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Olfacto-tactile integration in mouse barrel cortex

Anthony Renard

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Anthony Renard. Olfacto-tactile integration in mouse barrel cortex. *Neurons and Cognition* [q-bio.NC]. Sorbonne Université, 2021. English. NNT : 2021SORUS117 . tel-03432268

HAL Id: tel-03432268

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Dynamique du système auditif et perception multisensorielle

Intégration multisensorielle olfacto-tactile dans le cortex à tonneaux chez la souris

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Thèse de doctorat de neurosciences

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Présentée et soutenue publiquement le 14 septembre 2021

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Remerciements

Cette thèse est le fruit de plusieurs années de travail mené au sein de l'UNIC et plus récemment de l'Institut de l'audition. Elle doit beaucoup aux échanges stimulants avec les membres des équipes qui m'ont grandement enrichi intellectuellement et personnellement.

J'ai une dette envers Brice Bathellier qui m'a accueilli dans l'équipe avant de devenir mon directeur de thèse, ainsi qu'envers Evan Harrell, tout deux ayant témoigné d'une disponibilité et d'un soutien continu, et consacré un temps conséquent à suivre et conseiller mon travail. Qu'ils en soient ici vivement remerciés.

Je remercie également tous mes collègues et amis pour leur bonne compagnie durant cette période. Sebastian Ceballo, Étienne Gosselin, Sara Jamali et Alexandre Kempf pour avoir traversé les tribulations de la thèse ensemble; Dorian Goueytes et notre groupe de grimpeurs pour m'avoir transmis la passion de l'escalade; Sophie Hubatz, Timothé Jost-Mousseau et Yannick Passarelli pour les dîners japonisants; Sophie Bagur pour la bonne entente et sa passion émulative; Jacques Bourg et Joanna Schwenkgrub pour les conversations sociologiques et psychologiques.

Enfin, j'ai une pensée particulière pour ma famille et mes amis d'enfance, à qui je dédie ce travail.

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Chapter 1

Introduction

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1.1 Multisensory perception

1.1.1 Enrichment and disambiguation

It is no secret to Foley artists that perception is a multisensory phenomenon. They pull off the illusionist trick of reproducing the sounds of movie scenes by using surprisingly simple objects. One would not guess that the footsteps of a horse pacing are simply coconuts knocking on the floor, that the sound of a flying bird is just a shaking pair of gloves, or that a terrifying roar is nothing but a concrete block sliding along the floor. Although fairly different than their intended meanings, these sound effects blend in with the visual scene to form a robust percept. This seamless process illustrates that the combination of coincident sensory cues results in a significant enrichment of unisensory processing and facilitates the disambiguation of the perceptual scene.

Our perceptual system has to deal with incomplete information that can be ambiguous to some extent. The Rubin vase and the Necker cube are famous examples of ambiguous figures leading to multistable perception. Although mostly studied in vision, multistable perception also exists in audition (Bregman, 1994; Deutsch, 1974), touch in the context of apparent motion (Carter et al., 2008; Liaci et al., 2016), and even olfaction in the context of binaral rivalry (Zhou and Chen, 2009). Collecting more information is helpful to reach a stable interpretation; for example, the sense of depth created by adding shadows is often sufficient to resolve ambiguous figures (Kersten et al., 1996). However, when a single modality is not enough, disambiguation can be achieved by combining several sensory streams. A vivid demonstration of this process is well exemplified by the *streaming/bouncing effect* (Metzger, 1934; Sekuler et al., 1997). Inspired from Metzger's motion display, two identical disks move uniformly towards each other from opposite sides of a screen, overlap, and end up at their starting position. The disks can therefore be perceived as streaming through or bouncing off. Already pointed out by Gestalt psychologists to show that grouping by continuity also applies to movement, the majority of observers

(80% or more) report perceiving the disks streaming through (Shimojo et al., 2001). However, adding a brief auditory stimulus at the crossing point is enough to strongly switch the dominant percept towards bouncing (Sekuler et al., 1997). Although the effect was initially studied with simple click sounds, the efficiency of audition to disambiguate visual motion was shown to be more pronounced by using ecologically relevant sounds (Grassi and Casco, 2009, 2010). Moreover, Grassi and Casco (2010) showed that the congruence of the sound has a significant effect on the streaming/bouncing effect: the sound of the impact of billiard balls induced more bouncing perception than that of a drop of water or a firework, even when the latter were categorized as more salient or played 20 dB louder.

Even though vision was traditionally seen as impenetrable to the other senses, cross-modal disambiguation of visual perception is not limited to auditory influences. Indeed, a recent study showed that the visual motions used in the streaming/bouncing effect can also be disambiguated with a tactile stimulus presented on the hand of the observer in a way that correlates with a decrease in perceived overlap between the disks (*touch-induced illusory crescent*) (Meyerhoff et al., 2018). Surprisingly, olfaction, often considered as vestigial in humans, has been shown to display a similar influence on binocular rivalry. Zhou et al. (2010) presented images of a rose and of marker pens dichoptically, so that they engage in binocular rivalry. To assess whether olfactory cues could impact binocular rivalry, the subjects were simultaneously stimulated with odorants carrying the smells of roses or markers. They found that the dominance times before switch were 20% longer and shorter when the congruent and incongruent odorants, respectively, were paired with the visual stimuli. A clear role of cross-modal congruency in modulating binocular rivalry has also been shown for audition (Ee et al., 2009) and touch (Lunghi et al., 2010).

Rather than disambiguating, the integration of complementary information can enrich perception by providing missing information or enhancing detection and discrimination abilities. For example, Newell et al. (2001) showed that both vision and touch are dependent on viewpoint and that haptic information was crucial to the recognition

of 3D objects when visual information was incomplete. In other circumstances, cross-modal cues can increase the sensitivity of a stimulus presented in another modality even when the added cue is irrelevant to the task. Among the first studies addressing this phenomenon, [Gescheider et al. \(1974\)](#) showed that the detection of faint tactile vibrations was enhanced when a task-irrelevant tone was presented synchronously. More recently, the converse effect has been shown where a task-irrelevant haptic stimulus is presented during a tone discrimination with a specific increase in sensitivity without change in the propensity of response ([Gillmeister and Eimer, 2007](#)). Analogous auditory-somatosensory interactions exist for response times in the context of fastened detection ([Murray et al., 2005](#)). In similar studies, the detectability of sounds was improved when accompanied with irrelevant light flashes ([Lovelace et al., 2003](#)) and perceived loudness was increased ([Odgaard et al., 2004](#)), as well as the detectability and perceived brightness of flashes, when coinciding with tones ([Frassinetti et al., 2002](#); [Stein et al., 1996](#)).

These findings were also extended to the context of discrimination tasks. For instance, [Berger et al. \(2003\)](#) showed that auditory induced illusory flashes could facilitate an increase in orientation discrimination. Participants had to indicate which of two flashed oriented gratings played at 4 Hz were slightly tilted by 1° from vertical. When more beeps were played than flashes, participants reported seeing more flashes than were actually played, an effect known as the *sound-induced flash illusion* ([Shams et al., 2000, 2002](#)), and discrimination performance increased with more beeps, so that performance was matching with the number of perceived flashes whether illusory or not. Similar effects on speed and accuracy were obtained with tactile ([Arabzadeh et al., 2008](#)) and olfactory discrimination tasks ([Gottfried and Dolan, 2003](#)).

The line of research on complementary interactions reviewed in this section provides evidence that cross-modal interactions and semantic relations influence the combination of signals from the different sensory streams, and that inputs from one modality can facilitate sensory processing and enhance perception in another modality. As far as it could

be tested, some form of perceptual enhancement resulting from task-irrelevant cues seem to hold between every pair of modalities that have been investigated, even, for example, between olfaction and vision (Gottfried and Dolan, 2003; Zhou et al., 2010). Moreover, besides signals delivered to the auditory modality, tactile stimuli are also able to induce the *streaming/bouncing effect* (Meyerhoff et al., 2018), as well as the *sound-induced flash illusion* (Violentyev et al., 2005). While this interplay can suggest that multisensory interactions are generalized between the senses, this level of ubiquity may also reflect a generic attentional or decisional process unspecific of the modalities in question.

To date, this issue is still hotly debated, but a growing body of evidence indicates that these phenomena are at least partly sensory rather than fully cognitive. Firstly, the studies considered above rely on the framework of signal detection theory (Tanner Jr. and Swets, 1954) according to which sensory decisions unfold in a two stages: the signal undergoes sensory processing, and then the output is triggered by a decision threshold. Sensory processing is thus distinguished from response biases by a measure of sensitivity, d' , independent of the decision threshold, *criterion*. Although some work has suggested that improved detection results from shifts in criterion rather than increases in sensitivity (Gescheider et al., 1970; Gescheider and Niblette, 1967), the effects mentioned above on enhancement of sensory specific judgments by irrelevant cross-modal cues showed an increase in sensitivity while criterion stayed unaffected, which argues in favor of an interaction at the level of sensory processing. Others authors argue against high-level decisional mechanisms by using task design that are criterion-free (Arabzadeh et al., 2008; Gillmeister and Eimer, 2007). Secondly, the fact that the behavior gain in terms of speed, detection and discrimination strongly depend on the temporal alignment of the integrated signals (e.g., Gillmeister and Eimer (2007)) suggesting that it is based on low-level sensory interactions rather later decisional processes. In addition, some effects can influence independent aspects of perception as with the *streaming/bouncing effect*. Meyerhoff and Scholl (2018) showed that a secondary cue not only leads to increased bouncing, but

also induced the perception of large illusory crescents as if the disks did not fully overlap, which could be partly responsible for driving the bouncing percept.

Finally, some cross-modal illusions resulting from the integration of complementary information, such as the well-known *McGurk-MacDonald effect*, have such potent impacts on our perception that there is little doubt about their sensory nature. The role of congruence as a component that increases or is necessary for these cross-modal effects (cf. section 1.2.2) suggests that associations between sensory features from different modalities, that could be mediated by direct connections from sensory areas or by feedback connections from convergence zones to sensory specific areas (Driver and Noesselt, 2008). We will come back to this when discussing the physiology of multisensory perception. Before reaching that point, we will review the perceptual effects resulting from the integration of redundant information, either driving misleading dominance effects or sharper perception.

1.1.2 Spatial and temporal dominance

So far, we focused on beneficial multimodal interactions resulting from the combination complementary and synchronous sensory inputs. But when conflicts arise, the transfer of information between the senses is so pervasive that incongruent inputs are still combined in order to resolve the conflict, often with one modality dominating and sometimes leading to various illusions. In this section, we will describe the multisensory effects resulting from the presentation of conflicting information with a focus on mismatch in the spatial and temporal domains.

The best known and compelling illusion resulting from a spatial mismatch is the *ventriloquist effect*. When the performing artist speaks without moving his lips while animating the mouth of a puppet in synchrony, the puppet appears to spring into life as the visual stimulus “captures” the location of the sound. Luckily for the physiologist, the ef-

fect also works with simpler flashes and tones: as long as they are aligned in time, the location of the auditory stimulus will be shifted toward the visual stimulus (Bertelson and Radeau (1981); Howard and Templeton (1966); see Chen and Vroomen (2013) for a review). The effect works over surprisingly large distances; even if too large to result in a proper capture, there is still a clear bias in perceived location (Welch and Warren, 1980). The reverse effect of sound attracting vision can also happen, but seems to require the visual stimulus to be difficult to localize (Alais and Burr, 2004). Nonetheless, Hidaka et al. (2009) exploited this effect to uncover a clear impact of audition on visual location. They presented a blinking visual stimulus at a fixed location which they showed was perceived as moving laterally when the flash onset was synchronized with a sound alternating between left and right. This effect of spatial attraction is not limited to audition and vision. A similar spatial capture of audition has been reported when synchronous vibrations are presented on the fingertips (Bruns and Röder, 2010; Caclin et al., 2002), as well as spatial attraction exerted by vision on touch (Dionne et al., 2010; Taylor-Clarke et al., 2002).

Less well-known, a similar illusion occurs in the temporal domain where auditory and visual signals appear synchronous despite delays between them. Referred to as the *temporal ventriloquist effect*, Fendrich and Corballis (2001) asked participants to report the onset of a flash by pointing at a marker rotating around a visual display. The flash onset was perceived as earlier in time when a click was leading and later in time when it was lagging, as if the flash was dragged towards the click. To a smaller extent, the authors also found a reverse effect when participants were told to report the onset of the click.

Another well-documented influence of audition on vision in the temporal domain is known as *auditory driving* (Gebhard and Mowbray, 1959; Shipley, 1964). This phenomenon occurs when presented a flickering light together with hearing a fluttering sound. When a mismatch is introduced between the rates, the flicker is perceived as following the flutter, both upwards and downwards. When the two rates are initially matched, the flutter rate can be increased surprisingly high (up to 22 Hz for a flickering light at 10 Hz) be-

fore an asynchrony is felt (Shiple, 1964). The *sound-induced flash illusion* that we mentioned in the previous section is a more recent striking case of auditory driving, where the presentation of a single light flash accompanied by multiple beeps leads to the perception of extra flashes that are not there Shams et al. (2000, 2002). Reverse effects of auditory driving have not been found however, suggesting the dominance of audition over vision in the temporal domain.

Given that the temporal ventriloquist effect is rather subtle (the flash onset is judged to occur 40 ms earlier when the click is leading by 100 ms in Fendrich and Corballis (2001)) and that early studies relied on subjective reports, concerns can be raised about response bias that question the perceptual nature of the effect. For example, the short latency between clicks and flashes might make it hard to ignore the sound. A study addressed that concern with a temporal order judgment task (Scheier et al., 1999). They asked observers to tell which of two flashes located at a different place on a display appeared first (top or bottom). Two sounds bearing no information about the location of the flash could be played, flanking the flashes either outwards (AVVA) or inwards (VAAV). As the task is to report the order of appearance of the flashes and does not have to do with timing, the sounds could be more easily ignored. However, participants performance, measured in terms of just noticeable difference, was significantly improved when the sounds were played before and after the first and second flashes (AVVA) in an interval of 200 ms, seemingly pulling the flashes away from each other. Conversely, the task was more difficult when the sounds were played in between the flashes (VAAV). Using a similar approach, touch could also be shown to induce temporal capture over vision (Keetels and Vroomen, 2008), as well as over audition in a bidirectional way (Ley et al., 2009).

The perceptual effects in situations of multisensory conflicts described above illustrate the ubiquity and flexibility of multisensory processes. Although spatial and temporal coincidences are major requirements for the integration of multisensory inputs (Stein and Meredith (1993) and see section 1.2.1), two sensory streams that disagree in the spatial or

temporal domains still tend to be bound into a common percept. These phenomena of capture suggest a certain specialization in the synergy of cross-modal interactions. The reversal of dominance from vision to audition in the temporal domain lead [Welch and Warren \(1980\)](#) to put forward what they termed the *modality appropriateness hypothesis*. They argued that vision is especially designed for the processing of spatial information while audition specializes in temporal processing. Each modality is therefore given the primacy in their respective domain and stimuli from other senses are adjusted to resolve the conflict. Although much cited, this traditional description appears as a first order simplification rather than a universal principle. It does not account for sensory conflicts involving other modalities such as the influence of touch over vision and audition, which suggests a more intricate multisensory logic. Moreover, dominance in one domain is not absolute as [Alais and Burr \(2004\)](#) could show that visual dominance is reversed in the spatial ventriloquist effect when using degraded visual stimuli.

More recently, a much more generic and popular ideal observer model was introduced by [Ernst and Banks \(2002\)](#) as a theory of sensory dominance and more generally of redundant cue integration, on which we will focus in the next section.

1.1.3 Redundant cue integration

We have seen that disambiguation and cooperation lead to a beneficial enrichment of perception as a result of multisensory interactions between complementary aspects of the sensory environment. By contrast, a single feature perceived through multiple sensory channels gives rise to a redundancy of sensory information. For instance, in the ventriloquist effect discussed above, location is estimated through visual and auditory signals. In the same way, when judging the size of an object, both vision and touch can be used simultaneously. Although informative about the same property, this redundancy is used by the nervous system to increase the robustness of perception.

In this context, psychophysical studies in humans, monkeys and rodents have demonstrated that the accuracy of perceptual discrimination increases when redundant cues from several modalities are combined (Battaglia et al., 2003; Ernst and Banks, 2002; Fetsch et al., 2009; Nikbakht et al., 2018; Raposo et al., 2012). The multisensory gain in performance is especially strong when the discriminations are rendered difficult with degraded stimuli close to the perceptual threshold, an observation that finds its neural counterpart in the *principle of inverse effectiveness* – as we will see in section 1.2.1.

The multisensory improvement resulting from the integration of multiple sensory cues is often tested against ideal observer models and has been found to closely approximate the theoretical optimum in various combinations of modalities (Alais and Burr, 2004; Battaglia et al., 2003; Ernst and Banks, 2002; Fetsch et al., 2012; Gu et al., 2008). This approach was first applied in a multisensory context by Ernst and Banks (2002) in a visual-haptic study where participants had to judge the height of a ridge that could be seen or touched. They first measured the variance of the estimate in each modality separately by presenting ridges of different sizes. Then, in the bimodal conditions, they presented ridges of the same or different sizes while manipulating the reliability of the visual stimuli independently of the haptic ones, in order to create intersensory conflicts and assess their impact on the joint estimate of the height. The authors demonstrated that the resolution of the conflict was crucially determined by the variance associated with each estimate in such a way that the combined estimation closely matched with the sum of the unimodal estimate weighted by their reliability – defined as inverse of variance. The result was optimal in the sense that it produced an estimate with the lowest possible variance obtainable from a linear combination, in accordance with maximum-likelihood estimation (MLE) or Bayesian decision making.

More precisely, let \hat{s}_1 and \hat{s}_2 be two sensory estimates of some external property s by two modalities (e.g., the height of the bar though touch and vision). They may not be the same and vary from one sampling to another, partly due to the variability in the external

signal and its transduction into neural impulses. A simple way to reduce this uncertainty is to make a linear combination \hat{s}_c of these estimates such that

$$\hat{s}_c = w_1 \hat{s}_1 + w_2 \hat{s}_2 \quad (1.1)$$

where w_i is the weight attributed to modality i . Applying MLE, one can show that the \hat{s}_c with lowest variance is given by

$$w_i = \frac{1/\sigma_i^2}{\sum_i 1/\sigma_i^2} \quad (1.2)$$

with σ_i^2 the variance of \hat{s}_i , that is, when each modality is weighted according to the inverse of its variance (Clark and Yuille, 1990). Equivalently, the problem can be formalized in terms of Bayesian inference, where the point is to find the posterior distribution of s conditional on \hat{s}_i . According to Bayes' law, we have

$$p(s|\hat{s}_1, \hat{s}_2) = \frac{p(\hat{s}_1, \hat{s}_2|s)p(s)}{p(\hat{s}_1)p(\hat{s}_2)} = \frac{p(\hat{s}_1|s)p(\hat{s}_2|s)p(s)}{p(\hat{s}_1)p(\hat{s}_2)}. \quad (1.3)$$

Either way, if we assume, to ease the derivation, that the estimates are gaussian, and, crucially, that the noise corrupting the measurements are independent (used for the second equality in 1.3), and that the prior $p(s)$ is uniform, then \hat{s}_c is also gaussian, with mean and variance given as

$$\mu_c = \frac{1/\sigma_1^2}{1/\sigma_1^2 + 1/\sigma_2^2} \hat{s}_1 + \frac{1/\sigma_2^2}{1/\sigma_1^2 + 1/\sigma_2^2} \hat{s}_2, \quad (1.4)$$

$$\sigma_c = \frac{\sigma_1^2 \sigma_2^2}{\sigma_1^2 + \sigma_2^2}. \quad (1.5)$$

It follows from 1.5 that the variance of the combined estimate is always smaller than that of the unimodal ones – its reliability, or inverse of variance, is actually the sum of the reliabilities of the unimodal estimates. This model captures well the intuitions of the modality appropriateness hypothesis (see Section 1.1.2) in the sense that the combined estimation is a compromise between modalities and that the most reliable one in a given situation

will dominate. Moreover, it gives a quantitative account of sensory dominance that has the advantage of being more flexible and can explain, for example, that the traditional visual capture of audition in the spatial domain can be reversed when the visual stimuli are degraded (Alais and Burr, 2004). This flexibility accounts for multisensory interactions resulting both in illusions or sharpened perceptions with a single principle.

Arguably, this approach established itself as the most popular model in the literature on redundant cue integration. However, it is not specific to multisensory integration as the same idea holds within modalities. Indeed, MLE was already applied to visual cue integration a decades before the classic study of Ernst and Banks (2002) to show that the sense of depth results from a weighted linear summation of object motion and texture gradient that is dynamically adjusted from trial to trial when the reliability of the cues was manipulated (Landy et al., 1991; Young et al., 1993). The picture of multisensory integration according to this account is somewhat limited by the assumption of independent noise which denies the possibility of interaction in the first place. Therefore, synergies that would rely on underlying associations between the senses are not accounted for by this approach. We will see later on that this point is supported by reports of deviations from the predicted integration that go beyond the theoretical optimum. Furthermore, the Bayesian account of neural computations in general (Knill and Pouget, 2004) has been recently criticized for making fundamental confusions about computational levels and for lacking explanatory power (Bowers and Davis, 2012a,b; Brette, 2018; Griffiths et al., 2012; Rahnev and Denison, 2018). Yet, restricted to the context of multisensory integration, one has to acknowledge that the Bayesian model matches remarkably well with the psychophysical data in a variety of tasks and combinations of senses, even when three modalities are considered at the same time (Wozny et al., 2008).

The goal of this part was to give a broad description of the multisensory literature that was faithful to its diversity. The plethora of cross-modal effects makes it hard to resort to comprehensive principles that are informative about the underlying neural mechanisms.

Overall, it feels that evidence are still needed to give a strong basis to the perceptual nature of some multisensory effects and disentangle the involvement of cognitive and sensory factors. To this end, physiological investigations will be instrumental to localize the sites of integration and characterize the contribution of the different stages of sensory processing involved in the integration of multimodal signals. We will now review what has been done on the side of the physiology in the second part of this introduction.

1.2 The physiology of multisensory integration

It is not an overstatement to say that the interest in the physiology of multisensory integration was born with the work of Stein and colleagues on the superior colliculus of cats (Stein and Meredith, 1993). This work established some key principles on the integration of multisensory signals that guided later research into the cortex and laid a convincing neural basis to behavioral and perceptual effects that motivated the development of psychophysical work (Alais et al., 2010). In what follows, we will review the physiological facets of multisensory integration and the extent to which the current neurophysiological findings give a neural basis to the perceptual phenomena reviewed in the previous section. Starting with the superior colliculus, we will then see how the principles found in this structure apply to the associative cortex and finish with the recent multisensory influences in low-level sensory areas.

