visuelles
etc
Voies descendantes
Somesthésiques
Informations sensorielles
Moelle épinière
GCP locomoteurs
Motoneurones
Muscles
Locomotion
Pallium/ Cortex
Cortico-spinale
Thalamus
Présent chez la lamproie
Absent chez la lamproie
Ganglions de la base
TP/ SNc/VTA
DA
RLD
RLM
Cervelet
Formation réticulée
Noyaux vestibulaires
Noyau rouge
Voies descendantes
Réticulo-spinale
Vestibulo-spinale
Rubro-spinale
Spino-Cérébelleuse
Cortico-spinale
Moelle épinière

Figure 4. Organisation générale du contrôle locomoteur chez les vertébrés

(Haut) Chez les vertébrés, les centres supraspinaux modulent la locomotion en recrutant les générateurs centraux de patrons (GCP) locomoteurs au niveau de la moelle épinière via différentes voies descendantes. Les motoneurones sont recrutés par ces réseaux selon une séquence permettant la contraction des muscles appropriés pour générer la locomotion. Le retour sensoriel produit par les mouvements ainsi que les informations sensorielles provenant de l'environnement peuvent influencer l'activité des centres supraspinaux pour ajuster la locomotion tandis que les informations somesthésiques peuvent déclencher des réflexes spinaux. **(Bas)** Structures du système nerveux central impliquées dans le contrôle locomoteur chez les mammifères (bleu et orange) et la lamproie (bleu). Les régions locomotrices diencéphalique (RLD) et mésencéphalique (RLM) modulent indirectement la locomotion par leurs projections à la formation réticulée. Ces deux régions sont toniquement inhibées par les ganglions de la base et peuvent être recrutées par le pallium chez la lamproie ou le cortex chez les mammifères. Par ailleurs, l'activité du pallium et du cortex sont influencées par la boucle cortico-striato-pallido-thalamo-corticale. De plus, les neurones dopaminergiques (DA, rouge) du tubercule postérieur (TP) et de la substance noire pars compacta/aire tegmentaire ventrale (SNc/ATV) modulent à la fois l'activité des ganglions de la base et de la RLM pour influencer la locomotion. Parmi les régions absentes chez la lamproie, une place importante est occupée par le cervelet, qui reçoit des informations sensorielles par la voie spino-cérébelleuse pour moduler la locomotion via la formation réticulée, les noyaux vestibulaires et le noyau rouge. Ce dernier est aussi absent chez la lamproie. La voie corticospinale, qui est impliquée dans le contrôle volontaire de la locomotion chez les mammifères, n'a pas été identifiée chez la lamproie.

Bien que la moelle épinière puisse à elle-seule produire des patrons complexes d'activations musculaires permettant la locomotion, les influx sensoriels acheminent des informations essentielles directement à la moelle épinière et aux centres supraspinaux pour adapter la locomotion aux conditions externes (environnement) et internes (proprioception). Les informations extéroceptives provenant de récepteurs situés dans la peau, l'oreille interne, l'œil ou d'autres organes sensoriels sont acheminées au niveau des centres supérieurs qui permettent d'influencer l'activité motrice pour éviter des obstacles et se diriger vers un but. De plus, les informations

somesthésiques (cutanées, articulaires, musculaires) sont aussi acheminées directement au niveau de la moelle épinière pour induire des réflexes spinaux. Enfin, les structures supraspinales amorcent et contrôlent la locomotion via des projections descendantes à la moelle épinière. Puisque l'encéphale a évolué pour devenir très sophistiquée chez les mammifères, c'est à ce niveau qu'on retrouve des différences importantes dans le contrôle de la locomotion chez les vertébrés. Les centres supraspinaux impliqués dans le contrôle locomoteur de la lamproie et des mammifères sont décrits dans les sections suivantes ainsi que dans la figure 4.

1.4.2 Centres supraspinaux: Régions présentes chez la lamproie

Les structures supraspinales sont responsables d'initier, de diriger, d'ajuster et d'arrêter la locomotion et ces fonctions sont réalisées par des projections descendantes qui influencent l'activité des GCP locomoteurs au niveau de la moelle épinière. Chez les vertébrés, il existe un total de cinq voies descendantes directement impliquées dans le le contrôle moteur qui sont regroupées en deux grands systèmes: un système ventromédian pour le contrôle de la musculature axiale et proximale ainsi qu'un système latéral impliqué dans le contrôle de la motricité fine des membres (Lawrence et Kuypers, 1968a; Lawrence et Kuypers, 1968b). Le système ventromédian, regroupant les projections descendantes de la formation réticulée (RS), des noyaux vestibulaires (vestibulospinales) et du tectum optique/collicule supérieur (tectospinales) est présent chez la lamproie. Le système latéral, regroupant les projections descendantes du noyau rouge (rubrospinales) et du cortex (corticospinales) est quant à lui absent chez la lamproie. Ainsi, toutes les régions connues du système ventromédian existaient chez l'ancêtre commun des lamproies et des mammifères et ont été conservées, tandis que d'autres projections associées au contrôle précis des membres sont apparues plus tard durant l'évolution des vertébrés.

1.4.2.1 Voie réticulospinale et région locomotrice mésencéphalique

Chez la lamproie, les projections RS ont un rôle clef dans la locomotion puisqu'elles constituent la voie descendante commune et principale pour l'activation des GCP locomoteurs spinaux (Rovainen, 1974; Buchanan et Cohen, 1982; McClellan, 1988; Viana Di Prisco et al., 1997) en recrutant des motoneurones et des interneurones prémoteurs (Ohta et Grillner, 1989). Les différents noyaux de cellules RS ont des contributions spécifiques au contrôle locomoteur (Brocard et Dubuc, 2003) et sont impliqués dans la vitesse, la direction ainsi que le contrôle de l'équilibre durant la locomotion (Deliagina et al., 2000). Des données récentes ont aussi démontré que des populations

distinctes de cellules RS sont impliquées dans l'initiation, le maintien et l'arrêt de la locomotion (Juvin et al., 2016). Chez les mammifères, les cellules RS jouent un rôle semblable et activent directement les motoneurones associés à la musculature des membres pour moduler la locomotion (pour une revue, voir Shapovalov, 1972). La formation réticulée serait aussi impliquée dans la vitesse et la direction de l'activité locomotrice (Oueghlani et al., 2018).

Dans le contexte de la locomotion, deux sources d'excitation sont déterminantes pour la formation réticulée: la région locomotrice diencéphalique et la RLM. Chez la lamproie, la stimulation de ces deux régions induit l'activation de cellules RS qui recrutent les GCP locomoteurs spinaux (El Manira et al., 1997; Sirota et al., 2000; Brocard et al., 2010). Chez les mammifères, la RLM recrute aussi les projections RS pour induire de la locomotion (Noga et al., 2003; Bachmann et al., 2013) et elle contrôle la vitesse et le mode de locomotion chez tous les vertébrés testés (pour une revue, voir Ryczko et Dubuc, 2013). La région locomotrice diencéphalique permet également de contrôler la vitesse et le mode de locomotion des mammifères (Orlovskiĭ, 1969) et sa localisation exacte a été identifiée dans la zona incerta (Parker et Sinnamon, 1983; Kasicki et al., 1991). Cependant, il n'est pas clair si la région locomotrice diencéphalique recrute aussi la formation réticulée pour induire de la locomotion chez les mammifères (pour une revue, voir Kim et al., 2017).

1.4.2.2 Voie vestibulospinale

Chez les vertébrés, la voie vestibulospinale connecte les noyaux vestibulaires du tronc cérébral à la moelle épinière pour influencer la posture et l'équilibre durant la locomotion (Orlovskiĭ, 1972b). Les résultats chez la lamproie indiquent que l'organisation du système vestibulaire est similaire aux autres vertébrés (Bussières et al., 1999; Pflieger et Dubuc, 2000), à l'exception que les projections vestibulospinales sont restreintes à la moelle épinière rostrale (Rovainen, 1979a; Ronan, 1989).

1.4.2.3 Voie tectospinale

La voie tectospinale, une autre voie descendante présente chez tous les vertébrés, provient du tectum optique ou du collicule supérieur et est importante pour les mouvements de la tête et des yeux mais ne serait pas directement impliquée dans la locomotion. Bien que la stimulation du tectum optique ou du collicule supérieur produise de la locomotion chez la lamproie (Saitoh et al., 2007) et chez le rat (Dean et al., 1986), certaines études suggèrent que ces effets soient plutôt réalisés via des projections tecto-réticulo-spinales (Dean et al., 1986; Kardamakis et al., 2015).

1.4.2.4 Ganglions de la base

Les ganglions de la base sont constitués de plusieurs noyaux et jouent un rôle déterminant sur le comportement des animaux. Les projections GABAergiques des ganglions de la base sont spontanément actives et maintiennent sous inhibition tonique les régions locomotrices diencéphalique et mésencéphalique. Par exemple, chez la souris (Roseberry et al., 2016), l'activation ou l'inhibition de neurones glutamatergiques de la RLM par la voie directe ou indirecte des ganglions de la base permet d'initier ou d'arrêter la locomotion. Ces réseaux neuronaux, incluant les voies directes et indirectes, ont été conservées chez les vertébrés puisqu'ils existent aussi chez la lamproie (pour des revues, voir Grillner et al., 2013; Grillner et Robertson, 2016). Par ailleurs, la boucle cortico-striato-pallido-corticale, impliquée dans l'initiation et le déroulement des programmes moteurs, a également été identifiée chez la lamproie (Stephenson-Jones et al., 2012). Ainsi, chez l'ensemble des vertébrés, les ganglions de la base joueraient un rôle dans la sélection de l'action et peuvent initier ou freiner la locomotion via des projections inhibitrices à la région locomotrice diencéphalique et mésencéphalique.

1.4.2.5 Tubercule postérieur et SNc/ATV

Le niveau d'excitabilité des ganglions de la base est déterminant pour l'activité motrice et est régulé par les projections DA du TP chez la lamproie ou de la SNc/ATV chez les mammifères. Au niveau du striatum, une transmission DA trop élevée entraîne de l'hyperkinésie tandis qu'une transmission DA trop faible entraîne de l'hypokinésie, tel qu'observé chez les individus atteints de la maladie de Parkinson. Les projections DA du TP au striatum ont également été observées chez la lamproie (Pombal et al., 1997; Ryczko et al., 2013; von Twickel et al., 2019) mais ces neurones ont aussi des projections descendantes vers divers centres moteurs, incluant la RLM. Les projections DA du TP à la RLM ont été décrites plus haut (Section 1.3.3) et ont un rôle facilitateur sur la locomotion par l'activation de récepteurs D1 (Ryczko et al., 2013). Comme l'ensemble des structures nerveuses décrites dans la présente section, cette projection DA descendante a conservé un rôle important dans la locomotion des vertébrés puisqu'elle est aussi observée chez les amphibiens et les mammifères (Ryczko et al., 2016).

1.4.3 Centres supraspinaux: Régions absentes chez la lamproie

1.4.3.1 Voie corticospinale

Le développement du cortex a permis l'apparition de plusieurs fonctions motrices qui étaient absentes chez la lamproie, telle que la production de mouvements permettant la parole, par exemple. Il existe tout de même beaucoup de similitudes entre le pallium latéral (PL) de la lamproie et le cortex moteur des mammifères en ce qui concerne les projections efférentes et le contrôle moteur. Par exemple, la stimulation du PL induit des mouvements du disque oral, des yeux et du tronc ainsi que de la locomotion. Ses projections ciblent les ganglions de la base, la RLM et les cellules RS (Ocaña et al., 2015). Cependant, un élément important qui est absent chez la lamproie est une voie descendante entre le PL et la moelle épinière (Ocaña et al., 2015). Chez les mammifères, les projections corticospinales sont primordiales dans le contexte de la locomotion puisqu'elles permettent notamment d'intégrer les signaux visuels pour positionner précisément les membres et franchir des obstacles (pour une revue, voir Drew et al., 2004). La lésion des projections corticospinales chez le chat induit des déficits persistants dans le contrôle des mouvements distaux, incluant un trainement de la patte (Jiang et Drew, 1996) et l'incapacité de positionner correctement ses membres sur une échelle horizontale (Liddell et Phillips, 1944). L'activité de neurones corticospinaux durant la locomotion est augmentée lorsque des obstacles sont enjambés en fonction des informations visuelles perçues (Drew, 1988; Drew, 1993; Widajewicz et al., 1994). Chez les quadrupèdes, le cortex moteur ne serait toutefois pas nécessaire pour produire un rythme locomoteur de base (Bjursten et al., 1976). Chez l'humain, la voie corticospinale contribue directement à l'activité musculaire durant la marche (Fukuyama et al., 1997; Petersen et al., 2012). De plus, les accidents cérébro-vasculaires peuvent entrainer des lésions corticales qui affectent sévèrement la capacité à marcher. Chez ces patients, l'intégrité de la voie corticospinale spinale est importante pour la récupération de la marche (Preston et al., 2021). De plus, suivant une lésion spinale incomplète, l'activité du cortex moteur est importante pour récupérer les fonctions locomotrices (Thomas et Gorassini, 2005; Pulverenti et al., 2021). Par conséquent, chez l'humain, la voie corticospinale aurait plus d'importance dans la locomotion que chez les mammifères quadrupèdes.

1.4.3.2 Cervelet

Une différence importante entre la lamproie et les mammifères est le développement du cervelet. Chez les mammifères, le cervelet joue un rôle important dans la locomotion en permettant l'apprentissage de plusieurs tâches motrices telles que la production de patrons appropriés de mouvements des membres ainsi que la régulation de l'équilibre et de la posture (pour une revue, voir Morton et Bastian, 2004). Bien que la locomotion soit générée par la moelle épinière, le cervelet reçoit de l'information dynamique sur le mouvement en cours ainsi que le mouvement planifié et peut induire des corrections dans le mouvement en recrutant différentes voies descendantes (pour une revue, voir Grillner et El Manira, 2020). Durant la locomotion, le cervelet reçoit les informations sensorielles intégrées de différents muscles et articulations par la voie spino-cérébelleuse dorsale (Poppele et al., 2002) et reçoit également une copie d'efférences du GCP locomoteur spinal via la voie spino-cérébelleuse ventrale (Arshavsky et al., 1972) et par la formation réticulée via la voie spino-réticulo-cérébelleuse (Arshavsky et al., 1978). Ces informations permettent au cervelet de monitorer continuellement l'activité locomotrice et d'y apporter rapidement des corrections par ses projections aux noyaux de cellules RS (Orlovskiĭ, 1972a), vestibulospinales (Orlovskiĭ, 1972b) et rubrospinales (Orlovskiĭ, 1972c). Pour sa part, la lamproie possède un petit cervelet de forme aplatie où des cellules granulaires ont été observées (Nieuwenhuys, 1967). Cependant, la présence de cellules de Purkinje et d'un cortex cérébelleux constitué de plusieurs couches n'a pas été démontrée (Lannoo et Hawkes, 1997). Chez la lamproie, la correction rapide des mouvements et de la posture peut cependant être produite durant la locomotion via des projections ascendantes de la moelle épinière aux cellules RS (Kasicki et al., 1989; Dubuc et al., 1993; Vinay et al., 1998; Buchanan, 2011).

1.4.3.3 Voie rubrospinale

Le noyau rouge est un noyau mésencéphalique à l'origine de la voie rubrospinale. Au cours de l'évolution des vertébrés, ses fonctions et sa connectivité ont subi des changements majeurs qui coïncident avec des modifications importantes du patron locomoteur (pour des revues, voir ten Donkelaar, 1988; Gruber et Gould, 2010; Basile et al., 2021). Premièrement, l'apparition des membres chez les vertébrés aurait amené le développement de la voie rubrospinale comme système de contrôle parallèle permettant de coordonner leurs mouvements (Edwards, 1977). En effet, l'identification anatomique de la voie rubrospinale chez différentes espèces montre qu'elle serait directement liée à l'utilisation des membres dans la locomotion (ten Donkelaar, 1976a; ten

Donkelaar, 1976b; Smeets et Timerick, 1981; ten Donkelaar et Bangma, 1983; ten Donkelaar et al., 1983). Par exemple, il n'y a pas de voie rubrospinale chez le python royal (ten Donkelaar et Bangma, 1983), un vertébré dépourvu de membre, ni chez la lamproie (Nieuwenhuys, 1977; Rovainen, 1979b; Ronan, 1981; Pombal et Megías, 2011). De plus, le rôle principal du système rubrospinal dans la locomotion et la posture observé chez les vertébrés quadrupèdes disparait presque complètement chez les primates bipèdes où l'on voit apparaître un rôle dans le contrôle des mouvements fins de la main (Basile et al., 2021), ce qui serait lié à la perte des fonctions locomotrices des membres antérieurs (Massion, 1988). Le noyau rouge jouerait donc un rôle plus important dans la locomotion chez les quadrupèdes. Durant la marche du chat, les neurones du noyau rouge sont actifs surtout durant la phase de balancement (Orlovskiĭ, 1972c; Arshavsky et al., 1988) et leur stimulation modifie l'activité musculaire (Rho et al., 1999). De plus, lors de tâches d'enjambement d'obstacles, leur activité est augmentée, à l'instar des neurones corticospinaux (Lavoie et Drew, 2002). Un rôle dans le maintien de la posture a été proposé chez le chat (Zelenin et al., 2010; Herter et al., 2015). Le noyau rouge serait par conséquent impliqué dans la locomotion chez les quadrupèdes mais son rôle chez l'humain est encore peu connu.

Ensemble, les régions décrites dans cette section permettent le contrôle de la locomotion chez les vertébrés (Fig. 4). Toutes les régions impliquées dans la nage ondulatoire de la lamproie ont été conservées chez les vertébrés et sont aussi impliquées dans les différents modes de locomotion des mammifères. De plus, de nouvelles régions supraspinales sont apparues durant l'évolution des vertébrés et permettent un contrôle fin du mouvement.

1.5 Questions de recherche

L'objectif principal de ce projet de doctorat était de caractériser le rôle des neurones DA durant l'intégration de l'information olfactive dans le cerveau de la lamproie, en particulier dans 1) le BO ainsi que 2) le TP.

Partie 1 – Modulation dopaminergique du bulbe olfactif

1.1 Caractériser anatomiquement l'innervation DA du BO.

- 1.1.1 Quelles sont les sources de modulation DA du BO?
- 1.1.2 Quelles sous-régions du BO sont ciblées par l'innervation DA?
- 1.1.3 Y a-t-il des différences anatomiques entre les stades développementaux de la lamproie?

1.2 Caractériser physiologiquement la modulation DA du BO ainsi que son impact sur la transmission olfactomotrice.

- 1.2.1 Quel est l'impact de la modulation DA dans le BO sur la transmission du signal olfactomoteur au tronc cérébral?
- 1.2.2 Quels sont les récepteurs DA impliqués dans la modulation du bulbe olfactif?
- 1.2.3 Cette modulation existe-t-elle chez différents stades développementaux de la lamproie?

Partie 2 – Intégration du signal olfactif dans le tubercule postérieur

2.1 Caractériser anatomiquement les projections du BO médian au TP.

- 2.1.1 Quelles sous-régions et populations cellulaires du TP sont ciblées par l'innervation du BO médian?
- 2.1.2 Quelles populations cellulaires du TP relaient le signal olfactif à la RLM?

2.2 Caractériser physiologiquement l'activité du TP en réponse à la stimulation des voies olfactomotrices médiale et latérale.

- 2.2.1 Comment se compare l'activité du TP au repos et à la stimulation de la voie olfactomotrice médiale ou latérale?

2.2.2 Est-ce que les neurones du TP relaient le signal olfactif à la RLM?

2.2.3 Comment le TP est-il recruté lors de la nage spontanée ou induite par la stimulation des voies olfactomotrices médiale ou latérale?

1.6 Hypothèse générale

Les neurones dopaminergiques du tubercule postérieur sont impliqués dans la production et la modulation des comportements olfactifs

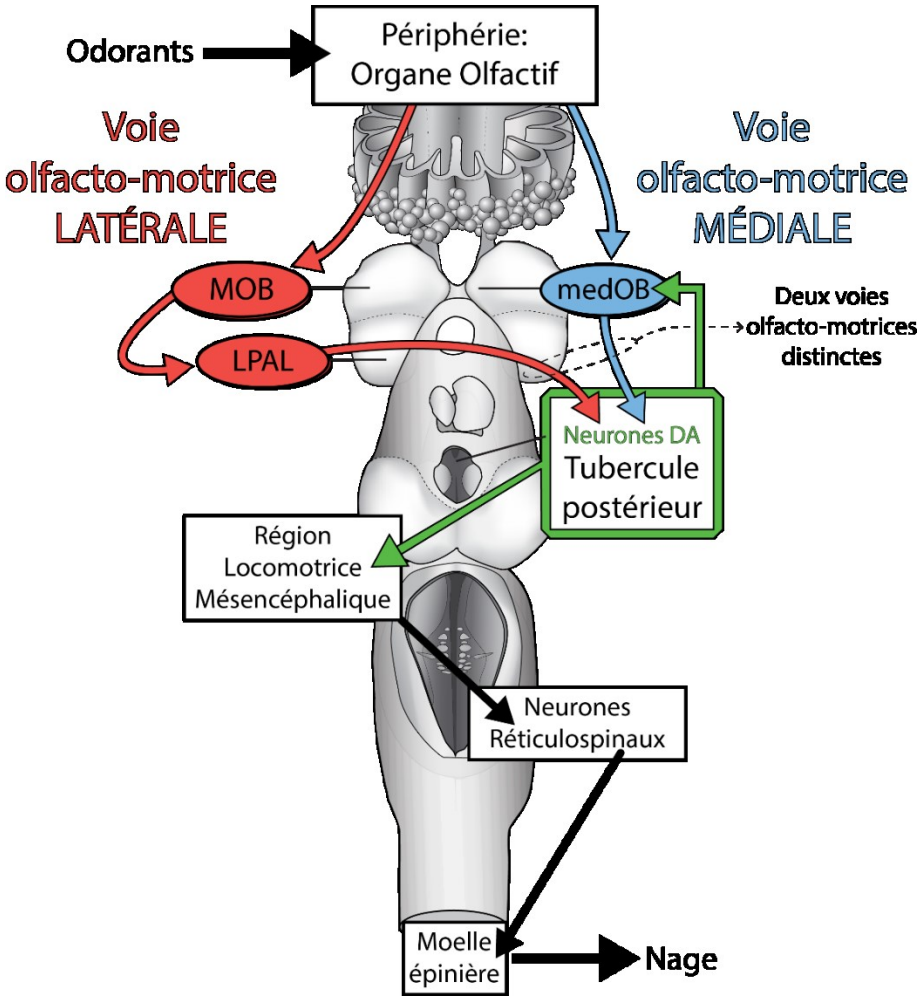

Figure 5. Hypothèse générale.

Schéma de la vue dorsale du cerveau de la lamproie adulte illustrant les voies olfactomotrices médiale (bleu) et latérale (rouge) ainsi que la localisation du tubercule postérieur (vert). Ses projections ascendantes au bulbe olfactif et descendantes à la région locomotrice mésencéphalique pourraient contribuer aux réponses olfactomotrices. LPAL: Pallium latéral; medOB: Bulbe olfactif médian; MOB: bulbe olfactif principal.

1.7 Objectifs spécifiques

Partie 1 – Modulation dopaminergique du bulbe olfactif

Hypothèse spécifique 1: Une innervation DA présente dans le BO de la lamproie module l'activité synaptique pour influencer les comportements olfactifs.

L'objectif principal de la première partie du projet était de caractériser l'innervation DA dans le système olfactif de la lamproie marine, en particulier dans le BO médian. Cette étude a été réalisée chez des animaux à trois différents stades du cycle de vie pour observer le développement du système DA dans le système olfactif. Une combinaison de techniques anatomiques et physiologiques a été réalisée dans l'organe olfactif ainsi que le cerveau isolé afin d'y observer la présence de cellules et de fibres DA dans le BO et de caractériser leur effet sur la transmission du signal olfactif aux régions motrices du tronc cérébral. Les résultats de cette étude sont compilés dans un article de recherche à la section 2.1.

Les objectifs spécifiques de cette partie du projet sont:

- **1.1** Caractériser l'innervation DA (immunofluorescence ciblant la tyrosine hydroxylase ou la DA) dans le système olfactif de la lamproie à plusieurs stades développementaux (larvaire, jeune adulte, adulte reproductrice).
- **1.2** Cartographier et caractériser l'innervation DA spécifiquement au niveau du BO médian et observer la relation des terminaisons axonales DA avec les neurones de projection du BO médian et les afférences olfactives primaires.
- **1.3** Identifier le rôle et les mécanismes de la modulation DA dans le BO médian (injection locale d'agonistes et d'antagonistes des récepteurs DA) durant la transmission du signal olfactomoteur (stimulation électrique du nerf olfactif et enregistrements intracellulaires de cellules RS, dans le cerveau entier isolé *in vitro*).
- **1.4** Localiser anatomiquement les neurones à l'origine de l'innervation DA du BO médian via des injections de traceurs rétrogrades dans le BO médian couplées avec de l'immunofluorescence ciblant la DA ou la tyrosine hydroxylase.

Ces expériences nous ont permis de montrer pour la première fois un rôle fonctionnel de la modulation DA dans le BO de la lamproie, où la transmission DA joue un rôle de filtre en modulant

le signal olfactif. Cette modulation provient en partie d'une projection DA du TP dans le BO médian pour y diminuer la transmission du signal olfactif aux cellules RS via l'activation des récepteurs D2.

PARTIE 2 – Intégration du signal olfactif dans le tubercule postérieur

Hypothèse spécifique 2: Les odeurs activent le TP et induisent la nage via deux voies olfactomotrices distinctes (médiale et latérale) chez la lamproie.

L'objectif de cette deuxième partie du projet était de caractériser anatomiquement et physiologiquement les mécanismes par lesquels le TP intègre l'information provenant des voies olfactomotrices médiale et latérale. Plus spécifiquement, une population de neurones DA bien documentée est présente dans le TP et leur participation dans le relai du signal olfactif a été spécifiquement évaluée. Cette étude a été réalisée chez des animaux au stade jeune adulte principalement et une combinaison de techniques anatomiques et physiologiques a été utilisée. Les données physiologiques ont été obtenues en partie dans des préparations semi-intactes, permettant de monitorer la nage de l'animal tout en enregistrant l'activité synaptique, et des prosencéphales isolés *in vitro* (cerveau où le tronc cérébral a été retiré), permettant un accès intéressant au TP tout en gardant intact les circuits olfactifs périphériques et centraux. Les résultats de cette étude sont compilés dans le manuscrit à la section 2.2.

Les objectifs spécifiques de cette partie du projet sont:

2.1 Caractériser anatomiquement les projections du BO médian au TP en localisant et en identifiant les cellules du TP innervées par les projections olfactives (injections de traceur axonaux antérogrades et immunofluorescence dirigée contre la DA, le glutamate et le GABA).

2.2 Déterminer si les neurones DA du TP répondent à la stimulation olfactive de la voie médiale (stimulation du BO médian) et de la voie latérale (stimulation du BO principal et du pallium latéral) en enregistrant l'activité des neurones du TP (enregistrements extracellulaires, enregistrements intracellulaires en patch-clamp et imagerie calcique).

2.3 Déterminer si les neurones DA du TP sont un relai dans la transmission du signal olfactomoteur spécifiquement vers la RLM par des expériences anatomiques (injection de traceurs rétrogrades dans la RLM) et physiologiques (imagerie de neurones du TP marqués par l'injection d'un indicateur calcique dans la RLM)

2.4 Caractériser physiologiquement l'activité du TP durant la nage induite par la stimulation des voies olfactomotrices médiale (BO médian) et latérale (pallium latéral) dans la préparation semi-intacte par des enregistrements extracellulaires simultanément à l'enregistrement intracellulaire de cellules RS et l'enregistrement vidéo des mouvements de nage.

Ces expériences ont mis en évidence que le TP est activé par les afférences olfactives des voies olfactomotrices médiale et latérale et fournissent une indication claire de son rôle dans la nage induite par la stimulation de ces deux voies. Les neurones DA y sont innervés par les afférences du BO médian et activeraient la RLM pour initier un épisode de nage en réponse à la stimulation olfactive.

2. Résultats

Les résultats contiennent deux études distinctes qui répondent aux questions et objectifs mentionnés plus haut. Le premier article (Beauséjour et al., 2020; Section 2.1) répond spécifiquement à la partie 1 des objectifs spécifiques et porte sur les projections ascendantes du TP qui modulent l'activité dans le BO médian. Ce manuscrit a été publié dans Journal of Comparative Neurology. La partie 2 des objectifs spécifiques est abordée dans un deuxième manuscrit (Section 2.2) qui est prêt pour une soumission chez Frontiers in Neural Circuits. Dans cette seconde étude, l'intégration des signaux olfactifs dans le TP est analysée avec des méthodes neuroanatomiques et neurophysiologiques.

2.1 Dopaminergic modulation of olfactory-evoked motor output in sea lampreys (*Petromyzon marinus L.*)

Le manuscrit suivant a été publié dans le Journal of Comparative Neurology en 2020. Une étude précédemment réalisée dans le laboratoire du Dr Réjean Dubuc (Derjean et al., 2010) a identifié le BO médian comme étant une région impliquée dans le relai de l'information olfactive vers des régions motrices à même de déclencher une réponse de nage. Nous avons étudié la possibilité que la modulation du BO médian par une innervation DA puisse influencer l'activité olfactomotrice. Nous avons observé que des neurones DA situés dans le TP innervent le BO médian où des fibres DA sont observées à proximité de neurones de projection et d'afférences olfactives primaires. De plus, des expériences physiologiques ont révélé que la DA a un effet inhibiteur sur l'activité de la voie olfactomotrice. Ce manuscrit répond aux objectifs spécifiques de la Section 1.7, partie 1.

Contributions des auteurs:

Beauséjour, Philippe-Antoine:	Conception des expériences, Mise-au-point méthodologique, Collecte de données, Analyse des résultats, Interprétation des résultats, Conception des figures, Rédaction de la première version du manuscrit, Révision du manuscrit, Financement.
Auclair, François:	Contribution à la conception des expériences, à l'interprétation des résultats et à la révision du manuscrit.
Daghfous, Gheylen:	Contribution à la conception des expériences, Révision mineure du manuscrit.
Ngovandan, Catherine:	Contribution à la collecte de données (3 jours, Figure 13).
Veilleux, Danielle:	Contribution à la collecte de données (2 jours, Figure 13).
Zielinski, Barbara:	Révision mineure du manuscrit, Financement.
Dubuc, Réjean:	Révision mineure du manuscrit, Financement.

TITLE: Dopaminergic modulation of olfactory-evoked motor output in sea lampreys (*Petromyzon marinus* L.).

RUNNING TITLE: Dopaminergic modulation of olfactory transmission.

AUTHORS:

Philippe-Antoine Beauséjour[1], François Auclair[1], Gheylen Daghfous[1,2], Catherine Ngovandan[1], Danielle Veilleux[1], Barbara Zielinski[3], Réjean Dubuc[1,2].

INSTITUTIONAL AFFILIATIONS:

[1]Université de Montréal, Département de neurosciences, Montréal, QC, Canada;

[2]Université du Québec à Montréal, Département des sciences de l'activité physique, Montréal, QC, Canada;

[3]University of Windsor, Department of Biological Sciences, Windsor, ON, Canada.

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DATA AVAILABILITY STATEMENT:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

2.1.1 Abstract

Detection of chemical cues is important to guide locomotion in association with feeding and sexual behavior. Two neural pathways responsible for odor-evoked locomotion have been characterized in the sea lamprey (*Petromyzon marinus* L.), a basal vertebrate. There is a medial pathway originating in the medial olfactory bulb (OB) and a lateral pathway originating from the rest of the OB. These olfactomotor pathways are present throughout the life cycle of lampreys, but olfactory-driven behaviors differ according to the developmental stage. Amongst possible mechanisms, dopaminergic (DA) modulation in the OB might explain the behavioral changes. Here, we examined DA modulation of olfactory transmission in lampreys.

Immunofluorescence against DA revealed immunoreactivity in the OB that was denser in the medial part (medOB), where processes were observed close to primary olfactory afferents and projection neurons. Dopaminergic neurons labeled by tracer injections in the medOB were located in the OB, the posterior tuberculum (PT), and the dorsal hypothalamic nucleus, suggesting the presence of both intrinsic and extrinsic DA innervation. Electrical stimulation of the olfactory nerve in an *in vitro* whole-brain preparation elicited synaptic responses in reticulospinal cells that were modulated by DA. Local injection of DA agonists in the medOB decreased the reticulospinal cell responses whereas the D2 receptor antagonist raclopride increased the response amplitude. These observations suggest that DA in the medOB could modulate odor-evoked locomotion. Altogether, these results show the presence of a DA innervation within the medOB that may play a role in modulating olfactory inputs to the motor command system of lampreys.

KEYWORDS: Dopamine, Olfactory bulb, Olfaction, Locomotion, Lamprey, Anatomical tracing, Immunofluorescence, Electrophysiology.

2.1.2 Abbreviations

- BSA: Bovine serum albumin
- DA: Dopamine
- DHN: Dorsal hypothalamic nucleus
- EPSPs: Excitatory postsynaptic potentials
- G: Glutaraldehyde
- GSIB4: *Griffonia simplicifolia* isolectin β4
- LPal: Lateral pallium
- medOB: Medial olfactory bulb
- MLR: Mesencephalic locomotor region
- MRRN: Middle rhombencephalic reticular nucleus
- MOB: Main olfactory bulb
- OB: Olfactory bulb
- ON: Olfactory nerve
- PBS: Phosphate-buffered saline
- PT: Posterior tuberculum
- RS: Reticulospinal
- SNc: Substantia nigra, pars compacta
- TBS-m: Tris 0.05 M with 1.0% sodium-metabisulfite
- TH: Tyrosine hydroxylase
- VTA: Ventral tegmental area

2.1.3 Introduction

Lampreys represent the oldest extant group of vertebrates and their behavior is strongly influenced by olfactory inputs. Sea lampreys (*Petromyzon marinus* L.) rely heavily on the detection of chemical cues for feeding (Kleerekoper et Mogensen, 1963) and sexual behaviors (Buchinger et al., 2015). Since odorant perfusion on the olfactory epithelium of lampreys from prolarval (Zielinski et al., 2005) to spawning (Li et al., 1995) stages activates sensory neurons, olfaction is thought to induce motor behavior throughout life. During the transformation from larva to young adult, the peripheral olfactory apparatus becomes well developed with a lamellar olfactory epithelium and an anatomically distinct accessory olfactory organ (Ren et al., 2009). Moreover, compared to other vertebrate species, lampreys have a large proportion of their brain dedicated to processing olfactory inputs, the size of the olfactory bulbs (OB) even exceeding that of the cerebral hemispheres (Nieuwenhuys, 1977). Furthermore, secondary olfactory neurons have extensive projections throughout the prosencephalon (Northcutt et Puzdrowski, 1988).

Our research group has previously identified a neural substrate responsible for generating locomotion in response to olfactory inputs (Derjean et al., 2010). Odorant detection is carried out by olfactory sensory neurons that project to the medial part of the OB (medOB; Green et al., 2017). There, a unique population of projection neurons located inside a single glomerulus (medOB glomerulus) conveys the inputs to the posterior tuberculum (PT; Derjean et al., 2010; Green et al., 2013; Pérez-Fernández et al., 2014; Daghfous et al., 2018). The PT then sends descending dopaminergic and glutamatergic inputs to the mesencephalic locomotor region (MLR; Ryczko et al., 2013; Ryczko et al., 2016; Ryczko et al., 2017), a structure known to play a crucial role in the control of locomotion in all vertebrate species tested so far. The MLR then activates reticulospinal (RS) cells (Sirota et al., 2000), which provide the main descending inputs to the central pattern generators for locomotion in the spinal cord.

Recent findings in our lab have revealed that olfactory projections from the rest of the OB (main OB: MOB) can also activate the PT, but via projections to the lateral pallium (Daghfous et al., 2018). The well-characterized, locomotion-controlling neural circuits that are part of an axis from the PT to the spinal cord can thus be activated by both a medial (medOB-PT) and a lateral (MOB-lateral pallium-PT) olfactomotor pathways to generate locomotor responses to olfactory cues. These two parallel olfactomotor pathways convey information from the olfactory sensory organ in

the periphery, which is activated by various naturally occurring food-related or reproductive olfactory cues such as amino acids, bile acids and pheromones (Green et al., 2017). Thus, they may be equally involved in food-seeking and mate-finding.

Multiple odorants that can trigger locomotion elicit responses in the medOB (Green et al., 2017). Moreover, the medial olfactomotor pathway is functional throughout life, but odor-driven behaviors differ among larval, parasitic (Kleerekoper et Mogensen, 1963; Silva et al., 2013) and spawning (Johnson et al., 2012) developmental stages. Hence, the activity in this neural circuit must be adjusted to various conditions, external and internal. Modulatory mechanisms upstream of the PT could efficiently regulate motor responses to odorants. The medOB would thus be a good target for modulation of the medial olfactomotor pathway.

Here, the possibility that dopamine (DA) could modulate olfactory processing taking place in the medOB was examined. Dopaminergic processes and cells were previously observed in the OB of lampreys (Pierre et al., 1994; Pierre et al., 1997; Pombal et al., 1997; Barreiro-Iglesias et al., 2009; Fernández-López et al., 2017), as in every other vertebrate studied (Smeets et González, 2000). Also, cells expressing D1 or D2 receptors have been observed in the OB (Pérez-Fernandez, 2013; Pérez-Fernández et al., 2014). DA transmission may thus modulate odor processing in lampreys, such as in other vertebrates. In mammals for instance, DA modulation in the OB is typically associated with olfactory discrimination learning (Pavlis et al., 2006; Tillerson et al., 2006; Wei et al., 2006; Escanilla et al., 2009), and it has also been shown to regulate reproductive behaviors such as mating, parturition and suckling (Kendrick et al., 1988; Keverne et al., 1993; Serguera et al., 2008). Hence, DA transmission could be involved in the modulation of olfactory processing inducing odor-driven behaviors in lampreys. This study examines the presence of DA immunoreactivity in the OB and the modulatory actions of DA in the medOB on olfactomotor transmission.

2.1.4 Materials and Methods

Experiments were performed on 72 larvae, 11 newly-transformed adults, and 23 spawning-phase adult sea lampreys (*Petromyzon marinus*) of both sexes. Larval and newly-transformed specimens were collected from the Pike River (Pike River, QC, Canada) and the Morpion Stream (Notre-Dame-de-Stanbridge, QC, Canada). The Vermont *US Fish and Wildlife Service* collected spawning-phase adults. All procedures conformed to the guidelines of the Canadian Council on Animal Care and were approved by the Université de Montréal and the Université du Québec à Montréal ethics and animal care committees. Care was taken to minimize the number of animals used and their suffering.

2.1.4.1 Anatomical experiments

Anatomical experiments were performed to analyse the distribution of DA+ and TH+ somata and processes in the OB. Under tricaine methanesulfonate anesthesia (MS-222, 200 mg/L; Sigma Chemical), the animals were decapitated and their brain was isolated *in vitro* in cold, oxygenated (100% O_2) Ringer's solution with the following composition (in mM): NaCl, 130.0; KCl, 2.1; $CaCl_2$, 2.6; $MgCl_2$, 1.8; HEPES, 4.0; dextrose, 4.0; $NaHCO_3$, 1.0, adjusted to a pH of 7.4 with NaOH.

The brain tissue at the site of tracer injection was lesioned beforehand with an entomological needle to precisely cut the axons, allowing them to pick up the tracer. Biocytin crystals (Sigma-Aldrich, St-Louis, MO, USA) were inserted in the lesioned area to dissolve. The brains were then kept overnight under Ringer's solution perfusion at 8 °C to allow transport of the tracer to the cell body. To label projection neurons of the medOB, biocytin was injected in the PT. The roof of the caudal part of the third ventricle was cut open along the midline to gain access to the PT. Biocytin injections were also performed in the medOB to retrogradely label neurons projecting to this area.

2.1.4.2 Histology

2.1.4.2.1 Tyrosine hydroxylase immunofluorescence

The brains were fixed by immersion in 4% paraformaldehyde in phosphate-buffered saline (PBS; 0.1 M, pH 7.4 with 0.9% NaCl) for 24 hours at 4 °C, then rinsed in PBS and incubated in a 20% sucrose solution in PBS overnight for cryoprotection. The tissue was frozen in 2-methylbutane at -50 °C and cut transversally with a cryostat. The sections (25 µm thickness) were collected on

ColorFrost Plus microscope slides (Thermo Fisher Scientific) and allowed to dry on a warming plate at 37 °C for a minimum of 12 hours.

The sections were rinsed (three 10-minute immersions) in PBS, immerged for one hour in a permeabilizing solution (normal goat serum 10%, Triton X-100 0.3%, in PBS), and incubated overnight at 4 °C with a primary antibody targeting tyrosine hydroxylase (TH) (rabbit anti-TH, Millipore, Cat# AB152, RRID:AB_390204) diluted 1:400 in the permeabilizing solution. The next day, the sections were rinsed and incubated at room temperature with a goat anti-rabbit antibody conjugated to Alexa Fluor 594 (Molecular Probes, Cat# A-11012, RRID: AB_141359) diluted 1:400 in the permeabilizing solution. The sections were then rinsed, mounted with Vectashield® (with or without DAPI), and stored in the dark at 4 °C.

2.1.4.2.2 Dopamine immunofluorescence

The brains were fixed by immersion in 2% glutaraldehyde (pH 7.4) in Tris-buffered saline with low sodium-metabisulfite (Tris 0.05 M with 0.1% sodium-metabisulfite and 0.8% NaCl, pH 7.4) for 60 minutes at 4 °C. The fixed brains were then incubated in a 20% sucrose solution in Tris-buffered saline with low sodium-metabisulfite overnight for cryoprotection, frozen in 2-methylbutane at -50 °C, and cut transversally with a cryostat. The sections (25 µm thickness) were collected on ColorFrost Plus microscope slides (Thermo Fisher Scientific) and dried on a warming plate at 37 °C for a minimum of 12 hours.

The sections were first rinsed in Tris-buffered saline with high sodium-metabisulfite (TBS-m: Tris 0.05 M with 1.0% sodium-metabisulfite, pH 7.4) and incubated in a reducing solution (sodium borohydride 0.2% in Tris-buffered saline 0.05 M with 0.9% NaCl, pH 7.4) for 45 minutes to decrease autofluorescence induced by the glutaraldehyde fixation. The glass slides were rinsed again and immerged for one hour in a permeabilizing solution (normal goat serum 10%, Triton X-100 0.3%, in TBS-m) for 60 minutes before overnight incubation at 4 °C with a monoclonal mouse anti-DA antibody (Millipore, Cat# MAB5300, RRID:AB_94817) diluted 1:300 in the permeabilizing solution. The next day, the sections were rinsed and incubated with a goat anti-mouse antibody conjugated to Alexa Fluor 594 (Jackson ImmunoResearch Labs, Cat# 115-585-146, RRID:AB_2338881) diluted 1:200 in the permeabilizing solution at room temperature. Sections were then rinsed, mounted with Vectashield® (with or without DAPI) and stored in the dark at 4 °C.

2.1.4.2.3 Antibody characterization

The antibodies used in this study are listed in Table 1. The rabbit anti-TH antibody has been used reliably on lamprey tissue in independent studies examining the presence of DA neurons (Barreiro-Iglesias et al., 2008b; Robertson et al., 2012). Additionally, our research group has previously used this antibody in lampreys and salamanders (Ryczko et al., 2013; Ryczko et al., 2016).

Tableau 1. – Antibodies used in this study

	Antibody	Immunogen	Source	Dilution
Primary antibodies	Anti-Dopamine Antibody, clone K56A	Dopamine-Gluteraldehyde-BSA	Chemicon, Cat# MAB5300 / RRID: AB_94817, Raised in mouse, monoclonal	1:300
	Anti-Tyrosine Hydroxylase Antibody	Denatured tyrosine hydroxylase from rat pheochromocytoma (denatured by sodium dodecyl sulfate)	Chemicon, Cat# AB_94817 / RRID: AB_390204, Raised in rabbit, polyclonal	1:400
Secondary antibodies	Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594	Rabbit IgG (H+L)	Invitrogen, Cat# A-11012 / RRID: AB_2534079, Raised in goat, polyclonal	1:400
	AffiniPure Goat Anti-Mouse IgG (H+L), Alexa Fluor 594	Mouse IgG (H+L)	Jackson ImmunoResearch Labs, Cat# 115-585-146, RRID: AB_2338881, Raised in goat, polyclonal	1:200
Other	Isolectin GS-IB4, Alexa Fluor 488	N/A	Life Technologies, Cat# L21411 / RRID: AB_2314665, Raised in *Griffonia simplicifolia*	1:100
	Streptavidin, Alexa Fluor 488	N/A	Invitrogen, Cat# S11223 RRID: AB_2336881	1:200
	Streptavidin, Alexa Fluor 350	N/A	Invitrogen, Cat# S11249	1:200

The pattern of labeling of the mouse antibody targeting DA in our material corresponded closely to that reported with other DA antibodies in the lamprey (Pierre et al., 1997; Abalo et al., 2005;

Barreiro-Iglesias et al., 2008a). Cross-reactivity of the mouse anti-DA antibody was determined by the manufacturer (Millipore) using an ELISA test with the following compounds: DA-glutaraldehyde (G)-bovine serum albumin (BSA) 1; Tyrosine-G-BSA 1:36000; L-DOPA-G-BSA 1:10000; Noradrenaline-G-BSA 1:>50000; Adrenaline-G-BSA 1:>50000. The specificity of the fluorescent secondary antibodies was verified by omitting the primary antibody from the procedures. In every case, no labeling was obtained under these conditions. BSA: Bovine serum albumin; IgG: Immunoglobulin G.

