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Perceptual Strategies and Neuronal Underpinnings underlying Pattern Recognition through Visual and Tactile Sensory Modalities in Rats

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Abstract

The aim of my PhD project was to investigate multisensory perception and multimodal recognition abilities in the rat, to better understand the underlying perceptual strategies and neuronal mechanisms.

I have chosen to carry out this project on the laboratory rat, for two reasons. First, the rat is a flexible and highly accessible experimental model, where it is possible to combine state-of-the-art neurophysiological approaches (such as multi-electrode neuronal recordings) with behavioral investigation of perception and (more in general) cognition. Second, extensive research concerning multimodal integration has already been conducted in this species, both at the neurophysiological and behavioral level.

My thesis work has been organized in two projects: a psychophysical assessment of object categorization abilities in rats, and a neurophysiological study of neuronal tuning in the primary visual cortex of anaesthetized rats. In both experiments, unisensory (visual and tactile) and multisensory (visuo-tactile) stimulation has been used for training and testing, depending on the task.

The first project has required development of a new experimental rig for the study of object categorization in rat, using solid objects, so as to be able to assess their recognition abilities under different modalities: vision, touch and both together.

The second project involved an electrophysiological study of rat primary visual cortex, during visual, tactile and visuo-tactile stimulation, with the aim of understanding whether any interaction between these modalities exists, in an area that is mainly deputed to one of them.

The results of both of the studies are still preliminary, but they already offer some interesting insights on the defining features of these abilities.

Chapter 1: Introduction

1.1 On the Use of Rats as Research Subjects

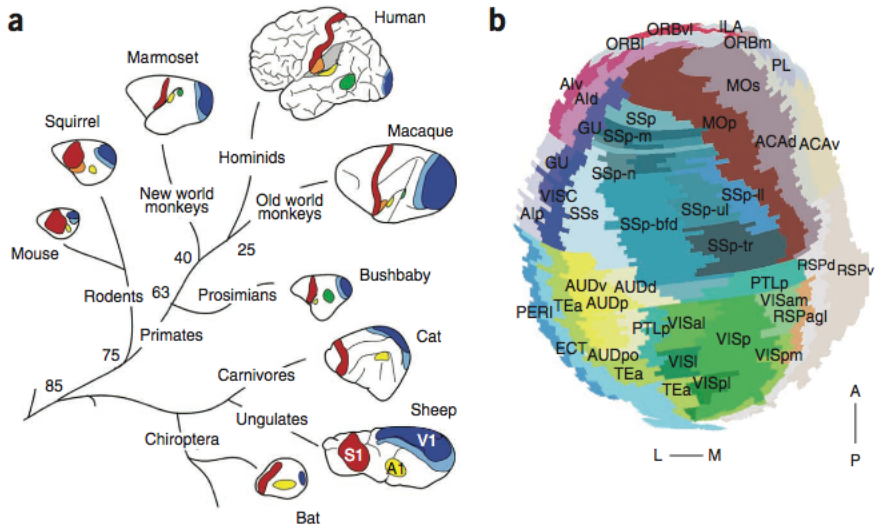
In psychology, the rat has been extensively used as an animal model to study spatial navigation and simple learning principles. During the past few years, the use of the rat as an experimental model has been broadened to studies of perceptual abilities and decision-making.

There are several important reasons at the root of this interest for rodents as animal models of cognitive abilities (Carandini & Churchland, 2013).

The main one is that they are not so distant from us on the evolutionary tree (Dawkins, 2004): our common ancestor dates back to 75 million years, 10 million years after the ancestor of other higher mammals (e.g., carnivores), and only 12 million years before the common ancestor of all primates (Fig. 1.1.A).

In practice, this means that rodents share many fundamental properties of the brain organization with other mammals, among which primates and humans (Krubitzer, 2007). The main similarity is a general common plan for cortex development and organization. Of interest to us is the fact that the rodent brain is organized in multiple areas, some of which are selectively responsible for processing of specific sensory information, according to the classical view (Fig. 1.1.B).

Figure 1.1: Evolutionary history and cortical areas in mammals.



A. Evolutionary tree and sensory cortical areas in mammals. Colors represent sensory areas in different species (V1, dark blue; V2, light blue; A1, yellow; S1, red; S2, orange). Numbers specify age of last common ancestor, in million of years.

B. Flattened map of the mouse cortex. Colors represent different functional areas, with sensory areas as in (A).

There are, however, two important caveats to this reasoning.

First, because of obvious anatomical differences, both at the brain and body level, it is not always possible to find a precise one-to-one mapping of these sensory areas between species.

Second, each species gives a different weight to each sensory modality, depending on its adaptation to the environment in which it lives. In practice, an animal will rely more on smell, vision or touch, depending on what are the more valuable stimuli in the natural selection process the species was subjected to.

Regarding the first caveat, the best answer is that, regardless of the anatomical differences, the working mechanisms that sustain the sensory processing might anyway be similar between species. This means that every new insight into one species adds a bit of information to the general knowledge about (e.g.) sensory processing.

Regarding the second caveat, different environmental constraints and evolutionary

pressure must be taken into consideration, when building research hypothesis and discussing any observed phenomenon. In the case of rodents, especially like mice and rats, it is indeed true that these species rely for many behaviors especially on olfaction and touch, and not mainly on vision. In fact, they use their olfaction during every interaction with conspecifics (D. G. Wallace, Gorny, & Whishaw, 2002) and are able to solve even "complex" tasks using it (Kepecs, Uchida, Zariwala, & Mainen, 2008), or their somatosensory ability with whiskers (Felsen & Mainen, 2012).This is reflected in the large amount of subcortical and cortical volume that is devoted to these senses: olfactory bulbs, barreloids in the thalamus and barrels in the S1 cortex (Petersen, 2007).

This does not mean that, as some might incorrectly argue, that they are simply nocturnal animals that do not pay any attention to the visual world around them. In fact, even if their spatial resolution is ~100 lower than that of primates (Huberman & Niell, 2011), their behavior is still widely influenced by vision, both in the natural environment and in the laboratory, when they are motivated or trained to do so.

In fact, rodents use vision as the main instrument to navigate in the environment, as has been proven in many studies (Chen, King, Burgess, & O'Keefe, 2013). More importantly, they are able to solve the "invariant problem" of vision, by correctly recognizing two-dimensional figures of three-dimensional objects, even when different visual transformations are applied on them (Alemi-Neissi, Rosselli, & Zoccolan, 2013; Tafazoli, Di Filippo, & Zoccolan, 2012; Zoccolan & Oertelt, 2009).

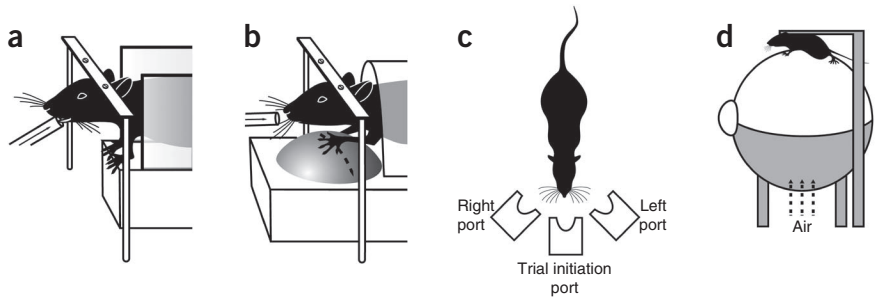
Apart from the evolutionary motivation for their use in research, there is a more practical one: rats and mice represent a flexible research model (Carandini & Churchland, 2013). First, nowadays, different techniques grant the researcher the opportunity to genetically manipulate the experimental subjects, especially in the case of mice. This means being able to genetically target the specific neurons of interest, and then observe and influence their activity through techniques like two-photons microscopy (Prakash et al., 2012), optogenetics (Peron & Svoboda, 2011; Scanziani & Häusser, 2009), and use of transgenic lines (Zeng & Madisen, 2012).

Second, the ease of practical organization and manipulation of these animals, especially when compared to primates, allows scientists to base their findings on a larger number of subjects and to avoid any interaction between different and successive experiments conducted on the same subjects. This is especially the case for monkeys and apes, where it is not uncommon to use the same subjects for different

subsequent experiments; interactions between these and possible influences are seldom if ever controlled or reported.

Moreover, thanks to their smaller physical dimensions, it is possible to use a variety of different experimental designs, sensory environments and physical apparatuses (Fig. 1.2). Experimental designs differ in the usage of one, two or more stimuli, paired with one, two or more responses. Experimental rigs, instead, diverge in the physical freedom left to the animal, the modalities under which stimuli are presented, and the ways the behavioral responses are collected. Depending on these choices, the link between the subject's perception and its observed behavior or neural activity will be more or less clear. On the other hand, the experimental rig and design will ultimately depend on the hypothesis to test and what aspect of the behavior and neuronal activity is necessary to observe.

Figure 1.2: Different techniques for rodent psychophysics.



A. Animal is head-fixed and indicates stimulus detection by interacting with a single licking sensor/reward spout.
B. As in (B), but animal interacts with a trackball to give different types of responses to stimuli.
C. Animal is free moving and interacts with three licking sensor/reward spouts to start a trial and give a response about stimulus identity.
D. Animal is head-fixed and walks on a floating ball to interact with images on screen.

Regarding the choice between rats and mice, instead, this strictly depends on the research hypothesis and on the methods used to confirm or falsify it. For example, from a genetic point of view, mice are more controllable, and are probably the better choice in studies centered on genetic manipulation. On the other hand, studying

perceptual decisions in rats offers many advantages, among which the ease of training, a wider spectrum of task complexity, and the possibility to collect larger amount of behavioral data. Moreover, different rat models of human diseases are currently being developed, among which autism (Umeda et al., 2010), schizophrenia (Chambers, Moore, McEvoy, Levin, & Andrew Chambers, 1996) and Alzheimer (Lecanu & Papadopoulos, 2013) , and any new information on these models is useful to better frame novel translational researches. For these reasons, the use of rodents as research subjects is, depending on the experimental hypothesis, one of the best tools to investigate core cognitive processes, such as perception.

1.2 Vision in Rats

1.2.1 Introduction

Until recent years, studying high-level vision in rats would have been considered an odd choice. This prejudice may sound surprising, considering how much work has been done in the past by researchers like Lashley (Lashley, 1938), Sutherland (N S Sutherland, 1968; N. S. Sutherland, Carr, & Mackintosh, 1962; N. S. Sutherland & Williams, 1969; N. Sutherland, 1961) and Dodwell (Dodwell, Ferguson, & Niemi, 1976; Dodwell, Niemi, & Ferguson, 1976; Dodwell, 1970) on the investigation of visual abilities in the rat. One possible reason is the established use of the rat as the animal model of choice for the study of tactile perception and decision making, which may have lead to an underestimation of his visual abilities (Diamond, von Heimendahl, Knutsen, Kleinfeld, & Ahissar, 2008; Fassihi, Akrami, Esmaeili, & Diamond, 2014).

Indeed, rats are particularly active under ground and in conditions of low luminosity, from sunset to sunrise, where their poor visual acuity might seem not enough to permit the use of vision (Douglas et al., 2005). Moreover, they lack a fovea (Euler & Wassle, 1995) and are unable to accommodate (Hughes, 1977, 1979). The retina is mostly composed by rods, around 99% of all photoreceptors (Green & Powers, 1982). Finally, their eyes are placed laterally, to grant them a panoramic field of view in front, to the side, above and behind the head (D. J. Wallace et al., 2013). These considerations likely led to the widespread belief that haptic abilities and sense of smell are the main sensory modalities used by rats to gather information about the environment.

However, the rat visual system may still be worth proper investigation (Sefton, Dreher, & Harvey, 2004). Indeed, visual acuity in pigmented rats, when measured both at the level of single neurons (Girman, Sauvé, & Lund, 1999) and in behavioral tasks (G. Prusky & Harker, 2002; G. T. Prusky, West, & Douglas, 2000), is still around 1.2 cycles per degree, making them suitable subjects in a variety of natural and experimental tasks (Forwood, Bartko, Saksida, & Bussey, 2007; Minini & Jeffery, 2006). Moreover, a small percentage of cones are present in the rat retina,

making both scotopic and photopic vision possible (Muntz, 1967). Rats are also able to perceive different colors, based on two types of cones (Szèl & Rohlich, 1992): 93% of these are tuned to 500-520 nm, in the blue-green part of the spectrum, while remaining 7% are tuned to 370 nm, in the violet-ultraviolet range (Akula, Lyubarsky, & Naarendorp, 2003; Jacobs, 2001). Finally, rats possess a theoretical binocular overlap of 40-60 degrees directly in front of them (Heffner & Heffner, 1992), which, together with head movement, could be used to retrieve the distance from objects in the environment (Legg & Lambert, 1990).

Regardless of this, the controversy about rat visual abilities is still open. Wallace et al. (D. J. Wallace et al., 2013) recently argued that the combination of low acuity and the lack of fovea and accommodation characterize the visual system of rats as fundamentally different from that of fovate mammals, rat vision being mainly specialized in detection and localization of potential dangers, by maintaining overlap of the monocular fields. Nevertheless, they still recognize the fact that rats are able to perform complex visual object recognition tasks, and that these disadvantages do not prevent rats from expressing detailed vision, binocular fusion and depth perception. They offer several explanations for this paradox, but no one conclusive.

The most probable hypothesis for this apparent conflict of results, is the experimental setup used by the authors: rats' performances were investigated during simple free roaming in an arena; there, they probably received very little pressure to use their visual ability for something different than overhead surveillance (D. J. Wallace et al., 2013). Rats are very adaptive animals, which naturally select the more economical way to perform a given task. This means that they will use their visual ability up to the maximum potential only if constrained by the environment or by the experimental design. In fact, visual object recognition is an "expensive ability": it requires a lot of cortical power and attention to handle the computations that are needed to recognize and coding an object, in spite of the transformations the object may undergo. This is true also in the case of other sensory modalities (as olfaction, touch, hearing), but in the case of visual modality, this process is even more difficult. The conclusion is that, only if the rat is put in a situation where its success is strictly dependent on the outcome of its visual abilities (as in object recognition tasks), will it exploit them to their maximum.

1.2.2 Primary Visual Cortex in Rats

The primary visual cortex of rats (V1 or area 17 or striate area) is located in the occipital cortex, in the most posterior surface of both hemispheres (Paxinos, 2004; Paxinos & Watson, 2007). It is the largest one between the seven visuotopically-organized areas found so far (Montero et al., 1981), and can be identified based on cytoarchitectonic analysis (Krieg, 1946a, 1946b).

V1 neurons can be distinguished between monocular and binocular, depending on if they receive inputs from one or both eyes, respectively. These neurons are roughly segregated in two different sub-regions of primary visual cortex: monocular neurons more medially (V1M) and binocular neurons more laterally (V1B). Binocular neurons constitute the majority, being around 80% of the total (Fagiolini, Pizzorusso, Berardi, Domenici, & Maffei, 1994; Sefton et al., 2004). Both of them show a retinotopic organization.

In contrast with what has been found in other species, no iso-orientation columns have been found in rat visual cortex (Ohki, Chung, Ch'ng, Kara, & Reid, 2005). Nonetheless, V1 neurons show a specific tuning to several features of the visual stimuli, like spatial frequency, temporal frequency, velocity, contrast, orientation and direction, as in higher mammals. The percentage of neurons with some form of orientation tuning is relatively high in V1, and the observed amount changes, depending on the study, from 60% up to 90% (see for former value: Ohki et al., 2005; see for latter value: Parnavelas et al., 1981; Girman et al., 1999).

These properties, together with the amount of background activity, have been found to change depending on the cortical layer. Superficial layer cells are usually more selective for stimulus parameter than deeper layer cells. Specifically, cells in layers II and III have been found to be more selective for orientation, to prefer high spatial frequencies and low temporal frequencies, and to have little background activity. Going deeper, cells in layer IV show less selectivity to orientation, a preference for smaller spatial frequencies and higher temporal frequencies, together with an increase in the background activity (Girman et al., 1999).

1.3 Touch in Rats

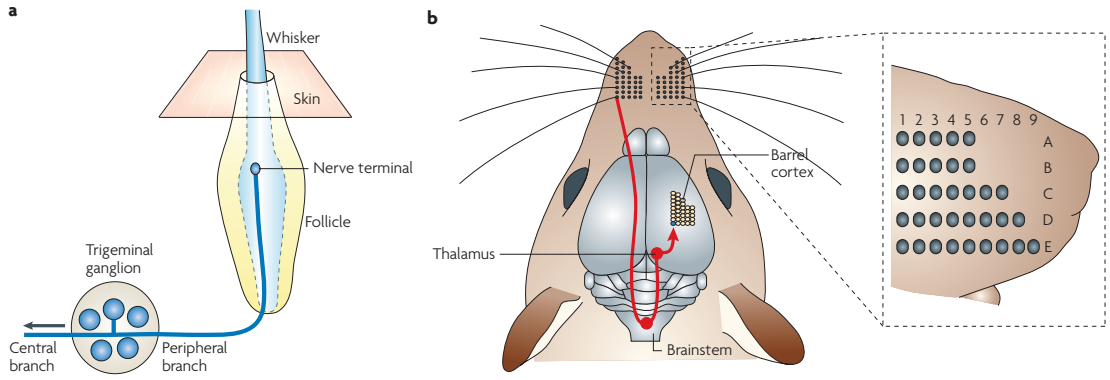
Rats are able to acquire tactile information through their paws, and use this in a variety of cases: feeding, fighting and interacting with the environment (I. Whishaw & Kolb, 2005). Simple observation of this ability is enough to define them as expert sensory processing organisms (I. Q. Whishaw & Coles, 1996). Nonetheless, most of the literature on tactile perception and cognition has focused its attention on the whiskers and not on the paws. Particularly, the long facial hairs, called macrovibrissae or commonly whiskers, have received more interest (Diamond & Arabzadeh, 2013; Diamond et al., 2008; Maravall & Diamond, 2014).

Rats possess a very organized array of long whiskers on their snout. These facial hairs differ from ordinary hair for several reasons, among which dimensions, structure, functionality and, especially, cortical representation (Fig. 1.3.A).

Whiskers are bigger than so-called "common hairs" with a size dependent on their position in the sensory array (Williams & Kramer, 2010) (Voges et al., 2012). Their follicle is highly innervated (Birdwell et al., 2007; Ebara, Kumamoto, Matsuura, Mazurkiewicz, & Rice, 2002), with receptors sending signals about the whisker movement to the brain stem and thalamus, and finally to the primary somatosensory cortex (Deschenes et al., 2005). They are able to acquire information about different features of whisker motion, like velocity, acceleration and position (Arabzadeh, Zorzin, & Diamond, 2005; Shoykhet, Doherty, & Simons, 2000).

In somatosensory primary cortex (S1), and specifically in a part of it called barrel cortex, each whisker has a direct and distinct cortical representation in a specific barrel (Fig. 1.3.B), a definite cluster of neurons (Woolsey & Van der Loos, 1970). This means, in neurophysiological terms, that it is easy while recording from a specific barrel, to correlate this activity with a specific tactile stimulation (Welker & Woolsey, 1974).

Figure 1.3: Layout of whisker sensory pathway.



A. Mechanoreceptors in each whisker follicle encode information about direction, velocity and duration of displacements and torques, due to external contacts.
B. This information arrives through brainstem and thalamus in primary sensory cortex, where a two-dimensional grid called barrel cortex encodes information singularly for each whisker.

Rats usually move these whiskers back and forth, in a movement commonly called "whisking" (Berg & Kleinfeld, 2003; Hill, Bermejo, Zeigler, & Kleinfeld, 2008), during normal locomotion and exploration (Hartmann, 2001; Mitchinson, Martin, Grant, & Prescott, 2007), with a frequency normally going from 3 to 25 cycles per second (Carvell & Simons, 1990). This movement is coordinated with those of the head and the body of the animal, and has a major role in the active sensing through which the animal locates and identifies stimuli in the environment (Ganguly & Kleinfeld, 2004; Knutsen, Pietr, & Ahissar, 2006).

This perceptual modality has been defined as "active" (Prescott, Diamond, & Wing, 2011) because of several findings. First, rats may be trained to modify some of its attributes, from frequency to amplitude, depending on the task. Second, head-restrained animals, nowadays widely used for in-vivo recording during behavioral task, do not show the usual pattern, but only a symmetric and synchronous one. Freely moving animals, on the opposite, have been observed to change timing, frequency and amplitude independently on both sides, depending on the context and the objective of the behavioral task to perform.

The literature contains numerous behavioral experiments addressing the role of whiskers, using several experimental designs and task, among which: gap

measurement and crossing (Harris, 1999), maze learning and navigation (Jenks, Vaziri, Boloori, & Stanley, 2010), detection, perception and elaboration of objects in space (A. Harvey, Roberto Bermejo, H. Phil, 2001). These experiments have shown that rats are able to use their whiskers to detect, orient to and track a meaningful stimulus in the space, and to discriminate between different types of textures (Itskov, Vinnik, & Diamond, 2011) and vibrations based on the tactile input (Diamond et al., 2008; Fassihi et al., 2014). Unexpectedly, shape and orientation discrimination has been poorly studied in rats, and apart from two studies (Brecht, Preilowski, & Merzenich, 1997; Polley, Rickert, & Frostig, 2005), the literature does not provide much information on this ability, and the roles of micro- and macro-vibrissae in this context.

1.4 Multisensory Integration

1.4.1 Introduction

Our environment can be just considered as information, transmitted through different types of energy, which each agent perceives and reacts to, to enhance its survival chances. The properties of this interaction depend on the aims of the organism and on how its perceptual and cognitive resources deal with affordances of the objects he may encounter. These affordances constitute the available information of the objects the agent may interact with and perceive through different ways of communication: the senses, each tuned to a different type of energy. The senses transduce the energy into neural signals trough specialized receptors. Each receptor is tuned to a specific range of stimuli.

Because of all the possible, different ecological niches in which organisms have adapted to live, sensory mechanisms and consequent capabilities greatly differ between the species. Usually, adaptive specializations either expand the dynamic range used by receptors, or change their role and function in the perception and behavior. The same environment is then perceived differently depending on the receptors used to sense it. What are in common, anyway, are the advantages that having different senses grant to multisensory species.

The environment is rarely discrete and unambiguous; most of the time it is noisy and continuously changing. Then, having different senses increases the probability of detecting and identifying any meaningful stimulus, because each sense is more reliable and successful in a different situation. Moreover, in case of "malfunction" of one of these senses, another can compensate for it.

The less obvious, but more important, advantage is the capability to use these senses synergistically, and combine the different sources of information in a unified percept: this integration usually unveils new features of the object and new ways to interact with it.

For these reasons, the ability to acquire information from different sensory modalities and use these bits to build a coherent multimodal percept constitutes, most of the time, a survival advantage: using in an optimal way different senses offers a

behavioral superiority to those organisms that are able to do it.

These are just some of the causes why these strategies, and the structures behind them, have been enforced from the beginning of the evolutionary run, up to the developments of the mammals. Indeed, they constitute one of the main reasons for the evolutionary success of the mammalian species (Stein & Meredith, 1993).

These hypotheses, and the investigations they encourage, are important not only because of the abstract knowledge they provide, but also because of growing evidence that various human cognitive disorders (schizophrenia, autism) show abnormal multisensory integration abilities (Foxe et al., 2013; Stone et al., 2011). Multisensory integration is the basis of several adaptive behavioral effects, and any deficit in it may represent the origin of some of the cognitive symptoms these patients show. Moreover, any information on cortical functional specificity may be useful for predicting and evaluating the result of sensory implants in humans, such as cochlear and retinal implants (Rauschecker & Shannon, 2002; Zrenner, 2002). Based on this, research on multisensory phenomena may provide insights not only about their working mechanisms, but also on ways to treat those cognitive and sensory impairments.

Thus, the study of how the mammalian brain integrates different sources of information under different modalities has always been of particular interest.

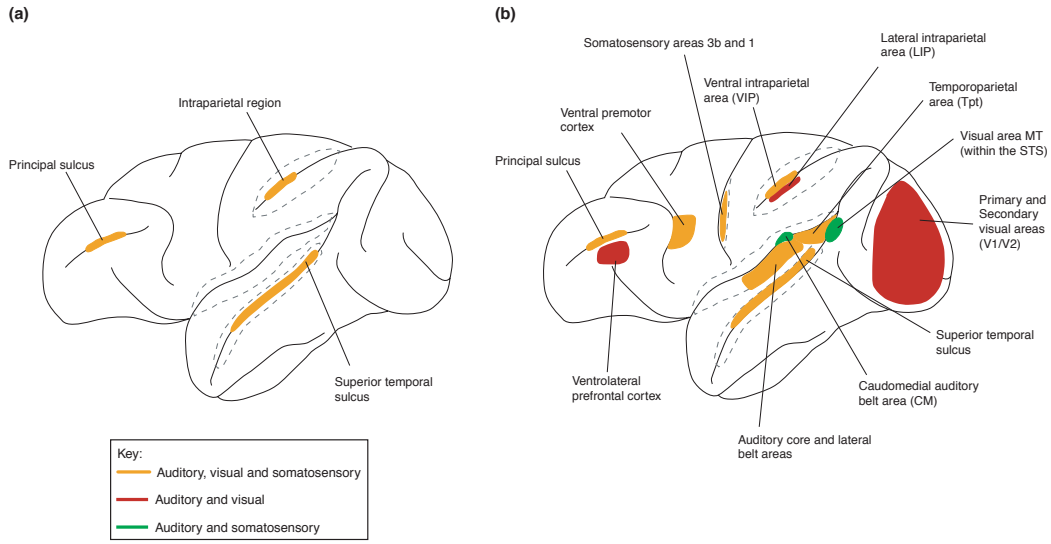
1.4.2 Multisensory Integration Models

The classical convergence theory of multisensory integration postulates a hierarchical model, composed of two levels of processing.

The first level is strictly unisensory, based on the assumption that each primary sensory area in the cortex is the final target of an isolated feed-forward communication through "labeled lines" from the sensory receptors. This notion originates from early neuroanatomical studies in monkeys and cats showing segregation between ascending sensory pathways (Kuypers et al., 1964), and from studies based on experimental lesions of discrete regions, which produced unimodal behavioral deficits (Massopust, Barnes and Verdura, 1965). Another support came form the absence of any strong anatomical links, at level of cortico-cortical communication, between primary sensory areas of different modalities (Jones & Powell, 1970).

The second level of processing was believed to take place only after this extensive unisensory processing was accomplished (Felleman & Van Essen, 1991). The multisensory integration is then postulated to happen only in higher-order associative areas. Some cortical areas were considered multisensory because of several reasons: 1) they have definite connections with primary sensory areas (Jones & Powell, 1970); 2) they show single neuron responses to multisensory stimulation (Bruce, Desimone, Gross, & Gross, 1981); and 3) in patients, lesions in these areas induce behavioral deficits that are attributable to an impairment of multisensory integration (Teuber, 1966). To date, the main cortical regions that, in primates, have been found to be involved in multisensory processing are the superior temporal sulcus, the intraparietal complex, and the frontal and prefrontal cortices. All of these regions are activated by auditory, visual and somatosensory stimuli, together or in any combination of two (Fig. 1.4).

Figure 1.4: Schematic of primate cortical anatomy.



A. Traditional scheme of multisensory areas in the primate brain.
B. Modern scheme of multisensory areas in the primate brain, based on anatomical and electrophysiological data.

This model has been challenged several times from its affirmation, in favor of a more widely distributed multisensory cortical processing (Calvert, Spence, & Stein, 2004; Lacey, Tal, Amedi, & Sathian, 2009; Pascual-Leone & Hamilton, 2001). In fact, the classical model was not able to account for many properties of multisensory processing (Driver & Spence, 2000). Multisensory integration, then, has been hypothesized to take place even before the higher-order associative areas (Cappe, Rouiller, & Barone, 2009; Falchier, Clavagnier, Barone, & Kennedy, 2002; Rockland & Ojima, 2003). Where this integration is supposed to take place (and to what extent) varies from study to study, with a spectrum going from a conservative notion that primary cortices have a only preference for a dominant modality, but are also capable of crossmodal processing (Driver & Noesselt, 2008; Sathian & Zangaladze, 2002), to a more radical one that all neocortex is in reality essentially multi-sensory (Ghazanfar & Schroeder, 2006).

Initially, feedback projections from higher associative areas to unisensory cortices were believed to be the only way of communication for crossmodal responses. In more recent models, the multisensory processing is supposed to take place at several levels of brain processing: 1) from thalamus to primary sensory areas, to higher-order associative areas (Miller & Vogt, 1984; Cappe & Barone, 2005); 2) at the level of

individual neurons or at the neuronal network level (Miller & D'Esposito, 2005); and 3) with communication including feed-forward, parallel or feedback mechanisms (Clavagnier, Falchier, & Kennedy, 2004; Foxe & Schroeder, 2005). All the differences in the observed mechanisms may be dependent on experimental modalities and task specifics.

Indeed, recent studies have found that crossmodal interaction may occur in primary sensory areas (Kayser, Petkov, & Logothetis, 2008; Martuzzi et al., 2007; C E Schroeder et al., 2001; Wang, Celebrini, Trotter, & Barone, 2008), at very short latencies (Foxe et al., 2000; Giard & Peronnet, 1999; Senkowski, Talsma, Grigutsch, Herrmann, & Woldorff, 2007). Effectively, direct, but sparse, connections between primary sensory cortices have been identified in many species, even in humans (Beer, Plank, & Greenlee, 2011).

If both higher-order association areas and hypothetical unisensory cortical areas are responsible for multisensory integration, up to a certain degree, then there must be a difference in how they are performing this task and what is accomplished by their operation.

Multisensory processes in association cortex may be responsible of building a complex representation of the external world, by linking together different aspects of a percept regardless of modality. Several findings seem to support this hypothesis: temporoparietal, lateral and ventral intraparietal areas possess a high degree of spatial correspondence between receptive field of stimuli coming form different senses (Andersen, Snyder, Bradley, & Xing, 1997; Schlack, Sterbing-D'Angelo, Hartung, Hoffmann, & Bremmer, 2005). Superior temporal sulcus and extrastriate areas seem to be activated for abstract concepts, like objects or motion, independent from modality (Charles E. Schroeder & Foxe, 2002). These characteristics are all needed to build a complete multisensory representation of the perceived stimulus.

Instead, low-level multisensory integration in primary sensory areas might be more limited in the purpose, and may be limited at influencing basic sensory perception, like simple detection of stimuli. One argument in favor of this is the absence of high spatial precision. Some examples are: 1) somatosensory input to auditory cortex lacks any precise somatotopic representation (Fu et al., 2003); and 2) connections between low-level unisensory auditory and visual areas are limited to the less precise peripheral visual field (Falchier et al., 2002; Rockland & Ojima, 2003). A second argument is the significant influence of temporal precision of multisensory

interactions in primary sensory areas. For example, the type of integration of auditory and visual stimuli during vocalization is strongly dependent on the interval between the two: less time produces a strong response, while longer time causes response suppression (Ghazanfar, Maier, Hoffman, & Logothetis, 2005).

1.4.3 General Principles of Multisensory Integration

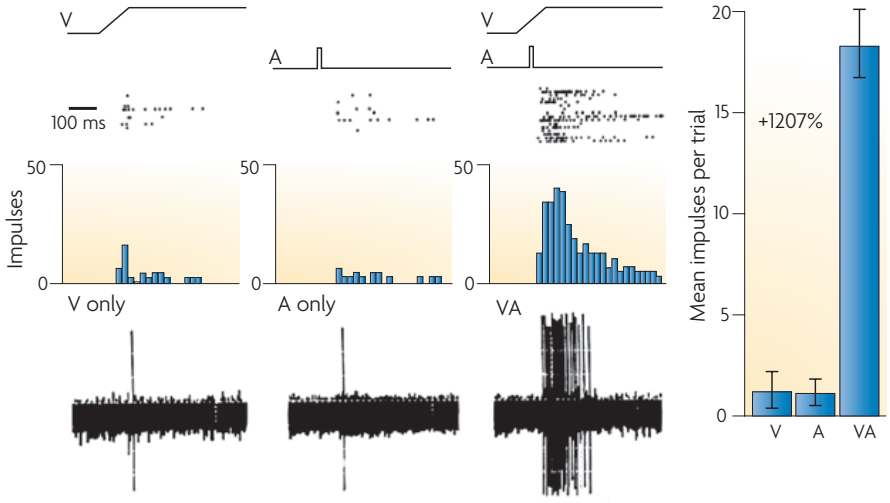
Multisensory integration refers to the mechanism by which information coming from two or more senses is combined, with two main consequences: it alters in some way the salience of the crossmodal events, and it produces a synergistic percept (Stein & Stanford, 2008). This new unitary experience is not just the sum of all its sensorial components, but it's someway a distinct and coherent form. The best example for human perception is the taste, which is built from the integration of gustatory, olfactory, tactile and visual stimuli (Auvray & Spence, 2008). This resulting sensation has its own dimension, as it's shown from the fact that consciously trying to separate its components may be difficult, if not impossible (Stein, Stanford, Ramachandran, Perrault, & Rowland, 2009).

This process may be rather complex: it has to consider the different information that is present in each modality, the fact that subjective impressions of them are not always translatable between modalities, and must be able to maintain these defining features during the integrative process. This act, often defined as "binding", is dependent on the animal's subjective development: it may need a prior experience with crossmodal combinations of different stimulations, during or not a specific critical period. Moreover, it has to tune the different sources' weights depending on how much information they add about the event under analysis (Stein, Stanford, & Rowland, 2014).

This process takes place in the mammals' brain seemingly without effort, and it's then easy to underestimate its complexity. In reality, every time the brain is confronted with two or more sensory signals, it must decide if and how integrating them, based on several conditions. Some of these are: 1) the stimuli have a common origin; 2) there are differences in their reference frames; 3) their relationship in the time dimension; 4) any previous experience on them; and 5) their reliability. Moreover, depending these on features, the outcome of the integration may severely change.

This process is usually investigated by quantifying the effect of crossmodal stimulation on an organism, and comparing it to those separately produced by its component unisensory stimuli (Fig. 1.5). This means finding any significant difference, between the response evoked by crossmodal stimulation, and that coming from the most effective of the unisensory stimulation (Meredith & Stein, 1983).

Figure 1.5: Multisensory enhancement in a single superior colliculus neuron.



- ***Left**: from top to bottom, visual (V), auditory (A) and combined (AV) stimuli, with relative raster plots, PSTH and single-trace oscillograms.*
- ***Right**: mean response of superior colliculus neuron to different stimuli, showing multisensory enhancement.*

At the level of neuronal activity, this means comparing between the number of impulses or the firing rates evoked by unisensory stimuli and their combination (Stein & Meredith, 1993). This multisensory integration may produce either enhancement or depression of the response, and its magnitude may greatly vary between different neurons, and even between different combinations of different sensory stimuli for the same neuron (Diederich & Colonius, 2004; Gillmeister & Eimer, 2007).

Together with these changes, the integration may decrease the latency of the multisensory response, and the time interval between sensory encoding and the eventual motor-command formation (Bell, Meredith, Van Opstal, & Munoz, 2005; Rowland & Stein, 2007). Then, at the level of behavior, investigating multisensory integration means analyzing any performance enhancement or decrease, by finding differences in speed and accuracy of detection, localization and identification of stimuli (Fetsch, Pouget, DeAngelis, & Angelaki, 2011; von Saldern & Noppeney, 2013).

Regarding physiological studies of multisensory neurons, much of the research has so

far been conducted especially in the midbrain and cerebral cortex of cats and monkeys, but in the last years rodents have slowly became another good model for this investigation (Raposo, Sheppard, Schrater, & Churchland, 2012; Sheppard, Raposo, & Churchland, 2013).

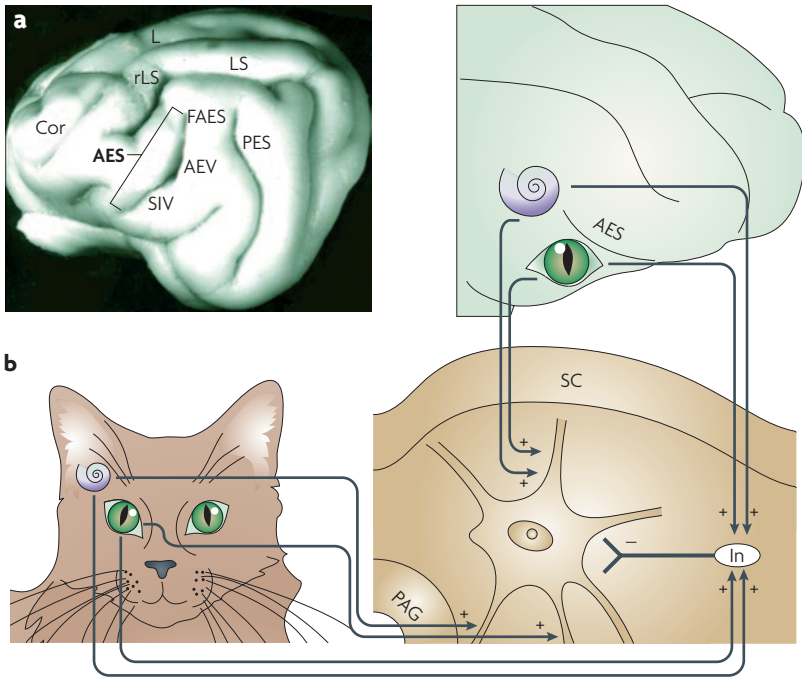
Depending on the species, different brain areas have been identified as models for studying multisensory integration, with the aim of finding common principles of working. These principles may not always be species-specific and region-specific, but just dependent on the function they support. Then, the different evolutionary histories, sensory capabilities and experiences must be taken into consideration when building the framework in which discussing any finding. Anyway, our hypothesis is that similar computational goals might produce similar integrative principles, to be weighted in such a framework.

1.4.4 Multisensory Integration in the Superior Colliculus of Cat

Many of the studies that have investigated the mechanisms of multisensory integration, its behavioral consequences and its development, started by using the superior colliculus of cats as a model (Fig. 1.6.A). This area constitutes a good model for studying these topics and highlighting some of the major principles of this phenomenon, which seem to be common among different mammal species (Chalupa & Rhoades, 1977; Dräger & Hubel, 1975; Knudsen, 1982).

The deep layers of the superior colliculus are particularly rich of neurons deputed to multisensory integration, and are responsible of a definite behavioral response: detecting and localizing external events (Fig. 1.6.B), and orienting toward them (Burnett, Stein, Perrault, & Wallace, 2007; Groh & Sparks, 1996; Jay & Sparks, 1987a, 1987b). This behavior shows clear steps of maturation in the life of the animal, permitting to investigate the development of any multisensory integration (Norton, 1974). Moreover, because of the high percentage of multisensory neurons, targeting them trough electrophysiological technique is not difficult. More importantly, these neurons also project to brainstem and spinal cord, to produce the orientation behavior: it is then possible to observe the direct effect of neuronal activation on the behavioral response (Goldberg & Wurtz, 1972a, 1972b; R H Wurtz & Goldberg, 1972; R.H. Wurtz & Goldberg, 1972).