1.2.1 Superior colliculus

The ability to orient and focus attention on surrounding events is fundamental for both predators and preys. The superior colliculus (SC), located on the dorsal surface of the midbrain, is the main structure involved in the flexible and rapid coordination of orienting movements of the eyes, ears, head and limbs (White and Munoz, 2011). It is laminated into seven anatomically defined layers which are grouped into two functionally distinct

regions. The first three layers receive direct projections from the retina and the primary visual cortex and sends outputs to the pulvinar complex and the lateral geniculate nucleus, which makes it specifically dedicated to the processing of visual information and is described functionally as a salience map (Fecteau and Munoz, 2006). The region of interest to us is constituted by the four deeper layers of SC¹. It is a multisensory region which receives visual, auditory and somatosensory inputs, and contains multisensory neurons that respond to all combinations of these modalities. These inputs emanates both from ascending pathways via a large number of subcortical structures, primarily the primary sensory nuclei of the thalamus, the inferior colliculus, the reticular areas and the cerebellum, as well as a broad set of descending corticotectal projections from most associative areas (Edwards et al., 1979; Harting et al., 1992).

The essential feature of SC that made it an excellent first model of multisensory integration is its topographic organization. The visual, auditory and somatosensory representations are laid out in a map-like manner with receptive fields that are in spatial register with each other (Meredith and Stein, 1990; Middlebrooks and Knudsen, 1984; Stein et al., 1975). As a result, the responses of neurons from a vertical portion of tissue are determined by the spatial location of incoming stimuli rather than by modality specific features. Overall, for all three maps, the upper space and body are represented medially while the lateral regions responds to the inferior space and body, the central space and the face are represented in the rostral part, and the peripheral space is represented in the caudal part. Most superior colliculus neurons project to premotor nuclei of the brainstem and to the spinal cord in a way that forms a premotor map in agreement with the sensory map. This correspondence likely allows SC to match incoming sensory inputs with the motor activity necessary to orient to them (Sparks and Nelson, 1987). Before reaching SC, stimulus location is computed with modality-specific features. Visual location is computed with respect to the center of gaze, audition is centered to the head, and somatosensation

¹Referred to as “superior colliculus” for simplicity.

is relative to the position of the body. Therefore, independent movement of the sensory organs would pose the problem of misalignment of the maps. Strikingly, it was shown that to account for this problem, the receptive fields of SC neurons make compensatory shifts to maintain the registration in retinotopic coordinates (Hartline et al., 1995). This general plan is well conserved among vertebrates and is valid for the superior colliculus of mammals as well as the optic tectum of birds (Knudsen, 1982), reptiles, sometimes involving infrared perception (Hartline et al., 1978), and fish, sometimes involving electrosensation (Kardamakis et al., 2016).

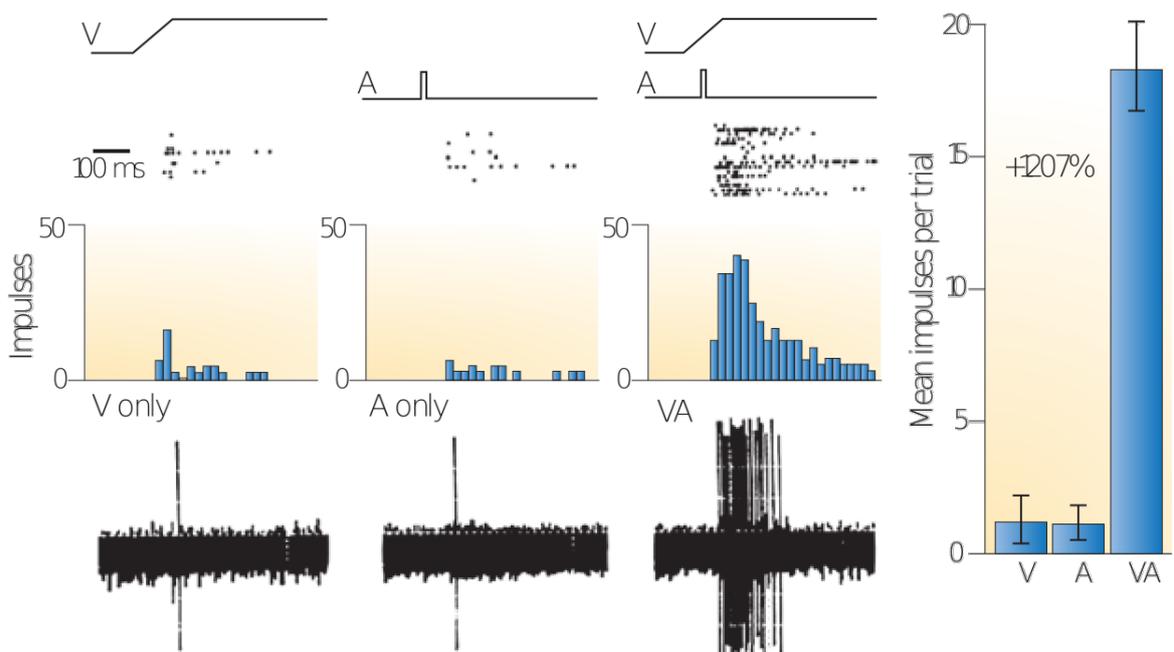


Figure 1.1 – Example of strong multisensory enhancement in a single superior colliculus neuron. From Stein and Stanford (2008).

Stein, Meredith and their colleges have extensively studied the principles that rule the integration of multisensory signals in SC, which inspired later investigations in other areas such as the cortex. A priori, a neuron could be multisensory without displaying proper integration. For instance, it could act as a simple relay from different sensory inputs that are treated as independent, even when related to the same event, and could respond similarly to both multisensory and unisensory stimuli (Stein et al., 2009). Therefore, from

the perspective of the single neuron, multisensory integration is defined as a significant change in firing rate to multisensory stimuli compared to the response evoked by the most effective modality alone. An alternative criterion could be to require the multisensory response to be greater than the sum of the unisensory ones. But this definition would deny that summation can be the result of a proper integration and is often considered too restrictive to be retained as the signature of multisensory enhancement (Stein et al., 2009).

One of the two principles that was firmly established has to do with spatio-temporal coincidence. The *spatial-temporal rule* states that multisensory enhancement is conditioned to a sufficient amount of overlap between cross-modal stimuli in space and time. The spatial aspect follows from the spatial registration of the sensory maps that we described above. As illustrated in Figure 1.1, neurons of SC fire significantly more when presented with a cross-modal combination of stimuli compared to the strongest modality alone (Meredith and Stein, 1983, 1986). When the spatial coincidence is broken however, this enhancement does not occur and neurons tend to be suppressed with the same combination of stimuli. Strong responses to salient stimuli alone can even be totally abolished by a mismatch with a second modality (Stein et al., 1988). Besides being colocalized, the cross-modal inputs must also be synchronous in time. This aspect is intuitive as it is the main information used to determine which of several disparate signals come from the same physical event. However, the window of integration is relatively large and allows for latencies of about ± 200 ms (Meredith et al., 1987; Royal et al., 2009). These latencies likely allow SC to accommodate for various delays in the transduction and processing between modalities.

The second principal of integration in SC relates to its tendency to exhibit non-linear responses. When cross-modal stimuli coincide in space and time, the resulting multimodal response tends to be significantly stronger than the sum of its unimodal parts, sometimes referred to as *superadditivity*. Stein and collaborators found that this type of enhancement increases as the responses to the unisensory signals decreases (Mered-

ith and Stein, 1986; Stanford et al., 2005). The functional interpretation of this principle, known as *inverse effectiveness*, is that the relative gain of multisensory integration is maximized in situations of degraded perception. Indeed, very unambiguous unisensory signals can be easily detected and attended to, so it makes sense that their combination only results in a modest enhancement of neural activity. In contrast, weak signals, in particular those at the limit of the detection threshold, elicit little firing alone and evoke a substantial increase in activity when combined. Interestingly, these physiological measurements are in line with the perceptual improvements in detection and discrimination tasks observed in multisensory psychophysics, that is particularly strong when subjects are brought to the limit of detection (Rowland et al., 2007).

In conclusion, SC generates orienting behavior thanks to a multisensory topography. Localization of external events is improved by multisensory enhancement in case of spatio-temporal coincidence, while response depression occurs in situations of incongruence. Inverse effectiveness further sharpens this process when single cues are ambiguous or close to the limit of detection. This physiological characterization offered an inviting neural basis to multisensory effects at the behavioral level, and became the guiding principles for further investigations. Yet, despite being so influential, it remains to be seen whether the rules of integration that apply to the very specific circuit and function of SC are valid for broader computations in the cortex. We have seen examples of intersensory conflicts, such as the ventriloquist effects, where cross-modal binding occurs even though the spatio-temporal coincidence is largely broken. Further, perceptual effects relying on the integration of non-redundant information, such as the streaming/bouncing effect or the McGurk-MacDonald effect, crucially depend on semantic congruence which is not rooted in spatio-temporal relationships and are likely implemented in cortical brain areas with their own mechanisms of integration – which we will review later in this section.

1.2.2 Association areas in the cortex

Different methodologies such as single-cell electrophysiology, neuroanatomical tracing and functional neuroimaging contributed to establish the existence of multiple zones of sensory convergence in the cortex (Kaas and Collins, 2004). We will start by considering studies in the ectosylvian sulcus of the cat (AES) and the posterior parietal cortex (PPC) in primates where strong representations of space exist and the principles of multisensory integration found in SC still tend to apply. We will then turn to the results from the superior temporal sulcus (STS) and the prefrontal cortex where more complex forms of multisensory integration depart from those found in SC in the sense that enhancement requires semantic congruence and is not crucially dependent on spatio-temporal coincidence.

The anterior ectosylvian sulcus in cats

As a continuation of their investigations in SC, extensive work has been led by Stein and his collaborators in the anterior ectosylvian sulcus of the cat (AES). SC receives broad connections from the associative cortex with strong inputs originating from the areas surrounding the sylvian sulcus, in particular from AES. The link between this structure and SC started with the observation that the influence of these inputs is critical to the integrative properties of SC. When reversibly deactivating AES by cryogenic blockade, most multisensory neurons are not affected in their amplitude of response to unimodal signals, but responses to cross-modal combinations of stimuli fail to go beyond the most effective modality, thus eliminating multisensory enhancement (Jiang et al., 2001; Wallace and Stein, 1994).

AES is subdivided into three modality specific subregions, a visual (Mucke et al., 1982), an auditory (Clarey and Irvine, 1986) and a somatosensory (Clemo and Stein, 1982) subregion, which all project heavily to SC. Spread over these subregions, with more density

on their borders, many multisensory neurons have been found. Moreover, the receptive fields of these multisensory cells are in spatial correspondence and show multisensory enhancement that depends on spatial coincidence and timing. Response depression associated with spatial mismatch was also found, as well as the characteristic inverse relation between the magnitude of multisensory improvement and the efficiency of unimodal signals (Wallace et al., 1992, 1993).

This role in multisensory integration was latter tested at the behavioral level in the context of an orientation task and found to match well with the single cell physiology. Wilkinson et al. (1996) trained cats to orient toward and approach a visual stimulus close to their detection threshold while ignoring a concomitant low-intensity auditory stimulus. Silencing of the AES, with local injection of lidocaine, did not influence the ability of the cat to orient to the visual stimulus when presented alone, but had a clear impact on the response to multimodal stimuli. The distinctive enhancement in performance in coincident bimodal conditions was significantly reduced and disparate stimuli lost their disorienting effects. These observations were apparent when AES was silenced, but could not be observed with the silencing of V1, A1, or the lateral part of the ectosylvian sulcus.

Quite similar behavioral effects were obtained by lesioning SC (Burnett et al., 2004). With the fact that silencing of AES entails a loss of integration in SC, these observations suggested that SC actually inherits its multisensory properties from that of AES. Wallace et al. (1992) addressed that question and reported, using orthodromic and antidromic stimulations, that the source of inputs from AES was constituted predominantly of unisensory neurons. The study concluded that the integrative properties of multisensory SC neurons would then not be inherited from AES, but rather reflect the integration of modality specific inputs originating from AES.

The function of the multisensory processing in AES then remains to be clarified. Despite parallels with SC, projections from AES to premotor areas involved in orienting behaviors have not been reported. It is likely that this associative structure is involved in

richer behaviors than orientation. Indeed, AES receptive fields are considerably larger than in SC. Moreover, except for a rough somatosensory map, the auditory and visual subregions do not show signs of topographic organization. Even though the spatial coincidence of combinations of stimuli tend to increase the activation of multisensory neurons, they are less sensitive to spatial disparities than SC and non-linear enhancement and depression as well as inverse effectiveness seem to be the exception rather than the rule (Stein and Wallace, 1996). It would be interesting to study the integrative properties of AES in different contexts, such as object recognition, where responsiveness to the semantic congruence of more complex stimuli could be assessed, and which might have been overlooked by the focus on spatial alignment in order to parallel the studies in SC.

Peripersonal space in the posterior parietal cortex

Rather than AES, where a homologous area has not been identified, related studies in humans and monkeys have focused on the *posterior parietal cortex* (PPC). The characterization of the functions of this area started from observations in patients recovering from strokes or head injuries. The first clinical studies were done by Austro-Hungarian physician Rezső Bálint, who gave his name to Bálint's syndrome. He observed that patients with bilateral strokes in PPC suffered from three main deficits: they had difficulties to perceive more than one object at a time in their field of view, were no longer able to make saccades towards defined targets, and were unable to make visually guiding reaching movements (Bálint, 1909; Whitlock, 2017). Other patients with PPC injuries were robustly found to lack awareness of their body parts or unable to localize them in space. Rare cases showed a full lack of awareness about their paralyzed limbs or a total neglect of their visual hemifield (Critchley, 1953). These observations contributed to establish that PPC is involved in the representation of peripersonal space, the coordination of actions between modalities within this space, as well as our sense of body awareness and self-localization which likely relies on integration across the senses.

This picture was then strengthened by further work on macaque that went on to detail the physiology of PPC in support of these functions. Most neurons in the ventral sub-region of PPC (VIP) were found to be multisensory with strongly selective responses to the direction of visual, auditory, somatosensory and vestibular stimuli (Bremmer et al., 2002; Duhamel et al., 1998; Schlack et al., 2005). Interestingly, studies designed to investigate a common reference frame among these modalities found that the receptive fields of visuo-tactile, audio-visual and visuo-vestibular neurons were in spatial agreement, with most tactile receptive fields located on the head. The visuo-tactile cells were moreover selective to the same direction, and, unlike most visual areas, their reference frame was centered on the head with visual receptive fields at least partially shifting with eye movements (Avillac et al., 2005; Duhamel et al., 1998).

Besides VIP, considerable work was also performed in the lateral and medial sub-regions of intraparietal cortex (LIP and MIP). LIP is specialized for saccadic eye movement, while MIP, which is part of the functionally defined parietal reach region (PRR), is dedicated to reaching. Recordings from these areas while monkeys performed delayed-saccade and delayed-reach tasks showed that they code for the planning of movements rather than for motor commands or spatial attention (Snyder et al., 1997). This is done by coding the location of the target across the visual and auditory modalities with eye-centered reference frames. Like in VIP, the shifts in receptive fields is only partial, with a subpopulation of auditory neurons staying in alignment with gaze direction, while the remaining neurons keep their head-centered coordinates or an intermediate version between eye-centered and head-centered (Cohen and Andersen, 2000; Stricanne et al., 1996).

These findings suggest parallels with multisensory processing in SC, where the maintenance of an eye-centered coordinate frame is robust also in awake monkeys (Jay and Sparks, 1984). To date, it seems that a single study addressed the question from the perspective of multisensory integration as in SC (Avillac et al., 2007). They did find cross-modal enhancement for spatially coincident visuo-tactile stimuli, but with the particular-

ity that, in coincident situations, depression of the most effective modality was as likely as enhancement. Superadditivity and inverse effectiveness were however not reported. This suggests that the functions of PPC are more diverse than SC and are not limited to the maintenance of a common reference frame. This interpretation is in agreement with the only partial re-mapping and the coexistence of several coexisting reference frames (Avillac et al., 2005).

Congruence in the superior temporal sulcus

The superior temporal sulcus (STS) is another key area for multisensory integration. It was first ascribed a linguistic function and is considered to be central to the processing of various aspects of social cognition including the processing of speech, visual and haptic aspects of communication, faces, conspecifics' actions and attribution of intentionality, with spatial processing clusters that are not clearly established (Deen et al., 2015).

Social cognition being essentially multisensory, it does not come as a surprise that the first electrophysiological studies on macaque STS found that most neurons respond to combinations of visual, auditory and somatosensory stimuli (Bruce et al., 1981; Desimone and Gross, 1979). However, these first studies used simple stimuli such as flashes and tones, and did not investigate the integration of concomitant stimuli. Although, multisensory enhancement can be found with simple stimuli (Calvert, 2001), subsequent work, including neuroimaging studies in humans, made use of complex meaningful stimuli which induce much greater responses (Beauchamp et al., 2002). Using functional magnetic resonance imaging (fMRI) in humans, one study presented visually depicted letters, spoken syllables or their combinations to passively viewing and listening participants (van Atteveldt et al., 2004). Regions of STS showed multisensory enhancement in bimodal conditions and specific regions only responsive to audiovisual combinations. Importantly, whether enhancement or depression was obtained depended on the congruence or incongruence, respectively, of the presented pairs. These conclusions were confirmed by

another study relying on the same methodology who presented images of graspable objects and animals associated with congruent or incongruent sounds, such as the image and sound of a hammer or the meowing of a cat (Beauchamp et al., 2004). Similarly, extracellular recordings in macaque during the presentation vocalizations together with human facial expressions showed that multisensory enhancement and depression occurs in STS as a function of the semantic congruency (Barracough* et al., 2005). Similar studies found superadditivity in the context of auditory-tactile and audiovisual integration (Calvert et al., 2000; Foxe et al., 2002), and some using weak stimuli even reported inverse effectiveness effects (Stevenson et al., 2007).

Yet, reports of superadditivity and inverse effectiveness are rare with enhancement that is reliable but not strong enough to go beyond the sum unimodal responses. This could be due to the fact that this literature rely mostly on fMRI which contrasts with single cells study because blood-oxygenation level-dependent signal is indicative of the activity of millions of neurons per voxel. Although the limits of temporal integration necessarily require a dependency on the temporal coincidence of cross-modal stimuli, multisensory enhancement and depression seem resilient to temporal and spatial asynchronies in STS (Rajj et al., 2000), but this question has not been studied extensively.

Departing further from SC, multisensory processing in STS seems unique in its dependency on congruence that has been shown for various types of stimuli. As one can orient to only a single direction at a time, enhancement and depression make sense in SC to reinforce consistent locations and suppress disparate stimuli. Regarding STS however, the contributions of enhancement and depression to the computations of a highly associative cortical area still remain to be clarified. Although some concerns about attention cast doubt on the relationship between congruence and STS activity by finding stronger responses to incongruent pairs of stimuli (Hocking and Price, 2008), the studies that we mentioned all support the idea that semantic congruence between multimodal signals dictates the level of multisensory integration, possibly serving a key role in improving or

degrading the intelligibility of social situations processed in STS.

1.2.3 Sensory areas

The multisensory physiology that we reviewed in the superior colliculus and the associative areas of the cortex was consistent with the traditional picture of sensory processing. According to the classic model inherited from the seminal work on the mammalian visual system, raw inputs go through a number of subcortical relays before reaching sensory specific areas in the cortex. These sensory areas are thought to apply a succession of filters that consecutively ramify the inputs and lead to the extraction of increasingly complex sensory features. The integration of sensory information is then deferred to the latest stages of the hierarchy where amodal object representations are built.

Contrasting with this hierarchical picture, increasing attention is given to sensory convergence and integration that occur at the early stages of processing, even down to the primary areas (Ghazanfar and Schroeder, 2006). The first suggestions about the existence of multisensory neurons in areas regarded as unisensory actually date back to the early days of sensory systems physiology, but the field was probably not ripe enough for these questions to be tackled yet (Lomo and Mollica, 1959; Murata et al., 1965).

Subsequent evidence came from diverse functional imaging studies where the modulation of sensory areas could be observed in response to bimodal stimulations and sometimes by secondary stimuli alone (Kayser and Logothetis, 2007). For instance, the primary auditory cortex can be activated by silent lipreading (Calvert et al., 1997; Pekkola et al., 2005), by simple visual stimuli (Lehmann et al., 2006) as well as cutaneous stimulations (Fu et al., 2003; Kayser et al., 2005) when synchronized with a sound. Some of these fMRI studies have impressive spatial resolutions which allow to make confident claims about the loci of integration, in particular when the primary regions are involved (Kayser et al., 2005, 2007). In complement, some studies based on electroencephalography report mod-

ulation occurring in a few tens of milliseconds as early as the onset response of unimodal stimulations [Molholm et al. \(2002\)](#); [Murray et al. \(2005\)](#).

Among these rapidly accumulating evidence, a strong case for low-level interactions comes from anatomical studies. In monkeys and cats, a host of tracing studies have identified descending projections from association to sensory areas that are neglected by feed-forward models ([Markov et al., 2013](#)). Although these top-down connections might convey contextual information about behavior and cognitive variables such as reward, prediction, attention or motor activity ([Busse et al., 2005](#); [Stringer et al., 2019](#)), they might also be implicated in a form of sensory processing that supports multisensory integration at an early stage. Besides top-down influences, horizontal projections directly connecting sensory cortices also exist. In monkeys, strong projections from the core and belt regions of the auditory cortex have been found to target the peripheral visual field regions which are as dense as those descending from the visual motion areas MT/V5 ([Falchier et al., 2002](#)). Conversely, the primary and higher visual areas project to the auditory cortex in ferrets and gerbils ([Bizley et al., 2007](#); [Budinger et al., 2006](#)), and there is also bidirectional projections from the visual and auditory cortices to the somatosensory cortex of macaques ([Cappe and Barone, 2005](#); [Smiley et al., 2007](#)).