2.1.4.2.4 Additional labeling

Streptavidin conjugated to Alexa Fluor 488 or 350 (diluted 1:200, S11223 or S11249, Thermo Fisher Scientific) was added to the secondary antibody solution to visualize biocytin. The primary olfactory afferents were stained with *Griffonia simplicifolia* isolectin β4 (GSIB4), which binds to galactosyl residues present on axons of olfactory sensory neurons, as previously done by others (Tobet et al., 1996) and us (Daghfous et al., 2018). This labeling was carried out after the immunofluorescence protocol by incubating the slides with GSIB4 conjugated to Alexa Fluor 488 (121411, Life Technologies) diluted 1:100 in the appropriate rinsing solution (TBS-m or PBS) for 60 minutes at room temperature. The sections were then rinsed and fixed in 4% paraformaldehyde in PBS for one hour at room temperature before they were rinsed again and mounted.

2.1.4.2.5 Fluorescence microscopy

The sections were observed and photographed on an E600 epifluorescence microscope equipped with a DMX1200 digital camera driven by the *Automatic Camera Tamer* software (Nikon Canada, Mississauga, ON, Canada). Photoshop CS5 software (Adobe Systems, San Jose, CA) was used to merge the photomicrographs. Photomicrographs taken with a 20X objective were assembled with the *Photomerge* function in Photoshop CS5. The sections were then drawn from the photomontage. The outline of the sections and OB glomeruli (GSIB4 labeling), as well as DA+ processes and cells were then drawn with precision on Illustrator CS5 software (Adobe Systems). The accuracy of the illustrations was validated under the microscope. The red labeling was transformed to magenta in Photoshop CS5. The only other changes made to the images were brightness and contrast adjustments.

2.1.4.3 Electrophysiological experiments

Whole brain preparations from larval specimens were isolated *in vitro* as described above and pinned down in an experimental chamber (total volume = 50 ml) continuously perfused with a Ringer's solution at a rate of 4 ml/min and maintained between 8 and 13 °C. A minimum of 60 min of postoperative recovery preceded the electrophysiological recordings. Intracellular recordings of RS cells were performed with sharp glass microelectrodes (filled with KAc 4 M; 80-120 MΩ). The electrical signal was amplified using an Axoclamp 2A amplifier (Axon Instruments, Union City, CA, USA) coupled to a Digidata 1200 (Axon Instruments) and stored on a computer using *Axoscope* software (Axon Instruments, Version 9.2.1.8). The RS cells analysed in this study had a membrane potential under -70 mV that was stable for a minimum of 15 minutes after impaling the cell. The larger Müller cells (B1, B3 and B4) were usually the cells that were recorded for reproducibility of the results. The ON was electrically stimulated with homemade glass-coated tungsten microelectrodes (4-5 MΩ; 30-50 µm tip exposure) connected to a Grass S88 stimulator coupled to a Grass PSIU6 photoelectric isolation unit for controlling the stimulation intensity (Astro-Med Inc., Longueuil, Canada). Trains of 1-3 pulses (50 Hz, 2 ms duration, 2-30 µA) were applied during resting activity of RS cells. To prevent desensitization, a minimum of 50 s was allocated between each train and the stimulation intensity was kept at threshold level to generate responses.

2.1.4.3.1 Drug application

Drugs were pressure ejected through glass micropipettes (tip diameter: 10-20 µm) positioned directly in the OB tissue. The ejection micropipette was inserted from the lateral OB and pressure ejections were delivered by a *Picospritzer II* (Parker Hannifin, General Valve Division, Fairfield, NJ, USA). An average of 31 ± 30 pulses of 20-30 ms duration at about 4 psi were delivered, yielding mean ejection volumes of 1.96 ± 1.90 nl. The volume ejected by a single pressure pulse was estimated by measuring the radius of a droplet ejected in the air from the tip of the pipette and using the equation for the volume of a sphere. Adding the inactive dye Fast Green to the drug solution allowed to visually monitor the diffusion in the tissue. The injections were centered on the medOB and did not exceed 300 µm in diameter.

The following drugs were used in this study: Dopamine hydrochloride (1.0 mM; non-selective DA receptor agonist; Sigma-Aldrich, St-Louis, MO); Dihydrexidine hydrochloride (0.1 mM; selective

D1 receptor agonist; Tocris Bioscience, Bristol, UK); (−)-Quinpirole hydrochloride (0.1 mM; selective D2 receptor agonist; Sigma-Aldrich, St-Louis, MO); R(+)-SCH-23390-hydrochloride (0.5 mM; selective D1 receptor antagonist; Sigma-Aldrich, St-Louis, MO); Raclopride (0.1 mM; selective D2/D3 receptor antagonist; Tocris Bioscience, Bristol, UK); Gabazine (local: 0.1 mM; selective $GABA_A$ receptor antagonist; Tocris Bioscience, Bristol, UK). All drugs were dissolved in Ringer's solution and kept at -20 °C (or 4 °C for less than 7 days) until application.

2.1.4.3.2 Data analysis

Electrophysiological data were analyzed with Spike2 software (Cambridge Electronic Design, Version 5.19) and a homemade script for excitatory postsynaptic potentials (EPSPs). Statistical analyses were carried out on Sigmaplot (Systat Software Inc., Version 11.0). One-way ANOVA for repeated measures or Friedman ANOVA on ranks for repeated measures were used and followed by multiple comparison procedures (Holm-Sidak or Tukey) to test equality of means in the different treatments (Control – Drug – Washout). For all statistical analyses, a significance level of 0.05 was adopted. Results are presented as the mean ± standard deviation.

2.1.5 Results

2.1.5.1 Dopamine immunofluorescence in the olfactory bulb

An immunofluorescence procedure was performed in 12 larval, 6 newly-transformed adult, and 11 spawning-phase adult lampreys of both sexes to analyze the distribution of DA and TH immunoreactivity in the OB. The most notable feature brought to light was the presence of two distinct types of processes that were differentially distributed in the OB (arrows in Fig. 6).

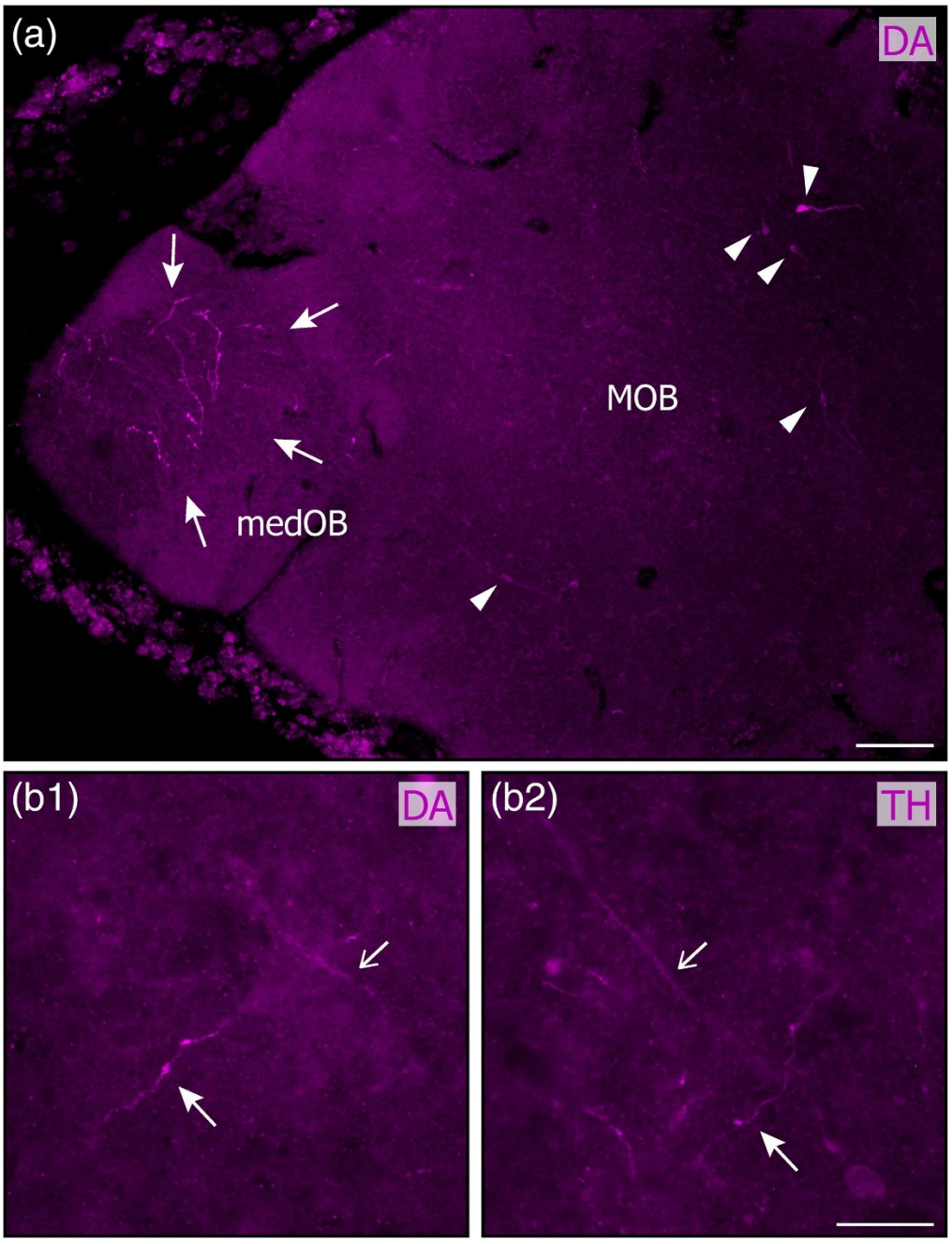

Figure 6. Dopaminergic processes in the olfactory bulb of lampreys.

(**a**) Low magnification photomicrograph showing immunofluorescence against dopamine (DA) on an olfactory bulb transverse section from a spawning-phase adult sea lamprey. Strongly labeled DA+ processes are mostly present in the medial part of the olfactory bulb (medOB) particularly in the area delimited by arrows. Immunoreactive somata (arrowheads) with weakly labeled processes are observed in the main olfactory bulb (MOB). (**b**) Photomicrographs illustrating two types of processes in the same picture frame (weakly labeled: empty arrow, strongly labeled: solid arrow) in the granular layer, after DA (**b1**) and tyrosine hydroxylase (TH; **b2**) immunofluorescence. Scale bar for **(a)**: 100 µm; Scale bar for **(b)**: 25 µm.

The first type of processes possessed varicose and were strongly labeled. They were readily seen under epifluorescence microscopy, even at low magnification (arrowheads in Fig. 6). These strongly labeled processes were preferentially located medially and caudally within the OB, although scarce and isolated processes were found in all bulbar regions (Figs. 6 and 7). Interestingly, these processes were found at all life stages, from larvae to spawning-phase adults, with very similar characteristics and distribution in each case. Moreover, processes with the same characteristics were labeled after immunofluorescence against TH (arrows in Fig. 6b). Most of the strongly labeled DA+ and TH+ processes were located around and inside the medOB, close to projection neurons and primary olfactory afferents. Projection neurons of the medOB were then retrogradely labeled to examine their relationship with DA+ processes (blue in Figs. 7, 8 and 9). Following injection in the PT, biocytin-labeled projection neurons were restricted to the medOB, although in larvae most were clustered more caudally than in adults (Figs. 7 and 8). Varicose DA+ processes were observed in the periphery and inside of the medOB, among the GSIB4-labeled primary olfactory afferents and in close proximity to projection neuron somata and dendrites. Caudal to the medOB, the DA+ processes coursed along the axons of the medOB projection neurons (Fig. 9). It was impossible to follow them distinctly in the septum, which contains a dense plexus of similarly labeled DA+ processes. No DA+ processes co-labeled by biocytin injection in the PT were seen in the OB. The combined staining of primary olfactory afferents, projection neurons and DA in the medOB suggests strongly that DA+ processes innervate elements of the medOB at every developmental stage.

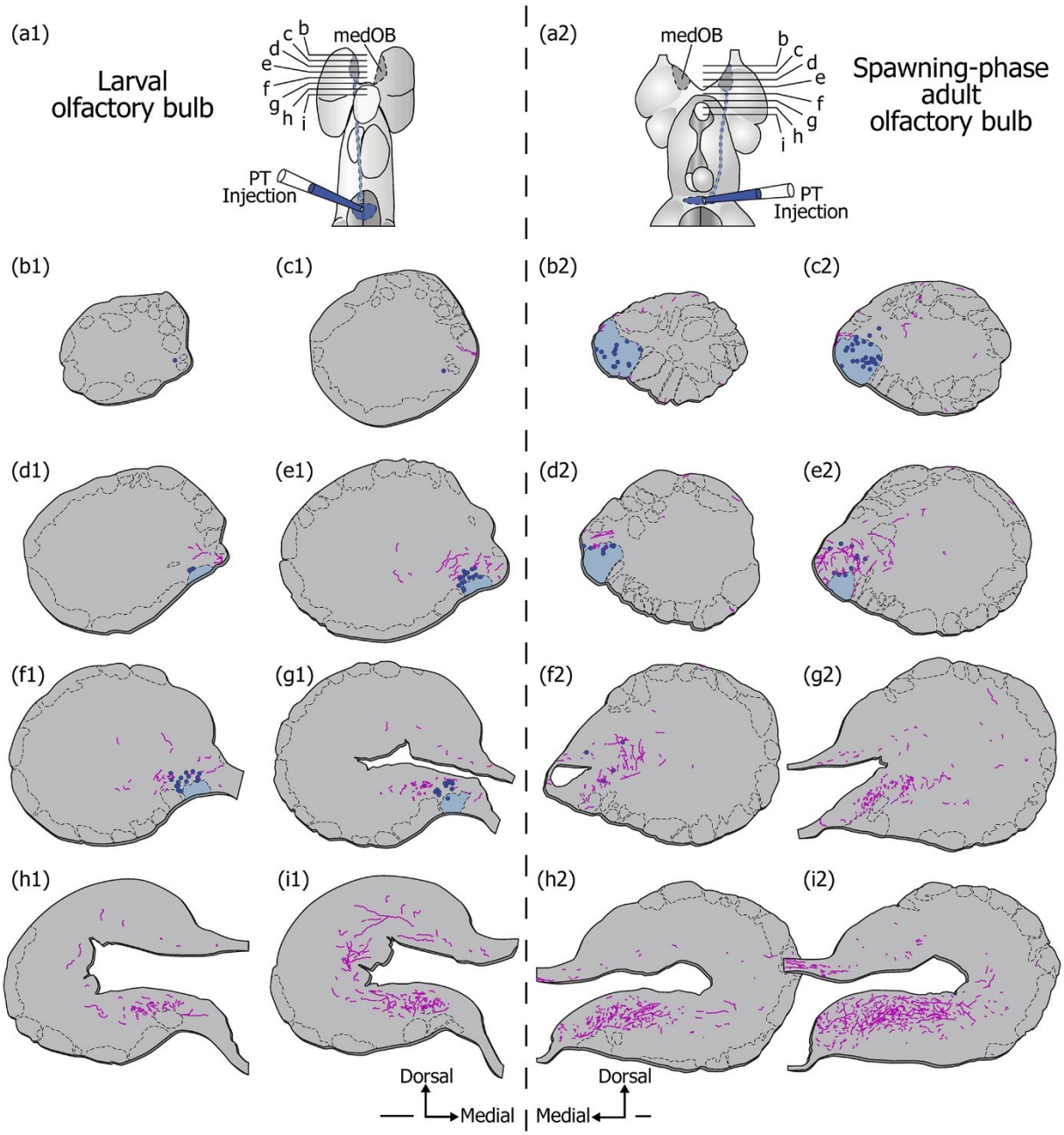

Figure 7. Localization of strongly labeled dopaminergic processes in the olfactory bulb of larval and adult lampreys.

Drawings of serial transverse representative hemi-sections of the larval (left) and spawning-phase adult olfactory bulb at corresponding rostro-caudal levels. The schematic dorsal view of the prosencephalon (**a**) shows the rostro-caudal level of the sections represented (**b-i**). Strongly labeled dopaminergic (DA) processes were drawn as red lines and the olfactory glomeruli, visualised with GSIB4 labeling, are delimited with black dashed lines. Biocytin injection in the posterior tuberculum (PT) backfilled projection neurons (enlarged blue dots) in the medial olfactory bulb

(medOB). The medOB glomerulus (colored in blue) corresponds to the overlapping of the GSIB4 labeling and the arborization of the dendrites from the backfilled projection neurons. Scale bars: 100 µm; Distance between sections: 25 µm (larva) and 75 µm (spawning-phase adult).

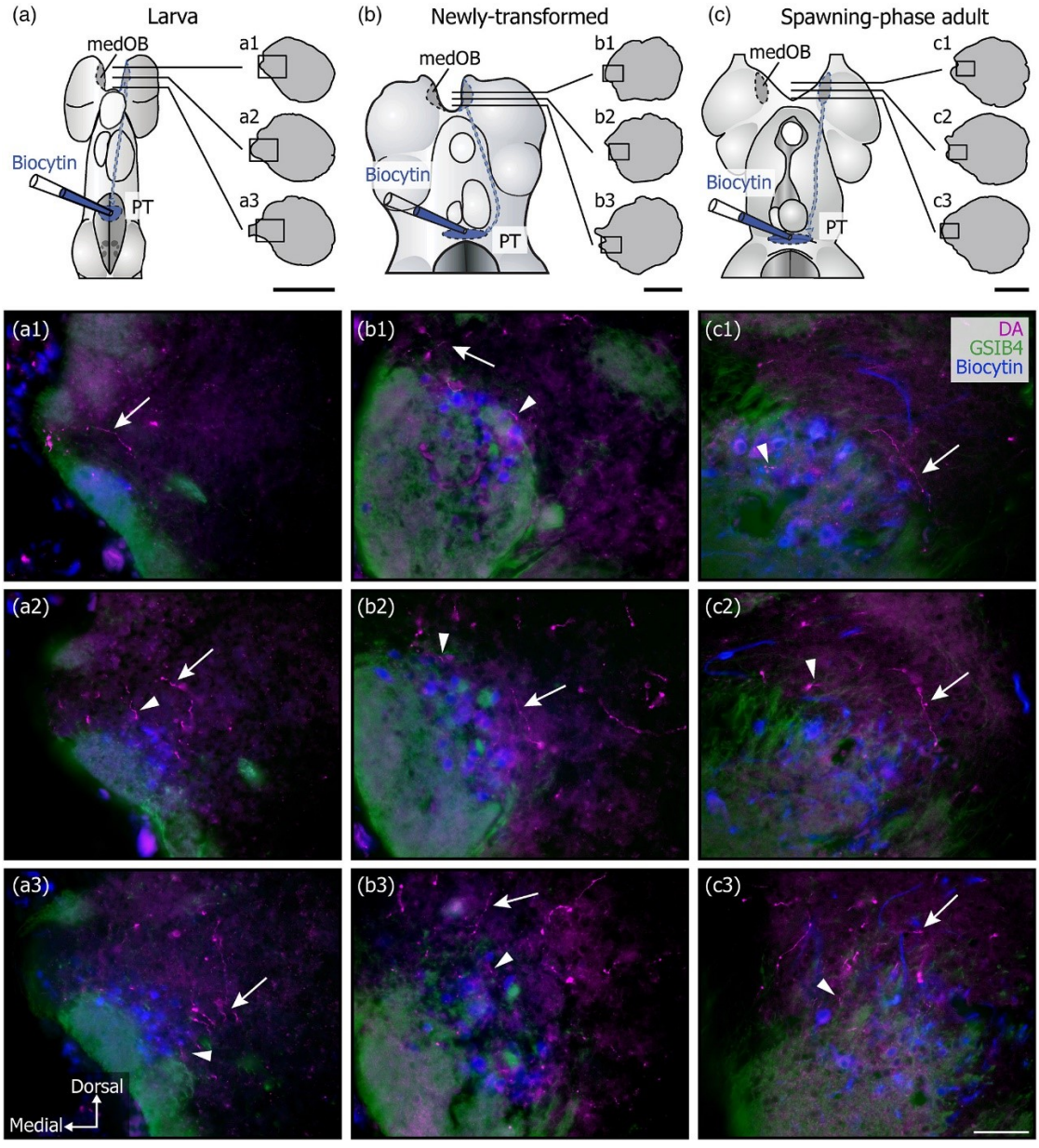

Figure 8. Strongly labeled dopaminergic processes in the medOB.

Photomicrographs of the medial olfactory bulb at three different developmental stages: larval (**a**), newly-transformed (**b**) and spawning-phase adult (**c**), at three different rostro-caudal levels (**1**, **2**,

3). The top three panels contain a schematic dorsal view of the prosencephalon showing the levels at which the photomicrographs were taken (black boxes on the corresponding hemi-section drawings). Photomicrographs are the result of merging three different labelings: dopamine (DA) immunofluorescence (magenta); GSIB4 binding to primary olfactory afferents that form the glomeruli (green); and biocytin labeling (blue) of medOB projection neurons and their dendrites following injection in the posterior tuberculum (PT). Strongly labeled dopaminergic processes are seen within (arrowheads) and in the periphery of (arrows) the medOB glomerulus, close to projection neurons and primary olfactory afferents. Scale bars for hemi-section drawings: 500 µm; Scale bar for photomicrographs (**a1-c3**): 50 µm.

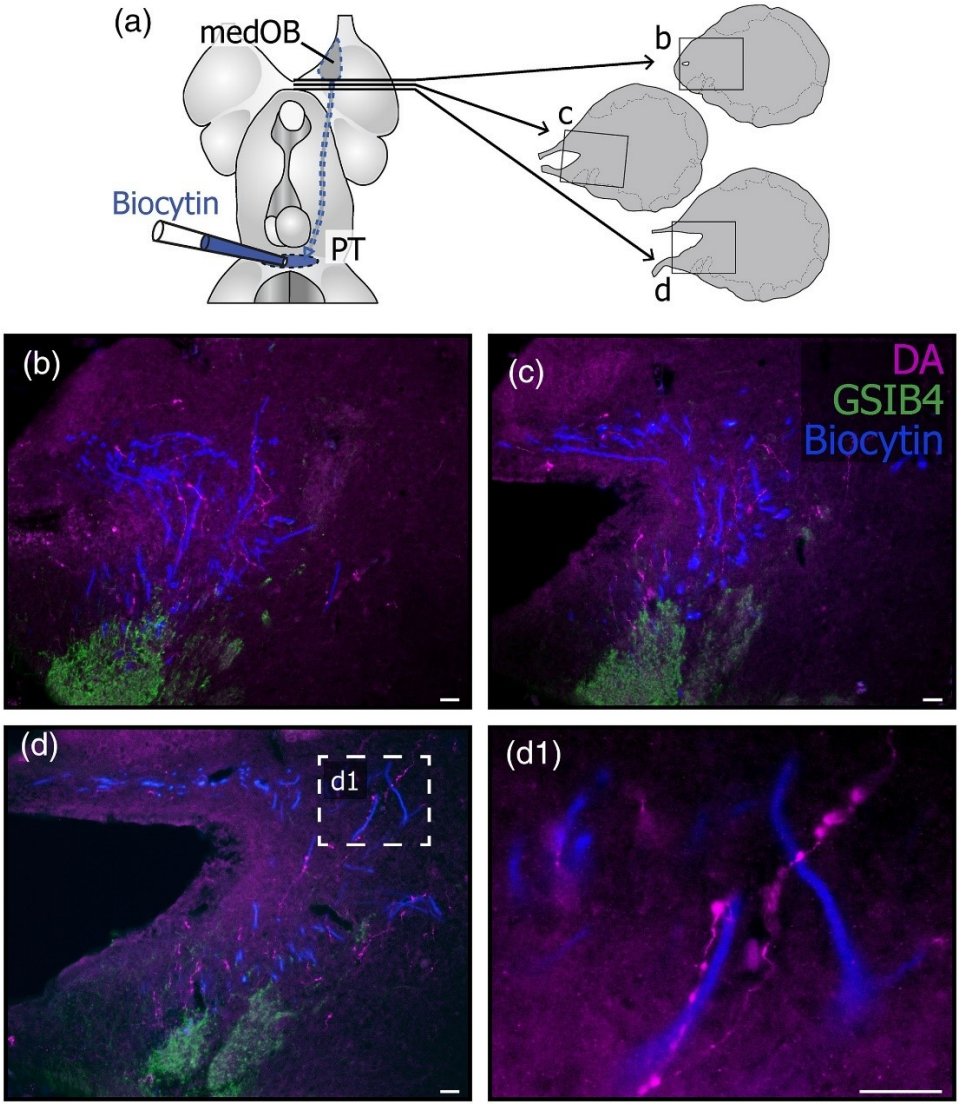

Figure 9. Descending axons from medOB projection neurons and their relationship with strongly labeled dopaminergic processes.

Projection neurons of the medOB send large diameter descending axons (blue in **b-d**) to the posterior tuberculum (PT). Those descending axons travel alongside strongly labeled dopaminergic (DA) processes (magenta in **b-d**) presumably ascending to the OB. Primary olfactory afferents were labeled in green with GSIB4. (**a**) Schematic dorsal view of the spawning-phase adult prosencephalon showing the biocytin injection site and the rostro-caudal levels (black lines) corresponding to transverse hemi-sections illustrated in **b-d**. (**b**-**c**) Photomicrographs showing descending large axons (blue) that originate from more rostrally-located projection neurons of the medOB. Upon leaving the medOB caudally, these axons are accompanied by DA+ processes (magenta). (**d**) Photomicrograph of a transverse section, slightly more caudal than those in **b**-**c**. The large axons from the medOB projection neurons (blue) are now divided into a dorsal and a ventral tract, both containing varicose, strongly labeled DA+ processes (magenta). (**d1**) High magnification of the boxed area in (**d**) shows the proximity of the two types of processes. Scale bars: 25 μm.

In the OB of newly-transformed and spawning-phase adults, DA immunofluorescence yielded additional labeling. Notably in adult lampreys, a second type of DA+ processes was detected (Fig. 6 and 10). These processes were in sharp contrast with those previously described: they were weakly labeled, did not display varicosities, and were spread homogeneously across the granular layer. Isolated processes also occasionally reached the glomerular layer (arrows in Fig. 10c2, d2).

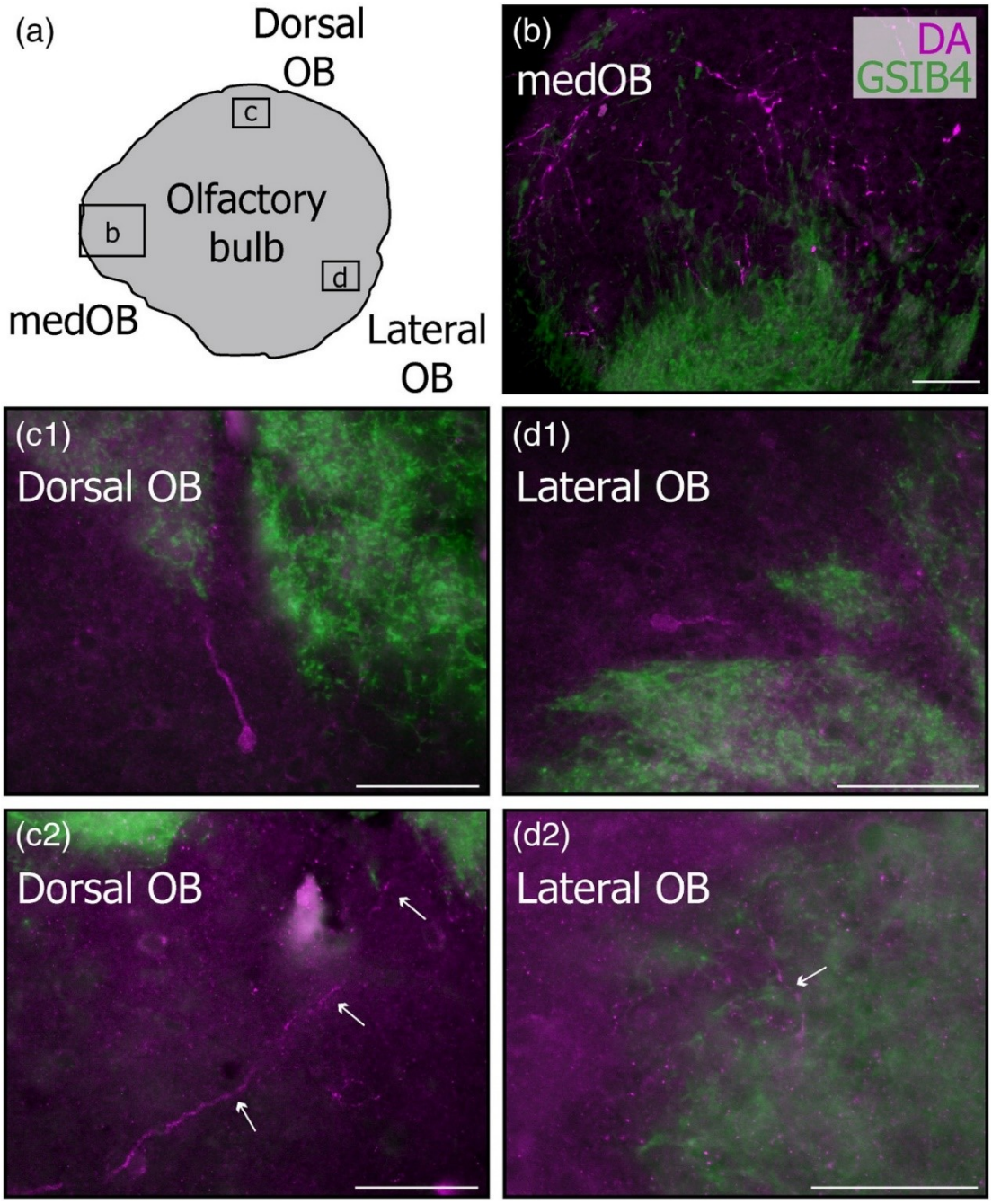

Figure 10. Dopaminergic processes in glomerular territories of the olfactory bulb.

(**a**) Drawing of a transverse section of a spawning-phase adult right olfactory bulb, illustrating the approximate localizations (boxed areas) of the photomicrographs in **b-d**, which were taken from different animals. Dopaminergic (DA) cells (**c1**, **d1**) and processes (**b**, **c2**, **d2**; arrows) are seen close to olfactory glomeruli. (**b-d**) are merged photomicrographs showing labeled olfactory

primary afferents in glomeruli (GSIB4, green) in combination with DA immunofluorescence (magenta). Scale bars: 50µm.

In addition, processes with same characteristics were often seen originating from local cell bodies, which were also exclusively observed in newly transformed and spawning phase adults. These somata had a similar, weak labeling intensity, and were small-sized (10-15 µm), round or ovoid and often bipolar (Fig. 11). From the most caudal to the most rostral levels, they were homogeneously scattered throughout the granular cell layer. They were occasionally seen bordering glomeruli in the dorsal and lateral OB, extending a process into the glomerular neuropil (Fig. 10c1, d1). After immunofluorescence against TH, very similar neurons were consistently observed; they showed a more intense labeling, making them easier to visualize. They exhibited similar size, shape, and distribution when compared to the DA+ cell bodies described above (Fig. 11), the only difference being that a greater number of TH somata could be visualized. Moreover, despite the absence of DA+ somata and weakly labeled processes in larval specimens, both were observed consistently in larvae following TH immunofluorescence.

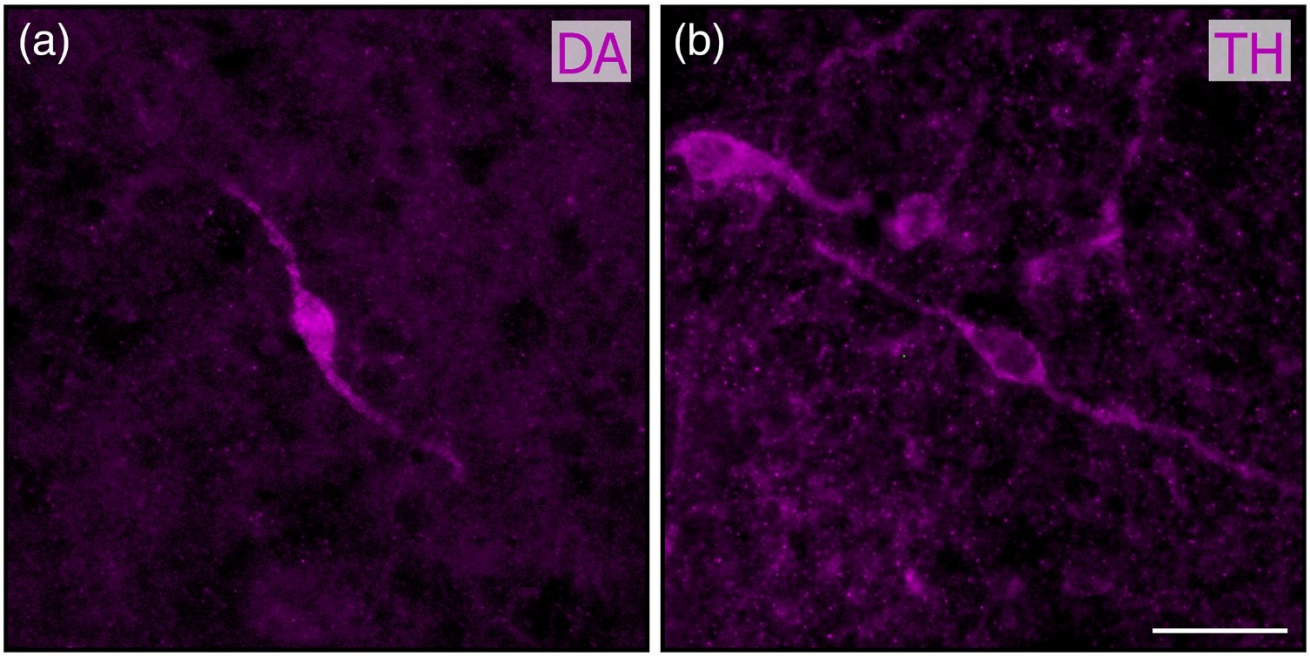

Figure 11. Dopamine and tyrosine hydroxylase immunoreactive cell bodies in the olfactory bulb.

High magnification photomicrographs showing neuronal cell bodies labeled after immunofluorescence against dopamine (DA; **a**) and tyrosine hydroxylase (TH; **b**) in the granular layer of the olfactory bulb in spawning-phase adults. Scale bar: 25 μm.

2.1.5.2 Dopaminergic afferents to the medial olfactory bulb

In the adult OB, the weakly labeled DA+ processes could stem from local neurons, since they displayed the same labeling intensity than the DA+ cell bodies and their associated processes in the granular layer. However, no labeled cell bodies were found to be as intensely labeled as the strongly labeled processes. It is possible that an extrinsic source of DA innervation to the OB exists. To determine the presence of such an extrinsic source of DA+ processes, biocytin injections in the medOB were combined with immunofluorescence targeting DA. In spawning-phase adults (n = 12), retrogradely-labeled DA+ neurons were detected in the granular layer of the OB (Fig. 12a), in the dorsal hypothalamic nucleus (DHN, Fig. 12b) and in the PT (Fig. 12c). In larvae (n = 13), DA+ neurons were also retrogradely-labeled in the DHN (Fig. 13b) and the PT (Fig. 13c) but not in the OB, since they are undetected at this developmental stage (see above). In combination with TH immunofluorescence (n = 12 larvae), medOB injections labeled numerous TH+ neurons in the OB (Fig. 13a). In the DHN and PT, TH+ neurons were also labeled by biocytin injections and were similar in morphology and localization to those observed with immunofluorescence against DA. The double-labeled neurons in the DHN were cerebrospinal fluid-contacting cells. Those observed in the PT were within the DA cell population described as homologous to the mammalian substantia nigra, pars compacta and ventral tegmental area (SNc/VTA; Pombal et al., 1997).

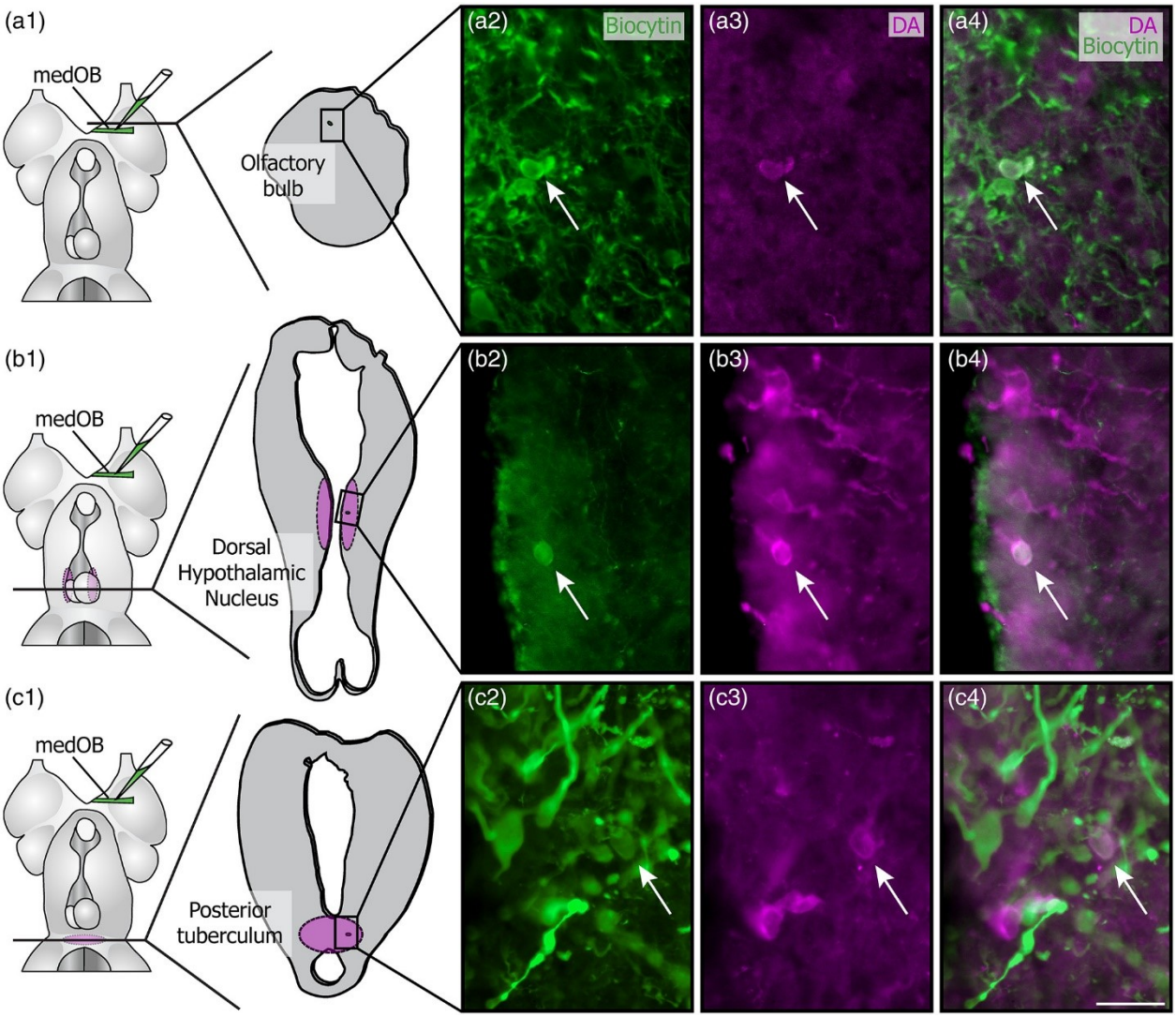

Figure 12. Origins of the dopaminergic projections to the medOB in spawning-phase adults.

Retrograde axonal tracing from the medial olfactory bulb (medOB) was combined with dopamine (DA) immunofluorescence to label neurons responsible for the DA+ processes in the medOB. The schematic dorsal views of the spawning-phase adult lamprey prosencephalon (**a1-c1**) illustrate the biocytin injection site and the levels at which double-labeled neurons were observed. The drawings of the corresponding transverse sections also show the frame of the photomicrographs to the right. Pictures in **a4**, **b4** and **c4** are a merge of **a2-a3**, **b2-b3** and **c2-c3**, respectively. These images show examples of neurons (arrows) in the olfactory bulb (**a**), dorsal hypothalamic nucleus (**b**) and posterior tuberculum (**c**) labeled by both DA immunofluorescence and biocytin injection in the medOB. Scale bars: 25µm.

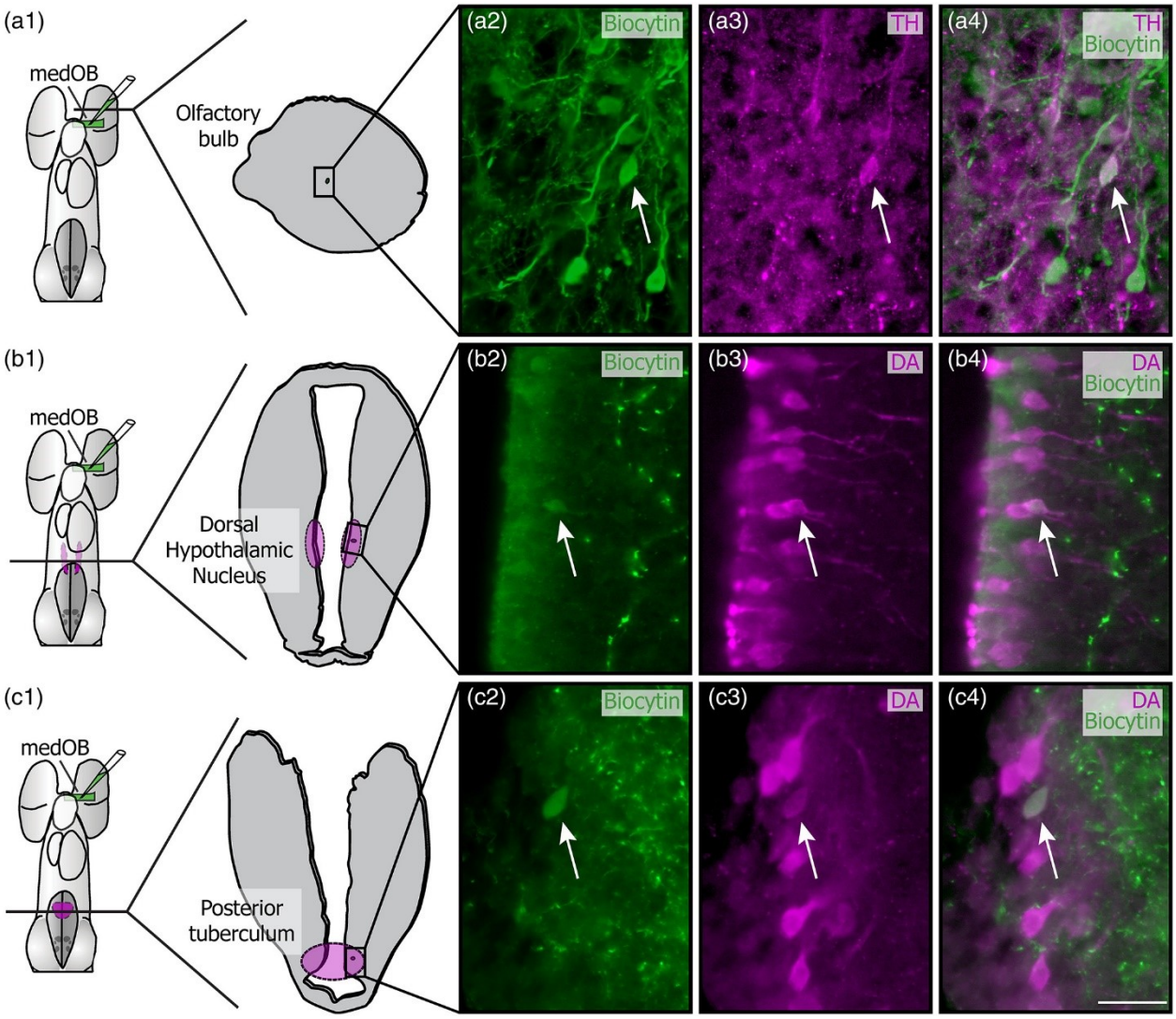

Figure 13. Origins of the dopaminergic projections to the medOB in larvae.

Retrograde axonal tracing from the medial olfactory bulb (medOB) was combined with tyrosine hydroxylase (TH; **a**) or dopamine (DA; **b**, **c**) immunofluorescence to label neurons responsible for the DA+ processes in the medOB. Immunofluorescence against TH was used in the olfactory bulb of larvae because immunofluorescence against DA did not yield any labeling of local cell bodies. The schematic dorsal views of the larval prosencephalon (**a1-c1**) illustrate the biocytin injection site and the levels at which double-labeled neurons were observed. The drawings of the corresponding transverse sections also show the frame of the photomicrographs to the right. Pictures in **a4**, **b4** and **c4** are a merge of **a2-a3**, **b2-b3** and **c2-c3**, respectively. These images show examples of neurons (arrows) in the olfactory bulb (**a**), dorsal hypothalamic nucleus (**b**) and

posterior tuberculum (**c**) labeled by both DA (or TH) immunofluorescence and biocytin injection in the medOB. Scale bars: 25µm.