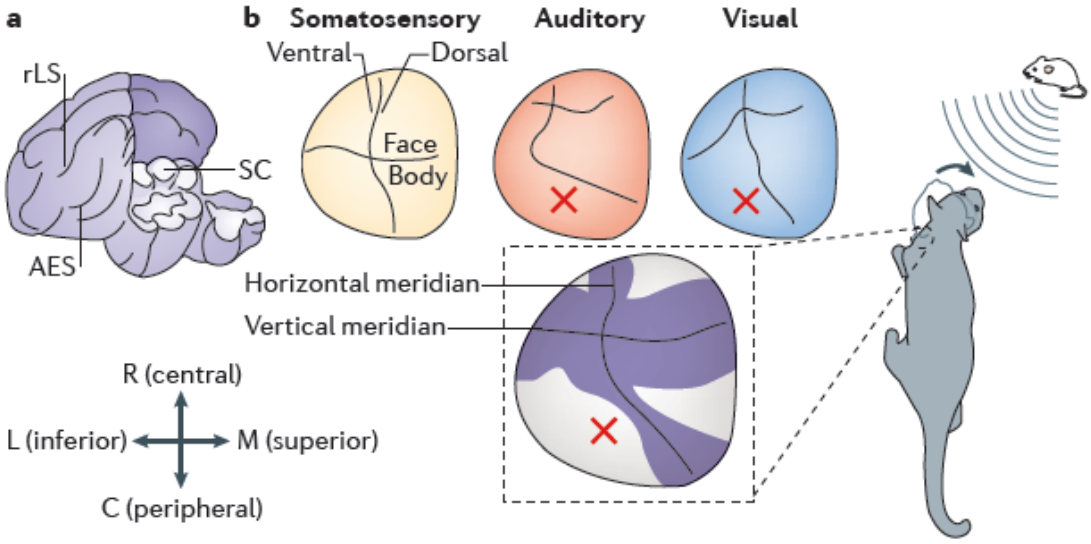
Figure 1.6: Superior Colliculus of cat and multisensory integration.



A. Lateral view of the cat brain, highlighting relationship of anterior ectosylvian sulcus (AES) with different sensory areas: somatosensory (SIV), visual (AEV) and auditory (AEV).
B. Schematic of visual and auditory integration in a superior colliculus neuron, with information coming from ascending (sensory receptors) and descending areas (AES).

The most relevant feature of multisensory neurons in the superior colliculus, is that they are characterized by multiple excitatory receptive fields, one for each sensory modality to which they respond to (visual, auditory and somatosensory; Fig. 1.7). These receptive fields are developed in a way that permits them to be overlapping, in register with each other, so that neuronal activation for an event is more dependent on its location, than on its modalities (Meredith & Stein, 1990, 1996). These sensory maps overlap with a common motor map, so that a salient event could in turn elicit the appropriate multisensory cortical and behavioral responses (Stein & Meredith, 1993).

Figure 1.7: Organization of receptive fields of neurons in Superior Colliculus of cat.



A. Location of superior colliculus (SC) and association cortex (AES and rLS), from which SC receives cortico-collicular inputs.
B. Overlapping multisensory topographic map (shown in grey) of somatosensory, auditory and visual representations (shown at top) during orientation to external events (purple regions show variations in meridians' orientation between sensory maps).

The research on superior colliculus has identified three general operating principles of multisensory integration, depending on which the result will be either multisensory enhancement or multisensory depression. These principles must be interpreted in the light of this area's purpose, which is to detect an external stimulus, localize it in the external environment and direct physical attention toward it. Any change in the neuronal activity, because of multisensory integration, must then increase the success of this area in performing this function (Cuppini, Ursino, Magosso, Rowland, & Stein, 2010; Patton & Anastasio, 2003).

First, the "spatial principle" deals with the physical location of the stimuli: crossmodal cues close in space generally increase the response of multisensory neurons, and also the opposite is true. This spatial proximity strictly depends on the receptive field map for every sense.

Second, the "temporal principle" deals with the temporal distance between the stimuli: any integration is dependent on the proximity in time of the stimuli. The time

window where this integration takes place may be comparatively long, to enable this process regardless of the different response latency and stimulus onset, for each modality. The response magnitude is then affected by the temporal overlap of the responses, and it is usually higher when their peaks are almost simultaneous.

Third, the "principle of inverse effectiveness" claims that multisensory enhancement is inversely related to the effectiveness of the individual stimuli that are being integrated. This principle usually shows up as a translation from superadditivity (more than the sum of the unisensory responses) to subadditivity (less than the sum of the unisensory responses), as the unimodal stimuli become stronger and more salient. The aim is to increase the probability of detecting stimuli that are weak in the two or three separate modalities. This mechanism has probably been selected as an optimal solution to deal with noise, both in sensory world and in the brain.

These three aspects of multisensory interaction, space, time and effectiveness, are strongly intertwined, and it is highly difficult to manipulate one without influencing the others.

Finally, as said before, all these principles may only be typical of the superior colliculus: their organization may then not always be present in other species and brain areas, and for functions much different from this one.

1.5 Crossmodal Perception and Cognition

1.5.1 Introduction

Inside multisensory integration, a specific field of research has been the study of crossmodal perception and cognition. This topic can be addressed in two ways. First, by studying neuronal activation in primary sensory areas in response to crossmodal unisensory stimulation. Second, by investigating it at the behavioral level, and, specifically, the ability of a subject to recognize an object across sensory modalities.
Both these lines of research have been developed on humans, but it is the work done on non-human primates, and recently on rodents, which produced information about the neurobiological bases of this phenomenon.
Different designs have been tried to assess this capability, and the most successful one in non-human primates, so far, has been the Crossmodal Matching to Sample (CMMS): the subject has to correctly recognize an object in one modality that matches the sample object, previously presented in another modality. Achieving a correct performance requires the subject to learn the features of the object that are significant not only in one modality, but also helpful for the recognition in in the other. Depending on the design, subjects are trained to select either the matching or the not-matching object, to receive a reward.
Every design that involves tactile recognition with real objects, and so manipulation of those, involves several practical drawbacks. The main one, anyway, is the high number of stimuli needed to exclude any general effect due to objects' intrinsic characteristics on the results. The most common solution to this problem, so far, has been the use of a numerous set of "junk" objects, of similar size and characteristics, to permit enough variety between series of trials.
Most of the initial studies involved a simultaneous presentation of the stimuli in the two modalities. The solving strategy usually varies depending on the number of items used. For a small set, a simple memorization of the rewarded couples of crossmodal stimuli is usually enough to solve the task in an acceptable way. For a bigger set, a more complex feature-by-feature comparison is needed, but the subject may avoid storing any information between trials, apart from the rule of the task.

The adding of a variable delay between different phases of testing, paired or not to manipulations like lesioning a specific area, permit the researcher to study memory involvement in the task, and to investigate which areas are responsible of perceiving, coding, storing and retrieving the multisensory representation.

1.5.2 Crossmodal Tasks in Non-Human Primates

Early studies on crossmodal object recognition in non-human primates, especially monkeys, were not very successful, often yielding negative results about the capacity of these animals to use crossmodal recognition (Burton & Ettlinger, 1960; Ettlinger & Blakemore, 1966; Wegener, 1965).

The first research to find positive results was conducted by Davenport and Rogers on apes (Davenport & Rogers, 1970). In their study, subjects were trained to solve a CMMS task, with both objects present at the same time. Specifically, apes were trained to view a sample object behind a transparent panel, while touching, at the same time, two other stimuli, which were visually hidden, and to select one of these two to be rewarded. Following this phase, subjects were tested with new objects and in both crossmodal directions: visual-to-tactile and tactile-to-visual (Davenport, Rogers & Russell, 1973). In successive experiments, apes were found to be able to solve the task even with delays between the sample and choice tasks, ranging from zero to twenty seconds (Davenport, Rogers & Russell, 1975).

The discrepancy of the results between these experiments on apes and the previous ones on monkeys was initially believed to be just due to species differences. It was later found that the difference was instead in the design used in the task (Cowey & Weiskrantz, 1975).

Previous research on monkeys, in fact, was mainly conducted using a "transfer task": there, subjects were extensively trained to select one among two presented objects in one modality, to receive a reward; after this training phase, they were moved to a testing phase, where they had to perform the same task with the same objects, but in another modality.

The lack of any crossmodal effect, like immediate or fast learning, in the main group, when compared to a control group with new objects, may be due to different reasons. The most probable ones are: 1) the simple fact that subjects were not explicitly trained to associate stimuli across modalities, and do not do it instinctively in a high demanding cognitive task; and 2) the more extreme assumption that crossmodal tasks use distinct neural systems from the ones used for unimodal recognition.

A support to first argument came from Jarvis and Ettlinger study (Jarvis & Ettlinger, 1977), which trained monkeys in a crossmodal object recognition test with junk

objects: this time, subjects were first trained in a discrimination phase with both modalities (vision and touch), and only after this moved to the usual crossmodal phase. According to the authors, this manipulation ensured that subjects correctly understood the requirement of the task. Another hypothesis, anyway, is that subjects actually need a prior experience with multisensory reasoning, to be able to link information in two different modalities to solve complex cognitive tasks. Regardless of the reason, monkeys showed a performance in crossmodal tasks comparable to the one found previously in apes (Bolster, 1978; Murray & Mishkin, 1985).

This is just one of the many examples in literature, demonstrating how a task must be carefully designed to deal with the species' perceptive and cognitive characteristics and resources.

1.5.3 Crossmodal Tasks in Rodents

Study of crossmodal object perception and recognition in rodents started just recently, probably because of the difficulties in studying these topics in primates.

From the literature, it is evident that researches approached this topic from several points of view, using disparate task designs: 1) detection of level of intensity of a visual and/or an auditory stimulus (Over & Mackintosh, 1969); 2) discrimination of the degree of thickness of a string, using tactile and olfactory cues (Tomie & Whishaw, 1990); and 3) recognition of the correct well to dig, based on tactile texture and olfactory cues (Botly & De Rosa, 2012), just to name a few. All these studies found that rats are indeed capable of performing judgments based on multisensory integration, but, because of their peculiarities, were hardly comparable with the studies performed on non-human primates.

An important step forward in this direction was the implementation of the "spontaneous object recognition" task in rodents (SOR). This gave researcher the opportunity to compare any finding with previous studies on other species, and to study perception and recognition of real three-dimensional objects (Ennaceur & Delacour, 1988).

The SOR task is usually divided in two phases. The first one is the sample phase, during which subjects are free to explore two identical objects, in a specific modality, depending on the research hypothesis. The second one is the choice phase, which takes place after a variable delay, from zero seconds to several days, and during which subjects are free to explore the familiar object paired with a novel one. The underlying mechanism is the rats' preference for unfamiliar objects, which usually produces a difference in the exploring times, in favor of the novel object. This task is usually carried out in an open arena, divided into quadrants, but other researchers prefer to use a Y-maze (Forwood, Winters, & Bussey, 2005; Winters, Forwood, Cowell, Saksida, & Bussey, 2004): this design limits the influence of spatial cues and better defines the choice of the subject and the way it explores the objects.

Usually, the tactile modality is investigated using a red light to illuminate the object, to only permit the tactile exploration. Instead, the visual modality uses visible light and a transparent panel, to only enable the visual exploration of the object.

The independent variables are often the time spent in exploring an object, usually

acquired either by simply recording where in the arena the subject is roaming, or from the distance between the subject and the object, and the direction he is facing. In some cases, and only when the Y-maze rig is used, researchers are able to obtain a someway-discrete response: the explicit answer of choosing one arm of the maze.

Thanks to this design, several studies found that rats are capable of using a tactile representation of an object to correctly recognize the same object when presented in the visual modality, and this even with a delay of one hour between the sample and choice phases (Reid, Jacklin, & Winters, 2014; Winters & Reid, 2010). Moreover, this crossmodal recognition ability was found to be unidirectional: performance in the visual-to-tactile condition was on chance.

On important aspect of this task is the use of junk objects as stimuli, so that each trial is performed on new ones. This characteristic of the task permits the researchers to conclude that the observed crossmodal performance relies on the rats' ability to find the intrinsic geometric similarities between the tactile and visual features of the sample objects, like some aspects of the shape or the texture. This may also mean that the coded neural representation was not strictly multisensory, but more like a unimodal percept composed by features translatable in another modality.

For this reasons, in a new task the unisensory phases were preceded by a multisensory pre-exposure phase (PE-CMOR), in which subject were free to inspect the object in every modality, and so either to build a multisensory representation or to acquire rules of translation between modalities. This modification had as a result the facilitation of PE-CMOR performance across longer delays, up to 24 hours (Reid, Jacklin, & Winters, 2012).

Another improvement to this class of designs was the introduction of a bow-tie maze as experimental rig (Albasser, Poirier, & Aggleton, 2010). This is composed by two sides, each divided in two parts: two objects are placed in each of these parts, each object baited with a sucrose pellet; the subject has to explore the two sides one after the other. The task, as the previous ones, exploits the rats' natural tendency to explore novel objects, but, in contrast with others, permits a continuous collection of trials. Albasser (Albasser et al., 2011) conducted each of the task phases in the dark or in the light, to study crossmodal and unimodal recognition. They found that not only rats were able to do the task in both conditions, but also that crossmodal recognition was bidirectional, meaning that it worked form visual-to-tactile and vice versa. The most probable explanation of this discrepancy with previous studies is the fact that rats

were not prevented from touching the object also in the light condition. Moreover, it has to be noted that olfaction was never considered in neither of these studies, as in the previous ones.

1.5.4 Limits of Spontaneous Object Recognition Task

Many of the studies previously shown have used spontaneous one-trial object recognition task: these tasks use the subjects' unconditioned preference for a novel stimulus to prove if a representation of a previously explored object, the familiar one, has been stored or not, and if this representation matches the familiar object during the second exploration. If it is the case, there will be a significant difference in exploration time during the choice phase, between the familiar and the novel stimuli, in favor of the latter (A. Ennaceur & Delacour, 1988; A. Ennaceur, Michalikova, & Chazot, 2009; A. Ennaceur, 2010; A. Ennaceur, Neave, & Aggleton, 1997; D. G. Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002; D. Mumby, Pinel, & Wood, 1990; Winters, Saksida, & Bussey, 2008).

These experiments usually use a collection of junk objects, to avoid any repetition of the same stimuli: this way, in the intentions of the researchers, there is no need to check the specific features of the objects that were used. This reasoning underestimates the influence that the intrinsic characteristics of the objects have on their recognition. In fact, each object possesses its own affordances, defined as the relationships between its properties and the perceiving abilities of the animal. Depending on this, every interaction, and its outcome, may greatly vary (Norman & Eacott, 2004).

These object affordances influence what the animal is able to perceive, recognize and memorize: some objects may be seen from the animals as more interesting, or dangerous, or with characteristics that are difficult to remember in one modality but not in another one. These differences will all have their influence on the recorded subjects' performance (Chemero & Heyser, 2005).

Moreover, one specific affordance is rarely checked in thee experiments: objects' specific odor and interaction with rats' olfaction. The usual methods of using different copies of the same object or cleaning it between the sample and choice phase, doesn't eliminate the influence that such a modality has on the animal's performance. This is even truer in rodents, which use this sense as the primary one for important aspects of their life, like foraging and mating.

To complicate this situation even more, recent studies reported that, in several situations, novelty preference instinct is replaced by the opposite tendency, a

preference toward the familiar object (Houston-Price & Nakai, 2004; Richmond, Colombo, & Hayne, 2007). This has been found in rats both in a free exploration task and in an object recognition task (D. G. Mumby et al., 2002; D. G. Mumby, 2001).

Moreover, the time window during which this novelty preference effect can be seen is usually very short. After it, the animal may devolve his attention to either the familiar object or the preferred one because of its peculiarities; this difference is usually not taken into consideration. This argument practically means that every analysis must be greatly constrained in time to be reliable. The novelty preference is a brief phenomenon, which lasts until the animal has encoded the properties of the new stimulus.

To conclude, the most critical fact motivating this thesis is that this type of research is usually unable to add any information, regarding the way these animals perceive and encode the stimuli, both unimodally and crossmodally. One-trial object recognition involves the encoding of an object representation, to later compare with another one. What it is tested is just if and where in the cortex these representation are compared within or between different modalities, providing no information about how the building of such a representation is done.

1.5.5 Differences between Visual and Haptic Object Recognition

Visual and haptic object recognition differs in many aspects, among which speed and accuracy of recognition. These aspects are dependent on many parameters: 1) the interaction between object's features and different sensory perceptual capabilities (Helbig & Ernst, 2007; Helbig et al., 2012; Takahashi & Watt, 2014); 2) different sensory memory capacities and precision (Bliss and Hämäläinen, 2005); 3) object familiarity and expertise (Tarr & Pinker, 1989; Bulthoff & Newell, 2006); 4) individual differences in imagery and modality preference (Lacey & Sathian, 2011); and 5) aim of the task and instructions about it, if any (Lederman & Klatzky, 1987).

In humans, object categorization has been extensively studied in both modalities, separately, crossmodally and together (Kassuba, Klinge, Hölig, Röder, & Siebner, 2013).

An important distinction has to be made between use of featural and configural processing strategies in the two modalities. One of the most studied cases in this regard, has been the ability in recognizing faces. To start with, many studies have shown that, regardless of the familiarity and expertise, visual face recognition is always more successful than haptic face recognition (Dopjans, Bulthoff, & Wallraven, 2012; Kilgour & Lederman, 2002). This difference probably relies in the different strategy enforced by these modalities.

Haptic exploration, in fact, uses a featural strategy: this is a sequential processing of the object's parts or attributes, which then must be reconstructed into the object (Lederman & Klatzky, 1987; Loomis, Klatzky, & Lederman, 1991). Sometimes coding of just one feature is enough to solve the task, but in others, as is the case with face recognition, these features must be reconstructed in one coherent percept.

Visual perception, instead, uses a configural strategy: this refers to the parallel processing of the objects' features, together with the object's structure in which these are placed (Nassi & Callaway, 2009). This is a holistic perception, in which salient elements and their organization are coded in concert.

The hypothesis that any difference in the two modalities performances relies in the different used strategy, has been supported by a recent study by Dopjans (Dopjans et al., 2012): the authors restricted the visual access to a face through a moving window, which participants controlled to inspect a face, a small portion at a time. In this case,

recognition performances in the two modalities were similar.

A second aspect to take into consideration is view-dependence. Every recognition action must be able to deal with changes in the object orientation. Usually these modifications have a significant effect: in the visual modality, they change the retinal pattern, while in the tactile modality the haptic sensations. Being able to recognize this objects regardless of these transformations is one of the most important goals of every sensory system. In vision research it is called "the invariance problem" (DiCarlo & Cox, 2007; DiCarlo, Zoccolan, & Rust, 2012).

Researches have found that both modalities show view-independent or view-dependent characteristics, depending on several conditions: the more relevant are the familiarity with the object (Newell, 1998; Vuong & Tarr, 2006) and the presence of diagnostic features (Lawson, 1999; Michael J. Tarr, Bulthoff, Zabinski, & Blanz, 1997; Wilson & Farah, 2003). Also rotating an object away from its default orientation may disrupt its recognition (Peissig & Tarr, 2007). For visual exploration, this impairment is strictly dependent on the axis in which rotation is performed and on the amount of it (Gauthier et al., 2002). Instead, in haptic exploration, this effect is the same for every axis of rotation (Lacey, Peters, & Sathian, 2007).

The constancy in perceiving an object must be maintained not only across orientation changes, but also in size. Visual system is able to deal with this issue, by elaborating the change in retinal images together with other cues, coming from depth or motion perception (Biederman & Cooper, 1992). Haptic system, instead, must integrate between cutaneous (contact area and force) and proprioceptive (finger spread and position) information during the contact (Berryman, Yau, & Hsiao, 2006). Both modalities may then possess a preference for canonical size for familiar objects (Konkle & Oliva, 2011).

Moreover, canonical views in two modalities rarely coincide. Preferred view in vision is the one where the object is aligned 45 degrees to the observer (Palmer, Rosch & Chase, 1981), while for touch it is aligned either parallel or orthogonal to the body midline (Woods, Moore, & Newell, 2008).

These differences may be particularly important in the interpretation of results coming from crossmodal experiments, where the presentation of one stimulus may be optimal in one modality, but not in the other (Woods, O'Modhrain, & Newell, 2004).

A final aspect is that all these objects' properties are differentially weighted in each modality, depending on the differential perceptual salience (Helbig et al., 2012;

Takahashi & Watt, 2014). For example, shape information is usually more influent than texture in visual categorization, while in haptic categorization they either have the same weight or are reversed in the priority (Klatzky, Lederman, & Reed, 1987). This may be one of the reasons why, in humans, crossmodal performance in visuo-tactile direction is usually better than in tactile-visual one (Jones, 1981; Streri & Molina, 1994; Lacey & Cambell, 2006). A sensory system, as a matter of fact, will select the type of information which is better for it, and not always for another sense: shape information, for example, will be discarded in favor of either texture or even hardness of the object. Especially in the last case, this difference will be evident: this is in fact an attribute perceivable only by haptic modality for static objects.

During simultaneous perception, instead, the two modalities may still weight differently the stimulus properties, but in the end they might be able to combine these on the basis of maximum likelihood estimates (Ernst & Banks, 2002; Helbig & Hernst, 2007).

1.6 Physiology of Crossmodal Perception in Rats

1.6.1 Areas Involved in Crossmodal Perception

1.6.1.1 Prefrontal cortex

The prefrontal cortex constitutes a known multisensory convergence area, and its involvement in crossmodal recognition has been deemed to be highly probable (Ongür & Price, 2000; Uylings, Groenewegen, & Kolb, 2003).

Reid (Reid et al., 2014) have investigated this area using the CMOR task, and have found that bilateral PFC lesions produce a selective impairment of the crossmodal recognition ability, in both directions, leaving intact the unimodal visual and tactile recognition performances. Moreover, these lesions negate any benefit from the multisensory pre-exposure phase to the crossmodal recognition.

Further investigations have then been conducted to better characterize the separate components of this area, by selectively lesioning medial prefrontal cortex and lateral and ventral regions of orbitofrontal cortex (OFC). First, it was found an involvement of the OFC just in the mnemonic part of the crossmodal processing. Second, and more important, performance in PE-CMOR task was unscathed for lesions of only one of these areas, showing a cortical form of compensation.

These results again show how selectivity of processing and cortical plasticity are defining features of the brain, which make sometimes hard to interpret any result coming from lesioning studies.

1.6.1.2 Parietal cortex

Much of the parietal lobe consists of association cortex: its regions receive input from many unimodal areas, and have been found to be responsible for multisensory integration (Reep et al., 1994; Reep & Corwin, 2009). The area that has received more attention in regard to this is the posterior parietal cortex (PPC) (Lippert, Takagaki, Kayser, & Ohl, 2013).

A study by Tees et al. (1999) showed that PPC in rats is responsible for linking visual and auditory spatial information: rats trained to orient themselves in a water maze-based task, by using either auditory or visual place-object pairing, were unable to do it after a bilateral PPC lesion.

Another study by Winters and Reid (Winters & Reid, 2010) found that rats with bilateral lesion of PPC were impaired in a CMOR and tactile unimodal task, while performance in unimodal visual task was maintained. One possible conclusion is then that PPC is especially involved in the tactile processing of stimulation, needed for crossmodal recognition.

1.6.1.3 Temporal lobe

The temporal lobe contains several areas implicated in crossmodal processing: perirhinal cortex (PRh), hippocampus, amygdala.

The role of the rhinal cortex in integrating information within and across different modalities, for object representation and recognition, has been proved in different species, from humans to monkeys to rodents (Bartko, Winters, Cowell, Saksida, & Bussey, 2007a, 2007b; Murray, Bussey, & Saksida, 2007; Winters & Bussey, 2005; Winters et al., 2004, 2008).

In rats, a study by Winters and Reid (Winters & Reid, 2010) found that bilateral lesion to PRh produce an impairment in the CMOR task and in the unimodal visual task, but not in the unimodal tactile task. This result, also found by Albasser (Albasser et al., 2011), states that PRh contribution in CMOR task is especially limited to visual processing.

1.6.1.4 Cortical Interactions

The binding of information between different modalities depends on the requirements of the undergoing task. Integrating between modalities, and so building a multisensory representation, may not be needed in the case of a crossmodal judgment: the regions of the cortex responsible of the unimodal response may just communicate between them, or even influence each other's neuronal activity, without storing any multimodal percept in a polymodal region of the brain (Lacey et al., 2007; Reid et al., 2012).

In the Winters and Reid study (Winters & Reid, 2010), parallel contributions of PRh and PPC were found respectively in the visual and tactile processing. These areas may either interact between them or with one or more multimodal areas, to solve the CMOR task. Hippocampus was initially believed to be one of the most probable candidates as an associative area, but bilateral lesions of it have failed to influence rats' performances in both CMOR and PE/CMOR tasks. Lesions in the connections between the two areas, instead, produced a selective impairment of the CMOR task, leaving unimodal task unaffected. Interestingly, this effect was delay dependent: no decrease in performance was found for absence of delay, but was present with one-hour delay.

So far, no conclusive results have been found to completely define the mechanisms of binding in the cortex, and many questions remain open.

1.6.2 Relevant Studies on Areas Involved in Crossmodal Perception

In the last years, several studies have started to challenge the established paradigm for strictly unisensory primary cortices (Driver & Noesselt, 2008; Stein & Stanford, 2008). Even though pure crossmodal responses have rarely been found in primary areas, the activity of these areas has been found to be modulated by a concurrent activation of a different sensory modality. These crossmodal modulatory effects act on the spontaneous and evoked activity of the recorded area, and are mainly composed by sub threshold responses (Bizley, Nodal, Bajo, Nelken, & King, 2007; Ghazanfar et al., 2005).

These crossmodal stimulations usually provoke a phase resetting of local network

fluctuations mainly in supragranular layers. Depending on the timing of the two different stimulations, the consequent neuronal activity may be suppressed or enhanced. Up to today, most of the studies have reported a suppressive effect on the dominant sensory activity in case of a crossmodal concurrent stimulation (Kayser et al., 2008; Lakatos, Chen, O'Connell, Mills, & Schroeder, 2007).

These interactions may occur either at the level of single neurons or/and of neural networks. At both levels, they are able to modulate the power and phase of oscillatory activity. The complex interplay of these phenomena, the characterization of resulting neural activity and its interaction with any behavioral response, are slowly being uncovered by researches.

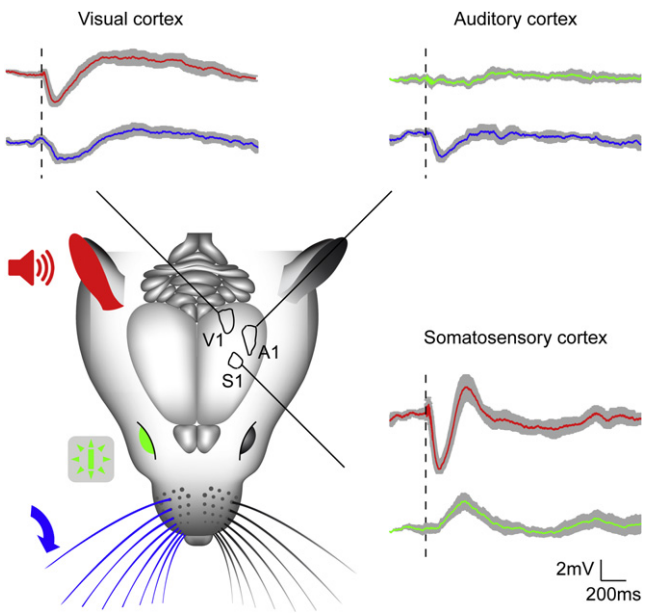
Most of these studies have been conducted on anaesthetized rats. This constitutes the standard procedure for several reasons. First of all, from a practical point of view, an acute recording is usually easier to perform. More importantly, the sleep-like conditions induced by the anesthesia prevent any influence by the level of consciousness and attention. Finally, the absence of any spontaneous whisking means a better control on the actual tactile activation. Anyway, this also means a less natural stimulation.

The effect of crossmodal stimulation on the spontaneous and evoked activity by the dominant modality has been recently explored in rat's primary sensory cortices.

A study by Iurilli (Iurilli et al., 2012) has especially investigated the crossmodal interactions between auditory and visual cortices. Their study characterized neuronal response of primary sensory areas (V1, A1, S1) in anaesthetized rats, through intracellular recordings, during different crossmodal stimulation (Fig. 1.8). The stimuli used in this study were: a flash of light (in the central binocular field), a noise burst (50 ms, 72 dB) and a multi-whisker back deflection (through a piezo-electric motor).

The authors observed all the possible interactions between these stimuli and primary sensory cortices, focusing their attention on V1. The auditory stimulation was found to elicit hyperpolarization in layer 2/3 pyramidal neurons of V1 and of barrel-related column in S1; the tactile stimulation provoked hyperpolarization in L2/3Ps of V1 and of A1; the transient visual stimulation, instead, was unable to cause any visible effect in L2/3Ps of A1, while it caused a depolarization in L2/3Ps of S1.

Figure 1.8: Neuronal responses in primary sensory cortices to crossmodal stimuli.



- *Visual stimulation (green) caused depolarization in S1 and no detectable response in A1.*
- *Auditory stimulation (red) caused hyperpolarization in V1 and S1.*
- *Tactile stimulation (blue) caused hyperpolarization in V1 and A1.*

The authors then concluded that neuronal response of a primary area might be inhibited by the activity of other primary cortices. This effect is achieved, at least in the case of A1-V1 communication, through cortico-cortical link, which activate an inhibitory circuit in the deep layers of V1. Interareal inhibition may then be a distinctive feature not only of higher associative areas, but also of primary sensory cortices.

The authors hypothesized that this mechanism might be used to modulate subthreshold neural activity, and so phase of excitability, in primary sensory areas. The aims may be to provide a common temporal frame to the concurrent stimulations, or to modulate attention levels toward them. It has to be noted, again, that the neuronal and behavioral outcome strongly depended on the relationship in time between the two stimuli.

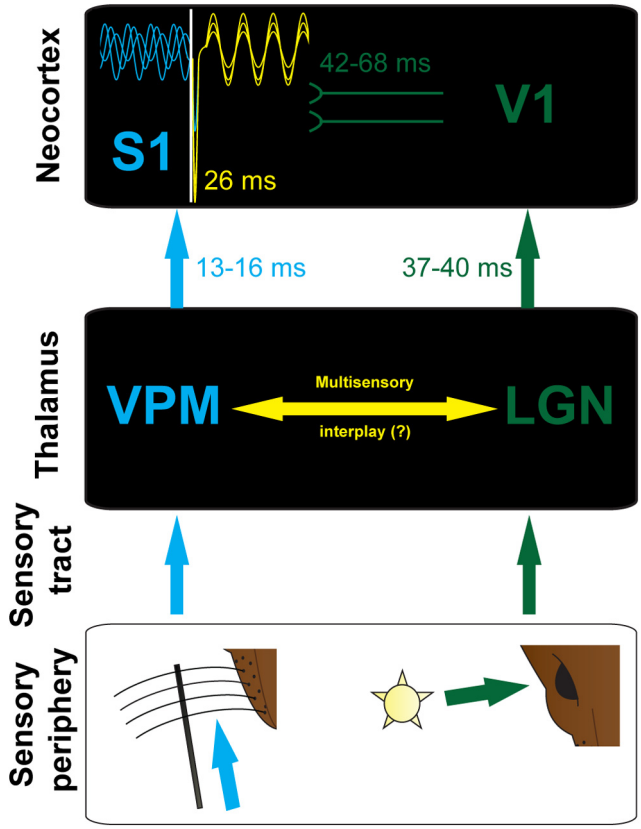
Finally, according to authors, the lack of any crossmodal influence in the combination visual stimulus – auditory area recording may constitute a hint of the different weight these senses have in the animal's life.

Another study by Sieben (Sieben, Röder, & Hanganu-Opatz, 2013) has focused the attention on visual-somatosensory interactions. They used multisite extracellular recording, paired with pharmacological manipulations, in V1 and S1 of anaesthetized rats, during unimodal or crossmodal stimulation. Visual and tactile stimuli were the same as in the previous experiment: a flash of light and a whisker deflection.

Authors found evidence that the supra-additive increase of tactile responses in unisensory tactile cortex, occurring when paired with visual crossmodal stimulation, was strongly due to the signal coming from the precedent subcortical sensory tract. Moreover, they also found that the reset of neuronal oscillations in S1, due to the visual stimulation, required an intact and working communication tract between the two unisensory cortices.

The authors, based on these results, proposed that crossmodal interaction takes place at two levels (Fig. 1.9). The first one is the processing by subcortical regions between sensory receptors and unisensory cortices, where thalamic nuclei are the ones more involved in the multisensory communication. Their involvement may take place in different ways: 1) by separately processing and sending information to the different unisensory cortices; 2) by integrating this information before sending it; and 3) by acting as a intermediate link of communication between cortical areas (corticothalamo-cortical route). The second level is the modulation of phase of network oscillations in the unisensory cortices, which crossmodal stimulation is able to reset. Most probably, this process takes place thanks to direct projections between unisensory cortices. The sparseness of these connections and the high magnitude of this effect, anyway, suggest that other ways, like the ones from higher order areas to primary ones, may be used (Ex.: S1 to V2 to V1).

Figure 1.9. Crossmodal interaction between visual and somatosensory information at neocortical and subcortical level.



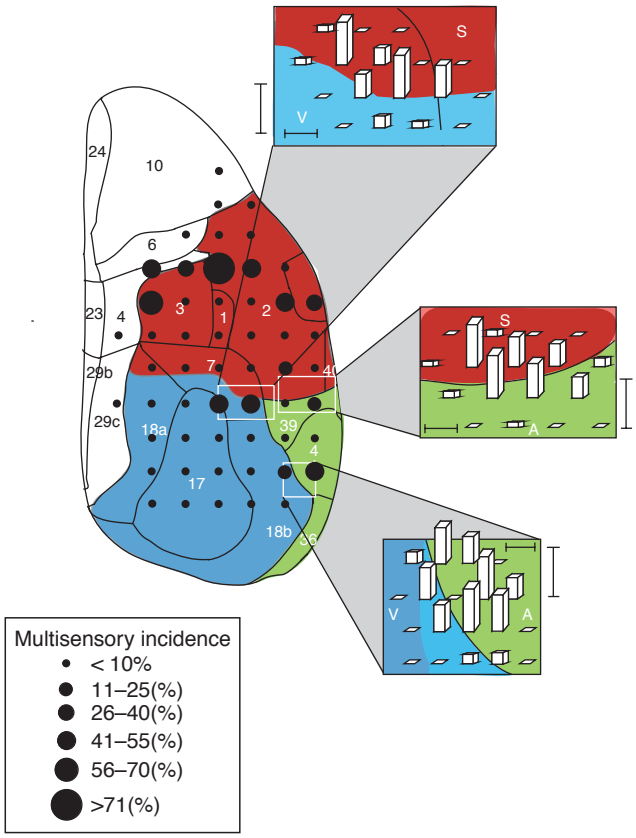
- *Sensory periphery: visual and somatosensory information are acquired at the same time.*
- *Thalamus: thalamic structures send information to unisensory cortices, and are able to integrate this information before sending it.*
- *Neocortex: unisensory cortices communicate between them through direct projections or other ways.*

A study by Wallace (M. T. Wallace, Ramachandran, & Stein, 2004) has shifted the focus of investigation from unisensory primary areas to multisensory high-order regions, specifically the parietal cortex of rats (PtA). They mapped single neuron responses across all this area, together with neighboring primary visual, auditory and somatosensory areas (Fig. 1.10).

Again, they found a certain percentage of neurons inside primary sensory areas responding to crossmodal stimulation. The main result, any way, was the finding of clusters of neurons in the space between sensory specific areas, which not only responded to stimuli of different sensory modalities, but also seemed to integrate

between modalities (as seen in difference between response to multisensory stimulus, and response elicited by the most effective single modality stimulus). In these borders, neuronal responses were found to be enhanced or suppressed depending on temporal proximity between crossmodal stimuli.

Figure 1.10: Distribution of multisensory neurons in rat neocortex.

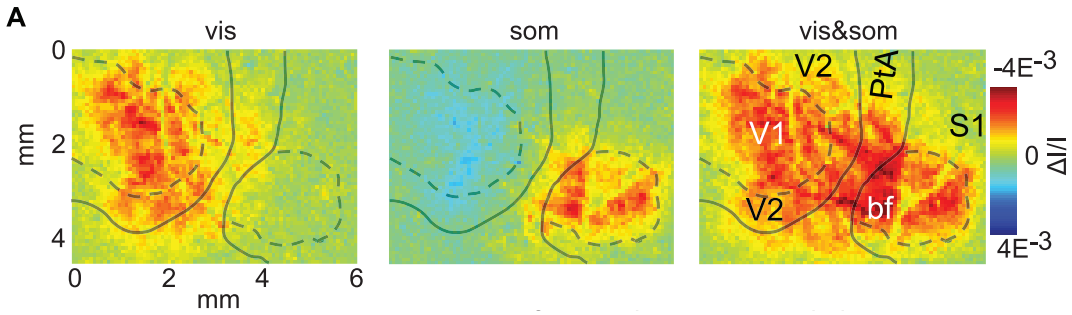


- *Colors depict cortex major subdivision in sensory areas (visual, blue; auditory, green; somatosensory, red). Circle size shows relative incidence of multisensory neurons at each penetration site.*
- *Insets show results for higher resolution sampling on transitional regions between major areas. Bar height shows relative incidence of multisensory neurons at each penetration site (Vertical scale bar shows 50% of multisensory incidence).*

A study by Lippert (Lippert et al., 2013) has investigated this area more in depth. The authors used optical-imaging, laminar electrophysiology and pharmacological manipulations to investigate any multisensory interaction that may take place there. As in previous studies, authors used light flashes and whisker deflections as visual and tactile stimulations.

The PtA region, as previously said, has been identified in several species, among which the rat, as the convergence site of multisensory information (Fig. 1.11). This association cortex is placed in the space between somatosensory and visual primary regions, and has a strong influence on the behavior.

Figure 1.11: Functional localization of multisensory parietal area in rat.



Localization of responses evoked by visual, somatosensory and multisensory stimuli in PtA, from intrinsic optical-imaging data.

Authors observed a non-linear interaction between visual and tactile responses in the neuronal activity of this region. This interaction was observed both at level of sub-threshold (sinks, or local depolarizing currents) and supra-threshold (multi-unit responses).

Importantly, the outcome of this interaction was dependent on the timing of the two individual stimuli. While a preceding visual response produced depression for the somatosensory sink, the opposite happened when was the tactile stimulus to precede the visual one. Authors were careful in defining this sequence, by discriminating between absolute "stimulus onset asynchrony" (SOA), expressed in terms of the physical stimulus presentation, and relative SOA, expressed in terms of the latency

needed for the unisensory stimuli to elicit a cortical response. The asymmetry in multisensory response was not centered on the absolute SOA, but on the relative SOA (a difference of around 33ms between modalities delays). Consequently, the sign of the interaction in the sinks depends on the relative order with which the two unisensory stimuli activate PtA. This difference underlines how important is to consider the relative timing of the two crossmodal stimuli, both in terms of experimental design and of cortical response delay.

This phenomenon, anyway, was affecting only local field potential activity, while multiunit activity was independent of it. Then, according to the authors, multisensory interactions in the evoked current sources are not immediately transferred at the level of neural spiking activity.

Chapter 2: Behavioral Projects

2.1 First Behavioral Project

2.1.1 Introduction

The main goal of my first project was to investigate the perceptual strategies underlying rat discrimination of solid, 3-dimensional objects under different sensing modalities: visual, tactile and visuo-tactile. My secondary goal was to test rat ability to form abstract representations of two categories of objects, by mean of a continuous exposure to different members of each category.