In mice, direct connections between the primary auditory and visual cortex also exist and their functional role is starting to be investigated at the cellular level. Projections from A1 to V1 are causally involved in boosting visual responses concomitant with sound presentations and sharpening orientation tuning curves in a context-dependent manner that could act as a low-level mechanism of sensory binding. ([Deneux et al., 2019](#); [Ibrahim et al., 2016](#); [Iurilli et al., 2012](#)). Spiking responses in primary auditory cortex to visual stimuli also exist in ferrets ([Bizley et al., 2007](#)), in gerbils ([Kobayasi et al., 2013](#)) and mice ([Banks et al., 2011](#)). In the ferret, [Atilgan et al. \(2018\)](#) presented a mixture of two auditory streams together with a visual stream whose luminance changes followed the amplitude of one of the two auditory streams. When the visual stream was present, neurons in the primary

auditory cortex were biased toward representing the associated sound. This is reminiscent of the streaming/bouncing effect and provides interesting evidence for audiovisual binding and disambiguation at the level the primary auditory cortex. In mice, robust projections exist from visual areas to A1 (Banks et al., 2011). A1 neurons residing in the deep layers, mostly layer 6, were found to be modulated by the presentation of synchronous flashes or drifting gratings (Morrill and Hasenstaub, 2018).

Although we have only considered the superior colliculus and the cortex as the sites of multisensory integration, further contributions from subcortical structures, in particular the thalamus, should also be considered. While communication between cortical areas are often assumed to be mediated by corticocortical connections, the role in cortical processing of cortico-thalamo-cortical routes may be underappreciated (Sherman, 2007; Tyll et al., 2011) and could be suitable for multisensory integration via the convergence of different sensory areas onto common thalamic nuclei. Along these lines, Cappe et al. (2009) carried a tracing study in macaque that revealed the existence of pathways bridging the auditory, somatosensory and premotor cortices involving several thalamic regions of which the pulvinar, usually considered as a high-level visual nucleus, showed the most prominent overlapping of retrogradely labeled neurons. Long before these tracing, it was already known in rats, cats and macaques that the medial and dorsal regions of the medial geniculate body, the major auditory relay, was responsive to visual, somatosensory and vestibular inputs (Calford and Aitkin, 1983; Wepsic, 1966). More recently, this influence on auditory thalamus in rats was confirmed by a study reporting gain modulation in about ~15% auditory neurons induced by visual cues (Komura et al., 2005). Similarly, the presentation of flashes concomitant with whisker stimulations enhances activity in the ventral posteromedial nucleus of rats (Bieler et al., 2018). These evidence hints at the potential multisensory nature of the thalamic structures. Taking them into account implies to consider the possibility that the multisensory effects reported at the cortical level could actually be inherited from integration in subcortical inputs. A recent study indeed

showed that a whisker-induced suppression of the auditory cortex was mediated by the suppression of the auditory thalamus via descending projections from the barrel cortex to the inferior colliculus (Lohse et al., 2021).

Taken together, the multisensory influences exerted on sensory specific areas provide strong evidence in favor of the sensory nature of multisensory effects. In particular, the direct connections that have been described between different sensory systems demonstrate that early cross-talk occur even at the level of the primary areas. However, the presence of these projections does not prove by itself that they induce the observed multisensory responses. Descending connections from association areas could mediate these effects despite direct projections. While the timing of some effects are consistent with top-down influences, not every effect can be explained by feedbacks. First, some fMRI studies on anesthetized animals show multisensory enhancement in the sensory areas without significant activation of the associative regions such as PPC, STS or prefrontal, excluding the possibility of top-down influences (Kayser et al., 2005). Second, although most of this recent work did not manipulate timing, spatial location and semantic congruence, some studies were conceived in the spirit of the work on SC and showed that bimodal enhancement decreases when the presented stimuli are desynchronized in time (Kayser et al., 2005; McDonald et al., 2005). This dependency on the timing of presentation, according to one of the key principles of multisensory integration, also rule out non-specific attentional effects mediated by top-down projections as well as low-dimensional motor activity that would trigger cortical activity by itself (Stringer et al., 2019). Finally, direct projections from A1 to V1 have been causally implicated in auditory-driven responses by specific chemogenetic silencing (Deneux et al., 2019). Future studies in mice will benefit from the availability of these circuit dissection tools not only to address confounding factors, but also to test specific hypotheses about the functional roles and behavioral advantages of low-level multisensory interactions which, to this date, remains a matter of speculation.

1.3 The whisker system under the influence of olfaction

1.3.1 Organization of the whisker system

Rodents are nocturnal and subterranean animals that evolved in dark tunnels. To alleviate for the inaptness of vision to dark burrows, rodents have an elaborate and highly sensitive tactile apparatus at their disposal, known as whiskers or mystacial vibrissae. They constitute the youngest sensory system acquired and shared by nearly all mammals, except humans, and are thought to share a common phylogenetic origin (Brecht et al., 1997). Besides being crucial in rodents' natural habitat, whiskers are also beneficial in a variety of other contexts. Notably, marine mammals such as the harbor seal strongly rely on their impressive set of whiskers – an example is displayed in Figure 1.2 along with whiskers from other species – to track the turbulences produced by their preys, which they can easily catch blindfolded and with ears plugged (Dehnhardt et al., 2001). Whiskers also happens to be useful in the air. Bats use specialized hairs implanted in the membrane of their wings to sense airflow, and they lose their ability to perform aerial maneuvers when depilated (Sterbing-D'Angelo et al., 2011).

The relevance of whiskers to rodents lives was recognized more than a century ago by a study of Vincent (1912) which showed that clipping whiskers of rats disoriented their navigation in mazes and made them prone to errors. Some rodents, together with a few other species like shrews, possess a major difference with other mammals in that they actively move their whiskers, a rhythmic process termed “whisking”, which they carry out systematically during navigation and search behaviors. Since the classic study of Vincent, rats and mice have been put in challenging situations which demonstrated their ability to execute complex object localizations, shape and texture discriminations while performing this active displacement, at a level of acuity that has been compared to the finger tips of primates (Carvell and Simons, 1990).

On top of being one of their dominant sensory channels, together with olfaction, by



Figure 1.2 – Examples of whiskers across mammals. From left to right: a mouse, a cat, a shrew, and a harbor seal.

which rodents collect information about their surroundings, the organization of the whisker system bears a valuable advantage for the physiologist. The processing pathways forwarding information from single whiskers to the somatosensory cortex are spatially segregated in remarkable anatomical maps layed out almost identically as on the snout (see Figure 1.3), where each whisker is thought to be represented by a discrete module localized in layer 4 of the cortical column, termed *barrel* by Woolsey and Van der Loos (1970) in analogy to a detail from an etching after Bruegel's *Fair of St George's Day*.

Each whisker is anchored to the snout by a follicle which is innervated by the terminal branches of about 200 receptor neurons in the trigeminal ganglion of rats. The endings of these cells transduce mechanical energy into action potentials via the opening of mechanosensitive ion channels (Dörfl, 1985). The principal neurons, located in the trigeminal ganglion, innervate a unique whisker and transmit their outputs, particularly selective of position, direction, speed and amplitude of displacement depending on the structure of their nerve endings (Stüttgen et al., 2006), to the trigeminal nuclei of the brainstem. Trigeminothalamic neurons are organized into *barrelettes* which respond preferentially to a single corresponding whisker. The principal trigeminal neurons then project to the ventral posterior medial nucleus (VPM) of the thalamus which is also somatotopically organized, into *barreloids*. Finally, VPM neurons reach layer 4 of the primary somatosensory cortex (wS1) to form what is known as the barrel map. This succession of processing stages constitutes the so-called *lemniscal pathway* which is conceived as the main route conveying information about touch and whisker positions to the cortex.

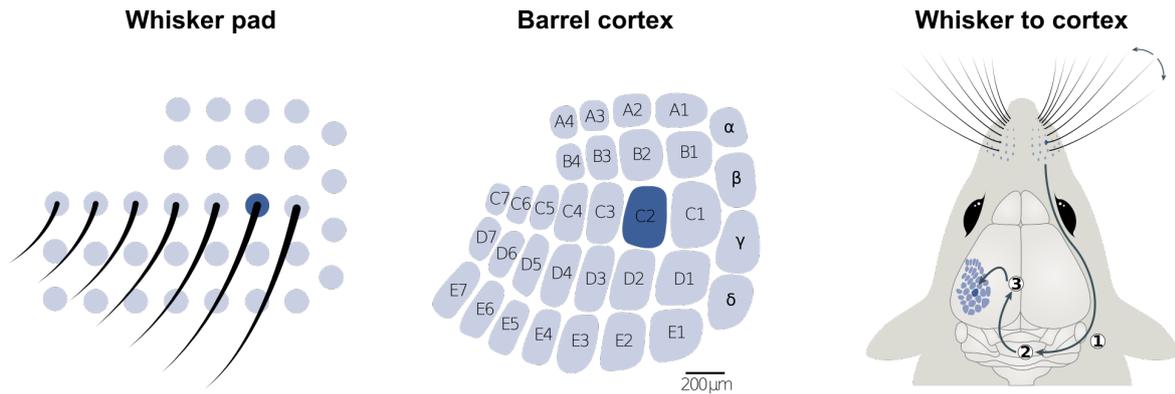


Figure 1.3 – Organization of the whisker pathways from whiskers to barrels. From [Petersen \(2019\)](#).

Such elegant labeling from the snout to the cortex is instrumental to uncover the computations carried out at each processing stages. Yet, there are marked differences between the barrel cortex and the periphery. While the trigeminal ganglion cells respond unambiguously to a single whisker with very little spontaneous activity ([Jones et al., 2004](#)), responses obtained from the cortex, even though dominated, especially in layer 4, by stimuli applied to the principal whisker, show much more variability, with receptive fields that are larger and selective for complex multiwhiskers stimuli ([Simons, 1978](#)). Further, the lemniscal pathway is not the only entryway to the cortex. At least two other equally important parallel pathways exist, the *extralemniscal pathway* and the *paralemniscal pathway*, which have been described in great details in the work of Deschênes on rats ([Deschenes and Urbain, 2009](#)). As a result, multiwhiskers responses can already be found at the level of the trigeminal nucleus ([Veinante and Deschênes, 1999](#)), where whisker representations are part of complex sensorimotor circuits under the influence of descending cortical projections ([Furuta et al., 2010](#)). These considerations imply that the barrel cortex does not simply represent the movements of the whiskers in a discrete manner within well-defined landmarks, but rather suggests that it associates ongoing sensory activity with past history in a highly contextual way.

1.3.2 Interactions between whisker touch and olfaction

The recent multisensory work in primary sensory areas in mice mostly emphasized interactions between vision and audition. However, as we already mentioned, rodents evolved in narrow and dark burrows, where these modalities, and especially vision, are less relevant. In contrast, whisker touch and olfaction are more prominent during navigation and object recognition in their natural environment, and are also both major means of social interactions with conspecifics (Wolfe et al., 2011). It is known that rodents do not readily use complex visual features (Minini and Jeffery, 2006), and reports of successful visual recognition invariant to substantial variability in view point, illumination or size, require extensive training (Zoccolan et al., 2009). This differs strikingly with the smooth acquisition of olfactory tasks that can reach significant performance after a single training session (Abraham et al., 2012). It was even shown, in the context of the visual cliff, that rats simply disregard visual information in conflict with whisker touch (Schiffman et al., 1970). It is then plausible that flexible olfacto-tactile associations play an important role in a rodent's life.

The creation of these associations could rely on the tight coupling between olfaction and whisker touch at the motor level. Indeed, these rhythmic orofacial behaviors share common muscles and constraints, such as the nasolabial muscle that contribute both to the extension of the nostrils during sniffing and the retraction of the whiskers during whisking (Berg and Kleinfeld, 2003). In his description of sniffing in rats, Welker already noticed that this behavior shared the same temporal relationship than whisking (Welker, 1964). Subsequent studies revisited his seminal work and confirmed the precise phase-locking of these movements (Deschênes et al., 2012; Ranade et al., 2013). Given that decorticated as well as sensory denervated rats still readily engage in their characteristic sniffing and whisking search behaviors when placed in novel environments, it was suggested that whisking could be driven by a central pattern generator in the brainstem

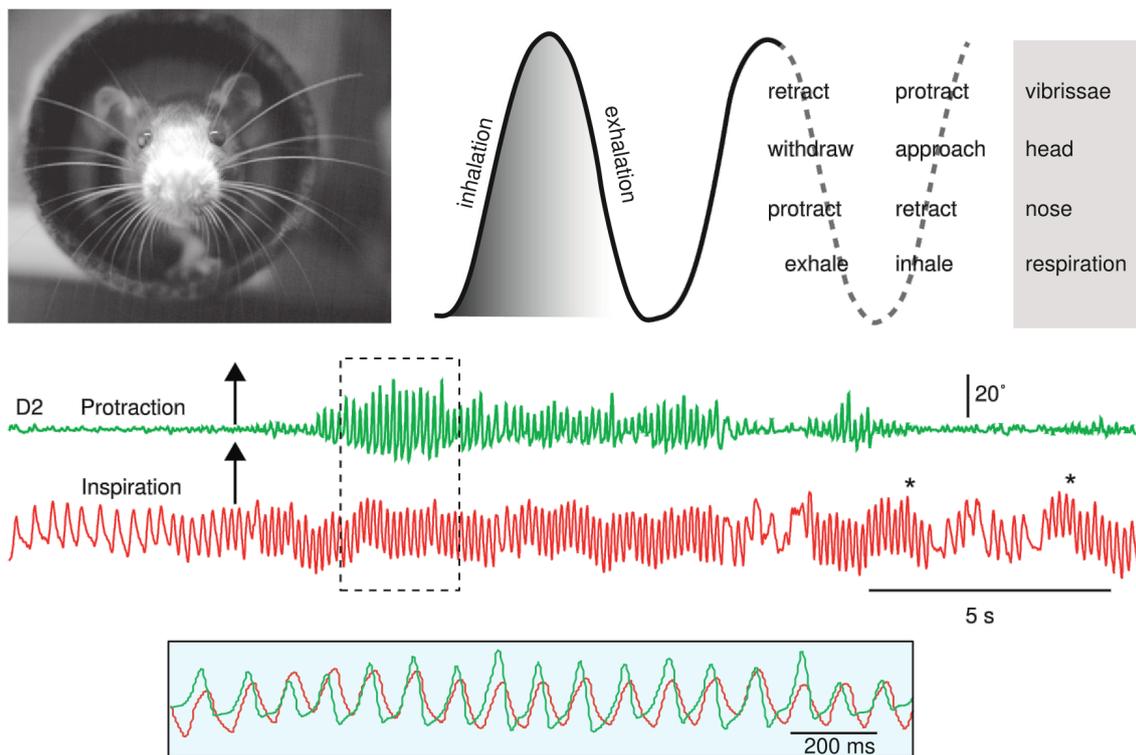


Figure 1.4 – Coordination of sniffing and whisking in rodents. From [Deschênes et al. \(2012\)](#).

and share motor plans with the pre-Bötzinger complex, the well described rhythmic generator that initiates every breath. A study led by Deschênes found that it is indeed the case ([Moore et al., 2013](#)). They found premotor neurons in a region of the medulla that oscillate in phase with motions of the whiskers and are under the direct monosynaptic control of the pre-Bötzinger complex which resets their phase, thereby elucidating both the question of the central patterned generator for whisking and its synchrony with sniffing.

On the basis of their findings, the authors of the previous study speculated that the tight coupling between whisking and sniffing orchestrated by the breathing centers could serve a perceptual role in shaping and binding the olfactory and tactile inputs in the cortex ([Kleinfeld et al., 2014](#)). However, contrasting with the work done at the periphery, little attention has been given to uncover a possible interaction at the cortical level. In human, tactile perception of fabric can be biased toward a softer or rougher texture depending on the type of associated odors ([Demattè et al., 2006](#)). This kind of perceptual

influences, abundant in the psychophysical literature, could be explained by the existence of associations learned by repeated exposures to specific combinations in the passed experience, which leads to biased perception. If such biases can be obtained in humans, it is reasonable to think that olfaction and touch would also interact in rodents.

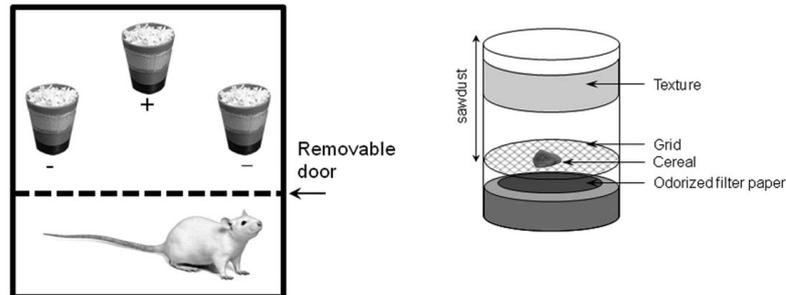


Figure 1.5 – Experimental setup of the foraging olfacto-tactile discrimination task of [Boisselier et al. \(2014\)](#).

At the behavioral level, a first olfacto-tactile task was introduced by [Whishaw and Tomie \(1991\)](#). It required rats positioned on an elevated platform to pull up ropes presenting different diameters and smells in order to obtain a food reward attached at their ends. The authors showed that rats could learn the task, but the tactile exploration relied on the paws rather than the whiskers to evaluate rope diameters, which likely explains the extensive training of about 50 days required to reach expert level. A foraging task was designed in subsequent work investigating the role of attention in cross-modal binding where rats were presented with bowls covert with textures and scented with odorants. Two studies showed that rats could readily use both cues to find a baited combination among other pairs made with the same unisensory stimuli. Moreover, systemic muscarinic blockade and lesions of the basal forebrain showed that the cholinergic system was necessary for the acquisition of the task, but not for a control unimodal version ([Botly and De Rosa, 2007, 2009](#)). These last studies were then improved by [Boisselier et al. \(2014\)](#) who designed a similar foraging task to investigate the contribution of specific areas, in particular the lateral entorhinal cortex (LEC), in the acquisition, retention and recombination of olfacto-tactile associations. In their protocol, rats were presented with textured

and scented baited buckets where the baits were accessible in only one of them (Figure 1.5). A first task consisted in learning a first rewarded association and showed that rats can achieve the criteria for expert level in just three sessions of 20 trials. Then rats had to learn a second task with new sensory features and a third one involving reversal learning where the stimuli used in the two previous tasks constituted a new reward combination. Applying bilateral injection of the NMDA antagonist d-APV, they could show that LEC is involved in the acquisition of the second and third tasks, but not in the retention of the first one. As learning in an unimodal version of the task was not affected by NMDA blockade, this study shows that LEC plays a specific role in the creation of multimodal associations.

Taken together, these studies show that rodents, at least rats, can easily make use of the combination of olfactory and tactile information. This indicates that olfacto-tactile integration in rodents might be a particularly good model for the study of multisensory integration. As far as can be judged, beside the contributions mentioned above, the literature has not addressed further the possible anatomical substrates that mediate the emergence of olfacto-tactile associations. As noticed by Boisselier et al. (2014), LEC receives its inputs from S1 via the perirhinal cortex. This area could be an interesting alternative candidate for early olfacto-tactile interactions as it sends and receives reciprocal connections with both the piriform and the barrel cortex (this hypothesis is detailed and tested in Chapter 3). Besides the question of the regions functionally involved, the physiology of these associations remains unexplored. This thesis is a contribution to bridge that gap.

1.4 References

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Chapter 2

Olfactory modulation of barrel cortex activity during active whisking and passive whisker stimulation

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ACTIVE WHISKING AND PASSIVE WHISKER STIMULATION

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2.1 Introduction

Perception occurs through the coordinated evaluation of information from multiple senses (Stein and Stanford, 2008). This coordination is evident when considering the well documented cross-modal illusions in humans (Alais and Burr, 2004; Botvinick and Cohen, 1998; McGurk and Macdonald, 1976), such as the ventriloquist illusion, in which erroneous sound localization is generated by visual cues. Illusions reveal strong associations across particular cues from different sensory modalities that can be maintained even if the resulting perception breaks with the physical reality. Such associations are generally useful to improve sensory judgments when information from each sense is scarce or ambiguous. In line with this, multisensory interactions are strongest when unisensory ambiguity is highest. (Alais and Burr, 2004; Ernst and Banks, 2002). This inverse-effectiveness phenomenon is interpreted as an optimal integration of available information (Ernst and Banks, 2002). Neurons were identified in the associative cortical areas of monkeys (Fetsch et al., 2012; Gu et al., 2008) and rodents (Raposo et al., 2014, 2012) which sum inputs representing congruent information from distinct modalities. This simple multisensory integration mechanism can explain inverse effectiveness and most perceptual observations, but not all (Nikbakht et al., 2018). A causal link between multisensory integration and associative integration has also been established (Raposo et al., 2014).

While neurons in association cortex do exhibit multimodal coding properties, several studies suggest that cross-modal connections already exist in the primary sensory cortical areas both in primates and rodents (Cappe et al., 2009b). These connections are functional. Most recently, it was shown that direct projections from the primary auditory cortex modulate supragranular primary visual cortex neurons in a context-dependent manner (Deneux et al., 2019; Ibrahim et al., 2016; Iurilli et al., 2012). Even if the perceptual impact of such low level auditory-visual interactions remains a puzzle, there is convergent evidence that they sharpen and emphasize cortical representations of the visual stimuli

that are coincident with startling sounds (Deneux et al., 2019; Ibrahim et al., 2016), without a particular congruence of visual and auditory cues. This suggests the existence of multi-sensory mechanisms that are complementary to the classical cross-modal integration observed at associative levels. However, so far, it is unclear whether such interactions represent a generic computational process in primary sensory cortical areas or a feature specific to audition and vision in rodents.

To investigate this question, we focused on another pair of sensory modalities. While primates and humans rely primarily on vision and audition for survival, rodents depend to a much larger degree on olfaction and whisker touch. This reliance is underscored by an exquisite coupling between sniffing and whisking rhythms (Moore et al., 2013) and by observations that rats can easily solve a task that requires the integration of olfactory and tactile cues (Boisselier et al., 2014). Nevertheless, it is unknown how these two crucial modalities interact in the brain. Activity in barrel cortex is modulated by breathing, and this modulation depends on an intact olfactory pathway (Ito et al., 2014), but there is no evidence that odor-related information arrives to barrel cortex. To examine the impact of odors on touch processing, we performed 2-photon calcium imaging in barrel cortex of awake mice during precisely controlled olfactory and tactile stimulations. We found that both barrel cortex activity and whisking behavior is impacted by odor stimulation. However, neither abolition of whisking by facial nerve sectioning nor pharmacological blockade of local cholinergic signaling could eliminate odor-related activity in barrel cortex. This indicates that it depends on olfactory-related, secondary projections to the somatosensory system which do not include the cholinergic attentional system. In support of this, odor identity could be readily decoded from barrel cortex population activity, but the presence of olfactory information did not impact the quality of tactile representations. Therefore, our study reveals that, in the absence of learned associations, barrel cortex activity contains olfactory information encoded in dimensions of the neural representations that are orthogonal to the tactile representations.

