Appel d'offre Interfaces Pour le Vivant 2014 (UPMC)

Title : Differential adhesion among axons : how does the olfactory system get wired ?

A collaborative project proposed by :
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Abstract :

In the mouse olfactory system, adhesion is initially among heterogeneous olfactory sensory neuron (OSN) axons as they exit the epithelium. In the olfactory bulb (OB), however, about 1,000 subpopulations reorganize extensively and coalesce homogeneously to form glomeruli. During this process, initial apposition followed by reorganization, are thought to depend crucially on differential adhesion. We will evaluate this mechanism using complementary neurobiological and biophysical methods. The Trembleau lab has developed explant cultures from the olfactory epithelium (OE) of embryonic wild-type or transgenic mice, enabling visualization of the extension, fasciculation and defasciculation of individual axons. Pairs of labeled explants dissected from different areas along the antero-posterior, dorso-ventral and medio-lateral axes of the OE will be co-cultured to determine if adhesion is differentially affected by the topographical source of an explant. The biophysical analysis of axon interactions will be performed in the Pincet lab using the Biomembrane Force Probe technique, that involves a streptavidin-coated bead bound to a biotinylated axon, and attached to a nanodynameter made of a micromanipulated biotinylated red blood cell (videomicroscopy). The information that we will gain from these measurements are both static and dynamic. First, by applying a fixed force, the system will reach equilibrium. The contact angles observed in a picture at equilibrium will provide a direct measurement of the tension of the axons. This tension can be related to the adhesion energy. Forces of various magnitude will be used to check the consistency of the results. We will also analyze the dynamic unzipping of axons either at constant force or at increasing forces. This is an alternate measurement of the adhesion energy of the axons. The final result is a systematic quantification of the axon:axon adhesion in the various explants studied here.

Scientific project :

Background :

The perception of odors begins when odorous ligands bind with receptors on the surface of olfactory sensory neurons (OSNs). A molecular basis for odor transduction emerged when Buck & Axel (1991) identified a large family genes encoding odorant receptors (ORs; there are about 1000 OR genes in mice). A single OSN expresses only one OR gene, and thus the olfactory epithelium comprises more than 1000 types of OSNs, defined by the OR they express. Strikingly, whereas OSNs expressing the same OR are scattered stochastically in large epithelial zones, they project their axons to topographically fixed glomeruli in the OB (reviewed in Mombaerts, 2006). A glomerulus receives input exclusively from OSNs expressing a specific OR (Treloar et al., 2002). From a functional point of view, the consequence of this homotypic axon convergence is that the OB is topographically organized, with glomeruli in stereotyped positions representing specific OR activation.

Conservatively, each side of the mouse nose has ~12 million OSNs, each of which sends an unbranched axon unilaterally to one glomerulus. Most axonal paths in the central nervous system are topographically organized such that the axons of neighboring sensory neurons remain in close proximity throughout their path, as occurs in the visual and auditory systems. However, in the olfactory path each axon follows a distinct trajectory that is dependent upon its point of origin in the epithelium, the stochastic location of the OSN within a zone. Figure 1 illustrates some important features of the anatomical organization of the olfactory sensory projections from the OE to the OB. Axons emerging from the OE assemble in tightly packed heterotypic fascicles and form branches of the olfactory nerve. The sorting of OSN axons according to their type, leading to the formation of homotypic bundles and their coalescence in homogeneously innervated glomeruli, only takes place in the outer layer of the OB, called olfactory nerve layer (Treolar et al., 2002; Miller et al., 2010).

In this process of axon guidance and targeting, initial apposition of axons followed by their reorganization and homotypic fasciculation are thought to depend crucially on **differential adhesion**. Interestingly, the sorting of these axons depends on the OR themselves, that presumably provide OSN axons with specific adhesion properties (reviewed in Mombaerts, 2006; Imai and Sakano, 2011; Lodovichi and Bellucio, 2012; Dubacq et al., 2014). In the context of a collaboration between our two teams, we recently

demonstrated that OR overexpression in heterologous cells provides these cells with homotypic and heterotypic adhesion properties in the nN range (Richard et al., 2013). The aim of the present project is to go a step further by assessing **axon:axon adhesive properties**.