Regarding the first goal, it is worth mentioning that, to date, unimodal tactile discrimination studies with rodents have mostly tested recognition of textures or vibrations, with the exception of a single study that has investigated shape discrimination. On the other hand, unimodal visual discrimination studies have started, in recent years, to investigate rodents' higher order visual abilities – namely object recognition in spite of identity-preserving image transformation (i.e., size and position changes, rotation about different axes, partial occlusion, etc.).

In particular, two recent experiments have applied, for the first time, a classification image method (known as the Bubbles) to uncover which features rats rely upon when discriminating two visual objects (Alemi-Neissi et al., 2013; Vermaercke & Op de Beeck, 2012). This method consists in superimposing to the visual objects the rats have to discriminate opaque masks punctured by a number of semi-transparent circular windows (the bubbles), whose location and number is randomly set in each presentation trial. The loss of discriminatory information produced by the bubble masks allows inferring what part of an object is critical (i.e., must be visible/present) for the object to be correctly recognized.

In my study, I took inspiration from these experiments and I tried to devise a classification image approach that would work with solid objects. My goal was to make it possible to change the available object discriminatory information on a trial-by-trial basis, by transforming the physical appearance of the stimuli. To this aim, I

designed two solid objects that are made of a common central body and several attachments, with the latter serving as the discriminatory features of object identity/category. By changing the position of these informative features over the objects' central bodies, it was possible to produce many different exemplars of the same objects, still maintaining their categorical identity. The aim was twofold: 1) to test, in each session, the recognition of different exemplars of the same category; and 2) to identify which specific parts of the stimuli were used by the rats to perceive and recognize the objects (as in classification image approaches).

Regarding crossmodal and multi-modal recognition studies, the literature offers a remarkable variety of experiments done on rodents, mostly using Spontaneous Object Recognition tasks (see the Introduction). Despite their differences in terms of experimental design and results, most of these studies lack the power to investigate the perceptual processes underlying rodent unimodal and multi-modal object recognition. My study aimed at addressing these issues, by devising a discrimination task that was able: 1) to obtain explicit responses from the rats about the identity of the stimuli; and 2) to uncover how this recognition was accomplished, by comparing rat performance for different exemplars of the same stimulus category.

My plan was to test first rat recognition in the visuo-tactile modality, and then have the same animals accomplishing the task in the visual and the tactile unisensory modalities separately. Ideally, comparing the outcomes of unisensory and multisensory sessions would have allowed discovering to what extent the perceptual strategies used by rats to perceive and categorize an object are modality-dependent. In addition, these experiments would have shown if there was any multimodal benefit in sensing the object with both touch and vision.

To achieve these aims, I designed and built a new experimental rig to test rat discrimination of solid objects, under different sensory modalities. This meant not only building and putting together the physical and electrical equipment, but also designing and creating the stimuli and the software to control the experiment. The main challenge was the use of solid objects as stimuli, since it was difficult not only to control how the rats interacted with the objects, but also to ameliorate the experimental rig, in case of any modification was necessary to adjust or optimize the behavioral training.

To summarize, my specific aims were: 1) to extend the study of rat recognition abilities to more natural settings, in which stimuli are solid objects, so as to allow

multimodal sensing (visual and tactile); 2) to test rat capability to categorize different members of two classes of objects, rather than different objects or different appearances of the same objects; and 3) to test and to compare rat discrimination abilities in different modalities (visuo-tactile, then visual and tactile). My overarching goal was to investigate whether rats would be able to form supramodal, categorical object representations that are invariant with respect to the appearance of individual category members.

2.1.2 Materials and Methods

2.1.2.1 Subjects

Six adult male Long Evans rats were used for this experiment. Animals were 8 weeks old at their arrival, weighted approximately 250 g at the onset of training and grew to 500 g. Rats received a constant amount of food each day and were water-deprived throughout the experiments. Each day, rats were trained and tested on a precise sequence, maintaining the same order: this way, the amount of hours of water deprivation, and so the motivation, was the same for each rat. During each experimental session, they received an amount of 4-8 ml of four parts of water and one part of pear juice as reward during the training. After each experimental session, but not immediately, they were dispensed with half an hour of water. During the weekends, they received free water.

All animal procedures were conducted in accordance with the National Institutes of Health, International, and Institutional Standards for the Care and Use of Animals in Research and after consulting with a veterinarian.

2.1.2.2 Experimental Rig

The training apparatus was custom built. It consisted of two main parts: the 'rat box', that is the space where the rat was kept, and the 'stimulus box', that is the space where the stimuli were presented, one at a time, during the experimental session (Fig. 2.1.1.A, 2.1.1.B).

The two parts were placed one next to the other, and the rat was able to extend his head out of the box and into the stimulus space through a hole on the wall. The stimulus was placed at a distance of seven centimeters from the hole, on a radial support. The hole was put at a height, so as to be centered on the center of the stimulus. All these elements were arranged according to this same criterion, so as to make everything accessible to the rat with easiness, regardless of the modality of interaction/exploration.

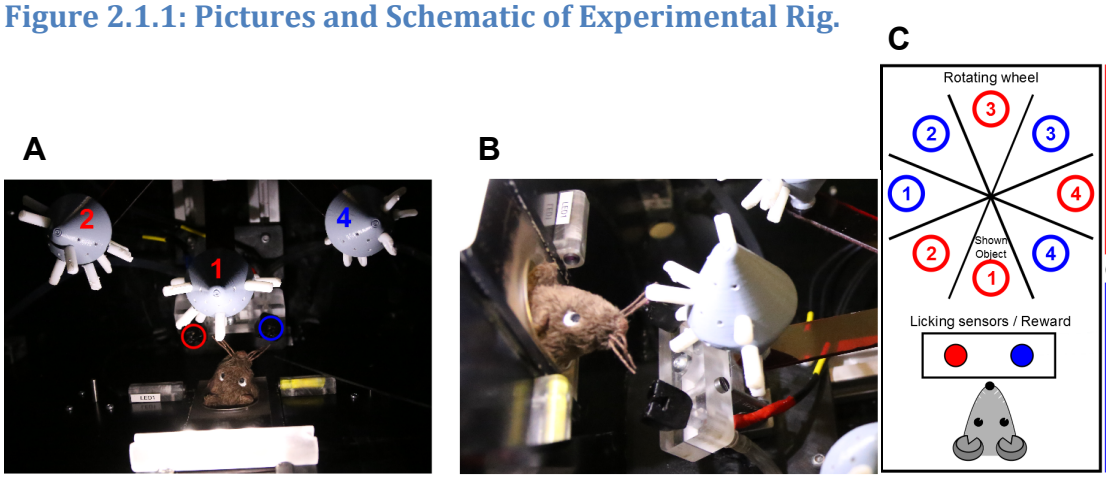
Rat actions and behavioral responses inside the experimental rig were collected using several components. An IR diode-photodiode couple was placed at the sides of the hole, to signal the start time of the trial, when the rat extended his head out of his space into the stimulus area. Two licking sensors, again made with IR diode-photodiode couples, were placed at five centimeters one from the other, between the hole and the stimulus, to collect the response of the animal about the stimulus identity. A high-speed IR camera, paired with an IR emitter, was placed on top of the experimental rig, to permit researcher to observe and record rat behavior from the top, even in the dark. A normal USB camera oriented toward the hole was placed inside the stimulus box, to permit researchers to observe rat behavior from the side.

The experiment could be controlled and managed by the researchers in several different ways, inside the experimental rig. A motor-controlled black panel was placed just after the hole: by moving this panel up and down, it was possible to prevent any activation of the licking sensors and any exploration or interaction with the stimulus, before the start and after the end of the trial. A motor-controlled rotating wheel with eight arms was placed at the center of the stimulus box; on top of the arms were placed the stimuli, which were presented to the rat one at the time on every trial (Fig. 2.1.1.C). Two motor-controlled syringe pumps were placed outside the rat and stimulus boxes, and connected through plastic tubes to the licking sensors: in this way, it was possible to give a precise quantity of water/pear juice as reward to the animals during the training. A series of LED white lights were placed on top of the hole, oriented toward the stimulus: by turning them on or off, it was possible to illuminate the stimulus only in specific trials, and so to permit only visual or only tactile exploration of the object. A support for a glass was placed between the licking sensors and the stimulus: this way, by placing a transparent glass it was possible to permit only visual exploration of the stimulus to the rat. Two speakers were placed on the left and right walls inside the stimulus box, to give reinforcement sound cues about the rat's choice outcome.

The sensors, the motor-controlled equipment, the lights and the speakers, were all connected to a National Instrument signal acquisition card (NI 6353), which was linked to a Windows computer and controlled through a custom-made program in LabView. This made it possible to acquire information in real time from all sensors, and to control the remaining equipment according to the implemented algorithm.

This experimental rig thus allowed: 1) presentation of solid objects that can be sensed, at the same close range, through both the visual and tactile modalities in repeated, consecutive trials; 2) fast-pace collection of behavioral responses; and 3) easy manipulation of object attributes on a session-by-session basis. Crucially, in our apparatus, the physical object and its distance from the rat were kept constant on each trial.

Figure 2.1.1: Pictures and Schematic of Experimental Rig.



A. Top View of Stimulus Box: red and blue circles indicate left and right licking sensors/reward spouts, and their relationship with object identity; numbers on top of object indicate their identity relatively to rotating wheel shown in Panel C.

B. Side View of Stimulus Box: stimulus and licking sensors/reward spouts are shown in relation to rat position.

C. Schematic of Rotating Wheel: red and blue colors indicate object category, and their connection to licking sensors/reward spouts.

2.1.2.3 Stimuli

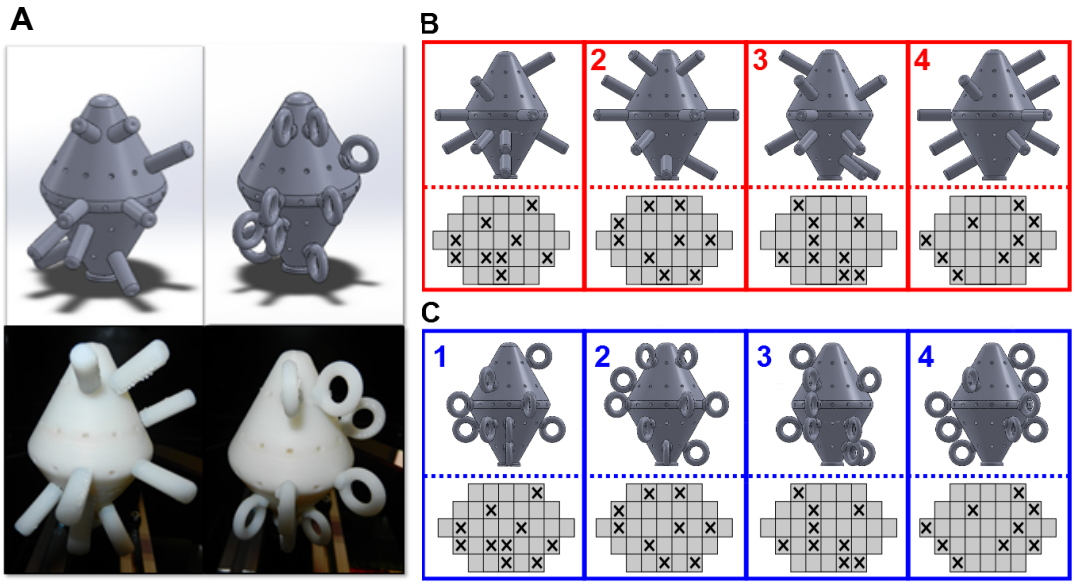
The stimuli have been custom designed. They have been first designed with a CAD software (Solidworks 2013) and then built with a 3D printer (3D Touch, 3D System), with the same printing material (PLA). The objects were built in this way for two reasons: first, to ensure that they all had a reproducible shape; second, to ensure that there was no information coming from the object's distinctive smell, helping rats to solve the task (Fig. 2.1.2.A).

The objects consist of a common central body, where several attachments of two types may be placed in different positions (Fig. 2.1.2.B-C).

The body is created by combining two cones by their bases. Each cone is 3.5cm high, with a diameter for the base of 5cm; the whole object is then 7cm high and 5cm large. Thirty-three holes were made on the surface of the central body, on the side facing the rat (90 degrees to the left and 90 degrees to the right, with 0 degree as the medial line of the rat, for the top and bottom parts of the body). These holes are organized in seven rows, each with a different number of holes (5-7-9-7-5, from top to bottom). The attachments may be either peg- or ring-like, distinctive for each category. Each attachment could be screwed in one of the holes. The two shapes of the attachments have been chosen to resemble the two objects with which our rats may have had more experience – the nozzles of the water bottles and the cereal treats they received as reward at the end of the session, respectively.

The shape of the body was chosen so that each attachment, depending on its placement, changes both its visual and tactile appearance. These changes were due to the rotation in the three axes and to the different distance of the attachment from the rat, producing a relevant change in the perceived dimension and shape of the attachment. We chose to use a fixed number of nine attachments for each category as the feature standard set to place on each stimulus, as the best compromise to have a category-informative object without using all the possible positions.

Figure 2.1.2: Pictures and Schematic of Stimuli.



A. Top: 3D CAD images of two stimuli samples; Bottom: actual printed objects.
B. Top: 3D CAD images of 4 different object configurations for Peg category; Bottom: schematic of attachment placement on object body.
C. As for (B), but for Ring category.

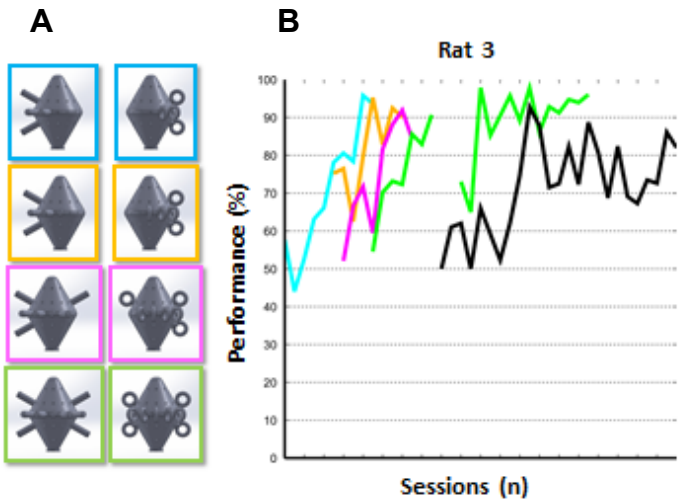
2.1.2.4 Experimental Design

2.1.2.4.1 Shaping Phase

Six rats were initially trained to identify the category of each object by licking the correct sensor associated to it. The training procedure was as following. The animals learn to trigger the start of each trial by extending their snout out of the hole from their space into the stimulus one. This movement interrupts the light between the diode and photodiode placed at the sides of the hole, which in turn raises a black panel placed between the hole on one side, and the licking sensors and the object on the other. After this, the animal is free to explore the object for a fixed amount of time (around 3 seconds). Within this time window, but not after, the animal can interact with the licking sensors/reward spouts. By touching the correct one, he receives a fixed amount of water/pear juice from it, and then the current trials stops. By activating the wrong one, he receives no reward; instead, a timeout period is enforced, before the end of the current trial. At the end of the trial, and any time the rat doesn't respond before the end of the allowed time window, the panel slides down, and the trial stops. After this, and after the positioning of a new object, a new trial starts.
During the Shaping Phase, the rats were allowed to sense the objects using the visuo-tactile modality: the animals were free to see and touch the objects on every trial. To train the animals to correctly associate each category to one of the sensors, we devised a specific shaping strategy (Fig. 2.1.3.A): during the initial sessions, the set of attachments for a specific category was placed on each stimulus only on the side toward the licking sensor that was associated to that category (see the blue framed objects in Fig. 2.1.3.A). In other words, for the object defined by the peg-like attachments, these attachments were all located to the left side of the object (since this category required a response to the left sensor), while, for the object defined by the ring-like attachments, these attachments were all located to the right side of the object (since this category required a response to the right sensor). The aim was to initially train the animals in a task of lower complexity: the rat did not need to identify the category (i.e., the shape) of the attachments, but just learn to touch the sensor that was nearer to the attachments. Session by session (Fig. 2.1.3.B), more attachments were added to the stimuli and were gradually moved toward the center of the object and

then on the other side of the object, for both the categories (see the yellow and magenta framed objects in Fig. 2.1.3.A). The final outcome of this training strategy was to use two final objects, where the two sets of category-specific attachments were placed in the same positions, symmetrically over the central body, for both objects (see the green framed objects in Fig. 2.1.3.A). At this point of the Shaping Phase, the two objects were not recognizable anymore based on the side of placement of the attachments, but only based on the shape of the attachments. Only after the animals were able to correctly recognize these final objects, they were moved to the Testing Phase.

Figure 2.1.3: Stimuli and Relative Session-by-Session Recognition Performance of Shaping Phase.



A. Different stimuli used in Shaping Phase, for both peg- and ring-category; colored frames indicate relative recognition performances in B.

B. Session-by-Session recognition performances on different shaping stimuli, regardless of category; black line indicates recognition performance on Testing Phase stimuli.

2.1.2.4.2 Testing Phase

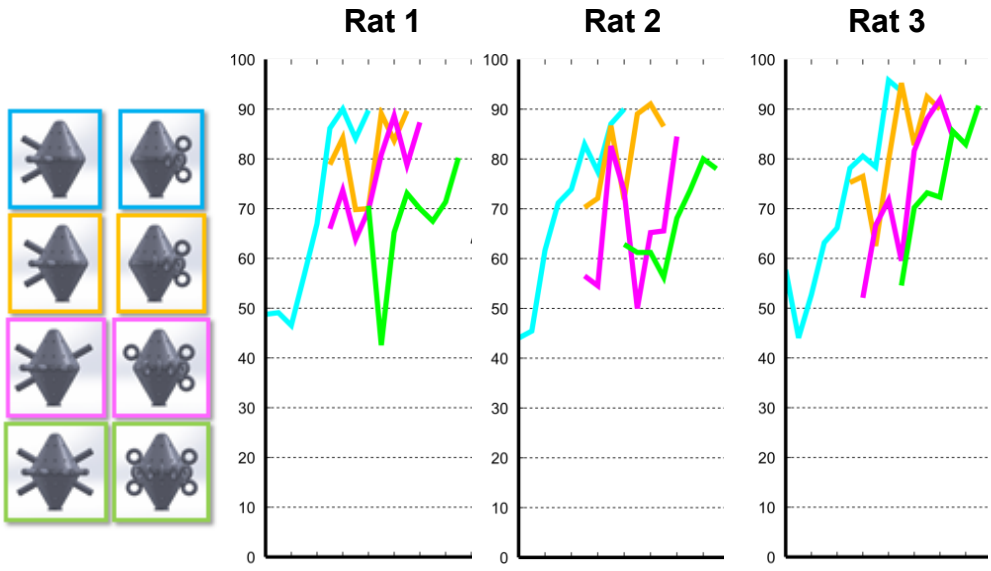
During the Testing Phase, the rats kept performing the same object discrimination task as during the Shaping Phase (still in the visuo-tactile modality). The only difference was in the stimuli they had to discriminate. In fact, in this phase, the placement of the attachments was pseudo randomly changed between each session, with four new configurations tested in each session (see an example in Fig. 2.1.2.B-C). Simultaneously with the random configurations, the final objects of the previous Shaping Phase were also tested for some session, to facilitate the passage between the two Phases. Three rats were tested for about 60 sessions with approximately 250 different configurations (i.e., 250 different exemplars of the two categories), in the visuo-tactile modality. These configurations were selected among all the possible random ones, to control that no specific position of the attachments was used more than the others. The session-by-session random variations in the location of the attachments had the aim of uncovering the most informative feature locations used by the rats to categorize the objects (as done in classification image approaches). This was possible by analyzing each configuration of attachments in connection with the recognition performance of the rats with such configurations.

2.1.3 Results

2.1.3.1 Shaping Phase

Only three rats out of six were able to complete the Shaping Phase. These three rats successfully went through the different training stages described in Section 1.2.4.1, where, at each stage, the overlap between the locations of the attachments for the two objects became progressively larger (rat performance across these shaping stages is shown in Fig. 2.1.3 in light blue, yellow and magenta curves). Eventually, these rats learned to correctly solve the task with the final stimuli (i.e., when the attachments were placed in exactly the same locations for both objects), with a performance reliably around 70% (see Fig. 2.1.3, green curve). This shows that my training strategy was successful in guiding the rats from the rule of just choosing the licking sensor near to the attachments placed at one of the sides of the object, to a recognition based on judging the identity of the attachments, regardless of their position.

Figure 2.1.3: Shaping Phase Session-by-Session Recognition Performances.



Session-by-session recognition performances, regardless of category, for the three rats that were able to complete shaping phase; colors indicates recognition performances relative to each shaping stimulus.

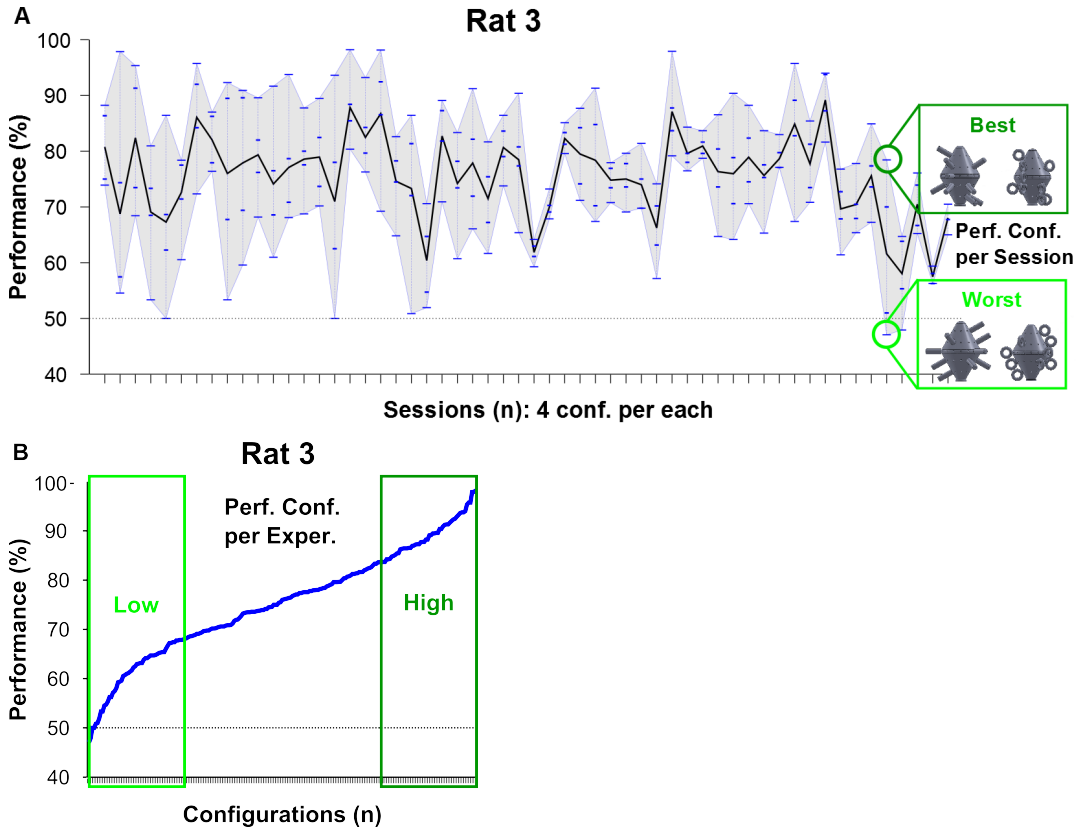
2.1.3.2 Testing Phase

During this phase, I have collected complete data sets for the three rats that completed the Shaping Phase. These animals were tested in the visuo-tactile sensing condition, across ~60 sessions. This allowed testing ~250 pseudorandom configurations of attachments (i.e., different members of the two object categories), with at least 50 trials collected per configuration. The pseudorandom selection of the attachments' locations was such as to guarantee that each of the 33 possible locations was used in about the same amount of trials.

For each rat, the average recognition performance of the test objects was between 70% and 80% correct (see one example rat in Fig. 2.1.4.A; solid line), showing that rats were able to solve the task above chance in a reliable way, regardless of the use of different configurations (i.e., different category exemplars) in each session. However, as expected, rat average recognition performance appeared to highly oscillate between sessions. The reason for this oscillation is evident once rat recognition performance is analyzed for each different configuration (see the blue ticks and dots in Fig. 2.1.4.A, showing the performance for each of the four distinct configurations that were tested in each session). The difference between the best and the worst recognition performances in each session (i.e., between the top and bottom ticks, for each data point in Fig. 2.1.4.A) could be as large as 40%, showing that the different placement of the attachments, for each configuration, had a great influence on the recognition abilities of rats.

To investigate how each configuration influenced the recognition performance, and so rat perception of the target objects, we built a rank plot of the complete configuration data set, depending on the associated recognition performance, for every rat (see example in Fig. 2.1.4.B).

Figure 2.1.4: Session-by-Session Recognition Performance and Configuration Rank Plot of Testing Phase.



A. Average recognition performance (thick line) for Rat 3, across 60 daily sessions, regardless of category. Each tick mark shows performance on a different configuration, during each session. Light green and dark green insets show the configurations corresponding to, respectively, worst and best performance during one exemplar session.

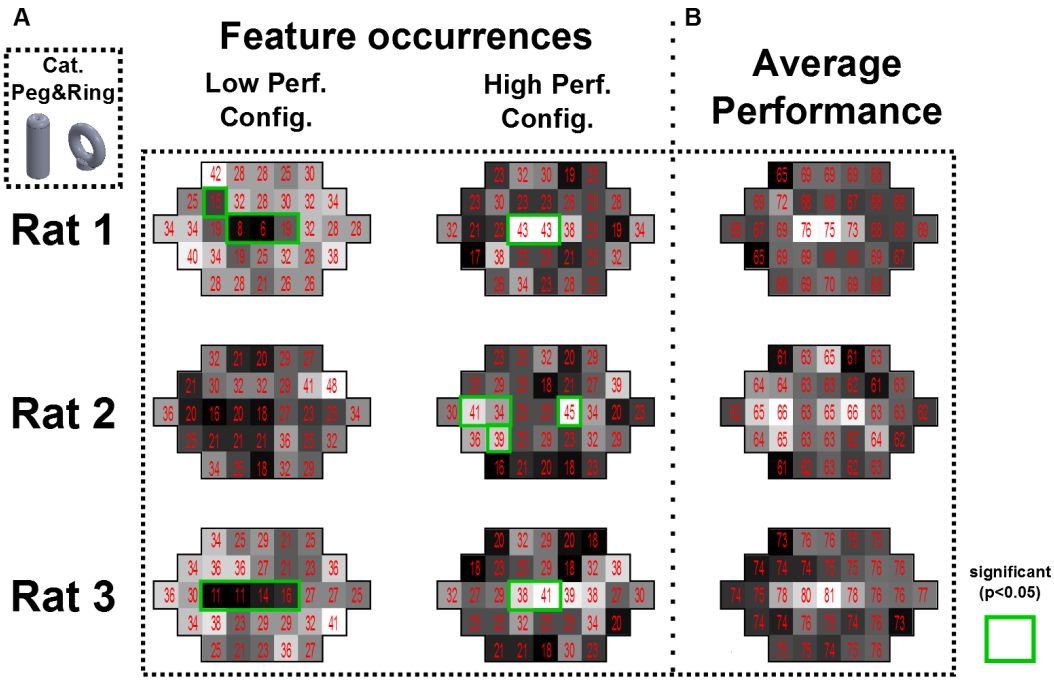
B. Object configurations, in abscissa, are ranked according to the corresponding recognition performance. Configurations in the first and last quartile of this distribution were selected to carry out final analysis.

To perform a classification image analysis, we decided to first process the configurations in the lower and upper quartile of this distribution. Namely, we computed the occurrence of the features in each of the 33 possible positions, for configurations leading to either the lowest or the highest quartile of the performance range. These are the configurations that influence more the recognition performance of the rats, in one direction or another, and so are probably the most informative about the strategy rats use to perceive and recognize the stimuli. In practice, those features that were used by the rats to correctly solve the task are likely to be more present in the high-performance quartile than in the low-performance one.

This analysis yielded the occurrence maps shown in Fig. 2.1.5.A (significance of each occurrence value was computed with a permutation test; see green frames). For rats 1 and 3, a narrow stripe of positions located in the central part of the objects was significant both in the low performance configurations (where these positions were significantly more absent) and in the high performance configurations (where they were significantly more present), thus leading to the conclusion that this stripe of features was particularly influential in determining the outcome of rat recognition). Rat 2, on the other hand, showed a way more scattered pattern of significantly salient locations (only in the highest quartile) and no consistent pattern between the highest and lowest quartile.

In addition, I also computed the average performance of a rat when a feature (peg or ring) was placed at any given location, to estimate the influence of that location on rat's recognition behavior (see Fig. 2.1.5.B). Namely, we computed for each specific position the average performance across all the configurations in which that position was used. Compared to the previous analysis (shown in Fig. 2.1.5.A), this approach was meant to: 1) use all the data I gathered, instead of just the extreme quartiles; and 2) confirm any result found in the previous analysis, in spite of taking now into consideration a bigger amount of configurations. As shown by comparing Fig. 2.1.5.A and b, both analyses showed a similar trend (that was consistent among the three rats), with a narrow, central span of locations being the most informative about object category. That is, all three rats, when allowed to sense the objects both haptically and visually, showed a tendency to rely mostly on those feature locations that were closer to their snout/vibrissae.

Figure 2.1.5: Salient Feature underlying Object Recognition for all Rats, regardless of Object Category.



A. *Values in the matrices show the occurrence of features (%) in each position, for configurations leading to either low or high performance. Features used by rats to correctly solve the task are likely to be more present in the high-performance configurations than in the low-performance ones.*
B. *Each cell shows the average performances of a rat when a feature (peg or ring) was placed at the location. Overall, the plot illustrates the effect that the presence of a feature in any given location had on rat recognition ability.*

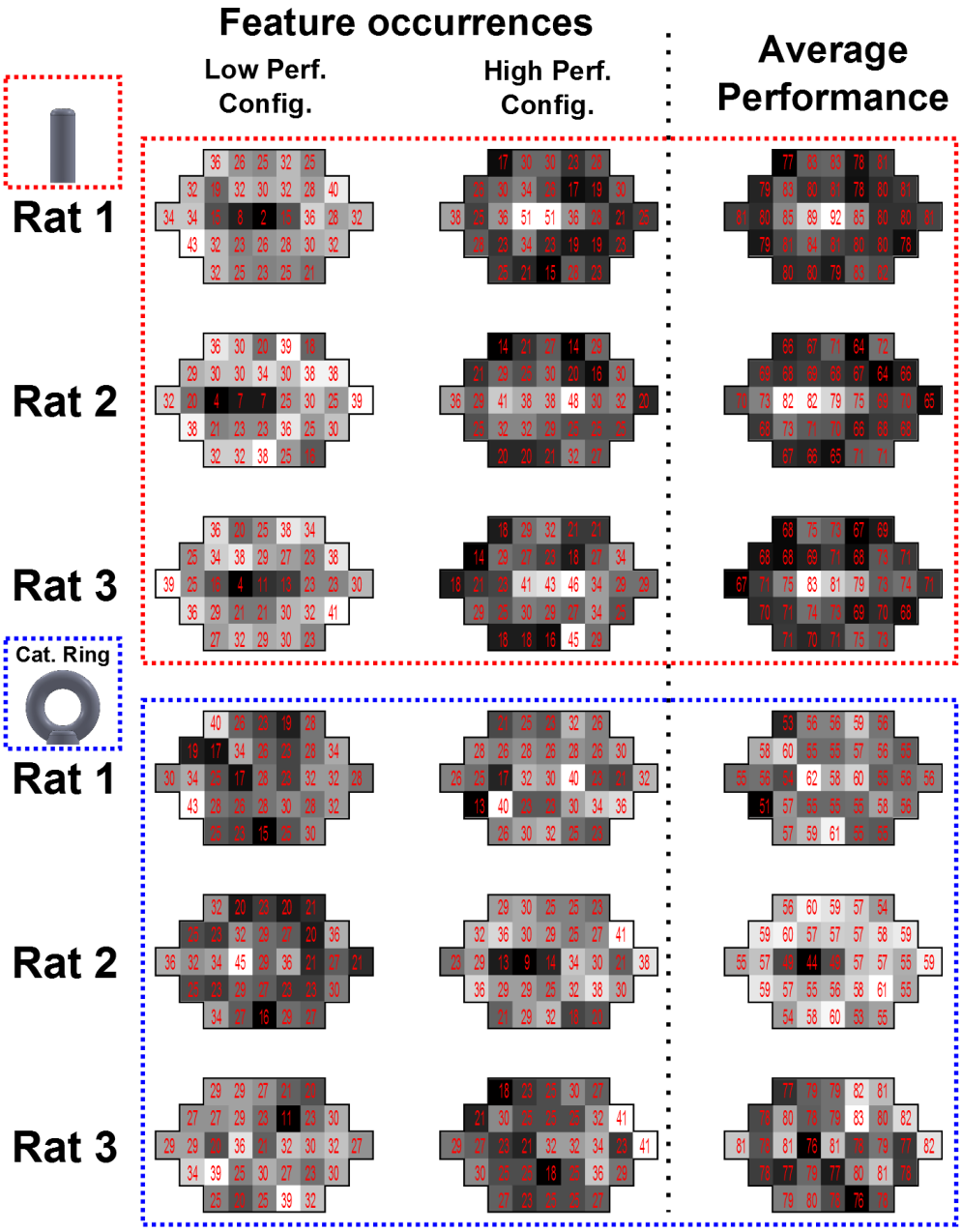
Significance of values, marked by green line around the cell, was assessed by a permutation test.

Both the analyses shown in Fig. 2.1.5.A-B were performed regardless of the category of the attachments (i.e., by pooling together the trials obtained from the exemplars of both categories). Subsequently, I decided to perform the same analysis but, this time, looking at the effect of each position, depending on the category of the attachment. My aim was to find if there was any category-dependent recognition strategy.

Indeed, this analysis reveled that the three rats solved the recognition task in different ways. Rat 1 appeared to consistently rely on the central, narrow stripe of feature locations, regardless of the category, although this pattern was more apparent for the peg category (see the top plot in Fig. 2.1.6.A). Rat 2 showed a completely different

strategy: this animal classified the objects according to whether any feature (no matter whether peg or ring) was located in the central part of the stimulus or not. In other word, this rat did not perform the categorization task he was supposed to. Instead, following the Shaping Phase, he started to perform a present-no present feature task (with regard to the central part of the objects), which did not take any longer into account the shape/identity of the features/attachments. Rat 3 showed a strategy that was similar to rat 1, although he seemed able to use more than just the central part of the object to solve the task, thus integrating information across a larger span of the stimulus. This same conclusion could be reached by looking at the performance maps shown in Fig. 2.1.6.B (obtained in the same way as the maps of Fig. 2.1.5, but considering the trials collected for each category separately). Moreover, all three rats showed a preference/bias toward a specific category of attachments (the pegs). This could have happened because of several possible reasons: 1) a general instinctive preference toward them, because they resembled the nozzles of the water bottles; 2) a higher perceptual saliency of this particular shape; or 3) the fact that, when placed in the central part of the object, these attachments were the ones protruding the most toward the rat.

Figure 2.1.6: Salient Features underlying Object Recognition for all Rats, depending on Object Category.



Values in the matrices are calculated as in before. The results show that different rats are solving the task in different ways, with some rat showing a category-dependent recognition strategy.

- *Rat 1 uses only a restricted part of the object (the central one), probably the most visually and tactically salient, basing his choice on roughly the same features in both categories.*
- *Rat 2 uses a completely different strategy, responding according to the presence or absence of features in a specific section of the object (the central part), regardless of category.*
- *Rat 3 exhibits the best performance, using localized features to recognize one category and more scattered features to recognize the other one.*

2.1.3.3 Attempts at testing the unimodal recognition strategies

After the end of the visuo-tactile testing phase, several attempts were done to test the three animals also in the visual modality, by either putting a transparent glass or increasing the distance between the rat and the stimulus. Unfortunately, none of these attempts succeeded. All three animals failed to perform above chance in any of the tested visual sessions.

Because of this, it was then decided to test the three animals in the tactile modality, by having the rats performing the recognition task in the dark. In contrast with the visual modality, this time the three animals were able to solve the task, after an initial training. Some preliminary analysis confirmed that the strategies used to recognize the object category in the tactile modality, were similar to the ones used before in the visuo-tactile modality: rats were again highly dependent on the presence or absence of attachments in the central part of the objects. It was anyway decided to stop the experiment, because some of the initial aims were impossible to fulfill (namely, the comparison between the perceptual strategies under the visual and tactile modalities). The stimuli were redesigned to improve their affordance (both visually and haptically) and a new set of experiments was carried with a new batch of rats. This new study is described in Section 2.2, after the results obtained with the original objects are discussed in Sections 2.1.4 and 2.1.5.

2.1.4 Discussion

In this experiment we have been able to test object categorization in rats, using an explicit, rather than a spontaneous, object recognition task, and presenting the animals with different exemplars of the same categories of 3-dimensional, solid objects. However, we have been able to successfully test rats' abilities only in the visuo-tactile and tactile modalities. Moreover, the recognition strategies used by the rats were found to be rat-dependent and, to a lesser extent, category-dependent. We found that the presence of features in specific positions of the objects' central bodies was correlated with high performance in the task in most of the subjects. Rats were able to categorize an object, in some cases at least (i.e., rats 1 and 3), by recognizing one or more of its attachments in some specific positions, in a way that tolerated only minor variation in the features' location.

The patterns of correct/incorrect choices associated with different attachment configurations were processed using classification image approaches to uncover what features' locations were critical to allow the correct identification of the objects. This revealed that, for each rat, the most informative feature locations about object category were restricted to a narrow, central band, directly in front of the animal's snout/vibrissae. It was unclear whether this is due to the fact that these positions are the ones where one or more of the attachments, for both or just one of the categories, were more salient, or simply more approachable by the animals. The latter option seems to be the more likely, suggesting a preference, in such a close-range object recognition task, for a perceptual strategy relying mostly on tactile information, in spite of visual information also being available. This conclusion was confirmed by the later failure in testing these animals in the visual modality and the success in accomplishing the training in the tactile modality. It should also be taken into account that, visually, the contrast of the attachments over the central body of the objects was probably quite low, given rat contrast sensitivity function, thus reducing the saliency of the features in the visual modality – a shortcoming that I corrected in designing the stimuli of the next experiments (described in Section 2.2). Under these circumstances, relying on the more approachable and more salient tactile information would have granted these animals the capability to solve the task in the fastest acceptable way,

from a performance point of view, and to invest the least possible cognitive resources in dealing with the stimulus perceptual variability.

The animals most probably focused their attention on just one part of the stimulus (the central band), using what appeared to be a featural perceptual strategy, not only because of perceptual or cognitive reasons, but also because of our experimental design. First, it is possible that some consequences of the design choices made during the Shaping Phase (namely the prominent use of the central band of the object in the "ambiguous objects") were able to later influence the results of the Testing Phase, by directing the rats' attention particularly toward this part of the object. Second, the random configurations tested in each session almost always used at least some of these positions, stably providing rats the optimal way to solve the task, in a fast and simple way.