2.2 Results

2.2.1 Calcium imaging of L2/3 barrel cortex neurons during olfacto-tactile stimulation

The first difficulty in investigating potential cross-talk between olfactory and tactile processing is to precisely control coincident olfactory and tactile stimulations. To achieve this, we coupled a motorized tactile-object-presenting wheel with a custom-made olfactometer to synchronously present oriented tactile gratings (0° or 90°) and odors (amyl acetate or ethyl butyrate diluted at 0.1%; Figure 2.1a) to the snout of a mouse. The olfactometer was calibrated with a photoionization detector (PID; Supplementary figure 2.1). Awake mice were head-fixed with their nose confined in a constant and isolated air stream to prevent the air flow from causing movements of the whiskers. In dark conditions with infrared backlighting, oriented tactile gratings were brought within range of the whiskers using a linear stage. Whisker interactions with the gratings were recorded with a high-speed infrared camera and breathing was monitored with a pressure sensor placed perpendicular to the odor stream (Figure 2.1d).

We considered two stimulation contexts (Figure 2.1b): in the first one, the active context, the animal was free to explore a grating that came into reach of its whiskers; in the second one, the passive context, the inferior and superior buccal branches of the facial nerve were sectioned bilaterally, abolishing whisking, and the tactile stimulation consisted of a single back-and-forth sweep of the grating against the whiskers. In both stimulation contexts, the onset of odor presentation to the nose was precisely synchronized with the time when the grating reached its fixed position (active context) or at first possible contact with the whiskers (passive context).

To record large populations of barrel cortex neurons during presentation of odors and tactile gratings, we performed stereotactic injections of AAV1-syn-GCaMP6s at 3-4 loca-

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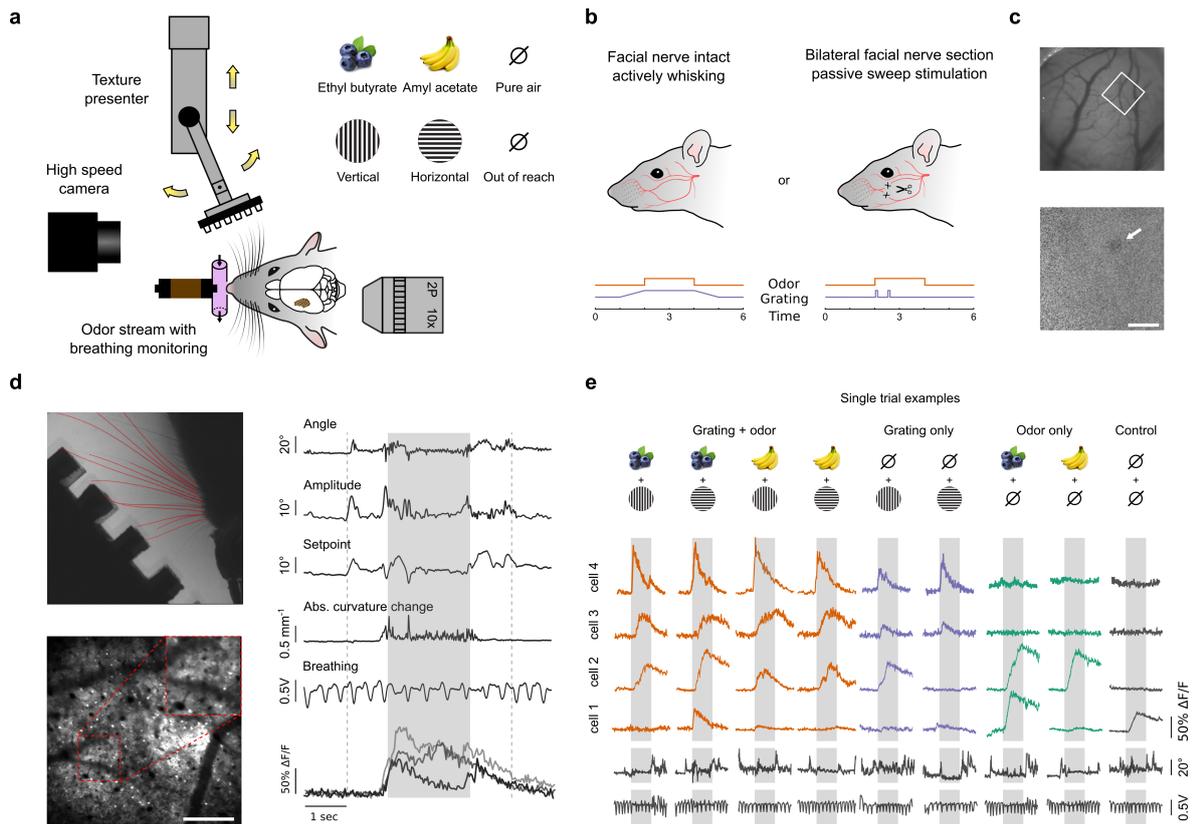


Figure 2.1 – Calcium imaging in L2/3 of mouse barrel cortex during olfacto-tactile stimulation. **a** Schematic of olfacto-tactile stimulation setup. A grating was moved in contact with the whiskers in either the vertical or horizontal orientation, while ethyl butyrate or amyl acetate was presented through a constant air stream. Whisking was recorded with high-speed videography (500Hz) and breathing was monitored with a pressure sensor. **b** Schematics of the two recording conditions. Left: facial nerve intact, mice whisked freely on the grating brought to their whiskers together with stimulation timing. Right: facial nerve sectioned, the grating was swept on the whiskers while whisking was abolished. **c** Example of C2 barrel localization with intrinsic signal imaging used to confirm the location of GCaMP6s expression in barrel cortex (scale bar: 1 mm, white square: two photon field of view, arrow: C2 response). **d** Top left: tracking of average whisker pad position and curvature. Bottom left: image of in vivo GCaMP6s expression in L2/3 (scale bar: 250 μ m). Right: example of simultaneously recorded whisker pad kinematics, breathing, and raw $\Delta F/F_0$ traces. Dashed lines indicate onset and offset of grating approach; gray shading indicates epoch of stimulation during which odors were present in the air stream. **e** Illustration of the nine combinations of tactile and olfactory stimuli with single trial example $\Delta F/F_0$ traces from four neurons showing olfactory responses.

tions in barrel cortex centered around the C2 barrel (AP -1.6, ML -3.3, DV 0.5) (Knutsen et al., 2016). After 4 weeks, location of the GCaMP6s expression locus in barrel cortex was validated using intrinsic imaging (Figure 2.1c), and mice were placed under a 2-photon microscope for calcium imaging of large populations of supragranular neurons (imaging depth from 100 μ m to 250 μ m). A total of 9714 neurons were recorded in the active whisk-

ing context from 20 sessions with 12 mice; and 14408 neurons from 19 sessions with 6 mice in the passive context. Among those populations, 1907 (19.6%) and 5162 (35%) neurons from the active and passive contexts, respectively, were responsive to at least one of the nine stimulation conditions (significance level = 5%; Kruskal-Wallis test) and were kept for analysis. One or two populations at different cortical depths were imaged per mouse in the active context and 1-4 locations in the passive context. Calcium traces from four example neurons illustrate responses to the nine unique stimulus conditions, which include uni- and bimodal stimulus conditions as well as a blank (Figure 2.1e), and show that odors can evoke responses in barrel cortex cells (neurons 1 and 2), or can modulate responses to tactile gratings (neurons 3 and 4).

2.2.2 Odors modulate responses in L2/3 barrel cortex in freely whisking head-fixed mice

We first analyzed how odors impact barrel cortex activity in an active whisking context when they are presented with or without tactile gratings (Figure 2.2a). Diverse response types were present in barrel cortex population (Figure 2.2b). In the presence of gratings, odors could either enhance or suppress tactile responses (Figure 2.2b, first and second examples), and when presented alone odors could evoke excitatory or inhibitory responses (Figure 2.2b, third and fourth examples). In some neurons, odors had the same impact whether a grating was present or not (Figure 2.2b, fourth example).

To quantify these effects at the population level, we compared the average population activity elicited by the gratings presented alone or paired with odors (Figure 2.2c). For simplicity, these analyses were done by pooling trials with different odors together (for odor specific analysis, see Figure 2.6). We found no difference in population activity levels when odors were paired with gratings computed over the entire stimulation epoch. However, when we looked at single cells and compared the average activity to gratings alone

and gratings with odors in the 2 sec time window following stimulus onset, we found that odors modulated the activity of 21.7% of the neurons (Figure 2.2d), with both enhanced and suppressed response types. The fraction of cells whose activity was affected by odors was clearly above a 5% false positive rate. Comparing the proportion of neurons impacted by odors with the proportion obtained from shuffling the trial labels confirmed that the proportion is well above chance level (Figure 2.2e). To account for differences in activity levels between cells regardless of their tactile responsiveness, we computed a modulation index (Figure 2.2f). Consistently, the distribution of indices revealed a significant impact of odors when compared with the distribution obtained from shuffled data (Figure 2.2f) and an equal proportion of cells were enhanced or inhibited by odors (Figure 2.2f, inset).

Similarly, odors presented alone did not impact average responses at the population level compared to double blank stimulation (no odor no grating) (Figure 2.2g) but at the single cell level, odor responses could be detected in 18.34% (Figure 2.2h-j) of the neurons, again with an equal proportion of enhanced and inhibited cells (Figure 2.2j, inset). Note that due to the sound of the tactile presentation wheel, which was present in double blank trials, mice increase whisking at the beginning of double blank trials yielding a weak increase in neuronal activity in barrel cortex (e.g. see Figure 2.3). Together, these results show that in an active tactile context, odors modulate the activity of some barrel cortex neurons both during free whisking and during whisking into tactile gratings.

2.2.3 Odors impact whisker dynamics

The mechanisms by which odors can potentially impact barrel cortex activity are numerous. One possibility is that odors motivate whisker movements which generate tactile inputs to barrel cortex as refferent sensory signals. To explore this possibility, we tracked whisker movements using high-speed videography and an automated whisker tracking algorithm (Clack et al., 2012), which allowed us to robustly detect and trace ~12 individ-

2.2. RESULTS

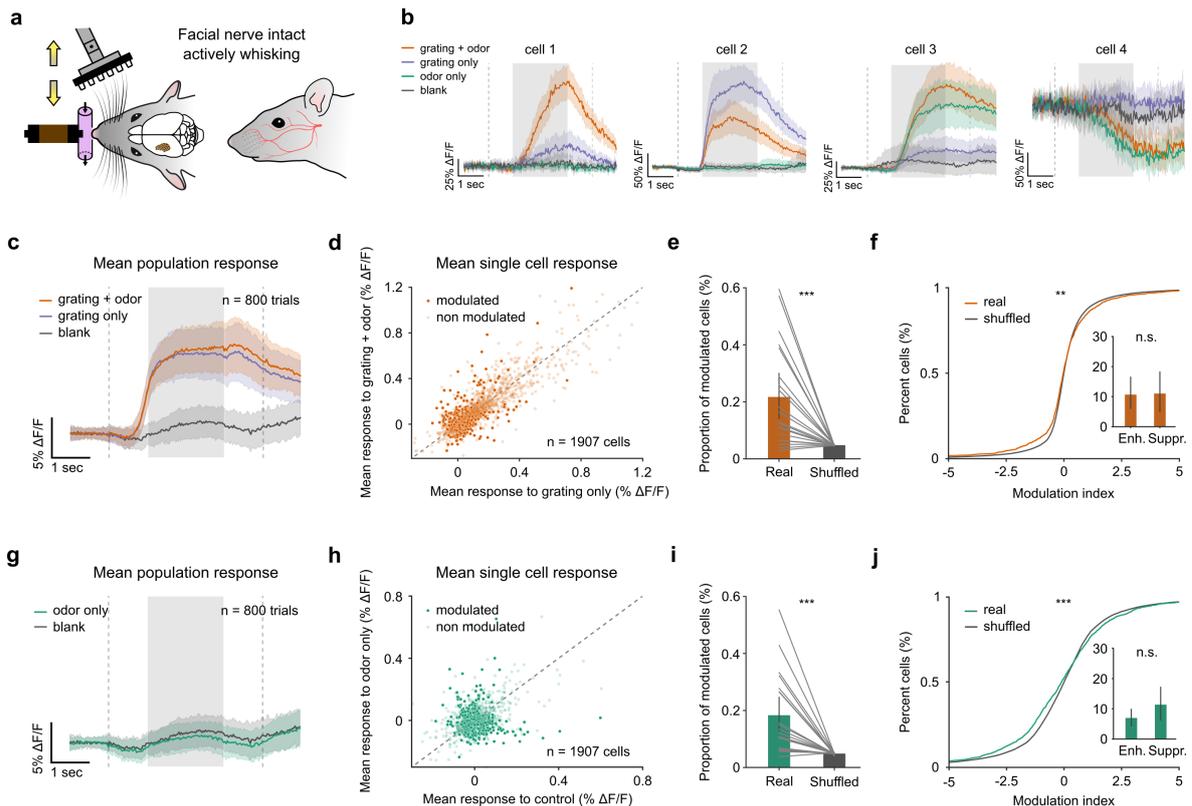


Figure 2.2 – Odor-induced responses of L2/3 barrel cortex cells in active stimulation context. **a** Schematic of the stimulation condition with facial nerve intact. Gratings were brought in contact with the whiskers. **b** Average $\Delta F/F_0$ for four example neurons displaying diverse odor responses. **c-f** Responses to grating+odor stimulation compared with grating only. Stimuli were grouped into four categories: grating+odor (orange), grating only (blue), odor only (green), and control (gray). **c** Population averages to grating+odor trials (both orientations and both odors grouped together; orange) and grating only trials (both grating orientations grouped together; blue); average to blank (gray) is shown for comparison. **d** Scatter plot of mean $\Delta F/F_0$ to grating+odor versus grating only for 1907 neurons. Neurons plotted in dark were significantly modulated ($p < 0.05$, Mann-Whitney U test). Dashed line indicates diagonal. **e** Proportion of modulated neurons across sessions compared with shuffled conditions (21.71% (14.69, 29.58) for real data; 4.84% (4.80, 4.87) for shuffled data; $p = 2 \times 10^{-5}$; Wilcoxon signed-rank test). **f** Cumulative distribution of modulation index for grating+odor versus grating alone ($p = 0.109$; Kolmogorov-Smirnov test). Inset: proportion of enhanced and suppressed cells (mean enhanced = 10.35% (5.60, 15.77); mean suppressed = 11.36% (6.06, 17.60); $p = 0.793$; Wilcoxon signed-rank test). **g-j** Response to olfactory stimulations alone compared with control. **g** Population averages to odor only trials (green) and control (gray) (mean of odors only trials = 0.57% $\Delta F/F_0$ (-0.64, 2.01), mean of blank trials = 1% $\Delta F/F_0$ (0.28, 1.81) computed over stimulation epoch; $p = 0.394$; $n = 800$ trials from 20 sessions; Mann-Whitney U test). **h** Analog to **d**. **i** Analog to **e** (18.34% (12.78, 24.76) for real data; 4.89% (4.84, 4.94) for shuffled data; $p = 1 \times 10^{-5}$; Wilcoxon signed-rank test). **j** Analog to **f** ($p = 2.3 \times 10^{-4}$; Kolmogorov-Smirnov test; inset: mean enhanced = 6.97% (4.48, 9.98); mean suppressed = 11.36% (6.10, 17.52); $p = 0.398$; Wilcoxon signed-rank test).

ual whiskers within the full pad at each time point. From this tracking, we calculated the average amplitude, set point, and absolute curvature change across time (Figure 2.3a).

During trials without tactile gratings, odors caused a slight but significant increase in average amplitude, setpoint, and absolute curvature change both within sessions (Figure 2.3b-d) and between sessions (Figure 2.3b-d). Similarly, in the presence of tactile gratings, odors caused a significant increase in average amplitude and absolute curvature change (Figure 2.3b-d). In this condition, setpoint did not change significantly in the presence of odors despite a tendency towards an increase. This is likely due to the restriction of the full whisking range by the gratings. This analysis shows that odors lead to significant changes in whisking parameters. As there is an intricate link between whisking behavior and sniffing, we also checked if breathing was impacted by odors. The amplitude (Figure 2.3e) and frequency (Figure 2.3f) of breathing, measured in a 2 sec time window starting at odor onset, was significantly reduced in the presence of odors compared to conditions without odors, even though there was large variability across sessions for breathing frequency (Figure 2.3f, bottom). Together, these data show that mice modify their orofacial motor programs when presented with odors. Because whisking impacts barrel cortex activity (Chakrabarti and Schwarz, 2018; Urbain et al., 2015), this implies that at least a part of the odor-related activity in barrel cortex could be due to modulations of whisking.

2.2.4 Odor-induced responses persist in the absence of peripheral interaction

To examine if the impact of odors on barrel activity depends on mechanisms that go beyond the peripheral modulation of whisking by odors, we performed recordings in a passive stimulation context in which whisking was prevented by bilateral sectioning of the inferior and superior buccal branches of the facial nerve (Supplementary figure 2.2). In this case, because the mice were not able to explore the tactile stimuli, the gratings were swept once forward and backward through the whisker pad (Figure 2.4a). This experimental approach generated robust tactile responses in S1 while removing any potential

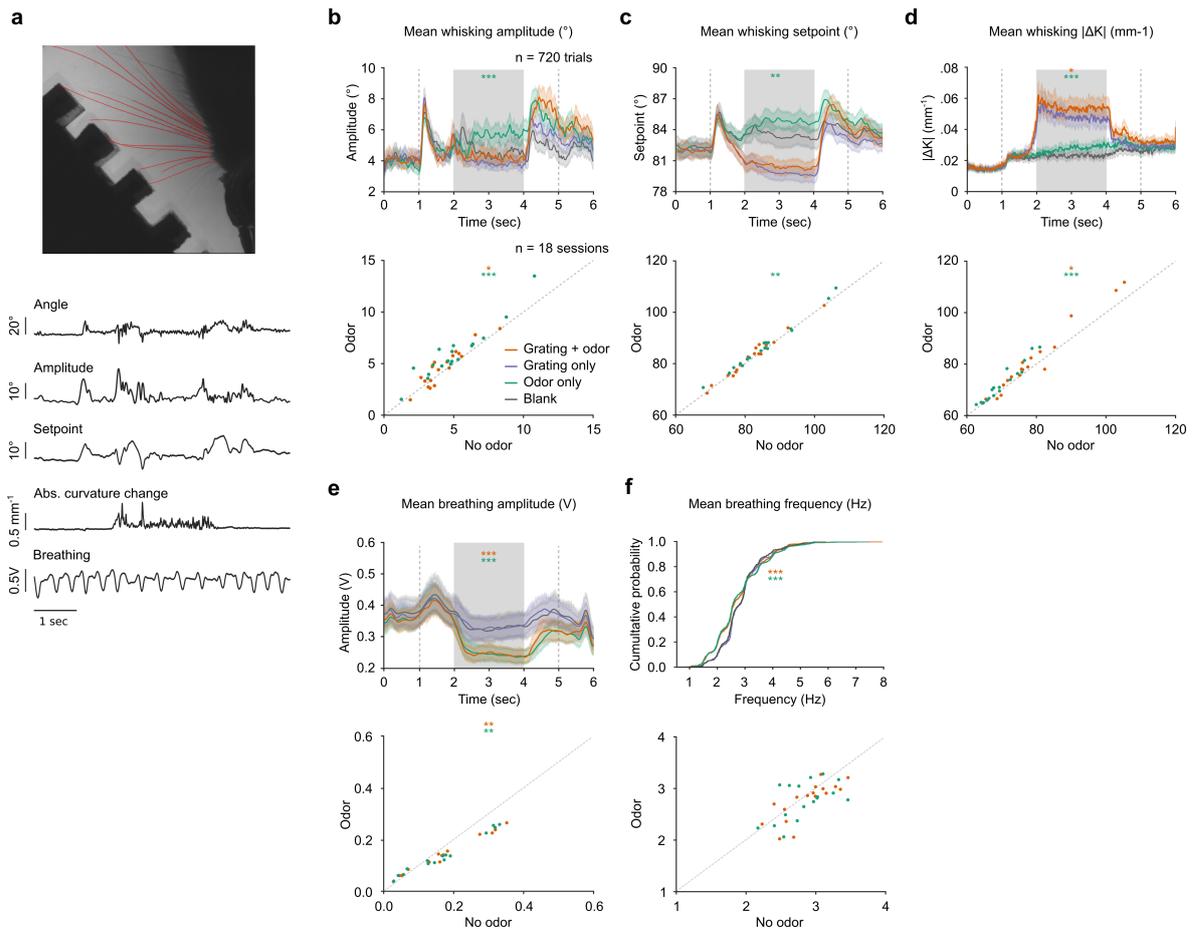


Figure 2.3 – Olfactory stimulations decrease breathing and increase whisking kinematics. **a** Example image of whisker pad tracking with traces of derived whisking kinematic parameters and breathing signal shown below. **b** Top: mean whisking amplitude within sessions. Bottom: mean whisking amplitude between sessions. Whisking was analyzed over the 2 sec stimulation epoch indicated by gray shading (orange: bimodal versus grating only; green: odor alone versus blank). **c** Top: mean setpoint within sessions. Bottom: mean setpoint between sessions. **d** Mean absolute curvature change within sessions. Bottom: mean absolute curvature change between sessions. **e** Mean breathing amplitude within sessions. Bottom: mean breathing amplitude between sessions. **f** Cumulative distribution of breathing frequency within sessions. Bottom: mean breathing frequency between sessions. $n = 18$ sessions from 10 mice; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; Wilcoxon signed-rank test; see Table 2.1 and 2.2 for detailed statistics.

for odor-induced whisking that results in reafferent sensory signals in barrel cortex. Similarly to the active context (Figure 2.2b), odors impacted barrel cortex activity with both enhancement and suppression (Figure 2.4b). At the population level, odors still had no effect on the trial-averaged total activity, except for a small increase that was significant within sessions (Figure 2.4c, g), but not robustly observed between sessions (Figure 2.4c, g). At the level of single cells, again, we observed significant modulation of responses by

odors with equal proportions of enhanced and suppressed cells. For each cell, we compared the average activity to the grating sweeps alone versus the sweeps with odors. In the absence of whisking, average $\Delta F/F_0$'s were still significantly modulated by odors in 8.99% of the neurons (Figure 2.4d, e; significance level = 5%; Mann-Whitney U test), a proportion that is still significantly above chance level (Figure 2.4e) and with a significant bias towards enhancement (Figure 2.4f, inlet). Consistently, the distribution of modulation indices showed a significant impact of odors when compared to shuffled data (Figure 2.4f). Interestingly, the fraction of odor-modulated neurons in the absence of whisking was significantly lower than in freely whisking mice (8.99% against 21.7%, $p = 0.012$, Mann-Whitney U test), indicating that the peripheral modulation of whisking by odors generates a significant fraction of odor-related activity in barrel cortex but not all of it.