2.1.5.3 The effect of dopamine on reticulospinal cell responses induced by olfactory inputs

Our anatomical results altogether suggest that DA+ processes from extrinsic sources (DHN and PT) innervate the medOB in addition to intrinsic sources (OB). The next step was to investigate whether the DA innervation modulates the transmission in the OB. In the larval OB, which did not contain DA+ somata or weakly labeled processes, strongly labeled DA+ processes were more densely localized close to and inside the medOB. Hence, electrophysiological experiments were carried out to characterize the physiological effects of DA on the medial olfactomotor pathway (Derjean et al., 2010). Projection neurons in the medOB send direct output to the PT and to the MLR, which relay the signal to RS cells to generate locomotor activity (Derjean et al., 2010; Daghfous et al., 2018). The recording of RS cells, which act as command cells for locomotion, can monitor this activity. Experiments were performed in the *in vitro* isolated larval brain to test the effects of DA microinjection in the medOB on RS cell synaptic responses to olfactory nerve (ON) stimulation.

Reticulospinal cell responses to electrical stimulation (1-3 pulses at 50 Hz, 2 ms duration, 10-30 µA) of the ON were recorded intracellularly. Local pressure microinjection of DA (1 mM) in the medOB (Fig. 14a-c) induced a significant reduction in the amplitude (to 63.4 ± 24.9%, F = 43.996, df = 2, $P < 0.001$, n = 9 cells in 9 larvae) and the area (to 52.8 ± 66.6%, $\chi^2 = 42.481$, df = 2, $P < 0.001$, n = 9 cells in 9 larvae) of the EPSPs. The responses recovered to control level after washout (amplitude: t = 0. 581, $P = 0.563$; area: q = 0.680, $P \geq 0.05$). Similar experiments were then performed in newly-transformed animals to determine if DA modulation also occurs in adults (Fig. 14d-f). As in larval animals, the RS cell responses were significantly reduced in amplitude (to 56.6 ± 30.4%; F = 29.825; df = 2; $P < 0.001$, n = 5 cells in 5 newly-transformed adults) and area (to 43.5 ± 45.4%; F = 22.153; df = 2; $P < 0.001$, n = 5 cells in 5 newly-transformed adults) by DA microinjection in the medOB. There were no significant differences between control and washout (amplitude: t = 0.125, $P = 0.901$; area: t = 0.557, $P = 0.580$).

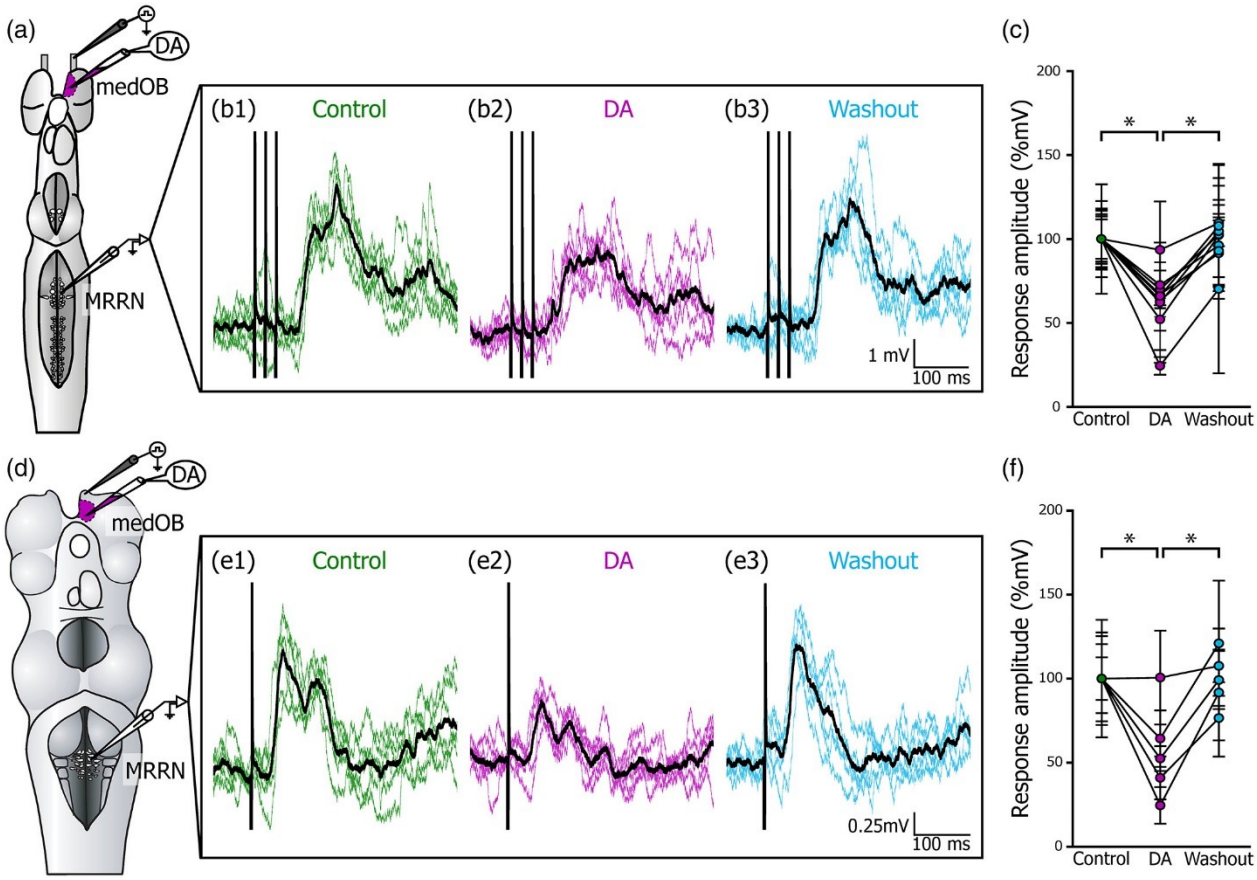

Figure 14. Effect of dopamine injection in the medOB on RS cell responses.

The schematic dorsal views of the isolated larval (**a**) and newly-transformed adult (**d**) brains illustrate the preparation where synaptic responses to electrical stimulation of the olfactory nerve were intracellularly recorded in ipsilateral reticulospinal neurons. (**b1-b3**; **e1-e3**) Responses evoked by electrical stimulation (black vertical bars: stimulation artifact) are represented as six superimposed traces (colored) and their mean (thick black trace). Compared to control conditions (**b1**, **e1**), responses were decreased following dopamine (DA) injection (1 mM) in the medial olfactory bulb (medOB) (**b2**, **e2**), and this effect was reversed after washout (**b3**, **e3**). (**c**, **f**) Mean response amplitudes during each condition are plotted as a line graph for every recorded RS cell (**c**, n = 9 cells in 9 larvae; **f**, n = 5 cells in 5 newly-transformed adults). MRRN: Middle rhombencephalic reticular nucleus. * $p < 0.001$.

We also tested the effects of DA on suprathreshold responses to get a better indication on whether DA also affects motor behavior. Previous work from our group has shown that blocking $GABA_A$

receptors in the OB with gabazine amplifies considerably RS cell responses to electrical stimulation of the ON (Daghfous et al., 2018). Therefore, the effects of DA were tested on amplified RS responses to stimulation of the ON (Fig. 15). Microinjection of gabazine (0.1 mM) in the medOB markedly amplified RS cell responses to ON stimulation, as previously described (Daghfous et al., 2018). A subsequent DA microinjection in the medOB significantly reduced the synaptic response amplitude (to 49.2 ± 16.8%; $\chi^2 = 42.467$, df = 2, $P < 0.001$, n = 5 cells in 5 larvae) and area (to 30.2 ± 18.3%; $\chi^2 = 46.067$, df = 2, $P < 0.001$, n = 5 cells in 5 larvae) (Fig. 15b-c) and the responses recovered to control values after DA washout (amplitude: q = 0.913, $P \geq 0.05$; area: q = 1.461, $P \geq 0.05$). Moreover, after gabazine microinjection, RS cells often displayed spiking activity in response to ON stimulation (Fig. 15d). Under these conditions, microinjection of DA in the medOB could reduce the excitatory responses in RS cells to the point of eliminating spiking activity. Therefore, the local effects of DA in the medOB may significantly affect motor output.

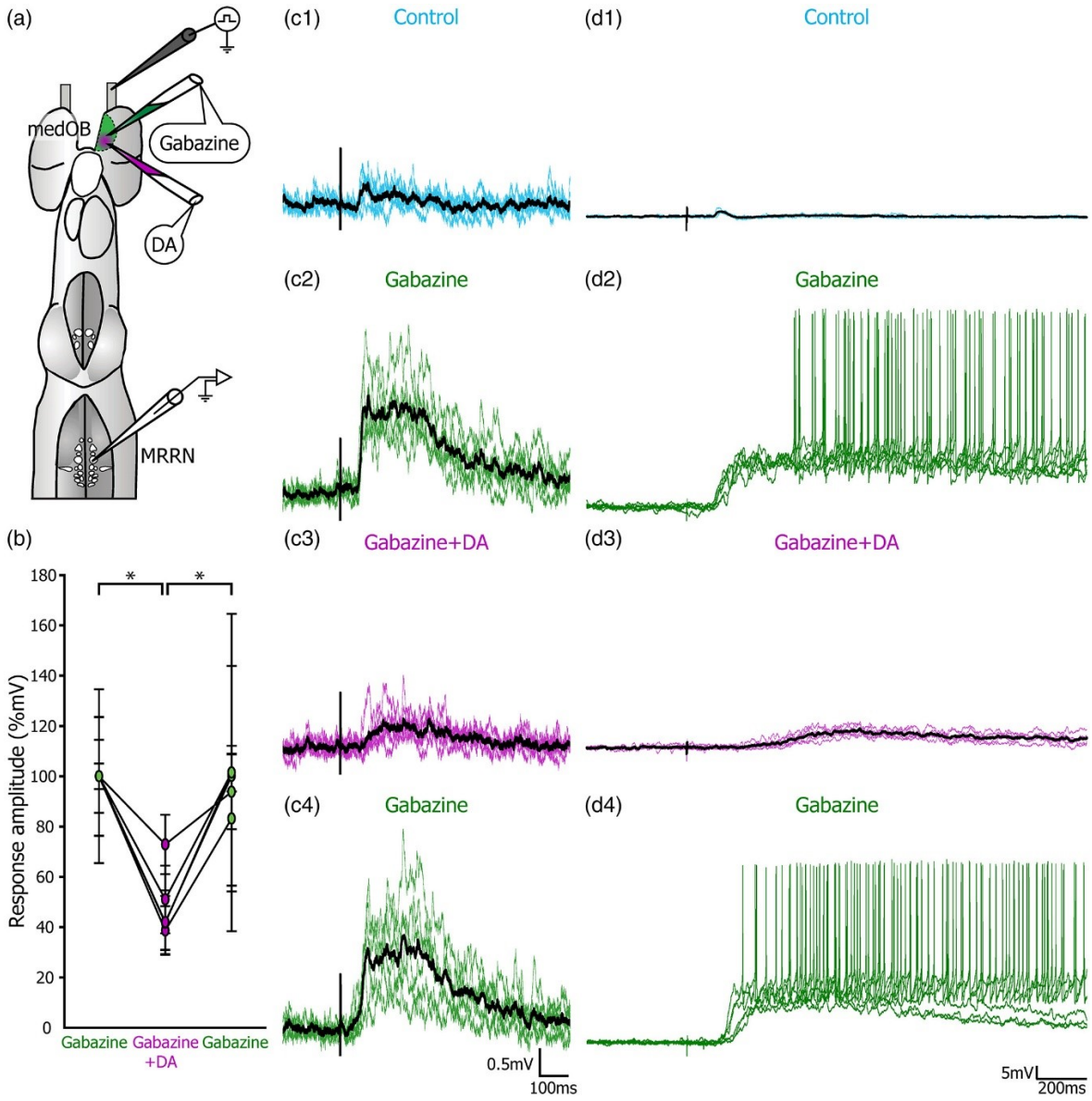

Figure 15. Effect of dopamine injection in the medOB on RS cell responses with prior removal of local medOB GABAergic inhibition with gabazine.

(**a**) Schematic illustration showing the experimental procedure where $GABA_A$ receptor antagonist gabazine (0.1 mM) was periodically injected in the medial OB (medOB) to disinhibit olfactory nerve (ON) stimulation-evoked synaptic responses in reticulospinal (RS) cells. When dopamine (DA) and gabazine are simultaneously injected in the medOB, the mean response amplitude is significantly decreased (n = 5 cells in 5 larvae). (b) Mean response amplitudes during each condition are plotted as a line graph for every recorded RS cell. **c** illustrates a representative example of RS cell sub-threshold responses under control conditions (**c1**), gabazine injection (**c2**),

gabazine and DA injection (**c3**), and after washout of DA but still under gabazine (**c4**). Another example shows RS cell supra-threshold responses under the same conditions (**d1-d4**). Colored traces are a superimposition of six responses to ON stimulation and their mean is represented by a thicker black trace. Although local injection of gabazine in the medOB amplifies RS cell synaptic responses, a combined DA injection induces a marked decrease of these sub-threshold (**c**) or supra-threshold (**d**) responses, which is reversed after washout of DA. MRRN: Middle rhombencephalic reticular nucleus. * $p < 0.05$.

2.1.5.4 The role of D1 and D2 receptors in modulating olfactomotor transmission

To further characterize the action of DA on olfactomotor transmission, ligands selective for D1 or D2 receptor were pressure-injected in the medOB (Fig. 16). A local microinjection of a D1 receptor agonist, dihydrexidine (0.1 mM), caused a significant decrease of RS cell responses to ON stimulation (Fig. 16c). Depression of both amplitude (to 71.5 ± 36.0%; F = 11.180, df = 2, $P < 0.001$, n = 6 cells in 6 larvae) and area (to 64.7 ± 87.0%; $\chi^2 = 6.222$, df = 2, $P = 0.045$, n = 6 cells in 6 larvae) was observed and no significant differences were detected between control and washout of dihydrexidine (amplitude: t = 0.329, $P = 0.743$; area: q = 0.943, $P \geq 0.05$). However, local microinjection of a D1 receptor antagonist, SCH 23390 (0.5 mM), did not significantly change the amplitude (F = 0.263, df = 2, $P = 0.770$, n = 5 cells in 5 larvae) or the area (F = 2.269, df = 2, $P = 0.113$, n = 5 cells in 5 larvae) of the RS cell responses (Fig. 16d). D2 receptor ligands had more significant effects on RS cell responses. Indeed, a D2 receptor agonist, quinpirole (0.1 mM), induced a marked reduction of RS cell responses (Fig. 16e), depressing their amplitude (to 48.7 ± 18.3%; F = 43.081, df = 2, $P < 0.001$, n = 6 cells in 6 larvae) and area (to 15.8 ± 45.4%; $\chi^2 = 32.889$, df = 2, $P < 0.001$, n = 6 cells in 6 larvae). No significant differences were observed between control and washout of quinpirole (amplitude: t = 0.672, $P = 0.504$; area: q = 0.667, $P \geq 0.05$). Furthermore, a D2 receptor antagonist, raclopride (0.1 mM), had the opposite effect (Fig. 16f), increasing both response amplitude (to 149.4 ± 48.8%; F = 17.888, df = 2, $P < 0.001$, n = 5 cells in 4 larvae) and area (to 170.4 ± 82.3%; $\chi^2 = 8.467$, df = 2, $P = 0.015$, n = 5 cells in 4 larvae). No significant differences were observed between control and washout of raclopride (amplitude: t = 0.427, $P = 0.671$; area: q = 0.183, $P \geq 0.05$). While microinjection of DA receptor agonists (DA, dihydrexidine and quinpirole) in the medOB reduced the RS cell responses to electrical ON stimulation,

raclopride induced an increase of their amplitude. Taken together, these results suggest that DA modulates olfactomotor transmission in the medOB.

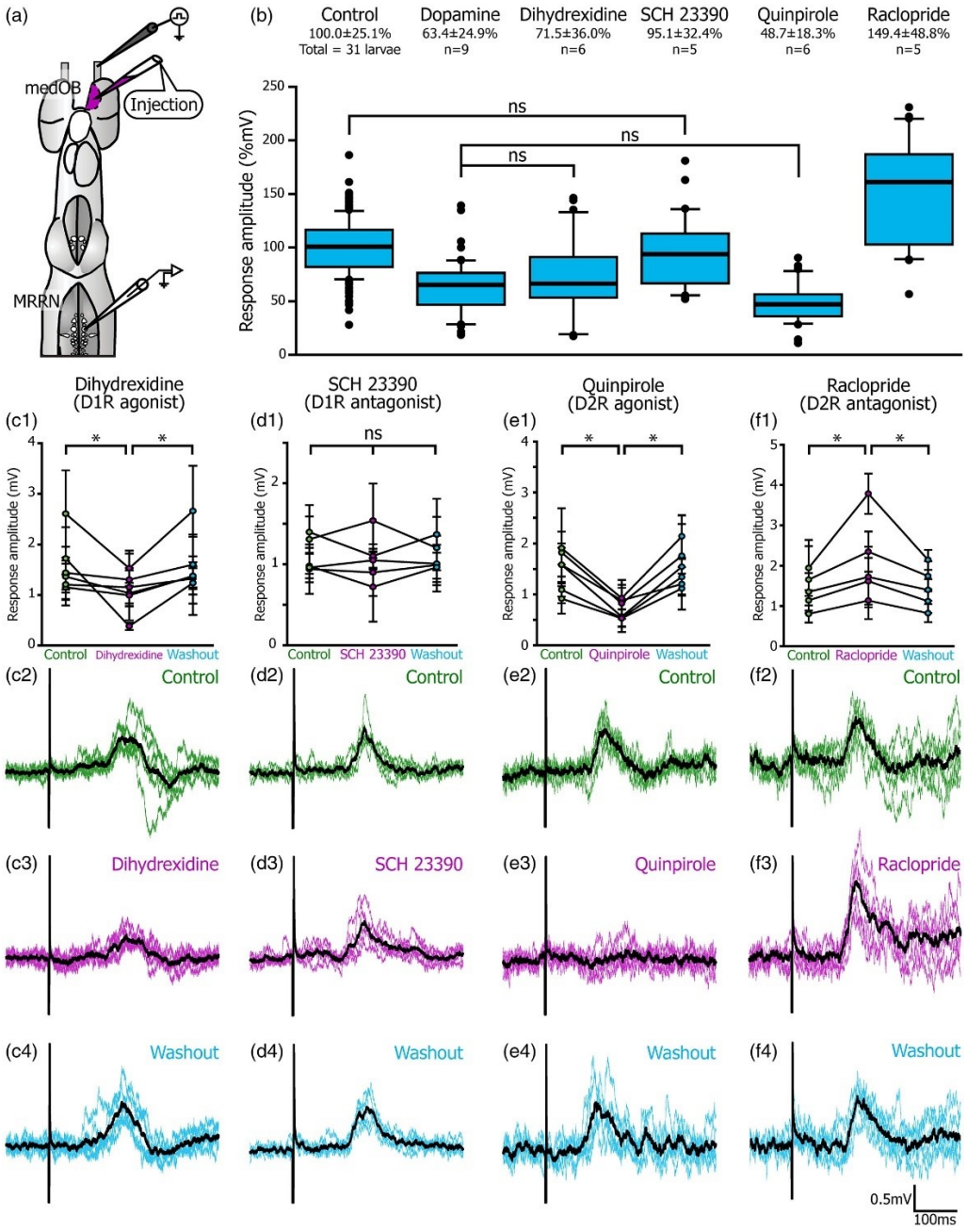

Figure 16. Effects of D1 and D2 receptor agonists and antagonists injection in the medOB on RS cell responses.

The physiological effects of selective dopamine (DA) receptor ligands injection in the medial olfactory bulb (medOB) on reticulospinal (RS) cells synaptic responses to olfactory nerve stimulation was studied in isolated larval brains (**a**). The data were compiled in a boxplot (**b**)

representing the relative response amplitude following local drug injections in comparison with control responses from every recording. In addition, the mean response amplitude of individual RS cells before, during and after injection of selective DA receptor agonists or antagonists was plotted as different line graphs (**c1**, **d1**, **e1** and **f1**). Evoked responses from representative recordings are exhibited as six superimposed traces and their mean (thick black trace) during each treatment (**c2-c4**; **d2-d4**; **e2-e4**; **f2-f4**). (**c**) D1 receptor agonist dihydrexidine (0.1 mM) decreased the mean response amplitude to 71.5 ± 36.0% of control (n = 6 cells in 6 larvae). (**d**) D1 receptor antagonist SCH 23390 (0.5 mM) did not produce robust effects or change significantly the mean response amplitude (95.1 ± 32.4% of control, n = 5 cells in 5 larvae). (**e**) Injection of D2 receptor agonist quinpirole (0.1 mM) significantly decreased evoked responses, with a mean amplitude of 48.7 ± 18.3% of control responses (n = 6 cells in 6 larvae). (**f**) D2 receptor antagonist raclopride (0.1 mM) significantly increased response amplitude over control values (mean = 149.4 ± 48.8%, n = 5 cells in 4 larvae). MRRN: Middle rhombencephalic reticular nucleus. * $p < 0.001$.

2.1.6 Discussion

Results from this study show that DA modulates the transmission of olfactory inputs to brainstem motor centers. Abundant DA+ processes were observed in the medial part of the OB and pharmacological manipulation of DA receptors in this region had physiological effects on olfactomotor activity. Local microinjection of DA agonists (DA, dihydrexidine or quinpirole) in the medOB decreased responses of RS cells to ON stimulation. Furthermore, microinjection of raclopride in the OB increased these responses, suggesting that D2 receptors are involved in the modulation of olfactory processing. Because DA+ neurons in the OB, DHN and PT were shown to project to the medOB, these different regions might control the activity of the medial olfactomotor pathway through DA transmission (Fig. 17).

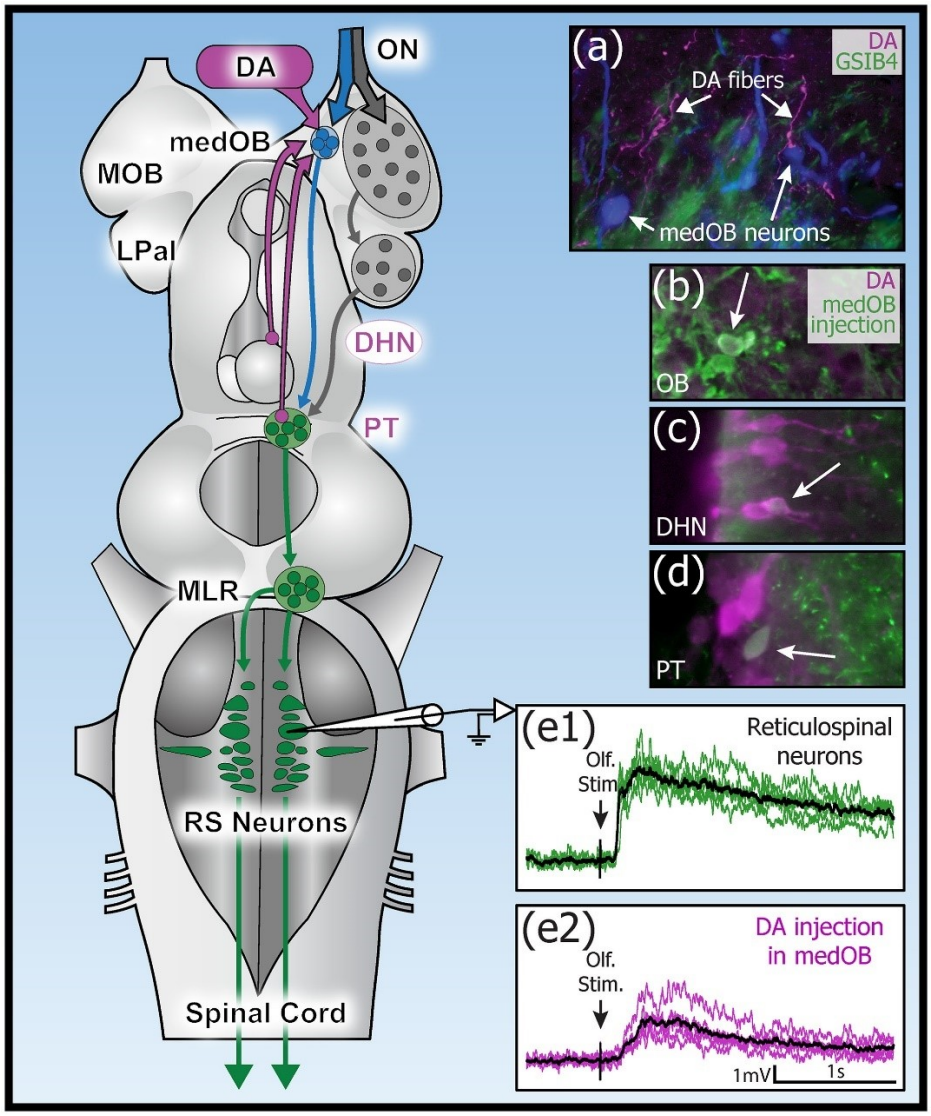

Figure 17. Dopaminergic modulation of the medial olfactory bulb.

Schematic representation of the brain illustrating a medial (blue; Derjean et al., 2010) and lateral (grey; Daghfous et al., 2018) olfactomotor pathway. Both pathways activate the PT which controls downstream locomotor circuitry (green). In the medial pathway, olfactory projection neurons are seen in close proximity to dopaminergic processes (**a**). The DA+ processes in the medial olfactory bulb (medOB) originated from neurons in the OB (**b**), DHN (**c**) and PT (**d**). The synaptic responses evoked by electrical stimulation of the olfactory nerve in RS cells are decreased following a localized dopaminergic agonist injection in the medOB (**e**), suggesting a gating of the transmission of olfactory inputs to the motor system in lamprey. DA, dopamine; DHN, dorsal hypothalamic nucleus; LPal, lateral pallium; medOB, medial olfactory bulb; MLR, mesencephalic locomotor region; MOB, main olfactory bulb; ON, olfactory nerve; PT, posterior tuberculum; RS, reticulospinal.

2.1.6.1 Dopaminergic processes in the lamprey olfactory bulb

Dopaminergic processes have been observed previously in the lamprey OB, from larval to adult stages, both in the river lamprey (*Lampetra fluviatilis* L.) (Baumgarten, 1972; Pierre et al., 1994; Pierre et al., 1997; Pombal et al., 1997; Pierre-Simons et al., 2002; Pérez-Fernández et al., 2014) and in *P. marinus* (Yáñez, et al., 1992; Abalo et al., 2005; Barreiro-Iglesias et al., 2009; Barreiro-Iglesias et al., 2010; Fernández-López et al., 2017). Compared to previous studies, a major difference in DA immunofluorescence observed in the OB was the presence of two distinct populations of DA+ processes with different developmental patterns and anatomical distributions. The processes from a first type were strongly labeled and denser in the medOB. The processes from a second type contrast sharply: they were weakly labeled and only observed in newly-transformed and spawning-phase adults. They seemed to arise from a local population of similarly labeled DA+ neurons, which are also detected only in adult animals.

Our results suggest that the strongly labeled processes of the first type do not originate from local cell bodies. Although it is possible that such strongly labeled processes arose from weakly labeled DA+ somata in the OB, it is more likely that they stemmed from one of the numerous DA cell groups in the diencephalon (Abalo et al., 2005). In our material, these processes appeared to reach the OB from its caudal aspect passing through the septum (see Fig. 7), close to axons from medOB

projection neurons exiting the OB (see Fig. 9). In larval lampreys, the strongly labeled DA+ processes were detected close to and inside the medOB despite the absence of (or failure to detect) local DA+ neurons in the OB. During prolarval development of *P. marinus*, the earliest detection of DA+ processes reaching the telencephalon coincides with that of DA+ neurons in the PT (Abalo et al., 2005). Most importantly, we provide evidence that DA+ neurons in the PT and DHN project to the medOB. However, PT injections did not allow us to observe anterogradely-labeled DA+ processes in the OB, which suggests otherwise. This may be due to a limited number of DA neurons projecting to the medOB from the PT. Although the DA innervation of the OB is traditionally considered to be exclusively local (Smeets & González, 2000), extrinsic projections to the OB were notably observed in rats, where a minor portion of SNc neurons send a direct DA projection to the OB (Höglinger et al., 2015). Furthermore, projections from the SNc and the VTA to the OB were observed in the sheep (Lévy et al., 1999) and PT-OB projections were detected in two species of shark (Yáñez et al., 2011). Also, monoaminergic projections modulating OB sensory processing is common in vertebrates, including noradrenergic fibers from the locus coeruleus (Shipley et al., 1985) and serotoninergic fibers from the raphe nuclei (Broadwell et Jacobowitz, 1976). Hence, in the medOB of lampreys, the DA innervation might originate from extrinsic DA afferents in addition to the intrinsic innervation.

2.1.6.2 Dopaminergic cell bodies in the lamprey olfactory bulb

Our study shows that DA+ somata were present in the granular layer of the OB in adult lampreys, but were not detected in larvae. The absence of DA+ cells in the OB of larval specimens was also reported previously (Yáñez et al., 1992; Pierre-Simons et al., 2002; Abalo et al., 2005), but studies on both *P. marinus* and *L. fluviatilis* have described DA+ neurons in adult specimens (Pierre et al., 1997; Pombal et al., 1997; Barreiro-Iglesias et al., 2009; Fernández-López et al., 2017). The phenotype of these cells was confirmed to be DA (TH+/DOPA decarboxylase+/DA+/dopamine β-hydroxylase-) in an immunoreactivity study (Pierre et al., 1997). Moreover, the same authors did not find somata containing dopamine-β-hydroxylase or phenylethanolamine-*N*-methyltransferase in the OB, suggesting the absence of other catecholaminergic (noradrenergic or adrenergic) neurons.

DA+ and TH+ cell groups had the same morphology: in both cases the cell bodies were small, round or ovoid, and often bipolar with processes that arose in opposite directions. Moreover, these

two cell groups appear late during development since TH+ cells are undetected in the OB during early larval stages (Pierre-Simons et al., 2002) and OB DA+ cells are not observed in larval specimens (Yáñez et al., 1992; Abalo et al., 2005; present results). This developmental pattern could be common in vertebrates, since DA cells of the OB, corresponding to the A16 DA cell group (Björklund et Dunnett, 2007), are among the last DA cell groups to be detected during brain development of lampreys (Pierre-Simons et al., 2002), fishes (Ekström et al., 1992; Manso et al., 1993), reptiles (Medina et al., 1994), birds (Puelles et Medina, 1994), mice (di Porzio et al., 1990), rats (Specht et al., 1981) and humans (Puelles et Verney, 1998). The late development of DA cells in the OB might be conserved in vertebrates and it could explain why DA+ cells are not detected in the lamprey's larval OB.

2.1.6.3 Dopaminergic modulation of the medial olfactory bulb

A single population of projection neurons located inside the medOB receive sensory inputs exclusively from chemosensory cells in the accessory olfactory organ (Green et al., 2017). The medOB projection neurons then project directly to the PT to drive swimming activity through activation of brainstem RS cells (Derjean et al., 2010; Daghfous et al., 2018). Direct medOB projections to the MLR were also observed (Daghfous et al., 2018). We now show that the injection of DA agonists in the medOB reduces the activation of RS cells in response to ON stimulation. The effects were even more powerful when gabazine, a $GABA_A$ receptor antagonist, was injected beforehand in the OB (see Daghfous et al., 2018). The spiking responses in RS cells were then totally suppressed. This suggests that activation of DA receptors in the medOB can lead to a substantially reduced motor output in response to olfactory inputs. This could explain the changes in motor responses to chemical cues that occur during the life cycle of the animal. For example, only during the spawning phase will lampreys respond to migratory pheromones released by larvae (Vrieze et Sorensen, 2001). However, despite dramatic effects in the isolated brain, the effects of DA modulation under more natural conditions are unknown. Future investigations are needed to define how the pharmacological manipulation of DA transmission in the medOB modulates the motor responses to odorants.

Expression of DA receptors was recently characterized in the OB of *P. marinus* and *L. fluviatilis* (Pérez-Fernández, 2013; Pérez-Fernández et al., 2014; Pérez-Fernández et al., 2015). D1 or D2 receptors are expressed on somata in the granular layer. In addition, no D4 receptor mRNA-

expressing cells were observed in the OB. Interestingly, cell bodies expressing D2 receptors were also observed in the glomerular layer of the medOB. In our material, microinjection of quinpirole in the medOB decreased RS cell responses to ON stimulation, suggesting that D2 receptor activation plays a role in the inhibitory action of DA on medOB output. Moreover, the D2 receptor antagonist, raclopride, had the opposite effect, suggesting that DA could be released endogenously. Whether DA is released tonically in the medOB or as a feedback mechanism in response to electrical ON stimulation during experimental procedures could not be ascertained in the present study. Additionally, microinjection of the D1 receptor antagonist, SCH 23390, did not significantly change the responses to ON stimulation, although this drug was shown to have physiological effects in the MLR of *P. marinus* (Ryczko et al., 2013). Moreover, D1 receptors have been detected in the OB (Pérez-Fernández, 2013). Dihydrexidine, a D1 receptor agonist, reduced olfactomotor response amplitude (28.5% decrease), but was less efficient than DA (36.6% decrease) or quinpirole (51.3% decrease), although it was reported that dihydrexidine exhibits more than ten-fold higher affinity and potency at the D1 receptor than DA (Rosell et al., 2015). Altogether, these physiological results demonstrate that DA exerts a strong modulatory effect on olfactomotor processing in the medOB via D2 and possibly D1 receptors.

Immunofluorescence revealed strongly labeled DA+ processes surrounding the medOB and entering the glomerular neuropil (see Fig. 7, 8 and 9). However, the cellular targets of DA modulation could not be identified. Dopamine receptors can be expressed on primary olfactory afferents, projection neurons and/or interneurons as in other vertebrates (Duchamp-Viret et al., 1997; Brünig et al., 1999; Ennis et al., 2001). Based on previous findings and the present results, we hypothesize that projection neurons are the main site of action of DA involved in the modulation of olfactomotor activity.

In rodents, projection neurons express D2 receptors on their dendrites (Gutièrrez-Mecinas et al., 2005), which are innervated by DA processes (Kasowski et al., 1999). Moreover, DA reduces the spontaneous and evoked activity of projection neurons (Davila et al., 2003). In zebrafish, DA has a direct hyperpolarizing effect on projection neurons and modulates their responses to odorants via D2 receptors (Bundschuh et al., 2012). Interestingly, D2 receptor-expressing cell bodies were detected in the glomerular layer of *L fluviatilis* (Pérez-Fernández et al., 2014) and these were exclusively located in the medOB. Although it could be expected that olfactory glomeruli are

devoid of cell bodies, the medOB glomerulus is definitely an exception and does contain the somata of PT-targeting projection neurons in its neuropil (Green et al., 2013; Daghfous et al., 2018; present results). Hence, D2 receptors might be present on the soma or dendrites of medOB projection neurons and directly modulate their activity and output to the PT. Moreover, their activity might also be modulated downstream, since their axons are observed in close proximity to DA+ processes caudal to the medOB (see Fig. 9). Indeed, axo-axonic contacts established by DA fibers have been detected in the striatum of lizards (Henselmans et Wouterlood, 1994) and rats (Bouyer et al., 1984; Freund et al., 1984; Pickel et Chan, 1990), in the median eminence of sheep (Kuljiš et Advis, 1989), and the cortex of monkeys (Sesack et al., 1995). Furthermore, in the ventral pallidum of rats, D2 receptors are detected mainly on axons or terminals of non-DA neurons, suggesting an effect on presynaptic release (Mengual et Pickel, 2002). In the striatum, the activity of cortical projections is presynaptically modulated via D2 receptors (Schwarcz et al., 1978; Garcia-Muñoz et al., 1991). Since DA neurons establish axo-axonic contacts, DA+ processes close to medOB projection axons may have modulatory effects on olfactomotor transmission through axo-axonic synapses.

Because somata expressing the D2 receptor are observed in the medOB in *L. fluviatilis* (Pérez-Fernández et al., 2014) and our electrophysiological data suggest an inhibition of the olfactomotor processing via D2 receptors in the medOB, we hypothesize that DA processes innervating the medOB act mainly on projection neurons via D2 receptors to regulate odor-driven locomotion. Future studies are needed to define the exact localization of DA receptors in the medOB.

2.1.6.4 Behavioral consequences

Lampreys rely extensively on olfaction to regulate their behavior. Furthermore, olfactory-evoked behaviors vary across life and are adapted to the developmental stage of the animal. For example, upon reaching the reproductive stage, lampreys stop feeding and are attracted to migratory pheromones (Sorensen et al., 2005; Vrieze et al., 2011). It has been suggested that the medial olfactomotor pathway is wired to quickly generate locomotion upon detection of numerous odorants (Derjean et al., 2010). A modulation of this pathway would thus allow for a wide variety of behavioral responses. Moreover, the olfactory sensory input originating from the accessory olfactory organ requires only two synapses to activate the motor systems. A modulation at the level of the OB would be an efficient way to regulate motor responses at the first relay of this pathway.

The evidence collected here suggests that DA modulation of the medOB exists from the larval to the spawning phase. Indeed, strongly labeled DA+ processes were present with a constant pattern of innervation of the medOB in larval, newly-transformed adult and spawning-phase adult lampreys. These processes could produce a physiological effect before the development – or detection – of DA+ somata in the OB and maintain their function after metamorphosis since DA microinjection in the larval medOB produced effects on olfactomotor transmission that persisted in the post-metamorphic stage. Dopaminergic modulation in the olfactomotor pathway could thus gate motor responses that would be inappropriate in relation to the life stage of the animal.

Another effect of DA may be the fine-tuning of medOB activity in response to odorants. The activation of the medial olfactomotor pathway could produce appetitive goal-directed locomotion, since the medOB is activated by amino acids, bile acids and pheromones (Green et al., 2017), all of which can elicit goal-directed swimming (Kleerekoper et Mogensen, 1963; Bjerselius et al., 2000; Li et al., 2002; Johnson et al., 2009). In the natural environment, these chemical cues are encountered in a wide range of concentrations and DA modulation in the medOB might contribute to olfactory processing during tracking behaviors. In rodents, DA modulation of glomerular activation was proposed to increase the range of odorant levels processed by the OB (Ennis et al., 2007). Dopaminergic inhibition in the medOB could thus dynamically adapt olfactory sensitivity, which would allow the animal to follow an olfactory target more efficiently. Such mechanism may increase the range of odorant concentrations inducing an appropriate locomotor response, which is to swim toward their source.

In addition to a local DA source of inputs in the medOB, the two diencephalic DA cell populations projecting to the medOB identified here could provide means for adapting olfactomotor behaviors in different contexts. First, one source of DA innervation originates from the PT, a DA nucleus homologous to the SNc/VTA (Baumgarten, 1972; Pombal et al., 1997). Since medOB projection neurons reach directly the PT to drive locomotion (Derjean et al., 2010; Daghfous et al., 2018), there are reciprocal connections between the medOB and the PT, which would thus allow PT neurons to control the inputs they receive. Interestingly, a reciprocal connection also exists between the PT and the tectum (Pérez-Fernández et al., 2014). It was recently found that DA neurons in the PT are activated by visual stimuli, coding saliency (Pérez-Fernández et al., 2017). In this study, authors reported that the PT modulates visuomotor transformations mediated in the tectum by

modifying tectal neuron responsiveness to visual stimuli via direct DA projections. Similarly, the DA projections of the PT to the medOB could modulate olfactory processing so that odorants generate a motor output less effectively. This mechanism would allow for flexibility in the motor output evoked by the medial olfactomotor pathway. Additionally, the DHN contained CSF-contacting DA+ cell bodies projecting to the medOB. Hypothalamic projections to the olfactory system are common in vertebrates and exert modulatory effects to control odor-driven behaviors (Gascuel et al., 2012). In lamprey, TH+ cells of the DHN are in contact with the CSF and give rise to long extrahypothalamic pathways reaching telencephalic structures (Pierre et al., 1994). Therefore, the CSF-contacting neurons may modulate the activity of the medOB to adjust the behavioral output according to the functional state of the hypothalamus or according to the rate of diverse hormones or other chemical substances in the CSF.

2.1.7 Conclusions

In mammals, DA transmission in the OB is important for odor discrimination (Pavlis et al., 2006; Tillerson et al., 2006; Wei et al., 2006) and learning (Escanilla et al., 2009) and DA interneurons are produced throughout life to maintain these functions. In lampreys, the role of DA modulation appears less complex with a more direct impact on motor output. We show here the presence of a powerful modulatory effect of DA on lamprey RS responses to olfactory inputs. The DA innervation is not only intrinsic but also originates from sources outside of the OB. Altogether, our results provide new insights into the control of a neural circuit transforming an olfactory input into a motor output in lampreys. Future directions could focus on the impact of DA transmission on motor behaviors that are induced by the direct application of odorants on olfactory sensory neurons.

2.1.8 References

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2.2 Olfactory projections to the posterior tuberculum in lampreys

Le manuscrit suivant est prêt depuis février 2022 pour une soumission chez Frontiers in Neural Circuits dans une édition spéciale nommée « Sensorimotor and Autonomic Contributions of the Brainstem in Physiological and Pathophysiological Conditions ». Des études réalisées précédemment dans notre laboratoire (Derjean et al., 2010; Daghfous et al., 2018) ont permis d'observer la présence de deux circuits neuronaux reliant anatomiquement l'organe olfactif périphérique aux réseaux locomoteurs spinaux. Présumément, ces deux circuits permettraient la production d'une réponse de nage lors de la détection d'une odeur. Puisque les deux voies olfactomotrices atteignent le TP et que cette région contient une population de neurones DA, nous avons évalué si cette population pourrait être impliquée dans la transmission du signal olfactomoteur. Ce manuscrit répond aux objectifs spécifiques de la Section 1.7, partie 2.

Contributions des auteurs:

Beauséjour, Philippe-Antoine:	Conception des expériences, Mise-au-point méthodologique, Collecte de données, Analyse des résultats, Interprétation des résultats, Conception des figures, Rédaction de la première version du manuscrit, Révision du manuscrit, Financement.
Veilleux, Jean-Christophe:	Collecte de données anatomiques (stage d'été, contribution pour les figures 18, 19 et 24), Contribution à l'analyse des résultats anatomiques.
Condamine, Steven:	Contribution à la mise-au-point méthodologique pour les expériences de patch-clamp (8 jours, figure 23)
Zielinski, Barbara:	Financement. (À venir: Révision du manuscrit)
Dubuc, Réjean:	Contribution à la conception des expériences, Validation de l'interprétation des résultats, Révisions mineures du manuscrit. Financement.

Olfactory projections to the posterior tuberculum in lampreys

by

Beauséjour, Philippe-Antoine[1]; Veilleux, Jean-Christophe[3]; Condamine, Steven[1]; Zielinski, Barbara[2]; Dubuc, Réjean*[1,3]

1: Université de Montréal
Department of Neurosciences
C.P. 6128, Succ. Centre-Ville
Montreal (Quebec) Canada H3C 3J7

2: University of Windsor
Department of Integrative Biology
401 Sunset Avenue
Windsor (Ontario) Canada N9B 3P4

3: Université du Québec à Montréal
Department of Exercise Sciences
Research Group in Adapted Physical Activity
C.P. 8888, Succ. Centre-Ville
Montreal (Quebec), Canada H3C 3P8

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Contribution to the field:

Our laboratory has previously uncovered two distinct neural circuits transforming an olfactory input into a motor output and synaptic modulatory mechanisms that may enable adaptation of these anatomical pathways (reviewed in Beauséjour et al. 2021, Cell Tissue Res 387: 13-27) in the lamprey, which is among the oldest extant vertebrates. The present manuscript builds on these prior findings to further our understanding of the neural circuits and describe the mechanisms producing olfactomotor behavior in the lamprey. Olfactory signals arise from two distinct olfactory organs in the periphery and reach segregated olfactory bulb subregions to eventually converge at the meso-diencephalic junction, where lies a dopaminergic cell nucleus. We provide compelling evidence that ‘’sensorimotor’’ neurons detect olfactory inputs from both subsystems, transmit the signal to brainstem locomotor regions and are activated during olfactory-induced behavior. Our results provide insight into cell-to-cell and neural systems connectivity, and on the resulting swimming movements produced by this neural circuitry. Our findings about the transformation of olfactory input into motor output may be relevant to other vertebrate species since the functions and general organization of the brainstem is conserved across the vertebrate lineage.

2.2.1 Abstract

The integration of sensory input into motor output is an essential function of the brain. Although olfaction is well-known to guide animal behavior, the neural circuits underlying the motor responses elicited by olfactory inputs are still not well understood. However, in the last decade, neural pathways from the olfactory bulb (OB) to the spinal cord have been identified in the lamprey, a basal vertebrate with a brain organisation very similar to that of mammals. Two distinct pathways were discovered from olfactory sensory neurons to the posterior tuberculum (PT). The first one reaches the PT directly from the medial OB (medOB), whereas the other reaches the PT indirectly from the main OB (MOB) and the lateral pallium (LPal).