Regarding the difference between rats on the categorization of the different attachments' categories, this could be due to several reasons. One of the two attachments' categories could have been less/more salient or less/more robust in helping rats solving the task. Alternatively, it is possible that the peg category was simply preferred by the animals because of its shape, or its similarity with the nozzle of the water bottles that rats were used to approach and interact with in their home cages.

Whatever the reasons for these differences could be, they posed an obvious problem to the continuation of this experiment, by making the collected results hard to interpret. This, together with the difficulties encountered in training animals in other modalities, brought us to the conclusion that a new experiment was needed in order to address some of the shortcomings of this old one.

2.1.5 Conclusion

In conclusion, we have been able to extend the study of rat recognition abilities to more natural settings, in which stimuli were solid objects, and to test it in an explicit way (by collecting discrete responses about the object's category) and in a multimodal and unimodal sensing (visuo-tactile and tactile).

Rats were able to successfully categorize different members of two classes of objects, by using a combination of shared and rat-specific perceptual and cognitive strategies. We saw a general tendency among all the subjects to select/use the same features to solve the task in the best way, where best meant simpler and faster for the rats.

These discrimination abilities have been tested and compared in different modalities. The aim was to study the mechanisms of supramodal and modality specific perceptual abilities, which are invariant with respect to the appearance of individual members. Anyway, it was not possible to come to clear and definite conclusions about this topic, because rats appeared to rely on just the tactile information, both in the visuo-tactile and in the tactile modalities. It was unclear if this strategy was due to the rats' intrinsic perceptual and cognitive dispositions and abilities, or to the characteristics of our experimental design.

2.2 Second Behavioral Project

2.2.1 Introduction

The experiments described in Section 1 accomplished only partially the expected aims and revealed several shortcoming in the experimental and stimulus design. The high risk of failure during the Shaping Phase (only 50% of the rats made it to the next phase), the excessive time needed to train the animals to solve the task (around three months), and the impossibility to successfully test all the desired modalities were some of the reasons not to consider further this choice of task and stimuli to investigate rat unimodal and multimodal recognition abilities. Instead, I addressed some of these issues by designing a new set of stimuli, in which, based on previous experience in our laboratory, I expected the issues encountered during the first experiment not to be present. In addition, I decided to train and test from the beginning separate group of animals, each with a different modality (visual, tactile and visuo-tactile), under the hypothesis that only in this way it is possible to appreciate differences in the discrimination abilities of rats under different sensing conditions. In fact, whenever these modalities are tested separately but in the same group, as by using an interleaved design for each session, it is not possible to control any crossmodal effect of learning between the modalities: this could take place, between each trial, at the cortical level in the subject brain, due to cortico-cortical or thalamo-cortical connections between different sensory primary areas. Moreover, by testing the animals in a single modality from the beginning, we can force the animals to base their recognition behavior on that specific sensory modality. In fact, whenever rats are trained in a visuo-tactile modality, it is hard to define which modality is mostly used: the different weight rats give to every information coming from each sensory modality, may depend on the stimulus characteristics, on the animals' perceptual capabilities, or on any interaction of these. Still, testing the animals in the visuo-tactile modality would be useful to uncover if they show any multimodal benefit in object recognition, or if they just rely on only one modality, also when exposed to both of them. This could be visible from the comparison of the multimodal strategy with the unimodal ones (visual and tactile).

My goal in the second experiment was the same as in the previous experiment: the study of rats' perceptual and cognitive abilities in discriminating solid objects. I was particularly interested to investigate these capacities in a scenario where rats are trained and tested in the recognition of different exemplars of the same category of objects, and under different modalities. My plan was to accomplish this: 1) by comparing the recognition performances, and so the perceptual mechanisms, within and between different groups of rats (and so different modalities); and 2) by comparing the recognition abilities within the same group of rats, by later switching the modality of testing.

In summary, my aim in this experiment was to develop a tool to investigate these topics in a fast and reliable way, where results are easier to interpret (compared to study described in Section 2.1), by taking advantage of the experience with the previous studies.

2.2.2 Materials and Methods

2.2.2.1 Subjects

Six adult male Long Evans rats were used for this experiment. Animals were 8 weeks old at their arrival, weighted approximately 250 g at the onset of training and grew to 500 g. Rats received a constant amount of food each day and were water-deprived throughout the experiments. Each day, rats were trained and tested on a precise sequence, maintaining the same order: this way, the amount of hours of water deprivation, and so the motivation, was the same for each rat. During each experimental session, they received an amount of 4-8 ml of four parts of water and one part of pear juice as reward during the training. After each experimental session, but not immediately, they were dispensed with fifteen minutes of water. During the weekends, they received two-three hours of free water.

All animal procedures were conducted in accordance with the National Institutes of Health, International, and Institutional Standards for the Care and Use of Animals in Research and after consulting with a veterinarian.

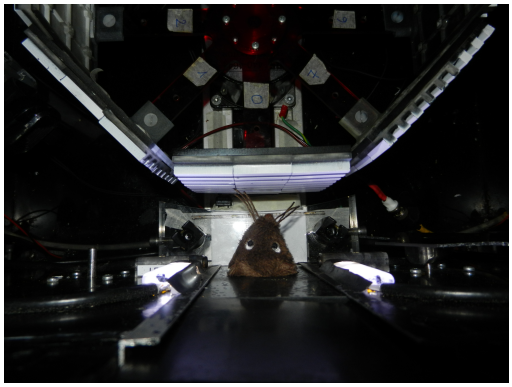
2.2.2.2 Experimental Rig

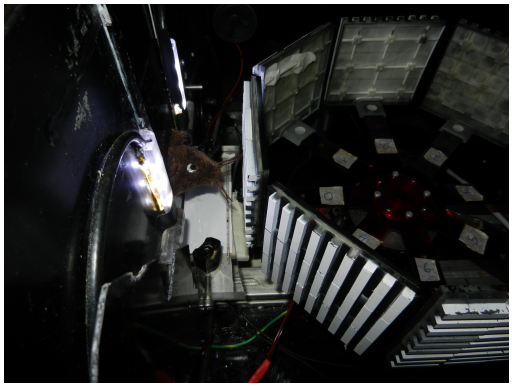
The experimental rig was the same used in the previous experiment, apart from some minor modification (see Section 2.1.2.2). Again, the training apparatus consisted of two main parts: the 'rat box', that is, the space where the rat was kept, and the 'stimulus box', that is, the space where the stimuli were presented, one at a time, during the experimental session. The two parts were placed one next to the other, and the rat was able to extend his head out of the box and into the stimulus space through a hole on the wall. Two changes were made to the previous experimental rig: first, the stimulus was placed at a distance of five centimeters from the hole, to put it nearer to the rat; second, the two licking sensors were placed at nine centimeters one from the

other, at the maximal possible distance, so as to avoid covering in any way the stimulus.

The distance between the hole of the rat box and the stimulus was kept constant across all the testing modalities (visual, tactile and visuo-tactile). As in the previous experiment, depending on the group, the rats had to explore the objects either haptically (i.e., with his whiskers and snout in the dark), or visually (i.e., with the object illuminated but not touchable, because of a transparent panel placed in front it), or visuo-haptically (i.e., with the object illuminated and reachable by the rat's whiskers and snout) to be able to solve the task. The rat actions and behavioral responses were monitored and collected as described in Section 2.1.2.2.

Figure 2.2.1: Pictures of Top and Side Views of Modified Experimental Rig Stimulus Box.





2.2.2.3 Stimuli

As in the previous experiment, the stimuli were first designed with a CAD software (Solidworks 2013), then built with a high-resolution 3D printer (ProJet 3510 HD Plus, 3D System), with the same printing material for all the stimuli (VisiJet M3 Plastic), and finally painted with same white and black paint for all stimuli. This granted us the same advantages of the previous experiment (high reproducibility of stimuli and absence of any odor informative cue).

The new stimuli were designed and created to address some of the issues of the previous experiment: 1) the high number of possible placements, which made too complicate any analysis of interactions between them; 2) the different distance between the attachments and the rat, depending on the location of the attachments (given that the objects' central body had a curved, barrel shape), which could have made some of the configurations very approachable (and, therefore, salient) and some others impossible to explore; and 3) the difference in the shape of the attachments, which could have made some of them simpler or harder to perceive, biasing in this way the animals from the beginning.

The new stimuli were much simpler (see Fig. 2.2.2). They were 3x3 square matrixes (9x9cm) composed of 9 tiles, with each tile being a solid grating (3x3cm) with 3 cycles (1x3cm) of white protrusions and black sinks, so as to form either a pattern of rows or columns, depending on whether a matrix was oriented horizontally or vertically. Each tile was removable, and, when not present, was substituted by a black smooth cap. In this experiment, then, the attachments were the same for both categories, with their orientation being the only informative feature for discriminating between them.

The stimuli were of two types. The so-called "Default" stimuli were the complete matrixes (i.e., all tiles present), in both orientations, and were used during the Shaping Phase and through all the Testing Phases. The "Modified" stimuli were only used during the Testing Phases, and were those in which some of the grating tiles were

substituted by black smooth caps, thus altering the discriminatory information afforded by the grating stimuli.

As in the previous experiment, all the parts of the stimuli (the supports and the tiles) were printed and painted with the same material. Moreover, the tiles were continuously swapped between positions and categories, between sessions. All these measures were taken in order to not give any cue about the identity of the stimulus, based on its smell, to the rats.

It must be noticed that rats of the visual and tactile groups have been trained with another intermediate set of stimuli, on the same orientation discrimination task, before adopting the final set of stimuli I have described above. These stimuli were composed each of a circular black base, on which nine white solid cubes were placed with horizontal or vertical orientation. The change to the final set of stimuli was considered necessary because of the absence of any learning effect in the rats' performances, after numerous sessions of training, due to unknown reasons. Rats in the visuo-tactile group, instead, have been trained from the beginning on the final set of stimuli.

Figure 2.2.2: Pictures of New Stimuli as visible from inside Rat Box, for Horizontal and Vertical Orientation.

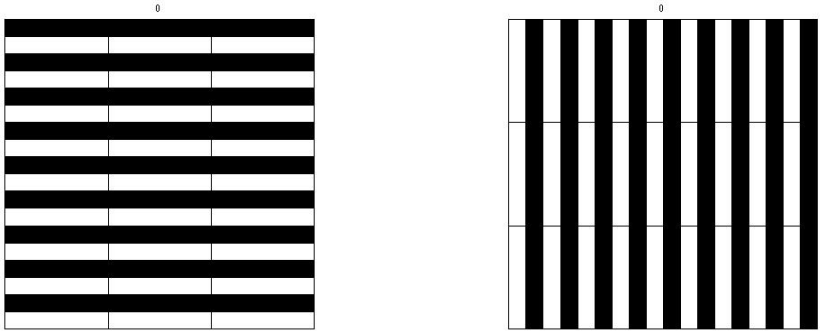
2.2.2.4 Experimental Design

2.2.2.4.1 Shaping Phase

The aim of the Shaping Phase was to find if rats are capable of discriminating the orientations of solid gratings, when trained in unimodal or multimodal modalities (visual, tactile, visuo-tactile). During the shaping phase, each rat was initially trained to discriminate the orientation of the "Default" stimuli (Fig. 2.2.3). The experimental design was the same as in the previous experiment, with two exceptions. This time, the six rats were divided from the beginning in three groups of two rats each: each group was trained in a different modality (visual, tactile, or visuo-tactile). Moreover, a different strategy was used to train the animals to solve the task.

First, a "Habituation Period" was used to get rats used to the experimental rig, by just keeping them inside for a variable amount of time. Second, a "Free Reward" strategy was used to teach them to interact with the reward spouts/licking sensors: at the start of each trial, before any choice, rats received reward from the correct reward spout, and had to retrieve it to proceed to the next trial. Third, a "Correction" strategy was used to train them to link each licking sensor with a given stimulus orientation: during each trial, rats had to solve the task by licking the correct sensor, but in case of an error, they had the opportunity to lick the other correct one, after a variable amount of time; the object stayed there during the whole correction trial. In these trials, only the first answer was recorded. Finally, rats had to solve the task without the possibility of correction: in case of correct response, they received a reward and could immediately go to the next trial; in case of wrong response, they received an error sound signal that was paired with a variable timeout before starting the next trial. The transitions from the first to the second, and from the second to the last shaping phases were not sharply separated between one session and another, but usually coexisted within each session, to have a smoother shaping course. Only after rats were able to solve the final version of the task (without correction allowed) with a performance above 70% correct for a consistent number of sessions, they were moved to the next Testing Phase.

Figure 2.2.3: Schematic of Default Stimuli for Horizontal and Vertical Orientation.

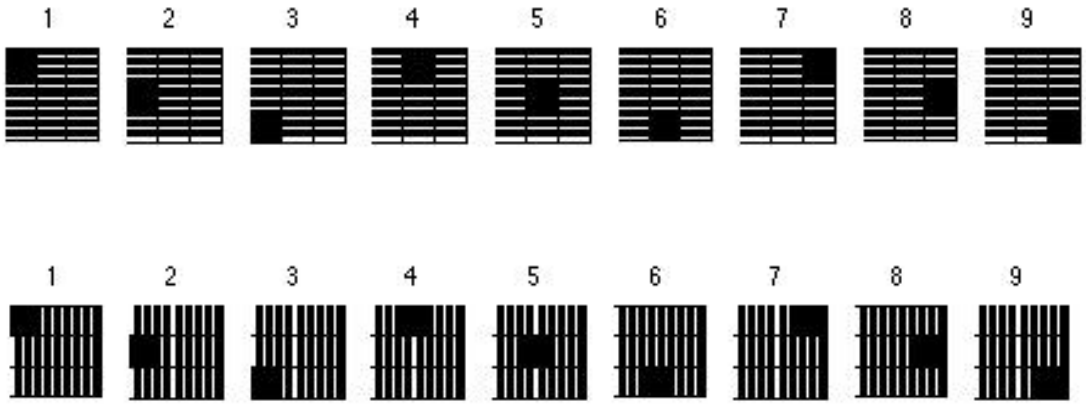


2.2.2.4.1 Testing Phase 1

During testing phase 1, rat ability to recognize the orientation of the "Default" and "Modified" stimuli was tested. The "Default" stimuli were the same as before. Nine different configurations, for each orientation, were tested as "Modified" stimuli. For each of these, one of the nine grating tiles was substituted with the black cap (see Fig. 2.2.4).

The aim of Testing Phase 1 was twofold. First, to inspect how robust was rat recognition ability to a minor change in the aspect of the stimulus and to the consequent small decrease of discriminatory information. Second, to investigate the possibility that rats' recognition ability was dependent on just some specific position of the tiles, regardless of, or depending on, the orientation. The influence of a specific grating tile on the recognition of the stimulus was assessed by the effect of its absence on the orientation discrimination performance.

Figure 2.2.4 Schematic of Modified Stimuli for Testing Phase 1, for Horizontal and Vertical Orientation, with Identification Number on Top.

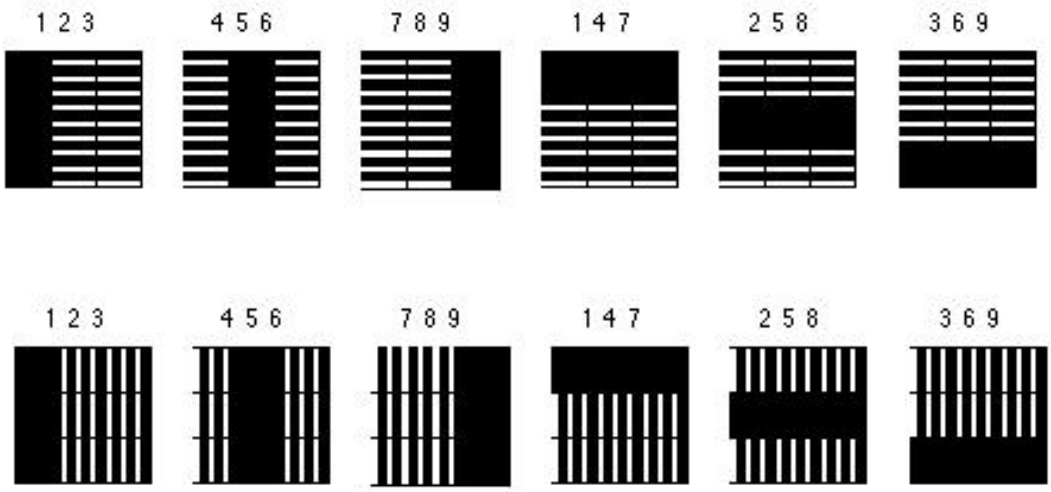


2.2.2.4.1 Testing Phase 2

During Testing Phase 2, the "Default" stimuli were the same as before. Six different configurations, for each orientation, were tested as "Modified" stimuli. For each of these, one of the three columns or one of the three rows of grating tiles was substituted with black caps (see Fig. 2.2.5).

The aim of Testing Phase 2 was again twofold. First, to inspect how robust was rat recognition to major changes in the aspect of the stimulus and to the consequent large decrease of stimulus discriminatory information. Second, to investigate the possibility that rats' recognition ability was dependent on the presence of specific coherent modules of information, organized either in rows or columns. As in the previous phase, the influence of a specific coherent group of grating tiles on the recognition of the stimulus was assessed by the effect of its absence on the orientation discrimination performance.

Figure 2.2.5: Schematic of Modified Stimuli for Testing Phase 2, for Horizontal and Vertical Orientation, with Identification Number on Top.

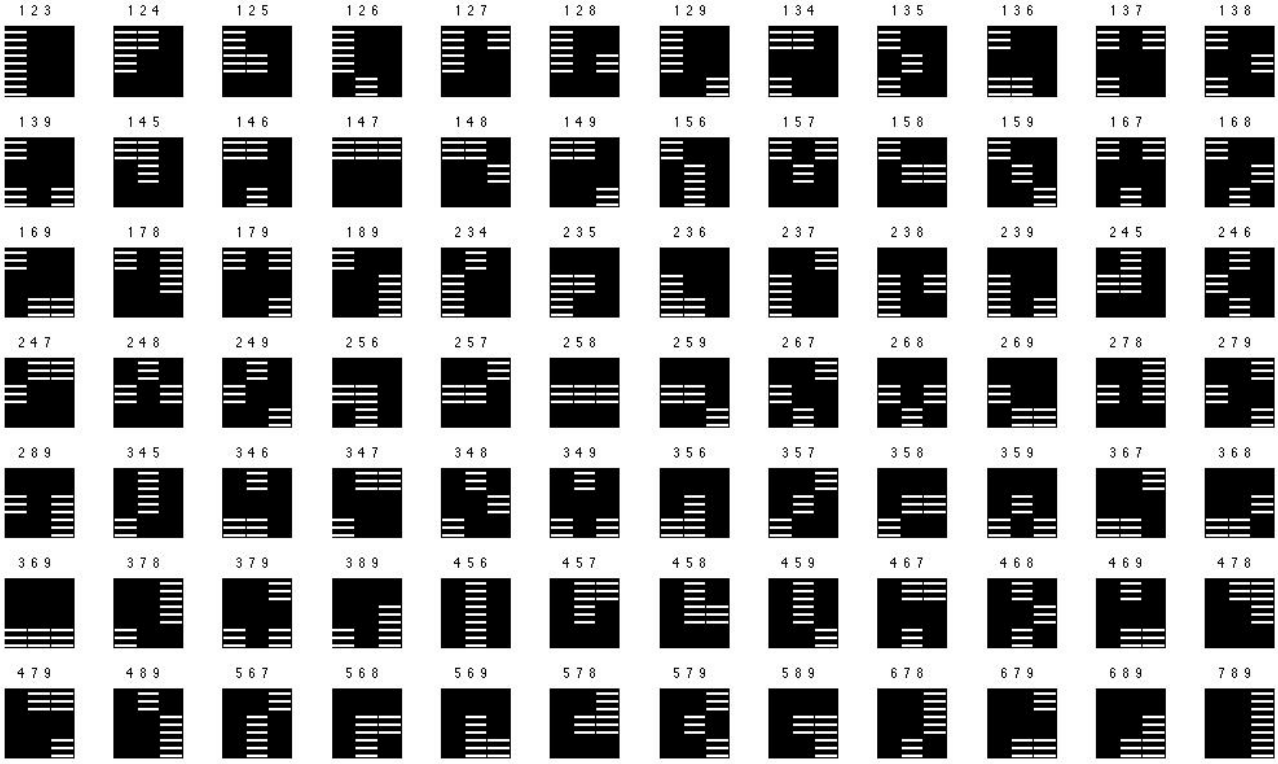


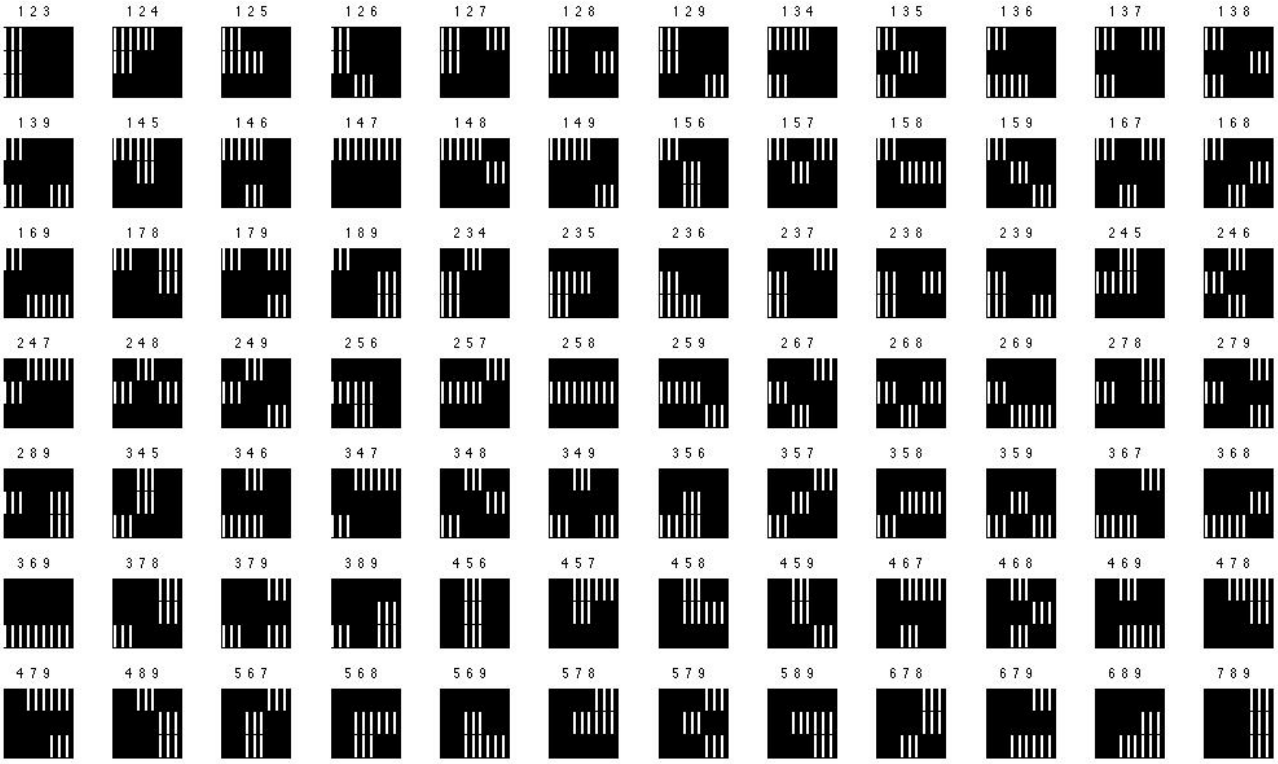
2.2.2.4.3 Testing Phase 3

During the Testing Phase 3, 84 different configurations, for each orientation, were tested as "Modified" stimuli. In each session, three of these Modified stimuli were shown along with the "Default" stimulus (a new set of stimuli was used in each session). For each of the Modified stimuli, only three grating tiles were shown on the stimulus, while the rest was replaced with black caps. These 84 configurations consisted of all the possible combinations of 3 elements on a 3x3 matrix (see Fig. 2.2.6).

The first aim of Testing Phase 3 was to build saliency maps (as in classification image approaches), showing the influence of each single grating tile on the rats' orientation perception and discrimination. The second aim was to find any effect of interaction between pairs of grating tiles on rats recognition. A third aim was to asses whether rat recognition was more affected by local features (i.e., the orientation of the pattern within the tiles) or by global stimulus properties (e.g., the overall group orientation of the produced by the arrangement of multiple tiles). This was possible because there were some configurations in which the group orientation, either in columns or rows, conflicted with the tiles orientation, either vertical or horizontal. In contrast with the previous phases, this time the influence of a single grating tile or a group of them on the recognition of the stimulus, was assessed by the effect of its presence on the orientation discrimination performance.

Figure 2.2.6: Schematic of Modified Stimuli for Testing Phase 3, for Horizontal and Vertical Orientation, with Identification Number on Top.





2.2.3 Results

2.2.3.1 Shaping Phase

During the initial Shaping Phase, all six rats have been successfully trained, trough the different sequential strategies described in Section 2.2.4.1, to recognize the orientation of nine coherent solid grating tiles placed on the object, in both unimodal and multimodal modalities, depending on the group (visual, tactile, visuo-tactile).

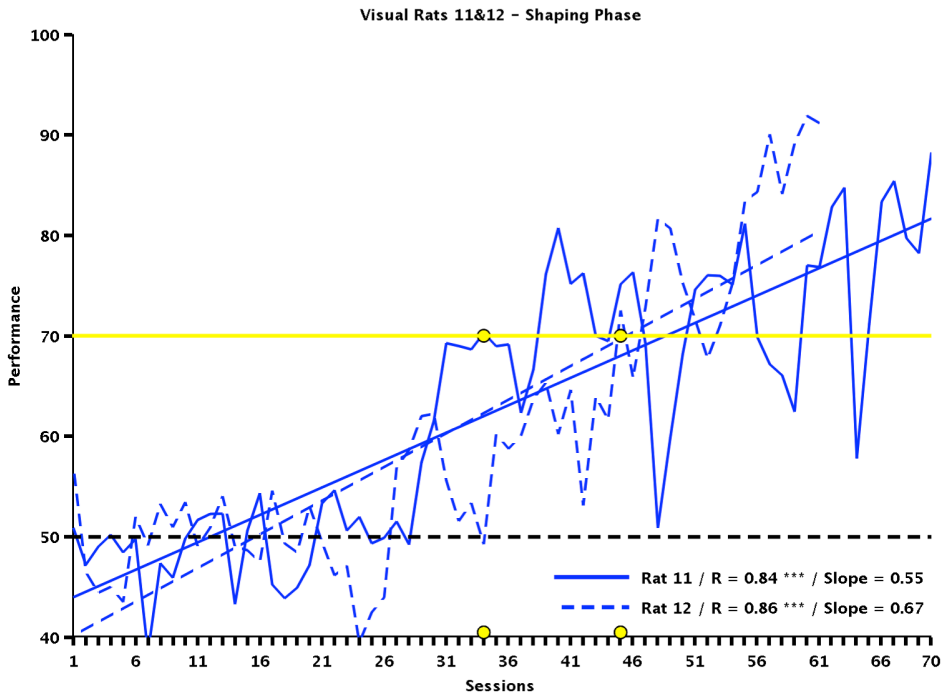
All the rats showed a distinctive saw-tooth shape in their session-by-session performance: the average discrimination performance widely oscillated across sessions. Nonetheless, a general trend toward an improvement of recognition ability was clearly visible: this was shown by the significant positive linear correlation between the session number and the recognition performances (Fig. 2.2.7.A-B-C; see the regression lines on top of rat performance curves, with corresponding Pearson correlation coefficients). Only after several sessions with a performance above the criterion, rats' behavioral responses started to stabilize, indicating the stable acquisition of the task rule. Moreover, even though the correlation coefficients are highly significant for all the groups, the slopes of the regression lines indicate a great difference in the speed of task rule acquisition, between the groups. Rats in the visual and tactile groups were quite slow in learning the task: rats in the visual group (labeled as rat 11 and 12) needed an average of 40 sessions to durably reach the criterion of 70% recognition performance, while rats in the tactile group (labeled as rat 13 and 14) required more than 50 sessions (see Fig. 2.2.7 A-B, where the yellow circles mark the first session in which criterion was surpassed). It must be noticed, however, that the initial 20 sessions of these two groups have been carried out using an intermediate set of stimuli, different from the final one, on the same discrimination task (see Section 2.2.2.3). The transition between the intermediate and the final set of stimuli was deemed necessary because of the lack of learning showed by rats. It is then hard to draw conclusions regarding the speed of training shown by these rats: the previous experience with the intermediate stimuli transition may have either eased or delayed the acquisition of the task with the final patterns. However, data obtained

from a new set of rats that are currently under training (not shown in this thesis), confirm that animals trained from the beginning in single modalities with the final stimuli have yet to learn the task, after more than 30 sessions. Indeed, unimodal learning of orientation discrimination task, within our experimental conditions, appear to be slower than multimodal learning for rats. In fact, consistently with the hypotheses about the benefits of multimodal perception (see Introduction), rats in the visuo-tactile group (labeled as rat 15 and 16) were quite fast to reach the performance criterion, needing an average of only ~12 sessions. Moreover, they were also considerably more stable in their recognition performance: contrary to what happened with the visual and tactile groups, their recognition performance never moved below criterion, once it was reached (see Fig. 2.2.7.C).

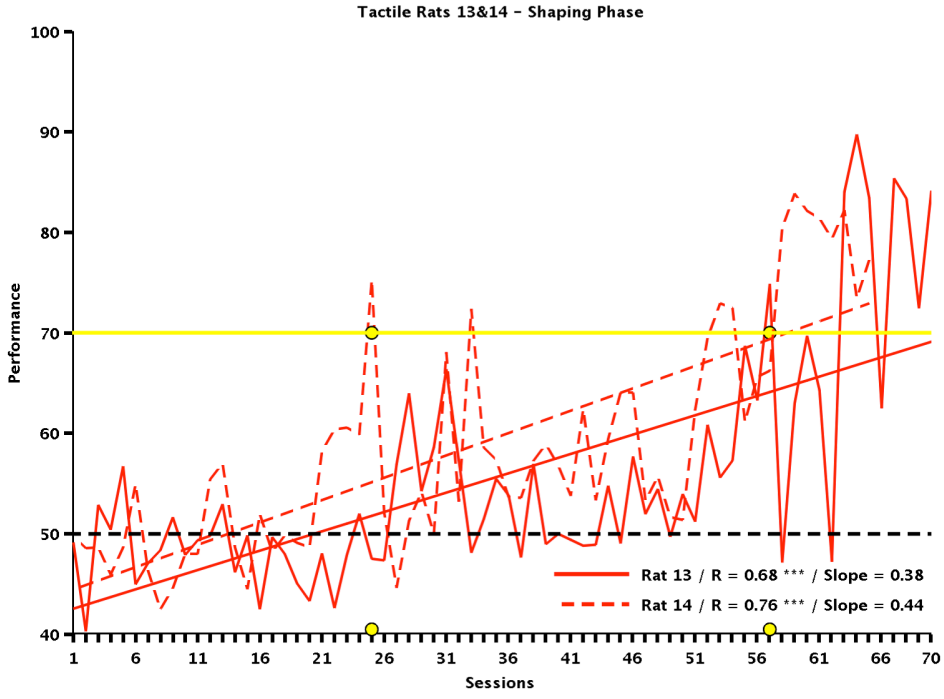
In summary, all rats were capable of discriminating the orientation of the gratings by the end of the Shaping Phase, with a different level of speed in acquiring the task rule, and a different stability in maintaining the required recognition performance level, depending on the group.

Figure 2.2.7: Session-by-Session Performance of all Rats in three Experimental Group on Recognition of "Default" Stimuli of Shaping Phase (A, Visual; B, Tactile; C, Visuo-Tactile).

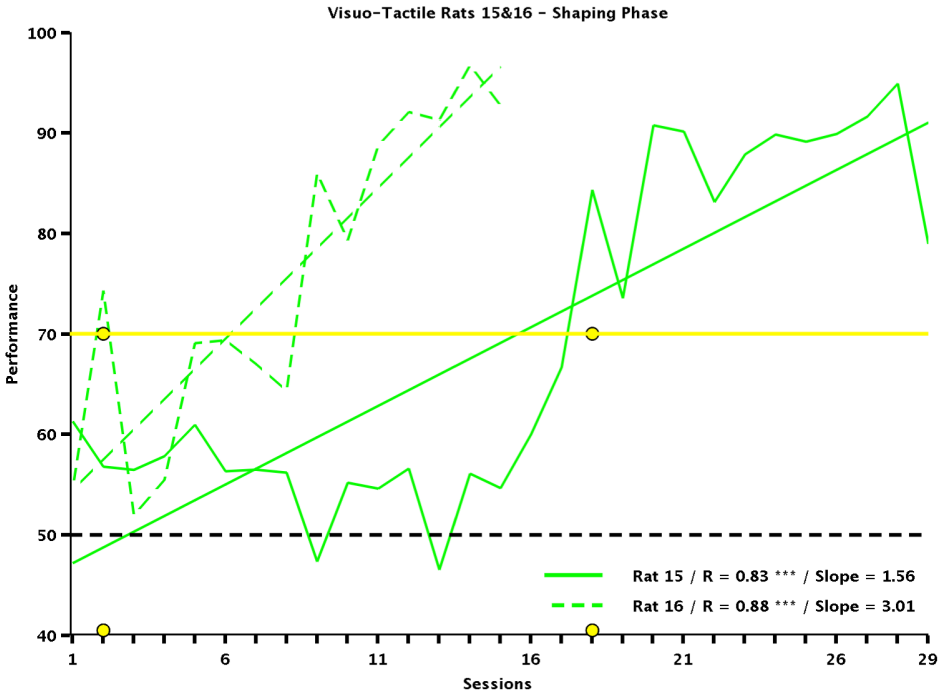
A



B



C


Visuo-Tactile Rats 15&16 - Shaping Phase
Performance
100
90
80
70
60
50
40
1
6
11
16
21
26
29
Sessions
Rat 15 / R = 0.83 *** / Slope = 1.56
Rat 16 / R = 0.88 *** / Slope = 3.01

2.2.3.2 Testing Phase 1

During Testing Phase 1, I tested whether rats were able to recognize not only the orientation of the "Default" stimuli (the complete objects with all the nine tiles placed on them), but also the orientation of the "Modified" ones. As explained in Section 1.2.4.1, the latter were objects built with nine different configurations of tiles, each missing a tile in one position (out of nine), replaced by a black smooth cap.

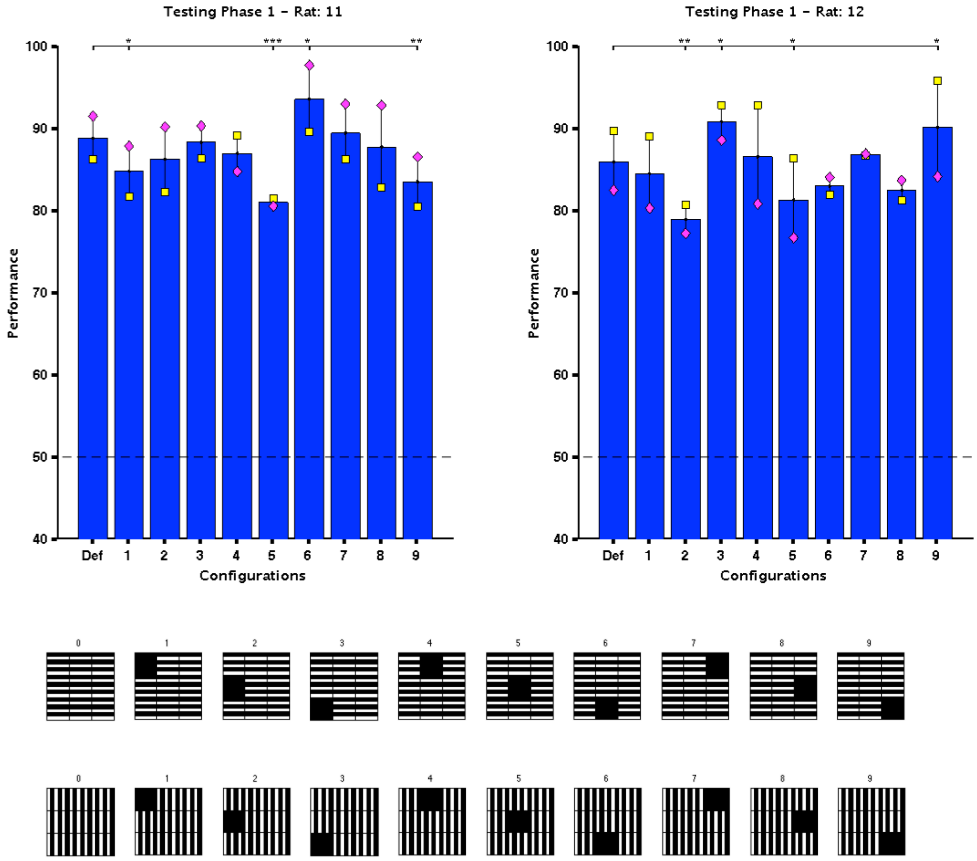
When tested with these configurations, rats of all groups showed a stable and robust recognition, thus displaying a substantial tolerance to such a minor stimulus modification. A Binomial test was used to compare the percentage of successful recognitions to chance level, showing that all recognition performances on "Default" and "Modified" stimuli were highly significant ($p < .001$ for all configurations; p-values or stars are not reported for clarity; see Fig. 2.2.8). At least ~300 trials were collected for each rat on each configuration, regardless of orientation.

In addition to show the overall recognition performances (computed on both vertical and horizontal gratings), Fig. 2.2.8 also reports the performances on each orientation separately: the yellow squares correspond to horizontal gratings, while the purple diamonds to vertical gratings. This information is useful to check if there is any specific or general tendency to classify a configuration more as vertical or horizontal, due to cognitive or perceptual reasons. Most rats showed a consistent tendency to report more frequently a given orientation (the vertical, in 5 out of 6 rats). However, this bias rarely resulted in performance differences between matching vertical and horizontal gratings that were larger than 10% (see the vertical lines connecting each pair of square and diamond), thus indicating that rat recognition was robust regardless of stimulus orientation.