In the same way, odors presented alone in the passive context also modulated barrel cortex activity with balanced enhancement and suppression. The average population response was equivalent for odor alone and double blank trials (Figure 2.4g) with a small enhancement within sessions (Figure 2.4g). A proportion of 10.81% neurons were significantly modulated by odors in this passive context (Figure 2.4h, j; significance threshold = 5%; Mann-Whitney U test), which was also significantly above chance level (Figure 2.4i). Modulation indices also showed a significant impact of odors presented alone on the population of recorded cells (Figure 2.4j). Again, the absence of whisking produced a decrease in the fraction of cells responsive in the odor-alone context, although not significantly due to variability across sessions (10.81% against 18.34%, $p = 0.118$, Mann-Whitney U test). Together, this observation shows that along with modulation of whisking behavior, there is a second mechanism by which odors impact barrel cortex activity that is still active when whisking is abolished.

2.2. RESULTS

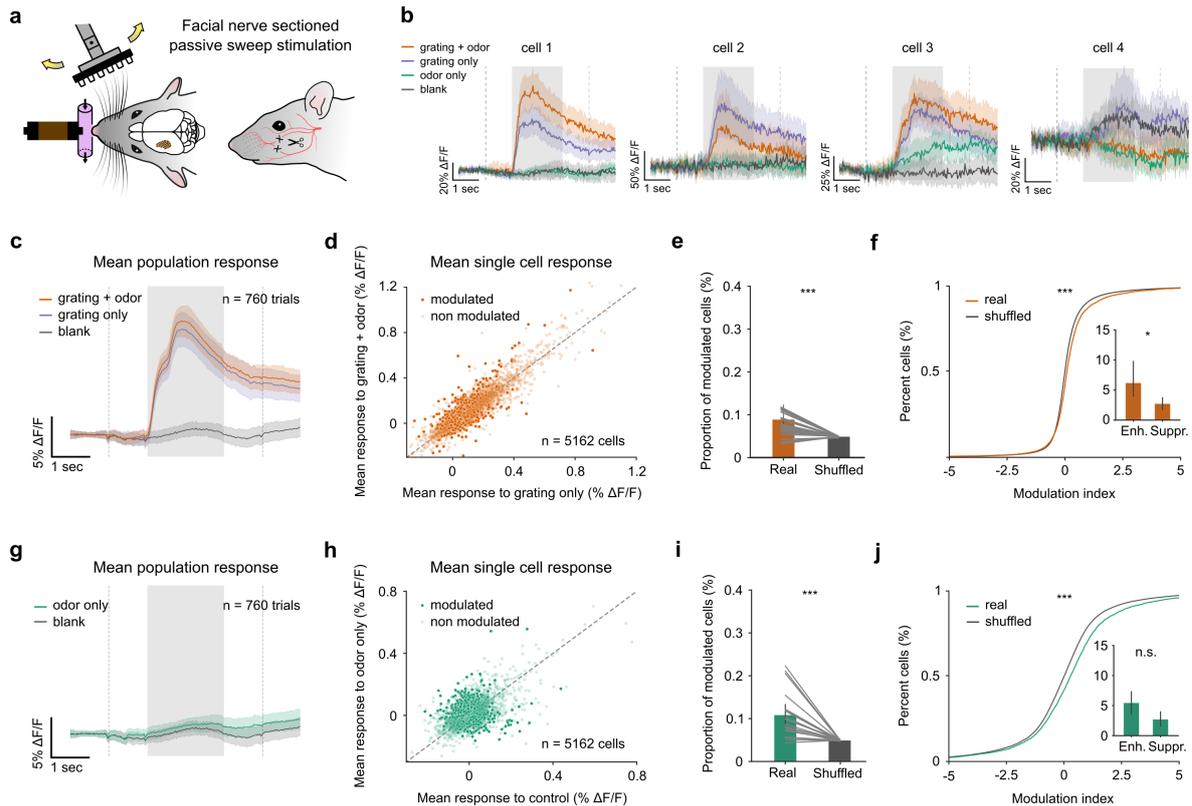


Figure 2.4 – Odor-induced activity in barrel cortex persists in the absence of whisking. **a** Schematic of the stimulation context with facial nerve sectioned. Gratings were brushed against the whiskers in a back and forth sweep. **b-j** As in Figure 2.1b-j. **b** Average $\Delta F/F_0$ for four example neurons displaying diverse odor responses. **c** Population averages to grating+odor trials (orange) and grating only trials (blue); average to control (gray) is shown for comparison (mean of bimodal trials = 11.22% $\Delta F/F_0$ (10.78, 11.66); mean of gratings only trials = 10.32% $\Delta F/F_0$ (9.81, 10.73); $p = 0.001$ within sessions; $n = 760$ trials; Mann-Whitney U test; $p = 0.28$ between sessions; $n = 19$ sessions; Wilcoxon signed-rank test). **d** Scatter plot of mean $\Delta F/F_0$ to grating+odor versus grating only for 5162 neurons. Neurons plotted in dark are significantly modulated ($p < 0.05$, Mann-Whitney U test). Dashed line indicates diagonal. **e** Proportion of modulated neurons across sessions compared with shuffled conditions (8.99% (7.79, 10.57) for real data; 4.81% (4.75, 4.87) for shuffled data; $p = 0.0006$; Wilcoxon signed-rank test). **f** Cumulative distribution of modulation index for grating+odor versus grating alone ($p = 1.15 \times 10^{-17}$, Kolmogorov-Smirnov test). Inset: proportion of enhanced and suppressed cells (mean enhanced = 6.16% (3.89, 9.70); mean inhibited = 2.83% (1.68, 3.91); $p = 0.047$; $n = 19$ sessions; Wilcoxon signed-rank test). **g** Population averages to odor only trials (green) and control (gray) (mean of bimodal trials = 0.91% $\Delta F/F_0$ (0.62, 1.2); mean of gratings only trials = 0.29% $\Delta F/F_0$ (0, 0.61); $p = 0.001$ within sessions; $n = 760$ trials; Mann-Whitney U test; $p = 0.25$ between sessions; $n = 19$ sessions; Wilcoxon signed-rank test). **h** Analog to **d**. **i** Analog to **e** (10.81% (8.55, 13.27) for real data; 4.89% (4.84, 4.94) for shuffled data; $p = 0.0001$; Wilcoxon signed-rank test). **j** Analog to **f** ($p = 5.91 \times 10^{-120}$; Kolmogorov-Smirnov test; inset: mean enhanced = 7.24% (4.94, 9.84); mean suppressed = 3.57% (2.08, 5.26); $p = 0.398$; $n = 19$ sessions; Wilcoxon signed-rank test).

2.2.5 Odor related activity in S1 does not reflect cholinergic inputs activity

Since whisking is not the sole factor explaining odor-related activity in S1, we examined if it could result from cholinergic inputs to the cortex. Cholinergic inputs to the cortex from the basal forebrain have very specific effects on excitatory and inhibitory neurons (Eggermann et al., 2014; Gil et al., 1997; Gullidge and Stuart, 2005; Oldford and Castro-Alamancos, 2003) and are known to play a role in attentional modulation based on the behavioral context (Buzsaki et al., 1988; Herrero et al., 2008; Kuchibhotla et al., 2017; Parikh et al., 2007). To assess whether they play a role in odor-related modulation of barrel cortex activity, we performed dual local injections of a nicotinic and a muscarinic receptor antagonist (1mM injection of atropine and mecamylamine, Figure 2.5a) in barrel cortex shortly before running our olfactory-tactile stimulation protocol in animals with their facial nerve sectioned. This pharmacological perturbation of cholinergic signaling did not have a significant effect on the number of cells modulated by odors presented with or without tactile stimulation (Figure 2.5b). We conclude that cholinergic signaling is not one of the mechanisms for modulating barrel cortex activity in the presence of odors. In summary, our results show that odors still modulate barrel cortex activity independently of whisking and cholinergic signaling.

2.2.6 Olfactory information in barrel cortex is mainly independent of tactile representations

Finally, we evaluated the type of information carried by odor-related activity in barrel cortex and to what extent this information interacts with tactile representations. For this, we used centroid classifiers with a stratified 20-fold cross-validation, trained on population activity vectors to discriminate various stimulation conditions. We first asked whether the presence of an odor was discernible in the population activity by training a classi-

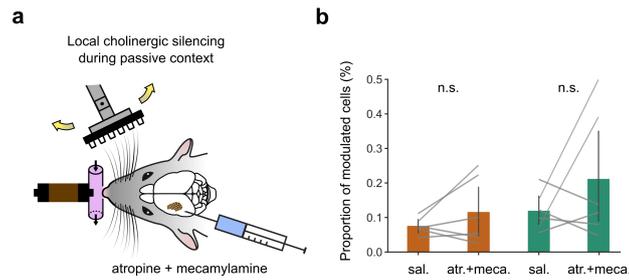


Figure 2.5 – Pharmacological blockade of cholinergic transmission in barrel cortex does not reduce the proportion of olfactory modulated cells. **a** Schematics of local silencing of cholinergic transmission with 1mM injections of atropine (atr.) and mecamylamine (meca.) in barrel cortex during the passive stimulation context. **b** Proportion of modulated cells with and without unilateral cholinergic silencing (mean saline = 7.57% (5.93, 9.34); mean atr.+meca. = 11.59% (4.87, 18.63); $p = 0.463$; grating+odor versus grating only (orange); mean saline = 11.98% (8.17, 16.28); mean atr.+meca. = 21.24% (8.93, 35.75); $p = 0.6$; odor only versus blank (green); $n = 12$ sessions from 3 mice; Wilcoxon signed-rank test).

fier to discriminate bimodal trials from tactile only trials or to discriminate odor only trials against blank trials in mice with facial nerve sectioning (Figure 2.6a, b; same analysis for the active whisking context shown in Supplementary figure 2.3). A proportion of 86.4% of the odor only against blank trials were correctly classified and 71.2% of the bimodal against tactile only trials correctly classified using activity from 1 to 2 seconds after stimulus presentation. This high performance of the classifier in both cases shows that information about the presence or absence of an odor is robustly encoded in barrel cortex. Next, we examined if the olfactory information present in barrel cortex was specific to the type of odor presented. Amyl acetate and ethyl butyrate are two very distinct chemicals which are well discriminated in circuits of the olfactory system (Bathellier et al., 2008). Interestingly, when training classifiers to discriminate amyl acetate and ethyl butyrate trials, classification performance was well above chance level with 68.5% trials correctly classified (Figure 2.6d). These effects were robust enough to be present across sessions (Supplementary figure 2.3) and olfactory information was also present during silencing of cholinergic inputs although less pronounced likely due to the reduced number of sessions (Supplementary figure 2.4). Hence, odor-related activity in barrel cortex is sufficiently precise to decode some information about odor identity.

This observation raises the question whether the presence of olfactory information in barrel cortex has any impact on the tactile representations or if the two representations are somewhat independent. To answer this question we quantified whether classifiers discriminating the two different tactile grating orientations presented to the animal were impacted by odor-driven activity. Tactile grating orientation classification could be easily performed by barrel cortex populations, especially in facial nerve sectioned animals (Figure 2.6c). To evaluate the impact of coincident odors, we trained a classifier to discriminate population activity for the two grating orientations using a training set of trials without odors. We then measured the performance of the classifier using a test set of trials with odors and a test set of trials without odors. When using a global neuronal population merging all recording sessions for this analysis (as in Figure 2.6c), the classifier score for discrimination was 100% in both cases, suggesting no strong impact of odors on tactile coding. For a more sensitive measurement, we also performed this analysis on single sessions (local populations). In this case again, we found no systematic difference between the classification scores obtained in the presence and the absence of odor (Figure 2.6e), confirming that odor-evoked activity does not strongly modify tactile representations along dimensions that are important for their discrimination (Figure 2.6f). As suggested previously (Bagur et al., 2018; Kaufman et al., 2014), independence of two representations suggests that they are contained in orthogonal spaces. To verify this for olfactory and tactile representations in barrel cortex, we measured the dot product between the vector separating the mean representations of the two odors and the vector separating the representation of the two grating orientations. The dot product was close to zero throughout time (Figure 2.6g, h) indicating that odor and tactile decoding axes are orthogonal. This shows that, in our experimental conditions, olfactory and tactile information are encoded in orthogonal subspaces of barrel cortex population activity.

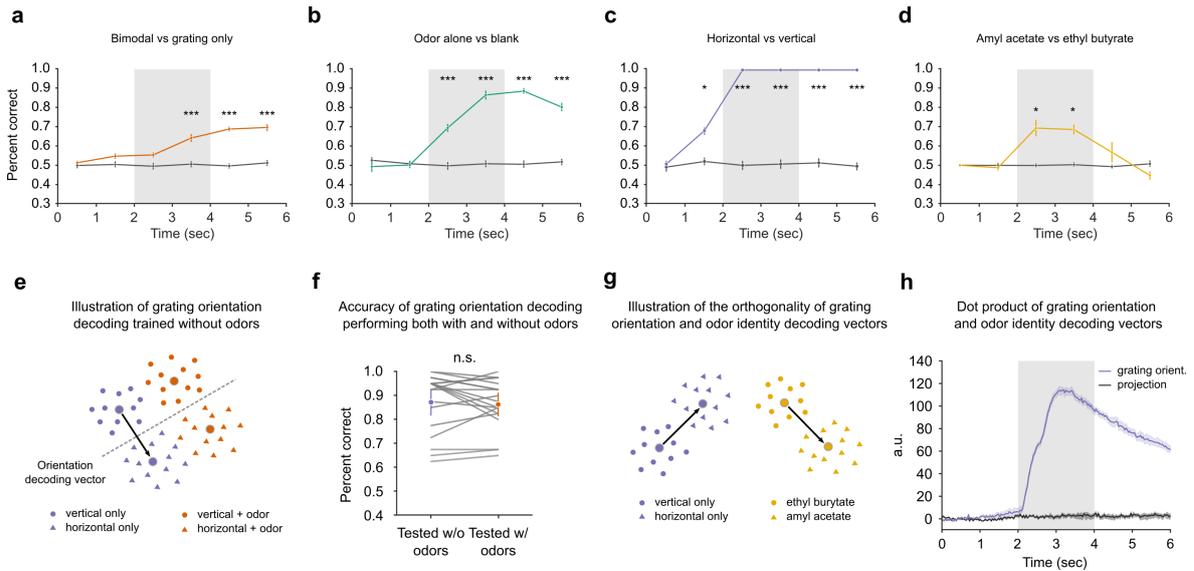


Figure 2.6 – Tactile and olfactory codes are independent in S1. **a** Accuracy of centroid classifiers decoding of grating orientation from S1 activity in the passive context averaged over 1 sec time bins. Performance for shuffled labels is shown in gray. P-values were obtained as the location of the mean accuracy in a distribution of 1000 shuffles; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Error bars indicate 95% c.i. with $n = 10$ for real data and $n = 100$ for shuffled data; each data point is the average of a 20-fold stratified cross-validation; shading indicates stimulus presentation. **b-d** As in **a** for decoding of bimodal and grating only trials, odor only and blank, and odor identity, respectively. **e** Illustration of grating orientation decoding where the classifier was trained without odors but was able to separate the orientations when odors were present. The black arrow represents the decoding vector between the two classes of stimuli computed as the difference between the average population responses over stimulus presentation. **f** Accuracy of centroid classifiers trained to decode grating orientations without odors and showing no impairment when tested in the presence of odors (mean without odors = 88.5% (83.4, 93.1); mean with odors = 86.3% (81.0, 91.0); $p = 0.401$; Wilcoxon signed-rank test). **g** Illustration of the orthogonality of the decoding vector separating grating orientations (**a**) with the decoding vector separating odor identities (**d**). **h** Dot product of grating orientation and odor identity decoding vectors (black). The scalar product was computed between the odor identity decoding vector across time and the grating orientation decoding vector fixed over the stimulus presentation. The same operation between the grating orientation decoding vector and itself is shown for comparison (blue). Shading indicates 95% c.i. over 10 random 2-fold train/test splits.

2.3 Discussion

Using 2-photon calcium imaging during olfactory-tactile stimulation in head-fixed mice with an intact whisker pad, we observed that barrel cortex encodes information about odor identity in parallel with the tactile representations of the proximal environment. We identify two mechanisms for odor-induced modulations of barrel cortex activity: a peripheral one, related to altered whisking when an odor is present (Figure 2.2 and 2.3),

and another, presumably of central origin, that is independent of whisking and cholinergic signalling (Figure 2.4 and 2.5). Together, these two mechanisms affect about one fifth of stimulus-responsive barrel cortex neurons in freely whisking animals, while the latter, central mechanism impacts only about one tenth of the stimulus-responsive neurons. Despite this modest proportion, whisking-independent odor representations are sufficient to reliably decode not only the presence of an odor, but also its identity, making it unlikely that these modulations reflect intact efference copies of whisking programs after facial nerve sectioning (Figure 2.6). In turn, it is unclear whether whisking-dependent effects themselves inform about odor identity as they could not be disentangled from the whisking-independent effects. However, odor decoding was not significantly improved when mice could whisk (Supplementary figure 2.3). Moreover, the relevance of whisking-dependent effects for behavior is difficult to interpret. In our experimental conditions, they reflect global changes in the dynamics of the whisker pad which most probably corresponds to an increase in the engagement of the animal to explore its near environment whether it contacts an object or not (Figure 2.3). Although these reafferent signals impact barrel cortex, it is possible that they are compensated by efference copies from motor centers (Bell, 1981; Fee et al., 1997; Hill et al., 2011). In a goal-directed context, these effects may also be canceled by a tighter control of whisking during behavior (Chen et al., 2013, 2015).

Whisking-independent effects may reflect in contrast a more central cross-modal signaling mechanism. Our pharmacological manipulations (Figure 2.5 and Supplementary figure 2.4) show that they do not depend on cholinergic signaling, indicating that they do not reflect attentional modulation mediated by this pathway. This does not rule out signaling by other neuromodulatory pathways which could play a role as suggested by the fact that both whisking and breathing are affected by the presence of an odor in our experimental conditions (Constantinople and Bruno, 2011; Hattox et al., 2003; Hirata et al., 2006; Hong et al., 2010). For these effects to be entirely driven by neuromodulatory sig-

naling, it would mean that neuromodulatory inputs to barrel cortex are odor-identity specific, as amyl acetate and ethyl butyrate trials can be discriminated based on barrel cortex activity (Figure 2.6 and Supplementary figure 2.3).

Outside of neuromodulatory sources, there are several other pathways that could introduce odor information into barrel cortex representation. Although there are no direct inputs to barrel cortex from the piriform cortex [Oh et al. \(2014\)](#); [Zingg et al. \(2014\)](#), they are bidirectionally connected to common associative areas, in particular the perirhinal cortex ([Aronoff et al., 2010](#); [Fabri and Burton, 1991](#); [Naber et al., 2000](#); [Zakiewicz et al., 2014](#); [Zingg et al., 2014](#)). The perirhinal cortex, which is located just above the piriform cortex on the ventro-dorsal axis, is an associative area that has been implicated in multimodal object recognition ([Brown et al., 1987](#); [Doan et al., 2019](#); [Meunier et al., 1993](#); [Xiang and Brown, 1998](#)). To the best of our knowledge, no study has addressed its potential role in olfactory-tactile integration, but in the rodent, it could be a multimodal hub that combines these two sensory modalities among others. In this case, the odor-evoked activity we observe in barrel cortex would represent feedback information from an associative area. Alternatively, barrel cortex receives inputs from several thalamic nuclei, including from secondary thalamic regions which themselves are known to receive multimodal inputs ([Cappe et al., 2009a](#)).

Independent of the pathway mediating it, the functional significance of olfactory signaling in barrel cortex remains an open question. We observed that olfactory activity in barrel cortex contains an equal amount of inhibitory and excitatory responses and builds neural representations orthogonal to tactile representations. Hence, for the odors and gratings that we presented in a naive behavioral context, information from the two modalities does not bias each other at barrel cortex level. Coincident olfacto-tactile stimulations neither sharpen tactile representations in barrel cortex as has been observed recently in the visual cortex with sounds ([Deneux et al., 2019](#); [Ibrahim et al., 2016](#)), nor do they improve detection of multimodal coincidence. The advantage of this coding scheme is that

barrel cortex can access olfactory information without perturbations of the tactile code. However, the presence of olfactory information in barrel cortex could enable the emergence of cross-modal associations, in specific behavioral contexts which require information from both modalities (Bobrov et al., 2014). The fact that odors have both inhibitory and excitatory effects (Figs. 2.2 and 2.4) suggests that odors could suppress some aspects of the tactile representations and boost others, but such mechanisms would not be engaged in the absence of specific associations.

Together our results demonstrate a novel site of convergence for olfactory and tactile information early in the sensory processing hierarchy and open interesting avenues to study the role of brain wide interactions in processing two sensory modalities that are crucial in rodents' daily life.

2.4 Methods

2.4.1 Cranial window implantation and viral injections

All procedures were conducted in accordance with protocols approved by the French Ethical Committee (authorization 00275.01). We used 8 to 12-weeks-old C57BL/6J mice housed 1-4 per cage, in normal light/dark cycle (12h/12h). Cranial window implantation and viral injections were performed under isoflurane anesthesia (1.3-1.7%) with body temperature maintained constant at 37°C using a regulated thermal blanket with a rectal probe (Rodent Warmer X1, Stoelting). A craniotomy of 4 mm in diameter was drilled over the barrel cortex on the left hemisphere. Four injections of 200 nl of AAV1-syn-GCaMP6s (1×10^{-12} vg.ml⁻¹) obtained from Vector Core (Philadelphia, PA, USA) with glass micropipettes and a programmable pump (Micro 4; World Precision Instruments) at 30 nl.min⁻¹ around the C2 barrel barrel column at AP -1.6, ML -3.3, DV 0.519. The craniotomy was sealed with a glass window comprising two circular coverslips bound together with

optical glue (5 and 3 mm diameter) and a metal post for head-fixation was implanted using dental cement (Super-Bond C&B, Sun Medical Co. Ltd.).

2.4.2 Facial nerve transection

For experiments in the passive context, whisking was prevented by bilateral sectioning of the inferior and superior buccal branches of the facial nerve (Dörfl, 1985). Mice were anesthetized under isoflurane with their temperature monitored as for cranial window implantation. A small 3 mm cut of the skin was performed to expose the buccal branches of the facial nerve which were sectioned with microsurgical scissors. The cut was then closed with a nylon suture.