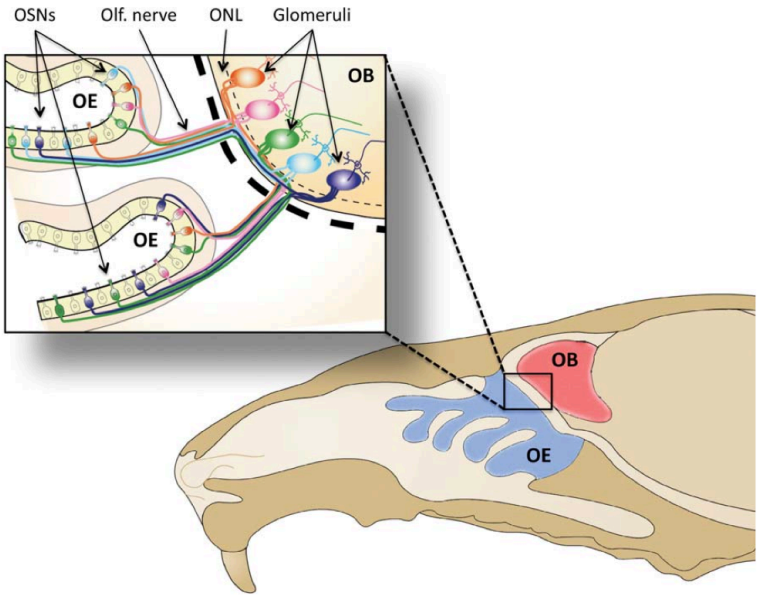
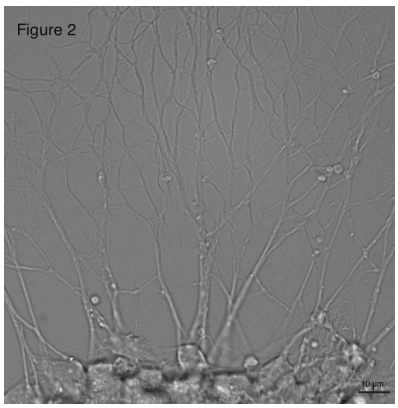


Figure 1 : Lower right drawing schematizes the organization of the rodent olfactory system, displayed here on a sagittal section of the head, with the olfactory epithelium (OE, represented in blue) lying in the nasal cavity, and the olfactory bulb (OB, represented in red) located at the anterior extremity of the brain. Inset represents a small area of the system (upper left part of the figure). Olfactory sensory neurons (OSNs) expressing the same odorant receptor (OR) are represented with the same color. Only five different populations are represented here but the mouse olfactory system actually comprises about 1000 OSN populations, each expressing a specific OR. OSNs expressing the same OR are scattered in large areas of the OE, but their axons converge on a small number of glomeruli. Olf. nerve : olfactory nerve branches. ONL: olfactory nerve layer of the OB. Thick broken line represents the cribriform plate; thin broken line delineates the limit of the olfactory nerve layer. *From Dubacq et al., 2014.*

Specific aim of the project :
The aim of this project is to address, using an explant culture paradigm and a biophysical approach, the adhesion energies between axons arising from different areas of the OE, and between axons of the same or different OR identities.

Methodology :
1) Olfactory epithelium explant cultures
The Trembleau lab has developed cultures of embryonic day 13.5 OE, grown for 48 hours on poly-lysin/Laminin substrate.
OSN axons grow from these explants, and interact with each other to form fascicles (Figure 2). Interestingly, these axons display dynamic lateral interactions along their shaft, with "zippering" and "unzippering" processing along axon shafts. These interactions are currently investigated in collaboration with Dr. Martin Zapotocky (theoretician physicist, Prague) and Daniel Smit (cotutelle PhD student), and preliminary measurements of axon tension were performed in collaboration with Frédéric Pincet (see below).



In the context of this proposal, we will first co-culture pairs of explants dissected from different areas along the antero-posterior, dorso-ventral and medio-lateral axes of the OE of wild-type mice, to determine if adhesion is differentially affected by the topographical source of an explant. Explants will be labeled with vital fluorescent dyes prior to their co-culture, allowing to distinguish the axons coming from explant A from the ones coming from explant 2.
Similar experiments will be performed using explants from transgenic mice expressing a GFP reporter in all neurons expressing the same OR, allowing to address more specifically homotypic and heterotypic interactions between axons coming from various areas of the epithelium.
Finally, since the cAMP pathway has been critically involved in the sorting of olfactory axons (reviewed in Zou et al., 2009; Nakashima et al., 2013), we will pharmacologically manipulate this pathway in order to test whether such manipulations (i.e. activation or inhibition of adenylate cyclases) modify the adhesion energies of the axons.

2) Biophysical analysis of axon:axon adhesion
The primary technique that will be use is a variation of the biomembrane force probe (Merkel et al., 1999). The neurons will be biotinylated using Sulfo-NHS-SS-Biotin. A 3 µm streptavidin-coated bead will be bound to an axon and attached to a nanodynamometer made of a micromanipulated erythrocyte. The stiffness of the spring is proportional to the membrane tension which is controlled by adjusting the

aspiration in the micropipette. Displacing the micropipette induces a force that tends to separate the individual axon from the others. The force is directly quantified by measuring the deformation of the erythrocyte. This is achieved in video microscopy by calculating variation of the distance the bead and the pipette. The information that we will gain from these measurements are both static and dynamic. First in the static situation, by applying a fixed force, the contact angles observed in a picture at equilibrium will provide a direct measurement of the tension of the axons. This tension can be related to the adhesion energy using models derived by Zapotocky. Forces of various magnitudes will be used to check the consistency of the results: it would make sense that the tension is independent of the force applied, and if not, we will have to explain the physical origin of this variation. We will also analyze the dynamic unzipping of axons either at constant force or at increasing forces. This is an alternate measurement of the adhesion energy of the axons. The final result is a systematic quantification of the axon:axon adhesion in the various explants studied here.

Expected results :

Overall, our project will characterize in an ex vivo system amendable for genetical, pharmacological and biophysical manipulations:

- the basic adhesion properties of OSN axons arising from different areas of the epithelium
- the homotypic vs. heterotypic ahesion between OSN axons
- the role of cAMP in these adhesive properties.

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