Here, we characterized the transmission of olfactory inputs to the PT, a dopaminergic (DA) nucleus that is homologous to the mammalian substantia nigra *pars compacta* and the ventral tegmental area that play a key role in motor control. We observed abundant projections from the medOB to the PT, with many terminals in close proximity to DA neurons. Intracellular and extracellular recordings in the PT revealed that neurons are activated by olfactory input. Furthermore, using calcium imaging, we showed that PT neurons relay the olfactory signal to the mesencephalic locomotor region. In semi-intact preparations, we demonstrated that stimulation of the medOB and LPal induces locomotion that is tightly associated with neural activity in the PT. Moreover, extracellular recordings in the PT displayed reliable bursts of activity throughout locomotor bouts occurring spontaneously. Altogether, our observations suggest that the medOB and LPal project to DA neurons of the PT, which in turn activate the brainstem motor command system to produce swimming activity.

Keywords: Olfaction, Locomotion, Dopamine, Lamprey, Neuroanatomy, Neurophysiology

2.2.2 List of non-standard abbreviations

ΔF/F: Relative changes in fluorescence

AOO: Accessory olfactory organ

AP5: 2-amino-5-phosphonopentanoic acid

CNQX: 6-cyano-7-nitroquinoxaline-2,3-dione

DA: Dopamine

DA+: Dopamine-immunopositive

GAD: Glutamic acid decarboxylase

LPal: Lateral pallium (corresponds to the evaginated part of the pallium)

medOB: medial olfactory bulb

MLR: Mesencephalic locomotor region

MOB: Main olfactory bulb (excludes the medOB)

MOE: Main olfactory epithelium

MRRN: Middle rhombencephalic reticular nucleus

OB: Olfactory bulb

ON: Olfactory nerve

PBS: Phosphate-buffered saline

PT: Posterior tuberculum

RS: Reticulospinal

SNc: Substantia nigra *pars compacta*

TBS: Tris-buffered saline

TBSm: Tris-buffered saline with low (0.1%) sodium metabisulfite

TBSM: Tris-buffered saline with high (1%) sodium metabisulfite

TH: Tyrosine Hydroxylase

TRDA: Texas Red-conjugated dextran amines, 3000 M.W.

vGluT: Vesicular glutamate transporter

VTA: Ventral tegmental area

2.2.3 Introduction

Meso-diencephalic dopaminergic (DA) neurons play an important role in motor control through modulation of the basal ganglia. In the brain of the lamprey, which occupies an important phylogenetic position as the oldest extant group of vertebrates, the substantia nigra pars compacta and ventral tegmental area (SNc/VTA) homolog corresponds to a nucleus of DA neurons located in the posterior tuberculum (PT). The considerable similitude of the lamprey PT to the SNc/VTA, including connectivity, cellular properties, and behavioral effects of DA, suggests that this DA system appeared before the separation of the lamprey and mammalian lineages (reviewed in Suryanarayana et al., 2021a). Notably, ascending DA projections to the direct and indirect basal ganglia pathways exist in lampreys (Robertson et al., 2012; Ericsson et al., 2013b; Pérez-Fernández et al., 2014; Pérez-Fernández et al., 2017), but also descending DA projections to brainstem motor nuclei such as the optic tectum (Pérez-Fernández et al., 2017; von Twickel et al., 2019), mesencephalic locomotor region (MLR; Ryczko et al., 2013; Ryczko et al., 2017), and reticulospinal (RS) cells (Ryczko et al., 2020) that directly modulate motor output. Importantly, electrical and pharmacological stimulation of the PT induces swimming in the semi-intact lamprey preparation with the tail freely moving in a recording chamber (Derjean et al., 2010; Gariépy et al., 2012; Ryczko et al., 2013; Ryczko et al., 2017; Ryczko et al., 2020), which suggests a crucial function in locomotor control. Furthermore, the PT receives axonal projections from many sensory regions (Pérez-Fernández et al., 2014) and was demonstrated to detect visual and electro-sensory inputs (Pérez-Fernández et al., 2017). Thus, as in mammals, activity of DA neurons in the PT (or SNc/VTA) may be influenced by multiple forms of sensory input and, in turn, exert a direct dopaminergic modulation of motor nuclei. Because the olfactory bulb (OB) projects to the PT (Heier, 1948; Northcutt et Puzdrowski, 1988; Polenova et Vesselkin, 1993; Derjean et al., 2010; Green et al., 2013; Pérez-Fernández et al., 2014; Daghfous et al., 2018; Beauséjour et al., 2020; Suryanarayana et al., 2021b), the PT may also detect olfactory inputs.

Olfactory inputs are crucial to guide the behavior of lampreys, such as feeding and reproduction. For instance, odor detection allows adult lampreys to locate preys (Kleerekoper et Mogensen, 1963) and spawning grounds (Bjerselius et al., 2000). Moreover, application of specific odor cues on the olfactory epithelium activates RS cells (Derjean et al., 2010), which provide excitatory input to the spinal locomotor networks (Viana di Prisco et al., 1997). Anatomical and physiological experiments from our research group laid the groundwork for the identification of neural pathways

linking odor detection to behavior in lampreys (reviewed in Beauséjour et al., 2022). First, in a medial pathway, peripheral input from the accessory olfactory organ is transmitted to the medial olfactory bulb (medOB), which has direct projections to the PT (Derjean et al., 2010). Second, in a lateral pathway, the main olfactory epithelium conveys information to the main olfactory bulb (MOB) that then reach the PT via projections to the lateral pallium (LPal; Daghfous et al., 2018). It is presumed that in both pathways, descending projections from the PT activate the MLR, located in the mesencephlic tegmentum, which exerts powerful control over RS cell activity (Sirota et al., 2000; Grätsch et al., 2019a; for reviews, see Dubuc et al., 2008; Ryczko et Dubuc, 2013; Grätsch et al., 2019b) and thus, locomotion. Indeed, since pharmacological inactivation of the PT decreases subthreshold EPSPs in RS cells induced by olfactory nerve (Derjean et al., 2010) or LPal (Daghfous et al., 2018) stimulation, it was hypothesized that the PT relays olfactory information to downstream brainstem motor regions that produce swimming behavior. However, the mechanisms through which the PT may contribute to olfactory-induced locomotion are not identified.

The aim of the present study was first to characterize secondary olfactory projections from the medOB to the PT and identify the cellular phenotype of neurons which receive these fibers since neuronal populations within the PT are heterogeneous in neurotransmitter content (Ryczko et al., 2017; von Twickel et al., 2019). Second, we tested whether the PT detects olfactory inputs and if they are indeed relayed to brainstem motor regions to produce locomotion. Our results show the presence of dense medOB terminals within the PT, close to DA, glutamatergic, and GABAergic neurons. Moreover, after confirming physiologically that PT neurons detect olfactory inputs, we demonstrated that both the medial and the lateral olfactomotor pathways activate the same PT neurons that project to the MLR. Dopaminergic, MLR-projecting PT neurons (Ryczko et al., 2013) are likely to be involved. Furthermore, in semi-intact preparations with the whole brain kept intact, it was demonstrated that medOB inputs induce locomotion. Interestingly, recordings of PT activity revealed that this region is robustly recruited during locomotion induced by either medOB or LPal stimulation. Surprisingly, we also observed that the PT is constantly activated throughout every swimming episode, including spontaneously occurring locomotion of the preparation, which suggests that PT activity plays an important role during locomotion. It was not ascertained whether ascending projections to the striatum, descending projections to brainstem motor regions, or both, are important to the production of locomotion. Altogether, our results show that activation of

neuronal populations in the PT upon stimulation of the medial and lateral olfactomotor pathways induces locomotion, presumably through projections to the MLR.

2.2.4 Material and Methods

2.2.4.1 Animals

Experiments were performed on 2 larvae, 67 newly transformed adult, and 16 spawning-phase adult sea lampreys (*Petromyzon marinus*) of both sexes. Larval animals were collected from the Pike River near Notre-Dame-de-Stanbridge, QC, Canada. Newly transformed adults were purchased from Acme Lamprey Co (Harrison, ME, United States). Spawning-phase adults were collected in the Great Chazy River (NY, United States) and kindly provided by the *US Fish and Wildlife Service* of Vermont. All animals were kept in aerated fresh water maintained at 4 °C. The sex of the animals was not taken into account. Procedures conformed to the guidelines of the Canadian Council on Animal Care and were approved by the Université de Montréal and the Université du Québec à Montréal ethics and animal care committees. Care was taken to minimize the number of animals used and their suffering.

2.2.4.2 Anatomical experiments

2.2.4.2.1 Isolated whole brain preparation

All animals were deeply anesthetized with tricaine methanesulfonate (MS-222, 200 mg/L, Sigma-Aldrich), and the brain was isolated *in vitro* in cold and oxygenated (100% O_2) Ringer's solution (NaCl: 130 mM; KCl: 2,1 mM; $CaCl_2$: 2,6 mM; $MgCl_2$: 1,8 mM; HEPES: 4,0 mM; dextrose: 4,0 mM; $NaHCO_3$: 1,0 mM, adjusted to a pH of 7,40 with NaOH). Animals were decapitated just caudal to the heart and all soft tissue ventral to the notochord was removed. The spinal cord and brain were then exposed by removing the dorsal part of the vertebrae, the dorsal and lateral parts of the cranium, and the mesencephalic and rhombencephalic choroid plexuses. All cranial nerves were cautiously sectioned, except for the olfactory nerve that was kept intact along with the peripheral olfactory organ to maintain connections between olfactory sensory neurons and the brain. Moreover, the skin of the head region was carefully removed because the skin-bound electrosensory receptive organs of the lateral line system evoke bursts of activity in PT neurons following detection of electric fields generated by brief pulses of current applied to the surrounding bath (Pérez-Fernández et al., 2017).

2.2.4.2.2 Tracer injection

To label anterogradely medOB projections to the PT or PT cell bodies with descending projections to the MLR, crystals of biocytin (Sigma-Aldrich) or Texas Red-conjugated dextran amines (TRDA, 3000 MW, Molecular Probes) were used. Tracer injection in the MLR was preceded by a midsagittal section of the rhombencephalic isthmus that allows appropriate visualization of the injection site (at the level of the I_1 Müller cell, see Sirota et al., 2000). Following careful lesion of the injection site with a fine entomological needle, crystals were immediately inserted and allowed to dissolve for 10 minutes. The preparation was then rinsed and transferred to a cooled chamber (8 °C) perfused with cold and oxygenated Ringer's solution to allow overnight transport of the tracer.

2.2.4.2.3 Tyrosine hydroxylase immunofluorescence

One day after the injection, the brains were fixed in paraformaldehyde (4% in phosphate-buffered saline, PBS 0,1 M; pH 7,40; with 0,9% NaCl, 24 hours at 4 °C), rinsed in PBS, and immersed in sucrose (20% in PBS) for cryoprotection. The tissue was then frozen in 2-methylbutane (-50 °C) and coronal sections (25 µm thickness) were produced with a cryostat, collected on ColorFrost Plus microscope slides (Thermo Fisher Scientific), and dried on a warming plate at 37 °C.

Immunofluorescence directed against tyrosine hydroxylase (TH) was performed according to Beauséjour et al., 2020. Briefly, sections were rinsed in PBS (3 x 10 minutes), incubated in a permeabilizing solution (normal goat serum 10% and Triton X-100 0,3%, in PBS, 60 minutes at room temperature), and immerged in a primary antibody solution (rabbit anti-TH, Millipore, 1:400 in the permeabilizing solution, overnight at 4 °C). The next day, sections were rinsed, incubated in a secondary antibody solution (goat anti-rabbit conjugated to Alexa Fluor 594, Molecular Probes, 1:400 in the permeabilizing solution, 60 minutes at room temperature), rinsed again, and mounted with Vectashield® antifade mounting medium without DAPI (Vector Laboratories). Biocytin was visualized by adding streptavidin (conjugated with Alexa Fluor 488, Thermo Fisher Scientific, 1:200) to the secondary antibody solution.

2.2.4.2.4 Dopamine, glutamate and GABA immunofluorescence

Immunofluorescence directed against DA, glutamate and GABA was performed according to procedures modified from Beauséjour et al., 2020. The day after the injection, brains were fixed in glutaraldehyde (2% in Tris-buffered saline with low sodium metabisulfite, TBSm: 0,1% sodium metabisulfite and 0,8% NaCl in Tris 0,05 M; pH 7,40; 60 minutes at 4 °C), rinsed in TBSm, and

incubated in sucrose (20% in TBSm) for cryoprotection. The tissue was then frozen in 2-methylbutane (-50 °C) and coronal sections (15-25 µm thickness) were produced with a cryostat, collected on ColorFrost Plus microscope slides (Thermo Fisher Scientific), and dried on a warming plate at 37 °C.

Sections were rinsed in Tris-buffered saline with high sodium metabisulfite (TBSM: 1,0% sodium metabisulfite in 0,05 M Tris, pH 7,40; 3 x 10 minutes), incubated in a reducing solution (sodium borohydride 0,2% in Tris-buffered saline 0,05 M with 0,9% NaCl, pH 7,40; 45 minutes at room temperature), rinsed again in TBSM, incubated in a permeabilizing solution (normal goat serum 10% and Triton X-100 1% in TBSM, 60 minutes at room temperature), and immersed in a primary antibody solution (mouse anti-DA, Millipore, 1:300; and/or rabbit anti-glutamate, Sigma-Aldrich, 1:600; and/or rabbit anti-GABA, Sigma-Aldrich, 1:300; in the permeabilizing solution, overnight at 4 °C). The next day, sections were rinsed in TBSM with 0,1% Triton X-100, incubated in a secondary antibody solution (for DA: goat anti-mouse conjugated with Alexa Fluor 594 (Jackson ImmunoResearch Laboratories, Inc) or Alexa Fluor 488 (Invitrogen), 1:200; for Glutamate and GABA: goat anti-rabbit conjugated with Alexa Fluor 488 (Invitrogen) or Alexa Fluor 350 (Invitrogen), 1:200; in the permeabilizing solution, 60 minutes at room temperature), rinsed again in TBSM with 0,1% Triton X-100, and mounted with Vectashield® antifade mounting medium with or without DAPI. Biocytin was visualized by adding streptavidin (conjugated with Alexa Fluor 488 or 350, Thermo Fisher Scientific, 1:200) to the secondary antibody solution.

2.2.4.2.5 Fluorescence and laser scanning microscopy

Sections were observed on an E600 epifluorescence microscope (Nikon) and photographed with a DXM1200 digital camera (Nikon) mounted on the microscope and driven by the Automatic Camera Tamer software (Nikon). Sections were also observed on a FluoView FV 1000 confocal laser scanning microscope (Olympus) and photographed with FluoView acquisition software (Olympus).

Photomicrographs were merged and adjusted for brightness and contrast with Photoshop CS6 software (Adobe) and ImageJ software. To produce schematized illustrations of immunolabeled brain sections, photomicrographs were taken with a 20X objective, assembled with the *Photomerge* function in Photoshop CS6, and the outline of sections and labelling were precisely drawn in

Illustrator CS6 software (Adobe). The accuracy of the illustrations was validated under the microscope.

2.2.4.2.6 Cleared brain whole mount

To visualize medOB projections to the PT in a whole brain, a unilateral TRDA injection was performed in the medOB and followed by overnight transport of the tracer in Ringer's solution at 8 °C. The brain was then fixed in paraformaldehyde (4% in PBS, 24 hours at 4 °C) and rinsed in PBS. Next, the tissue was dehydrated through successive incubations in ethanol (5 minutes at 50%, 5 minutes at 70%, 5 minutes at 85%, 5 minutes at 95%, 15 minutes at 100%). The dehydrated brains were then cleared and stored in methyl salicylate (Thermo Fisher Scientific). The brains were mounted ventral side up on a concave microscope glass slide for observation under a FluoView FV 1000 confocal laser scanning microscope equipped with a 20X water-immersion objective. Images were acquired with FluoView acquisition software (Olympus) and analyzed with ImageJ software.

2.2.4.3 Physiological experiments

2.2.4.3.1 Isolated forebrain preparation

The above-described dissection procedures for the isolated whole brain were used in newly transformed adults. The isolated whole brain was then glued to an angled ramp and cut in the coronal plane that leaves the habenula intact in a vibratome. As reported by Ericsson and colleagues (2007), high vibration amplitude and frequency combined with slow blade advance speed produces best cell survival and preparation durability. The isolated forebrain was then pinned down to a homemade ramp so that the rostral side is facing down and the caudal end is facing up. The ramp was then pinned down in an experimental chamber perfused continuously with cooled (8 - 10 °C) oxygenated (100% O_2) Ringer's solution at a rate of 4 mL/minute. A minimum of 1 hour of recovery time preceded experimental procedures. This preparation maintains intact connections between the olfactory bulbs and the PT, which allows for pharmacological or electrical stimulation of the ON, medOB, MOB, and LPal, while observing the PT under the microscope for calcium imaging and electrophysiological recording.

2.2.4.3.2 Extracellular recordings

Extracellular recordings of neural activity in the PT were performed with suction electrodes with borosilicate glass micropipettes (Sutter Instrument; 125 µm tip diameter) and filled with Ringer's solution. Light negative pressure was applied to the PT to increase the signal/noise ratio. Signals (100 - 500 Hz bandwidth) were amplified with a Model 1800 Microelectrode AC amplifier (A-M Systems) and acquired through a Digidata 1200 (Axon Instruments) coupled with Axoscope software (Axon Instruments, Version 9.2.1.8).

2.2.4.3.3 Electrical stimulation

Electrical stimulation was delivered to the nervous tissue with homemade glass-coated tungsten microelectrodes (4-5 MΩ; tip exposure 40-50 µm) connected to a S88 dual output square pulse stimulator (Grass Instruments) coupled to a Model PSIU6 photoelectric stimulus isolation unit (Grass Instruments). In the isolated forebrain and isolated brain preparations, single pulses (2 ms duration, 5-30 µA intensity) or trains of 2-3 pulses (50 Hz) were applied to the olfactory nerve, medOB, MOB, and LPal. In the semi-intact preparation, trains (2 s, 25 Hz, 5 - 30 µA) were bilaterally applied to olfactory nerves, medOBs, MOBs, and LPals with a 20 ms delay resulting in left-right alternating pulses. To prevent desensitization of the preparation, stimulation intensity was kept at the threshold intensity for eliciting responses. Moreover, a minimum of 50 s recovery time was allocated before stimulation of the isolated brain and forebrain preparations, and a minimum of 10 minutes recovery time was allocated before stimulation of semi-intact preparations.

2.2.4.3.4 Drug application

Drugs were pressure ejected (4 psi, 20 - 40 ms duration) through glass borosilicate glass micropipettes (tip diameter: 10 - 20 µm) positioned in the medOB or in the LPal. Reproducible pressure ejections were delivered by a Picospritzer II (Parker Hannifin) and drug solutions were colored with Fast Green FCF (Thermo Fisher Scientific), a pharmacologically inactive dye, to monitor diffusion in the tissue. For bath applications, drugs were simply added to the Ringer's solution continuously perfusing the recording chamber and a minimum of 15 minutes was allocated before further data collection. Drugs were stored at -20 °C and dissolved in Ringer's solution before application. The following drugs were used: gabazine (bath-applied at 10 µM, Tocris Bioscience), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, pressure-ejected at 1 mM, Tocris Bioscience), and 2-amino-5-phosphonopentanoic acid (AP5, pressure-ejected at 0,5 mM).

2.2.4.3.5 Calcium imaging

The whole brain of newly transformed animals was isolated as described above and a midsagittal section of the rhombencephalic isthmus was performed to allow visually guided injection of the calcium-sensitive indicator dye Calcium-Green dextran crystals (3000 MW, Invitrogen) in the MLR (anatomical landmark = I_1 Müller cell, see Sirota et al., 2000). The preparation was then carefully rinsed and transferred to a cooled (8 °C) chamber perfused continuously with cold and oxygenated (100% O_2) Ringer's solution for 3 - 20 hours to allow the dye to backfill PT neurons projecting to the MLR. An isolated forebrain preparation was then produced according to the above procedures and mounted under an Eclipse FN-1 epifluorescence microscope (Nikon) equipped with a 20X water-immersion objective and a CoolSNAP HQ CCD monochrome camera (Roper Scientific). Images were captured (2 Hz) with Metafluor® Fluorescence Ratio imaging software (Molecular Devices) and analyzed with ImageJ (Fiji) software. Briefly, stacks of images were first processed with the *Image stabilization* plugin (Kang Li), the *Bleach correction* function, and the *Subtract background* function. Mean fluorescence signal intensity of regions of interests (precisely hand-drawn over dye-filled PT neuron somata) was then measured with the ROI Manager tool. Relative changes in fluorescence ($\Delta F/F$) were calculated with the baseline (F) defined as the averaged fluorescence value for 50 s before stimulation.

2.2.4.3.6 Whole-cell patch clamp

An isolated forebrain preparation was then produced according to the above procedures and transferred under an Eclipse FN-1 epifluorescence microscope (Nikon) equipped with a 20X water-immersion objective. Whole-cell patch clamp recordings of neurons in the PT were made in voltage-clamp mode (-60 to -70 mV) or current-clamp mode (zero current) with a Model 2400 patch clamp amplifier (A-M Systems). Patch pipettes (tip resistance: 5-8 MΩ) were pulled from borosilicate glass capillaries (outer diameter: 1,5 mm; inner diameter: 0,75 mm; World Precision Instruments) on a P-87 flaming/brown micropipette puller (Sutter Instruments) and filled with patch pipette solution: cesium methane sulfonate: 102,5 mM; NaCl: 1 mM; $MgCl_2$: 1 mM; EGTA: 5 mM; HEPES: 5 mM; ATP: 0,3 mM; GTP: 0,1 mM. The pH was adjusted to 7,20 with cesium hydroxide and the osmolarity to 240 mOsm with water. Light positive pressure allowed better tissue penetration by the pipette. Bright field imaging allowed to target neuronal cell bodies within the DA nucleus of the PT. Signals were acquired through a Digidata 1200 (Axon Instruments)

coupled with Clampex 9.0 software (Axon Instruments). Data analysis was performed with Spike2 (Cambridge Electronic Design, Version 5.19).

2.2.4.3.7 Intracellular recordings (sharp glass microelectrodes)

Reticulospinal cells were impaled with sharp borosilicate glass pipettes (80-120 MΩ) pulled from borosilicate glass capillaries (outer diameter: 1,5 mm; inner diameter: 0,75 mm; World Precision Instruments) on a P-87 flaming/brown micropipette puller (Sutter Instruments) and filled with potassium acetate (4 M). Signals were amplified with an Axoclamp 2A amplifier (Axon Instruments) and acquired through a Digidata 1200 (Axon Instruments) coupled with Axoscope software. Reticulospinal cells included in this study had a stable resting membrane potential under -75 mV throughout the recording. For reproducibility of results, only the largest Müller cells of the MRRN (B1, B3, and B4) were recorded. Data analysis was performed with Spike2 (Cambridge Electronic Design, Version 5.19) and a homemade script for excitatory postsynaptic potentials (Jean-François Gariépy).

2.2.4.3.8 Semi-intact preparation

Semi-intact preparations were used to record neural activity in the PT during olfactory-induced locomotion. The brain was dissected with the above-described procedures for the isolated whole brain preparation, but the body caudal to the heart was left intact and free to swim in a second, deeper compartment of the video-monitored recording chamber. A minimum of 1 hour of recovery time was allowed before experimental procedures.

2.2.4.3.9 Kinematic analysis

Movements produced by the semi-intact preparations were recorded (30 frames per second) with an HDR-XR200 digital camcorder (Sony) positioned above the preparation. The analysis of videos was done with a 2-D motion tracking software (Tracker, Open Source Physics, Version 5.1.3). Briefly, we measured the lateral displacement of a body segment along a line perpendicular to the longitudinal axis of the animal. This lateral displacement was plotted over time and displayed graphically to represent body movement. Swimming was defined as travelling mechanical waves of lateral displacement propagating from head to tail (Sirota et al., 2000). Locomotion of fish may be considered as a typical undulating movement, its main feature being the waves of contractions propagating along the segments of the body musculatures (Gray, 1933).

2.2.5 Results

2.2.5.1 Olfactory bulb projections to the posterior tuberculum

To examine the possibility that the PT transmits the olfactomotor signal from the medOB to downstream motor centers, anatomical experiments were first carried out to analyze medOB projections to the PT. Texas Red-conjugated dextran amines injections were performed into the medOB and anterograde labelling confirmed descending projections to the PT (Fig. 18). In whole brains that were cleared using methyl salicylate (n = 2 adults, 1 newly transformed), unsectioned medOB projections could be observed at the level of the PT in the horizontal plane and z-projection images were produced (Fig. 18B), allowing to observe varicosed fibers terminating in the PT. While most medOB fibers terminated in the ipsilateral PT, others crossed the midline to terminate in the contralateral PT and hypothalamus. To determine if medOB projections terminate onto DA neurons in the PT, similarly to MOB (Suryanarayana et al., 2021b) and LPal (Pérez-Fernández et al., 2014) projections, anterograde tracer injections in the medOB were performed in combination with immunofluorescence directed against DA (red in Fig. 18C; n = 14 spawning-phase adults, 17 newly transformed, 2 larvae) or TH (8 newly transformed). Descending projections were much denser on the ipsilateral side as large varicosed fibers turned medially from the hypothalamus and terminated bilaterally within the PT, intermingled with DA neurons and neurites. Moreover, superposition of anterogradely-labeled medOB axons (varicosed terminals) and DA+ somata and neurites could be observed in z-projection images at the level of the PT (arrows in Fig. 19A). These results suggest that medOB projection neurons directly transmit the olfactory signal to DA neurons in the PT.

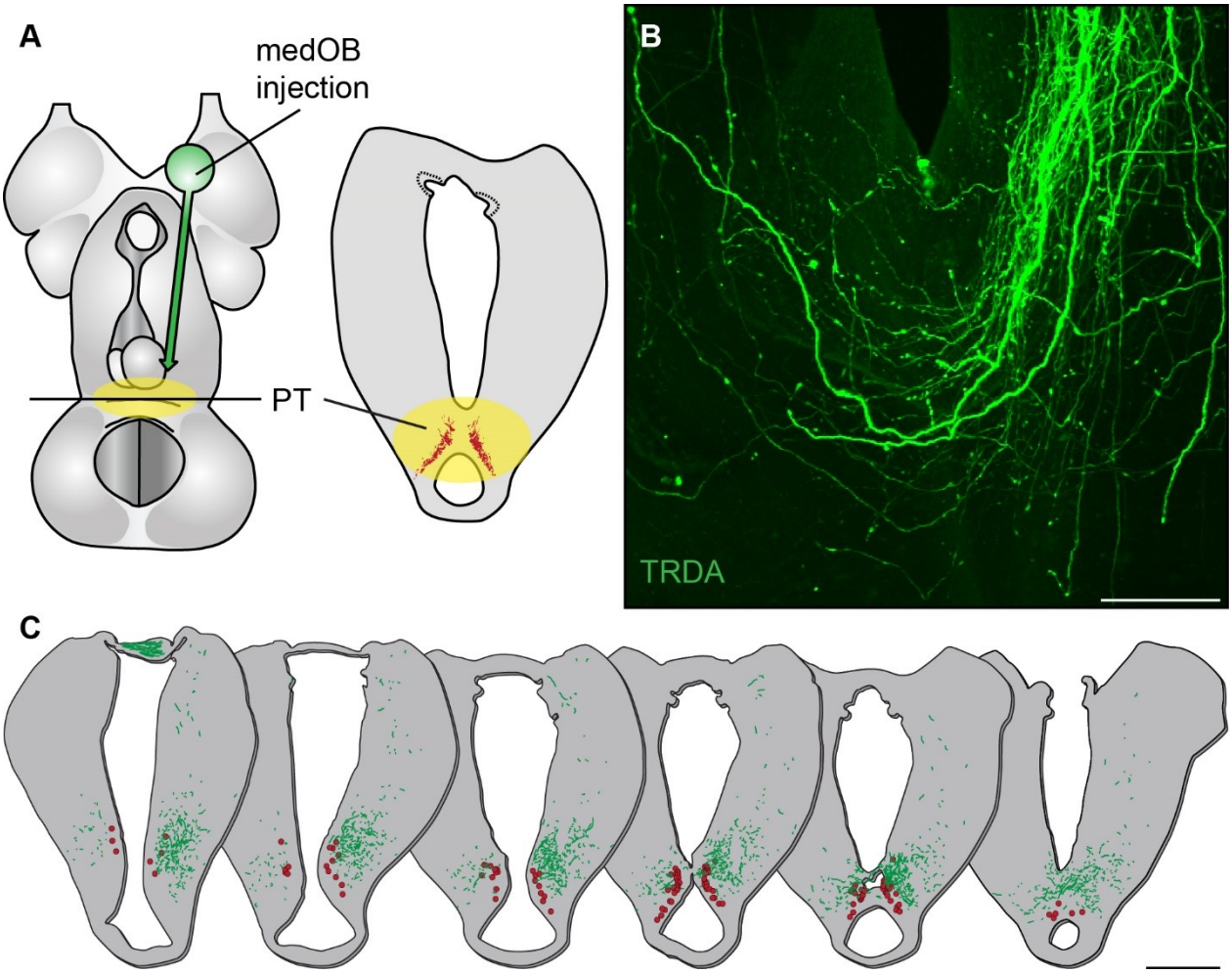

Figure 18. Localization of medial olfactory bulb projections close to dopaminergic neurons in the posterior tuberculum.

(A) The schematic dorsal view of the lamprey forebrain and mesencephalon shows the injection site in the medial olfactory bulb (medOB) and the rostro-caudal level of the posterior tuberculum (PT) at the meso-diencephalic junction. The adjacent schematized transverse section shows the location of dopaminergic neurons in the PT (red). **(B)** Confocal image representing a horizontal section at the level of the PT (rostral is to the top) in a reproductive adult. Photomicrographs were taken in a whole-brain that was cleared with methylsalycylate following Texas Red-conjugated dextran amine (TRDA; 3000 M.W.; depicted in green) injection in the medOB that labeled dense terminals in the PT. **(C)** Drawings of serial transverse sections at the level of the PT (distance between sections: 50 µm) with superimposed drawings of dopaminergic cell bodies (enlarged red

circles) and anterogradely-labeled medOB projections with TRDA (green). Scale bar in B: 100 µm; Scale bar in C: 500 µm.

Moreover, it was recently shown that DA neurons of the PT can co-store glutamate and/or GABA (Ryczko et al., 2017; von Twickel et al., 2019), such as in the mammalian SNc/VTA (Sulzer et al., 1998; Morales et Margolis, 2017). Thus, double immunofluorescence protocols directed against DA and glutamate (n = 2 spawning-phase adults; 4 newly transformed) or DA and GABA (n = 8 newly transformed) were performed to identify the neuronal phenotype of PT cells in relation with medOB projections. Anterograde tracing revealed that medOB projections terminate in close proximity to co-labeled DA+/Glut+ neurons (arrowheads in Fig. 19B) and DA+/GABA+ neurons (arrowheads in Fig. 19C). This suggests that DA neurons co-storing glutamate and/or GABA receive olfactory inputs from the medOB.

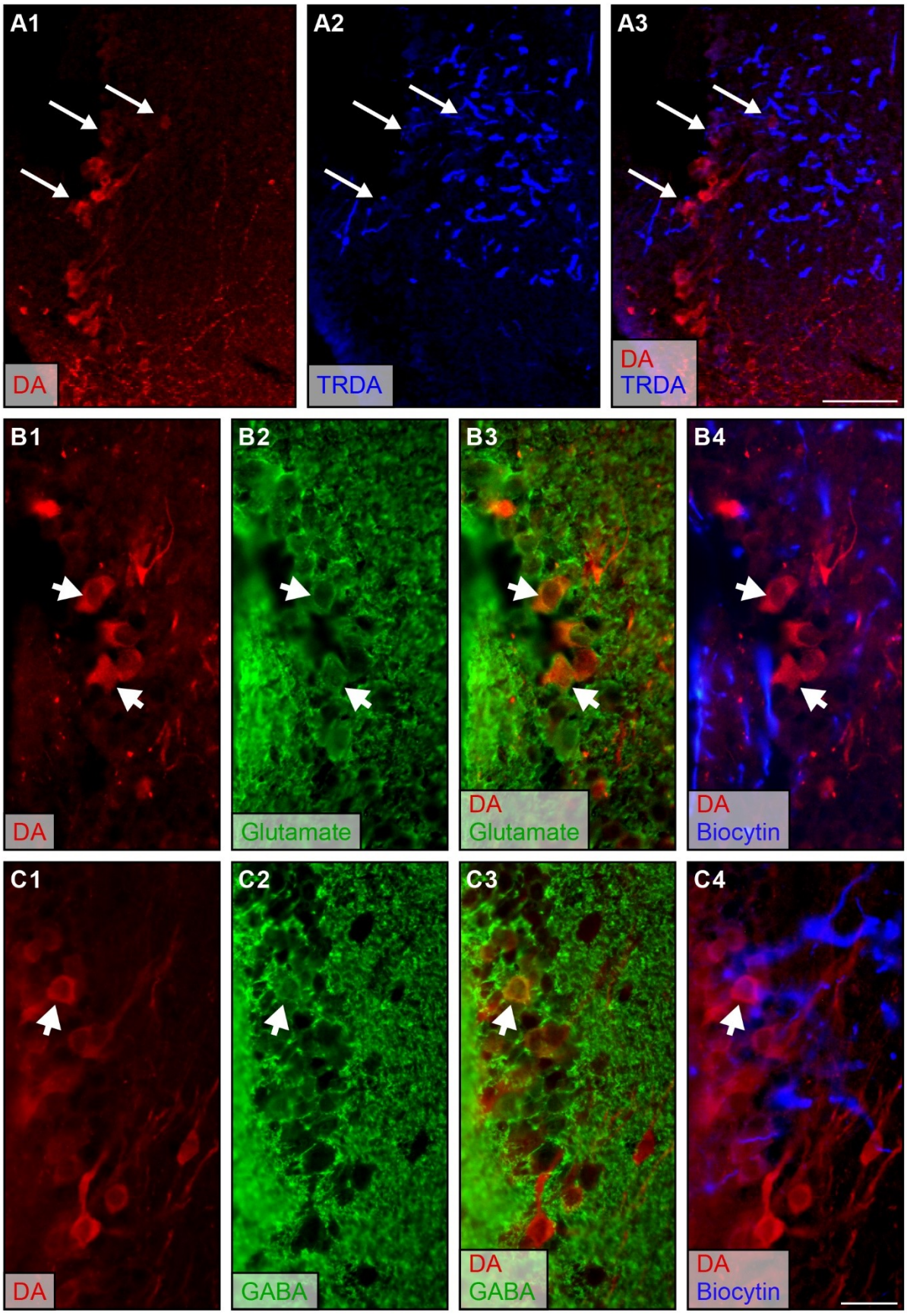
A1
DA
A2
TRDA
A3
DA
TRDA
B1
DA
B2
Glutamate
B3
DA
Glutamate
B4
DA
Biocytin
C1
DA
C2
GABA
C3
DA
GABA
C4
DA
Biocytin

Figure 19. Secondary olfactory projections from the medial olfactory bulb terminate in close proximity to dopaminergic, glutamatergic, and GABAergic neurons in the posterior tuberculum.

All photomicrographs were taken in transverse sections at the level of the posterior tuberculum (PT) in adult lamprey brains in which biocytin injection into the medial olfactory bulb (medOB) labeled projections to the PT that were observed in combination with immunofluorescence. **(A1-A3)** Confocal images representing dopamine (DA, depicted in red) immunofluorescence in the PT superimposed with TRDA-labeled (depicted in blue) medOB projections showing close proximity (white arrows). Thus, DA neurons are probable synaptic partners with axonal projections of the medOB. Moreover, **(B1-B4)** epifluorescence photomicrographs show the presence of DA-immunopositive (**B1**, red) and glutamate-immunopositive (**B2**, green) neurons, some of which are co-labeled (**B3**, white arrowheads) and in close proximity with axonal projections from the medOB (**B4**, blue, white arrowheads). Also, **(C1-C4)** epifluorescence photomicrographs show the presence of DA-immunopositive (**C1**, red) and GABA-immunopositive (**C2**, green) neurons, some of which are co-labeled (**C3**, white arrowheads) and in close proximity with axonal projections from the medOB (**C4**, blue, white arrowheads). Scale bar in A3: 50 μm; Scale bar in C4: 25 μm.

2.2.5.2 Neuronal responses in the posterior tuberculum to stimulation of olfactomotor circuitry

We then assessed whether neuronal responses are induced in the PT by olfactory nerve stimulation. For that, neural activity was recorded extracellularly in the PT of isolated forebrain preparations in response to electrical stimulation of the olfactory nerve (Fig. 20A). The recording electrode (tip diameter: 125 μm) was positioned over the population of DA neurons intermingled with descending projections of the medOB that we previously identified (see Figs. 18 and 19). Upon electrical stimulation (single 2 ms square pulse, 10-30 μA) of the olfactory nerve (n = 11), bursts of activity were evoked (Fig. 20B), which suggests that olfactory inputs are indeed detected by PT neurons. Moreover, when glutamate receptor antagonists (CNQX: 1 mM; AP5: 0.5 mM) were locally microinjected in the PT (n = 2), no response to electrical stimulation of the olfactory nerve could be observed (Fig. 20C). In contrast, when $GABA_A$ receptor antagonists (gabazine: 10 μM) were bath-applied (n = 7), a striking increase in PT activity induced by olfactory nerve stimulation

was observed. These results confirm that transmission from the OB to the PT is glutamatergic (Derjean et al., 2010) and that PT neurons are depolarized by olfactory stimulation.

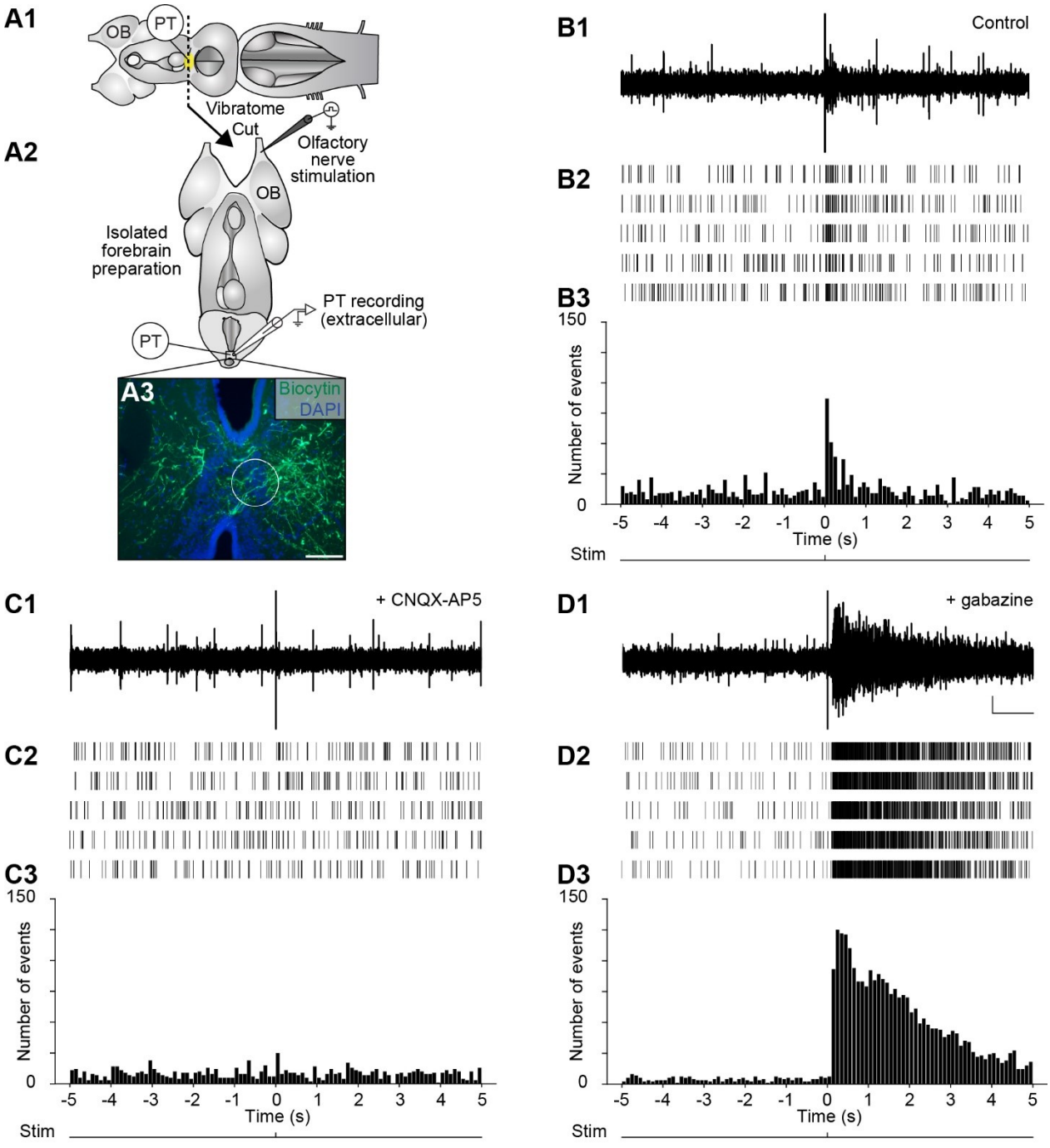

Figure 20. Extracellular responses in the posterior tuberculum to electrical stimulation of the olfactory nerve.

(A1) The schematic dorsal view of the isolated adult lamprey brain illustrates the rostro-caudal level at which a transverse section was performed to produce the isolated forebrain preparation **(A2)** that allows experimental access to the posterior tuberculum (PT). **(A3)** Photomicrograph of a transverse section at the level of the PT illustrating the approximative extracellular recording site (white circle; tip diameter: 125 µm). Cell populations within the PT are labeled with 4',6-Diamidino-2-Phenylindole (DAPI; blue) and axonal projections of the medial olfactory bulb (green) are anterogradely-labeled by a biocytin injection. **(B1)** Extracellular recording in the PT shows responses evoked by electrical stimulation of the ipsilateral olfactory nerve. In a representative animal, five responses are shown in a raster plot **(B2)** aligned on the time of stimulation (Time = 0) and summed in a vertical bar chart (**B3**, bar width: 100 ms). **(C1-C3)** The same representation is shown in the next column after local pressure-injection of a combination of glutamatergic receptor antagonists (6-cyano-7-nitroquinoxaline-2,3-dione, CNQX: 1 mM; 2-amino-5-phosphonopentanoic acid, AP5: 0.5 mM) in the PT, which abolishes responses induced by electrical olfactory nerve stimulation. **(D1-D3)** In a different animal, bath-perfusion of $GABA_A$ receptor antagonist (gabazine: 10 µM) dramatically increased extracellular responses evoked by the electrical stimulation of the olfactory nerve. Scale bar in A3: 100 µm; Scale bar in D1: 1 s and 50 µV.

Stimulation of the olfactory nerve recruits both the medial (Derjean et al., 2010) and the lateral (Daghfous et al., 2018) olfactomotor pathway by activating the medOB and the MOB. To compare the PT responses to stimulation of these pathways, the extracellular electrode was kept in the same PT recording site while consecutively stimulating the olfactory nerve, medOB, MOB and LPal (Fig. 21, n = 5). Upon electrical stimulation of the ipsilateral medOB (Fig. 21C), bursts of neural activity were observed in the PT, which confirms that medOB projections can indeed produce excitatory responses in the PT. Moreover, similar PT responses were also recorded following electrical stimulation of the LPal (Fig. 21E), which indicates that activity in the lateral olfactomotor pathway may also be processed in the PT. Interestingly, similar bursts of activity were also observed in the contralateral PT (Fig. S28) upon stimulation of the olfactory nerve (Fig. S28B; n = 5), medOB (Fig. S28C; n =3), and LPal (Fig. S28E). However, upon stimulation of the MOB (Fig. 21D, n = 5), which could be anatomically coupled to PT neurons through a relay in the LPal

(Daghfous et al., 2018), no responses could be observed in the PT. Previous results (Daghfous et al., 2018) showed that MOB stimulation does not induce RS cell responses because it is under a strong GABAergic inhibitory control. Hence, we tested the effect of bath-applied gabazine and found that large bursts of neural activity are induced following electrical stimulation of the MOB (Fig. 22D). In addition, responses to olfactory nerve (Fig. 22B), medOB (Fig. 22C) or LPal (Fig. 22E) stimulation were also increased during bath application of gabazine.