2.4.3 Intrinsic optical imaging

The location of the GCaMP6s expression locus in barrel cortex was validated with intrinsic optical imaging under isoflurane anesthesia (1%; SomnoSuite, Kent Scientific) on a thermal blanket. The signal was obtained under 625 nm LED illumination and images of the vasculature over the same field of view were taken under 480 nm LED illumination. Reflected light was acquired with a CCD camera (GC651MP, Smartek Vision) equipped with a 50 mm objective (Fujinon, HF50HA-1B, Fujifilm) on a 656 x 496 pixel region and resolution of 5.58 μm per pixel at 15 fps through a cranial window 1 to 2 weeks after implantation. Four seconds after imaging onset, the C2 whisker was deflected by a piezoelectric bender (PI PICMA Bender) at 10 Hz for 4 sec following a sinusoidal wave along the rostrocaudal axis, for twenty trials with 8 sec inter-trial intervals. Change in reflectance was computed as $(R_{\text{stim}} - R_{\text{base}}) / R_{\text{base}}$ where R_{stim} and R_{base} are averaged over the 4 sec of stimulus presentation and baseline, respectively. Response images were averaged across all deflections. Mice whose intrinsic response did not coincide with the GCaMP6s expression locus were excluded from further analysis.

2.4.4 Odor delivery and grating presentation

Odors (amyl acetate and ethyl butyrate) were from Sigma-Aldrich and delivered with a custom-made olfactometer (Supplementary figure 2.1). Mass flow controllers were used to pass air flow through small bottles (Wilmad ML-1490-702, SP Scienceware) filled with 20 ml of odor solution diluted at 0.1% in mineral oil. The total flow was constant ($1 \text{ l} \cdot \text{min}^{-1}$) and the snout of the animal was placed inside a confined air stream to prevent movement of the whiskers. To obtain a stable concentration during stimulus application, we ensured that the flow was stationary with a 5 sec bubbling period before the stimulus was presented. Photoionization detector measurements were made (Supplementary figure 2.1) to precisely synchronize the timing of arrival of the odorized air flow to the snout with the final position of the grating (active context) or the onset of the sweep (passive context). Orientated gratings were made of five ridges of 3.5 mm thickness and spacing on a 3d printed PLA disk of 35 mm diameter. Gratings were brought to the animal or swept using a custom-made presentation wheel consisting of two stepper motors (42BYG, Makeblock) mounted on a linear stage (eTrack, Newmark) and controlled with an Arduino. All parts of the stimulation system were timed and synchronized with a data acquisition card (USB-6343-BNC, National Instruments) and MATLAB scripts (Mathworks). Bimodal conditions were presented 10 times (40 total), unimodal conditions 20 times (80 total), and the blanks 30 times, for a total 150 pseudo-randomized trials with one stimulus presentation every 19 sec.

2.4.5 Two-photon calcium imaging in awake mice

One week before imaging, mice were trained to stand still, head-fixed under the microscope for five consecutive days for 15 min to 1 h per day. Then mice were imaged for 1h long sessions with up to four vertical depths imaged per mouse on different days. Imaging was performed using a two-photon microscope (Femtonics, Budapest, Hungary)

equipped with an 8 kHz resonant scanner combined with a pulsed laser (MaiTai-DS, SpectraPhysics, Santa Clara, CA, USA) tuned at 920 nm. The objective was a 10x Olympus (XLPLN10XSVMP), obtaining a field of view of 1000 x 1000 μm . Images were acquired at 31.5 Hz during blocks of 11 sec interleaved with 8 sec intervals.

2.4.6 Calcium imaging data analysis

Data analysis was performed using Python scripts. Motion artifacts, regions of interest selection, and the signal extraction was carried out using Suite2p (Pachitariu et al., 2017). Neuropil contamination was subtracted by applying the following equation: $F_{cor}(t) = F(t) - 0.7F_n(t)$. Then the change in fluorescence $\Delta F/F_0$ was calculated as $(F_{cor}(t) - F_0)/F_0$, where F_0 is estimated as the 8th percentile of F for each trial. To account for baseline fluctuations, the baseline activity two to one seconds before stimulus onset was subtracted for all cells on each trial. In total, 9714 neurons were recorded from 12 mice over 20 sessions in the active condition, and 14408 neurons from 6 mice over 19 sessions in the passive condition. Among those populations, 1907 (19.6%) and 5162 (35%) neurons in the active and passive contexts, respectively, were responsive to at least one of the nine stimulation conditions (significance level = 5%; Kruskal-Wallis test) and were kept for analysis. Single cell $\Delta F/F_0$ averages were computed over the 2 sec of stimulus presentation and were considered odor responsive if their mean response was significantly different in the comparison between bimodal trials with grating only trials or odor only trials with blanks. For simplicity, we first evaluated the modulation by grouping together trials with odors, regardless of odor identity, resulting in four condition categories (bimodal, grating only, odor only, and blank; Figs. 2.2 and 2.4). We defined the olfactory modulation index as $(\Delta F/F_{odor} - \Delta F/F_{no\ odor}) / \Delta F/F_{no\ odor}$ with $\Delta F/F_{odor}$ and $\Delta F/F_{no\ odor}$ the average response over stimulus presentation to bimodal and grating only conditions or odor only and blank, respectively (Stein et al., 2009).

2.4.7 Whisker tracking and breathing monitoring

The full whisker pad was monitored with a high-speed camera (HXC20, Baumer) at 500 Hz and 608 x 600 pixels per frame under infrared backlight. Automated tracing of the whiskers and extraction of angle and curvature was performed with Whisk (Clack et al., 2012). For each frame, angles and curvatures of detected whiskers (~12 per frame) were averaged to obtain a global measurement for the whisker pad. The phase of the whisking cycle was computed by applying a Hilbert transform to the band-pass filtered azimuthal angle (2nd order butterworth filter, 4-30 Hz) from which the time point of maximum protraction and retraction were retrieved (Hill et al., 2011). Amplitude and setpoint were then defined as the range and center of angular motion over a single cycle after quadratic interpolation. Absolute change in curvature was obtained by taking the absolute curvature and subtracting a baseline defined as the average absolute curvature two to one sec before stimulus presentation. Breathing was monitored with a microbridge mass airflow sensor (Honeywell AWM3300V, Morris Plains, NJ) positioned in front of the animal's snout and perpendicular to the air stream (Bolding and Franks, 2017). Negative change in voltage corresponds to inhalation. Traces were sampled at 1 kHz and bandpass filtered (2nd order butterworth filter, 4-20 Hz) and a Hilbert transform was used similarly to obtain breathing amplitude similarly to whisking. Breathing frequencies over a 2 sec duration before and after stimulus presentation were defined as the peak in the power spectrum computed with Welch's method.

2.4.8 Silencing of cholinergic inputs

For pharmacological manipulation of cholinergic signaling, 400 nl of 1 mM atropine and mecamylamine was injected into barrel cortex via a hole through the cranial window while the animal was awake. To allow for diffusion, we waited 30 min after injection before starting the recording. Controls were performed by injection of 400 nl of saline. Control

and silencing recordings were made with a two days interval starting with controls first for half the experiments and silencing first for the other half. A similar plane was imaged in control and silencing sessions but the identity of cells were not tracked across sessions.

2.4.9 Stimulus decoding and orthogonality

To evaluate the robustness of odor-evoked activity in barrel cortex, we tested the accuracy at which the presented stimulus could be decoded from single-trial population responses by training and testing a nearest centroid classifier with a stratified k-fold cross-validation procedure implemented in Scikit-learn. Single-trial global population vectors were constructed with neurons recorded from all sessions using $\Delta F/F_0$ averages in 1 sec time bins. Training and testing sets were created by randomly partitioning single trials in $k = 10$ pairwise disjoint groups with equal number of trials from the two tested conditions, each serving as a test set against the others. This procedure was repeated 10 times and the accuracy of the classification was defined at the average of these 10 cross-validations. To evaluate its significance, the same procedure was performed for $n = 1000$ shuffles of the conditions and the accuracy of the non-shuffled data was located in the distribution of shuffled accuracies to obtain a p-value. Note that the precision of the p-value was limited to 3 decimals as 1000 shuffles were performed. For decoding between sessions (Supplementary figure 2.3), population vectors were constructed with neurons recorded in single sessions using $\Delta F/F_0$ averages in 1 sec time bins. The same k-fold cross-validation procedure was applied. Each session was used to obtain a cross-validated accuracy with and without shuffling. Significance was assessed by comparing the mean accuracy of the real and shuffled data (Wilcoxon signed-rank test). The data was z-scored to reach significance in the results presented in Supplementary figure 2.3, in accordance with the usual machine learning prerequisite for classification: the average response of each cell to all conditions of stimulus presentation was subtracted and scaled by the standard deviation

over the same epoch. To study the independence of the tactile and olfactory information, we considered the decoding axes classifying grating orientation and odor identity (Figure 2.6c, d), defined as the difference vectors between the corresponding conditions, which we projected onto each other (Figure 2.6h). Specifically, we took the olfactory decoding axis that we projected across time onto the tactile decoding axis defined on the second time bin after stimulus presentation (during which olfactory information is maximal), we then took the norm of the resulting projection as our measure of orthogonality.

2.4.10 Statistical analysis

All quantification and statistical analysis were performed with Python scripts. Plotting relied on Matplotlib and Seaborn. Statistical assessment was performed with non-parametric tests implemented in the Statistical functions module reported in figures and legends together with mean and 95% confidence interval, the number of samples used for the test and the nature of the samples (number of sessions for between sessions assessment and number of trials for within sessions assessment). Hypotheses were two-sided and significance levels were set at 5%. Confidence intervals were computed by the bootstrap procedure implemented in Seaborn with $n = 1000$ bootstrap iterations. In all analyses, all subjects which underwent a particular protocol in the study were included.

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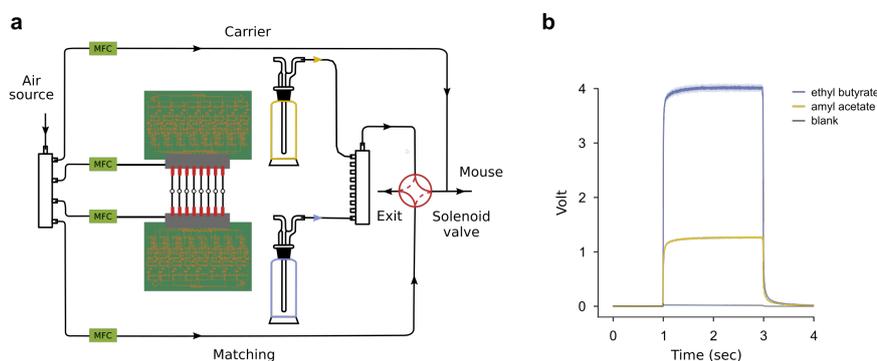
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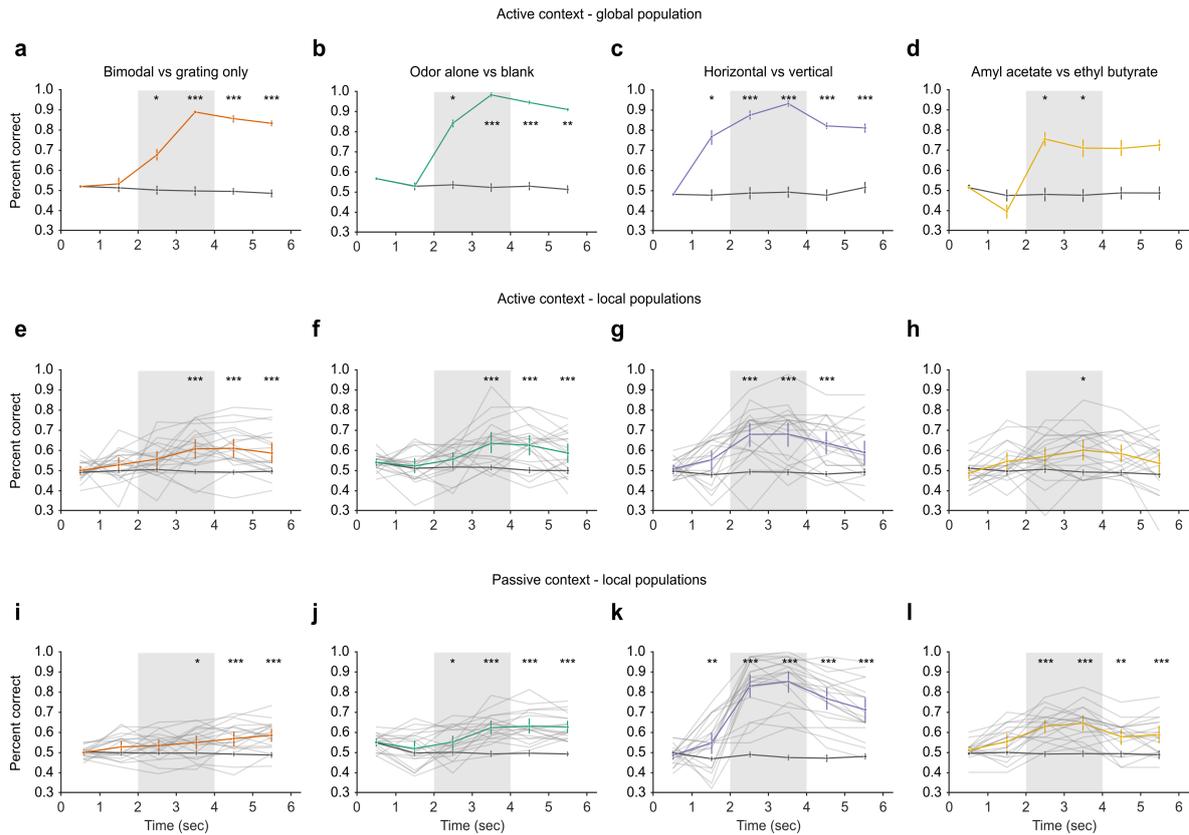
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2.6 Supplementary material



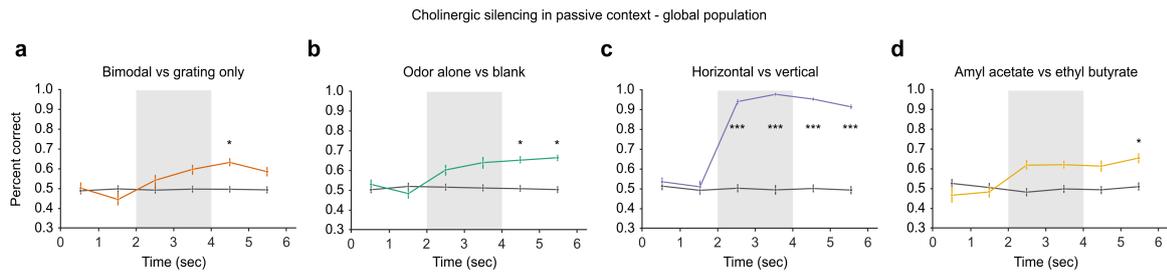
Supplementary figure 2.1 – Design and calibration of the custom-built olfactometer. **a** The olfactometer was constructed with chemically inert 1/16” and 1/32” inner diameter polytetrafluoroethylene tubing. The flow rates of four independent streams were regulated by mass flow controllers (MFC; SFC5400, Sensirion). Two MFC’s (250 ml/min each) controlled the flow producing odorized air by passing through 20 ml of odor solutions prediluted at 0.1% in mineral oil and contained in small bottles (Wilmad ML-1490-702, SP Scienceware). Two MFC’s controlled the carrier and matching streams (500 ml/min each). The carrier stream was constantly delivering 500 ml/min to the mouse. A shuttle valve was used to switch between matching and odorized streams with precise timing. The output flow to the animal was constant at 1 L/min. Valves to an empty bottle were actuated during trials without odors. **b** Photoionization detector (PID) measurements showing temporal precision of odor presentation. The PID was placed at the location of the mouse’s snout. Shading indicates standard deviation over $n = 10$ trials.

2.6. SUPPLEMENTARY MATERIAL

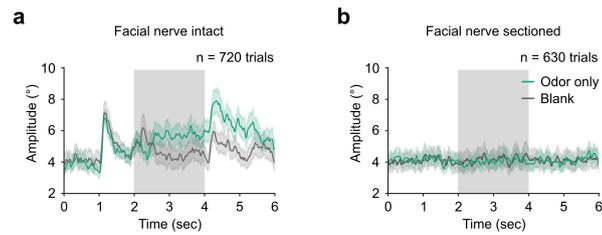


Supplementary figure 2.2 – Whisking is abolished by bilateral section of the facial nerve. **a** Average whisking amplitude within sessions for trials without tactile gratings for mice with intact facial nerves (active context). Shading indicates 95% c.i.; n = 720 trial from 18 sessions and 10 mice. **b** Analogous to a for mice with sectioned facial nerves (passive context); n = 630 trials from 9 sessions and 5 mice (some sessions from the passive context were not included as videos of the whiskers were not recorded for all sessions in this context). The baseline of 4° in amplitude is the result of an imprecision in whisker detection from frame to frame.

CHAPTER 2. OLFATORY MODULATION OF BARREL CORTEX ACTIVITY DURING ACTIVE WHISKING AND PASSIVE WHISKER STIMULATION



Supplementary figure 2.3 – Olfactory information is also present in the active context in S1 and between populations in both contexts. **a-d** Accuracy of centroid classifier decoding of stimulus from S1 activity in the active context averaged over 1 sec time bins; analogous to Figure 2.6a-d. Performance for shuffled labels is shown in gray. P-values were obtained as the location of the mean accuracy in a distribution of 1000 shuffles; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Error bars indicate 95% c.i. with $n = 10$ for real data and $n = 100$ for shuffled data; each data point is the average of a 20-fold stratified cross-validation; shading indicates stimulus presentation. **e-h** Analogous to **a-d** with decoding performance across sessions in the active context. Each data point is the average of a 20-fold stratified cross-validation with data from single sessions. Significance was assessed by comparing the mean accuracy of real and shuffled data with $n = 20$ and $n = 19$ sessions in the active and passive contexts, respectively (Wilcoxon signed-rank test). Grey lines indicate single session performance. **i-l** Analogous to **e-h** in the passive context. Note that the data was z-scored to reach significant performance in the results presented in this figure, which was not the case in Figure 2.6.



Supplementary figure 2.4 – Olfactory information is present during silencing of cholinergic input to S1. **a-d** Accuracy of centroid classifier decoding of stimulus during silencing of cholinergic inputs from S1 activity in the passive context averaged over 1 sec time bins; analogous to Figure 2.6a-d. Performance for shuffled labels is shown in gray. P-values were obtained as the location of the mean accuracy in a distribution of 1000 shuffles; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Error bars indicate 95% c.i. with $n = 10$ for real data and $n = 100$ for shuffled data; each data point is the average of a 20-fold stratified cross-validation; shading indicates stimulus presentation. The data was z-scored similarly to Supplementary figure 2.6

*CHAPTER 2. OLFACTORY MODULATION OF BARREL CORTEX ACTIVITY DURING
ACTIVE WHISKING AND PASSIVE WHISKER STIMULATION*

Within sessions			
	Condition	Mean (95% c.i.)	p-value
Amplitude	Bimodal	4.67 (4.37, 4.99)	0.081
	Grating only	4.31 (4.01, 4.63)	
	Odor only	6.01 (5.60, 6.43)	1.10 ⁻⁶
	Blank	5.08 (4.62, 5.59)	
Setpoint	Bimodal	82.51 (81.81, 83.19)	0.12
	Grating only	81.91 (81.20, 82.62)	
	Odor only	86.50 (85.71, 87.30)	0.014
	Blank	85.25 (84.34, 86.17)	
Curvature change	Bimodal	0.053 (0.048, 0.058)	0.04
	Grating only	0.048 (0.043, 0.052)	
	Odor only	0.030 (0.028, 0.032)	0.001
	Blank	0.024 (0.023, 0.026)	
Between sessions			
	Condition	Mean (95% c.i.)	p-value
Amplitude	Bimodal	4.65 (3.83, 5.49)	0.042
	Grating only	4.27 (3.62, 5.00)	
	Odor only	6.01 (5.6, 6.43)	1x10 ⁻⁴
	Blank	5.08 (4.62, 5.59)	
Setpoint	Bimodal	82.79 (79.17, 86.50)	0.111
	Grating only	82.18 (78.73, 85.89)	
	Odor only	86.64 (82.53, 91.27)	0.002
	Blank	85.44 (81.35, 89.82)	
Curvature change	Bimodal	0.053 (0.039, 0.069)	0.017
	Grating only	0.047 (0.036, 0.061)	
	Odor only	0.030 (0.022, 0.038)	0.001
	Blank	0.025 (0.019, 0.031)	

Table 2.1 – Mean (95% c.i.) of average whisking amplitude, setpoint, absolute curvature change, both within and between sessions, presented in Figure 2.3. Averages are computed over the 2 sec stimulus presentation epoch. P-values are from the statistical tests comparing bimodal versus grating only conditions and odor only versus blank (Mann-Whitney U test within sessions and Wilcoxon signed-rank test between sessions).

Within sessions			
	Condition	Mean (95% c.i.)	p-value
Amplitude	Bimodal	0.23 (0.21, 0.25)	1.10-5
	Grating only	0.30 (0.27, 0.34)	
	Odor only	0.23 (0.21, 0.26)	1.10-4
	Blank	0.30 (0.27, 0.34)	
Frequency	Bimodal	2.78 (2.71, 2.85)	0.002
	Grating only	2.89 (2.83, 2.96)	
	Odor only	2.79 (2.71, 2.86)	0.008
	Blank	2.85 (2.79, 2.92)	
Between sessions			
	Condition	Mean (95% c.i.)	p-value
Amplitude	Bimodal	0.23 (0.12, 0.37)	0.002
	Grating only	0.30 (0.15, 0.52)	
	Odor only	0.23 (0.12, 0.38)	0.003
	Blank	0.30 (0.15, 0.51)	
Frequency	Bimodal	2.76 (2.58, 2.92)	0.068
	Grating only	2.89 (2.72, 3.05)	
	Odor only	2.77 (2.59, 2.93)	0.355
	Blank	2.84 (2.68, 3.00)	

Table 2.2 – Mean (95% c.i.) of average breathing amplitude and frequency, both within and between sessions, presented in Figure 2.3. Averages are computed over the 2 sec stimulus presentation epoch. P-values are from the statistical tests comparing bimodal versus grating only conditions and odor only versus blank (Mann-Whitney U test within sessions and Wilcoxon signed-rank test between sessions).