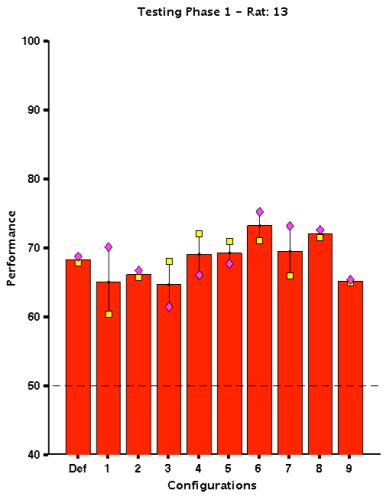
For each rat, the recognition performances on of the "Modified" stimuli were compared to the performance on the "Default" stimulus, to check if the absence of one tile in a specific position of the plate was enough to significantly alter rat recognition. A Fisher Exact Test was applied to pairs of vectors containing the trials' outcomes [0,1], with one vector referring to the "Default" stimulus and the other

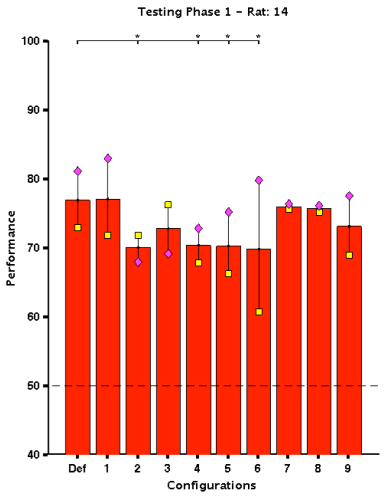
vector referring to each of "Modified" stimuli (the outcome of the test is reported above each bar: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). A few comparisons showed a significant decrement of recognition performance, suggesting that rats were preferentially relying on the information conveyed by the corresponding missing tile. Interestingly, there were also a few cases in which recognition performance was instead positively affected by the absence of information. One possibility is that the information that was present in those positions was detrimental for rats' judgment, but a more conservative hypothesis is that rat recognition was still unstable at this point of the training, leading to some spurious increase of performance. Finally, the tile positions corresponding to a significant decrement/increment of recognition performance were generally different among the rats of the same group, with a few exceptions in the case of the visual group (i.e., configurations 5 and 9). These exceptions are indicative of a possible preferential reliance of the "visual" rats on the tiles placed in the corresponding positions, i.e., the center of the plate (configuration 5) and the bottom-right corner (configuration 9; see Fig. 2.2.4).

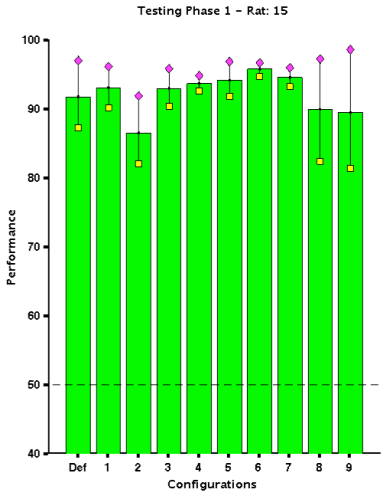
Figure 2.2.8: Performance of all Rats on Recognition of "Default" and "Modified" Stimuli of Testing Phase 1.

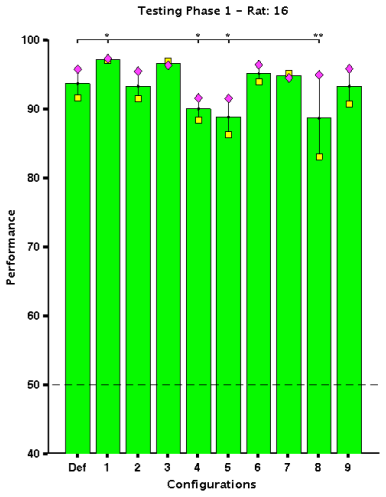


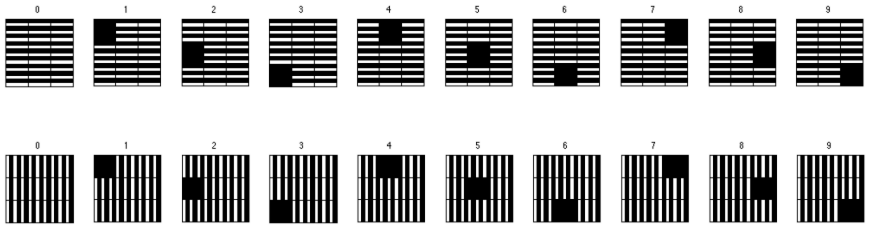
*Bar plots showing the performances of the rats in the three experimental groups (blue: visual; red: tactile; green: visuo-tactile) on the "Default" (first bar in each plot) and the "Modified" stimuli of Testing Phase 1 (bars labeled from 1 to 9; refer to the image at the bottom of each figure or to Fig. 2.2.4 for an image of the stimuli). All the performances were significantly larger than expected by chance, according to a Binomial test ($p < 0.001$). The stars refer to the significance of the comparisons between the performances observed for the Default stimulus and each of the Modified stimuli (Fisher Exact Test , * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).*


Testing Phase 1 – Rat: 13
Performance
Configurations
Def 1 2 3 4 5 6 7 8 9


Testing Phase 1 – Rat: 14
Performance
Configurations
Def 1 2 3 4 5 6 7 8 9

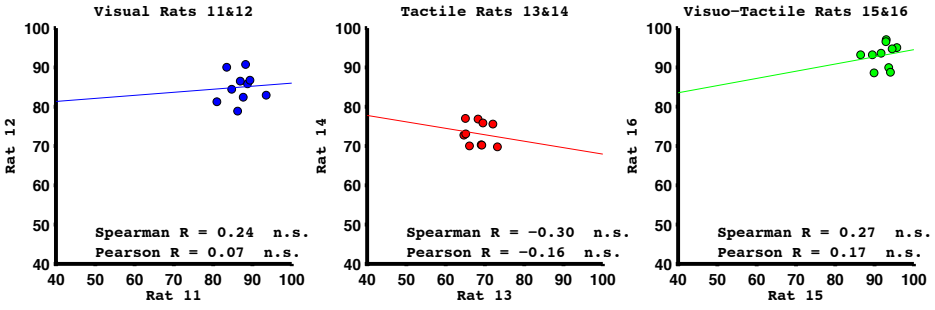

Testing Phase 1 – Rat: 15
Performance
Configurations
Def 1 2 3 4 5 6 7 8 9


Testing Phase 1 – Rat: 16
Performance
Configurations
Def 1 2 3 4 5 6 7 8 9


0 1 2 3 4 5 6 7 8 9
0 1 2 3 4 5 6 7 8 9

More in general, however, the performances on the "Modified" stimuli were not significantly correlated between the rats of the same group (see Fig. 2.2.9). This result does not necessarily imply that the alterations of the default stimuli had a different effect on the rats within a group. Rather, it is likely a consequence of the fact that rat performance on the Modified stimuli was only minimally altered, compared to the Default stimulus, and, as such, did not vary over a sufficiently wide range of values to allow a meaningful assessment of correlation (see how, in Fig. 2.2.9, all the dots are clustered in the same region of the performance plane).

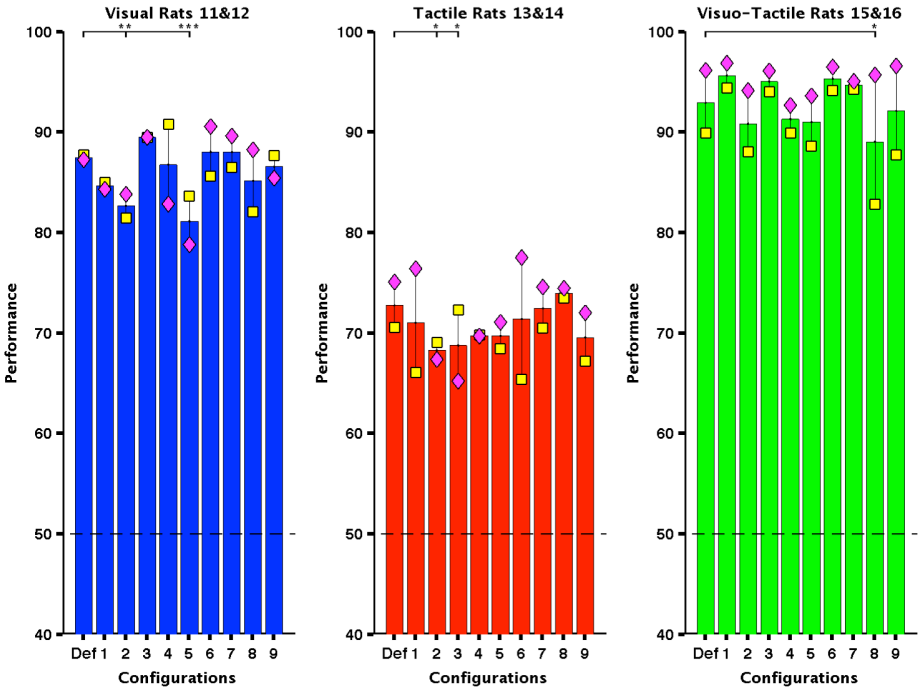
Figure 2.2.9: Correlation between Recognition Performances on "Modified" Stimuli of Testing Phase 1 between Rats of same Group.



Scatter plots showing the correlation between the performances obtained, on the "Modified" stimuli, for the two rats in each of the experimental groups (visual: blue dots; tactile: red dots; visuo-tactile: green dots).

Because of this overall similarity between the performances obtained for the rats within a group, I concatenated the vectors of trials' outcomes obtained for the animals within each group and I tested if any statistically significant difference was observable between the performances on the Default stimulus and each of the Modifies stimuli (Fisher Exact Test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, see Fig. 2.2.10). The result does not show any strong consistency across the three groups of rats, apart from a general tendency for the tiles in the central row (i.e., configurations 2, 5 and 8; see Fig. 2.2.4) to affect more the performance.

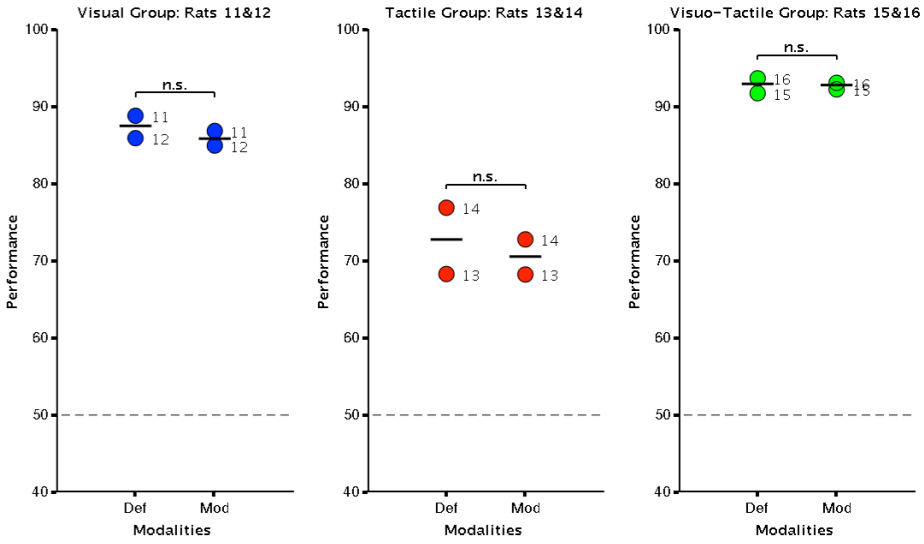
Figure 2.2.10: Performance of all Groups on Recognition of "Default" and "Modified" Stimuli of Testing Phase 1.



Same as Fig. 2.2.8, but after concatenating the responses of the rats within each experimental group, so as to achieve a more compact comparison between the visual (blue bars), tactile (red bars) and visuo-tactile groups (green).

To further check if stimulus' modification had any general effect on rat recognition, at the level of group and regardless of configuration identity, I concatenated the trials' outcomes of the rats within the same group and applied the Fisher Exact Test to check if there was a significant difference between the group performances on the Default stimulus and on the "Modified" stimuli (where the trials obtained for all the Modified" stimuli were concatenated). No statistically significant difference was found, thus showing that, overall, the novelty of the stimulus appearance and the small decrease of discriminatory information produced by the missing tile were not enough to produce any general effect on rat orientation discrimination.

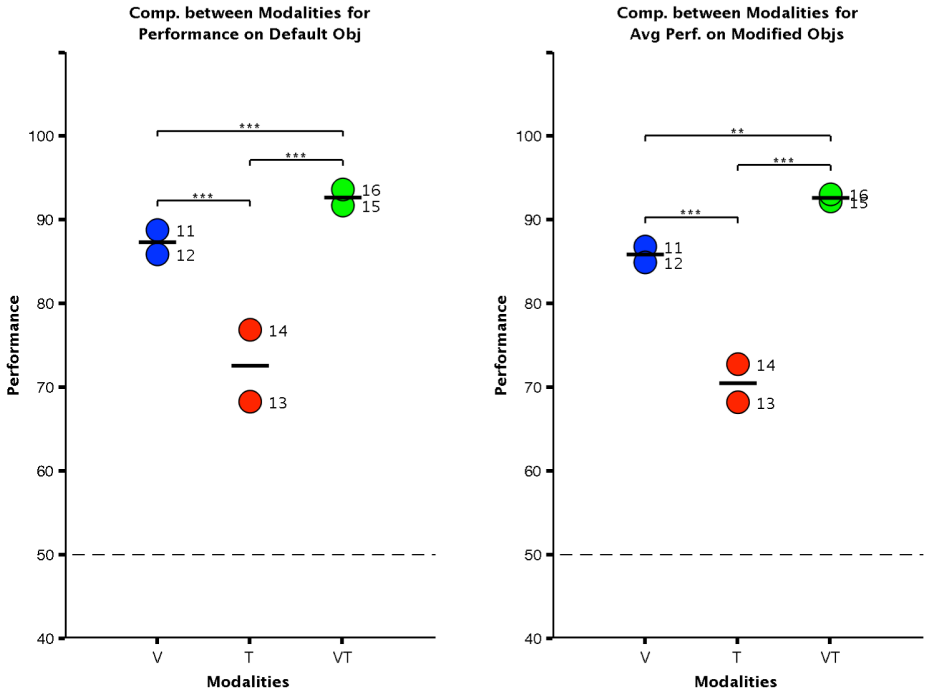
Figure 2.2.11: Comparison between Rats of same Group on Recognition Performance on "Default" and "Modified" Stimuli of Testing Phase 1.



Comparison between rat performances on the "Default" and "Modified" stimuli (where the latter were considered all together, without distinction among the 9 different configurations). The performance of each rat (colored dots) is reported, along with the overall performance obtained by concatenating the trials' outcomes of both rats within a group (horizontal lines). The Fisher Exact Test was applied to these overall group performances, yielding no statistically significant difference in any group.

Finally, I looked at the effect of perceptual modality on the recognition performance, by comparing the vectors of concatenated trials' outcomes that were obtained for each experimental group. This analysis was done, separately, for the "Default" and the "Modified" stimuli, by using the Fisher Exact Test as before (see, respectively, the left and right panels in Fig. 2.2.12). In both cases, rat performances were significantly different between the sensory groups, with the visuo-tactile group slightly outperforming the visual group and both the visual and visuo-tactile groups substantially outperforming the tactile group.

Figure 2.2.12: Comparison between Groups on Recognition Performance on "Default" and "Modified" Stimuli of Testing Phase 1.



Comparison among rat performances in the three experimental groups, for both the "Default" (left) and the "Modified" stimuli (right; the latter were considered all together, without distinction among the 9 different configurations). As in Fig. 2.2.11, the performance of each rat (colored dots) is reported, along with the overall performance obtained by concatenating the trials' outcomes of both rats within a group (horizontal lines). The Fisher Exact Test was applied to these overall group performances ($p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).*

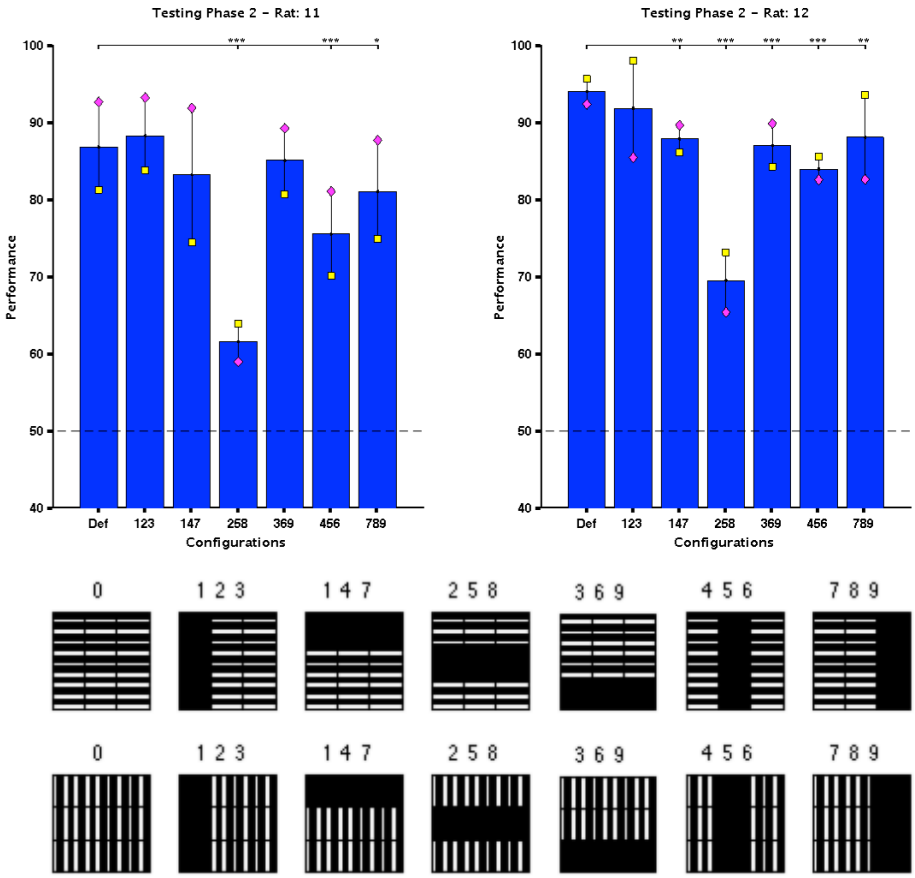
Summarizing, Testing Phase 1 produced three main results. First, rat recognition ability is robust enough to solve the orientation discrimination task in spite of a minor change in the appearance of the stimulus (i.e., the removal of a tile), and of the consequent small decrease of discriminatory information. Second, rat discrimination did not rely on just one specific position of the tiles, because they were able to solve the task no matter which tile was missing. Third, a general tendency was observed across the three groups of rats to be subject to a decrease of performance (although modest), whenever one grating tile was absent in one of the central row positions ("Modified" configurations 2, 5, 8), suggesting that these parts of the object were more salient (or more relied upon) to solve the orientation discrimination task.

2.2.3.2 Testing Phase 2

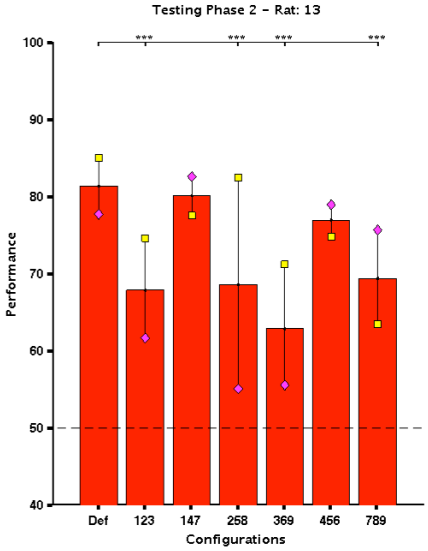
During Testing Phase 2, rats were tested on six different configurations, for each orientation, of a new set of "Modified" stimuli: in each of these, either one full row or one full column of three tiles was removed and substituted with black smooth caps (see Fig. 2.2.5). At least ~300 trials were collected for each rat on each configuration, regardless of orientation.

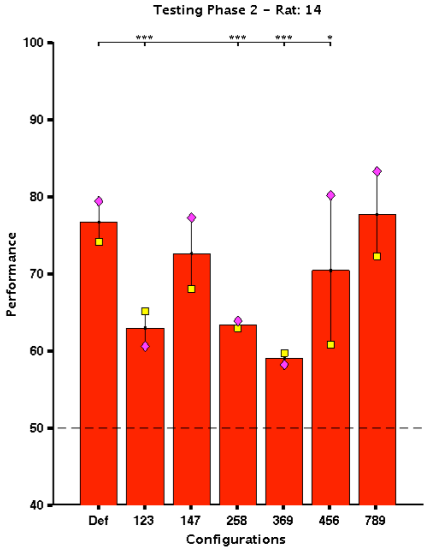
As in the previous Testing Phase, rats of all groups maintained an ability to discriminate the grating orientation, in spite of such a major stimulus modification (see Fig. 2.2.13). As before, a Binomial test was used to compare the percentage of observed successes to chance level (i.e., 50% correct responses): all recognition performances on the "Default" and "Modified" stimuli turned out to be significantly different from chance ($p < .001$ for all configurations; p-values or stars are not reported for clarity; see Fig. 2.2.13). In spite of this, the impact of the missing tiles on rat recognition performance was substantially larger than in the previous phase. For each rat, a Fisher Exact Test was carried out to compare the performance on the Default stimulus to the performances on each of the Modified stimuli. Several comparisons were significant for the visual and tactile groups, while just one comparison was significant for visuo-tactile group. Observing a substantial drop of the performance on a given Modified stimulus suggests that rat recognition preferentially relied on the discriminatory information afforded by the row or column of tiles that were missing in the Modified stimulus. On the other hand, the fact that recognition performance was still above chance, also when this information was missing, can be interpreted as an ability to compensate for the lack of this preferred feature by integrating the information that was present in remaining portions of the gratings.

Figure 2.2.13: Performance of all Rats on Recognition of "Default" and "Modified" Stimuli of Testing Phase 2.



*Bar plots showing the performances of the rats in the three experimental groups (blue: visual; red: tactile; green: visuo-tactile) for the "Default" (first bar in each plot) and the "Modified" stimuli of Testing Phase 2 (the numbers under the bars indicate what tiles were missing from each of the Modified stimuli; refer to the image at the bottom of the figure or to Fig. 2.2.5 for an image of the stimuli). All the performances were significantly larger than expected by chance, according to a Binomial test ($p < 0.001$). The stars refer to the significance of the comparisons between the performances observed for the Default stimulus and each of the Modified stimuli (Fisher Exact Test , * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).*


Testing Phase 2 – Rat: 13
***
***
***
***
100
90
80
70
60
50
40
Performance
Def
123
147
258
369
456
789
Configurations


Testing Phase 2 – Rat: 14
***
***
***
*
100
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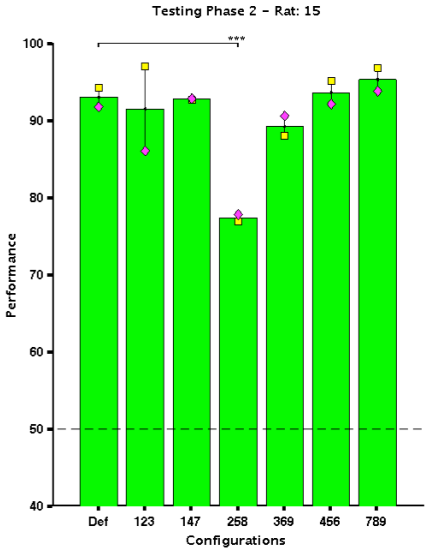
40
Performance
Def
123
147
258
369
456
789
Configurations


Testing Phase 2 – Rat: 15
***
100
90
80
70
60
50
40
Performance
Def
123
147
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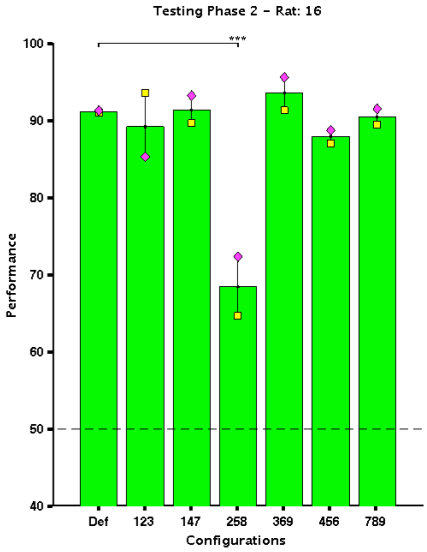
369
456
789
Configurations


Testing Phase 2 – Rat: 16
***
100
90
80
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50
40
Performance
Def
123
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258
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456
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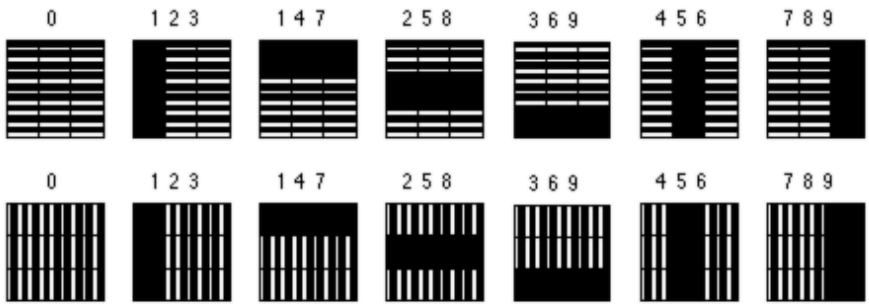
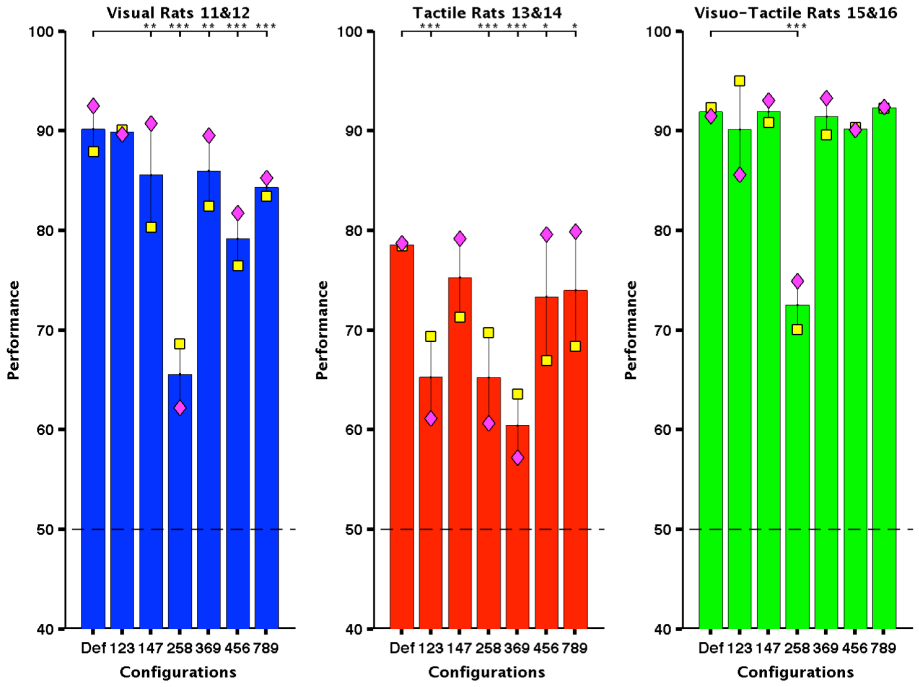
Configurations


0
1 2 3
1 4 7
2 5 8
3 6 9
4 5 6
7 8 9
0
1 2 3
1 4 7
2 5 8
3 6 9
4 5 6
7 8 9

The most prominent trend that emerged from looking at Fig. 2.2.13 is the consistency between the perceptual strategies of the rats belonging the same group, i.e., the consistency between the specific rows or columns of tiles each animal within a group preferentially relied upon. This can be easily appreciated in the case of the visual and visuo-tactile rats, where the removal of the central row of tiles produced the largest drop in recognition performance.

Given such a consistency, I concatenated the vectors of trials' outcomes that were obtained for the two rats within each group on any specific stimulus configuration, so as to assess the recognition strategy at the group level (see Fig. 2.2.14). Again, a Fisher Exact Test was performed to find out what Modified stimuli led to a significant decrement of the recognition performance. This was the case for 5 out of 6 stimuli in both the visual and the tactile groups, while only one stimulus configuration yielded a significant drop of the performance in the visuo-tactile group. Interestingly, this configuration (with missing tiles 2, 5 and 8) had a strong impact on the performance of all the groups, thus confirming the influence of the central row of tiles in determining rat recognition behavior, regardless of the sensory modality.

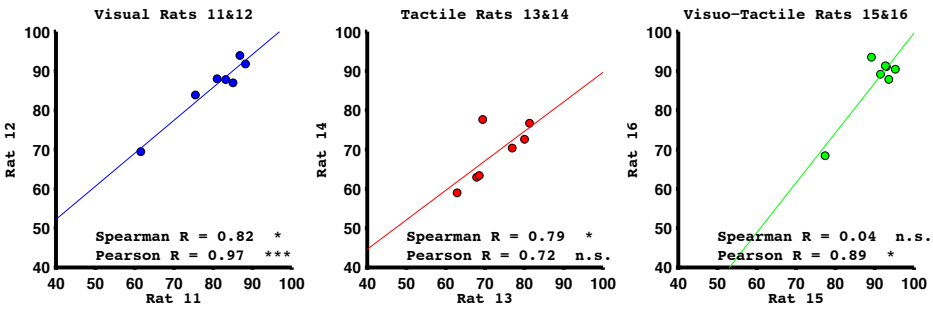
Figure 2.2.14: Performance of all Groups on Recognition of "Default" and "Modified" Stimuli of Testing Phase 2.



Same as Fig. 2.2.13, but after concatenating the responses of the rats within each experimental group, so as to achieve a more compact comparison between the visual (blue bars), tactile (red bars) and visuo-tactile groups (green).

To better check the consistency of rat recognition strategy within each group, and test whether it was justified to concatenate the responses of the animals belonging to the same group (as done in Fig. 2.2.14), I measured the correlation between the patterns of responses obtained, for the rats within a group, across all the Modified stimuli (see Fig. 2.2.15). Pearson's product-moment correlation test showed a linear positive correlation for all pairs of rats, which was significant for the visual and visuo-tactile groups. Instead, Spearman rank correlation test yielded a monotonic positive correlation only for the visual and tactile groups, likely because, in the case of the visuo-tactile group, the range of variation of the performance was too limited (with most conditions clustering in a small region of the performance plane; see Fig. 2.2.15; green dots) to yield a clean monotonic relationship between the patterns of performances obtained for the two rats. Taken together, these results suggest that the recognition strategies of the rats in each sensory group were highly congruent, i.e., were affected in the same way by the same "Modified" configurations. This implies that the rats in each group used similar a perceptual strategy to solve the discrimination task.

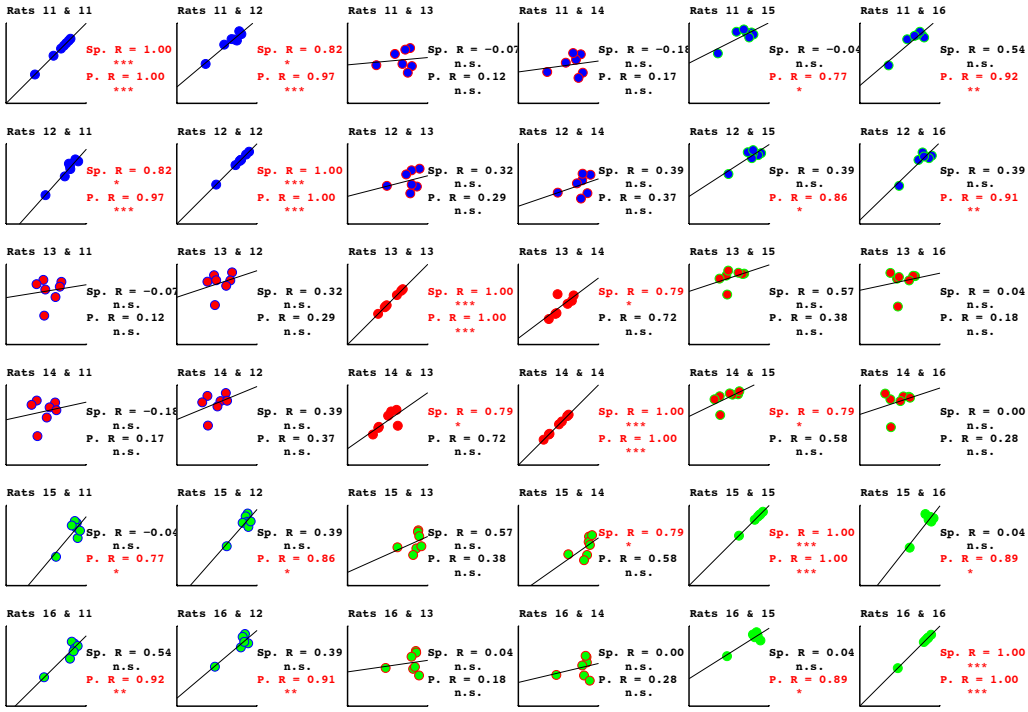
Figure 2.2.15: Correlation between Recognition Performances on "Modified" Stimuli of Testing Phase 2 between Rats of same Group.



Scatter plots showing the correlation between the performances obtained, on the "Modified" stimuli, for the two rats in each of the experimental groups (visual: blue dots; tactile: red dots; visuo-tactile: green dots).

Another interesting question is whether rats tested with different modalities, (i.e., animals in different groups) also applied a similar recognition strategy to solve the task. This hypothesis is supported by the fact that the central row of tiles had a similarly strong influence on rat recognition, regardless of the sensing modality. To address this question, I computed the correlation coefficients between the patterns of performances obtained for all possible pairs of rats, regardless of the group they belong to. The resulting correlation matrix (shown in Fig. 2.2.16 along with the corresponding scatter plots) shows that there was no correlation between the rats of the tactile group and the rats of the other groups, while, in the case of visual and visuo-tactile groups, the recognition performances were significantly linearly correlated for all the pairs of tested rats (the Spearman's rank correlations were not significant, likely because of the lack of variation in the range of performance values obtained for the visuo-tactile rats, as previously pointed out). These results suggest that the rats in the visuo-tactile group shared a common recognition strategy with the animal in the visual group, although the former had, overall, a more stable performance against the loss of discriminatory information produced by the tiles' removal. The rats in the tactile group, on the other hand, showed a strategy that was not consistent with that of the visual and visuo-tactile groups.

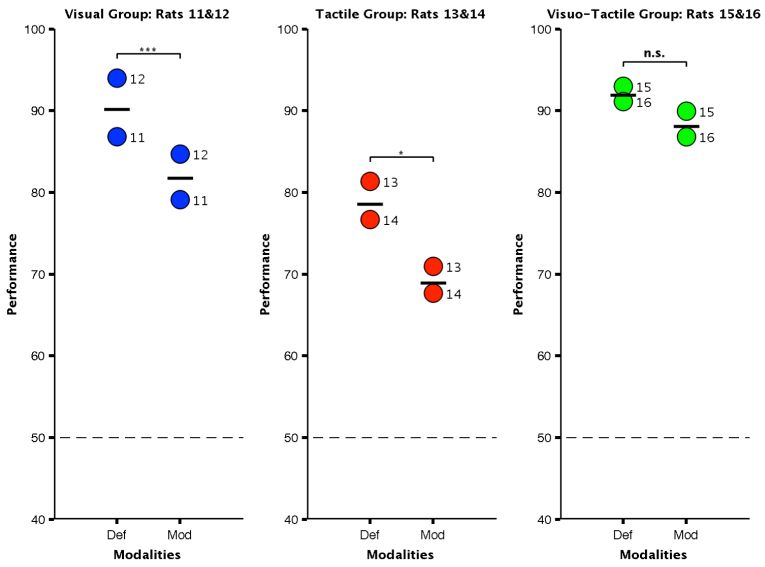
Figure 2.2.16: Correlation between Recognition Performances on "Modified" Stimuli of Testing Phase 2 between all Rats.



Scatter plots showing the correlation between the performances obtained, on the "Modified" stimuli, for each pair of rat that was tested in the experiment, regardless of the group the animal belong to. Note that the plots along the diagonal show the correlation of a rat with itself (so they are not meaningful) and that the matrix is symmetric along the diagonal. The plots showing the correlations between the rats of the same group (i.e., those in full blue, full red and full green) are the same as those already shown in Fig. 2.2.15.

To summarize, my analysis indicate that the use of the "Modified" stimuli had two main effects on rat discrimination responses: first, recognition performance was definitely lower for all the groups when the central band of tiles was absent (see Figs. 2.2.13 and 2.2.14); second, based on the correlation analysis, the perceptual strategies of the visual and visuo-tactile groups were roughly similar (see Figs. 2.2.15 and 2.2.16). On the other hand, it is also clear from Figs. 2.2.13 and 2.2.14 that the rats in the visuo-tactile group tolerated much better the alteration of the gratings, since they were not significantly affected by the "Modified" stimuli, with the exception of the configuration corresponding to the missing central band. To confirm this, I compared, inside each group, the recognition performance of the "Default" stimulus with the recognition performance of all the "Modified" stimuli together (again, a Fisher Exact Test was applied to the vectors of trials' outcomes, obtained by concatenating the responses of the two rats within each group to the two classes of stimuli). As expected, a significant difference between the performances on the "Default" and the "Modified" stimuli was found only for the rats in visual and tactile groups (see Fig. 2.2.17). This indicates that the rats in the visuo-tactile group were, overall, more tolerant to the variations introduced with the "Modified" stimuli.

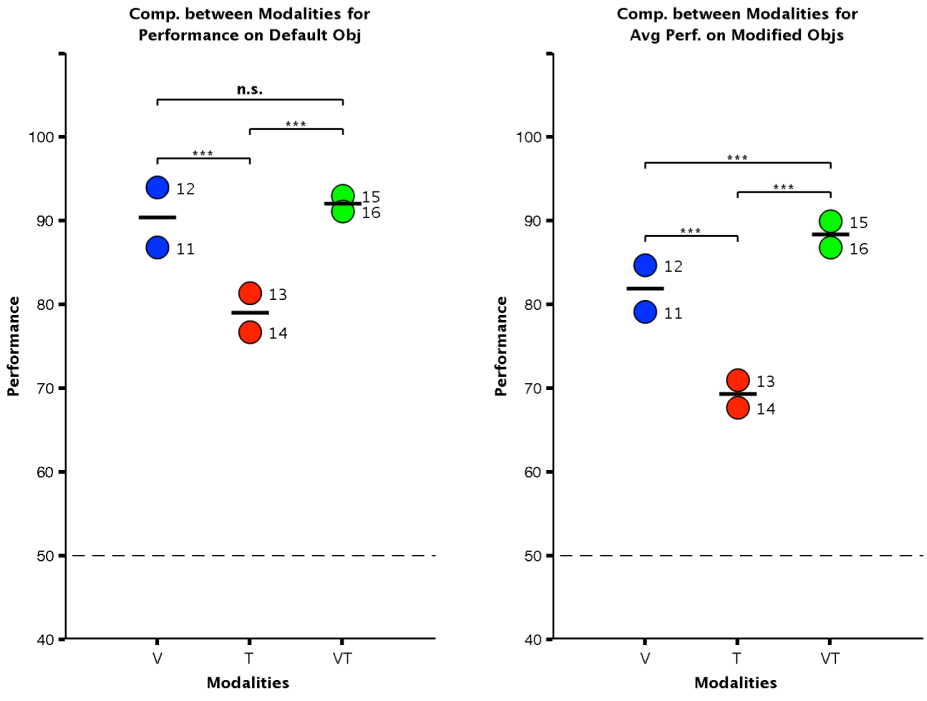
Figure 2.2.17: Comparison between Rats of same Group on Recognition Performance on "Default" and "Modified" Stimuli of Testing Phase 2.