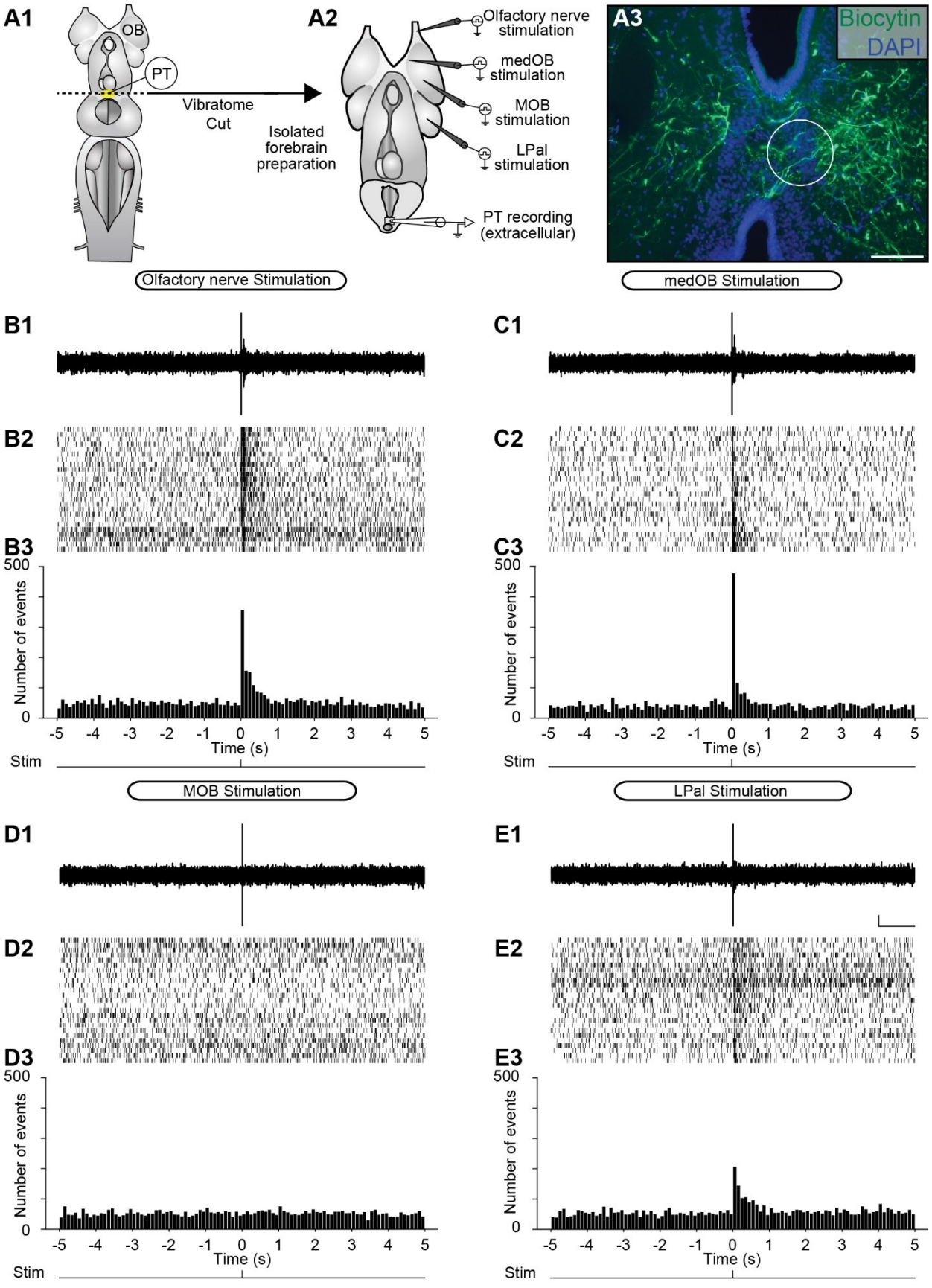
A1
OB
PT
Vibratome Cut
Isolated forebrain preparation
A2
Olfactory nerve stimulation
medOB stimulation
MOB stimulation
LPal stimulation
PT recording (extracellular)
A3
Biocytin
DAPI
Olfactory nerve Stimulation
medOB Stimulation
B1
B2
B3
C1
C2
C3
500
Number of events
0
-5 -4 -3 -2 -1 0 1 2 3 4 5
Time (s)
Stim
MOB Stimulation
LPal Stimulation
D1
D2
D3
E1
E2
E3

Figure 21. Extracellular responses in the posterior tuberculum to electrical stimulation of the olfactory nerve, medial olfactory bulb, main olfactory bulb, and lateral pallium.

(A1) The schematic dorsal view of the isolated forebrain preparation in adult lamprey illustrates the recording site in the PT and the multiple stimulation sites in the olfactory nerve **(B)**, medial olfactory bulb (medOB; **C**), main olfactory bulb (MOB; **D**), and lateral pallium (LPal; **E**). **(A2)** Photomicrograph of a transverse section at the level of the PT illustrating the approximative extracellular recording site (white circle; tip diameter: 125 µm). Cell populations within the PT are labeled with 4',6-Diamidino-2-Phenylindole (DAPI; blue) and axonal projections of the medOB (green) are anterogradely-labeled by a biocytin injection. **(B1)** Extracellular recording in the PT shows the response evoked by electrical stimulation of the ipsilateral olfactory nerve in a representative animal. **(B2)** In a raster plot, responses (n = 25, N = 5) are aligned on the time of stimulation (Time = 0) and summed in a vertical bar chart (**B3**, bar width: 100 ms). **(C1-C3)** The same organization is shown with the same representative animal after the stimulation electrode was repositioned in the ipsilateral medOB, which evokes extracellular responses in the PT. **(D1-D3)** The same organization is shown with the same representative animal after the stimulation electrode was repositioned in the ipsilateral MOB, which does not evoke extracellular responses in the PT. **(E1-E3)** The same organization is shown with the same representative animal after the stimulation electrode was repositioned in the ipsilateral LPal, which evokes extracellular responses in the PT. Scale bar in A3: 100 µm; Scale bars in E1: 1 s and 50 µV.

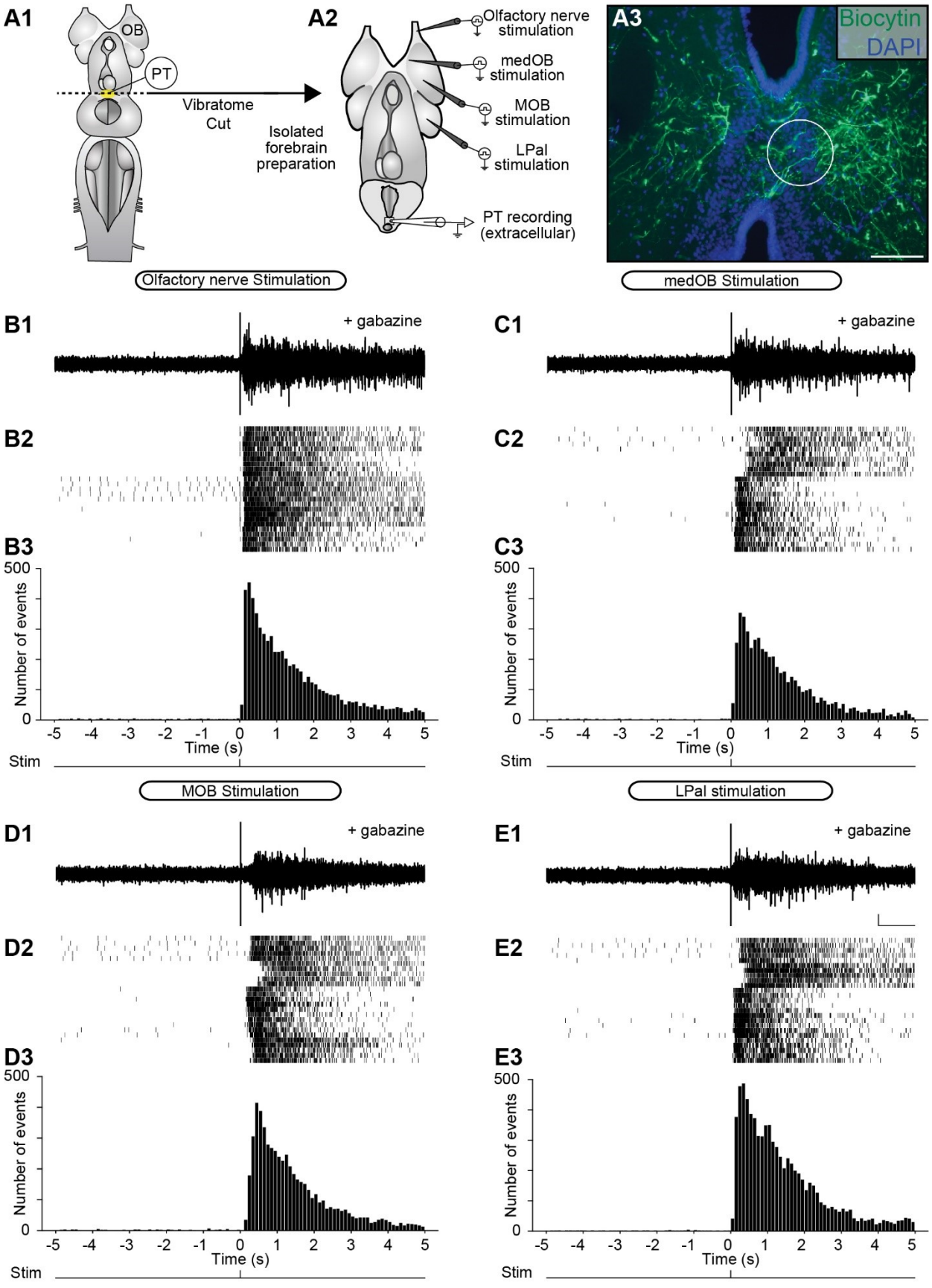
A1
OB
PT
Vibratome Cut
Isolated forebrain preparation
A2
Olfactory nerve stimulation
medOB stimulation
MOB stimulation
LPal stimulation
PT recording (extracellular)
A3
Biocytin
DAPI
Olfactory nerve Stimulation
medOB Stimulation
B1
+ gabazine
B2
B3
C1
+ gabazine
C2
C3
Number of events
Time (s)
Stim
MOB Stimulation
LPal stimulation
D1
+ gabazine
D2
D3
E1
+ gabazine
E2
E3

Figure 22. Extracellular responses in the posterior tuberculum to electrical stimulation of the olfactory nerve, medial olfactory bulb, main olfactory bulb, and lateral pallium during bath-application of gabazine.

(A1) The schematic dorsal view of the isolated forebrain preparation in adult lamprey illustrates the recording site in the PT and the stimulation sites in the olfactory nerve **(B)**, medial olfactory bulb (medOB; **C**), main olfactory bulb (MOB; **D**), and lateral pallium (LPal; **E**). (A2) Photomicrograph of a transverse section at the level of the PT illustrating the approximative extracellular recording site (white circle; tip diameter: 125 µm). Cell populations within the PT are labeled with 4',6-Diamidino-2-Phenylindole (DAPI; blue) and axonal projections of the medOB (green) are anterogradely-labeled by a biocytin injection. **(B1)** Extracellular recording in the PT shows the amplified response evoked by electrical stimulation of the ipsilateral olfactory nerve in a representative animal during bath perfusion of $GABA_A$ receptor antagonist (gabazine: 10 µM). **(B2)** In a raster plot, responses (n = 25, N = 5) are aligned on the time of stimulation (Time = 0) and summed in a vertical bar chart (**B3**, bar width: 100 ms). The same organization is shown with the same representative animal after the stimulation electrode was repositioned in the ipsilateral medOB **(C1-C3)**, MOB **(D1-D3)**, or LPal **(E1-E3)**, all of which also evoke amplified extracellular responses in the PT. Scale bar in A3: 100 µm; Scale bars in E1: 1 s and 50 µV.

Next, to observe how PT neurons react to olfactory inputs, intracellular recordings were performed in the PT of the isolated forebrain preparation (Fig. 23A). Whole-cell patch-clamp recordings of PT neurons (n = 45 neurons in N = 13 animals) were performed to assess the presence of synaptic responses to electrical stimulation of the olfactory nerve (trains of 1-5 pulses, 50 Hz, 7,5-20 µA). Synaptic responses were observed in 8 cells from 6 animals, whereas 37 cells from 12 animals did not respond to the stimulation (Fig. 23). Synaptic responses showed variation from cell to cell as both excitatory (6/8) and inhibitory (2/8) post-synaptic potentials or currents were recorded. This variability may be explained by the heterogeneity of neuronal populations in the PT (see Fig. 19; Ryczko et al., 2017; von Twickel et al., 2019).

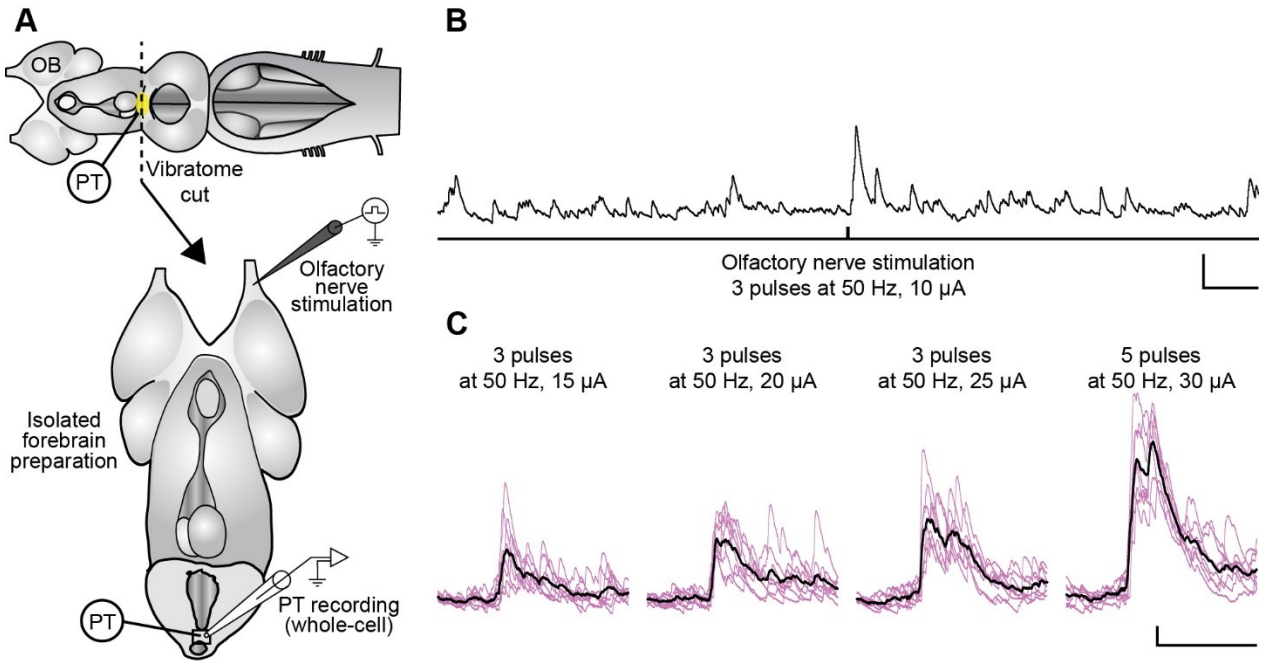

Figure 23. Whole-cell responses in the posterior tuberculum to electrical stimulation of the olfactory nerve.

(A, top) The schematic dorsal view of the isolated adult lamprey brain illustrates the rostro-caudal level at which a transverse section was performed to produce the isolated forebrain preparation **(A, bottom)** that allows access to the posterior tuberculum (PT). **(B)** Whole-cell patch-clamp recording (current-clamp mode, I = 0) of an unidentified neuron within the PT shows spontaneous synaptic activity and responses to electrical stimulation of the olfactory nerve. **(C)** EPSPs evoked by stimulations of increased intensities. Responses are represented as eight superimposed traces (colored) and their mean (thick black trace). Scale bars in B: 1 s and 10 mV; Scale bars in C: 1 s and 2 mV.

2.2.5.3 The posterior tuberculum recruits downstream locomotor circuitry following olfactory stimulation

Once we confirmed that PT neurons respond to olfactory inputs, we determined whether they may subsequently recruit downstream locomotor circuitry. Thus, we assessed whether MLR-projecting PT neurons could be recruited by descending projections of the medOB. In a series of anatomical

experiments (Fig. 24A), we injected biocytin crystals into the MLR to retrogradely label PT neurons that project to this region. Also, TRDA was injected into the medOB to label secondary olfactory projections that reach the PT, in combination with immunofluorescence directed against DA (12 adults, 3 newly transformed, 2 larvae) or TH (n = 8 newly transformed). First, we found that many DA-positive and TH-positive neurons were retrogradely-labeled at the level of the PT from a unilateral biocytin injection in the MLR. Similarly to Ryczko and colleagues (2013), retrogradely-labeled DA neurons were located exclusively in the DA nucleus of the PT, mostly on the ipsilateral side, and none were found in the adjacent mammillary area, thus confirming previous results from our lab. Furthermore, our experiments revealed that varicosed medOB projections terminate close to retrogradely-labeled DA-positive (arrows in Fig. 24A) or TH-positive neurons, which suggest that DA neurons in the PT may relay the olfactory signal from the medOB to the MLR to induce locomotion following odor-detection.

Physiological experiments were then designed to assess if PT neurons that project to the MLR respond to olfactory stimulation (Fig. 24B-D). Neurons in the PT were first retrogradely-labeled by an injection of Calcium-Green dextran crystals within the MLR, before a transverse section was performed to produce an isolated forebrain preparation that allows access to the PT. Also, because both the medial and lateral olfactomotor pathways are under tonic GABAergic inhibition (Daghfous et al., 2018), gabazine was bath-applied to the recording chamber. Electrical stimulation (1-3 pulses, 50 Hz, 5-30 µA) of the medOB (30 neurons in 5 animals) induced calcium responses in PT neurons (Figs. 24, S29 and S30). Although their cellular phenotype was not ascertained, responding neurons were located within the nucleus of DA neurons in the PT (red in Fig. 24C). Similarly, stimulation of the olfactory nerve (19 neurons in 4 animals) or the LPal (13 neurons in 2 animals) activated PT neurons, evoking bilateral calcium responses (Fig. S31). Interestingly, out of the 13 cells that responded to LPal stimulation, 10 also responded to medOB and/or olfactory nerve stimulation (Fig. S31), which suggests that individual PT neurons integrate activity from both the medial and lateral olfactomotor pathway. Hence, upon odorant detection, the medOB and LPal may recruit the same PT neurons that relay the olfactory signal to the MLR to induce locomotion.

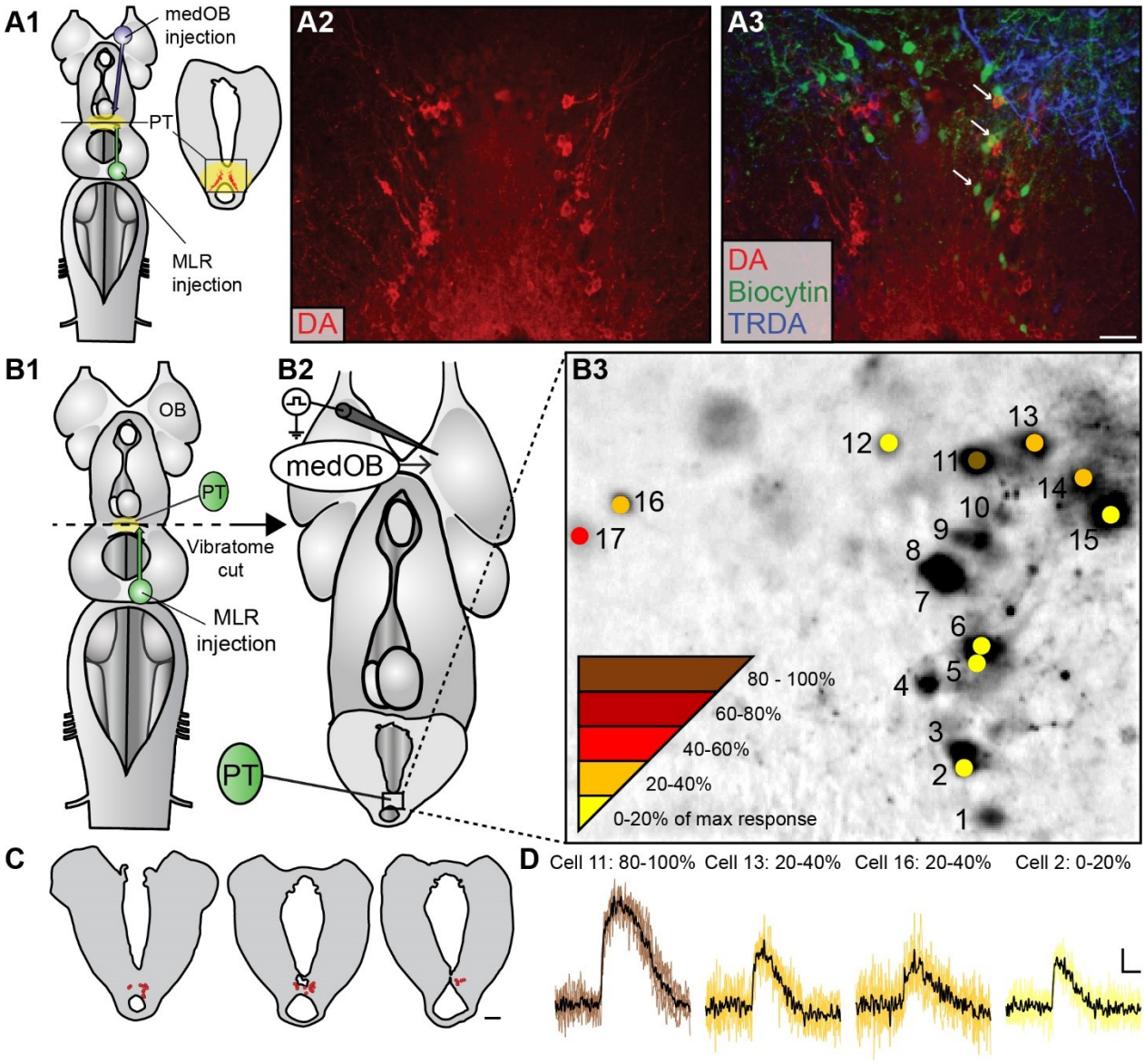

Figure 24. Calcium-imaging responses to electrical stimulation of the medial olfactory bulb in brainstem-projecting neurons of the posterior tuberculum.

(A1) The schematic dorsal view of the lamprey forebrain and mesencephalon shows the Texas Red-conjugated dextran amines (3000 M.W., TRDA; blue) injection site in the medial olfactory bulb (medOB), the biocytin (green) injection site in the mesencephalic locomotor region (MLR), and the level of the posterior tuberculum (PT) at the meso-diencephalic junction. The adjacent schematized transverse section shows the location of the dopaminergic (DA) nucleus of the PT and the black frame corresponds to the region illustrated in A2-A3. Immunofluorescence directed against DA (red; **A2**) labels neurons in the PT that are retrogradely-labeled by a biocytin injection

into the MLR (green) and are in close apposition with axon terminals of medOB projection neurons anterogradely-labeled by TRDA (blue; **A3**). **(B1)** The schematic dorsal view of the isolated adult lamprey brain illustrates the Calcium-Green dextran crystals (green) injection in the MLR and the rostro-caudal level at which a transverse section was performed to produce the isolated forebrain preparation **(B2)** that allows access to the PT. The black frame in B2 corresponds to the PT region that is imaged during electrical medOB stimulation following bath application of gabazine **(B3)**. From a representative animal, the image in B3 shows mean calcium signal (as shades of grey) during a 900 s acquisition to allow visualization of neurons that were retrogradely-labeled by the MLR injection. Out of 17 labeled cells, 10 respond to medOB stimulation and for each of these, the area under the curve (ΔF/F over time) was measured. The value of 100% was assigned to the cell with the maximal response (cell #11), other cells were color-coded according to their percentage of the maximal response (see the inset of B3). **(C)** Three schematized transverse sections at the level of the PT illustrating the approximate localization of MLR-projecting neurons that respond to medOB stimulation in all tested animals (N = 5 animals; n = 30 neurons). **(D)** Representative calcium-responses to the same electrical medOB stimulation of four distinct cells shown above in B3. Responses are represented as six superimposed traces (colored) and their mean (thick black trace). The cell number shown above each response in panel D corresponds to cell numbers shown in panel B3. Scale bar in A3: 50 µm; Scale bar in C: 200 µm; Scale bars in D: 10 s and 10 %ΔF/F).

Next, we sought to determine whether PT neural activity may contribute to locomotion induced by olfactory stimulation. In the semi-intact preparation (intact tail freely-swimming in the recording chamber, see Material and Methods), locomotion was monitored visually during intracellular recording of RS cells and extracellular recording of the PT (Fig. 25A). Bilateral electrical stimulation (25 Hz, 2 s, range: 5 – 30 µA) of the medOB induced coordinated bouts of sustained locomotion that was accompanied by RS cell spiking activity and neural activity in the PT (Figs. 25B-D and S31; n = 5 animals). The position of both stimulation electrodes in the medOBs were histologically confirmed after the experiments (white dashed lines in Fig. 25A4). Following medOB stimulation, neural activity in the PT was robustly recruited during locomotion in every

animal studied, which suggests that the PT is important to induce locomotion in the medial olfactomotor pathway.

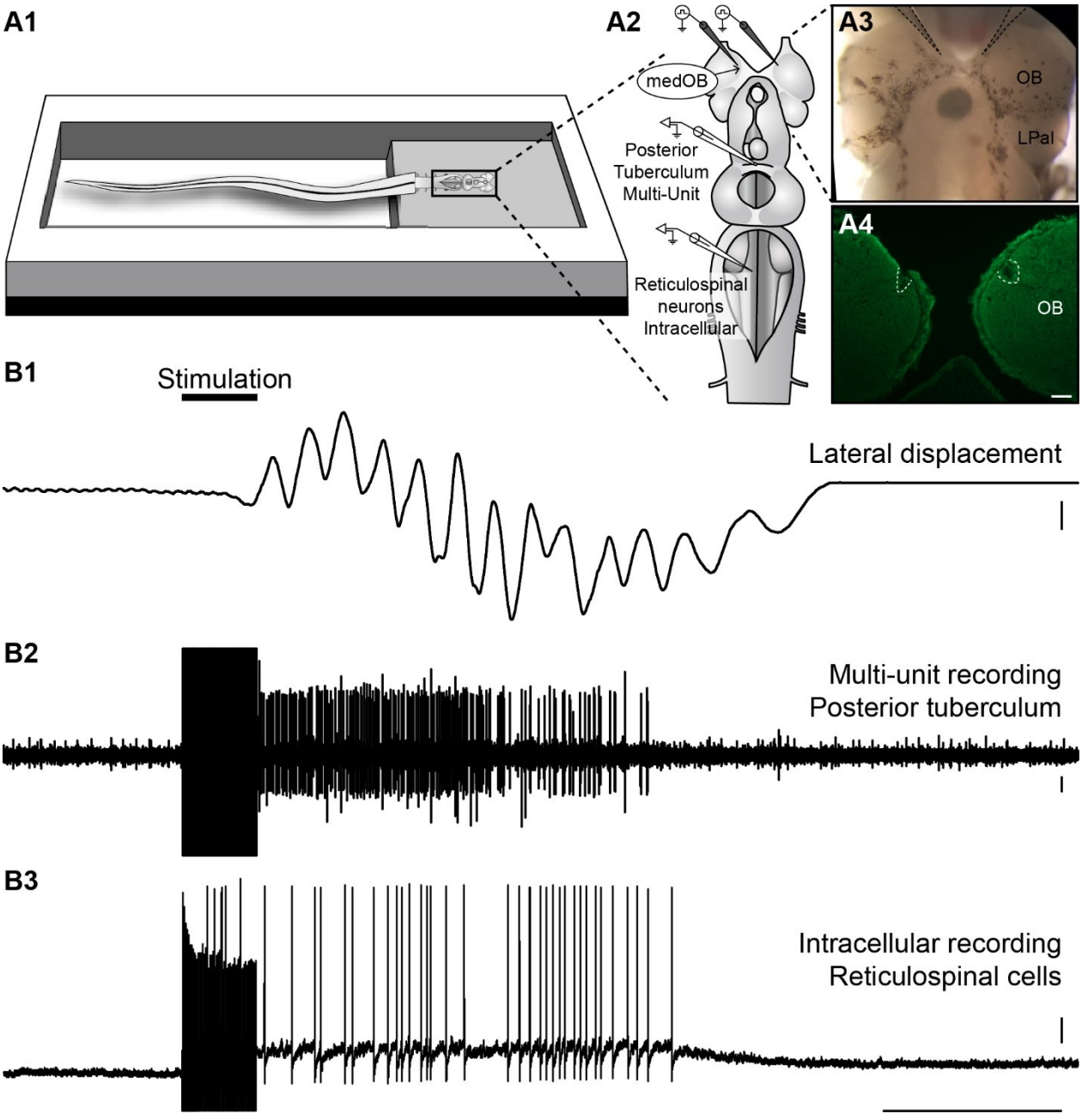

Figure 25. Electrical stimulation of the medial olfactory bulb produces swimming, extracellular activity in the posterior tuberculum and spiking activity in reticulospinal cells.

(A1) Schematized representation of the semi-intact lamprey preparation showing the isolated whole-brain (black frame) pinned to the bottom of the recording chamber, and the intact, freely-swimming body in a second, deeper compartment. **(A2)** The brain is schematized to show the bilateral medial olfactory bulb (medOB) stimulation site, the posterior tuberculum (PT) extracellular recording site, and the intracellular recording site of middle rhombencephalic reticulospinal (RS) cell . **(A3)** Photograph of the dorsal view of the telencephalon with stimulation electrodes (dashed lines) bilaterally positioned in the medOBs. **(A4)** Photomicrograph of a transverse section at the level of the olfactory bulb showing the damage caused by the stimulating electrodes (white dashed lines). This confirms that the tip of both stimulation electrodes was within the medOB. **(B)** Bilateral medOB stimulation (25 Hz, 2s, 5 – 30 µA), induced episodes of swimming activity that were accompanied by neural bursts of activity in the PT and RS cell spiking. **(B1)** Lateral displacement of a body segment was monitored with a video camera and plotted to illustrate swimming activity. Concurrently, extracellular activity was recorded in the PT **(B2)** and RS cell activity was intracellularly recorded **(B3)**. Scale bar in A4: 100 µm; Scale bar in B1: 20 mm; Scale bar in B2: 100 µV; Scale bars in B3: 5 s and 10 mV.

To confirm that these responses are specific of medOB activity, and not activated by fibers of passage or neurons that project to the medOB, such as known PT projections to the medOB (Beauséjour et al., 2020), the medOB was stimulated bilaterally with glutamate (3-5 mM; Fig. S33; n = 6 animals). Such stimulations induced concomitant bursts of activity in PT neurons, sustained depolarization with superimposed action potentials in RS cells, and swimming of the animal (Fig. S33). The position of glass capillaries used for glutamate injection were histologically confirmed to be in the medOBs (white dashed lines in Fig. S33A). These results demonstrate that activation of glutamatergic receptors in the medOB is sufficient to induce swimming and strongly supports our hypothesis that OB projections recruit the PT and downstream motor centers to produce locomotion upon odorant detection.

We then assessed whether the PT is also activated during locomotion induced by LPal stimulation. Bilateral electrical stimulation (25 Hz, 2 s, 30 µA) of the LPal reliably induced locomotion in the semi-intact preparation (n = 3), simultaneously with sustained depolarization in RS cells and neural activity in PT neurons (Figs. 26 and S34). The location of the stimulation electrodes in the LPal

was histologically confirmed after the experiments (white dashed lines in Fig. 26A4). Altogether, these results suggest that PT neurons are recruited by both the medOB and the LPal during olfactory-induced swimming and thus that the PT plays an important role in locomotion evoked by both the medial and lateral olfactomotor pathways.

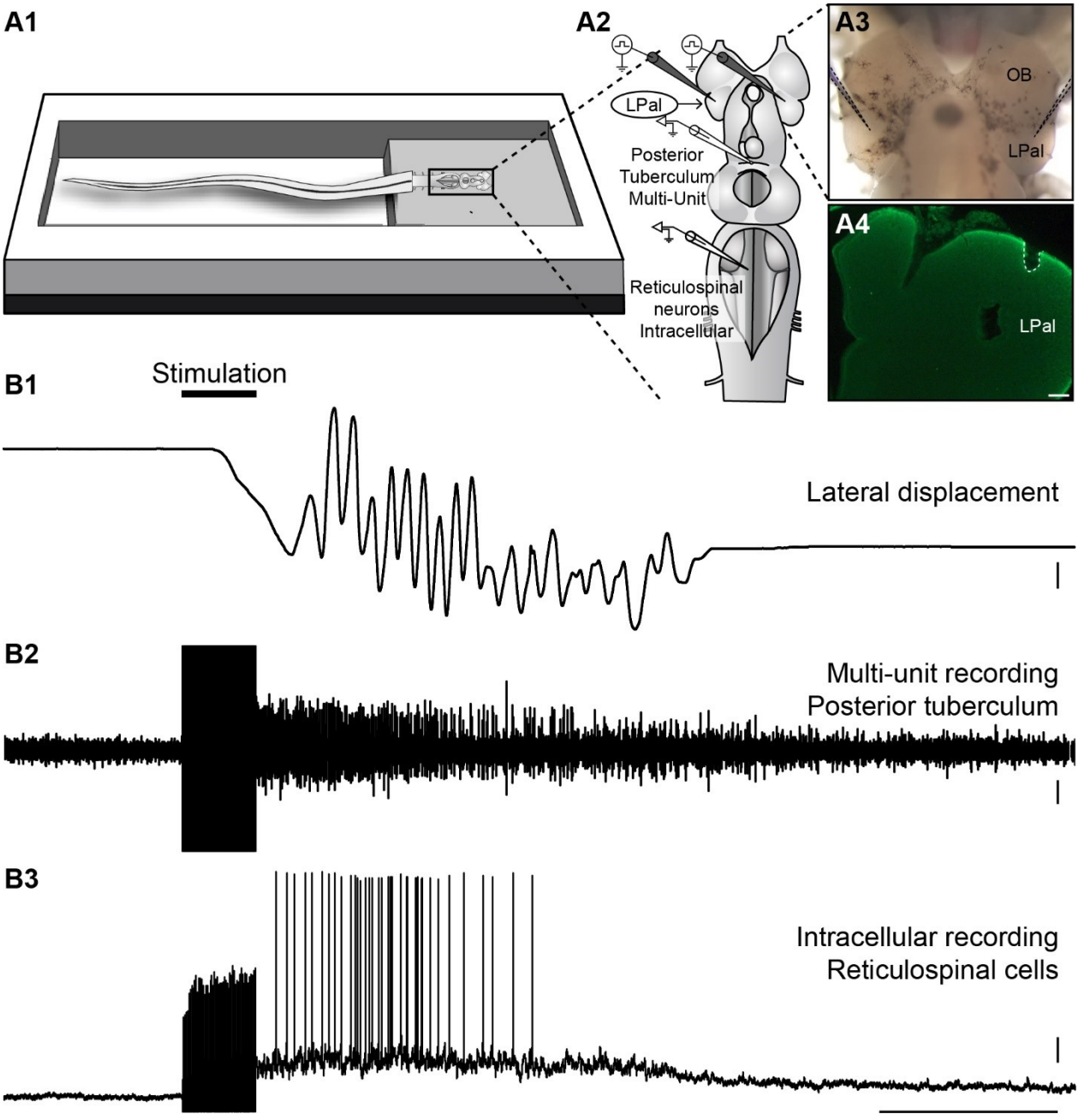

Figure 26. Electrical stimulation of the lateral pallium produces swimming, extracellular activity in the posterior tuberculum and spiking activity in reticulospinal cells.

(A1) Schematized representation of the semi-intact lamprey preparation showing the isolated whole-brain (black frame) pinned to the bottom of the recording chamber, and the intact, freely-swimming body in a second, deeper compartment. **(A2)** The brain is schematized to show the bilateral lateral pallium (LPal) stimulation site, the posterior tuberculum (PT) extracellular recording site, and the reticulospinal (RS) cell intracellular recording in the middle rhombencephalic reticular formation. **(A3)** Photograph of the dorsal view of the telencephalon with stimulation electrodes (dashed lines) bilaterally positioned in the LPals. **(A4)** Photomicrograph of a transverse section at the level of the right LPal showing the damage caused by the stimulating electrode (white dashed line). **(B)** Bilateral LPal stimulation induced episodes of swimming activity with neural bursts of activity in the PT and RS cell spiking. **(B1)** Lateral displacement of a body segment was monitored with a video camera and plotted to illustrate swimming activity. Extracellular activity was concurrently recorded in the PT **(B2)** and membrane potential was intracellularly recorded in RS cell **(B3)**. Scale bar in A4: 100 μm; Scale bar in B1: 20 mm; Scale bar in B2: 100 μV; Scale bars in B3: 5 s and 10 mV.

Finally, during resting periods of the experiments, spontaneous episodes of locomotion were observed in the semi-intact preparation (n = 11). These events occurred sporadically and allowed to record extracellular activity in the PT and intracellular activity of RS cells during spontaneous bouts of swimming (Fig. 27, n = 11). Interestingly, bursts of activity in PT neurons and sustained depolarization with superimposed action potentials in RS cells occurred concomitantly with spontaneous episodes of locomotor activity. Extracellular activity in the PT was observed during every swimming episode and was also robustly coupled to RS cell and locomotor activity, which suggests a critical role in the control of locomotion. Furthermore, neural activity was observed in the same PT recording site during both olfactory-induced (Figs. 25 and 26) and spontaneous (Fig. 27) locomotion, which suggests the existence of a common neuronal substrate at the level of the PT to induce locomotion.

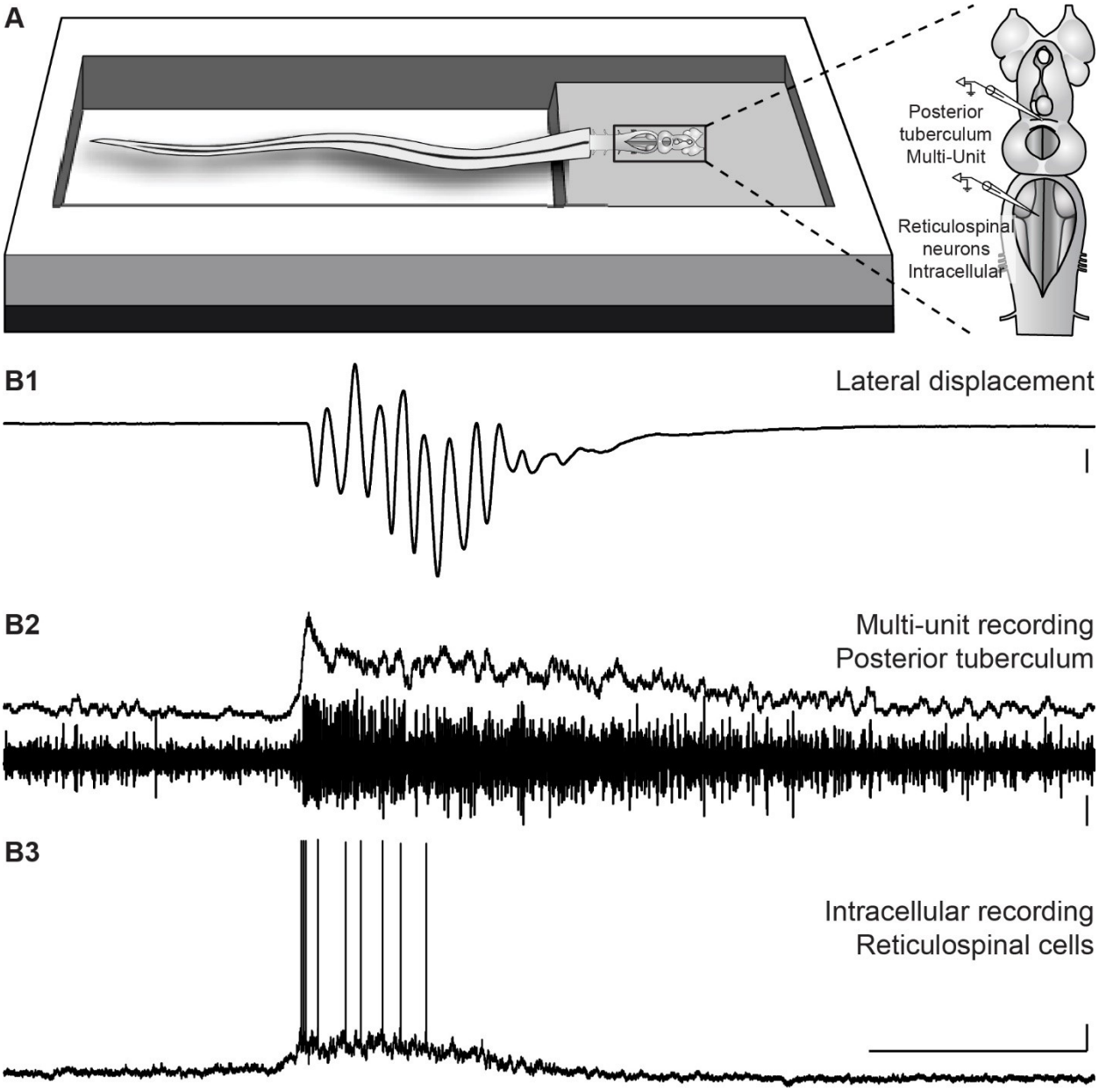

Figure 27. Extracellular activity in the posterior tuberculum and spiking activity in reticulospinal cells during spontaneous swimming in the semi-intact preparation.

(A, left) Schematized representation of the semi-intact lamprey preparation showing the isolated whole-brain (black frame) pinned to the bottom of the recording chamber, and the intact, freely-swimming body in a second, deeper compartment. **(A, right)** The brain is schematized to show the posterior tuberculum (PT) extracellular recording site, and the reticulospinal (RS) cell intracellular recording in the middle rhombencephalic reticular formation. **(B)** Episodes of spontaneous swimming were accompanied by neural bursts of activity in the PT and RS cell spiking. **(B1)**

Lateral displacement of a body segment was monitored with a video camera and plotted to illustrate swimming activity. **(B2)** Extracellular recording in the PT is shown and is additionally displayed directly above as a rectified and smoothed signal. **(B3)** Intracellular recording of a RS cell in the middle rhombencephalic reticular formation. Scale bar in B1: 20 mm; Scale bar in B2: 100 μV; Scale bars in B3: 5 s and 10 mV).

2.2.6 Discussion

Animals use olfaction to navigate in the environment, but the brain circuitry underlying olfactory behavior is not well characterized. In the lamprey, two distinct neural pathways that linked the peripheral olfactory apparatus to the spinal cord were studied (Ren et al., 2009; Derjean et al., 2010; Green et al., 2013; Green et al., 2017; Daghfous et al., 2018; Beauséjour et al., 2020; reviewed in Beauséjour et al., 2022). Here, we delve deeper into this circuitry, as we describe how olfactory inputs are transmitted to the posterior tuberculum and transformed into motor commands. In this study, we characterized secondary olfactory projections to the PT, demonstrated that it responds to olfactory inputs and provided evidence that neural activity in the PT is importantly involved in producing locomotion in response to sensory input. Within the DA nucleus of the PT, we found neurons that 1- respond to olfactory inputs and 2- project to the MLR, a brainstem region controlling locomotion (for review, see Ryczko et Dubuc, 2013). Moreover, we found individual neurons that are recruited by electrical stimulation of the olfactory nerve, medOB and LPal, which may constitute a common descending pathway for the medial and lateral olfactomotor pathways to activate the MLR and downstream locomotor circuitry. Since we found that neural activity in the PT is coupled to locomotion, a population of PT neurons may integrate sensory inputs and induce locomotion by activating the MLR.

2.2.6.1 Medial olfactory bulb projections to the posterior tuberculum

Direct anatomical OB projections to the PT were previously documented in several lamprey species (*Ichthyomyzon unicuspis*: Northcutt et Puzdrowski, 1988; *Lampetra fluviatilis*: Heier, 1948; Nieuwenhuys, 1977; Polenova et Vesselkin, 1993; Pérez-Fernández et al., 2014; Suryanarayana et al., 2021b; *Petromyzon marinus*: Derjean et al., 2010; Green et al., 2013; Daghfous et al., 2018; Beauséjour et al., 2020), cartilaginous fishes (Yáñez et al., 2011) and teleostean fishes (Rooney et al., 1992; Matz, 1995; von Bartheld, 1984; Northcutt, 2011; Northcutt et Rink, 2012; Miyasaka et al., 2014). However, it was never confirmed physiologically whether olfactory activity in the OB is transmitted to the PT. In the lamprey, as mentioned above, two distinct neural pathways linking the OB to the PT exists: the medial (medOB – PT) and lateral (MOB – LPal – PT) pathways. In the latter, projections to the PT from the MOB (Suryanarayana et al., 2021b) and the LPal (Pérez-Fernández et al., 2014; Ocaña et al., 2015) were previously detailed and were not investigated in our study. On the other hand, characterization of specific medOB projections to the PT was lacking until now.