Chapter 3

The perirhinal cortex as a possible source of modulation

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3.1 The perirhinal areas as a bridge between the piriform and barrel cortex

3.1.1 Evidence from anatomical studies

If the modulation that we have described is indeed induced by olfactory inputs reaching the barrel cortex, uncovering the pathway that mediates this transfer of information would be an important step to go beyond a qualitative description. In that regard, large scale connectivity studies have been performed in mice that delineate the areas that could bridge the relatively distant piriform and barrel cortex. Among the possible candidates, two studies in the barrel cortex (Aronoff et al., 2010; Zakiewicz et al., 2014) and one in the piriform cortex (Haberly, 2001) indicate strong reciprocal connections with the perirhinal cortex (defined as Brodmann's areas 35 and 36; Figure 3.1). Further, these tracing results are corroborated by the connectivity maps of the Allen Institute (Oh et al., 2014) as well as the mouse connectome project (Zingg et al., 2014) – from which retrograde tracing consistent with a bisynaptic projection from the piriform to the barrel cortex is displayed in Figure 3.2.

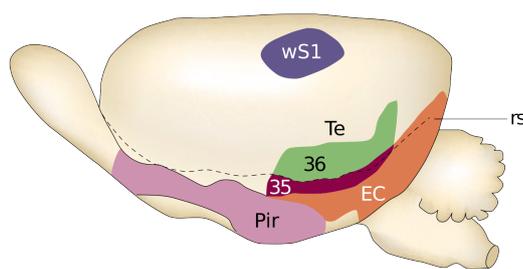


Figure 3.1 – Perirhinal cortex location in the rat brain defined as Brodmann's areas 35 and 36. EC, entorhinal cortex; rs, rhinal sulcus; Te, temporal association area; Pir, piriform cortex; wS1, barrel cortex. Adapted from Brown and Aggleton (2001).

Besides the perirhinal cortex, mutual connections with the orbitofrontal cortex have been reported (Haberly, 2001; Zakiewicz et al., 2014), although weaker than with the perirhinal cortex, as well as a convergence onto the entorhinal cortex which receives direct in-

puts from the piriform cortex and indirect inputs from the barrel cortex via the perirhinal areas (Kerr et al., 2007). In addition, two studies by the same group reported the existence of monosynaptic connections directly linking the two cortical areas of interest; however, the histological evidence provided were scarce and unconvincing, so this claim might be best restricted as a possibility (Wang et al., 2015; Ye et al., 2012). Overall, we can conclude from these studies that associative areas connect the piriform and barrel cortices in a bidirectional and monosynaptic way. Among those areas, the strength of these projections points toward the perirhinal cortex as the best candidate for the anatomical substrate of the association between olfaction and whisker touch at the cortical level.

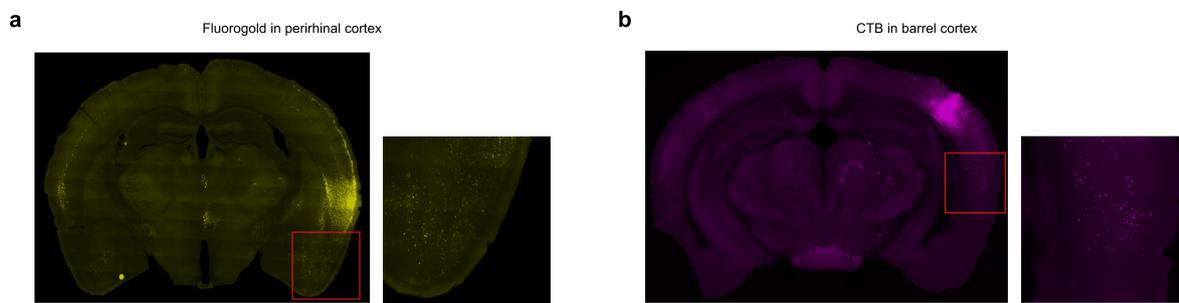


Figure 3.2 – Retrograde tracing consistent with a bisynaptic projection from the piriform cortex to the barrel cortex. **a** Fluorogold-based retrograde tracing from the perirhinal cortex showing dense labeling of cell bodies in the piriform cortex and the top of layer 2/3 and 5a of the barrel cortex. **b** CTB-based retrograde tracing from the barrel cortex showing labeling of cell bodies in the perirhinal cortex. From the mouse connectome project (Zingg et al., 2014); experiments SW120118-04B and SW110418-03A, respectively.

3.1.2 Evidence from physiological studies

At the functional level, the perirhinal cortex has traditionally been implicated in novelty detection and recognition of single objects. It is a distinctive feature of perirhinal neurons to display selective responses to novel stimuli which are reduced with repeated presentations (Brown, 1996). This reduction has been interpreted as signaling stimulus familiarity or recency, and generally occurs after a single exposure in both rats and monkeys. Consistent with neuronal recordings, the surgical removal of the perirhinal cortex in these animals severely and permanently impairs object recognition (Meunier et al., 1993; Mumby

3.1. THE PERIRHINAL AREAS AS A BRIDGE BETWEEN THE PIRIFORM AND BARREL CORTEX

and Pinel, 1994). By contrast, while object familiarity has also been linked with hippocampal circuits, in this case, spatial context is a vitally important aspect of the object's identity. The evidence for this is that, while lesions of the hippocampus strongly impair spatial memory tasks, individual object recognition abilities tend to be spared (Aggleton and Brown, 1999), and the effects on entorhinal cortex are milder and more transient (Leonard et al., 1995).

These observations have been further confirmed with immediate-early-gene imaging, functional imaging in humans and clinical studies. Together, this provides a convincing evidence in favor of a functional dissociation between the perirhinal and hippocampal structures, where the former is considered essential for novelty detection, whereas the latter is involved in associational forms of object recognition in spatial context. To date, this proposal of dissociation and the subtleties of the memory processes in play are still not universally accepted (Brown and Aggleton, 2001; Kealy and Commins, 2011).

Besides its role as a novelty detector, parts of the work on the perirhinal cortex focus on sensory processing rather than mnemonic functions. It was suggested that the perirhinal cortex takes part in the processing of the ventral visual stream, in particular in discriminating complex stimuli. For example, rats with lesions affecting the perirhinal cortex have been shown to be impaired in memory-independent visual discrimination tasks (Eacott et al., 2001; Gaffan et al., 2000). Although most mnemonic studies rely on vision, the perirhinal cortex is not limited to this modality and is clearly involved in olfactory and tactile discrimination tasks (Otto and Eichenbaum, 1992; Suzuki et al., 1993). Interestingly, rats trained to discriminate complex visuo-tactile stimuli were not able to perform after perirhinal lesions, whereas hippocampal lesioned rats were not impaired (Moran and Dalrymple-Alford, 2003). Another two studies in a multisensory context showed that rats with lesioned perirhinal cortex could not recognize an object when visually re-explored after having been exposed in the dark with the tactile and olfactory modalities (Winters and Reid, 2010), or conversely (Albasser et al., 2011).

In light of these findings, the perirhinal cortex might be an important site for the acquisition, transfer and consolidation of cross-modal associations, and, together with the connectivity data, stands out as a plausible candidate to support olfacto-tactile interactions.

3.2 Olfactory modulation of the barrel cortex during perirhinal silencing

3.2.1 Silencing approach

To test the hypothesis that the reported olfactory modulation of the barrel cortex was mediated via the perirhinal cortex. We reproduced the olfacto-tactile experiments presented in the previous chapter in the passive stimulation condition during chemogenetic silencing of the perirhinal cortex. In addition to GCaMP6f injections in wS1 executed as described in Section 2.4, three injections of 250 nl of pure pAAV-hSyn-DIO-hM4D(Gi)-mCherry (AAV8; 7×10^{-12} vg/ml) obtained from Addgene were performed in the perirhinal cortex. The first injection was located at AP -1.4, ML -4.3, DV 0.5 and the other two were $\pm 300 \mu\text{m}$ away in the anterior posterior axis, in order to target the densest areas of projection from the perirhinal to the barrel cortex.

Out of 5 mice, DREADDs were injected unilaterally in the ipsilateral hemisphere of 3 mice, while 2 mice were injected bilaterally. These two groups are analyzed together in what follows. Recordings consisted of 2 sessions over two days in each of the 5 mice. The day of the first session, 200 nl of saline was injected intraperitoneally 30 min before the recording started. The next day, mice were injected with 200 ml of clozapine-N-oxide (CNO) at a concentration of 3 mg/kg. Each recording sessions lasted 45 min.

3.2.2 Results

To quantify the effect of the inhibition at the population level, we compared the average population activity elicited during bimodal stimulations in the saline and CNO conditions (Figure 3.3a). We found no effect at the population level within or across sessions, and the population activity was actually slightly higher in the CNO condition. Looking at single cells responses, we found an average across sessions of 11.7% and 19.1% modulated cells in the saline and CNO conditions, respectively (Figure 3.3b). The proportion in the saline condition was above chance level and consistent with our previous recordings in the passive stimulation condition. Unexpectedly, the proportion of modulated cells was higher in the CNO condition, but this difference was not significant.

Considering odors presented alone, the average population response was smaller in the CNO condition which was significant within sessions ($p=0.014$; Mann-Whitney U test; activity averaged over the 2 sec odor presentation), but not across sessions. At the single cell levels, we obtained 17.1% and 10.8% modulated cells in the saline and CNO conditions, respectively (Figure 3.3c). Despite a small decrease in proportion of modulated cells, this difference was not significant.

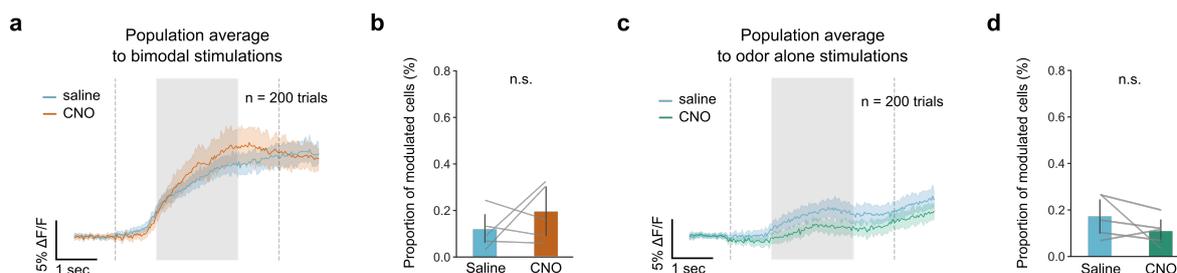


Figure 3.3 – Odor-induced activity in barrel cortex during perirhinal silencing. **a** Population average to bimodal trials for the saline (blue) and CNO (orange) conditions. Shadings indicate 95% c.i. within sessions. **b** Proportion of modulated cells for the saline (blue) and CNO (orange) conditions. Shadings indicate 95% c.i. across sessions. **c-d** Same as *a-b* for olfactory stimulations alone.

While the amount of odor-related activity was about the same with and without perirhinal silencing, it could be that the modulation carried less information about the odors during silencing. To quantify a possible reduction in information, we used centroid classi-

ifiers, with a 20-fold cross validation, trained on population activity vectors to discriminate the various stimulation conditions, in accordance with the decoding analysis presented in Figure 2.6.

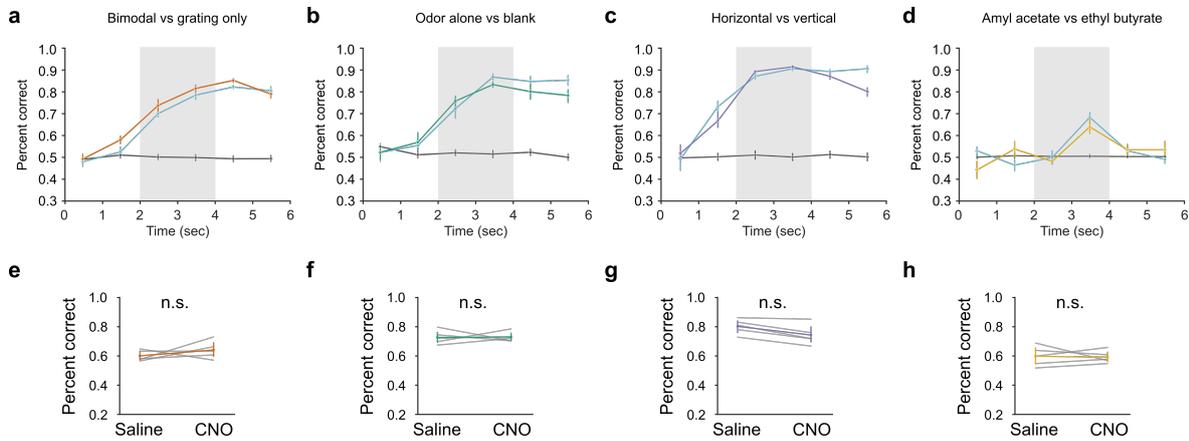


Figure 3.4 – Decoding of olfactory information in barrel cortex during perirhinal silencing. **a** Accuracy of centroid classifiers decoding of bimodal and grating alone trials from wS1 activity averaged over 1 sec time bins with the global population of cells recorded over all sessions for the saline (blue) and CNO (orange) conditions. Performance for shuffled labels in saline condition is shown in gray. Error bars indicate 95% c.i. with $n = 10$ for real data and $n = 100$ for shuffled data; each data point is the average of a 20-fold stratified cross-validation; shading indicates stimulus presentation. **b-d** As in **a** for decoding of odor alone and blank trials, grating orientation and odor identity, respectively. **e-f** As in **a-d** with local populations, with activity averaged between 1 and 2 sec after stimulus onset.

We asked whether the presence of odor was detectable by training classifiers to discriminate between the bimodal and grating alone stimuli, as well as the odor alone and blank stimuli. We also evaluated the discrimination of the grating orientation and the odor identity. Overall, whether we quantified the information among the global population of recorded cells (Figure 3.4a-d) or among the local single session populations (Figure 3.4e-h), no difference could be found during perirhinal silencing.

The conclusions and shortcomings of these experiments, in particular the efficiency of the silencing approach and the comparison of different recording sessions, will be discussed in the following chapter.

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Chapter 4

Discussion and perspectives

Contents

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Through the course of this work, we envisaged the barrel cortex beyond the pure sensory extraction of tactile features, as a site also involved in the processing of higher-order information. From recordings of large populations in the barrel cortex of awake mice, the main contribution of this thesis is to report an olfactory modulation of barrel cortex activity that is equally shared between enhancement and suppression and specific enough to be informative about odor identity without perturbation of the tactile code. In the following, we will discuss the relevance and shortcomings of this work in the context of the multisensory literature, elaborate on the possible origins and functional roles of the olfactory-related signals reaching the barrel cortex, and conclude with perspectives of future work to assess this functional role with behavioral tasks in a multisensory context.

4.1 Taking multisensory integration beyond a noise story

The picture of the state of the art in multisensory integration that we have sketched in the introduction of this thesis aimed at pointing towards a gap between the psychophysics of multisensory integration and its physiology. The classic work in the psychophysics of multisensory integration, reviewed in Section 1.1.1, emphasizes rich synergies and binding between complementary aspects of the sensory environment that have a holistic impact on perception, and are well exemplified by the variety of multimodal illusions existing in humans among all pairs of modalities.

By contrast, most of the work on the neurophysiology of multisensory integration, reviewed in Section 1.1.3, focuses on the redundancy of multisensory signals. It deals with situations where sensory cues processed in parallel by different sensory pathways are informative about a common parameter, such as the size of an object that can be touched and seen. It is considered that, on reaching an associative area, their integration produces a combined estimate that is more reliable than the unimodal ones – a combination that is often reported as optimal with respect to Bayesian modeling. The rationale is that

the unisensory estimations are corrupted by inherent noise stemming from the nature of the neuronal transformations; the increased reliability of the joined estimate is therefore achieved by a simple sum which cancels the noise – provided that it is independent between modalities. From this perspective, multisensory integration boils down to just a noise cancellation (e.g. see [Fetsch et al. \(2013\)](#)). One can argue that this statistical view is too limited to do full justice to the variety of multisensory effects, in particular to the richer non-redundant interactions. Moreover, it suffers from some technical and conceptual shortcomings that motivated our approach.

As mentioned in the introduction, besides the general good fit between the Bayesian model of sensory cue integration and the psychophysical data, deviations from the predicted integration are often reported with some going beyond the theoretical optimum ([Battaglia et al., 2003](#); [Fetsch et al., 2012](#); [Kiani et al., 2013](#); [Nikbakht et al., 2018](#); [Raposo et al., 2012](#); [Rosas et al., 2005](#)). For instance, a recent study by [Nikbakht et al. \(2018\)](#) reported strong examples of supralinear effects. They trained freely moving rats to discriminate oriented gratings that could be either seen, touched or both. They found that rats were able to discriminate the full psychometry of orientations both with vision and whisker touch, and that their performance showed strong multisensory enhancement in trials where both modalities could be used that were beyond the theoretical optimum of the Bayesian model, even for the less ambiguous orientations.

These intriguing supralinear effects might be explained by an enhanced motivation during bimodal trials. As it has been suggested in other studies ([Raposo et al., 2012](#)), animals are sensitive to the overall rate of reward. As bimodal trials yield higher average reward rates than do unimodal trials, rats could attend unimodal trials less. This would result in an underestimation of the reliability of the unimodal cues, and therefore the bimodal estimate would appear to exceed the expected sum. However, [Nikbakht et al. \(2018\)](#) checked that the exploration time was not reduced in unimodal trials, and they compared specific sessions where only unimodal or bimodal trials were presented to

arguably keep the motivation of the rats constant. Across these sessions, the enhancement was still largely beyond the optimal prediction. An alternative explanation most relevant to our discussion could be that these supralinear effects result from the violation of the crucial assumption of independent noise. Rats tended to solve this task by keeping their whiskers protracted, without rhythmic whisking, combined with movement of the head, suggesting that they evaluate the level of resistance of the gratings on the whiskers (Nikbakht, 2015). If the placement of the head happens to be an important parameter to solve the task with whiskers, it is plausible that the visual cues help sample the gratings more efficiently via a sensorimotor interaction between the two modalities – akin to the peripheral interaction between whisking and breathing that we report (Figure 2.3). Further, functional connections at the central level between the two processing pathways could exist and lead to synergies between cross-modal features as a result of associative learning.

These deviations from the best linear combination challenge the type of minimal interactions assumed by the Bayesian model of redundant cue integration. The need to broaden multisensory integration beyond a simple summation in associative areas was one of the main motivations for our project. We conducted our work in the primary areas to test whether sensory representations are affected by multisensory integration which would offer strong evidence of cross-modal synergies. Our results expand the variety of early cross-modal effects that have been recently reported and could be the substrate for the emergence of cross-modal associations that would develop in specific behavioral contexts.

4.2 Origin and functional role of the olfactory modulation of barrel cortex

To summarize the findings of this thesis, we have shown that the barrel cortex encodes information about the presence and identity of odors in parallel to tactile representations. We performed two-photon calcium imaging recordings of large populations of barrel cortex neurons in awake mice in a naive context without behavior. The tactile stimuli were presented either in a condition where mice could freely whisk on the gratings, termed the active stimulation condition (even though mice were not engaged in a task), or with the facial nerve sectioned, the passive stimulation condition. The modulation that we observed contrasts with the multisensory literature in two ways: first, it is shared between an equal proportion of enhancement and suppression, and second, it is specific enough to convey information about the identity of the presented odors to the barrel cortex.

The cross-modal influences in the sensory areas, that we reviewed in Section 1.2.3, tend to report either boosting or inhibition but not both (Deneux et al., 2019; Ibrahim et al., 2016; Lohse et al., 2021; Morrill and Hasenstaub, 2018). Although some effects depend on the context (Deneux et al., 2019), the unidirectional modulations of these reports are often suggested to rely on gain modulation mechanisms. The continuous distribution between enhancement and suppression that we observe does not fit in a straightforward way with gain modulation. Olfacto-tactile interactions could differ in that respect from interactions involving vision or audition. It may be tempting to think of multisensory interactions as generic, but they have their specific features. For example, auditory responsive cells in V1 are found in layer 2/3 and 5 (Ibrahim et al., 2016), while their reciprocals are restricted to layer 6 (Morrill and Hasenstaub, 2018). These distinctions likely serve different computational roles. Consequently, shared excitatory and inhibitory effects could be a specific characteristic of olfacto-tactile interactions that could boost some tactile features and suppress others so that associations emerge in appropriate contexts.

The second originality of our findings is that the olfactory information available to the barrel cortex was informative about odor identity. Indeed, it seems that previous reports managed to find reliable information in the primary areas about the presence of secondary stimuli but not about their identity. The claim that it is without precedent should be tempered however. Notable differences with the literature is that we repeatedly presented only two odors and recorded large populations. The conjunction of these facts does not make the decoding very challenging. Further, the accuracy of odor identity decoding was only ~70% (but reliably through out conditions) and we did not find single cells tuning to odors. In contrast, reports that checked for interaction specificity, such as [Morrill and Hasenstaub \(2018\)](#) and [Lemus et al. \(2010\)](#), presented more than two stimuli, recorded a small amount of neurons and limited their investigations to single cell tuning. It is likely that more information is to be found by relying on population vectors similarity measures combined with large population recordings.

While about 20% cells were significantly affected by odors in the active condition, this proportion dropped to 10% in the passive condition. We interpreted this difference as the contribution of two mechanisms for the odor-induced modulation of the barrel cortex: a peripheral one related to the increased whisking induced by the presentation of odors, and a second one, separate from tactile reafferences, presumably of central origin. Tracing the pathways by which these latter whisking-independent olfactory signals arrive to the barrel cortex is an important step in understanding their functional significance, and despite our efforts put in this direction, we have not yet established the connections responsible for this cross-talk. Besides controlling for odor-induced whisker movements, we have considered two possible sources: the cholinergic system and the perirhinal cortex.

We first examined the possibility of an attentional modulation that would be triggered by the presentation of odors. The cholinergic transmission from the basal forebrain to the cortex could carry this modulation as it is implicated in attentional modulation depend-

ing on the behavioral context and has diverse effects on excitatory and inhibitory neuron responses (Eggermann et al., 2014; Kuchibhotla et al., 2017). Moreover, global silencing of the cholinergic system by systemic muscarinic blockade and lesions of the basal forebrain has been reported to impair learning of a bimodal olfacto-tactile task (Botly and De Rosa, 2007, 2009). We tested that hypothesis by silencing the cholinergic inputs to the barrel cortex by local injections of atropine and mecamylamine, a muscarinic and a nicotinic receptor antagonist, respectively. As shown in Figure 2.5, this silencing did not have a significant impact on the proportion of modulated cells, and information decoding was similar to the control condition (data not shown). We concluded from these experiments that the modulation was not mediated by cholinergic signalling, but that did not exclude the possibility of an attentional modulation conveyed by other neuromodulators such as norepinephrine or serotonin (Constantinople and Bruno, 2011; Hattox et al., 2003).