Comparison between rat performances on the "Default" and "Modified" stimuli (where the latter were considered all together, without distinction among the 6 different configurations). The performance of each rat (colored dots) is reported, along with the overall performance obtained by concatenating the trials' outcomes of both rats within a group (horizontal lines). A Fisher Exact Test was applied to these overall group performances ($p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).*

Finally, I checked if the recognition performance was still significantly different, as observed during Testing Phase 1 (see Fig. 2.2.12), between the three different groups, separately for the "Default" and the "Modified" stimuli. A Fisher Exact Test was carried out on the group performances, obtained by concatenating the trials' outcomes of the rats within each group. All the group performances were significantly different from each other, with the exception of the comparison between the visual and visuo-tactile group for the "Default" stimulus (see Fig. 2.2.18). These results show that there was a clear perceptual advantage for the rats to rely on multimodal sensing, especially when the stimuli were altered, so as to miss part of the discriminatory information. At the same time, the relatively marginal increase of performance of the visuo-tactile group, as compared to the visual one, suggests that the former was mainly, but not exclusively, relying on visual information to solve the task.

Figure 2.2.18: Comparison between Groups on Recognition Performance on "Default" and "Modified" Stimuli of Testing Phase 2.



Comparison among rat performances in the three experimental groups, for both the "Default" (left) and the "Modified" stimuli (right; the latter were considered all together, without distinction among the 6 different configurations). As in Fig. 2.2.17, the performance of each rat (colored dots) is reported, along with the overall performance obtained by concatenating the trials' outcomes of both rats within a group (horizontal lines). A Fisher Exact Test was applied to these overall group performances ($p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).*

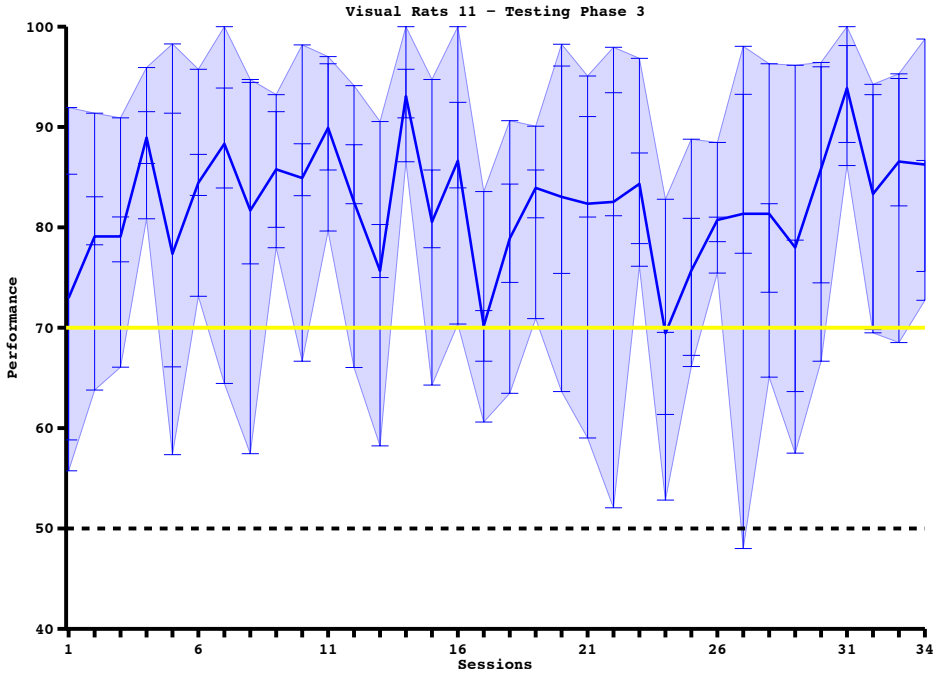
Summarizing, my results show that, in general, the discrimination abilities of the rats, especially in the visual and tactile groups, were strongly influenced by the manipulation of the stimuli. In these groups, rat recognition performance on the "Modified" stimuli was much more variable, when compared to that of the visuo-tactile group. Moreover, the magnitude of the performance drop was strongly dependent on what part of the stimulus was missing the tiles: rat recognition performance in all groups maximally decreased if all the grating tiles in the middle horizontal row were absent. This indicates that this specific region of the object is particularly important, because of its saliency or easiness of interaction, for the resolution of the task, regardless of the sensing modality.

2.2.3.2 Testing Phase 3

During Testing Phase 3, rats have been tested with a new type of "Modified" stimuli, each consisting of a different combination of three grating tiles placed on the object in different positions, for a total of eighty-four possible different configurations per orientation.

An initial observation is that rat recognition performance was strongly influenced by the identity of the "Modified" stimuli: even though the average recognition performance remained above the criterion level of 70% on almost every session, the performances on the single configurations could range between 100% and 50% (see, as example, the session-by-session average performance for rat 11 in Fig. 2.2.19, where the blue ticks show the performance for each of the four distinct configurations that were tested in each session).

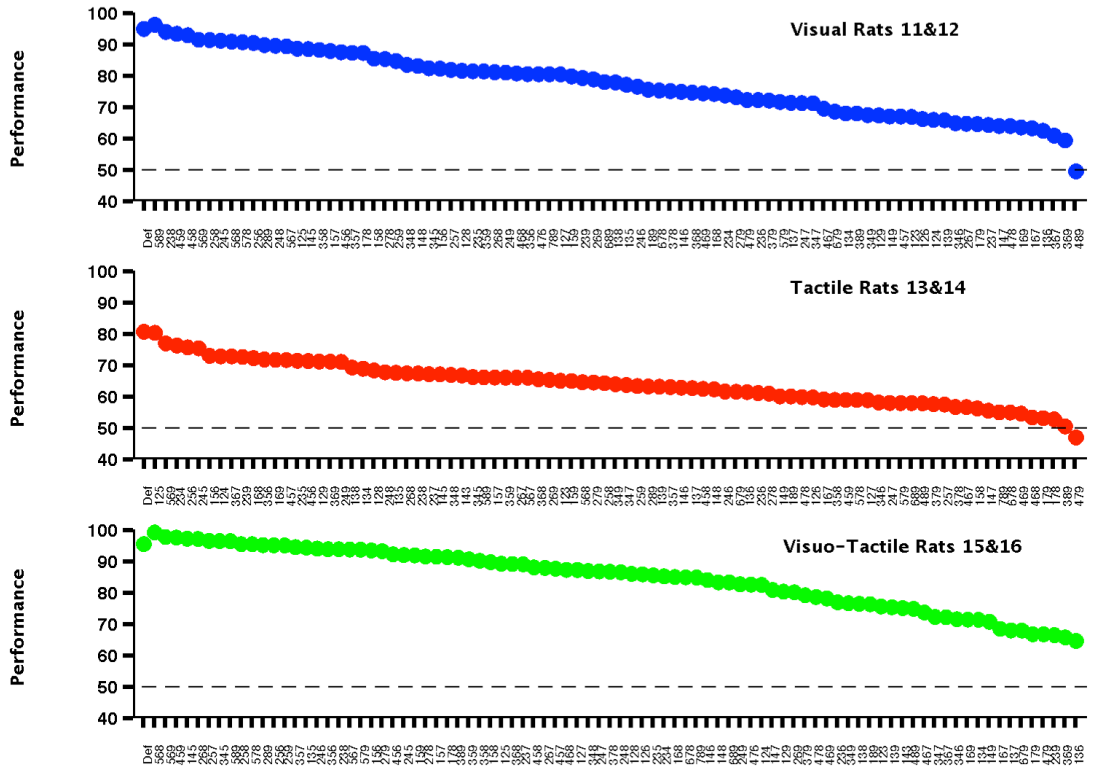
Figure 2.2.19: Session-by-Session Performance of Rat 11 on Recognition of "Default" and "Modified" Stimuli of Testing Phase 3.



An example of session-by-session performance for a rat of the visual group (rat 11). The thick line shows the average performance of the animal over the 4 different Modifed stimuli he was presented with, in each session. The performances on the individual stimuli are indicated by the horizontal ticks. The shaded area shows the spread between the highest and the lowest performances.

To investigate the influence of each configuration on the recognition performance, and so on rat perception of the objects, I built a rank-order plot of the Modified stimuli, depending on the associated recognition performance, for every group of rats (see Fig. 2.2.20; as done before, I concatenated the trials' outcomes for the two animals within each group to achieve a group performance). The plots clearly show how the recognition performances smoothly covered a wide interval of values, ranging, for instance, between 50% and 100% for the visual rats (top panel), depending on the configuration. This is an initial indication that stimulus manipulation had a great effect on rat recognition ability. The plots also show how the visuo-tactile rats had, overall, a higher, more stable performance (bottom panel), especially when compared to the tactile group, which instead, exhibited the steepest performance drop (middle panel).

Figure 2.2.20: Rank-order Performance of all Groups on Recognition of "Default" and "Modified" Stimuli of Testing Phase 3.

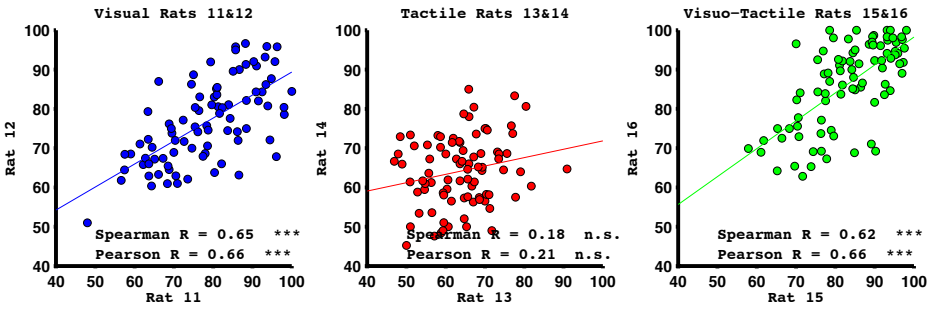


Rank-order plots showing how steeply rat recognition performance decreased across the set of Modified stimuli used in Testing Phase 3, as compared to what observed for the Default stimulus (first dot in each plot). The Modified stimuli are arranged along the abscissa according the performance they yielded (the labels of the Modified stimuli correspond to the images shown in Fig. 2.2.6).

Obviously, the large number of "Modified" configurations that was tested makes hard to understand, just by looking at the raw performances for each configuration, which recognition strategies were used by rats to solve the task, depending on sensory modality. To address this issue, I carried out a series of analyses, aimed at understanding what features of the grating stimuli played the most important role in determining rat recognition behavior.

As the first step of my analysis, I measured the correlation between the patterns of responses obtained, for the rats within a group, across all the Modified stimuli (see Fig. 2.2.21). Both the Pearson's product-moment and the Spearman rank correlation tests showed a linear and monotonic positive correlation for all the pairs of rats. Such correlations were significantly larger than expected by chance for the visual and visuo-tactile groups, but not for the tactile group. These results suggest that a common perceptual strategy was shared by the members of the visual and visuo-tactile groups. The absence of significance for the tactile rats may be due to the larger decrease of their recognition performance, which may have led to a perceptual strategy that was less stable across configurations and less reproducible across rats.

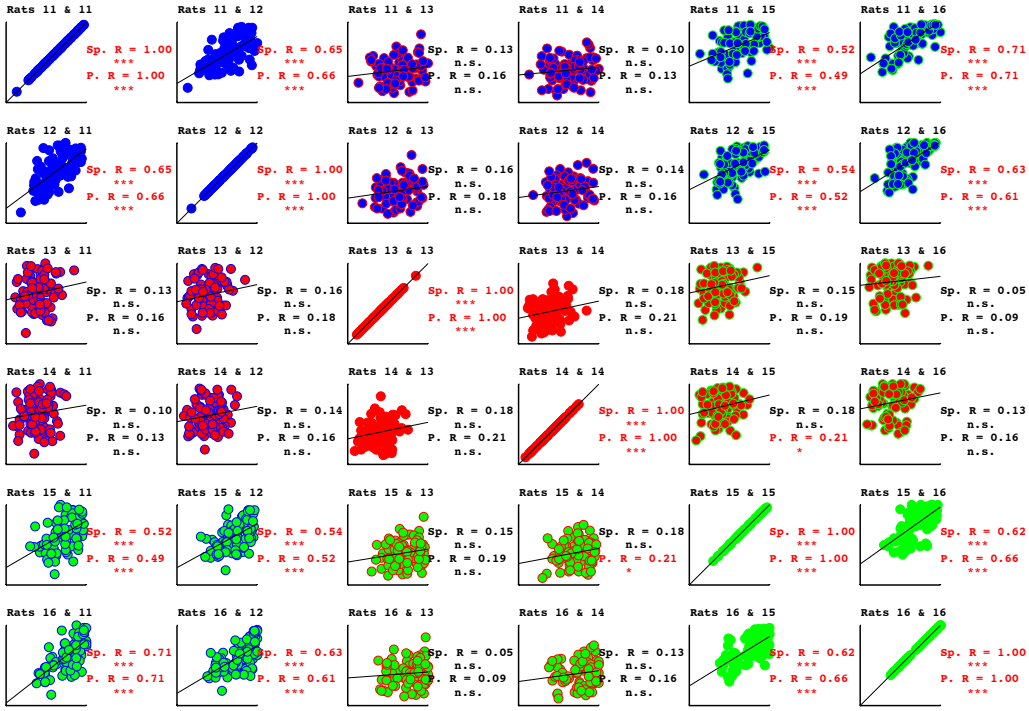
Figure 2.2.21: Correlation between Recognition Performances on "Modified" Stimuli of Testing Phase 3 between Rats of same Group.



Scatter plots showing the correlation between the performances obtained, on the "Modified" stimuli, for the two rats in each of the experimental groups (visual: blue dots; tactile: red dots; visuo-tactile: green dots).

To further investigate the relationships between sensory modalities and recognition, I also computed, as previously done for Testing Phase 2 (see Fig. 2.2.16), the correlation coefficients between the patterns of performances obtained for all possible pairs of rats, regardless of the group they belong to. The resulting correlation matrix (shown in Fig. 2.2.22, along with the corresponding scatter plots) indicates that the recognition performances were consistent between all the rats within the visual and visuo-tactile groups. This result strengthens the conclusions about the similarity of the perceptual strategies between these two groups of rats, and their dissimilarity from the strategy used by the tactile group.

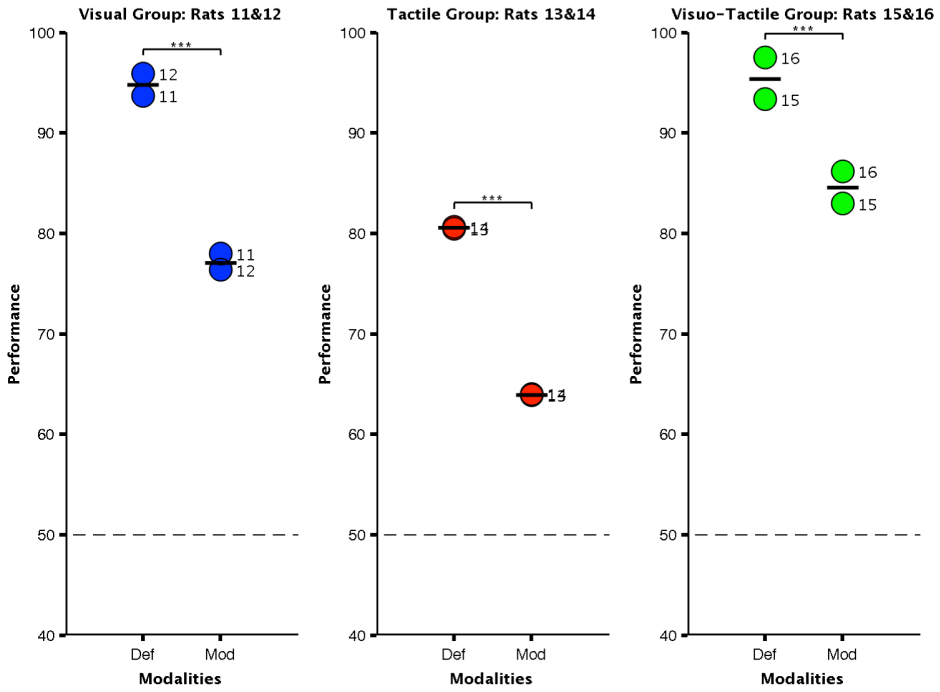
Figure 2.2.22: Correlation between Recognition Performances on "Modified" Stimuli of Testing Phase 3 between all Rats.



Scatter plots showing the correlation between the performances obtained, on the "Modified" stimuli, for each pair of rat that was tested in the experiment, regardless of the group the animal belong to. Note that the plots along the diagonal show the correlation of a rat with itself (so they are not meaningful) and that the matrix is symmetric along the diagonal. The plots showing the correlations between the rats of the same group (i.e., those in full blue, full red and full green) are the same as those already shown in Fig. 2.2.21.

Figure 2.2.20 already made clear that the manipulations yielding the "Modified" stimuli had a major effect on rat discrimination success. This was also confirmed by comparing, inside each group, the recognition performance on the "Default" stimulus and all the "Modified" stimuli taken together, by applying the Fisher Exact Test to the respective trials' outcomes vectors, as done previously (e.g., see Figs. 2.2.11 and 2.2.17). This analysis confirmed a strong and significant drop of performance for the Modified stimuli for all the groups (see Fig. 2.2.23). Noticeably, this is the first manipulation that is able to have a global, significant effect on the recognition performance of the rats belonging to the visuo-tactile group too (compare Fig. 2.2.23 to Figs. 2.2.11 and 2.2.17).

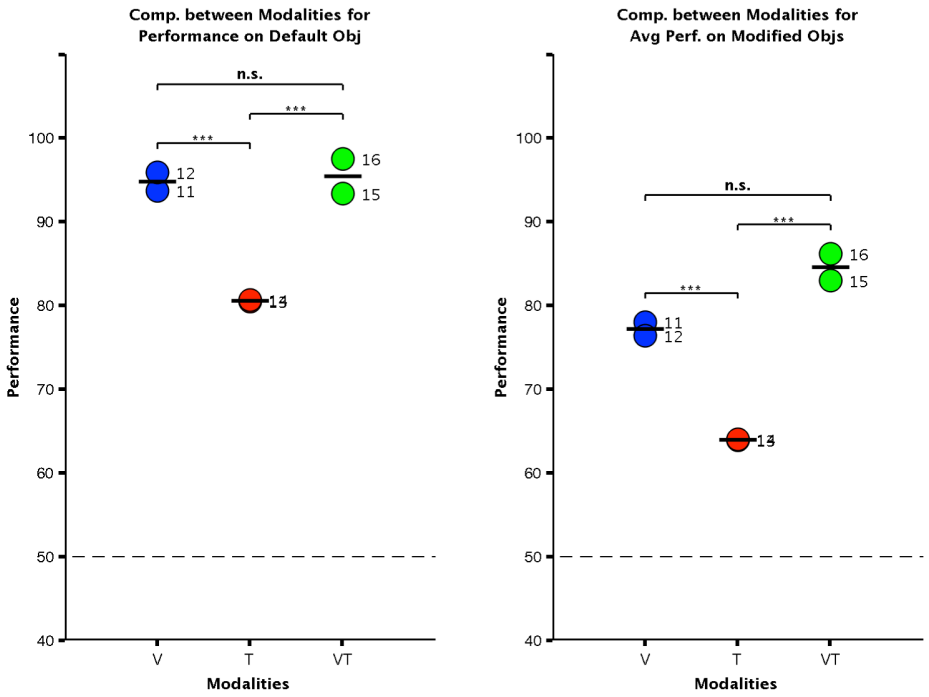
Figure 2.2.23: Comparison between Rats of same Group on Recognition Performance on "Default" and "Modified" Stimuli of Testing Phase 3.



Comparison between rat performances on the "Default" and "Modified" stimuli (where the latter were considered all together, without distinction among the 6 different configurations). The performance of each rat (colored dots) is reported, along with the overall performance obtained by concatenating the trials' outcomes of both rats within a group (horizontal lines). A Fisher Exact Test was applied to these overall group performances ($p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).*

The effect of the manipulation yielding the new "Modified" stimuli was visible not only within groups, but also between groups. A Fisher Exact Test was carried out, as before, to compare rats' performances on the Default and Modifies stimuli across the three experimental groups (see Fig. 2.2.24). For both classes of stimuli, the visual and visuo-tactile groups had performances that were not significantly different from each other, but were both significantly different from the performances of the tactile group. This confirms the conclusion of the correlation analysis (see Figs. 2.2.21 and 2.2.22), indicating a similarity in the way the visual and visuo-tactile stimuli were processed.

Figure 2.2.24: Comparison between Groups on Recognition Performance on "Default" and "Modified" Stimuli of Testing Phase 3.



Comparison among rat performances in the three experimental groups, for both the "Default" (left) and the "Modified" stimuli (right; the latter were considered all together, without distinction among the 6 different configurations). As in Fig. 2.2.23, the performance of each rat (colored dots) is reported, along with the overall performance obtained by concatenating the trials' outcomes of both rats within a group (horizontal lines). A Fisher Exact Test was applied to these overall group performances ($p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).*

2.2.3.3 Saliency maps revealing rat recognition strategy

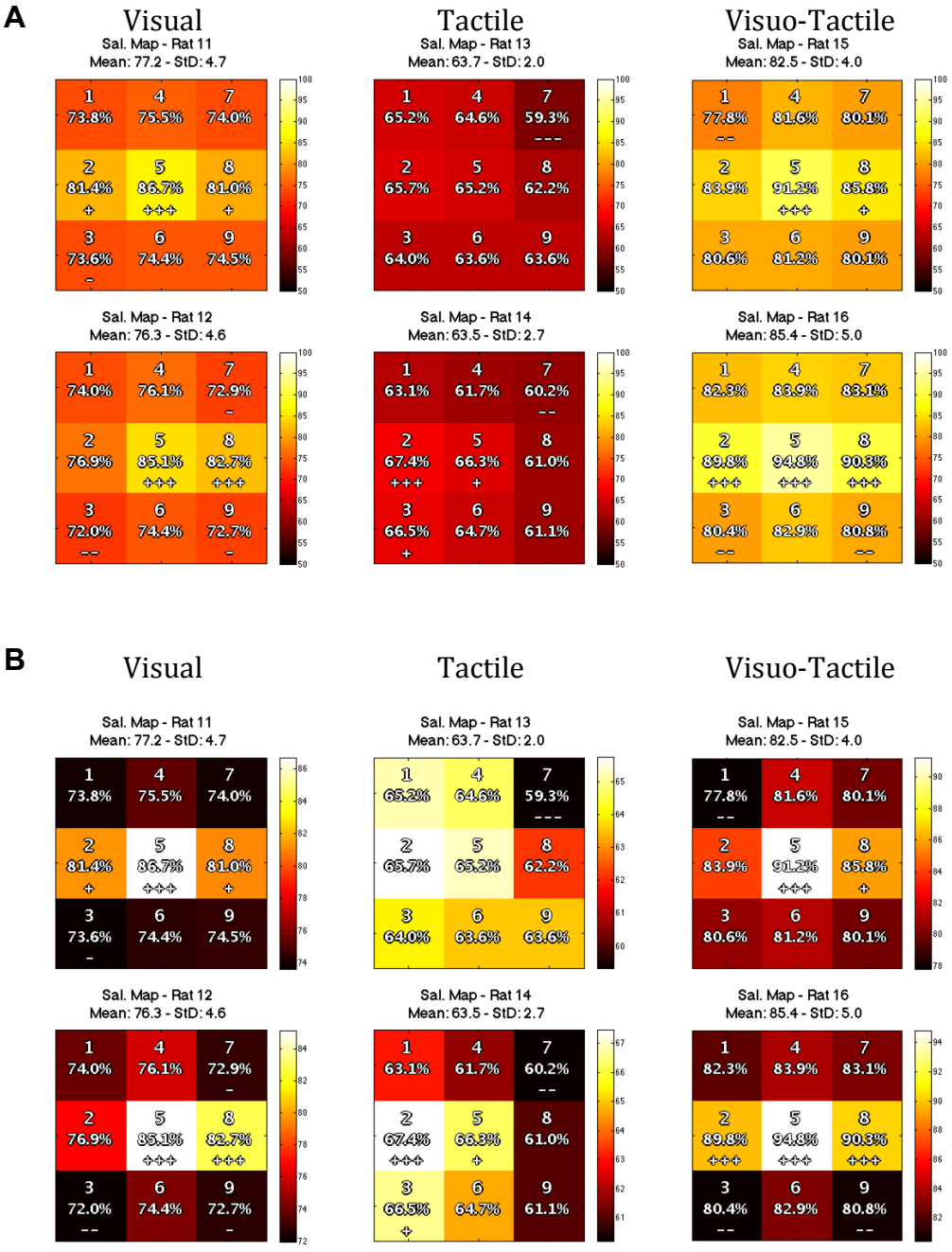
The final important step in my analysis was to infer the perceptual strategies used by each rat to process the grating stimuli and solve the task. To this aim, I built for each rat two different types of saliency maps of the stimulus (as in classification image approaches). My goal was to understand the relationship between the presence of one or more tiles at specific locations on the object and the chance for that specific stimulus configurations to be successfully discriminated. In other words, I tried to link each specific position within the stimulus with the recognition performance that was obtained when that position was filled by a tile, regardless or depending on the orientation of the tile. Looking from another point of view, this approach aimed at understanding where and how rats focused their attention to solve the task, regardless or depending on the sensory modality of perception.

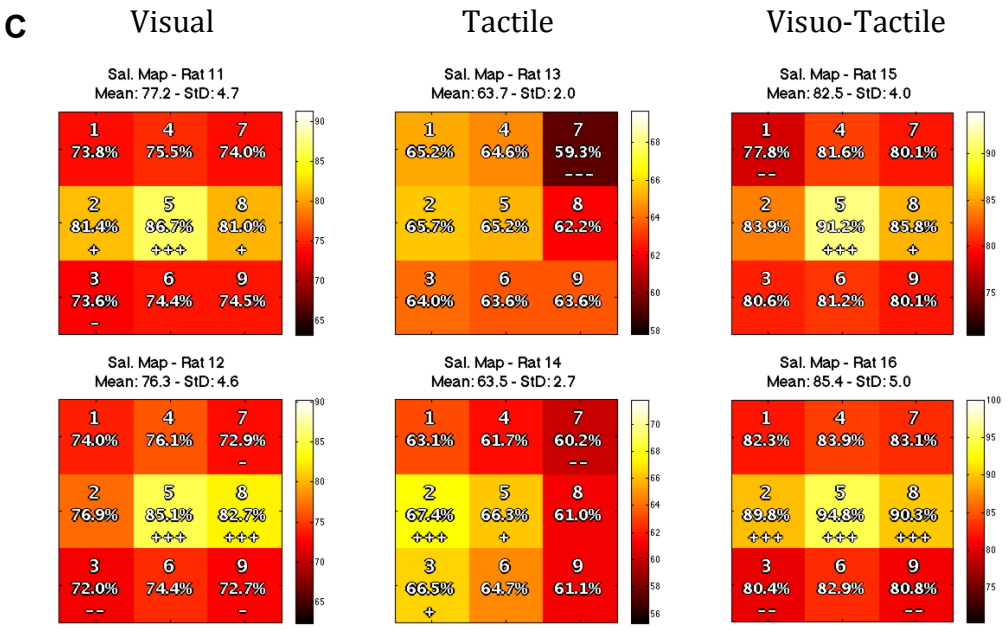
The first type of saliency map was built by taking into consideration all the trials' outcomes for all the 28 (out of 84) different "Modified" stimuli, where a given position (e.g., the central location or the top-left one, etc.) was used to place a tile. I concatenated all these trials, considering both orientations together, and I computed the recognition performance over these trials' outcomes (see Fig. 2.2.25). Each position, in fact, has been used in exactly 28 "Modified" configurations (see Fig. 2.2.6), where each of the other positions was used 7 times. The general idea was to extract the influence of a single tile on rat recognition performance, by filtering out the effect of the other tiles that were presented along this specific one, across the set of 28 Modified stimuli. To better visualize the results, the resulting saliency maps are reported with three different scales for the color-coding of the recognition performance. The first map (see Fig. 2.2.25.A) uses a fixed scale between 50 and 100 % correct, which is common to all the rats, so as to allow a better comparison within and between groups. The second map (see Fig. 2.2.25.B) uses a rat-specific scale, whose extremes are set to the minimum and maximum recognition performance of each individual animal (this is helpful to enhance any effect that is specific to a single rat). The last map tries to establish a compromise between the previous approaches, by setting the extremes of the scale to three standard deviations below and above the average recognition performance of a rat, across all the cells of the map.

To assess the statistical significance of each value, I carried out a permutation test. For each position (i.e., cell in the saliency map), I compared the actual performance to a null distribution of 5000 performance values. Each of these values was obtained by computing the recognition performance after permuting the association between the sets of trial outcomes obtained for the various Modified stimuli and the identity of the stimuli themselves. This comparison was done with a two-tailed hypothesis, checking if the actual performance was either in the leftmost or rightmost 5^{th} percentile of the null distribution. In the first case, the significance is marked by a "plus" sign in the saliency map, while, in the latter, by a "minus" sign.

Figure 2.2.25: Saliency Maps for all Rats.

Saliency maps showing, for each rat, the discrimination performance that was obtained when any given position of the grating stimulus was filled by a tile. Panels A-C show the same maps, but rendered with a different scaling, so as to either optimize the comparison among rats and groups (A) or among tile positions within the same map (B and D;(see the main text for details). The plus and minus signs indicate, respectively, whether the performance value associated to a given tile location was significantly larger or smaller than expected by chance (see the main text for details).





Many conclusions can be taken by looking at the saliency maps. First, they confirmed the overall tendency of the animals in the tactile group to have a lower performance (regardless of the tile that was present in the grating), as compared to the rats of the visual and visuo-tactile groups (see Fig. 2.2.25.A, left and right columns). When considering matching positions over the grid of tiles, the ranking of the discrimination performances was invariably: visuo-tactile, best; visual, second-best; tactile, last. Second, the maps obtained for the rats in visual and visuo-tactile groups were largely consistent, revealing a better recognition performance when the central band of the stimulus was used (see Fig. 2.2.25.B and C, left and right columns). This indicates that the animals relied mostly on this salient region to solve the task. Within this region, the highest recognition performance was reached when the tile placed in the middle of the band (and, therefore, of the stimulus) was present. On the contrary, the lower part of the objects turned out to be slightly anti-salient, i.e., when the tiles were located in that region, rat performance was often significantly lower than expected by chance (given the overall animal performance across all the tile positions). Finally, in the case of the tactile group, rats showed a more variable recognition strategy regarding which positions were more advantageous or disadvantageous for the resolution of the task (see Fig. 2.2.25, middle column). Still there was a general trend toward relying more on the central-lower positions at the left of the object, than on the top-right positions.

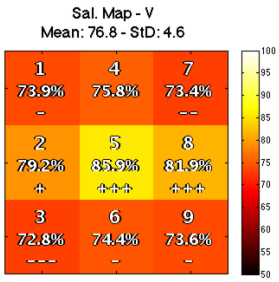
Given the similarity of the maps obtained for the rats belonging the same group (see also Fig. 2.2.21), I merged all the trials of the two animals within a group (for any given "Modified" stimulus) and carried out the same saliency map analysis. This allowed obtaining a better picture of the difference among the perceptual strategies at the group level. The resulting maps (see Fig. 2.2.26) were obviously consistent with those obtained for the individual rats, but showed an enhanced distinction between salient and anti-salient regions of the grating stimuli. For the rats in the visual and visuo-tactile groups, a salient, horizontal band was flanked by two anti-salient bands (one above and one below it). For the tactile rats, the salient region was located in the bottom-left corner of the stimulus matrix, while the other end of the stimulus (the top-right corner) was anti-salient. Interestingly, although the visuo-tactile rats presented a saliency map that was very similar to the map of the visual group, at the same time they were also generally better to solve the task, indicating an advantage given by multisensory perception. Some preliminary hypotheses are: first, they are better because they feel more confident, due to the fact that the interaction with the stimulus is more natural; second, they are using not only visual information to solve the task, but also the tactile one, maybe as a secondary tool to acquire certainty about the choice. In the latter case, it would be interesting to understand how the saliency map obtained for the visuo-tactile sensing modality can be derived from the maps obtained for the visual and tactile modalities.

Figure2.2.26: Saliency Maps for all Groups.

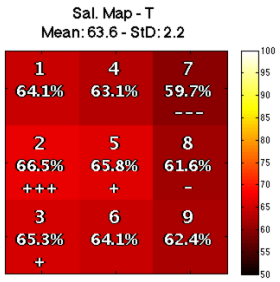
Saliency maps showing, for each group of rats, the discrimination performance that was obtained when any given position of the grating was filled by a tile. These maps are equivalent to those shown in Fig. 2.2.25, only the trials' outcomes of the rats within each group were merged. Same conventions as in Fig. 2.2.25.

A

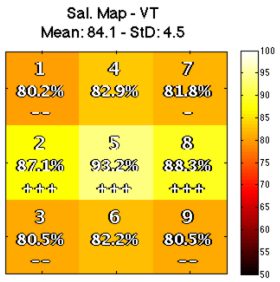
Visual



Tactile

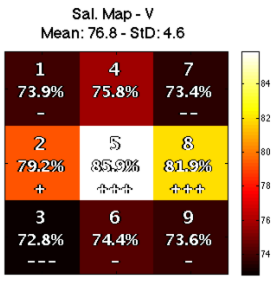


Visuo-Tactile

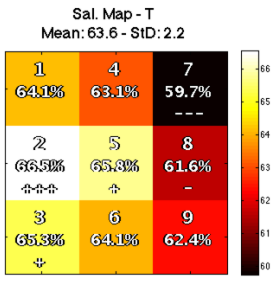


B

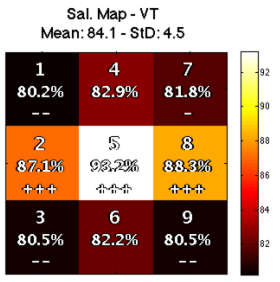
Visual



Tactile

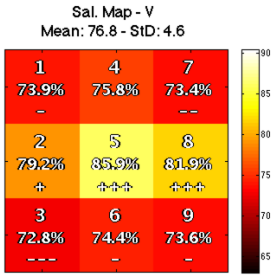


Visuo-Tactile

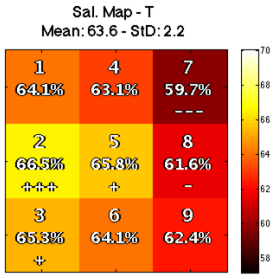


C

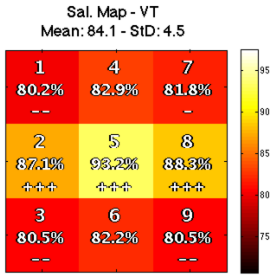
Visual



Tactile



Visuo-Tactile



In order to understand not only the effect of single grating positions on rat recognition, but also the effect of any possible interaction between different positions, I also built a different kind of saliency maps. Each cell in these maps reports the recognition performance over all the trials in which a given pair of positions was filled by the tiles (seven different "Modified" stimuli contributed to the performance associated to each pair of tiles). I computed the statistical significance of each performance in these maps using a permutation test (similar to the one described above) and I carried out this analysis for both the individual rats and the three sensory groups (see, respectively, Figs. 2.2.27 and 2.2.28). As done previously, these maps too rendered with different scales to either emphasize the comparison among the rats/groups or the comparison among the cells within each map (compare panels A, B and C). Note the cells along the diagonal of the map report the performances associated to a single position only, i.e., corresponding to the case where the tile at that position was indistinctly paired to any other tile within the grating (i.e., same data as in Figs. 2.2.25 and 2.2.26).

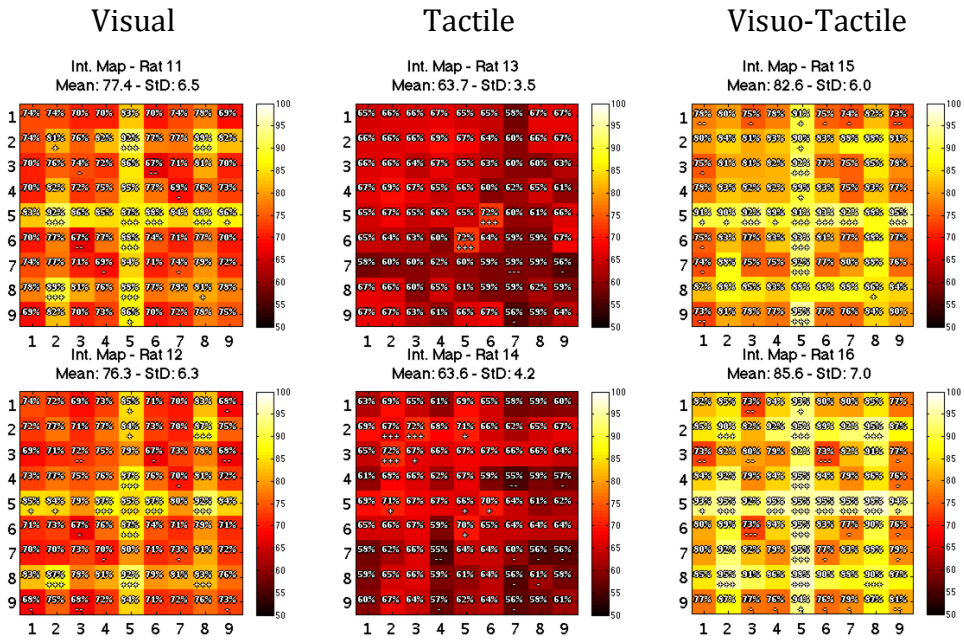
Again, I found a substantial congruency between the results of visual and visuo-tactile groups (compare the left and right columns in Figs. 2.2.27 and 2.2.28). Each of the positions belonging to the central band (i.e., positions 2, 5 and 8), and especially the middle one (position 5), were highly salient. This gave rise to a distinct patterns inside the saliency maps of the visual and visuo-tactile rats: 1) a central cross of very high performance values, corresponding to the configurations when a tile in position 5 was paired to a tile in any other position; and 2) a square of still relatively high performance values (although lower than those on the central cross), corresponding to the configurations when either a tile in position 2 or 8 was paired to a tile in any other position. Noticeably, the largest performances were observed when pairs of positions in the central band were simultaneously filled by the tiles. This was especially noticeable in the group maps of the visual animals (see Fig. 2.2.28, left), where the concomitant presence of the tiles at positions 2 and 8 yielded performances that were substantially higher than those obtained when each of these tiles was indistinctly paired to any other tile (see the diagonal elements). This trend was also observable for the visuo-tactile rats (see Fig. 2.2.28.B, right), although it was less prominent, possibly because of the ceiling effect produced by the very high performances attained by this group of animals. This ceiling effect was particularly strong when the

central position was involved. In fact, this position was the most salient and was able to support a successful stimulus categorization, regardless of the other position it was paired with (hence the cross shape). Nevertheless, a marginal performance increase was noticeable when this position was paired to one of the other two positions of the central band (see Fig. 2.2.28.B, left and right columns), as compared to when it was indistinctly paired to any other position (see the center of the maps). The results of rats in tactile group were harder to interpret, but it looked like the concomitant presence of two tiles in the positions at top-right corner of the grating led to a lower recognition performance rates.