One aim of the present study was to identify the cellular phenotype of PT neurons that receive medOB projections, with a particular emphasis on DA cells. In our material, anterograde tracing of medOB projections allowed us to observe dense innervation of the DA nucleus of the PT (Fig 18). Moreover, medOB tracer injection combined with confocal microscopy revealed that medOB terminals in the PT are colocalized with DA-immunoreactive somata and neurites. The juxtaposition of medOB terminals with DA-immunoreactive neurons means that medOB projections are positioned to directly recruit them and suggests that medOB projection neurons directly relay olfactory inputs to DA neurons of the PT. Moreover, as in the PT of zebrafish (Filippi et al., 2014) and mammalian SNc/VTA (Sulzer et al., 1998; Yamaguchi et al., 2011; Yamaguchi et al., 2013; Yamaguchi et al., 2015; Root et al., 2016; Morales et Margolis, 2017), DA neurons co-express other neurotransmitters such as glutamate (Ryczko et al., 2017; von Twickel et al., 2019; present results) and GABA (von Twickel et al., 2019; present results). Indeed, in the lamprey, a combination of immunofluorescence directed against tyrosine hydroxylase with *in situ* hybridization directed against the vesicular glutamate transporter (vGluT: glutamatergic neuron marker) or glutamic acid decarboxylase (GAD: GABAergic neuron marker) allowed von Twickel and colleagues (2019) to observe different neuronal types such as DA/Glutamate, DA/GABA, Glutamate/GABA and even triple-labeled DA/Glutamate/GABA neurons. Since other authors could not previously observe double-labeled DA/Glutamate (Fernández-López et al., 2017) or DA/GABA (Barreiro-Iglesias et al., 2009) neurons in the PT, it was stated that the low sensitivity of their method (immunofluorescence) may account for their negative results (von Twickel et al., 2019). However, in our material, immunofluorescence was successfully used to obtain both DA/Glutamate and DA/GABA co-labelling of neurons in the PT. Interestingly, we found that medOB projections terminate in close proximity to PT neurons co-expressing 1- DA/Glutamate (Fig. 19B) and 2- DA/GABA (Fig. 19C), which suggests that they detect olfactory signal from the medial olfactomotor pathway. Based on their transmitter content and anatomical projections, these neuronal populations may process olfactory inputs differently and have distinct functions. For example, it was shown that individual DA/Glutamate neurons project to both the striatum and the MLR (Ryczko et al., 2013) or the optic tectum (Pérez-Fernández et al., 2017).

In the isolated forebrain preparation, we were able to precisely position pipettes into the DA nucleus of the PT that is easily accessible for physiological recordings. This allowed us to record extracellular (multi-unit) and intracellular (patch-clamp, Ca2+ imaging) responses of PT neurons

following medOB stimulations. More importantly, in the semi-intact preparation, trains of electrical stimuli to the medOB induced sustained bursts of activity in the PT. Altogether, these results demonstrate anatomically and physiologically that PT neurons can indeed detect medOB activity. That DA neurons may be activated by medOB projections is supported by the following observations: 1 – our extracellular recording pipette was positioned over the DA nucleus of the PT (Figs. 21, 22 and 25) and 2 – calcium responses were obtained from neurons located within the DA nucleus of the PT (Fig. 24). Moreover, it is plausible that medOB neurons target DA neurons in the PT since projections neurons of the MOB do (Suryanarayana et al., 2021b). Furthermore, it is also important to note that DA neurons in the PT send projections to the medOB (Beauséjour et al., 2020) and that there could be reciprocal connections between the medOB and the PT that could allow these neurons to modulate the inputs they receive from the medOB. Olfactory projections to the PT may have been evolutionarily conserved since in zebrafish, all glomerular clusters send projections that are closely associated with DA neurons in the PT (Miyasaka et al., 2014). Hence, we propose that medOB projections transmit olfactory signals to some DA neurons of the PT. Further studies are required to determine the exact phenotypes of other PT neurons that may respond to olfactory inputs from the medOB, but also from the MOB, and the LPal.

2.2.6.2 Main olfactory bulb and lateral pallium projections to the posterior tuberculum

Anatomical projections from the lateral olfactomotor pathway to the PT were previously described. Projections of the MOB directly terminate on DA neurons in the PT (Suryanarayana et al., 2021b). However, MOB olfactory signal may reach the PT mainly through a relay in the LPal (Daghfous et al., 2018), which also sends efferent fibers that directly terminate on DA neurons (Pérez-Fernández et al., 2014; Ocaña et al., 2015). Since these anatomical projections were already detailed, they were not examined in our study. Extracellular recordings revealed that although MOB-PT connections exists, MOB stimulation failed to elicit responses in the PT except when gabazine, a $GABA_A$ receptor antagonist, is added to the bath. This result is consistent with the idea that a GABAergic tone exists in the OB that acts as a ‘’gatekeeper’’ and downregulates transmission in lateral olfactomotor pathway (Daghfous et al., 2018).

LPal stimulation reliably induced extracellular responses in the PT, which confirms previously characterized anatomical projections (Pérez-Fernández et al., 2014; Ocaña et al., 2015). Similarly to the medial olfactomotor pathway, single pulse LPal stimulations induce extracellular (Figs. 21

and 22) and calcium (Fig. 24) responses in the PT. In the semi-intact preparation, bilateral trains of electrical stimuli to the LPal elicited sustained bursts of extracellular activity in the PT (Fig. 26). Altogether, our data confirm physiologically that PT neurons are activated by LPal inputs.

In the light of recent results showing that the LPal is far more complex than originally thought and may even be considered as a homologue of the mammalian neocortex (reviewed in Suryanarayana et al., 2021a), the exact position of the stimulation electrode in the LPal during our experiments is a matter that must be addressed. Indeed, although the LPal is often considered as a region with mostly olfactory functions (Johnston, 1902; Edinger, 1905; Kappers et Theunissen, 1908; Herrick et Obenchain, 1913; Nieuwenhuys, 1967; Nieuwenhuys, 1977; Northcutt et Wicht, 1997; Daghfous et al., 2018), it can now be subdivided into multiple subregions with separate afferent and efferent connectivity. Mainly, the LPal can be segmented into a ventral sensory area where olfactory functions have been demonstrated (Suryanarayana et al., 2021b), and a dorsal area that projects to motor centers and contains distinct visual, somatosensory, and motor areas (Ocaña et al., 2015; Suryanarayana et al., 2020). Moreover, anatomical analysis has shown that neurons targeting the PT are located preferentially in the dorsolateral part of the LPal (Ocaña et al., 2015). Interestingly, our electrical stimulation site in the LPal (depicted in Fig. 26) corresponds this dorsolateral area where a high density of PT-projecting neurons was observed (Ocaña et al., 2015). These anatomical data support our physiological recordings of PT neurons activated by LPal stimulation.

2.2.6.3 Convergence of the medial and lateral olfactomotor pathways in the posterior tuberculum

In the lamprey, two segregated olfactory subsystems were previously identified (reviewed in Beauséjour et al., 2022). The first originates from the accessory olfactory organ, a distinct olfactory epithelium located in the nasal cavity that projects only the medOB (Ren et al., 2009; Green et al., 2017). The second originates from the main olfactory epithelium and projections span all over the MOB (Green et al., 2017). Interestingly, the olfactory signal from the accessory olfactory organ and the main olfactory epithelium may converge in the PT, presumably to induce locomotion. Our data show that electrical stimulation of the olfactory nerve, which is constituted of primary afferents from both the accessory olfactory organ and the main olfactory epithelium, also induces extracellular responses in the PT (Figs. 20, 21, 22 and S28). These responses were blocked by injection of glutamate receptor antagonists in the PT, which is coherent with the fact that OB

projection neurons (or mitral cells) are glutamatergic (Villar-Cerviño et al., 2011). The stimulation of the olfactory nerve may obviously recruit the medial and lateral olfactomotor pathways simultaneously, and the observed PT responses may correspond to the activity induced in both pathways. Indeed, when the stimulation electrode is repositioned from the olfactory nerve to the medOB or the LPal, with the same stimulation parameters, extracellular responses, albeit visibly smaller, are also observed within the same PT recording site (Figs. 21, 22, and S28). Moreover, in semi-intact preparations where the medOB and LPal were both stimulated, potentialized extracellular responses were recorded within the same PT recording site (Figs. 25 and 26). Furthermore, axon terminals from the medOB (Fig. 18), MOB (Suryanarayana et al., 2021b) and the LPal (Pérez-Fernández et al., 2014; Ocaña et al., 2015) colocalize with DA neurons in the PT. Thus, the signal induced by olfactory nerve stimulation may be carried via both the medial and lateral olfactomotor pathways to the same neuronal populations inside the DA nucleus of the PT. More importantly, Ca^{++}-imaging experiments demonstrated that specific PT neurons are activated by stimulation of (1) the olfactory nerve, (2) the medOB and (3) the LPal (Fig. S31). These data provide strong evidence for our hypothesis that olfactory inputs from both the accessory olfactory organ and main olfactory epithelium converge on the same DA neurons in the PT through the medial and lateral olfactomotor pathways.

Another interesting characteristic of olfactory projections to the PT is the bilateral inputs from the medOB and the LPal. Indeed, electrical stimulation of the contralateral olfactory nerve, medOB, or LPal, induced similar responses in the PT than ipsilateral stimulation (Fig. S28). Moreover, Ca^{++}-imaging showed that stimulation of the olfactory nerve, medOB, and LPal activate neurons in the contralateral DA nucleus of the PT (Figs. 24, S29, S30 and S31). These data are consistent with our observations that dense medOB projections decussate in the PT commissure and terminate in close proximity to DA neurons in the contralateral PT.

2.2.6.4 The role of the posterior tuberculum in olfactory-induced locomotion

In our material, we could robustly induce swimming in the semi-intact preparation by electrical and chemical stimulation of the medOB and the LPal that was coupled with RS cell spiking and neural bursts of activity in the PT. Indeed, stimulation of brain regions associated with the medial or lateral olfactomotor pathways induced neural activity in both the PT and RS cells that began and terminated concurrently. This provides strong evidence that PT activity plays an important role in

the production of olfactory-induced locomotion, presumably by recruiting downstream locomotor centers. Interestingly, we have additionally demonstrated here that PT neurons which are recruited by olfactory inputs also project to the MLR, a brainstem region involved in the initiation, maintenance, and stopping of locomotion (for reviews, see Dubuc et al., 2008; Le Ray et al., 2011; Ryczko et Dubuc, 2013; Grätsch et al., 2019b). Projections to the MLR were extensively characterized previously as PT neurons storing DA (Ryczko et al., 2013), glutamate and both DA/glutamate (Ryczko et al., 2017), or GABA (Ménard et al., 2007) were retrogradely-labeled after injections of anatomical tracer in the MLR. The glutamatergic projection produces a graded increase in MLR activity and swimming speed (Ryczko et al., 2017) while the DA projection provides additional excitation by activating D1 receptors in the MLR (Ryczko et al., 2013; Ryczko et al., 2017). The roles of GABAergic neurons projecting to the MLR were not investigated. Moreover, a DA projection from the PT was also shown to directly innervate every RS cell nucleus and, via D1 receptors, increase their activity and swimming speed evoked by the stimulation of the PT (Ryczko et al., 2020). Thus, the PT can initiate and modulate locomotor activity via DA, glutamatergic, and presumably GABAergic projections. Interestingly, we now show that projections from the medOB terminate in the DA nucleus of the PT in close proximity with neurons storing DA, glutamate, GABA, DA/glutamate and DA/GABA, which makes them probable synaptic partners for the transmission of the olfactomotor signal to the MLR and to RS cells. In the lateral olfactomotor pathway, it was also shown that DA neurons in the PT are in close proximity with MOB (Suryanarayana et al., 2021b) and LPal (Pérez-Fernández et al., 2014; Ocaña et al., 2015) axon terminals.

Altogether, the present results strongly support our hypothesis that DA neurons in the PT receive olfactory inputs and contribute to the production of motor output by recruiting and modulating downstream motor centers such as the MLR and RS cells. However, future experiments should test the inactivation of the PT on olfactory-induced swimming behavior to achieve a more thorough understanding of its exact role. Moreover, chemical stimulation of the olfactory epithelium with various odorants should be tested to investigate whether the PT is preferentially activated by specific odorants, and the resulting behavior in the semi-intact preparation. The semi-intact preparation used here (see material and methods) with the whole brain and peripheral olfactory apparatus left attached allowed us to demonstrate for the first time that swimming is induced by stimulating the medial olfactomotor pathway and may be an important tool for future studies of

olfactory-induced behaviors. Indeed, it provides experimental access to the brain and olfactory peripheral organ while allowing to analyze resulting movements of the body.

Our data reveal that the PT detects olfactory inputs and in turn, projects to regions involved in motor control. However, the PT is not dedicated only to processing olfactory inputs, as it receives information from several other of sensory channels (Pérez-Fernández et al., 2014), while its efferent projection pattern allows it to exert control over many motor nuclei. For instance, it was shown that the PT is activated by electrosensory stimulation applied in the surrounding bath and pulses of light delivered to the retina (Pérez-Fernández et al., 2017). Interestingly, Pérez-Fernandez and colleagues (2014; 2017) also showed that the PT sends DA projections to the optic tectum that are activated by visual stimuli. Moreover, in response to visual stimuli, the optic tectum induces distinct eye and head movements that are facilitated by the DA projections from the PT. Thus, visual input may recruit DA neurons in the PT, which then modulate activity in the tectal circuitry to adjust visuomotor responses. We believe that, similarly, olfactory inputs to the PT recruit descending DA projections to motor regions - such as the MLR (Ryczko et al., 2013; Ryczko et al., 2017) and RS cells (Ryczko et al., 2020) - to facilitate olfactomotor responses.

Furthermore, another mechanism through which the PT may affect the activity of motor nuclei is through ascending DA projections that exert control over the basal ganglia. Indeed, the basic organization of the basal ganglia was already present in lampreys and is very similar to that of mammals (Ericsson et al., 2011; Stephenson-Jones et al., 2011; Robertson et al., 2012; Stephenson-Jones et al., 2012; Ericsson et al., 2013a; Ericsson et al., 2013b; Stephenson-Jones et al., 2013; Pérez-Fernández et al., 2014; for reviews, see Grillner et al., 2013; Grillner et Robertson, 2016; Suryanarayana et al., 2021a). Thus, recruiting DA neurons in the PT may activate the lamprey homolog of the nigrostriatal pathway (Baumgarten, 1972; Pombal et al., 1997; Ericsson et al., 2013b) that will act through the direct or indirect pathway to relieve or increase the tonic GABAergic inhibition maintained by basal ganglia output neurons over motor regions, such as the optic tectum and the MLR. Thus, the PT may participate in locomotion either via descending and/or ascending projections. First, descending projections to the MLR may be part of a common locomotor pathway that is necessary, or at least facilitating, to induce locomotion. Second, ascending DA projections to the striatum may be necessary, or at least facilitating, for disinhibition of the MLR. Because the MLR is under tonic GABAergic inhibition by the basal ganglia output

nuclei (Stephenson-Jones et al., 2011), ascending DA projections would have to activate the direct pathway to allow disinhibition of the MLR. These two hypotheses are not mutually exclusive as both descending and ascending DA projections may be necessary to induce locomotion and also because individual DA neurons in the PT have dual projections to the striatum and the MLR (Ryczko et al., 2013). Due to the many efferent DA projections of the PT (Pérez-Fernández et al., 2014), much work still needs to be done to elucidate how olfactory inputs to the PT may shape behavior.

Finally, another interesting result is that we demonstrated that the PT is not only activated during sensory-evoked locomotion, but also during spontaneously occurring swimming in the semi-intact preparation (Fig. 27). This strongly suggests that the neuronal population in the PT that is active during locomotion in response to external stimuli may also be recruited when locomotion is needed to fulfill the internal needs of the animal (for example, hunger induces foraging). A study from Thompson and colleagues (2008) also supports the importance of the PT during spontaneously occurring locomotion. In lampreys injected with MPTP, a DA neuron-selective neurotoxin presumably damaging DA neurons in the PT, a dramatic decrease in striatal DA levels was observed in parallel with a reduction of spontaneous swimming. Remarkably, the initiation and maintenance of swimming activity induced by chemical stimulation of the olfactory epithelium was also severely weakened. Since non-selective DA receptor agonist apomorphine reduced these deficits (Thompson et al., 2008), the importance of DA in the initiation of spontaneous or olfactory-induced locomotion is very convincing.

2.2.6.5 Conclusion

In the lamprey, two distinct sensory epithelia exist in the peripheral olfactory organ and give rise to separate olfactory pathways that both have projections to the PT, the homolog of the mammalian SNc/VTA, which was proposed to produce to locomotor output in response to olfactory input (Derjean et al., 2010; Daghfous et al., 2018). The present study shows anatomically and physiologically how the PT integrates information from both the medial and lateral olfactomotor pathways. Neurons in the DA nucleus of the PT receive bilateral input from both the medOB and the LPal and project down to the MLR, a brainstem region well-known to induce locomotion. Glutamatergic, GABAergic, and mostly DA neurons may be involved. However, further experiments must be done to confirm this. Moreover, we now demonstrate that electrical and

chemical stimulation of the medOB elicits swimming in a semi-intact preparation. Interestingly, simultaneous recording of PT activity indicates that it is tightly coupled to RS cell activity and undulatory swimming movements of the preparation. The results presented here provide additional insight into the neural circuits that allow the production of an appropriate motor response following odorant detection.

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2.2.8 Supplemental figures

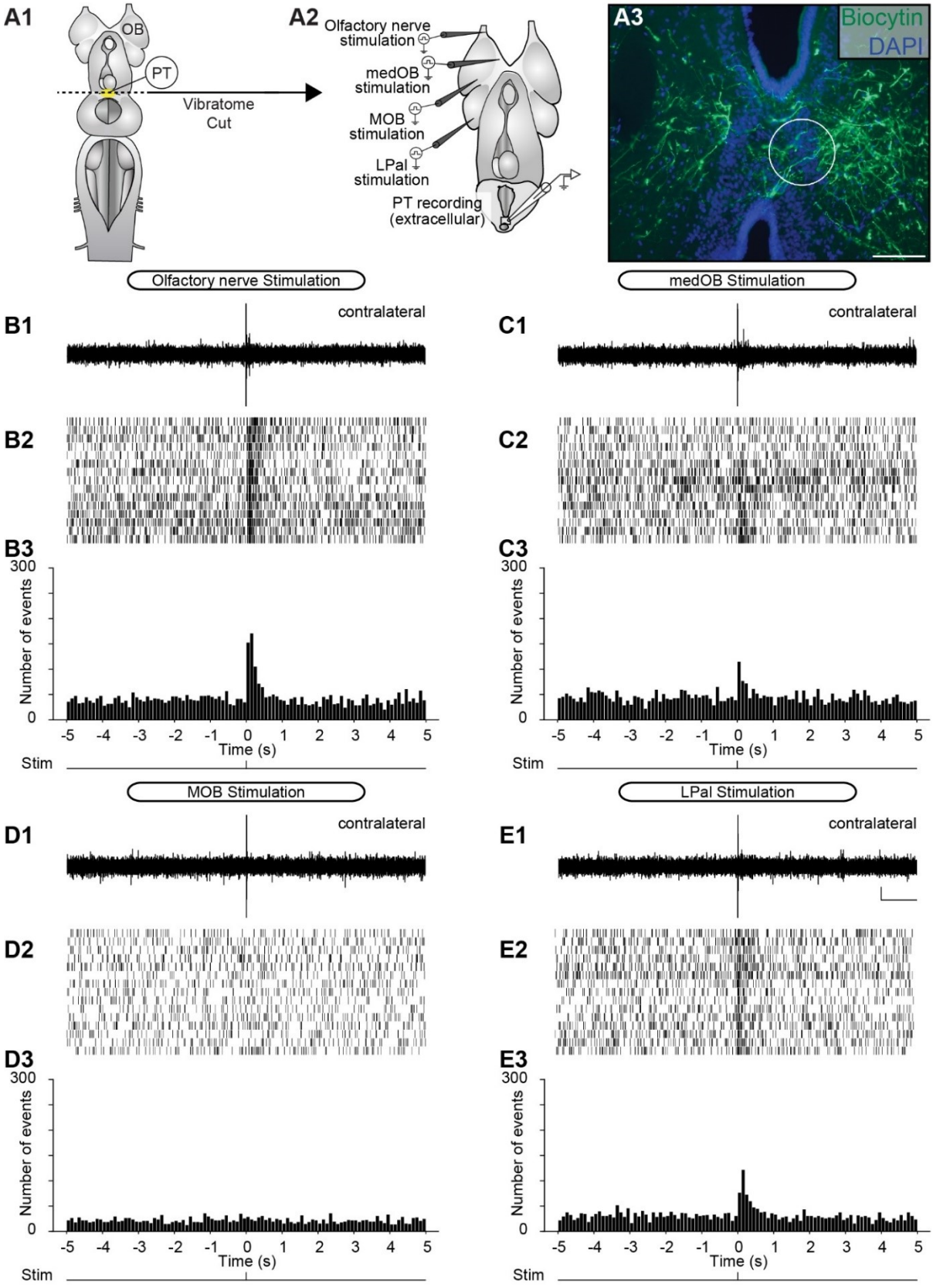

Figure S28. (Supplemental figure) Extracellular responses in the posterior tuberculum to electrical stimulation of the contralateral olfactory nerve, medial olfactory bulb, main olfactory bulb, and lateral pallium.

(A1) The schematic dorsal view of the isolated adult lamprey brain illustrates the rostro-caudal level at which a transverse section was performed to produce the isolated forebrain preparation **(A2)** that allows experimental access to the posterior tuberculum (PT). The schematic dorsal view of the isolated forebrain preparation illustrates the recording site in the PT and the multiple stimulation sites in the contralateral olfactory nerve (B), contralateral medial olfactory bulb (medOB; C), contralateral main olfactory bulb (MOB; D), and contralateral lateral pallium (LPal; E). **(A3)** Photomicrograph of a transverse section at the level of the PT illustrating the approximative extracellular recording site (white circle; tip diameter: 125 µm). Cell populations within the PT are labeled with 4',6-Diamidino-2-Phenylindole (DAPI; blue) and axonal projections of the medial olfactory bulb (green) are anterogradely-labeled by a biocytin injection. **(B1)** Extracellular recording in the PT shows the response evoked by electrical stimulation of the contralateral ipsilateral olfactory nerve in a representative animal. **(B2)** In a raster plot, responses (n = 25, N = 5) are aligned on the time of stimulation (Time = 0) and summed in a vertical bar chart (**B3**, bar width: 100 ms). **(C1-C3)** The same organization is shown with the same representative animal after the stimulation electrode was repositioned in the contralateral medOB, which evokes extracellular responses in the PT. **(D1-D3)** The same organization is shown with the same representative animal after the stimulation electrode was repositioned in the contralateral MOB, which does not evoke extracellular responses in the PT. **(E1-E3)** The same organization is shown with the same representative animal after the stimulation electrode was repositioned in the contralateral LPal, which evokes extracellular responses in the PT. Scale bar in A3: 100 µm; Scale bars in E1: 1 s and 50 µV.

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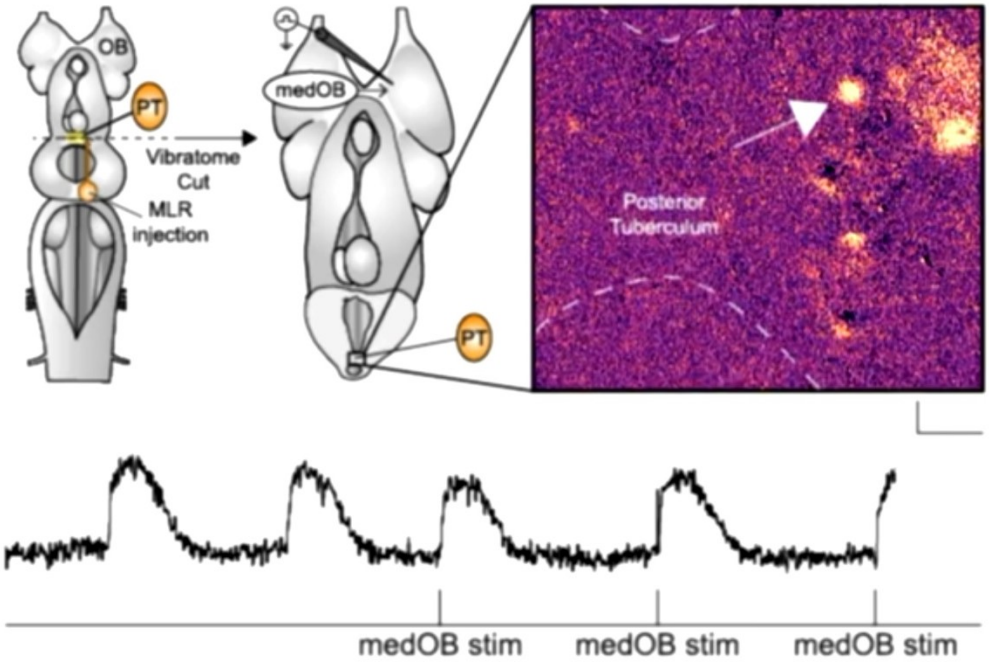

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Figure S29. (Supplemental figure) Calcium responses to medial olfactory bulb stimulation by neurons of the posterior tuberculum that project to the mesencephalic locomotor region, related to Figure 24.

(Top) Following Calcium Green-conjugated dextran amine crystals injection in the mesencephalic locomotor region (MLR), the brain was sectioned at the meso-diencephalic junction to produce the isolated forebrain preparation, which allows calcium-imaging in the posterior tuberculum (PT). The video in the top left panel represents the ΔF/F values measured from the calcium signal imaged in the PT during spontaneous activity and upon medial olfactory bulb (medOB) stimulation. White dashed lines over the video delineate the 3rd ventricle and the white arrow identifies the cell whose activity is shown below. **(Bottom)** The trace illustrates the ΔF/F activity of PT neurons to medOB stimulation after two episodes of spontaneous activity. The imaged cell corresponds to cell 11 in Figure 24. The video shows an acquisition bout that lasted 450 s (imaged at 2Hz) and compressed in 30 s (at 30 fps, played at 15x normal speed). Scale bars: 30 s and 20% ΔF/F.

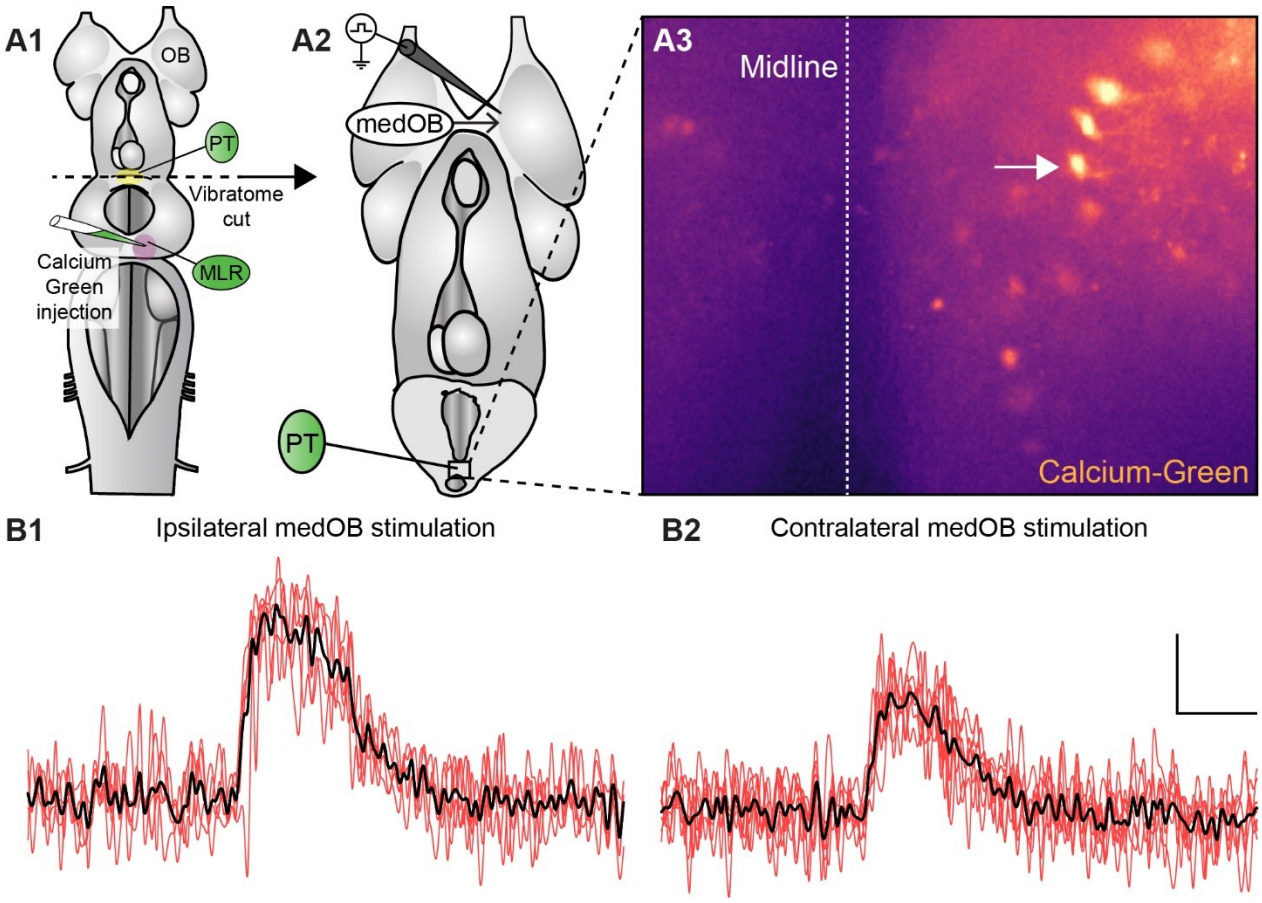

Figure S30. (Supplemental figure) Stimulation of the medial olfactory bulb induces bilateral calcium responses.

(A1) Schematic dorsal view of the adult lamprey brain illustrating the Calcium Green-conjugated dextran amine crystals injection site in the mesencephalic locomotor region (MLR). Also shown is the rostro-caudal level of the section performed to produce the isolated forebrain preparation **(A2)**, which allows calcium-imaging in the posterior tuberculum (PT) and electrical stimulation of the medial olfactory bulb (medOB). **(A3)** Colorized image of PT neurons retrogradely-labeled by a Calcium-green injection in MLR. Both traces in B1 and B2 were acquired in a single identified neuron (white arrow). **(B1-B2)** Calcium responses evoked by electrical stimulation of the ipsilateral **(B1)** and contralateral **(B2)** medOB are represented as five superimposed traces (red) and their mean (thick black trace). Scale bars in B2: 10 s and 10 % ΔF/F.

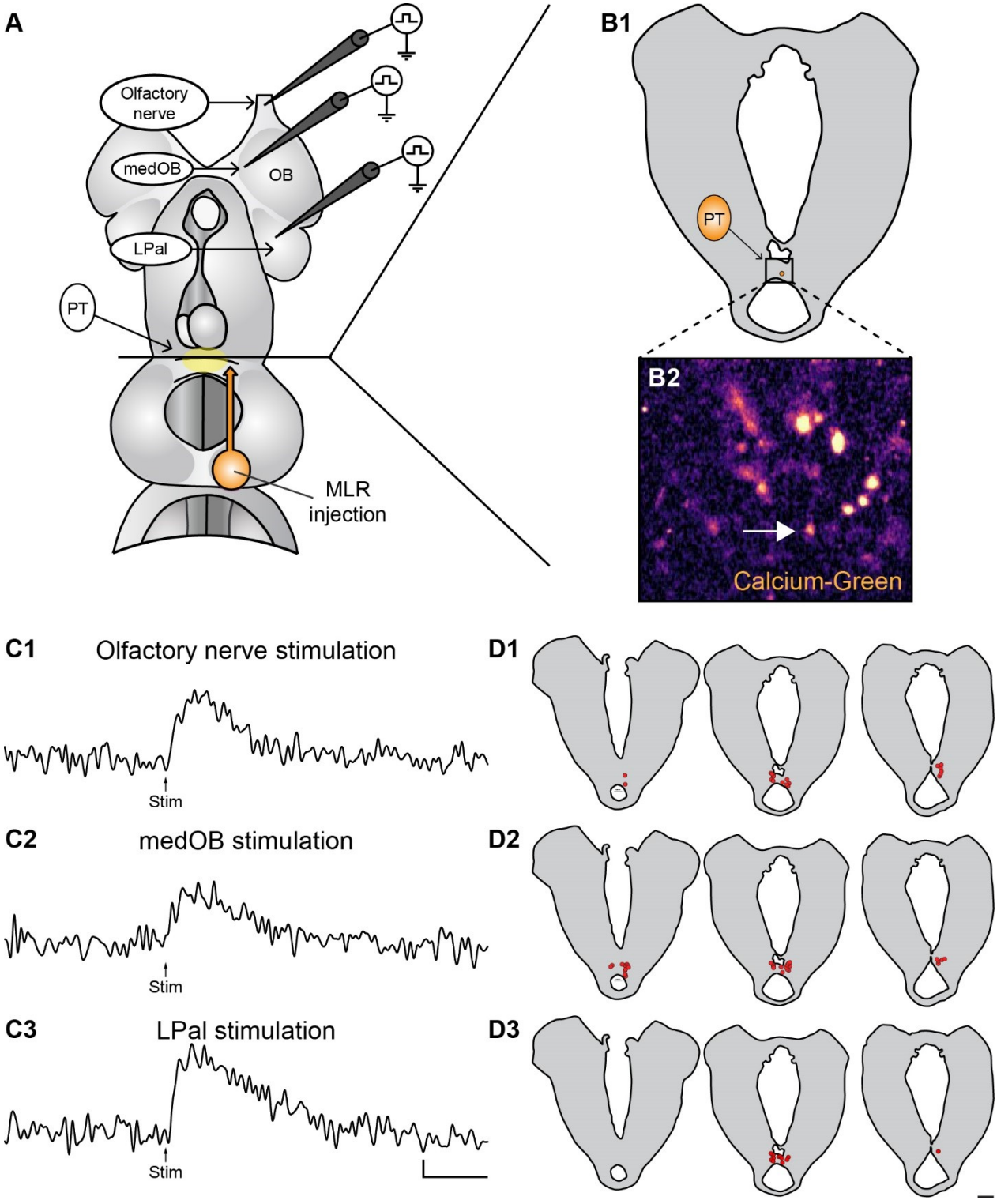

Figure S31. (Supplemental figure) Stimulation of the olfactory nerve, medial olfactory bulb and lateral pallium induces calcium responses in the same neurons.

(A) Schematic dorsal view of the adult lamprey brain illustrating the Calcium Green-conjugated dextran amine crystals injection site in the mesencephalic locomotor region (MLR) and the rostro-caudal level of the section performed to produce the isolated forebrain preparation. Moreover, the electrical stimulation sites in the olfactory nerve, medial olfactory bulb (medOB), and lateral pallium (LPal) are illustrated. **(B1)** Schematized transverse section at the level of the posterior tuberculum (PT), the black frame illustrates the calcium-imaging site within the PT **(B2)** where neurons are retrogradely-labeled by Calcium-Green (orange). The white arrow identifies the neuron from which the calcium responses in C were recorded. Following bath-perfusion of gabazine (10 µM), calcium responses to electrical stimulation of the ipsilateral olfactory nerve **(C1)**, medOB **(C2)**, and LPal **(C3)** were observed in an individual PT neuron that projects to the MLR and are shown as the mean of six responses. **(D)** Schematized transverse sections at the level of the PT that represents the approximate localization of neurons (enlarged red dots) responding to the electrical stimulation of the olfactory nerve (**D1**, N = 4 animals, n = 19 neurons), medOB (**D2**, N = 5 animals, n = 30 neurons), and LPal (**D3**, N = 2 animals, n = 13 neurons). Scale bars in C: 10 s and 10 % ΔF/F; Scale bar in D3: 200 µm.

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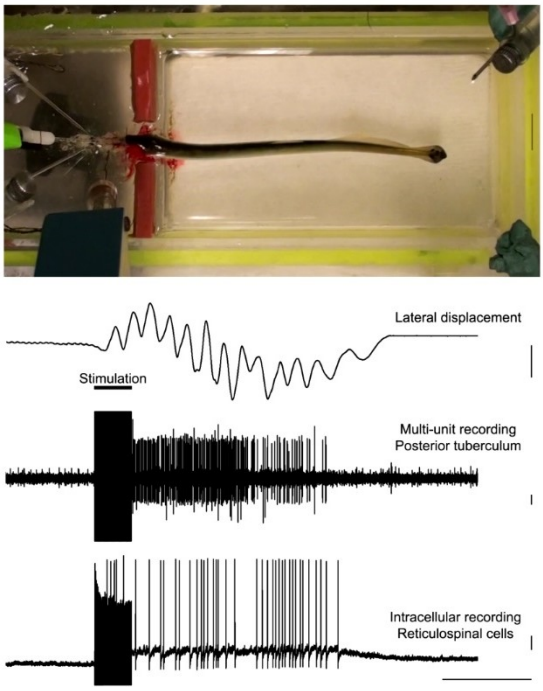

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Figure S32. (Supplemental figure) Swimming activity induced by electrical stimulation of the medial olfactory bulb, related to Figure 25.

(Top) Video recording of a semi-intact preparation in which the isolated whole-brain is pinned to the bottom of the recording chamber, and the intact body is freely-swimming in a second, deeper compartment. Bilateral electrical stimulation (25 Hz, 2s, 5 – 30 µA) of the medial olfactory bulb induces swimming activity **(top trace)**, concurrently with bursts of neural activity in the posterior tuberculum **(middle trace)** and spiking activity in intracellularly recorded reticulospinal cells of the middle rhombencephalic reticular formation **(bottom trace)**. Scale bar in top panel: 10 mm; Scale bar in top trace: 20 mm; Scale bar in middle trace: 100 µV; Scale bars in bottom trace: 5s and 10 mV.

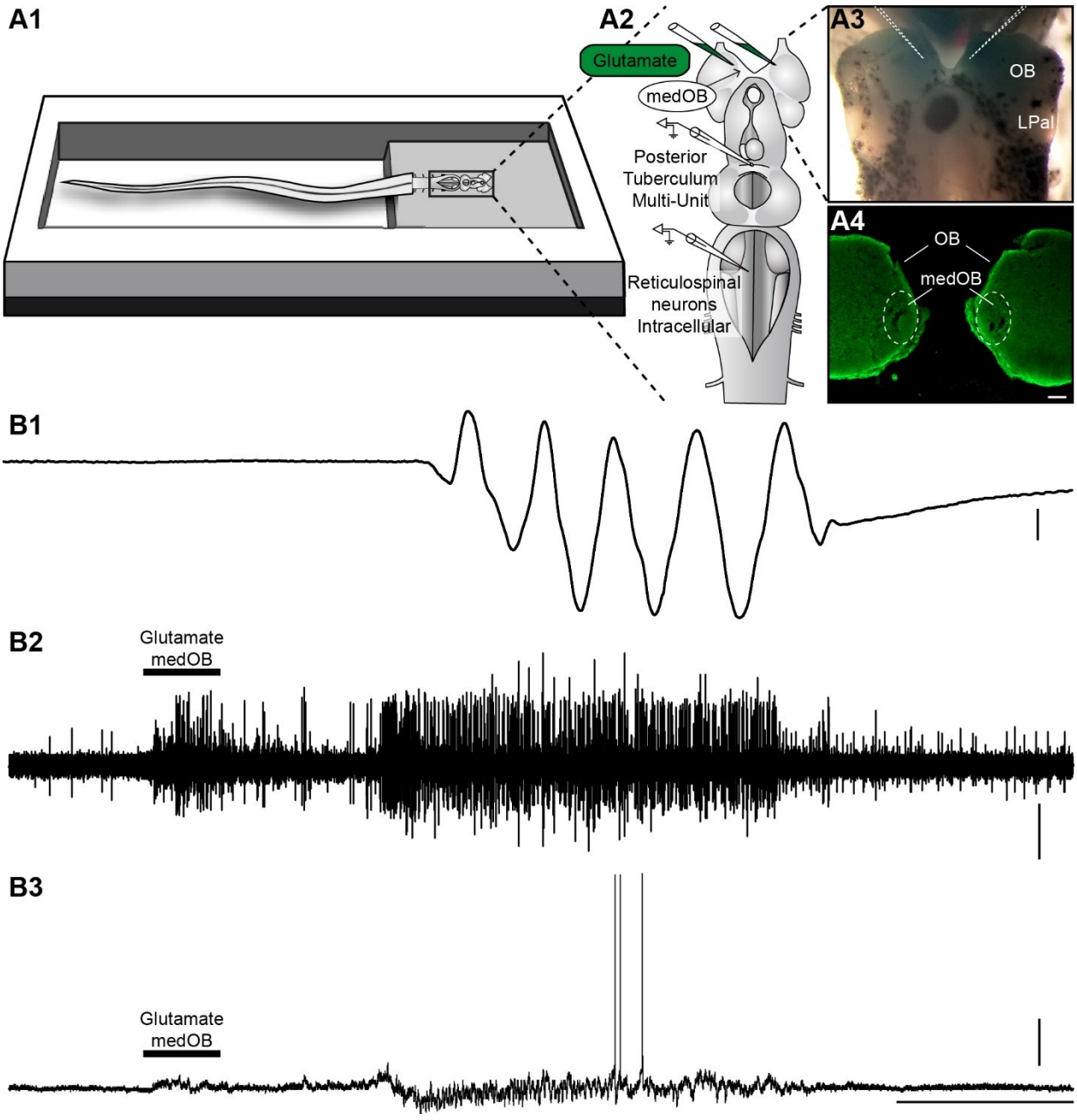

Figure S33. (Supplemental figure) Chemical stimulation of the medial olfactory bulb produces swimming, extracellular activity in the posterior tuberculum and spiking activity in reticulospinal cells.

(A1) Schematized representation of a semi-intact lamprey preparation showing the isolated whole-brain (black frame) pinned to the bottom of the recording chamber, and the intact, freely-swimming body in a second, deeper compartment. **(A2)** The brain is schematized to show the bilateral glutamate injection in the medial olfactory bulbs (medOB), the extracellular recording in the

posterior tuberculum (PT), and the intracellular recording of reticulospinal (RS) cell in the middle rhombencephalic reticular formation. **(A3)** Photograph of the dorsal view of the telencephalon with microinjection pipettes (white dashed lines) bilaterally positioned in the medOBs. **(A4)** Photomicrograph of a transverse section at the level of the olfactory bulbs showing the lesion (white dashed lines) produced by the insertion of the microinjection pipettes in the medOB. This confirms that the tip of both microinjection pipettes was within the medOB. **(B)** Bilateral glutamate (3 mM) injection in the medOB induced episodes of swimming activity that was accompanied by neural bursts of activity in the PT and RS cell spiking. **(B1)** The lateral displacement of a body segment was monitored with a video camera and plotted to illustrate swimming activity. Concurrently, extracellular activity was recorded in the PT **(B2)** and RS cell activity was intracellularly recorded **(B3)**. Scale bar in A4: 100 μm. Scale bar in B1: 10 mm; Scale bar in B2: 200 μV; Scale bars in B3: 5s and 20 mV.

VIDEO – VIDEO – VIDEO – VIDEO - VIDEO

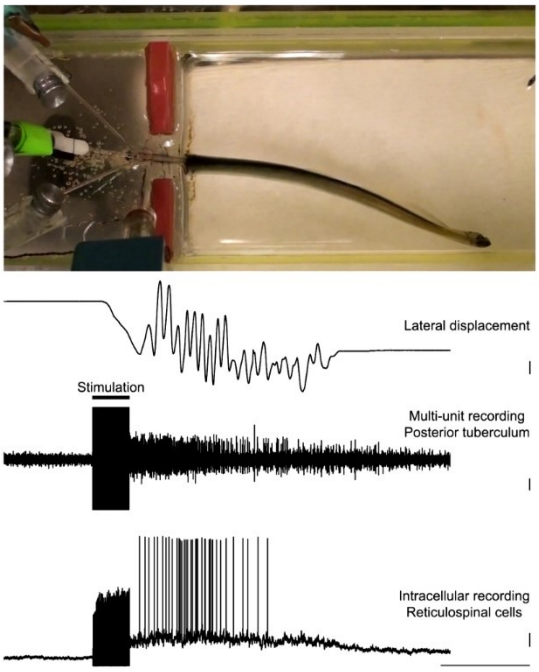

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Figure S34. (Supplemental figure) Swimming activity induced by electrical stimulation of the lateral pallium, related to Figure 26.

(Top) Video recording of a semi-intact preparation in which the isolated whole-brain is pinned to the bottom of the recording chamber, and the intact body is freely-swimming in a second, deeper compartment. (B) Bilateral electrical stimulation (25 Hz, 2s, 5 – 30 µA) of the lateral palliums induces swimming activity **(top trace)**, concurrently with bursts of neural activity in the posterior tuberculum **(middle trace)** and spiking activity in intracellularly recorded reticulospinal cells of the middle rhombencephalic reticular formation **(bottom trace)**. Scale bar in top trace: 5 mm; Scale bar in middle trace: 100 µV; Scale bars in bottom trace: 5s and 10 mV).

3. Discussion

3.1 Résumé des résultats

La recherche décrite dans cette thèse a pour objectif de mieux comprendre les circuits par lesquels des comportements sont déclenchés par la détection de molécules odorantes. Bien que l'odorat ait un impact important sur le comportement, le substrat neuronal permettant la transformation d'un signal olfactif en une réponse motrice demeure méconnu chez la vaste majorité des animaux. Dû à la complexité de ces circuits chez les mammifères, un modèle plus simple a été utilisé pour permettre l'étude de ces circuits à l'échelle cellulaire: la lamproie. Les recherches effectuées chez ce vertébré basal ont d'abord permis d'observer un large répertoire de comportements olfactifs ainsi que de détailler l'organisation de ses systèmes olfactif et moteur, ce qui en fait un excellent modèle pour établir un lien entre ces deux systèmes. Des études précédemment réalisées dans le laboratoire du Dr Dubuc (Derjean et al., 2010; Daghfous et al., 2018) suggèrent que le TP participerait à la transmission des signaux olfactifs vers le système moteur, mais la fonction qu'il occupe dans ce circuit n'a pas encore été caractérisée. L'objectif principal de ce projet de doctorat était donc de clarifier le rôle du TP dans le traitement des informations olfactives, notamment 1) de déterminer si le TP détecte effectivement les signaux olfactifs ainsi que 2) de caractériser ses projections ascendantes qui pourraient moduler l'activité du BO et 3) ses projections descendantes qui pourraient recruter des régions impliquées dans la production de locomotion.