Next, we tested the hypothesis that the perirhinal cortex acts as a bridge between the piriform and barrel cortex. We have elaborated on this possibility in chapter 3.1 based on the bidirectional anatomical connections that are well documented between these areas and on the functional role of the perirhinal cortex in object recognition as well as in the consolidation of cross-modal associations. The silencing approach that we employed in this case was based on chemogenetics. We expressed the inhibitory DREADD hM4Di in the perirhinal areas and injected clozapine N-oxide (CNO) actuator systemically via intraperitoneal injections. The silencing of perirhinal circuits did not alter the proportion of modulated cells or the decoding of olfactory information.

Both silencing experiments have some shortcomings that could be addressed by readjusting our methodology. First, while the local injections of antagonists directly in the cortex is an efficient blockade method, the same cannot yet be said with confidence about chemogenetic silencing. Although hM4Di silencing has been used successfully at the behavioral levels (e.g. (Banerjee et al., 2020)), its efficacy at reducing neural activity seems only partial (e.g. (Deneux et al., 2019)). The silencing mechanism of hM4Di first described

works by the activation of G-protein inward rectifying potassium channels, leading to an attenuation of neural activity (Armbruster et al., 2007). However, it was shown more recently that a potentially more potent silencing can be obtained by activation of hM4Di at the presynaptic terminal acting via the suppression of glutamate release (Stachniak et al., 2014). Nowadays, silencing experiments relying on DREADDs tend to apply microinfusion of the actuator directly in the area of interest (Takahashi et al., 2020), preferentially using compound 21 over CNO to avoid non-specific activation of endogenous G-protein coupled receptors due to the reverse metabolization of CNO into clozapine. Reproducing our silencing experiments of the perirhinal cortex with microinfusion of compound 21 rather than systemic injection of CNO would likely result in a more potent silencing, and might give different results. This would have to be done by making sure that the DREADDs are expressed along as much as the perirhinal cortex as possible, whose elongated anatomy does not ease the silencing process. Although care was taken to inject the DREADDs at the location where the projections to the barrel cortex are the densest (see Section 3.2.1), these projections exist along most of the perirhinal cortex and the silencing of a more substantial proportion of the area might be required to detect an effect.

A second limit that is relevant to both silencing experiments comes from the current impossibility to assess the silencing within single sessions. Both manipulations were analyzed by comparing a first control session with injection of saline with a second session with injection of the drugs. It allowed to compare the proportion of modulated cells and of odor-related information in between each sessions. However, the experimental conditions made it difficult to come back to the same field of views to image the same cells. The cells were therefore not identified in between sessions, and a significant turn over in responsiveness from one day to the next was observed for those that could be identified (Rule et al., 2019; Schoonover et al., 2021). To be able to assess the effect of the silencing at the single cell level within sessions and thereby avoid the comparison of recording sessions performed on different days and increase statistical power, implementing a silenc-

ing strategy based on optogenetics would be beneficial – which we have not done due to the technical difficulty in performing simultaneous two-photon imaging acquisition and optogenetic stimulation.

Outside of our main hypotheses, strong relationships have also been established between neural activity in the primary areas and motor activity, such as locomotion and orofacial movements, both during passive stimulation and engagement in decision-making tasks (Musall et al., 2019; Stringer et al., 2019). These non-sensory variables have been reported to account for large fractions of the variance in neural activity and to be restricted to low dimensions orthogonal to the encoding of sensory variables. Whisking was shown to be a major component and, during passive viewing, explains by itself ~24% of the neural variance in V1 (Stringer et al., 2019). Although these studies do not provide a mechanism by which motor activity is reflected in the cortex, we have accounted for this bias by removing whisking reafferences via the sectioning of the facial nerve, which indeed reduced the proportion of odor-modulated cells from 20% down to 10%. Based on the mentioned reports, it can be argued that the remaining part of the modulation corresponds to movements of other body parts that we did not monitor (or even of efference copies of whisking commands which are likely not removed by the section of the facial nerve). For instance, strong locomotion responses in barrel cortex layer 2/3 and 5, independent of whisking, have been recently reported (Ayaz et al., 2019). Along those lines, if the totality of modulated cells fell in that category and were not genuinely related to odors, one would expect that abolishing whisking would reduce the information about odor information available to the barrel cortex compared to the active condition where the facial is intact. This prediction is not reflected in the decoding of odor presence and identity, which is stable between the two conditions. Further, the decoding of odor identity would require the remaining behavioral variables to be discriminative of the odors. Monitoring the entire face as well as movements of the paws would be valuable in that respect. Although in our experiments the tip of the snout is confined in the olfactometer

air stream, part of it is visible on whisking videos and can be analyzed. Importantly, more attention should be paid to breathing as respiration signals propagate to the cortex via olfactory bulb reafferences (Bagur et al., 2021; Ito et al., 2014; Sobel and Tank, 1993). This is another possible confound and it should be checked that odors do not induce different breathing modulations that would ease the decoding of odor presence and identity – this analysis will appear in a revised version of our current manuscript (Renard et al., 2021).

Finally, other pathways that we have not considered could mediate the modulation. For instance, it would be interesting to investigate the cross-modal integration properties of S2, which has been suggested to be more responsive to decisional variables than S1 and to be involved in multiwhisker processing and extraction of global sensory features that could support multisensory integration (Hubatz et al., 2020; Kwon et al., 2016; Yang et al., 2016). Also, as we described in Section 1.2.3, even though the olfactory processing pathways are distinctively segregated from other senses at the subcortical level, which made us favor hypotheses based on cortocortical projections, subcortical routes, for example involving the amygdala and the thalamus, cannot be excluded straightaway (Lohse et al., 2021).

Irrespective of their anatomical origin, the functional role of cross-modal signals in the primary sensory areas remains an open question. Similarly to studies showing that early encoding of behavioral and cognitive variables are independent of sensory information, we found that the olfactory modulation of barrel cortex lies in a dimension orthogonal to that of the tactile representations, and so does not perturb the tactile code. What could this mixing of information be good for? One can speculate that this interaction, independent of tactile coding in a passive context, might be the substrate by which cross-modal associations emerge in a behavioral context where this information is made useful. This is particularly relevant if the barrel cortex is viewed as implementing an internal model of the whiskers, rather than as a pure sensory map, whose function is to perform simulations to evaluate actions and their outcomes (Brecht, 2017). This view fits with the framework

of predictive coding where action selection could be achieved by a dynamic comparison of internal expectations generated by the model and incoming sensory inputs. There is room to accommodate multisensory processes in this picture. If these models can be updated by experience, cross-modal associations could develop to inform simulated body actions with other sensory modalities; for instance, to decide that a red stove is too hot to be touched. Body ownership illusions such as the rubber hand illusion (Botvinick and Cohen, 1998) suggests that internal models of the body can be animated by multisensory information rather than only by sensory afferents (Shokur et al., 2013).

To assess the functional relevance of the olfactory modulation of barrel cortex, further experimental work, including bimodal discrimination tasks, will be needed to evaluate the emergence of early cross-modal associations and their causal role in perception. Possible directions toward this end will be described in the last section of this thesis.

4.3 Future perspectives

Several limitations have been discussed in this chapter that can be addressed by extending our work with more complex stimuli and a behavioral task in which odor/texture pairs are given meaning. As discussed above, the evidence that odor identity can be decoded from barrel cortex activity is weakened by only using two odors at a single concentration. To provide stronger evidence that olfactory inputs are the source of the modulation, a straightforward improvement of our experiments would be to record barrel cortex responses to the presentation of more odors with varied concentrations. In this configuration, the levels of olfactory information available to the barrel cortex could be assessed with more challenging decoding. A significant performance would stand as a strong confirmation that the modulation originates from olfactory inputs, and could be compared against classifications based on confounding sources such as motor activity or respiration.

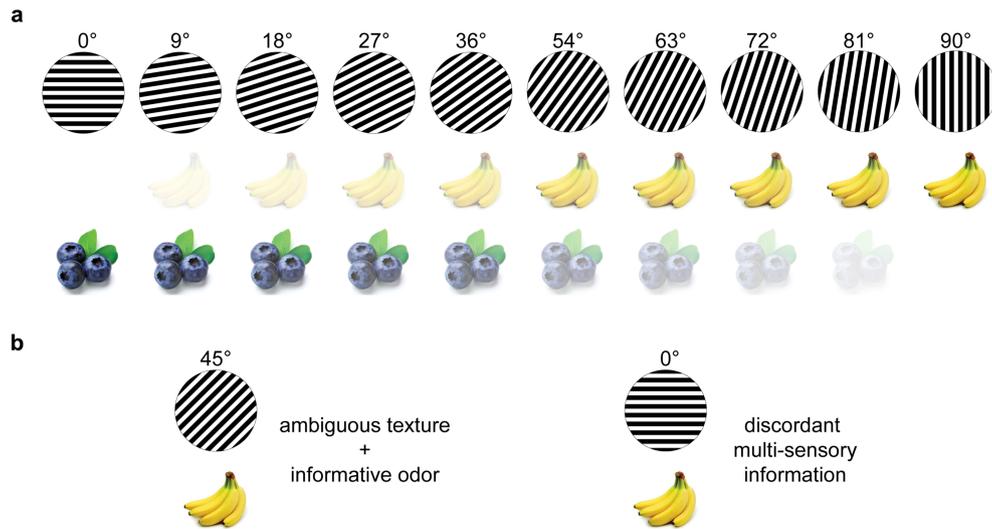


Figure 4.1 – Stimulus design of a bimodal discrimination task. **a** Two continuous series of tactile oriented gratings and odor mixing are associated to form two multimodal objects with independent ambiguities. **b** Schematic of two types of catch trials that would be introduced to probe synergy effects and multisensory illusions.

Although increasing the number of odors would be useful to evaluate the refinement of olfactory information available to the barrel cortex, stimulations in naive animals are limited to investigate how multisensory information is used, due to the lack of relevance of the stimuli. To contribute to the question of the functional role of multisensory interactions in the sensory areas, a behavioral context is required. A bimodal discrimination task can be developed by presenting odor/texture pairs from two full continuous series as depicted on Figure 4.1a. The tactile series will consist in incremented grating orientations from horizontal to vertical. A similar series can be achieved for odors with mixing that linearly transition to full ethyl butyrate to full amyl acetate. To assign a value to these stimuli, water deprived mice would then be trained in a go/no-go operant conditioning protocol (or ideally in a two-alternative forced choice protocol if training time allows it) to associate one half of the series with licking and the other half with no licking.

The purpose of continuously transitioning between two multisensory stimuli is to introduce ambiguities. Multisensory integration being stronger when signals are degraded, these ambiguities would force the animal to make use of both senses and avoid the dom-

inance of one on the other. The first phase in this protocol would be to learn the two unisensory tasks in parallel to match the psychophysics curves by adjusting parametrically the difficulty of the two tasks. This would permit to verify that both information are used and can be done by adjusting the odor mixing until the olfactory discrimination becomes as hard as the orientation discrimination. In a second phase, multisensory trials would be introduced and two crucial questions could then be addressed. First, introducing congruent multisensory pairs with the same levels of ambiguity alongside unimodal stimuli would permit to assess if multisensory enhancement occurs behaviorally and if it matches with enhanced and stabilized neural representations. Second and most importantly, we would test whether the introduction of discordant multimodal information is able to bias sensory representations. Assuming that neural activity is recorded in the somatosensory cortex, this could be achieved in two ways. Either by presenting an ambiguous texture together with an informative odor, thereby creating potential synergies where the olfactory information helps resolve the ambiguity by shifting the tactile representation towards its associated orientation (Figure 4.1b (right)); or by presenting discordant stimuli to test whether a multisensory illusion can be created where the tactile representation is shifted toward the orientation associated with the discordant odor (Figure 4.1b (left)).

Training mice in a double discrimination task matching in difficulty while recording neural activity would be admittedly hard, however, observing shifts in neural representations resulting from cross-modal influences would constitute a decisive demonstration of the involvement of low-level sensory areas in multisensory processes and contribute to uncovering their functional role.

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Appendix A

Methodological appendix on whisker detection and tracking

The purpose of this appendix is to provide methodological details on the approaches that we used to tackle the problem of quantifying whisking. Quantifying whisker movements is crucial to interpret neural activity in the whisker system, and the tight coupling between whisking and sniffing emphasized this need in our work due to the impact of odor sampling on whisking documented in Figure 2.3.

The fast speed of whisker movements together with the low contrast of whiskers on their background make whisker detection and tracking a computationally demanding problem. To alleviate for this difficulty, in many studies the whiskers are clipped down to a single row or a single whisker (Jadhav et al., 2009; O'Connor et al., 2010). Even though rodents still perform well in various tasks under these conditions, ablation of this sensory organ is not ideal behaviorally and the impact of sensory deprivation on barrel cortex is not known. Other approaches rely on small markers or fluorescent paint (Rigosa et al., 2017; Roy et al., 2011), but these methods add significant weight to the whiskers which likely impacts perception and behavior and are still restricted to the tracking of a few whiskers.

In this work, we chose to keep the whisker pad intact during the active stimulation condition to maintain a natural exploration of the gratings as much as possible, maximize the amount of activity and cells susceptible to be modulated by odors and stay consistent with an orientation discrimination task developed in parallel in the team (Harrell et al., 2020). To date, tracking the identity of all whiskers across time without trimming is still out of reach, but such precise tracking was not necessary in our case. Our need was primarily limited to a global measurement of whisking in order to assess the impact of odor presentations.

To obtain a global measure of displacement of the whisker pad, we developed a simple method to compute the centroid of the pad across time. It consisted, after gaussian filtering of the image in the frequency domain to enhance the detection of the whiskers, in projecting the binarized pixels of a small segment of the whisker shafts, from a manually drawn region of interest close to the base, onto a whisking axis tangent to the whisker pad (Figure A.1a (middle)). This simple method gave a reliable measurement of the position of the centroid of the whisker pad on the whisking axis at a very low computational cost. However, this computational efficiency comes at the expense of precision. Rather than computing a single angle value for each portion of whisker shaft detected in the region of interest, all pixels are projected on the axis which bias the measurement toward the thickest and longest whisker shafts. Moreover, this method simplifies whisking as a translation along a line, whereas it is a rotational motion that is better approximated by the azimuth in the horizontal plane. Finally, as the detected shafts are not modeled, no measure of curvature can be recovered. Yet, curvature, as the best read out of the forces applied to the follicle, is a valuable information to verify if sampling odors affects barrel cortex activity.

To address the limits mentioned above, we turned to the methods that had been developed previously. As far as can be judged, only the BIOTACT Whisker Tracking Tool (BWTT) is designed to track untrimmed whiskers without labels (Perkon et al., 2011). It

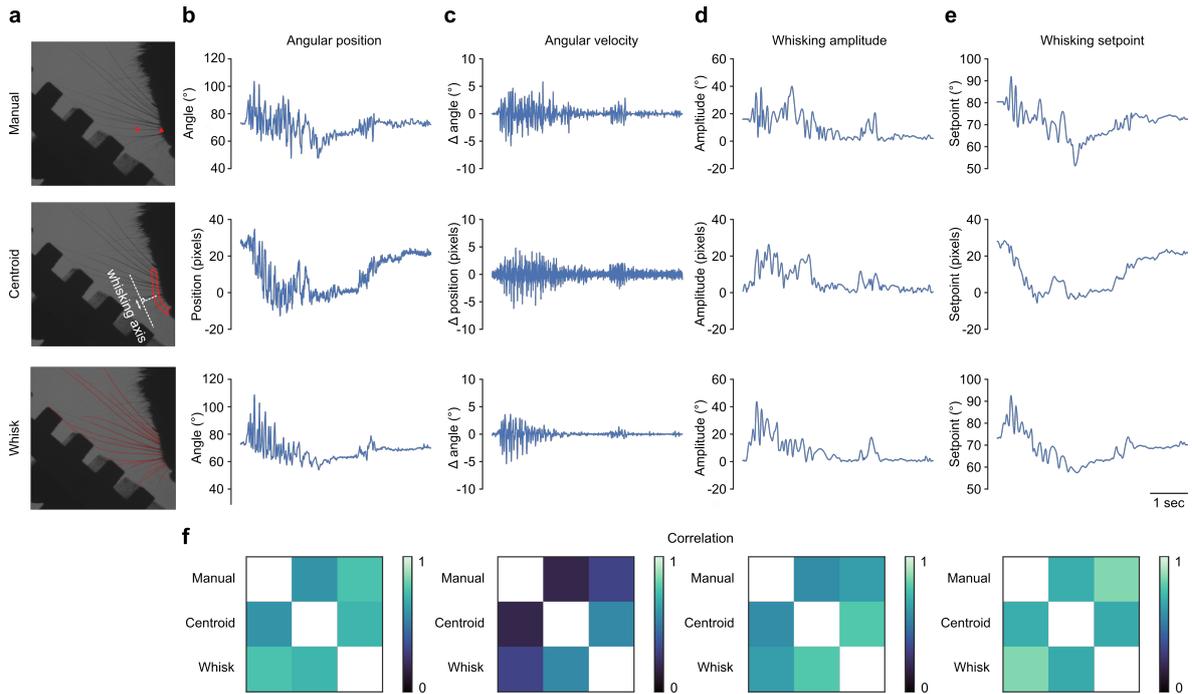


Figure A.1 – Comparison of whisking quantification methods for a single trial. **a** Illustration of the tracking methods. Top: manual tracking of a single whisker at the base and haft length. Middle: centroid method showing the whisker segments detected in a manually drawn region of interest and the whisking axis on which they are projected. Bottom: whisker detection with Whisk. **b-e** Angular position, velocity, whisking amplitude and whisking setpoint of the full whisker pad for the three methods. Computations are based on angular position as described in Section 2.4.7. **f** Correlation between the three methods for the four parameters showing higher correlation of the manual tracking with Whisk than the centroid method.

allows to measure the angle of all detected whiskers at a user-defined distance from the head and so would provide of a more precise measure of the global movement of the whisker pad. However, the whisker shafts are modeled as rigid segments which prevent curvature measurement, and the algorithm has exponential complexity in the number of detected whiskers (but see [Ma et al. \(2017\)](#) for a significantly sped up version). Besides BWTT, Janelia Whisk is another software that is popular in the whisker community. Its approach has the advantage of taking the shape of the whiskers into account by modeling them as parametric curves which allows to measure the force applied at the follicle. Although it does not aim at tracking the full whisker pad, it does a very good job at detecting non-trimmed whiskers with a fast processing time. Moreover, it is designed to track the identity of a row of whiskers which we considered an extra benefit in case single row

experiments with more precise whisker tracking turned out to be needed.

For these reason, we decided to rely on Whisk rather than the centroid method to quantify whisking in this work. To validate this choice, we benchmarked Whisk against the centroid method with the manual tracking of a single whisker as ground truth. From the angular position of the whisker pad, obtained by averaging the angle of all detected whiskers at each time point (after a curation based on the location of the base and the length of the traced curve), velocity as well as the slower parameters of whisking amplitude and setpoint (Figure A.1b-e). Amplitude and setpoint were obtained by applying a Hilbert transform to the band-pass filtered angular position (2nd order butterworth filter, 4-30 Hz) from which the instantaneous phase was obtained and used to recover the times of maximum protraction and retraction (Hill et al., 2011). Although the two methods gave sensibly similar results, correlations with the manually tracked trial was higher for all four parameters (Figure A.1f) and validated Whisk as a superior method in intact whisker pad tracking. The remaining discrepancy between Whisk and the manual trial is likely due to differences in tracking the whole pad against a single whisker.

Recently, a new tracking tool was developed based on BWTT and Whisk that outperforms both of them in whisker detection and tracking (Betting et al., 2020). Besides methods relying on computer vision, deep learning tools such as DeepLabCut could be promising, but, in our experience, full pad tracking was not reliable, and the tracking of a row of four whiskers, with a manually labeled training set of 200 images with five dots on each of the four whiskers, still showed a substantial amount of errors. An adapted version of figure A.1 will appear in a revised version of Harrell et al. (2020).

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Intégration multisensorielle olfacto-tactile dans le cortex à tonneaux chez la souris

Résumé

L'intégration de l'information provenant de différents sens est traditionnellement considérée comme relevant des étages associatifs du traitement sensoriel. Cette vue est remise en cause par des effets multisensoriels opérants dès les aires corticales primaires. Chez la souris, les études se sont focalisées sur la vision et l'audition, alors que les rongeurs se fient principalement à l'odorat et au touché vibrissal. La pertinence éthologique de ces modalités, fortement couplées au niveau moteur, offre un potentiel d'interactions intéressant. Nous avons étudié la modulation du cortex à tonneaux en réponse à des stimulations olfactives chez la souris naïve en contention. En combinant la présentation de grilles orientées à des odeurs durant l'enregistrement de larges populations, nous avons établi que 20% des cellules actives de la couche 2/3 sont significativement modulées par les stimulations olfactives, avec une part égale d'augmentation et de suppression des réponses. Cette proportion est réduite à 10% après abolition du balayage vibrissal par section du nerf facial. Dans ces deux conditions, la présence ainsi que l'identité des odeurs peuvent être décodées de l'activité du cortex à tonneaux. Le blocage de la transmission cholinergique n'affecte ni l'ampleur de la modulation, ni la quantité d'information olfactive. Les connexions réciproques avec le cortex perirhinal ont été étudiées, mais leur implication n'a pas pu être démontrée. Ces résultats établissent une nouvelle interaction multisensorielle et contribuent à renouveler notre conception des aires primaires. Le substrat anatomique ainsi que le rôle fonctionnel de cette modulation restent toutefois à déterminer.

Mots clés : neurosciences intégrative, multisensoriel, cortex à tonneaux, olfaction

Olfacto-tactile integration in mouse barrel cortex

Abstract

The integration of information from different senses is traditionally considered as relevant to the associative stages of sensory processing. This view has recently been challenged by multisensory effects occurring at the level of the primary cortical areas. In mice, studies focused on vision and audition, while rodents rely mainly on smell and whisker touch. The ethological relevance of these modalities, strongly coupled at the motor level, offers potential for interesting interactions. We have studied the modulation of the barrel cortex in response to olfactory stimulation in naive head-fixed mice. By combining the presentation of oriented gratings with odors during the recording of large populations, we have established that 20% of layer 2/3 active cells are significantly modulated by olfactory stimuli, with an equal part of enhancement and suppression. This proportion is reduced to 10% after whisking abolition by section of the facial nerve. Under these two conditions, the presence as well as the identity of odors can be decoded from barrel cortex activity. Blockade of the cholinergic transmission does not affect the extent of the modulation or the amount of olfactory information available to the barrel cortex. Reciprocal connections with the perirhinal cortex have been studied, but their involvement could not be demonstrated. These results establish a new multisensory interaction and contribute to renewing how we think about the role of primary areas. The anatomical substrate as well as the functional role of this modulation remain to be determined.

Keywords: system neuroscience, multisensory, barrel cortex, olfaction