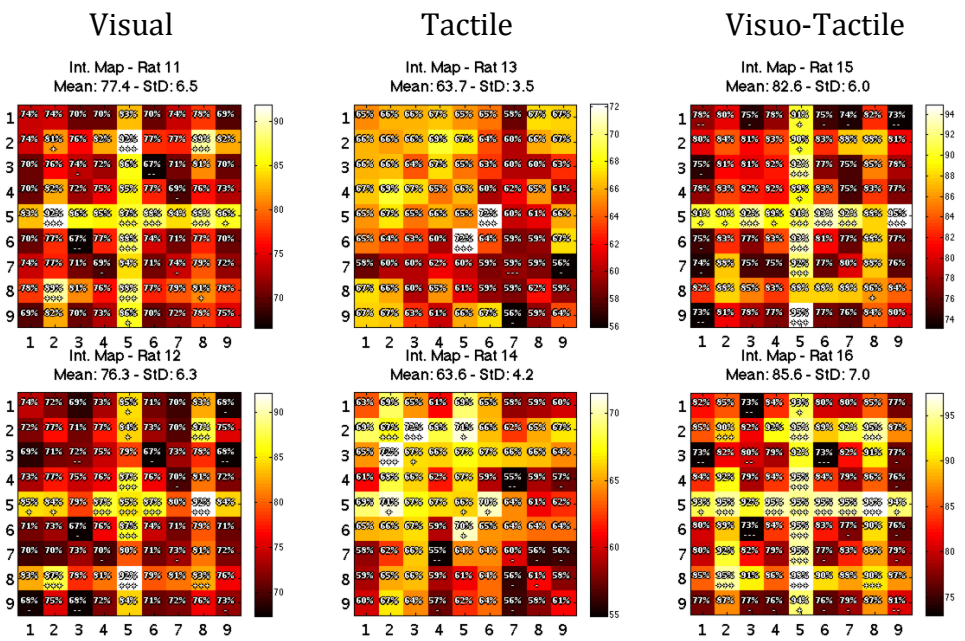
Figure 2.2.27: Saliency Maps of Interaction for all Rats.

Saliency maps showing, for each rat, the discrimination performance that was obtained when any given pair of positions of the grating stimulus was filled by a tile. Panels A-C show the same maps, but rendered with a different scaling, so as to either optimize the comparison among rats and groups (A) or among tile positions within the same map (B and D;(see the main text for details). The plus and minus signs indicate, respectively, whether the performance value associated to a given pair of tile locations was significantly larger or smaller than expected by chance (see the main text for details).

A



B


Visual
Tactile
Visuo-Tactile
Int. Map - Rat 11
Mean: 77.4 - StD: 6.5
Int. Map - Rat 13
Mean: 63.7 - StD: 3.5
Int. Map - Rat 15
Mean: 82.6 - StD: 6.0
Int. Map - Rat 12
Mean: 76.3 - StD: 6.3
Int. Map - Rat 14
Mean: 63.6 - StD: 4.2
Int. Map - Rat 16
Mean: 85.6 - StD: 7.0

C

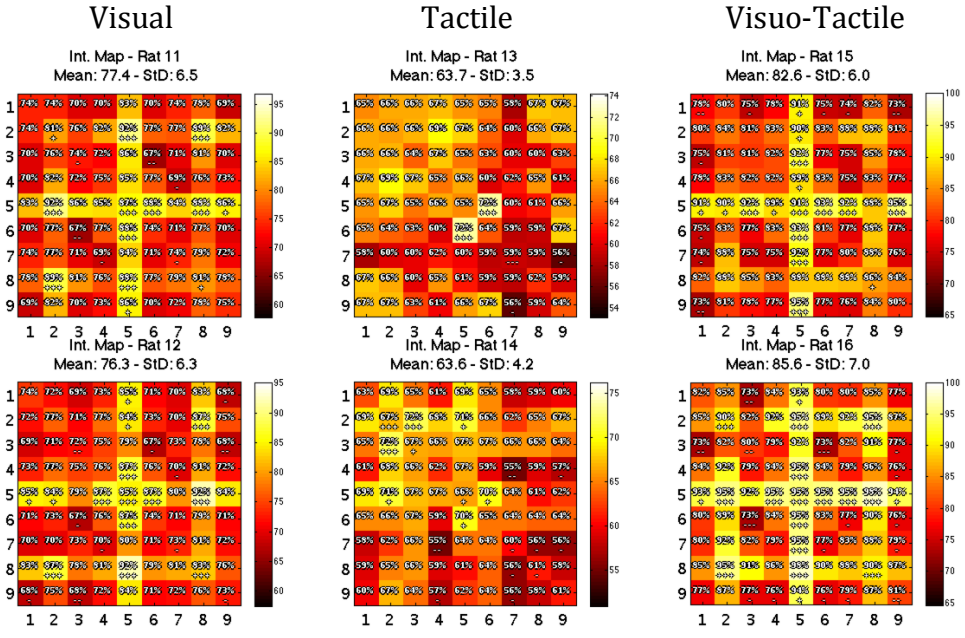
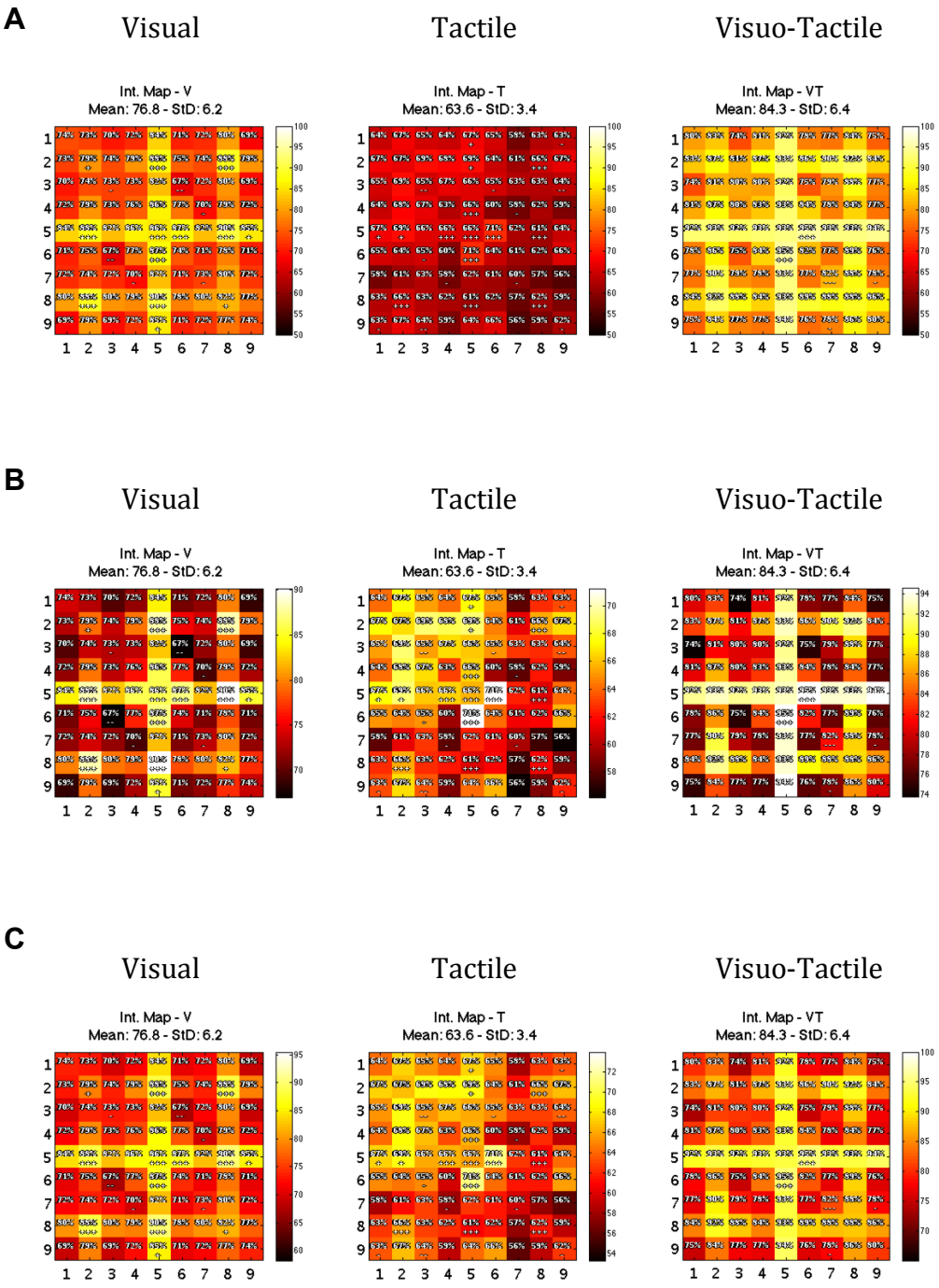

Visual
Tactile
Visuo-Tactile
Int. Map - Rat 11
Mean: 77.4 - StD: 6.5
Int. Map - Rat 13
Mean: 63.7 - StD: 3.5
Int. Map - Rat 15
Mean: 82.6 - StD: 6.0
Int. Map - Rat 12
Mean: 76.3 - StD: 6.3
Int. Map - Rat 14
Mean: 63.6 - StD: 4.2
Int. Map - Rat 16
Mean: 85.6 - StD: 7.0

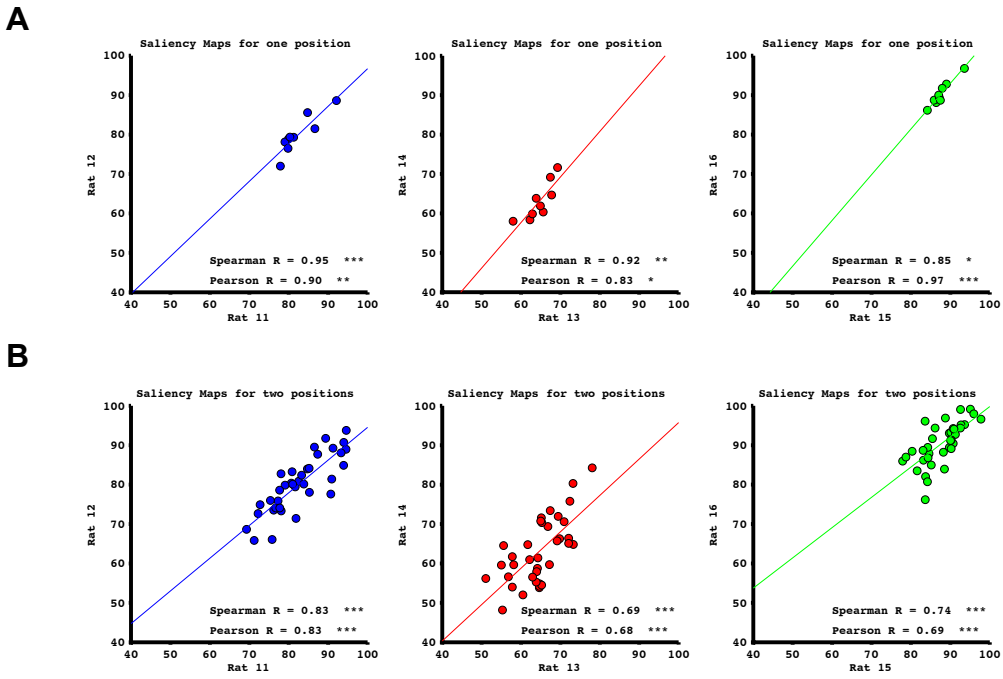
Figure2.2.28: Saliency Maps of Interaction for all Groups.

Saliency maps showing, for each group of rats, the discrimination performance that was obtained when any given pair of positions of the grating was filled by a tile. These maps are equivalent to those shown in Fig. 2.2.27, only the trials' outcomes of the rats within each group were merged. Same conventions as in Fig. 2.2.27.



Another way to look at these data was to calculate the correlation between the saliency maps values obtained for the rats of same group (as previously done for the recognition performances on "Modified" stimuli; see Fig. 2.2.21). I carried out this analysis by taking each pair of rats within a group and plotting against each other the performance values reported, for those rats, in corresponding cells of the saliency maps shown in Figs. 2.2.25 and 2.2.27 (see, respectively, Figs. 2.2.29.A-B). Pearson's product-moment and Spearman rank correlation coefficients were also computed and their significance assessed. The resulting scatter plots showed that there was a significant positive linear correlation (and, therefore, a strong congruency) between the saliency maps obtained for the rats within each group, and, therefore, between their recognition strategies. Observing these correlations also justifies having merged the data of the rats within each group to achieve the group saliency maps shown in Figs. 2.2.26 and 2.2.28.

Figure 2.2.29: Correlation between Saliency Map Values between Rats of same Group.



Correlation between the saliency maps values obtained for the rats within each sensory group (blue, visual; red, tactile; green, visuo-tactile). Panels A and B refer, respectively to the values obtained for single positions (i.e., the values shown in the maps of Fig. 2.2.25) and pairs of positions (i.e., the values shown in the maps of Fig. 2.2.27 (see min text for details).

To further assess the dependence of rat recognition performance on the presence of one or more tiles in the central band of the grating stimuli (i.e., positions 2, 5 and 8), I also carried out the following analysis. I divided the "Modified" stimuli in four different categories, depending on the number of grating tiles they had in their central band (either 0, 1, 2 or 3), and, for each rat, I plotted the corresponding performances as a function of the category label (see colored dots in Fig. 2.2.30). In addition, I also computed the averages of all the performances within each category (see black crosses in Fig. 2.2.30). Finally, I computed the Pearson's correlation coefficient between the performances and their category labels, to check if any linear relationship was observable between the number of tiles in the central band and rat discrimination accuracy (see regression lines in Fig. 2.2.30).

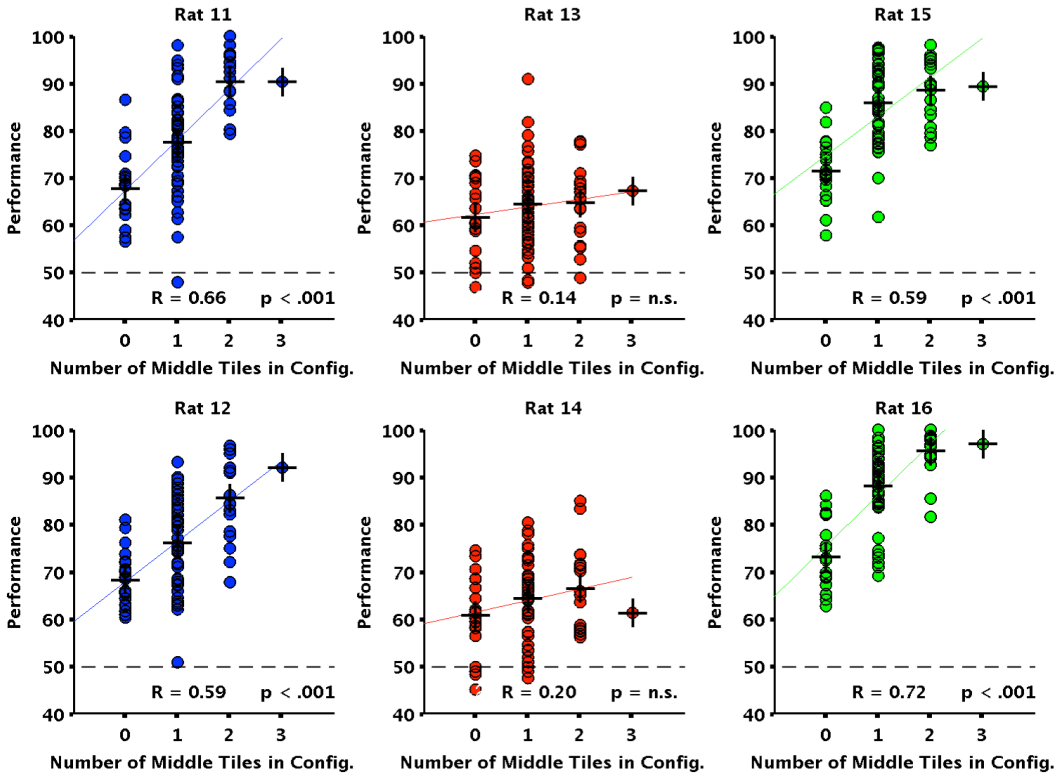
In general, rat performance varied widely across the Modified stimuli that were included in each category (see the vertical spread of dots in Fig. 2.2.30), thus showing that the number of tiles inside the central band was not the only predictor of rat performance – the specific positions of the tiles within and outside the central band also played a major role in determining rat accuracy.

Nevertheless, for both the visual and visuo-tactile groups, it was possible to find a significant positive linear correlation between the number of tiles in the central band and rat performance. In addition, for the visual rats (and also one of the tactile rats), the relationship between average performance within a category and category label (see the crosses in Fig. 2.2.30) followed a trend that pretty much overlapped with the regression line that was obtained through a least-square fit to the individual performances (i.e., to the dots in Fig. 2.2.30). This means that the category label (i.e., how many tiles were present in the central band) definitely accounted for a part of the variation of rat recognition performance across the Modified stimuli.

In particular, these results suggest that, on average, there is an additive positive effect of including progressively more tiles in the central band of the grating stimuli on rat discrimination ability. This relationship appears to be very linear for the visual rats (first column in Fig. 2.2.30), while it looks like saturating for the visuo-tactile rats (last column in Fig. 2.2.30), probably because of a ceiling effect in the performances due the overall higher discrimination accuracy in this group of animals. In the tactile group, instead, coherently with what has been found in the saliency maps, there was only a mild and not significant linear correlation, which could be interpreted as the

fact that the presence of the grating tiles in the central positions was not so influential. Still, for one of the rats (14), the average performances within the categories overlapped well with the regression line, suggesting a marginal influence of the number of tiles in the central band on rat recognition strategy.

Figure 2.2.30: Performance of all Rats on Recognition on selected "Modified" Configuration.



Plots showing rat recognition performance as a function of the number of tiles that were located inside the central band of the Modified stimuli (i.e., either 0, 1, 2 or 3, as shown by the labels in the abscissa). The dots refer to the performances on individual stimuli (the lines are the result of a regression through such points), while the crosses are the averages of such performances.

So far, the analyses I have presented in this section have all focused on saliency maps that were obtained by considering together the responses to the vertical and to the horizontal grating stimuli. Still, it is possible to compute the saliency maps separately for the horizontal and the vertical categories, by taking into consideration only the trials' outcomes when the stimulus was presented with a given orientation. These maps are shown in Figs. 2.2.31 and 2.2.32 for the three different experimental groups (after concatenating the responses of the rats within each group). Fig. 2.2.31 refers to the case where the impact of a single tile's position on rat recognition performance was assessed, while Fig. 2.2.32 refers to the case where the position of pairs of tiles was considered. This time, the color-code refers to the standard scale, which was fixed between 50% and 100%, to allow a better comparison among the groups.

The resulting maps show that the rats in tactile group did not perform differently depending on the stimulus orientation, displaying the same overall performance and the same saliency pattern of for both the horizontal and vertical gratings. On the contrary, the rats in visual and visuo-tactile groups performed generally better when the stimulus was shown with a vertical orientation than when it was shown with a horizontal one. However, the pattern of salient and anti-salient tiles was pretty much preserved between the two orientations, thus showing that the perceptual strategy used by the rats to process the two orientations was similar (if not identical), despite the larger overall preference for the vertical gratings.

Figure 2.2.31: Saliency Maps for all Groups for Different Orientation.

Saliency maps showing, for each group of rats, the discrimination performance that was obtained when any given position of the grating was filled by a tile. These maps are equivalent to those shown in Fig. 2.2.26, only the trials' outcomes of the rats were processed separately for the horizontal (panels A-C) and the vertical (panels D-F) gratings.

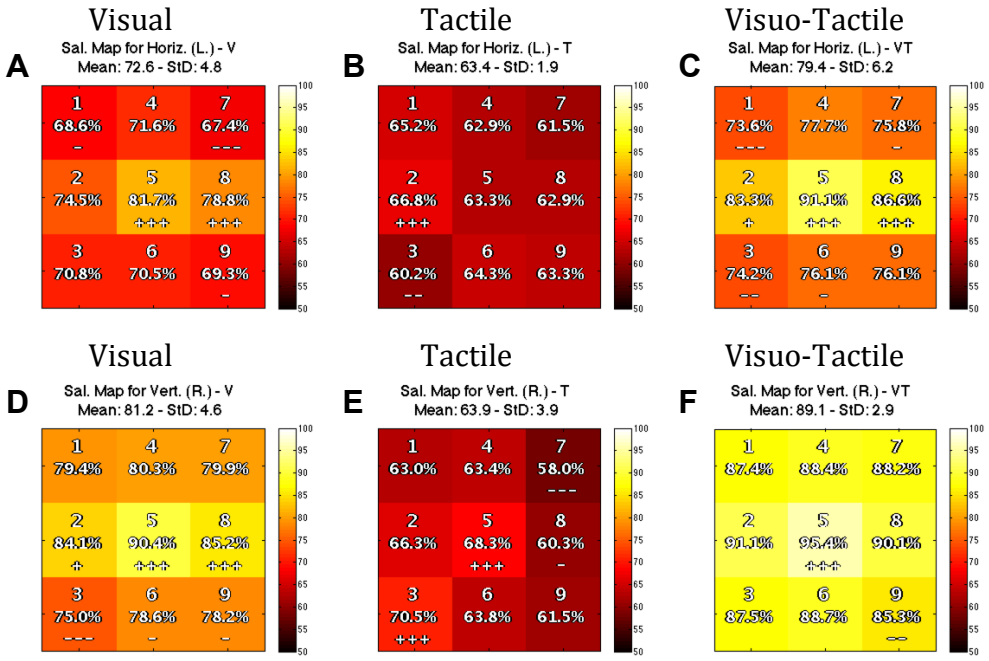
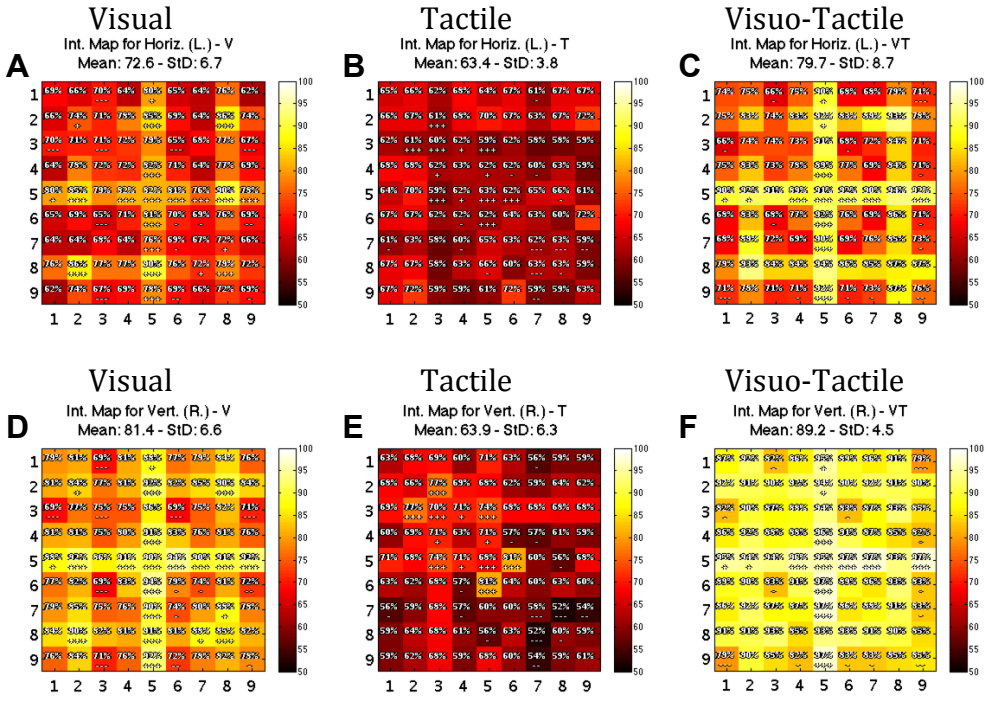


Figure 2.2.32: Saliency Maps of Interaction for all Groups for Different Orientations.

Saliency maps showing, for each group of rats, the discrimination performance that was obtained when any given pair of positions of the grating was filled by a tile. These maps are equivalent to those shown in Fig. 2.2.28, only the trials' outcomes of the rats were processed separately for the horizontal (panels A-C) and the vertical (panels D-F) gratings.

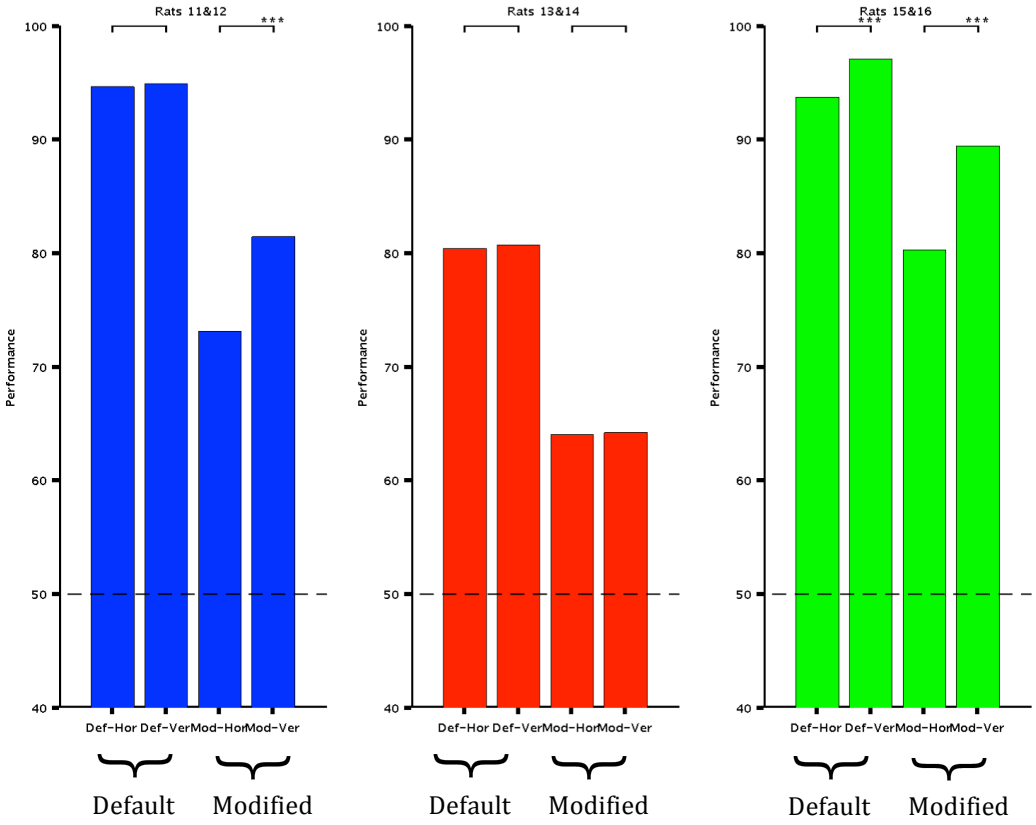


To better understand whether this preference could be attributed to an overall bias for the vertical stimuli, I compared the recognition performances for the "Default" and the "Modified" stimuli, depending on stimulus orientation. To do this, I concatenated the vectors of trials' outcomes obtained for the rats of the same group on the "Default" stimuli, separately for each orientation, and I did the same for the Modified stimuli. I then compared the resulting performances using a Fisher Exact Test (see Fig. 2.2.33). These comparisons show that, for both the visual and the visuo-tactile groups, but not for the tactile group, there was a significant difference in the recognition performances of the "Modified" stimuli, where the recognition of the vertical orientation was always better than that of the horizontal orientation.

However, this effect was likely not due to a general bias, since in the case of the "Default" stimuli, these differences were much smaller (see the first two green bars, corresponding to the visuo-tactile group) or even absent (see the first two blue bars, corresponding to the visual group).

The most likely explanation is that there is a decision bias that emerges only in case of uncertainty (i.e., with the more difficult "Modified" stimuli, but not with the simpler "Default" ones). In any event, because of this tendency to categorize the "Modified" stimuli more as vertically oriented, it was not possible to test whether an interaction existed between the overall orientation of the arrangement of the three tiles and the orientation of the individual tiles. In fact, during trials with "Modified" stimuli, rats have a slight tendency to categorize the stimuli as more vertically oriented, regardless of the specific configuration.

Figure 2.2.33: Comparison between Performance of all Groups on Different Orientations of "Default" and "Modified" Stimuli of Testing Phase 3.



Plots showing the recognition performances for the three groups (blue, visual; red, tactile; green, visuo-tactile) on the "Default" and the "Modified" stimuli, separately for each orientation. A Fisher Exact Test was applied to compare the performances in the two orientations overall ($p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).*

To summarize, the results of Testing Phase 3 showed that the specific position of the tiles in the "Modified" stimuli had a great influence on the rats' discrimination ability, with different effects, some common and some other exclusive, to each sensory group. Among the main effects that were common to all the groups, there is the fact that the "Modified" stimuli strongly challenged rat discrimination ability, to a different extent depending on the interaction between the sensory modality and the specific configuration. Nonetheless, all the rats have been able to solve the task, confirming their recognition abilities were robust enough to the lack of part of the discriminatory information. Among the main effects that were exclusive to the visual and visuo-tactile group, there were: 1) the reliance of these rats on the tiles that were located in some specific positions, namely the central band of the grating (see Figs. 2.2.25, 2.2.26, 2.2.27 and 2.2.28); and 2) the influence that the number of grating tiles that was placed in these positions had on the recognition success (see Figs. 2.2.27, 2.2.28 and 2.2.30).

2.2.4 Discussion

In this experiment we have been able to study rats' perceptual and cognitive abilities in discriminating orientation of solid objects, in different sensory modalities, by improving the previous experimental design.

We investigated these capacities by training each group of rats in a different sensory modality from the beginning, and by testing them in the recognition of different exemplars of the same category of objects (the so-called "Modified stimuli").

So far, we compared the consequent recognition performances, and so the perceptual mechanisms, within and between different groups of rats (and so different modalities).

Thanks to the experience with the previous experiment, we were successful in developing a new experimental design, able to test our hypotheses in a more reliable, faster and simpler way.

In this discussion I will mainly talk about results in terms of sensory groups' perceptual strategies, which were indeed shared between rats tested in the same modality: this was repeatedly confirmed by similarity of performances on "Default" stimuli and significant correlations between performances on "Modified" stimuli, between rats of the same sensory group.

The choice of which sensory modality was used for training and testing had several effects on rats' recognition abilities; the most clear was a difference in the orientation discrimination success not only on "Default" stimuli, but also on "Modified" ones: rats in visual and visuo-tactile groups always performed similarly better than rats in the tactile group on the "Default" orientation discrimination; instead recognition of "Modified" stimuli showed a definite hierarchy of performances, with visuo-tactile group at the top, than visual in the middle and finally tactile group at the bottom, which remained constant across all the different Testing Phases.

Then, even if rats in visual and visuo-tactile groups were both successful at performing the orientation discrimination task in normal conditions (as with the "Default" stimuli), there is a clear difference in their ability to deal with the stimulus modifications (as with the "Modified" stimuli), and so in their invariant recognition ability: rats in visuo-tactile group are able to better compensate for the difference or lack of information of the altered stimuli, either by collecting and using information in both visual and tactile sensory modalities, or because of higher confidence in their

recognition ability. This was confirmed by results of the average absolute distance analysis, of each "Modified" performance from the "Default" one, which were significantly lower in visuo-tactile group than in visual and tactile groups, in Testing Phases 3.

The multimodal discrimination responses of visuo-tactile group seem to be mainly based on visual information, as it could be hypothesized from the significant difference in recognition performance between the visual and tactile groups. The multimodal benefit found in these rats' responses might then arise by confirming during each trial the chosen decision, mainly taken thanks to visual information, with the concurrent or subsequent tactile cues.

It must be noted, in fact, that the two investigated sensory modalities (vision and touch) operate not only with a probable different perceptual strategy, but also with a different timing, during the trial succession of events: visual information is acquired (passively or actively) directly from the start of the trial, as soon as the panel slides up, while tactile information collection must wait for the rats' micro or macro vibrissae to enter in contact with the object, either at the beginning by actively approaching the object, or later in a passive way while going toward one of the licking sensors.

It may be necessary to point out (as done in the Introduction), that Rats possess two different systems of tactile perception on their snout, micro and macro vibrissae, which according to literature could be used in different roles. Micro vibrissae (together with snout skin) probably acquire information through simple touch of the object, while macro vibrissae need a difference in the frequency or amplitude of deflection of each whisker, to gather information about the object identity. These two types of information could then be used to perceive shapes, textures and movement. In our case, only the shape feature could be used to help rats in the discrimination: micro vibrissae could detect the orientation of edges of white protrusions, while macro vibrissae could detect a difference in the frequency of each contact with the rows or columns for some/all of the whiskers (in practice, whiskers either slide in the horizontal sinks or repeatedly hit each vertical protrusion).

Our observation during the recorded trials suggest that, during the initial frontal exploration, micro vibrissae may be best suited in gathering information about the stimulus orientation; in fact, macro vibrissae, even though are able through whisking

movement to engage the grating tiles in all of the positions, might not be able to acquire enough information to be meaningful. Instead, during the subsequent choice movement, when the rat moves the snout left or right to activate the sensor to respond, micro vibrissae are unusable, while macro vibrissae acquire information in the most salient way for them (absence or presence of deflections of all or some whiskers, with different frequency and amplitude).

Going back to the analysis of each sensory modality, it is possible that differences in the specific perceptual strategies employed by each group could be one of the reasons why rats, always in search of a way to solve the task in the fastest optimal manner, show different performances depending on the sensory modality, especially in the case of the "Modified" stimuli: rats in visual and visuo-tactile groups acquire information from the start of the trial, are able to immediately take a decision, and then move faster toward the sensors; moreover, rats in visuo-tactile group may use tactile information acquired while moving toward the sensors, to validate or veto their choice.

Instead, rats in tactile groups must first approach and explore the object to locate where and what is the information, before being able to take their decision; otherwise, they can decide from the start to go toward one of the two sensors, and successively validate or veto their choice.

The veto strategy, anyway, might be too difficult for animals like rats, especially in a condition of water deprivation; most probably, the effort and slowing down involved in the search do no constitute a natural choice for rats tested in tactile modality, regardless of any consequent benefit in performance.

Another hypothesis regarding rats' lower recognition performances in tactile group is that "Modified" stimuli scatter too much the available information, by using separated positions for the grating tiles: the subsequent lack of coherent nearby tactile information may be the cause for the significant decrease in performance, especially when compared to that on "Default" stimuli.

All of these results could help to better understand the rejection of the most probable hypothesis, about which position were more salient for rats in tactile group: namely, the positions on the central band were considerate to be the best candidate, because of easiness for rat to interact with them through the whiskers, during both the initial

exploration and the choice movements toward the sensors (as shown in the previous experiment).

Contrary to our expectations, again, the hypothesis of central positions being more influential than the others for the rats' discrimination was instead confirmed for rats in visual and visuo-tactile groups. Apparently rats in these groups focused their attention on a specific part of the objects, regardless of orientation: the central band. This was not expected, especially for the visual group, considering that literature offers several different hypotheses regarding which parts of objects/visual space are more probably visually perceived by rats in experimental settings, none of which is the central part (some examples are: bottom part for Minini & Jeffery, 2006; top part for Wallace et al., 2013).

Moreover, this recognition tendency was not a simplistic featural strategy: presence of more than just one grating tile in the central band was linked to an increase in the recognition performance, which is more coherent with a configural, rather than featural, visual recognition strategy. Rats were able to better ground their judgment about orientation of the stimulus, when more coherent information was present in the preferred positions.

These hypotheses concern rats in both visual and visuo-tactile groups. As said before, rats in visuo-tactile group were probably especially dependent on visual information, but were able to be more invariant to object identity-preserving transformations thanks to acquisition of additional tactile information.

Anyway, there was an interesting difference between visual and visuo-tactile group in the results coming from the analysis of recognition performances in terms of 2-grating tiles interaction, when analyzed separately for orientation: rats in visual group show very small interaction in the horizontal category, but enough of it, especially for central band positions, in the vertical category; rats in visuo-tactile group, instead, show an interaction between grating tiles in the central positions already for horizontal orientation; moreover, when tested with vertical oriented stimuli, the effect of interaction between different positions spread above the whole matrix.

It then appears that rats in visuo-tactile group, thanks to the benefit of multimodal perception (either because of tactile information or just increased confidence in the decision outcome), were able to better integrate information coming from all positions of the stimulus, especially in the case of vertical orientation. A simpler explanation

could be that this phenomenon is just due to an increased performance during trials performed on vertical stimuli rather than on horizontal ones: this explanation, anyway, has to be rejected, because both visual and visuo-tactile groups showed an increase in recognition performance for vertical stimuli, but only the latter showed this interaction effect.

Indeed, rats in both visual and visuo-tactile groups showed a significant perceptual bias toward categorizing the grating tiles on "Modified" stimuli as more vertically oriented, regardless of real orientation. This bias might be only perceptual, and not decisional, as it can be seen from the concurrent unbiased performance on "Default" stimuli.

In the case of visuo-tactile group, a possible explanation could be this: in those cases in which a grating tile is placed with no other at the sides, even if it has an horizontal orientation, it may still perceived more as vertical. This happens because its rows may be hit at the sides by the rat's macro vibrissae, during the explorative whisking movement: this contact may produce a tactile feeling that is more similar to the one encountered during perception of vertically oriented grating tiles.

This hypothesis, anyway, doesn't explain why this phenomenon was present also in the case of visual group rats; in this case, the only possible hypothesis is a perceptual one: in case of decrease of information, vertical orientation is more visually perceivable than horizontal one. This may be due to either a general perceptual phenomenon (for which we don't have any insights coming from literature), or to our specific experimental rig characteristics, like for example the shadows originating from the interaction of the LED lights with the stimulus.

We still haven't decided about the next phase of the experiment, but most probably this will involve the testing of all groups on different modalities, from the ones on which rats have been trained and tested so far, not only to enrich our analysis of the different sensory perceptual strategies, but also to better understand the phenomena of both multimodal and crossmodal perception.

2.2.5 Conclusion

In conclusion, we have been able to further develop the previous experimental design, to study rat recognition abilities with solid objects, under different sensory modalities and in spite of stimulus modifications, in a fast and reliable way.

We found that rats in different sensory groups are indeed able to successfully categorize the orientation of both "Default" and "Modified" stimuli, with a combination of common and specific, from the sensory point of view, perceptual and cognitive strategies.

Our results about which positions were more influential for the categorization success, depending on sensory modality, provide some interesting new insights on how solid objects, differing in just a shape feature (their orientation), are perceived and recognized by rats: visual and visuo-tactile groups seemed to be both dependent on the central band positions, and not just on one of these, while tactile group failed so far to show any preference, probably due to some of the reasons previously depicted.

Moreover we were able to appreciate a distinct multimodal effect in the characteristics of visuo-tactile group's recognition performances, not only in the degree and robustness of categorization, but also in its capability to integrate more information, especially for one orientation category.

Concluding, our experimental design proved to be a valid tool to further unveil the characteristics of rats' perception capabilities under different sensing modalities.

Chapter 3: Electrophysiological Project

3.1 Introduction

In recent years, research on the relationship between unisensory primary areas and crossmodal or multimodal stimulation has produced many novel insights on the way the brain perceives and builds sensory representations. In particular, there is accumulating evidence against the notion of strictly unimodal primary sensory areas, and in favor, instead, of the existence of a multimodal modulation also at primary level, produced by the interaction between different primary sensory areas (through several ways of communication: cortico-cortical, cortico-thalamo-cortical, or thalamo-cortical; Driver & Noesselt, 2008; Ghazanfar & Schroeder, 2006; Sathian & Zangaladze, 2002; Stein & Stanford, 2008). As a result, even though these areas are mainly associated to a specific modality, they can still be influenced by other modalities, especially in the cases where the different stimulations are linked in space and/or time, as with a congruent percept.