Premièrement, des projections du BO au TP ont été observées anatomiquement à plusieurs occasions chez la lamproie et plusieurs autres espèces de poissons (voir section 3.2), ce qui suggère qu'il s'agit d'une connexion qui aurait été évolutivement conservée. Cependant, il n'a jamais été démontré que le TP répond aux informations olfactives, jusqu'à maintenant. Nos résultats démontrent que les neurones du TP sont recrutés à la fois par la stimulation de la voie olfactomotrice médiale (BO médian-TP) et latérale (BO principal-Pallium latéral-TP). Ces données fournissent un soutien important à l'hypothèse proposée par Derjean et al. (2010) selon laquelle le TP pourrait relayer l'activité olfactive à des régions motrices pour induire de la locomotion. De plus, l'innervation du TP par le BO médian a été caractérisée et les neurones DA ont été identifiés comme une cible probable de ces projections.

Ensuite, sachant que le TP est recruté par les informations olfactives, la prochaine étape était d'identifier comment son activité peut influencer les circuits olfactomoteurs. Des expériences anatomiques ont d'abord permis d'observer que des neurones DA du TP projettent spécifiquement au BO médian. Pour caractériser en détail l'innervation DA du BO, un nouveau protocole d'immunofluorescence ciblant directement la molécule de DA a dû être mis au point pour rehausser la qualité du marquage. Cette amélioration a permis de révéler la présence de deux innervations distinctes dans le BO: une innervation du BO principal par des interneurones DA locaux ainsi qu'une innervation du BO médian par des fibres extrinsèques en provenance du TP. Ensuite, des expériences physiologiques ont révélé que la transmission d'informations olfactives vers le système moteur est inhibée par l'activation de récepteurs D2 dans le BO médian, ce qui diminuerait la production de mouvements en réponse à la détection d'odeurs. Donc, le TP reçoit de l'information olfactive directement du BO médian et, en retour, envoie des projections DA qui pourraient être impliquées dans la modulation du traitement olfactif produisant des comportements olfactomoteurs.

Le TP reçoit des informations olfactives encodées dans le BO et en retour, il a été proposé que le signal est transmis vers les cellules RS pour initier la locomotion (Derjean et al., 2010). Cependant, les mécanismes par lesquels les neurones du TP acheminent le signal olfactif à la formation réticulée n'avaient pas encore été confirmés. Le TP possède des projections DA vers de multiples régions impliquées dans le contrôle moteur (Pérez-Fernández et al., 2014), mais parmi celles-ci, la plus susceptible de produire de la nage est sa projection à la MLR (Ryczko et al., 2013; Ryczko et al., 2017), une région bien connue chez les vertébrés pour contrôler la locomotion par ses projections directes à la formation réticulée (Sirota et al., 2000; Ryczko et Dubuc, 2013; Caggiano et al., 2018; Noga et Whelan, 2022). Des expériences anatomiques ont alors permis d'observer que les projections du BO médian innervent des neurones DA du TP qui projettent à la RLM. De plus, des expériences d'imagerie calcique ont permis de confirmer que les neurones du TP associés à la RLM sont robustement recrutés par la stimulation des deux voies olfactomotrices, médiale (BO médian-TP) et latérale (BO principal-Pallium latéral-TP). Finalement, nous avons montré pour la première fois que la stimulation olfactive induit effectivement la nage, mais aussi que le TP est invariablement actif durant cette activité locomotrice. En effet, la stimulation de la voie olfactomotrice médiale ou latérale mène à une activité potentialisée dans le TP qui est couplée avec l'activité des cellules RS ainsi que les mouvements de nage de l'animal. De plus, nos résultats

indiquent que le TP est également activé durant les épisodes de nage qui surviennent spontanément. Ainsi, des neurones DA au sein du TP pourraient constituer une voie descendante commune induisant des réponses motrices aux stimuli externes (par exemple: la détection d'une proie entraîne un comportement d'approche) ou aux besoins internes de l'animal (par exemple: la faim déclenche un comportement d'exploration).

3.2 Le tubercule postérieur détecte les stimuli olfactifs

Le TP reçoit des projections axonales de multiples régions sensorielles (Pérez-Fernández et al., 2014) et il a été démontré qu'il est recruté par les systèmes visuel et électrosensoriel (Pérez-Fernández et al., 2017). Ainsi, l'activité du TP pourrait être influencée par plusieurs formes d'informations sensorielles, incluant les stimuli olfactifs. En effet, il est connu depuis longtemps que le TP reçoit des projections axonales en provenance du BO chez plusieurs espèces de lamproies (Heier, 1948; Nieuwenhuys, 1977; Northcutt et Puzdrowski, 1988; Polenova et Vesselkin, 1993), chez plusieurs espèces de requins (Yáñez et al., 2011) ainsi que chez le poisson rouge (von Bartheld et al., 1984), la morue (Rooney et al., 1992), le saumon (Matz, 1995), l'esturgeon (Northcutt, 2011), le dipneuste (Northcutt et Rink, 2012) et le poisson-zèbre (Miyasaka et al., 2014). Ces données suggèrent qu'il s'agirait d'une projection évolutivement conservée qui serait donc importante pour la survie et la reproduction de l'animal. Par ailleurs, des études récentes du laboratoire de Réjean Dubuc en collaboration avec le laboratoire de Barbara Zielinski ont permis de proposer deux voies neuronales par lesquelles les informations olfactives seraient véhiculées jusqu'au TP chez la lamproie (Derjean et al., 2010; Green et al., 2013; Daghfous et al., 2018; pour une revue, voir Beauséjour et al., 2022). En périphérie, deux organes olfactifs contiennent des neurones chimio-sensibles dont les axones forment le nerf olfactif et projettent au BO en innervant des territoires glomérulaires différents (Ren et al., 2009; Green et al., 2013; Green et al., 2017). Dans un premier temps, l'organe olfactif accessoire (OOA) innerve uniquement le BO médian, tandis que l'épithélium olfactif principal (ÉOP) distribue ses projections dans l'ensemble du BO principal (Ren et al., 2009; Green et al., 2017). Le BO médian et le BO principal contiennent deux populations distinctes de neurones de projection (Green et al., 2013). Notamment, le patron de projection diffère entre les neurones de projection du BO médian qui innervent principalement le TP et les neurones de projection du BO principal qui innervent principalement le pallium latéral (PL; Derjean et al., 2010; Green et al., 2013). De plus, il a été proposé que les neurones de projection du PL qui reçoivent le signal du BO principal innerveraient à leur tour le TP (Pérez-Fernández et al., 2014; Ocaña et al., 2015; Daghfous et al., 2018). Ainsi, l'information olfactive détectée par l'OOA et l'ÉOP serait relayée en parallèle dans des circuits qui convergent dans le TP. Cependant, il n'existait pas de preuve directe que les stimuli olfactifs puissent recruter les neurones du TP. En conséquence, une des hypothèses de travail de la présente thèse était que le TP soit activé par la stimulation olfactive. Des expériences anatomiques et physiologiques nous ont

permis de caractériser les projections du BO médian dans le TP et de montrer que des neurones individuels y sont recrutés à la fois par la stimulation de la voie médiale et de la voie latérale.

L'innervation du TP par le BO médian a déjà été démontrée par l'injections de traceurs qui marquent rétrogradement les neurones de projection du BO médian (Derjean et al., 2010; Green et al., 2013; Pérez-Fernández et al., 2014; Daghfous et al., 2018; Figures 7, 8 et 9). À l'inverse, le traçage antérograde à partir du medOB a permis d'observer que certaines projections s'arrêtent dans le TP ipsilatéral tandis que quelques-unes traversent la ligne médiane (Derjean et al., 2010). Cependant, une caractérisation détaillée de cette projection n'avait jamais été réalisée avant les travaux de cette thèse. Premièrement, cette innervation a été cartographiée précisément (Figure 18), ce qui a permis d'observer quels territoires du TP sont susceptibles de recevoir l'information du BO médian. Notamment, le TP contient un noyau de neurones DA (Baumgarten, 1972) qui est divisé en deux sous-populations: dorso-médiane et ventro-latérale (Pierre et al., 1997). Dans nos résultats et d'autres résultats précédents du laboratoire (Ryczko et al., 2013), les neurones de la sous-population ventro-latérale sont marqués intensément par l'immunofluorescence ciblant la TH, mais très peu par l'immunofluorescence ciblant la DA. Pour expliquer le nombre moins élévé de neurones marqués par l'immunofluorescence ciblant la DA, il a été proposé que la pénétration des anticorps dans le tissu est limitée par la fixation au glutaraldéhyde (Ryczko et al., 2013). Nous pensons aussi que les neurones de la sous-population ventro-latérale du TP contiennent une concentration plus faible de DA au niveau du corps cellulaire que ceux de la sous-population dorso-médiane qui sont marqués beaucoup plus intensément par l'immunofluorescence ciblant la DA. Par conséquent, bien que la sous-population ventro-médiane soit marquée intensément par l'immunofluorescence ciblant la TH, elle n'apparait pas clairement dans la figure 18 qui illustre le marquage de la DA. Les résultats présentés ici montrent que les terminaisons axonales des neurones de projection du BO médian sont situées spécifiquement à l'intérieur et autour de la population dorso-médiane de neurones DA et ce, des deux côtés du TP. De plus, nous montrons en microscopie confocale que ces projections sont colocalisées avec les neurones DA du TP (Figure 19). Cependant, comme dans le TP du poisson-zèbre (Filippi et al., 2014) et la SNc/ATV du mammifère (Sulzer et al., 1998; Yamaguchi et al., 2011; Yamaguchi et al., 2013; Yamaguchi et al., 2015; Root et al., 2016; Morales et Margolis, 2017), les neurones DA contiennent d'autres neurotransmetteurs comme le glutamate (Figure 19; Ryczko et al., 2017; von Twickel et al., 2019) ou le GABA (Figure 19; von Twickel et al., 2019). En effet, l'utilisation d'un protocole d'immunofluorescence

développé pour obtenir les données de cette thèse a permis une qualité de marquage suffisante pour observer dans le TP des neurones qui contiennent à la fois de la DA et du glutamate (Ryczko et al., 2017). De plus, l'utilisation de l'hybridation *in situ* en combinaison avec l'immunofluorescence dirigée contre la tyrosine hydroxylase a permis de confirmer ce résultat chez la lamproie, en plus d'observer des neurones qui produisent à la fois de la DA et du GABA (von Twickel et al., 2019). Des neurones triplement marqués DA+/Glutamate+/GABA+ ont aussi été observés dans le TP (von Twickel et al., 2019). Ces résultats sont intéressants parce qu'ils montrent que la transmission d'autres neurotransmetteurs en combinaison avec la DA serait apparue tôt dans l'évolution des vertébrés et serait un trait conservé évolutivement jusqu'aux mammifères (Pérez-Fernández et al., 2021), peut-être même chez l'humain (Tiklová et al., 2019). Dans cette thèse, nous montrons que les terminaisons axonales des neurones de projection du BO médian sont juxtaposées avec des neurones du TP contenant à la fois de la DA et du glutamate ainsi que des neurones contenant à la fois de la DA et du GABA, ce qui suggère que ces populations neuronales pourraient recevoir le signal olfactif. Cependant, les techniques n'ont pas permis de savoir si les projections du BO médian peuvent aussi atteindre des neurones triplement marqués (DA/glutamate/GABA). Puisqu'elles contiennent des neurotransmetteurs différents, il est possible que ces populations neuronales occupent des rôles distincts dans le traitement du signal olfactif.

Pour déterminer si le TP peut effectivement être recruté par l'activité olfactive dans le BO médian, une nouvelle préparation expérimentale, le prosencéphale isolé, a été développée pour permettre la stimulation électrique des régions olfactives simultanément à l'enregistrement de l'activité neuronale dans le TP. Basé sur nos résultats anatomiques précédents, le noyau dorso-médian de neurones DA a été ciblé pour caractériser son activité en réponse à la stimulation olfactive. Cette méthode a révélé que la stimulation du nerf olfactif entraîne des réponses extracellulaires dans le TP (Figures 20, 21, 22 et S28) ainsi que des réponses de neurones individuels en imagerie calcique (Figure 31) et en enregistrements intracellulaires (Figure 23). Puisque le nerf olfactif est composé d'afférences primaires en provenance à la fois de l'OOA et de l'ÉOP, ces réponses pourraient correspondre à de l'activité produite simultanément dans les voies olfactomotrices médiale et latérale. Donc, pour évaluer plus spécifiquement la contribution de la voie olfactomotrice médiale, la contribution du BO médian a été étudiée. Ces expériences ont révélé que la stimulation électrique du BO médian déclenche robustement des réponses extracellulaires (Figures 21, 22 et S28) et des réponses calciques (Figures 24, S29, S30 et S31) dans le TP. De plus, ces résultats ont été confirmés

dans la préparation semi-intacte, où des trains de stimulation électrique induisent des réponses extracellulaires potentialisées et prolongées dans les neurones du TP. Cependant, puisque le TP envoie aussi des projections au BO médian (Figures 12 et 13), les réponses observées pourraient être causées par l'activation antidromique de ses axones durant la stimulation électrique du BO médian. Puisque la stimulation électrique du nerf olfactif (Figures 20, 21, 22, 23, S28 et S31), qui relâche du glutamate dans le BO médian, et l'injection de glutamate précisément dans le BO médian (Figure S33) ont toutes deux robustement induit des réponses, on peut en déduire que l'activité olfactive du BO médian recrute effectivement des neurones dans le TP. Ainsi, les résultats anatomiques et physiologiques de cette thèse indiquent que dans la voie olfactomotrice médiale, les signaux olfactifs venant de l'OOA sont transmis du BO médian aux neurones dans le noyau DA dorso-médian du TP.

Dans la voie olfactomotrice latérale, les signaux en provenance de l'ÉOP sont acheminés dans le BO principal (Ren et al., 2009; Green et al., 2017). Nous avons étudié la possibilité que ces informations olfactives puissent atteindre le TP et y induire de l'activité, similairement au BO médian. D'abord, il est connu depuis longtemps chez la lamproie que les projections du BO principal ciblent principalement le PL (Heier, 1948; Nieuwenhuys, 1977; Northcutt et Puzdrowski, 1988; Polenova et Vesselkin, 1993; Northcutt et Wicht, 1997). Récemment, ces projections ont été davantage caractérisées et deux populations distinctes de neurones de projections du BO principal, semblables aux cellules mitrales et touffues de mammifères (Suryanarayana et al., 2021a), ont été observées. Ces deux types de cellules ont des projections glutamatergiques qui ciblent les neurones de projection du PL directement ou indirectement via un relai dans le noyau télencéphalique dorso-médian (Suryanarayana et al., 2017; Suryanarayana et al., 2020; Suryanarayana et al., 2021a), ce qui suggère que le signal du BO principal peut être relayé en dehors du PL. Puisqu'une sous-population de neurones de la partie dorsale du PL projette au TP (Pérez-Fernández et al., 2014; Ocaña et al., 2015), ces auteurs ont proposé que le PL relaie l'information olfactive du BO principal au TP (Pérez-Fernández et al., 2014; Ocaña et al., 2015). Plus récemment, cette idée a été supportée par des expériences anatomiques qui ont montré que des axones du BO principal se terminent à proximité des dendrites de neurones de projection du PL qui ont été rétrogradement marqués par une injection dans le TP (Daghfous et al., 2018). De plus, le phénotype des neurones susceptibles de recevoir le signal du PL a été étudié anatomiquement. Il a été observé que les neurones DA de la population dorso-médiane du TP sont colocalisés avec des terminaisons axonales en provenance

du PL (Pérez-Fernández et al., 2014; Ocaña et al., 2015), mais aussi directement du BO principal (Suryanarayana et al., 2021a).

La préparation de prosencéphale isolé a aussi été employée pour enregistrer dans le TP les réponses extracellulaires et calciques à la stimulation du BO principal et du PL. Dans ces expériences, la stimulation électrique du BO principal n'a pas induit de réponses (Figures 21 et S28). Ceci pourrait s'expliquer par le fait que contrairement au BO médian et au PL qui contiennent une grande densité de neurones de projections innervant le TP, ce n'est pas le cas pour le BO principal et sa stimulation électrique ne permettrait pas de recruter suffisamment de neurones pour déclencher des réponses mesurables en enregistrements extracellulaires. Par ailleurs, l'activité du BO est soumise à une importante modulation GABAergique (Daghfous et al., 2018) qui pourrait empêcher l'activité dans le BO principal d'être suffisante pour recruter le TP. C'est aussi ce que suggèrent les expériences réalisées dans cette thèse, en montrant que le retrait pharmacologique de cette inhibition permet de déclencher des bouffées d'activité prolongées dans le TP en réponse à la stimulation du BO principal. Cependant, aucune preuve physiologique ne montre qu'il existe un contact direct du BO principal vers le TP et il est plus probable que la levée de l'inhibition favorise surtout la transmission du BO principal vers le PL qui pourrait ensuite transmettre ce signal au TP (Daghfous 2018). De plus, la stimulation du PL induit aussi des réponses extracellulaires et calciques dans la sous-population dorso-médiane de neurones DA du TP. Ainsi, nos résultats montrent qu'il est très probable que l'activité olfactive du BO principal soit relayée dans le PL pour recruter le TP, particulièrement les neurones DA.

Les résultats de cette thèse montrent aussi que le TP est recruté par le nerf olfactif, le BO médian et le PL des deux côtés du cerveau. En effet, pour un même site d'enregistrement extracellulaire dans le TP, la stimulation du nerf olfactif, du BO médian ou du PL controlatéral induit des réponses comparables à la stimulation ipsilatérale (Figure S28). De même, des neurones du TP montrent des réponses calciques à la stimulation du nerf olfactif, du BO médian ou du PL controlatéraux (Figures 24, S29, S30 et S31). Ces données physiologiques concordent avec nos données anatomiques montrant l'innervation bilatérale du TP par le BO médian (Figure 18; Derjean et al., 2010), ainsi que celles d'autres auteurs montrant l'innervation bilatérale du TP par le PL (Ocaña et al., 2015). Dans l'ensemble, les données présentées ici démontrent pour la première fois que le TP est recruté par les afférences olfactives. En effet, des neurones du TP sont dépolarisés par la stimulation du

nerf olfactif, qui achemine au cerveau le signal des neurones chimio-sensoriels de l'ÉOP et de l'OOA. L'activité du TP est aussi observée en réponse à la stimulation du BO principal et du PL (qui reçoivent le signal de l'ÉOP) ainsi que du BO médian (qui reçoit le signal de l'OOA), ce qui montre que les voies médiale et latérale peuvent toutes deux recruter le TP.

Bien que les signaux provenant respectivement de l'OOA et de l'ÉOP sont transmis en parallèle dans les voies médiale et latérale, ils convergent au niveau du TP. Pourrait-il y avoir un substrat neuronal commun aux deux voies olfactomotrices dans le TP? Les résultats présentés dans cette thèse montrent que oui. D'abord, des expériences anatomiques ont révélé que des projections axonales du BO médian (Figures 18 et 19), du BO principal (Suryanarayana et al., 2021a) et du PL (Pérez-Fernández et al., 2014; Ocaña et al., 2015) innervent les neurones de la sous-population dorso-médiane de neurones DA du TP (Pierre et al., 1997), ce qui en fait des cibles synaptiques très probables pour la transmission du signal olfactif. Lorsque l'activité extracellulaire de cette région est enregistrée, la stimulation du BO médian ou du PL entraîne des réponses dans un même site d'enregistrement (Figures 21, 22 et S28). Surtout, des expériences d'imagerie calcique ont montré que des neurones individuels du noyau DA du TP sont activés à la fois par la stimulation (1) du nerf olfactif, (2) du BO médian et (3) du PL (Figure S31). Puisque des cellules sont recrutées à la fois par le BO médian et le PL, il est proposé que certains neurones DA du TP intègrent le signal de l'OOA et de l'ÉOP transmis en parallèle par les voies olfactomotrices médiale et latérale.

Une limite importante des résultats obtenus dans cette thèse est que le phénotype des neurones du TP qui reçoivent le signal olfactif n'a pas été confirmé physiologiquement. En effet, les résultats présentés ici ne fournissent pas de preuve directe que les neurones DA répondent à la stimulation olfactive. Plusieurs expériences complémentaires pourraient être réalisées pour confirmer qu'ils sont recrutés par le BO médian et le PL. Par exemple, suivant l'enregistrement en cellule-entière d'un neurone du TP qui répond à la stimulation olfactive, il serait possible d'aspirer le contenu cellulaire dans la pipette d'enregistrement et de l'analyser pour déterminer le phénotype de la cellule enregistrée (Cadwell et al., 2016; Fuzik et al., 2016). Ainsi, il serait possible d'analyser son transcriptome et de détecter l'expression de gènes associés à la transmission pré-synaptique de DA, de glutamate ou de GABA. Cette méthode de Patch-seq permettrait donc d'identifier le phénotype des neurones enregistrés dans le TP. Par ailleurs, l'imagerie calcique offre aussi l'opportunité de phénotyper plusieurs neurones à la fois en imageant simultanément leur activité en réponse à la

stimulation olfactive, suivi d'une identification histologique à l'aide d'immunofluorescence dirigée contre la DA, le glutamate et le GABA. Les expériences proposées permettraient ainsi d'identifier les neurones du TP recevant l'information olfactive.

Les résultats de cette thèse constituent la première démonstration que les neurones du TP sont activés par la stimulation olfactive. Puisque la lamproie est un vertébré basal dont les ancêtres sont apparus avant les Gnathostomes, ce phénomène pourrait être un caractère symplésiomorphique au sein des vertébrés. D'ailleurs, chez le poisson-zèbre, le marquage de neurones de projection individuels a permis d'observer que le TP reçoit des axones en provenance de tous les regroupements glomérulaires du BO (Miyasaka et al., 2014). Ainsi, contrairement à ce qui a été montré chez la lamproie (Derjean et al., 2010), des projections de l'ensemble du BO convergent directement vers le TP, ce qui suggère la possibilité qu'une grande diversité d'odeurs ou de combinaisons d'odeurs peuvent le recruter. Par ailleurs, similairement à ce qui a été observé dans cette thèse, ces projections olfactives secondaires étaient bilatérales, traversant la ligne médiane au niveau de la commissure du TP, et étaient situées tout près des neurones DA (Miyasaka et al., 2014). De plus, l'identification des sites d'activité pré-synaptique des neurones de projection par la synthèse d'une protéine de fusion liant la synaptophysine avec la protéine fluorescente verte a permis l'observation de marquage en apposition avec les neurites de neurones DA, ce qui indique de façon convaincante que ces neurones pourraient être recrutés par l'activité du BO. Ainsi, le TP pourrait détecter l'activité olfactive chez le poisson-zèbre aussi.

En plus de l'olfaction, le TP de la lamproie pourrait être sensible à plusieurs autres modalités sensorielles (Pérez-Fernández et al., 2017). Premièrement, le TP reçoit des projections de plusieurs régions sensorielles telles que le BO (olfaction), le tectum optique (vision), l'aire octavolatérale (vibrations mécaniques, champs électriques), le torus semi-circulaire (vibrations mécaniques, champs électriques) et le noyau des colonnes dorsales (somesthésie), en plus de recevoir des projections du PL, du thalamus et du pré-tectum qui reçoivent tous des informations sensorielles de plusieurs modalités (Pérez-Fernández et al., 2014). De plus, des bouffées d'activité extracellulaire sont induites dans le TP en réponse à la présentation d'un stimulus visuel ou d'un stimulus électrique appliqué à proximité de la tête (Pérez-Fernández et al., 2017). Ensuite, ces auteurs ont aussi étudié la possibilité que certaines propriétés du stimulus visuel puissent être encodées dans la réponse neuronale. Pour ce faire, différents stimuli visuels ont été employés tels

que des points noirs grandissant à différentes vitesses d'expansion, ce qui pourrait être interprété comme un objet s'approchant de l'animal. Il a ainsi été observé que la taille des réponses du TP augmente en fonction de la vitesse d'expansion, soit de la saillance du stimulus. Cette observation intéressante a porté Pérez-Fernández et collaborateurs (2017) à proposer que, comme dans la SNc du mammifère (Schultz, 2016), les neurones DA du TP encodent les événements saillants chez la lamproie. Ensuite, des études très intéressantes réalisées chez le poisson-zèbre ont révélé que les neurones DA du TP et d'autres noyaux DA du diencéphale sont aussi sensibles à plusieurs autres modalités sensorielles, telles que les stimuli visuels, tactiles et auditifs. La larve de poisson-zèbre, un animal translucide, est un organisme-modèle idéal pour ces travaux, étant donné la possibilité d'enregistrer in vivo l'activité de neurones préalablement identifiés génétiquement. Ainsi, en utilisant un spécimen transgénique où les neurones DA expriment la protéine fluorescente verte ou un indicateur calcique, l'enregistrement électrophysiologique ou la visualisation de leur activité peuvent être réalisés dans un animal intact. Ces méthodes ont permis d'observer que l'exposition à un stimulus visuel saillant entraîne de l'activité soutenue dans les neurones DA de l'hypothalamus (Mu et al., 2012) et du TP (Reinig et al., 2017). De plus, les neurones DA du TP répondent aussi aux stimuli tactiles (Reinig et al., 2017) et auditifs (Barrios et al., 2020). Dans l'ensemble, ces expériences indiquent que chez le poisson-zèbre, les neurones DA du TP intègrent au moins les stimuli visuels, tactiles et auditifs. Ceci pourrait aussi être le cas chez d'autres espèces de poissons, telles que *Porichthys notatus*, un téléostéen comme le poisson-zèbre, où il a été constaté que le TP est aussi recruté par la stimulation auditive. Chez cette espèce, le mâle émet un son pour attirer des femelles gravides durant la période reproductive et l'exposition à ce son chez les mâles (Petersen et al., 2013; Ghahramani et al., 2018) comme chez les femelles (Forlano et al., 2017) active les neurones DA du TP. Ainsi, chez différentes espèces de poissons, les signaux sensoriels de plusieurs modalités convergent vers les neurones DA du TP et ceux-ci pourraient donc constituer un substrat neuronal commun pour l'intégration de plusieurs modalités sensorielles.

3.3 Le tubercule postérieur module la transmission olfactomotrice au niveau du bulbe olfactif

La détection de molécules odorantes dans son environnement exerce une influence profonde sur le comportement de la lamproie (pour une revue, voir section 1.2). Par exemple, la détection olfactive d'acides aminés associés à une source de nourriture induit une réponse de nage vigoureuse chez des lamproies en captivité (Kleerekoper et Mogensen, 1963). Les comportements olfactifs de la lamproie se produisent durant tout le cycle vital, soit au stade larvaire (Perrault et al., 2014; Wagner et al., 2016), au stade de jeune adulte (Kleerekoper et Mogensen, 1963; Silva et al., 2013) et au stade reproducteur (Johnson et al., 2012). Cependant, les comportements induits par l'olfaction varient durant la vie de l'animal et sont adaptés aux besoins de chaque stade développemental. Par exemple, la lamproie adulte qui est d'abord attirée par les odeurs de proies cesse de se nourrir et est attirée par des phéromones migratoires lorsqu'elle atteint le stade reproducteur (Sorensen et al., 2005; Vrieze et al., 2011). Donc, l'activité olfactive doit être ajustée à des conditions variables pour produire des comportements appropriés en réponse à la détection d'odeurs. Par ailleurs, dans la voie olfactomotrice médiale, un seul glomérule situé dans le BO médian est innervé exclusivement par l'OOA (Ren et al., 2009; Green et al., 2013) qui contient plusieurs types de chimiorécepteurs tels que des récepteurs olfactifs, des récepteurs associés à une amine à l'état de trace et des récepteurs voméronasaux de type 1 (Chang et al., 2013). Ainsi, l'OOA peut détecter plusieurs types d'odeurs et pourrait déclencher la locomotion même lorsque cette réponse est inadéquate. Un système de modulation inhibitrice doit donc être présent pour réguler les réponses olfactomotrices. Ici, nous nous penchons spécifiquement sur la possibilité que le TP puisse moduler l'activité du BO, sachant que des neurones de cette région détectent l'activité bulbaire (voir Section 3.2). Bien que des projections du TP aient été observées dans plusieurs régions sensorielles (Pérez-Fernández et al., 2014), des projections au niveau du BO n'avaient pas été observées avant les travaux de cette thèse.

Plusieurs études ont analysé la présence de DA ou d'enzymes catécholaminergiques dans le système nerveux central et ont constaté la présence de marquage dans le BO chez toutes les espèces de lamproie étudiées (Baumgarten, 1972; Pierre et al., 1994; Pierre et al., 1997; Pombal et al., 1997; Pierre-Simons et al., 2002; Abalo et al., 2005; Barreiro-Iglesias et al., 2009; Barreiro-Iglesias et al., 2010; Fernández-López et al., 2017). De plus, la présence de cellules exprimant le récepteur

D1 ou D2 ont aussi été observés dans le BO de la lamproie (Pérez Fernández, 2013; Pérez-Fernández et al., 2014). L'innervation DA du BO est un trait commun chez tous les vertébrés (Smeets et González, 2000), mais elle n'avait jamais été caractérisée en détails chez la lamproie. Les résultats présentés ici ont mis en évidence chez la lamproie adulte une population locale de neurones DA dans le BO principal qui innervent la couche glomérulaire. Sachant que d'autres auteurs n'ont pas observé de neurones DA dans le BO au stade larvaire (Yáñez et al., 1992; Pierre-Simons et al., 2002; Abalo et al., 2005), les résultats présentés ici suggèrent que les neurones DA du BO apparaissent tardivement dans le développement de la lamproie. Ce phénomène pourrait avoir été évolutivement conservé dans la lignée des vertébrés puisque les cellules DA du BO comptent parmi les dernières à être détectées durant le développement du cerveau chez les lamproies (Pierre-Simons et al., 2002), les poissons (Ekström et al., 1992; Manso et al., 1993), les reptiles (Medina et al., 1994), les oiseaux (Puelles et Medina, 1994), les souris (di Porzio et al., 1990), les rats (Specht et al., 1981) et les humains (Puelles et Verney, 1998).

Dans le BO médian, des projections DA du TP sont observées à tous les stades développementaux. Deux types distincts de fibres DA ont été montrés (Figure 6): des fibres larges et faiblement marquées qui sont associées à des corps cellulaires innervent le BO principal tandis que des fibres minces et fortement marquées sont observées dans le BO médian uniquement. Puisque ces dernières ne sont pas associées à des corps cellulaires locaux et peuvent être suivies en-dehors du BO, la possibilité qu'elles proviennent d'une source extrinsèque a été examinée. Ainsi, nos expériences anatomiques ont révélé que ces fibres proviennent en partie du TP, et ce, à tous les stades développementaux étudiés (Figures 12 et 13), ce qui suggère l'importance de cette projection pour le traitement olfactif durant toute la vie de la lamproie. Ainsi, les neurones DA du TP pourraient recevoir le signal olfactif et, en retour, moduler ce signal via une projection réciproque.

L'observation détaillée de l'innervation du BO médian à tous les stades développementaux (Figures 7 et 8) a révélé que des fibres DA portant des varicosités se situaient à proximité des neurones de projection et aussi des axones du nerf olfactif, ce qui suggère que ces deux cibles sont susceptibles d'être modulées par le TP. Cependant, la ou les cibles exactes de cette innervation n'ont pas été vérifiées avec certitude. Cela pourrait se faire, par exemple, sur une préparation permettant la stimulation du nerf olfactif, l'enregistrement de neurones et l'application de DA dans le BO médian pour déterminer quel type de cellule est ciblé par l'innervation DA. Des

enregistrements en patch-clamp des neurones de projection ou même des terminaisons axonales des afférences primaires pourraient nous renseigner non seulement sur l'identité des cellules modulées, mais aussi sur l'effet que la DA exerce sur l'activité au repos et en réponse à la stimulation du nerf olfactif.

Après avoir détaillé l'innervation DA provenant du TP dans le BO médian, nous avons analysé son rôle dans la transmission olfactomotrice. Plus spécifiquement, l'impact de la modulation DA sur la transmission du signal aux cellules RS, qui sont responsables d'activer les réseaux locomoteurs spinaux, a été examiné. Dans la préparation de cerveau isolé, qui permet la stimulation électrique du nerf olfactif et l'enregistrement intracellulaire de cellules RS, l'injection de DA dans le BO médian réduit la taille des réponses enregistrées (Figure 14). Cet effet est encore plus marqué lorsque l'inhibition tonique GABAergique qui atténue les réponses à la stimulation du nerf olfactif est levée à l'aide d'antagonistes pharmacologiques (Figure 15). Ceci montre que l'innervation DA du BO médian joue un rôle inhibiteur sur la transmission du signal olfactomoteur vers la formation réticulée. Ainsi, ces expériences suggèrent que la transmission DA dans le BO médian produit une modulation du traitement olfactif qui mène à des réponses locomotrices considérablement réduites lors de la détection d'odeurs. Ensuite, pour déterminer le type de récepteur DA impliqué dans cette modulation, des injections d'agonistes et d'antagonistes ont été réalisées dans le BO médian (Figure 16), ce qui a révélé que l'activation de récepteurs D2 dans le BO médian restreint la transmission olfactomotrice. Puisqu'à l'inverse, l'injection d'un antagoniste des récepteurs D2 dans le BO médian induit une augmentation des réponses dans les cellules RS, ceci suggère que cette injection bloque les effets de la DA qui est relâchée de façon endogène durant l'expérience. Ce résultat supporte nos hypothèses de travail selon lesquelles la stimulation olfactive recrute les neurones DA du TP (voir Section 3.2) qui, en retour, relâchent de la DA dans le BO médian. Par ailleurs, la localisation des récepteurs DA exprimés dans le BO de la lamproie (Pérez Fernández, 2013; Pérez-Fernández et al., 2014; Pérez-Fernández et al., 2015) a révélé au niveau de la couche glomérulaire la présence de corps cellulaires exprimant le récepteur D2 uniquement au niveau du BO médian, qui contient les neurones de projections innervant le TP. En combinaison avec nos résultats anatomiques et physiologiques, nous avons proposé que l'activité du TP entraîne la relâche de DA qui active des récepteurs D2 présents sur les neurones de projection du BO médian. Ceci permettrait d'inhiber la transmission olfactomotrice vers les cellules RS et ainsi, la locomotion induite par la détection d'odeurs dans le milieu naturel. Cependant, la présence de récepteurs D2

sur les neurones de projection du BO médian est encore spéculative et devrait être vérifiée par des expériences complémentaires. Par exemple, l'observation du BO en microscopie électronique a déjà permis de détecter la présence de récepteurs D2 sur les terminaisons axonales du nerf olfactif ainsi que sur les dendrites de neurones de projection et de neurones périglomérulaires chez le rongeur (Gutièrrez-Mecinas et al., 2005). Des expériences similaires chez la lamproie permettraient d'identifier avec exactitude le compartiment cellulaire où sont exprimés ces récepteurs en plus d'identifier les neurones susceptibles d'être modulés par l'innervation DA dans le BO médian.

Chez les mammifères, le rôle de la DA dans le BO est de diminuer la transmission du signal olfactif en agissant sur plusieurs types de cellules (Wilson et Sullivan, 1995), notamment sur les terminaisons axonales des afférences primaires olfactives (Hsia et al., 1999; Ennis et al., 2001; McGann, 2013; Vaaga et al., 2017; Liu, 2020), les neurones de projection (Davila et al., 2003; Kiyokage et al., 2010) et les interneurones locaux (Brünig et al., 1999; Maher et Westbrook, 2008; Liu et al., 2013; Banerjee et al., 2015; Vaaga et al., 2017; Liu, 2020). Cependant, des travaux récents (Kosaka et al., 2020) ont montré que les sous-populations de neurones DA du BO sont beaucoup plus hétérogènes qu'observé précédemment (Halász et al., 1981; Pignatelli et al., 2005; Kosaka et Kosaka, 2007; Kosaka et Kosaka, 2008; Kosaka et Kosaka, 2009; Kosaka et Kosaka, 2011; Chand et al., 2015; Kosaka et Kosaka, 2016; Pignatelli et Belluzzi, 2017; Galliano et al., 2018; Korshunov et al., 2020a; Korshunov et al., 2020b) et que leurs connexions synaptiques ainsi que leurs fonctions physiologiques sont encore méconnues (Capsoni et al., 2021). Cependant, l'injection d'agonistes et d'antagonistes DA *in vivo* révèle que la modulation du BO est associée à l'apprentissage (Escanilla et al., 2009) et la discrimination olfactive (Pavlis et al., 2006; Tillerson et al., 2006; Wei et al., 2006) et a aussi été liée à la régulation de comportements reproducteurs tels que l'accouplement, la parturition et l'allaitement (Kendrick et al., 1988; Keverne et al., 1993; Serguera et al., 2008). Chez la lamproie, bien que l'innervation DA ait été démontrée anatomiquement bien avant les résultats de cette thèse, un rôle fonctionnel de la modulation DA dans le BO est montré ici pour la première fois. Dans notre matériel, la DA joue un rôle inhibiteur dans le traitement olfactif et cette fonction pourrait représenter un mécanisme primitif qui a été conservé évolutivement jusqu'aux mammifères.

Par ailleurs, les données présentées dans cette section suggèrent que la modulation DA du BO est présente durant toute la vie de la lamproie, puisque des fibres DA sont observées dans le BO médian

avec un patron d'innervation constant entre les larves, les jeunes adultes et les adultes reproductives (Figure 8). Aussi, chez la larve (Figure 14a-c), la modulation DA du BO médian est présente avant même qu'il soit possible de détecter (avec nos méthodes) des neurones contenant de la DA dans le reste du BO. Ces effets modulateurs de la DA sur la transmission olfactomotrice sont conservés chez les animaux adultes (Figure 14d-f). Pour ces raisons, il a été suggéré que la modulation DA du BO médian filtre le signal pour que les odeurs induisent des réponses motrices moins efficacement, étant donné que des réponses motrices pourraient être inappropriées en fonction du stade développemental de l'animal (Beauséjour et al., 2020). Ainsi, ce mécanisme permettrait une certaine flexibilité dans l'activité motrice induite par la voie olfactomotrice médiale et pourrait être responsable des changements majeurs qui surviennent dans le comportement olfactif à différents stades du cycle vital, tels que la migration qui se produit uniquement durant le stade reproducteur (Vrieze et Sorensen, 2001). En effet, plusieurs types d'odeurs (acides aminés, acides biliaires, phéromones) peuvent être détectés par l'OOA (Chang et al., 2013) et déclencher de l'activité dans le BO médian (Green et al., 2017) et dans les cellules RS (Derjean et al., 2010), en plus d'induire de la nage dirigée (Kleerekoper et Mogensen, 1963; Bjerselius et al., 2000; Li et al., 2002; Johnson et al., 2009). Puisque dans son environnement aquatique la lamproie est exposée à différentes combinaisons et concentrations de ces odeurs, la modulation des afférences olfactives au niveau du BO est nécessaire pour ajuster la réponse olfactomotrice en fonction du stade développemental et des besoins vitaux de l'animal.

Bien que nos résultats indiquent un effet modulateur des projections DA dans le BO médian, nos expériences ont été réalisées dans un cerveau isolé et les effets de cette modulation dans le milieu naturel ne sont pas encore déterminés. Une façon de se rapprocher de ces conditions idéales serait de tester l'impact de la modulation DA du BO médian sur l'activité locomotrice dans la préparation semi-intacte. En effet, l'injection d'agonistes ou d'antagonistes DA dans le BO médian permettrait de vérifier si la réponse motrice à la stimulation du nerf olfactif diffère avec les réponses en conditions témoin. Ces expériences n'ont malheureusement pas été réalisées parce que la collecte de données (Beauséjour et al., 2020) a précédé la mise-au-point d'un protocole de stimulation efficace pour induire des réponses de nage à la stimulation olfactive (voir Section 3.4). Cependant, l'injection simultanée d'antagonistes GABAergiques a révélé que la modulation DA pouvait inhiber l'activité du BO médian au point de supprimer les plateaux de dépolarisation enregistrés dans les cellules RS suite à la stimulation du nerf olfactif (Figure 15). Enfin, pour avoir un portrait

plus complet de l'impact de cette modulation sur le traitement olfactif, des expériences remplaçant la stimulation électrique du nerf olfactif par l'application précise de molécules odorantes sur l'épithélium de l'OOA devraient être réalisées. Ceci permettrait de vérifier si la modulation DA du BO médian aurait un effet spécifique pour des odeurs particulières ou plutôt si elle agit en tant que « commande de gain » pour toutes les odeurs.

La modulation DA du système olfactif par le TP pourrait aussi exister dans les autres systèmes sensoriels de la lamproie. En effet, le TP a des projections DA qui atteignent le tectum optique (vision) et le torus semi-circulaire (vibrations mécaniques, champs électriques), en plus d'envoyer des projections au thalamus et au pré-tectum qui reçoivent des informations sensorielles de plusieurs modalités (Pérez-Fernández et al., 2014). D'ailleurs, il a été observé qu'une connexion réciproque existe entre le TP et le tectum optique (Pérez-Fernández et al., 2014; Pérez-Fernández et al., 2017), dans laquelle les neurones DA seraient activés par des stimuli visuels et innerveraient le tectum optique en retour. Cette projection DA exerce une influence modulatrice sur les neurones du tectum optique via les récepteurs D1 et D2 pour modifier leur excitabilité ainsi que les comportements induits en réponse aux stimuli visuels. Ainsi, la modulation DA au niveau du tectum optique serait très similaire à ce que nous observons au niveau du BO, où la modulation DA modifie les réponses à la stimulation olfactive. Le TP pourrait donc jouer un rôle global de modulation des informations sensorielles dans le cerveau. Chez le poisson-zèbre, le TP répond non seulement à la stimulation sensorielle de plusieurs modalités (voir Section 3.2), mais envoie aussi des projections DA vers les régions sensorielles qui l'innervent. Par exemple, la ligne latérale contient des neuromastes cutanés qui détectent les déplacements de l'eau, et ces organes sensoriels sont innervés par des cellules ganglionnaire qui transmettent les signaux vers l'aire octavolatérale rhombencéphalique, ce qui permet à l'animal de percevoir le mouvement à une courte distance (Hofer, 1908; Dijkgraaf, 1963). Les neurones DA du TP chez le poisson-zèbre sont activés par les signaux mécanosensoriels de la ligne latérale (Reinig et al., 2017) et envoient des projections 1) dans les noyaux rhombencéphaliques recevant les afférences de la ligne latérale (Bricaud et al., 2001; Haehnel-Taguchi et al., 2018) et en périphérie 2) dans les ganglions de la ligne latérale (Bricaud et al., 2001; Haehnel-Taguchi et al., 2018) et 3) dans les neuromastes (Metcalfe et al., 1985; Bricaud et al., 2001; Jay et al., 2015; Toro et al., 2015; Haehnel-Taguchi et al., 2018). Il a d'ailleurs été démontré que les cellules ciliées des neuromastes expriment le récepteur D1 via lequel leur activité est augmentée (Toro et al., 2015). Ainsi, chez le poisson-zèbre, les projections

DA du TP atteignent plusieurs régions associées à la ligne latérale et pourraient ainsi moduler à plusieurs niveaux les afférences mécanosensorielles reçues. Similairement, chez *Porichthys notatus*, un autre poisson téléostéen, non seulement les neurones DA du TP sont sensibles aux stimuli auditifs (Petersen et al., 2013), mais ils établissent aussi des projections réciproques vers le système auditif central et périphérique (Forlano et al., 2014). En effet, les projections DA du TP ont été observées dans le noyau octavolatéral, dédié entre autres à l'audition, ainsi que dans le nerf VIII pour atteindre les cellules ciliées dans le saccule de l'oreille interne, l'organe auditif principal chez ces poissons. Ces projections réciproques avec le système auditif en font d'excellents candidats pour la modulation des signaux auditifs dans le cerveau (Forlano et al., 2017). En résumé, il a été observé chez plusieurs espèces de poissons que le TP détecte différents types de modalités sensorielles, tels que l'olfaction (Section 3.2), la vision (Pérez-Fernández et al., 2017), l'audition (Petersen et al., 2013), et envoie des projections DA à des régions sensorielles en amont pour moduler l'entrée d'informations dans le cerveau. Puisque les neurones du TP ont des projections distribuées dans plusieurs régions sensorielles (Metcalfe et al., 1985; Pérez-Fernández et al., 2014) et que les neurones DA s'activent de façon synchronisée entre eux (Reinig et al., 2017), toutes leurs cibles efférentes recevraient de la DA en même temps. Ainsi, les neurones DA du TP joueraient un rôle de modulation globale de plusieurs systèmes sensoriels simultanément. Cette théorie intéressante a été proposée indépendamment par plusieurs auteurs travaillant sur les poissons téléostéens (Metcalfe et al., 1985; Reinig et al., 2017; Haehnel-Taguchi et al., 2018) et est supportée par nos résultats (Section 2.2) et d'autres (Pérez-Fernández et al., 2014; Pérez-Fernández et al., 2017) chez la lamproie.

3.4 Le tubercule postérieur est impliqué dans la réponse motrice aux stimuli olfactifs

Chez la lamproie, un lien important existe entre le système olfactif et le système moteur. Premièrement, la détection d'odeurs induit et guide la locomotion de lamproies se déplaçant librement en laboratoire (Kleerekoper et Mogensen, 1963) et dans leur habitat naturel (Bjerselius et al., 2000). Par ailleurs, cela fait longtemps qu'il a été démontré que les cellules RS sont dépolarisées par la stimulation du nerf olfactif (Wickelgren, 1977a; Wickelgren, 1977b). En se fiant au patron de projections des neurones du BO médian, El Manira et al. (1997) ont proposé que les signaux olfactifs puissent déclencher la nage en recrutant les cellules RS via une voie dédiée à l'initiation rapide de la locomotion en réponse à la détection d'odeurs. En effet, il a été montré que la stimulation chimique de l'épithélium olfactif produit un signal qui est transmis aux cellules RS et qui induit robustement de la locomotion chez des animaux pouvant nager librement (Thompson et al., 2008). Par ailleurs, des données du laboratoire Dubuc montrent que similairement à la stimulation électrique du nerf olfactif, l'application de molécules odorantes sur l'épithélium olfactif peut aussi déclencher de l'activité dans les cellules RS (Derjean et al., 2010). De plus, l'injection de glutamate dans le BO entier peut provoquer de l'activité alternée dans les racines ventrales qui rappelle l'activité associée à la locomotion (Derjean et al., 2010).

Plusieurs éléments de la littérature portent à croire que le TP soit impliqué dans la production de locomotion suivant la détection olfactive. Par exemple, chez des animaux ayant subis une lésion sélective des neurones DA (Langston et al., 1983) par l'injection de l-méthyl-4-phényl-l,2,3,6-tétrahydropyridine (MPTP), le seuil requis pour induire de la locomotion en réponse à la stimulation de la périphérie olfactive est augmenté de façon importante (Thompson et al., 2008). De plus, dans le cerveau isolé, l'inactivation du TP par une injection locale d'antagonistes glutamatergiques induit une diminution de l'amplitude des PPSEs enregistrés dans les cellules RS à la stimulation du nerf olfactif (Derjean et al., 2010) ou du PL (Daghfous et al., 2018). Ainsi, ces résultats ont mené à l'hypothèse que le TP est impliqué dans la locomotion induite par l'olfaction (Thompson et al., 2008; Derjean et al., 2010; Daghfous et al., 2018). De plus, il a été montré que la stimulation électrique ou chimique du TP provoque de la nage chez la préparation semi-intacte suivant une décérébration complète (Derjean et al., 2010; Gariépy et al., 2012; Ryczko et al., 2013; Ryczko et al., 2017; Ryczko et al., 2020), ce qui suggère l'existence de connexions excitatrices

avec des régions motrices du tronc cérébral. Sachant que des neurones du TP peuvent détecter les signaux olfactifs (section 3.2) et que l'activité de cette région peut aussi déclencher de la nage, existe-t-il des projections efférentes du TP qui pourraient relayer le signal olfactif pour entraîner la locomotion? Il est connu que le TP innerve plusieurs régions motrices via des projections DA descendantes dans le tronc cérébral ciblant le tectum optique (Pérez-Fernández et al., 2014; Pérez-Fernández et al., 2017; von Twickel et al., 2019), la RLM (Ryczko et al., 2013; Ryczko et al., 2017) ainsi que les cellules RS (Ryczko et al., 2020). Puisque l'inactivation de la RLM par une injection locale d'antagonistes glutamatergiques induit une diminution de l'amplitude des PPSEs enregistrés dans les cellules RS en réponse à la stimulation du nerf olfactif (Derjean et al., 2010) et que le TP possède une projection GABAergique atteignant la RLM (Ménard et al., 2007), il a été proposé que le TP relaie le signal olfactif à la RLM pour induire de la locomotion (Derjean et al., 2010). En effet, par ses projections aux cellules RS chez la lamproie (Sirota et al., 2000; Brocard et Dubuc, 2003; Le Ray et al., 2003; Smetana et al., 2007; Brocard et al., 2010; Smetana et al., 2010; Grätsch et al., 2019a), la RLM contrôle l'initiation, le maintien et l'arrêt de la locomotion (pour des revues, voir Dubuc et al., 2008; Le Ray et al., 2011; Ryczko et Dubuc, 2013; Grätsch et al., 2019b). Ainsi, une des hypothèses de travail de cette thèse était que le TP soit impliqué dans la locomotion induite par l'olfaction et, plus spécifiquement, en transmettant le signal olfactif à la RLM.

Pour tester cette hypothèse, nous avons commencé par vérifier si des neurones individuels du TP peuvent à la fois 1) recevoir les projections du BO et 2) projeter à la RLM. De plus, ces expériences de traçage anatomique ont été combinées avec un protocole d'immunofluorescence ciblant la DA, puisqu'il est connu que les projections DA du TP à la RLM amplifient la durée et la vitesse de la nage (Ryczko et al., 2013; Ryczko et al., 2017). Les résultats de cette thèse ont révélé que les neurones de projection du BO médian ont des terminaisons axonales à proximité de neurones DA du TP projetant à la RLM (Figures 18, 19 et 24). Par ailleurs, ces résultats ont été observés de la larve à l'adulte reproducteur, ce qui suggère que cette connexion (BO médian-TP-RLM) serait conservée durant toute la vie de l'animal. Dans la voie olfactomotrice latérale, les neurones DA du TP sont contactés par des neurones du PL (Pérez-Fernández et al., 2014; Ocaña et al., 2015). Cependant, la possibilité que ces neurones DA puissent transmettre le signal olfactif du PL à la RLM était inexplorée. Pour confirmer nos données anatomiques, les neurones du TP ont été rétrogradement marqués par l'injection d'un indicateur calcique dans la RLM et ont été imagés durant la stimulation des voies médiales et latérales. Ces expériences physiologiques ont démontré

que des neurones parmi le noyau DA du TP qui projettent à la RLM répondent à la stimulation du nerf olfactif, du BO médian et du PL (Figure S31). Les résultats de cette thèse montrent que le TP transmet l'information olfactive de la voie médiale et de la voie latérale directement à la RLM, présumément pour induire de la locomotion, et soutiennent notre hypothèse de travail initialement formulée par Derjean et collaborateurs (2010).

Par ailleurs, puisque le noyau DA du TP est situé très près de la ligne médiane, les réponses calciques à la stimulation olfactive ont pu être enregistrées simultanément des deux côtés du cerveau. Ceci a premièrement permis d'observer que les neurones du TP projettent aux deux côtés de la RLM (Ryczko et al., 2013) et que ces neurones sont activés par la stimulation du nerf olfactif, BO médian et PL ipsi- et controlatéraux. Ainsi, les deux voies olfactives recrutent le TP bilatéralement, qui recrute à son tour la RLM bilatéralement. L'activation de la RLM recrute les cellules RS bilatéralement (Brocard et al., 2010), ce qui permettrait de générer efficacement une réponse locomotrice à la stimulation olfactive. De plus, certains neurones individuels sont recrutés à la fois par la stimulation du nerf olfactif, du BO médian ainsi que du PL, et pourraient représenter un substrat neuronal commun pour transmettre le signal des voies olfactomotrices médiale et latérale vers la RLM. Ainsi, il existe une population de neurones à l'intérieur du noyau DA du TP qui intègre le signal en provenance de l'ÉOP et de l'OOA et projette vers les réseaux locomoteurs du tronc cérébral.

Par quels mécanismes les signaux olfactifs reçus par les neurones du TP peuvent-ils être transmis à la RLM et quel peut être l'effet sur son activité? D'abord, nos résultats anatomiques (Section 3.2) révèlent que les projections du BO atteignent les neurones DA, glutamatergiques et GABAergiques ainsi que des neurones contenant à la fois de la DA et du glutamate ou du GABA. Tous ces types de neurones pourraient être impliqués dans la transmission du signal olfactif à la RLM. Parmi ces différentes populations, il a été précédemment montré que des neurones DA (Ryczko et al., 2013), des neurones glutamatergiques (Ryczko et al., 2017), des neurones co-exprimant la DA et le glutamate (Ryczko et al., 2017), ainsi que des neurones GABAergiques (Ménard et al., 2007) projettent à la RLM. La stimulation du TP entraîne non seulement de la nage, mais aussi la relâche de DA dans la RLM (Ryczko et al., 2013). Cette projection DA descendante fournit une excitation additionnelle dans la RLM par l'activation de récepteurs D1, ce qui augmente la production de nage de l'animal (Ryczko et al., 2013; Ryczko et al., 2017). Ensuite, la projection glutamatergique

produit une augmentation graduelle - en fonction de l'intensité de stimulation électrique du TP - de l'activité neuronale dans la RLM ainsi que de la vitesse de la nage produite (Ryczko et al., 2017). Enfin, le rôle des neurones GABAergiques du TP n'a pas été étudié, mais on peut supposer que ces neurones pourraient exercer une influence inhibitrice dans la RLM, par exemple pour induire l'arrêt de la locomotion. Par ailleurs, le protocole d'immunofluorescence ciblant la DA, développé pour les travaux de cette thèse, a permis d'observer pour la première fois que les neurones DA du TP innervent tous les noyaux contenant des cellules RS (Ryczko et al., 2020). Les expériences que j'ai effectuées en collaboration avec plusieurs co-auteurs durant mon doctorat démontrent que ces projections jouent un rôle modulateur via le récepteur D1 qui amplifie l'activité des cellules RS ainsi que la vitesse de la nage induite par la stimulation du TP (Ryczko et al., 2020). Donc, les expériences réalisées durant cette thèse établissent que l'activité du BO médian et du PL recrute le TP et font le lien avec plusieurs mécanismes connus de cette région qui régulent la locomotion. Par ses connexions descendantes, notamment à la RLM, le TP peut déclencher une réponse locomotrice à la détection de stimuli olfactifs.

Une question importante demeure: le TP est-il recruté durant la nage induite par des signaux olfactifs? Bien qu'il ait été démontré plus haut que le TP répond à la stimulation olfactive, il n'est toujours pas connu si cette région est activée durant la nage induite par les afférences olfactives. Premièrement, son activité n'avait jamais été enregistrée en réponse à la stimulation des voies olfactomotrices avant les résultats de cette thèse. De plus, bien que plusieurs études aient permis l'enregistrement de l'activité cérébrale et spinale en réponse à la stimulation de la périphérie olfactive ou du BO (Wickelgren, 1977a; Wickelgren, 1977b; Derjean et al., 2010; Green et al., 2017; Daghfous et al., 2018; Section 2.1; pour une revue, voir Section 1.3), aucune n'a permis d'observer la réponse motrice résultante. Ainsi, de nouvelles méthodes ont été mises au point pour mesurer l'activité du TP durant la nage induite par l'olfaction. Un nouveau type de préparation semi-intacte incluant la muqueuse olfactive et le cerveau entier a permis d'observer la nage de l'animal tout en conservant les connexions intactes entre la périphérie olfactive et les muscles activés durant la nage. Traditionnellement, la préparation semi-intacte est décérébrée (Viana Di Prisco et al., 1997; Sirota et al., 2000; Viana Di Prisco et al., 2000; Brocard et Dubuc, 2003; Guimond et al., 2003; Le Ray et al., 2003; Brocard et al., 2005; Gravel et al., 2007; Martel et al., 2007; Brocard et al., 2010; Derjean et al., 2010; Smetana et al., 2010; Gariépy et al., 2012; Ryczko et al., 2013; Juvin et al., 2016; Ryczko et al., 2017; Grätsch et al., 2019a; Ryczko et al., 2020), ce

qui rompt la communication entre le nerf olfactif, le BO médian ainsi que le PL et le reste du cerveau. Aussi, un protocole de stimulation électrique qui permet de recruter efficacement les voies olfactomotrices pour induire de la nage a aussi dû être développé. En effet, malgré l'observation de réponses dans les cellules RS à la stimulation du nerf olfactif ou du BO (Wickelgren, 1977a; Wickelgren, 1977b; Derjean et al., 2010; Daghfous et al., 2018; Beauséjour et al., 2020), un protocole de stimulation induisant la nage de l'animal n'avait jamais été réalisé. Les résultats de cette thèse montrent maintenant qu'un train prolongé de stimulations dans le nerf olfactif ou le BO des deux côtés du cerveau (voir Section 2.2.3.3.3) permet d'induire robustement une activité locomotrice. Nous montrons donc pour la première fois que la lamproie nage en réponse à la stimulation du nerf olfactif ou du BO médian, ce qui constitue une preuve de concept importante pour supporter la théorie selon laquelle les circuits olfactomoteurs induisent la locomotion (Derjean et al., 2010). Nos résultats indiquent aussi que dans la voie latérale, la stimulation du PL induit la nage de la préparation semi-intacte, ce qui confirme les résultats préalablement observés par un autre groupe (Ocaña et al., 2015). Surtout, l'enregistrement simultané du TP a permis d'observer que son activité est immanquablement synchronisée avec l'activité des cellules RS et l'activité locomotrice du corps. En effet, la stimulation du BO médian (Figures 25, S32 et S33) ou du PL (Figures 26 et S34) déclenche de la nage et de l'activité neuronale dans le TP et les cellules RS qui commencent et terminent conjointement. Ces résultats fournissent une indication claire que l'activité du TP joue un rôle dans la production de locomotion induite par la stimulation olfactive à la fois dans la voie olfactomotrice médiale et la voie olfactomotrice latérale. Présumément, le TP pourrait jouer ce rôle via ses cellules DA et glutamatergiques qui reçoivent les projections olfactives et innervent la RLM (Figures 18, 19 et 24) puisque nous avons démontré que le TP relaie les informations olfactives à cette région (Figures 24, S29, S30 et S31). Ainsi, nous proposons que les neurones DA et/ou glutamatergiques du TP qui projettent à la RLM (Ryczko et al., 2013; Ryczko et al., 2017) pourraient être dépolarisés par le signal olfactif et déclencher, maintenir et amplifier l'activité locomotrice via cette projection.

Pour caractériser davantage le rôle du TP dans la production de locomotion induite par la stimulation olfactive, des expériences additionnelles pourraient être réalisées. Par exemple, étant donné qu'il existe des projections DA directes du TP aux cellules RS (Ryczko et al., 2020), l'injection d'un indicateur calcique dans cette région permettrait de déterminer si les neurones du TP qui y projettent sont aussi recrutés par le signal olfactif et pourraient contribuer à la nage en

réponse à la stimulation olfactive. De plus, l'inactivation du TP dans la préparation semi-intacte avant la stimulation du BO médian ou du PL permettrait d'observer son impact sur la nage produite par les deux voies olfactomotrices. De plus, il serait très intéressant de tester la stimulation de la périphérie olfactive par l'application directe de molécules odorantes, ce qui permettrait d'étudier si des odeurs spécifiques activent préférentiellement le TP ou déclenchent des comportements particuliers. La préparation semi-intacte utilisée ici pourrait être un outil important pour étudier davantage les liens entre différentes odeurs, les circuits olfactomoteurs du cerveau et le comportement de l'animal.

Enfin, le TP est activé non seulement durant la nage suivant la stimulation des voies olfactomotrices, mais aussi durant la nage se produisant spontanément dans la préparation semi-intacte (Figure 27). Cette observation suggère que le TP est activé constitutivement durant la nage et serait donc impliqué dans la production de locomotion. Pour un même site d'enregistrement extracellulaire localisé dans le noyau de neurones DA du TP, des neurones sont activés en synchronie avec la locomotion spontanée et avec la locomotion induite par la stimulation olfactive. Basé sur nos résultats et sur les rôles connus du TP dans la locomotion (Ryczko et al., 2013; Ryczko et al., 2017), nous proposons que des neurones DA y intègrent à la fois les stimuli extéroceptifs et intéroceptifs pour former une voie descendante commune vers la RLM qui module et déclenche la locomotion. Pour rappel, il a été montré que la lésion sélective des neurones DA avec une neurotoxine entraîne non seulement une diminution importante des épisodes de nage spontanée, mais aussi un affaiblissement des réponses de nage à la stimulation olfactive (Thompson et al., 2008). En combinaison avec nos résultats anatomiques (Figures 18, 19 et 24), ces données appuient notre hypothèse que les neurones DA du TP jouent un rôle important dans la production de nage en réponse aux signaux olfactifs.

Chez d'autres poissons tels que le poisson-zèbre, il semble que les neurones DA du TP, en plus d'être activés par plusieurs types de stimulations sensorielles (voir Section 3.2), soient aussi impliqués dans la réponse motrice à ces stimuli. La larve de poisson-zèbre permet de tester cette hypothèse puisqu'il est possible de fixer sa tête sous un microscope pour mesurer son activité motrice simultanément à l'activité neuronale dans des sous-populations identifiées, incluant les neurones DA du TP. À noter que tous les groupes connus de cellules DA ont été cartographiés chez le poisson-zèbre (Holzschuh et al., 2001; Rink et Wullimann, 2002; McLean et Fetcho, 2004; Chen

et al., 2009; Filippi et al., 2010; Yamamoto et al., 2010; Yamamoto et al., 2011) et que ces groupes sont largement conservés durant l'évolution entre les poissons et les mammifères (Smeets et González, 2000). Chez le poisson-zèbre, les neurones DA du TP sont localisés dans une région nommée *diencephalic cluster 2* (DC2) et DC4 (Tay et al., 2011) d'après les observations détaillées de Wullimann et Rink (2001). L'étendue des projections de ces neurones du TP révèle qu'ils seraient impliqués dans le contrôle moteur non seulement via des projections ascendantes vers le striatum (Rink et Wullimann, 2001; Kastenhuber et al., 2010), mais aussi via des projections descendantes vers plusieurs régions motrices (Tay et al., 2011). Ensuite, plusieurs expériences physiologiques ont montré de façon convaincante que ces neurones jouent un rôle dans la production de mouvement chez le poisson-zèbre aussi. D'abord, l'activité des neurones DA du TP est fortement corrélée avec l'activité locomotrice qui survient spontanément dans la préparation (Jay et al., 2015; Reinig et al., 2017; Barrios et al., 2020). Similairement aux observations chez la lamproie (Figures 25, 26, 27, S32, S33 et S34), des bouffées d'activité dans le TP précèdent l'initiation de la locomotion ou se poursuivent jusqu'à la fin de l'épisode moteur (Reinig et al., 2017; Barrios et al., 2020), ce qui suggère que les neurones DA pourraient participer à l'initiation et au maintien de la locomotion spontanée.

Le rôle de la DA dans la production d'activité motrice a aussi été étudié chez le poisson-zèbre. Il a été montré que la photostimulation spécifique des neurones DA est suffisante pour entraîner un épisode locomoteur (Barrios et al., 2020) ou augmenter la probabilité d'initier un épisode locomoteur (McPherson et al., 2016), ce qui suggère que ces neurones sont globalement impliqués dans la production de locomotion. De plus, ils pourraient aussi participer à la production de locomotion en réponse à la stimulation sensorielle. En effet, chez le poisson-zèbre, l'activité des neurones DA est corrélée avec la locomotion et la stimulation sensorielle visuelle (Mu et al., 2012; Reinig et al., 2017; Jha et Thirumalai, 2020), mécanique (Reinig et al., 2017) et auditive (Barrios et al., 2020). Ces neurones pourraient encoder l'information sensorielle et participer à la locomotion en recrutant ou en modulant des régions motrices comme nous le proposons chez la lamproie. Comme chez cette dernière (Ryczko et al., 2020), il a été montré anatomiquement que des neurones DA du poisson-zèbre projettent aux cellules RS (Barrios et al., 2020). De plus, les cellules RS, incluant la cellule de Mauthner, sont recrutées par la stimulation optogénétique des neurones DA (Mu et al., 2012; Barrios et al., 2020). Dans le cas de la cellule de Mauthner, il a été observé que la DA induit une augmentation de son excitabilité en réduisant son activité spontanée,

ce qui amplifie son ratio signal/bruit pour des stimulations sensorielles (Mu et al., 2012). De plus, la DA exerce aussi un effet important sur l'activité des motoneurones impliqués dans la locomotion (Jha et Thirumalai, 2020), soit en augmentant leur excitabilité, ce qui permet de recruter davantage de motoneurones et d'augmenter la fréquence des potentiels d'action des motoneurones actifs. Cette activité additionnelle permet d'augmenter l'amplitude des ondulations natatoires ce qui résulte en une nage plus rapide. Dans le cas de la cellule de Mauthner (Mu et al., 2012) et des motoneurones (Jha et Thirumalai, 2020), la DA agit via le récepteur D1 pour augmenter l'excitabilité cellulaire ainsi que la probabilité et la vitesse des réponses locomotrices déclenchées par la stimulation auditive (Mu et al., 2012) ou visuelle (Jha et Thirumalai, 2020). À l'inverse, l'ablation sélective des neurones DA entraîne une diminution de la locomotion spontanée (Jay et al., 2015; McPherson et al., 2016; Barrios et al., 2020) ainsi qu'une diminution des réponses de nage à la stimulation sensorielle (Mu et al., 2012; Jha et Thirumalai, 2020), ce qui confirme l'importance de ces neurones pour l'initiation de la locomotion. Une observation similaire a été faite chez la lamproie (Thompson et al., 2008), ce qui suggère que certains mécanismes par lesquels les neurones DA favorisent l'initiation de la locomotion auraient été évolutivement conservés.

La revue de littérature ci-haut permet d'apprécier plusieurs similitudes au niveau des neurones DA du TP entre le poisson-zèbre et la lamproie. Chez le poisson-zèbre, les neurones DA répondent à la stimulation sensorielle (Mu et al., 2012; Reinig et al., 2017; Barrios et al., 2020) et il a été proposé (Tay et al., 2011) qu'en retour, ils pourraient produire 1) une modulation ascendante des afférences sensorielles (Metcalfe et al., 1985; Bricaud et al., 2001; Jay et al., 2015; Toro et al., 2015; Haehnel-Taguchi et al., 2018) et 2) une modulation descendante des efférences motrices (Mu et al., 2012; McPherson et al., 2016; Reinig et al., 2017; Barrios et al., 2020; Jha et Thirumalai, 2020). Nos résultats chez la lamproie supportent cette hypothèse et suggèrent que les neurones DA du TP répondent à la stimulation olfactive (Section 3.2) et qu'en retour, leurs projections 1) modulent l'activité afférente au niveau du BO (Section 3.3); et transmettent l'information à la RLM pour participer dans la réponse motrice (Section 3.4). Le TP pourrait effectivement occuper un rôle important dans la locomotion chez la lamproie puisque comme chez le poisson-zèbre, il s'active durant la locomotion qui survient spontanément (Figure 27; Jay et al., 2015; Reinig et al., 2017; Barrios et al., 2020). Nous proposons donc que les neurones DA du TP occupent une position très importante pour l'intégration sensorimotrice chez la lamproie, soit à la jonction entre les afférences

sensorielles et les efférences motrices, et pourraient pratiquement être appelés des « neurones sensori-moteurs ».

Les neurones DA du TP intègrent l'information sensorielle de sources variées et participent à l'élaboration de la réponse motrice et donc, ils encodent à la fois de l'information sensorielle et de l'information motrice. Chez la lamproie, les neurones du TP sont recrutés par les signaux convergents de différents organes olfactifs (Section 3.2) en plus de la rétine et de la ligne latérale (Pérez-Fernández et al., 2017) et en retour, envoient des projections DA dans plusieurs régions sensorielles telles que le BO (Section 3.2) et le tectum optique (Pérez-Fernández et al., 2017). Dans les deux cas, ces projections sont modulatrices et influencent la réponse motrice aux stimuli sensoriels. Additionnellement, des neurones DA innervent plusieurs régions motrices qui peuvent amplifier la réponse comportementale (Ryczko et al., 2013; Pérez-Fernández et al., 2014;; Ryczko et al., 2017; Ryczko et al., 2020) subséquemment à la détection de stimuli saillants. Par ailleurs, via leurs projections DA aux voies directe et indirecte des ganglions de la base (Baumgarten, 1972; Pombal et al., 1997; Ericsson et al., 2013; Stephenson-Jones et al., 2013; pour une revue, voir Grillner et al., 2013) ces neurones pourraient aussi moduler le comportement via le rôle qui leur est traditionnellement associé, soit de retirer ou maintenir l'inhibition constitutive des régions motrices pour permettre ou inhiber le mouvement via les voies directe et indirecte des ganglions de la base. Donc, l'activation du TP par la stimulation olfactive ne devrait pas recruter uniquement la RLM pour produire de la locomotion, mais l'ensemble des régions ciblées par ses projections DA et ainsi, tout un réseau de régions motrices qui fonctionnent ensemble pour induire le comportement et aussi plusieurs régions sensorielles qui fournissent un retour d'informations essentiel à toute activité motrice. Par exemple, la détection d'un stimulus olfactif saillant qui indiquerait la présence d'une proie entraînerait l'activité des neurones DA du TP qui, à leur tour, moduleraient les signaux olfactifs, mais aussi les signaux visuels et électrosensoriels pour faciliter la localisation et l'identification de cette proie. Similairement, les signaux mécaniques et vestibulaires, qui sont nécessaires pour générer une locomotion adaptée à l'environnement, seraient aussi modulés pour faciliter un déplacement rapide permettant la capture de la proie. De plus, la modulation simultanée de plusieurs régions motrices permettrait de faciliter le mouvement en direction de la proie, par exemple en accélérant la locomotion via la RLM et les cellules RS (Ryczko et al., 2013; Ryczko et al., 2017; Ryczko et al., 2020) ou via le tectum optique en facilitant les mouvements d'orientation (Pérez-Fernández et al., 2017). À ces effets directs s'ajouteraient une

couche supplémentaire de contrôle moteur via les ganglions de la base qui maintiennent tous les centres moteurs sous inhibition et régulent donc l'activation des différents programmes moteurs (Grillner et al., 2005; Ménard et Grillner, 2008; Takakusaki, 2008; Kozlov et al., 2009). Puisque les neurones DA s'activent de façon synchronisée entre eux (Reinig et al., 2017), toutes leurs cibles efférentes recevraient de la DA simultanément, et ainsi, le TP jouerait un rôle de modulation globale de plusieurs systèmes sensoriels et moteurs qui permettrait ultimement de produire un comportement adapté aux stimulations perçues dans l'environnement. Nous proposons donc que les neurones DA du TP constituent un centre d'intégration multisensorielle exerçant une influence globale à la fois sur le traitement sensoriel en amont, mais aussi sur les efférences motrices en aval, ce qui permettrait d'ajuster le comportement de l'animal à son état interne (éveil/sommeil; appétit/satiété; statut reproducteur) et aux conditions externes (présence de proie ou prédateurs, jour/nuit, température de l'eau).

3.5 Comparaison phylogénétique avec les mammifères

Le comportement de la lamproie est en grande partie déterminé par les molécules odorantes détectées dans son environnement aquatique. Les résultats que nous avons obtenus dans ce projet de recherche ont permis de décrire en partie les réseaux de neurones qui sont responsables d'induire de la locomotion en réponse aux entrées olfactives provenant de la périphérie. Notamment, les projections du BO vers le TP, en particulier les neurones DA qui s'y trouvent, semblent occuper une position importante dans ces circuits. Chez les mammifères, les régions homologues au TP seraient constituées par la SNc/ATV, deux noyaux adjacents qui contiennent une haute densité de neurones DA. Ceci a d'abord été proposé lorsqu'une population de neurones DA a été observée à l'intérieur du TP de la lamproie (Baumgarten, 1972). Initialement, cette homologie était controversée dû à la position de ces neurones dans le diencéphale. Cependant, des neurones DA sont présents dans le diencéphale ventro-caudal chez la lamproie (Nieuwenhuys 1977, Pombal et al., 1997), les myxines (Wicht et Northcutt, 1994), les poissons cartilagineux (Stuesse et al., 1994), les poissons téléostéens (Rink et Wullimann, 2001), les amphibiens (González et Smeets, 1994), ainsi que chez les reptiles, les oiseaux et les mammifères (Marín et al., 2005; Björklund et Dunnett, 2007). Bien que chez les reptiles, les oiseaux et les mammifères les neurones DA de la SNc/ATV soient plus nombreux dans le mésencéphale, ils sont aussi présents au niveau du diencéphale ventral (pour une revue, voir Vernier et Wullimann, 2009). Comme chez les mammifères, ces neurones innervent le striatum (Pombal et al., 1997) et modulent l'activité des ganglions de la base via les voies directe et indirecte chez la lamproie (Robertson et al., 2012; Ericsson et al., 2013). Chez les poissons téléostéens, il existe aussi une population de neurones DA dans le TP qui innerve le striatum (Rink et Wulliman, 2001). Basé sur ces observations anatomiques et sur l'expression de marqueurs développementaux (Kapsimali et al., 2001; Blin et al., 2008; Wullimann et Umeasalugo, 2020), l'homologie entre les neurones DA du TP et la SNc/ATV est maintenant bien acceptée (Vernier et Wullimann, 2009; Yamamoto et Vernier, 2011; Wullimann, 2014). Il semblerait donc que ces populations de neurones DA seraient apparues avant la lamproie et que leur organisation générale aurait été conservée évolutivement. Bien que ces régions aient certainement gagné en complexité à travers de la généalogie des vertébrés, plusieurs études suggèrent que les projections anatomiques ainsi que les fonctions du TP et de la SNc/ATV sont demeurées semblables de la lamproie aux mammifères (pour une revue de littérature, voir Grillner et al., 2013; Suryanarayana et al., 2021b).

La projection descendante DA du TP à la RLM observée chez la lamproie (Ryczko et al., 2013) aurait aussi été conservée de la lamproie aux mammifères (Ryczko et al., 2016). Chez la salamandre et le rat, les projections du TP et de la SNc relâchent de la DA dans la RLM et contribueraient donc au contrôle locomoteur (Ryczko et al., 2016). Les expériences physiologiques chez la salamandre ont d'ailleurs confirmé que la concentration de DA dans la RLM était corrélée à l'activité des cellules RS. De plus, tel qu'observé chez la lamproie (Ryczko et al., 2013), plusieurs neurones DA du TP et de la SNc projettent à la fois au striatum et à la RLM chez la salamandre et le rat (Ryczko et al., 2016). Enfin, des fibres DA ont même été observées au niveau de la RLM chez l'humain, ce qui suggère qu'elle pourrait aussi être modulée par la SNc. Par ailleurs, ces projections SNc-RLM ont aussi été observées dans plusieurs espèces de mammifères (Beckstead et al., 1979; Gerfen et al., 1982; Edley et Graybiel, 1983; Scarnati et al., 1987; Semba et Fibiger, 1992). Nous pensons donc que cette voie descendante et son rôle dans la modulation de l'excitabilité des réseaux locomoteurs a été conservée phylogénétiquement de la lamproie aux mammifères (Ryczko et al., 2016) et qu'elle serait importante pour induire des réponses locomotrices à la détection d'odeurs dans l'ensemble des vertébrés.

Nos résultats chez la lamproie suggèrent que les signaux olfactifs activent les neurones DA du TP pour recruter les réseaux locomoteurs du tronc cérébral impliqués dans la locomotion. Il serait donc intéressant de déterminer si le signal olfactif est acheminé aux neurones DA de la SNc/ATV et si ces projections pourraient être impliquées dans les comportements olfactifs des mammifères. En premier lieu, les neurones DA sont-ils activés lors de la détection d'odeurs? Des résultats récents montrent que c'est le cas chez la souris, où l'on observe des réponses calciques dans les neurones DA de la SNc/ATV lorsqu'une odeur est présentée (Morrens et al., 2020). De façon intéressante, l'intensité de leurs réponses dépend de la « nouveauté » de l'odeur présentée. Chez les mammifères, il est connu que les neurones DA encodent la saillance d'un stimulus (Comoli et al., 2003; Schultz, 2016), incluant plusieurs paramètres d'un stimulus tels que l'intensité physique, la valeur de la récompense (Fiorillo et al., 2013), l'incertitude d'obtenir la récompense (Fiorillo et al., 2003) ainsi que la nouveauté du stimulus (Ljungberg et al., 1992; Lak et al., 2016). Chez la lamproie, il a été observé que des neurones dans le TP encodent aussi la saillance des stimuli (Pérez-Fernández et al., 2017). Ainsi, les odeurs saillantes pourraient produire un signal olfactif dans le BO qui serait transmis au TP où il serait transformé en signal de récompense, un rôle associé aux neurones DA de la SNc/ATV. Chez la souris, il a été montré que les odeurs attractives induisent de l'activité

spécifiquement dans le BO postérieur (Kermen et al., 2016) et que la stimulation de cette région induit un signal de récompense puisque l'animal est prêt à travailler pour recevoir la stimulation (Midroit et al., 2021) dans le contexte d'expériences d'auto-stimulation intracrânienne (Olds et Milner, 1954; Carlezon et Chartoff, 2007). De plus, la stimulation du BO postérieur induit de l'activité dans les neurones DA de l'ATV (Midroit et al., 2021). Cependant, contrairement à ce que nous observons chez la lamproie (pour une revue, voir la Section 1.3), les projections du BO n'atteignent pas directement la SNc/ATV (Watabe-Uchida et al., 2012). Comment le signal olfactif est-il acheminé à la SNc/ATV chez le mammifère? Une étude récente a identifié un circuit neuronal par lequel le signal olfactif atteint les neurones DA en passant par le tubercule olfactif (Midroit et al., 2021), une région du striatum contenant aussi des neurones épineux moyens. D'abord, le BO postérieur innerve densément le tubercule olfactif. De plus, l'activité neuronale est augmentée à la fois dans le tubercule olfactif et les neurones DA de l'ATV lorsque le BO postérieur est stimulé. Dans des expériences où les souris sont conditionnées à associer des odeurs avec différents lieux (préférence de place conditionnée), certaines odeurs sont considérées attractives puisqu'elles induisent une préférence de place chez les animaux. Tout comme la stimulation du BO postérieur, l'exposition à ces odeurs attractives augmente l'activité des neurones moyens épineux du tubercule olfactif et celle des neurones DA de l'ATV (Midroit et al., 2021). Ceci suggère que la voie BO postérieur – tubercule olfactif – ATV permet aux odeurs attractives de recruter le système de la récompense. Notamment, l'administration d'un antagoniste des récepteurs D1 supprime la préférence de place conditionnée induite par les odeurs attractives, ce qui suggère que la transmission DA de l'ATV est nécessaire pour les comportements d'attraction en réponse à des odeurs (Midroit et al., 2021). Ces résultats ont été validés chez l'humain (Midroit et al., 2021). Similairement à la souris, l'exposition à un odorant attractif induit une préférence de place conditionnée chez les participants, mettant en évidence que les odeurs peuvent recruter le système de récompense et renforcer des comportements chez l'humain. De plus, lors d'expériences d'imagerie par résonnance magnétique fonctionnelle, l'exposition des participants à des odeurs entraîne une réponse beaucoup plus élevée au niveau du tubercule olfactif si l'odeur est attractive (Midroit et al., 2021), ce qui suggère que cette région est importante pour relayer le signal des odeurs attractives chez l'humain aussi. Ainsi, un lien important entre le système olfactif et les neurones DA aurait été conservé chez les vertébrés de la lamproie aux mammifères. Cependant, la transmission directe du signal du BO aux neurones DA observée chez la lamproie n'aurait pas été

conservée chez les mammifères où un relai serait réalisé par le tubercule olfactif. Il est très plausible que le tubercule olfactif relaie le signal des odeurs attractives parce qu'il a été impliqué dans la perception olfactive (Zelano et al., 2007; Wesson et Wilson, 2010), les comportements guidés par les odeurs (Wesson et Wilson, 2011; Gadziola et al., 2015; Murata et al., 2015), les comportements sociaux et reproductifs (Hitt et al., 1973; Agustín-Pavón et al., 2014; DiBenedictis et al., 2015) ainsi que les comportements de recherche de récompense (Ikemoto, 2003; Ikemoto, 2005; de Araujo et al., 2009; Gadziola et Wesson, 2016). Par exemple, chez les souris femelles dont l'activité du tubercule olfactif est inhibée, la préférence normalement observée pour les phéromones de mâles n'est plus observable. Par ailleurs, le signal olfactif pourrait aussi être acheminé aux neurones DA en parallèle par d'autres régions du cerveau, tel que le cortex piriforme qui reçoit des projections directes du BO (Ojima et al., 1984) et innerve les neurones DA de l'ATV (Watabe-Uchida et al., 2012). De manière intéressante, l'homologue du cortex piriforme chez la lamproie serait contenu dans le PL (Heier, 1948; von Bartheld et al., 1984; Nieuwenhuys et Nicholson, 1998; Suryanarayana et al., 2021a), ce qui suggère que la voie olfactomotrice latérale aurait été conservée chez les mammifères. En comparaison avec les résultats de cette thèse chez la lamproie, les observations réalisées chez les mammifères suggèrent que la transmission du signal olfactif aux neurones DA méso-diencéphaliques a été conservée et a un impact déterminant pour les comportements olfactifs dirigés vers un but.

3.6 Perspectives futures

Un travail considérable reste à accomplir pour mieux comprendre les circuits neuronaux responsables des comportements olfactifs chez les animaux. Cette thèse permet de s'approcher de ce but en se penchant sur le cerveau de la lamproie, spécifiquement sur les interactions entre le BO médian et les neurones DA du TP. Évidemment, ces interactions ne suffisent pas à expliquer l'ensemble des comportements olfactifs et le rôle de plusieurs autres régions du système nerveux central doivent être étudiés pour mieux comprendre comment le signal olfactif est traité pour produire une réponse comportementale. En effet, les projections du BO médian ne ciblent pas uniquement le TP et pourraient recruter de nombreuses autres régions susceptibles d'être impliquées dans la réponse locomotrice. Des résultats qui n'ont pas été présentés dans cette thèse par mesure de concision montrent que le BO médian innerve entre autres le striatum, les régions locomotrices diencéphalique et mésencéphalique ainsi que plusieurs noyaux de cellules réticulospinales au niveau de la formation réticulée mésencéphalique et rhombencéphalique. Toutes ces régions sont connues pour leurs fonctions motrices et pourraient être recrutées directement par l'activité du BO médian. Une avenue possible pour la suite de ces travaux serait en premier lieu d'établir une cartographie détaillée des projections du BO médian dans l'ensemble du cerveau de la lamproie suivie d'expériences physiologiques pour caractériser individuellement le rôle des différentes régions qui reçoivent le signal olfactif. La préparation semi-intacte développée pour les expériences de cette thèse pourrait être employée pour évaluer l'impact de l'inactivation pharmacologique de ces régions sur la nage induite par la stimulation électrique du BO médian. Aussi, ces expériences devraient inclure l'inactivation du TP puisque l'effet de son activité sur la nage induite par l'olfaction reste encore à déterminer. Ces expériences permettraient d'acquérir une meilleure connaissance de l'ensemble des projections du BO médian essentielle pour comprendre comment cette région induit différents comportements olfactifs chez la lamproie.

Une autre perspective intéressante serait d'étudier la réponse à l'application d'odeurs en périphérie dans l'organe olfactif. En effet, dans l'ensemble des expériences physiologiques de cette thèse, l'activation des circuits olfactomoteurs est induite en stimulant électriquement ou pharmacologiquement le tissu cérébral de la lamproie. Nos résultats ont permis de caractériser davantage les réseaux olfactomoteurs, mais ne permettent pas d'observer si des différences existent dans les mécanismes et circuits impliqués pour le traitement d'odeurs individuelles. Par exemple, l'odeur de proies induit un comportement d'approche (Kleerekoper et Mogensen, 1963) tandis que

les nécromones produisent un comportement de fuite (Imre et al., 2014). Les mécanismes et/ou les régions du cerveau recrutés par la détection de ces molécules doivent certainement différer, mais le substrat neuronal exact n'est connu ni pour l'une ni pour l'autre. Pour mieux comprendre comment sont produits les différents comportements olfactifs, toutes les molécules chimiosensorielles connues pour induire de la locomotion (pour une revue, voir la Section 1.2) devraient être étudiées. Notamment, la préparation de prosencéphale isolé, développée pour les expériences de cette thèse, permettrait d'aborder plusieurs questions importantes qui demeurent sans réponse. D'abord, deux voies olfactomotrices anatomiquement distinctes (médiale et latérale) ont été identifiées, mais leurs rôles dans le traitement de différentes odeurs individuelles restent à déterminer. La mesure de l'activité du BO médian, du BO principal et du PL en réponse à l'application d'odeurs dans la périphérie olfactive permettrait d'évaluer si certaines odeurs sont traitées spécifiquement dans la voie médiale ou latérale ou en parallèle dans les deux. De plus, des expériences complémentaires dans la préparation semi-intacte permettraient d'évaluer si les réponses motrices aux odeurs diffèrent lorsqu'une des deux voies est inactivée (par exemple avec des lésions ou l'injection de substances pharmacologiques). Ensuite, la préparation de prosencéphale isolé fournit un accès sans précédent au TP qui permettrait de déterminer quelles odeurs y engendrent des réponses et d'identifier le phénotype (DA, glutamate, GABA), les projections (vers la MLR) ou d'autres caractéristiques des neurones recrutés dans le TP. De manière intéressante, les différentes odeurs à tester ont déjà été associées chez la lamproie à des comportements observés dans le milieu naturel. Donc, en identifiant les régions recrutées par chaque odeur testées (odeurs de proies, phéromones sexuelles, etc), il sera possible de déduire le contexte (prédation, reproduction, etc) dans lequel ces régions sont recrutées.

Enfin, un autre élément important à considérer est le sexe des lamproies utilisées parce que les individus féminins et masculins présentent des réponses comportementales différentes à la détection d'un stimulus olfactif identique (pour une revue, voir Section 1.2). Par exemple, la spermine, une molécule en forte concentration dans l'éjaculat du mâle, entraîne des comportements d'approche spécifiquement chez la femelle (Scott et al., 2019). Ceci suggère la présence de dimorphismes sexuels qui influencent la façon dont les signaux olfactifs sont traités dans le système nerveux central de la lamproie. Il serait intéressant d'identifier comment le traitement de certaines odeurs diffère entre les lamproies mâles et femelles pour bonifier notre compréhension des circuits olfactifs dans la production de comportements.

Finalement, bien que les circuits neuronaux permettant des réponses motrices aux signaux olfactifs soient relativement bien caractérisés chez la lamproie, beaucoup de travail est encore nécessaire chez les autres espèces de vertébrés. Par exemple, le poisson-zèbre, dont le BO contacte directement les neurones DA du TP (Miyasaka et al., 2014), serait un bon modèle pour vérifier si les circuits olfactomoteurs décrits dans cette thèse ont été conservées chez les poissons téléostéens. Les études réalisées chez la lamproie et d'autres vertébrés fourniront des renseignements importants sur la façon dont les signaux olfactifs permettent de générer des comportements essentiels pour la survie et la reproduction des animaux et aussi sur la façon dont ces réseaux ont été conservés ou modifiés au cours de l'évolution.

3.7 Conclusions

Dans cette thèse, nous avons étudié les circuits neuronaux permettant la transformation d'un signal olfactif en réponse de nage chez la lamproie. Dans l'ensemble, nous montrons que les neurones DA du TP jouent un rôle important dans la transmission olfactomotrice. Nous avons d'abord caractérisé l'innervation du TP par le BO ainsi que son activité neuronale en réponse à la stimulation olfactive. Notamment, nous avons observé la présence de neurones individuels recrutés à la fois par la voie olfactomotrice médiale et la voie olfactomotrice latérale. L'étude des effets possibles de cette activité neuronale a révélé que par des projections DA au BO, le TP peut contrôler la transmission du signal olfactif vers les centres moteurs. De plus, nous montrons que les neurones du TP qui répondent à la stimulation olfactive ont des projections vers la RLM, dont le rôle est de contrôler la locomotion. Justement, nous montrons aussi que l'activité du TP accompagne systématiquement la nage de la lamproie, incluant la locomotion survenant spontanément chez l'animal. Dans une préparation permettant de montrer pour la première fois que la stimulation des afférences olfactives induit de la nage, nous avons observé que l'activité du TP est couplée avec cette activité motrice. Nos résultats permettent d'avoir une compréhension plus approfondie des populations neuronales responsables de produire un comportement en réponse à la détection d'odeurs dans l'environnement, particulièrement les neurones DA du TP.

Bien que nos observations portent uniquement sur la lamproie, des études portant sur d'autres poissons y révèlent des rôles similaires pour les neurones DA du TP. Nous proposons donc que cette population neuronale ne jouerait pas un rôle spécifique au système olfactif, mais plutôt un rôle global permettant de moduler l’ensemble des entrées d’informations sensorielles et des réponses motrices, et jouerait donc un rôle plus important qu'anticipé dans l'intégration sensorimotrice.

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Veuillez noter que les références associées aux manuscrits contenus dans cette thèse se retrouvent dans une liste bibliographique propre à chaque article.

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Annexes

Trois fichiers vidéo sont annexés à cette thèse. Il s'agit de figures supplémentaires accompagnant le deuxième manuscrit de la section résultats, qui correspondent aux figures S29, S32 et S34 de la thèse.

La figure S29 illustre les variations intracellulaires de calcium dans les cellules du TP après stimulation électrique du BO médian. Cette figure vidéo permet de voir le décours temporel de l'activité calcique à partir des images produites durant les expériences et sous forme graphique en simultané.

Les figures S32 et S34 montrent les mouvements de nage produits dans la préparation semi-intacte à la stimulation électrique du BO médian et du PL, respectivement. Le décours temporel des potentiels enregistrés extracellulairement dans le PT et intracellulairement dans la formation réticulée apparait en synchronie avec l'image de la nage de l'animal et permet d'apprécier l'activité neuronale et le mouvement résultant du corps de la préparation.