The nature of these crossmodal modulatory influences is currently under investigation, but several effects, both at the level of single neuron and neural network, have already been reported. These effects influence both spontaneous and evoked sub-threshold neural activity (Bizley, Nodal, Bajo, Nelken, & King, 2007; Ghazanfar et al., 2005). For instance, a modulation of the oscillatory activity's power and phase has been reported in a given primary sensory area as a consequence of the concomitant stimulation of a different primary sensory area (Sieben, Röder, & Hanganu-Opatz, 2013). This phenomenon has been interpreted as a way to either enhance or suppress the perception of the stimulus properties in one modality, depending on the other, or to give a common temporal frame to concurrent stimulations via different sensory channels.

Several studies have started investigating these phenomena in anesthetized rats (Iurilli et al., 2012; Sieben, Röder, & Hanganu-Opatz, 2013), focusing on three primary sensory primary areas: visual cortex (V1), auditory cortex (A1) and somatosensory

cortex (S1). The goal of these investigations was mainly to characterize the nature of these cross-modal modulations and understand what are the cortical or sub-cortical communication ways that are responsible for them. To achieve these aims, these studies have relied on very brief and simple stimuli, such as flashes of light, multi-whisker deflections, and sound tones. This approach has been successful in revealing the presence of cross-modal modulation between (e.g.) V1 and S1. More specifically, it has been observed an hyperpolarization of pyramidal neurons in layers 2/3 of visual cortex, as well as phase resetting of local field potentials, following tactile stimulation, mainly due to cortico-cortical connections from S1, targeting inhibitory neurons in V1 deep layers (Iurilli et al., 2012).

Using these findings as a starting point, I wanted to further extend the current knowledge of cross-modal modulation in primary sensory areas, by investigating these phenomena at the level of neuronal tuning for relevant stimulus dimensions and in relation to longer and more complex stimuli. To this aim, I used as visual stimuli oriented drifting gratings. These were shown with different parameters (e.g., orientation, spatial frequency, etc.) and were either paired or not paired with a tactile stimulation, which was administered to the vibrissae of an anesthetized rat through a solid grating moving in two possible directions. My aim was to test whether the cross-modal modulation that has been reported in previous studies still existed, when a complex and very salient stimulus was used. More importantly, my goal was to assess if such a modulation was effective at altering the tuning of the recorded neurons for along specific dimensions (e.g., orientation). Moreover, I wanted to address three possible limits of previous studies. First, I wanted to look into the temporal characteristics of the cross-modal modulation, and, for this reason, I used a longer stimulus (1000ms), which gives the opportunity to look at the dynamics of the neuronal response over a wider scale. Second, I wanted to use stimuli that were someway coherent in the two different modalities, by showing visual drifting gratings and, at the same time, deflecting the whiskers of the rat through a solid drifting grating (i.e., not just a flash of light, paired to a multi-whisker deflection). Finally, I wanted to differentiate between two types of tactile stimulation, each deflecting the whisker in a different direction.

So far, I have been able to conduct an exploratory study, with several recording sessions, and I are still in the process of analyzing the data, to refine the experimental design and the recording protocol. In the following, I will illustrate the properties of the cross-modal response modulation I have been able to characterize so far. My current results already show that the neural activity in rat V1 may be modulated by a concurrent tactile stimulation, at least for a fraction of the recorded neurons.

3.2 Materials and Methods

3.2.1 Animal Preparation and Surgical Procedure

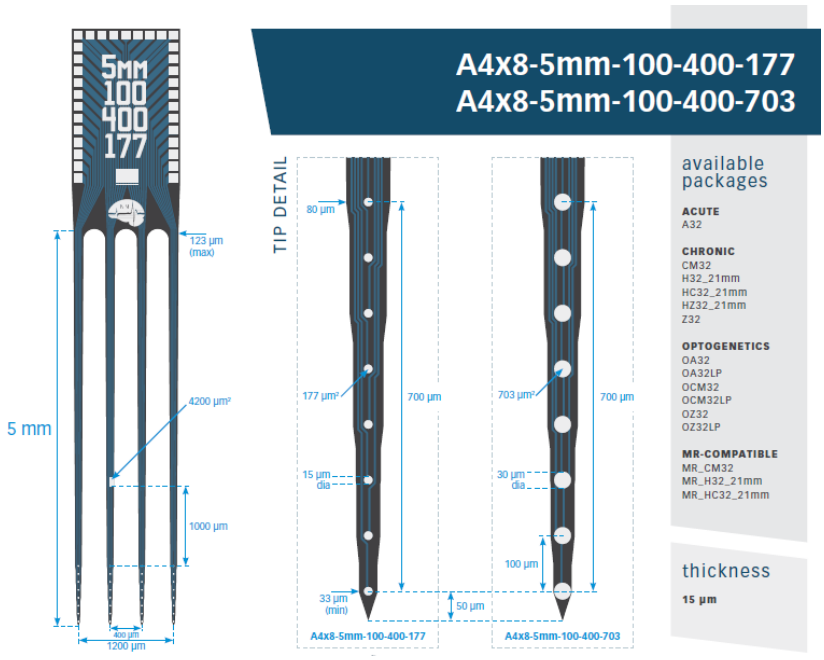
All animal procedures were conducted in accordance with the National Institute of Health, international and institutional standards for the care and use of animals in research. A Veterinarian has been consulted and informed on all the steps of animal preparation and surgery. I performed all the extracellular recordings on Naïve Long-Evans male rats with an age between 3 and 4 months (after the complete development of rats' visual system; see Fagiolini et al., 1993) and with a weight between 300 and 700 grams. Before surgery, all rats were anesthetized with an intraperitoneal injection of a solution of 0.3 g/kg of Fentanyl (Fentanest, Pfizer, 0.1mg/2ml) and 0.3g/kg of Medetomidin (Domitor, Orion Parma, 1mg/ml). Before surgery, the level of anesthesia was controlled by testing the absence of paw, tail and ear reflexes of retraction; this was also done during the whole recording. Depending on the level of anesthesia, maintenance anesthesia was administered between 30 minutes and 2 hour after the beginning until the end of recording (solution of 0.1g/kg/h Fentanyl and 0.1g/kg/h Medetomidin). During the whole duration of surgery and recording: a) body temperature was kept around 37^{o}C with a thermostatically controlled heating pad, to avoid anesthesia-induced hypothermia; b) heart rate and oxygen level were monitored through a pulse oximeter; c) a constant flow of oxygen was delivered to the rat, to avoid hypoxia. During surgery, rats' eyes were protected from direct light and damage with a cloth, and kept wet through administration of ophthalmic solution Epigel (Ceva Vetem). During recording, the eye facing the screen was kept wet through saline solution, while the eye facing away from the screen was covered with a wet cloth and black tape. The whiskers facing the screen were cut at a length of 10mm, while the whiskers not facing the screen were totally cut away. The animals were placed for surgery and recording on a modified stereotaxic apparatus. During the surgery, a craniotomy was made over left hemisphere in V1, at coordinates ~4 ML (medio-lateral axis) and ~6.5 AP (antero-posterior axis), with a size of 1.5x1.5mm around the target coordinates. Before inserting the electrode array, the rat was placed on a 10cm support base, so as to have the horizontal plane of the rat's right eye parallel to the base of the screen at 45cm from it, and rotated 45^{o} toward the left. The

rat's right eye was first rotated to have the pupil directed to the center of the screen, and then blocked through an eye-ring that was attached to the stereotaxic apparatus.

3.2.2 Recording Procedures

Recording were performed from area V1, using a 4 shanks, 32 channels Michigan probe (NeuroNexus Technologies, Ann Arbor, MI, USA), with the following configuration: 5mm of length, 100µ of site spacing, 200µ of distance between shanks, site area of 413μ^2, 15µ of thickness, and reference site on the second shank (see Fig. 3.1). My target initial depth was around ~900µ form the surface of the cortex, so as to have the probe spanning the whole 2/3 cortical layers, and part of layer 4. The probe was coated with Vybrant DiI cell-labeling solution (Invitrogen, Oregon, USA), as a long-term tracer for neuronal cells, to permit retrieval of the shanks' position through histological procedures. The probe was grounded through a wire to the animal's head skin. The reference site on the probe was used as a reference: this was placed directly at the level of the cortex surface, and was continuously covered with saline solution (to also keep the cortex wet). The probe was moved into place, at the chosen coordinates and depth, by means of a microdrive apparatus (Narishige, SM-11). Visual confirmation of the insertion of all sites inside the cortex was also performed through a dissection microscope that was placed over the craniotomy. After complete insertion of the probe, I waited 30-45 minutes before starting the recording session, so as to compensate for brain dimpling.

Figure 3.1 Recording Probe



3.2.3 Visual and Tactile Stimulation

Each recording session lasted between 4 and 8 hours, depending on the number of recording blocks it was possible to perform; each block lasted ~2h. The stimuli were displayed on a 47inches LCD monitor (SHARP model PNE471R, 1920x1080 pixel resolution, 1.200:1 contrast ratio), positioned at a distance of 45 cm from the right eye, spanning a visual field of 120° azimuth and 90° elevation. The data were acquired using a Tucker-Davies Technologies (TDT) recording system, which allowed real-time monitoring of brain activity in each of the 32 channels of the probe. Every experimental session consisted of two consecutive parts: first, the receptive field positions and sizes of the recorded neurons were measured; then, the visuo-tactile experimental block was run.

The receptive field mapping consisted in the presentation of 10^{o} wide moving bars at various orientations and 66 different positions over the screen (i.e., over a grid of 6 rows spanning vertically from -20° to +30°, and 11 columns spanning horizontally from -50° to +50°). Each bar was shown for 300ms, followed by a 250ms blank inter stimulus interval. This protocol was performed for at least 15 minutes, in order to collect at least ~10 trials per condition. A tangent screen approximation was implemented, in order to avoid distortions in the stimulus size at each retinal position (i.e., the bars were shown as if they were painted on flat, planar surfaces tangent to a sphere with a radius equal to the distance of the eye from the position on the monitor in front of the eye itself – 45 cm). After collecting enough trials, a brief check was carried out, to ensure that the units' receptive fields were indeed located on the central part of the screen, where the visual stimuli had to be presented in the ensuing visuo-tactile stimulation protocol.

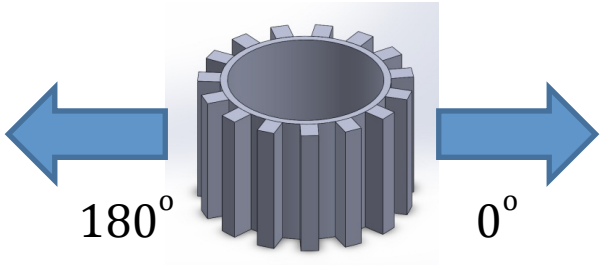
The visuo-tactile experimental block took place after the RF mapping, and had a duration of ~2 hours, in order to collect 30 trials per condition. Each trial consisted in the presentation of a 1000ms visual drifting grating on the screen, paired or not with a concomitant tactile stimulation (visual and visuo-tactile conditions, respectively). In addition, trials with only tactile stimulation were collected (tactile condition). An inter stimulus interval consisting in a black background, with 500ms of duration, followed each trial.

The visual drifting gratings were shown inside a circular transparent mask with 60° of radius, with 4 different spatial frequencies (0.025, 0.05, 0.1, 0.2 c/d) and 12 different directions (from 0° to 330° in steps of 30°). The speed was fixed at 60°/s, producing 4 different temporal frequencies, one for each spatial frequency (1.5, 3, 6, and 12 c/s). Around the mask, a black background was shown, covering the rest of the screen.

The tactile stimulation was administered through a solid circular grating (see Fig. 3.2) with vertical orientated ridges and grooves (5mm in width and 80 in height), all painted black, in direct contact with the rat's whiskers on right part of the snout and 10mm below the right eye (to leave the rat's visual field as much free as possible). The rotation of the motor caused the rat's whiskers to be bent by each ridge and stuck in every groove, one physical cycle after the other. Clockwise and counter-clockwise stimulations (named, respectively, direction 180º and 0º) were independently administered, with a speed of 180°/s. This speed was chosen as the best compromise to have a proper tactile stimulation and to have the motor work in the most reliable way, given the time precision constraint. The motor was controlled through an Arduino Uno equipped with a Motor Shield, linked to a photodiode placed on the screen: activation and deactivation of the motor was linked to appearance of a white pixel in the bottom right part of the screen, simultaneously with the appearance on the screen of a visual drifting grating or a black background (respectively, visuo-tactile or tactile conditions).

In summary, I had 48 different visual conditions (12 directions x 4 spatial frequencies), each paired or not with one of two different tactile stimulations (direction 0° or 180°), plus the two tactile stimulations alone, for a total of 146 conditions. On average, each condition was tested 30 times.

Figure 3.2 Model of Solid Circular Grating used for Tactile Stimulation



3.3 Data Analysis

The creation of the visual stimuli and the data analysis were carried out using MatLab (http://www.mathworks.com). The tactile stimulation was controlled through Arduino software IDE (http://www.arduino.cc/). The experimental protocol was created and played using MWorks (http://mworks-project.org/).

3.3.1 Spike Sorting

So far, all my data have been collected through extracellular recordings: each probe collected action potentials from the neurons near each of its 32 recording sites. The raw data acquired in this way may represent the extracellular potential of one or more neurons. It is therefore necessary to differentiate between single- and multi-units activity by mean of a spike sorting procedure.

To detect and sort the spikes, I used Waveclus (Quiroga et al., 2004): this algorithm uses both a wavelet transform, to localize distinctive spike features, and a superparamagnetic clustering, to classify the data without assumptions such as low variance or gaussian distributions. The results of the clustering are the sequence of spike times, the cluster membership and the spikes' shapes (Quiroga, 2012). So far, I have not applied yet quality metrics to assess the goodness of the spike sorting, but I plan to use such tools in my future analyses.

3.3.2 Spike Count Window and Latency of the Response Onset

The number of trials that was collected for each stimulus condition varied, depending on the condition, between 20 and 40 in the first recording sessions, and was instead fixed at 30 for all the conditions in the last sessions. The firing rate of a neuron was calculated in overlapping time bins of 25ms, each shifted in time of 1ms. Different ways to define the optimal spike count window were tried.

First I tried to select a fixed duration window of ~250ms, set around the unit's maximum average firing rate (calculated as the average of the unit's firing rate across all stimulus directions, at the spatial frequency where the unit was most responsive). This approach invariably led to choose the initial onset response for most of the units, but the spike count window selected in this way was found to be poorly informative about stimulus' identity.

Because of this, I tried a different approach, by selecting a fixed duration window of ~250ms set around the maximum absolute deviation of the firing rate (where each deviation was the absolute distance between the unit's firing rate on a specific direction, and the average of the unit's firing rates across all stimulus directions, at the spatial frequency where the unit was most responsive). This approach proved to be more successful in identifying the period during which the unit was more informative about the stimulus' identity. Anyway, this method produced a lot of variation in the chosen spike counting window coordinates between different units, making difficult any comparison between them.

At the end, both the methods described above were not found to be satisfactory. For this reason, I decided to use a much simpler (but unbiased) approach: a fixed duration spike count window of 750ms, starting 250ms after the onset of the visual and/or tactile stimulation (to discard the initial uninformative responses onset), and continuing until the end of the stimulus. Even though this procedure reduces the level of visual tuning that was computed for each unit, it is anyhow the best in terms of simplicity and reliability.

3.3.3 Unit's Preferred Direction and Spatial Frequency

To define the visual tuning for each unit, I proceeded in three steps. First, I assessed the general response characteristics of each unit, by building a matrix where each cell reported the unit's average firing rate for each condition (i.e., at a specific direction and spatial frequency of the drifting grating, together or not with the tactile stimulation; see, for instance Fig. 3.3A). Second, I selected the spatial frequency for

which the unit was more responsive, by calculating the mean of all the average firing rates across all the directions for that specific spatial frequency, and choosing the one with the maximum mean value (in terms of the response matrix shown in Fig. 3.3A, I chose the row with the maximum average response across the columns). Third, I selected the direction for which the unit was more responsive, by calculating the mean of all the average firing rates at each specific direction across all the spatial frequencies, and again choosing the one with the maximum mean value in the previously chosen spatial frequency (in terms of the response matrix shown in Fig. 3.3A, I chose the column with the maximum average response across the rows). The second and the third step were conducted on the visual sensory conditions only, i.e. the ones with only the visual stimulation, to use them as a benchmark to which compare the multimodal conditions.

In rare cases, a manual adjustment to the selected direction and spatial frequency was made, in order to better characterize the unit's response. Finally, the spontaneous, or background, activity was calculated by taking, as a spike count window, the 250ms before the onset of each stimulation, and then calculating the mean on all trials on all conditions.

3.3.4 Unimodal, Crossmodal and Multimodal Response Analysis

To characterize the presence of any crossmodal effect, at the level of single neurons, I first looked at the mean number of spikes evoked on each stimulus presentation, both in terms of average spike count and average firing rate, for the preferred condition (i.e., the neural responses to the drifting grating with the most effective direction and spatial frequency, with or without tactile stimulation). My aim was to measure the responses to the stimulation in both the single sensory modality (visual and tactile condition) and combined sensory modality (visuo-tactile) in terms of spiking neural activity, to find whether multisensory stimulation elicited a response depression, a response enhancement or the absence of any interaction. For this reason, I used an unpaired t-test to compare the units' average spike count (for trials collected at the preferred direction and spatial frequency) that was obtained in the visual condition

with the one that was obtained in each of the two visuo-tactile conditions. This comparison yielded, for each unit, a value of statistical significance and a sign, depending on which average spike count was higher. A positive, significant comparison would mean that the unit's average spike count on the preferred visual condition was significantly larger from the average spike count obtained for one or both the visuo-tactile conditions (the opposite was true, in case of a negative significant comparison). All the recorded units have then been categorized, depending on the outcome of this comparison, as *unaffected*, *inhibited* or *enhanced* in their best neuronal response by the concurrent tactile stimulation.

Moreover, two standard indexes were used to better define the multisensory integration: the interactive index and the multisensory contrast (Sarko, Ghose and Wallace, 2013). All these indexes have been applied to the units' spike count values on the best condition. The first index simply defines the multisensory interaction by calculating the decrease or increase in the neuronal response produced by a stimulus, due to the concurrent stimulation in a different sensory modality. It is calculated as:

Interaction Index (%) = [(VT - V) / V] x 100

Where VT is the max value between the mean numbers of spikes per trial evoked by the two different visuo-tactile stimulations and V is the mean number of spikes per trial evoked by the visual stimulation (all these metrics are always computed at the best direction and spatial frequency for the visual stimuli). This index, even though is a fast and simple way to ascertain the presence of any multisensory interaction, fails to take into consideration also the influence of the other sensory modality alone, in our case the tactile one.

The multisensory contrast index, instead, considers also the second sensory modality, and it is calculated as:

Multisensory Contrast Index = VT + SA - V – T

Where VT and V are defined as before, SA is mean number of spikes of spontaneous (or background) activity and T is the mean of the mean numbers of spikes per trial evoked by the two tactile stimulations. This model defines not only an enhancement or a suppression of the activity due to the concurrent stimulation, as with the previous formula, but also the type of integration: by considering both unisensory responses, it finds whether there is a super-additive (Index > 0) or sub-additive (Index < 0) effect.

These two formulas only rely on changes in the mean firing profile of the units, but there are other ways in which information can be encoded to quantify multisensory integration, such as mean response duration, response latency and peak firing rate. All these measures may provide further information about the units' temporal response dynamics and their effect on the multisensory integration, which may not be evident in studying firing rate changes alone. At the moment, however, I have just focused on these simple indexes.

Finally, I also computed the Fano Factor on all the spike count vectors for the best stimulus, separately by sensory condition, to compare the response variability between the visual condition and each of the visuo-tactile conditions:

$FF = \sigma^2/\mu$

The rationale for the use of this measure is that, the more a unit is variable in responding to a particular condition, the less reliable it is, and then less prone to be considered in the perception of a multisensory stimulus. Then, changes in response reliability may be used as a weighting factors in the neural processes responsible for sensory integration. Anyway, it must be considered that at high firing rates, responses are typically less variable, just because of the constraint due to refractory period.

3.4 Results

3.4.1 Example of Recorded Neurons

So far, I have performed 8 recording sessions, all from area V1 and at the same depth (~900μ), using the same probe configuration (see 3.2.2). I was able to extract 96 putative units (see 3.3.2).

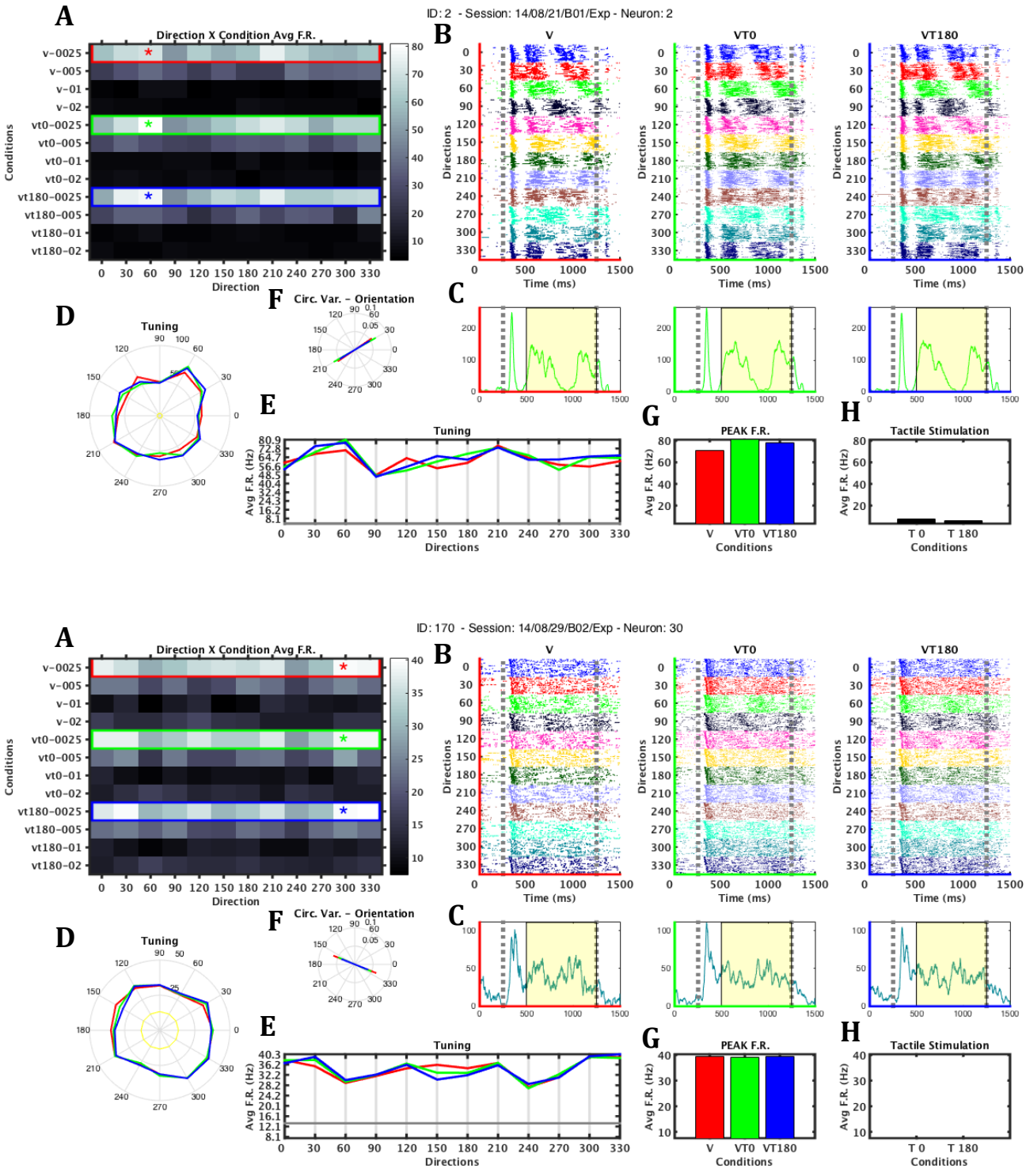
For each unit, I first tried to understand the general neuronal response, by looking at the average firing rates across all the stimulus conditions, and then, in more detail, to the responses obtained for the most effective spatial frequency and direction. The latter were selected on the basis of the neuronal activity in the visual condition only.

In general, I found a large variability across the recorded units, in terms of: 1) the amount of tuning they showed to presentation of the drifting gratings (calculated through Circular Variance Indexes for orientation and direction); 2) the level of activity (calculated through spike count or firing rate); and 3) the level of modulation due to concomitant tactile stimulation (calculated through comparison between sensory conditions). Based on the above mentioned properties, the units can be roughly categorized as: 1) visually responsive, but un-tuned (or weakly tuned) and unaffected by tactile stimulation (e.g., see Fig. 3.3); 2) visually responsive, direction- and/or orientation-tuned and unaffected by tactile stimulation (e.g., Fig. 3.4); 3) visually responsive, tuned and slightly suppressed by the tactile stimulation (e.g., Fig. 3.5); 4) visually responsive, tuned and enhanced by the tactile stimulation (e.g., Fig. 3.6).

In each of the above-mentioned figures, I have shown two examples neurons for each of these categories. For each neuron, I have reported several metrics/plots that are useful to understand its tuning and the extent of its cross-modal modulation. Panels A shows a matrix with the average firing rates on each stimulus condition, with the directions reported along the columns and the spatial frequencies reported along the rows (rows 1 to 4: visual stimuli; rows 5 to 8: visuo-tactile stimuli with the tactile stimulation in the 0^{o} direction; rows 9 to 12: visuo-tactile stimuli with the tactile stimulation in the 180^{o} direction). Panel B shows the raster plots corresponding to the

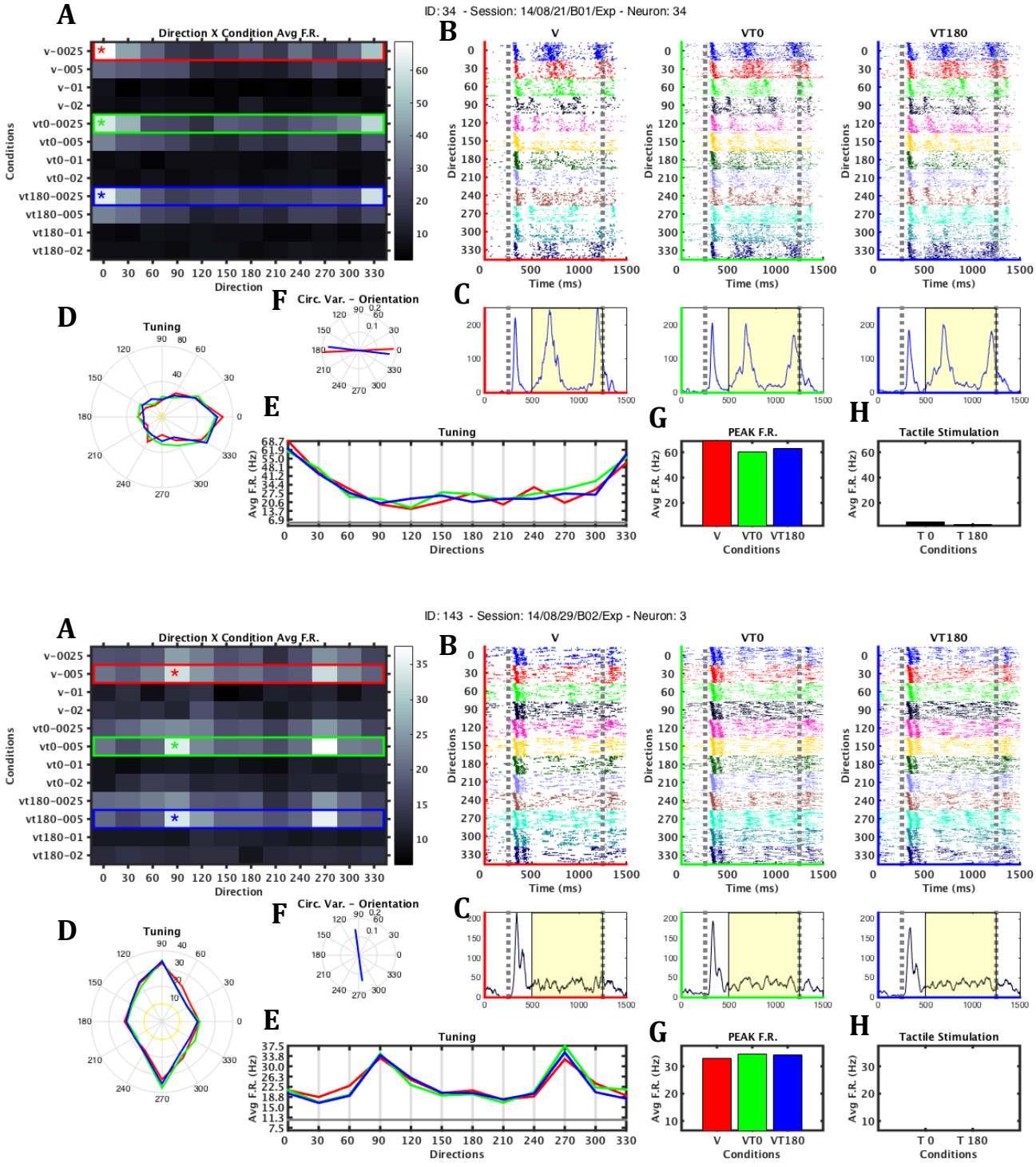
rows that are highlighted by the colored frames in A – that is, the responses of the neuron across all tested directions, at the preferred spatial frequency, for both the visual (V) and the two visuo-tactile conditions (VT0 and VT180). Note a different color is used to indicate the trials corresponding to each specific direction (the start and end of stimulation are indicated by the dashed gray lines). Panel C shows the PSTHs obtained, at the most effective direction and spatial frequency, for each of the three sensory conditions. The color of the line in the PSTH matches the color of the corresponding most effective direction in the rasters of B (the light yellow box indicates the spike count window). Panels D and E shows the tuning curves across directions (at the preferred spatial frequency) that were obtained for both the visual (red curve) and the two visuo-tactile conditions (green and blue curves). The tuning curves are reported both on a polar plot and a Cartesian plot (the background activity is shown as a yellow circle and gray line, respectively). Panel F shows the vectors resulting from computing the circular variance across directions (again, at the preferred spatial frequency) for the three sensory conditions (V: red; VT0: green; VT180: blue). Panel G shows the average firing rates for the selected direction and spatial frequencies, one for each sensory condition (V: red; VT0: green; VT180: blue). Finally, panel H shows the average firing rates for the tactile conditions. Note that each figure reports two example neurons, and that the labels of the panels are the same for the two neurons. As already mentioned in describing each panel, the visual, the visuo-tactile condition with tactile stimulation in the 0^o direction, and the visuo-tactile condition with tactile stimulation in the 180^o direction, are highlighted and pointed out in all panels with red, green and blue colors, respectively. The background activity has been subtracted from the average firing rates only in the case of tactile conditions (panel H).

Figure 3.3 Examples of visually responsive but un-tuned neurons



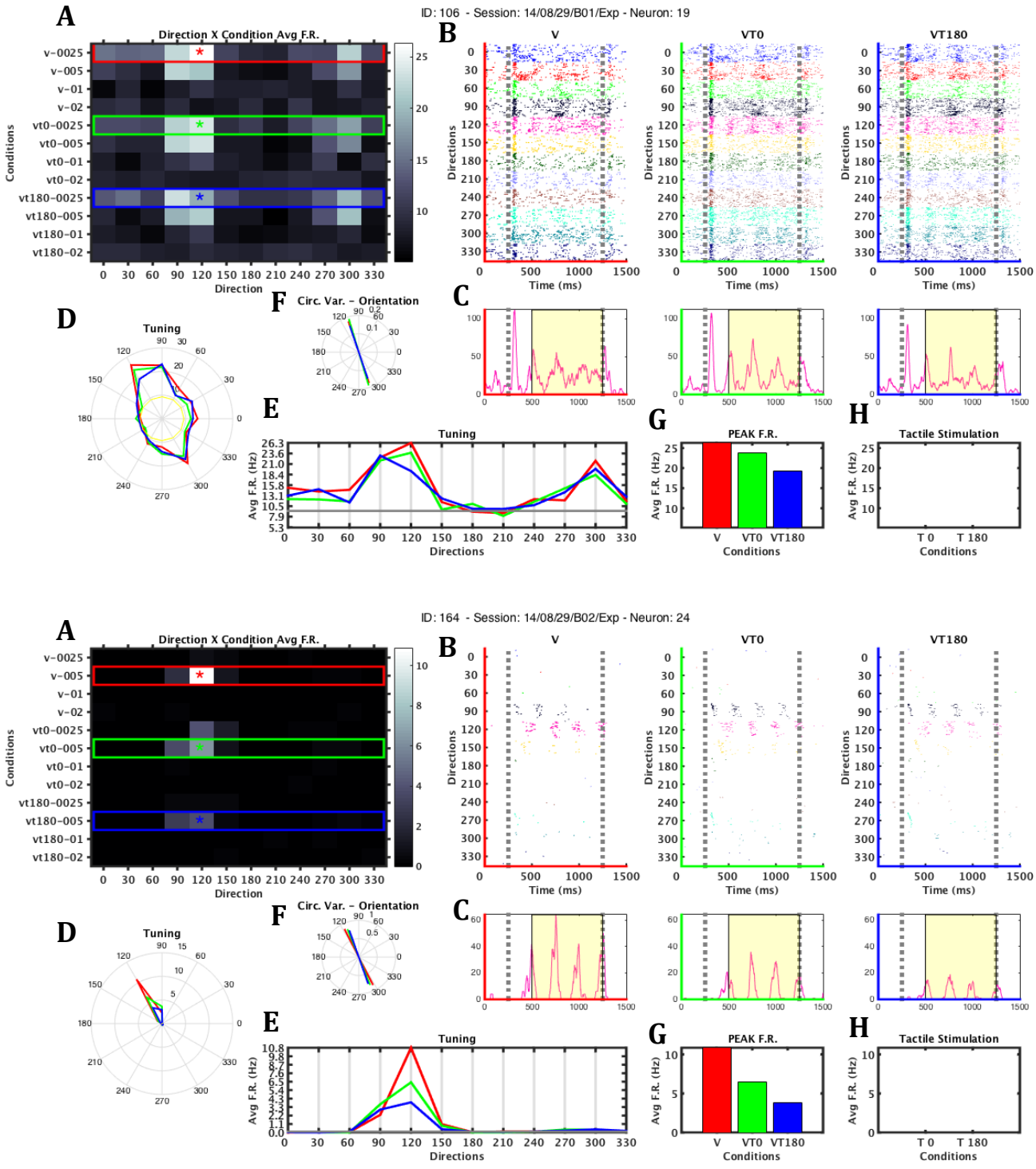
A) Matrix of Average Firing Rates on all Conditions;
B) Raster plot; C) PSTH; D-E) Tuning curves;
F) Circular Variance for Orientation;
G) Average Firing Rates Peaks on Visual and Visuo-Tactile Conditions;
H) Average Firing Rates on Tactile Conditions.

Figure 3.4 Examples of visually responsive and tuned neurons that are unaffected by the tactile stimulation



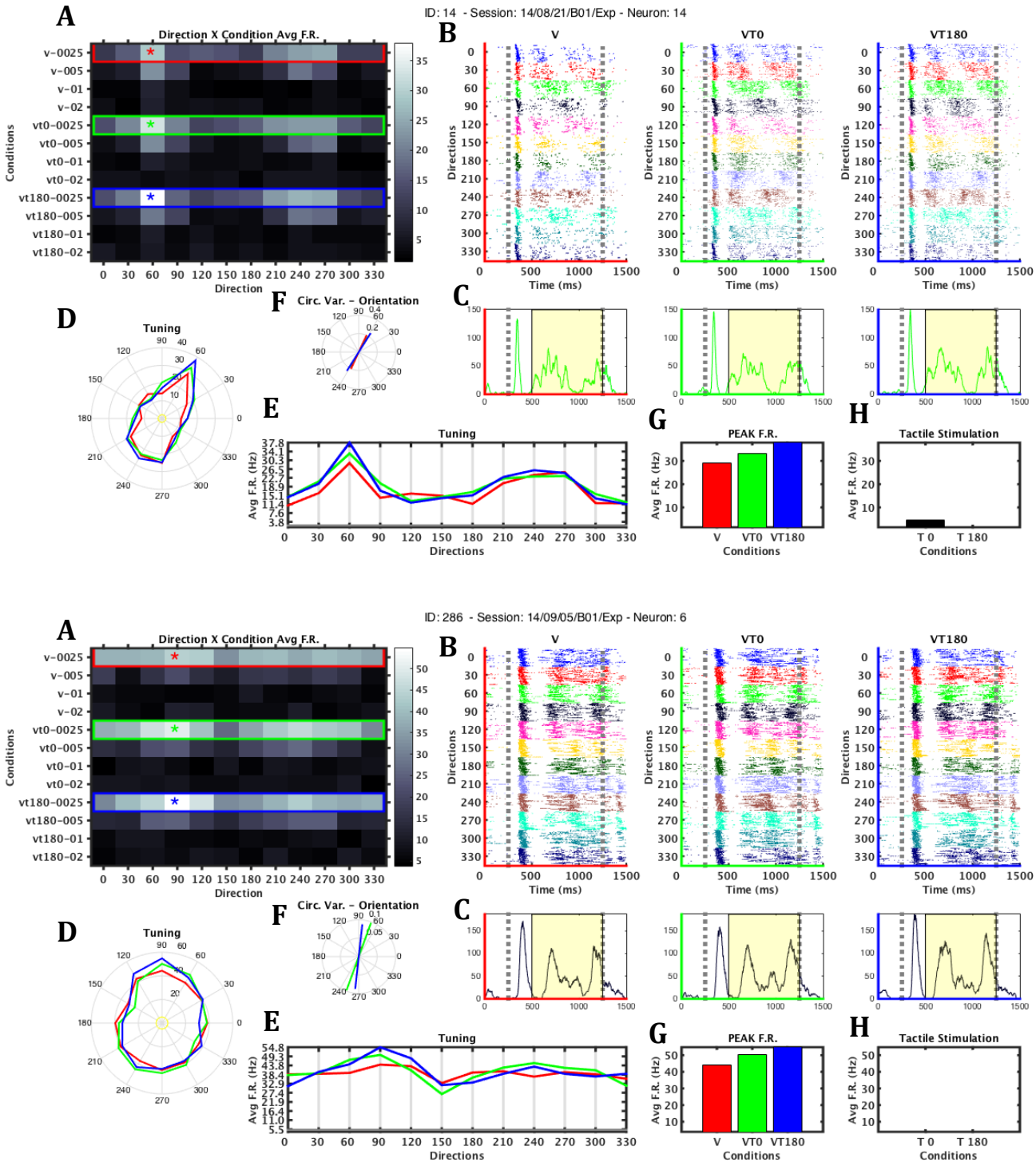
A) Matrix of Average Firing Rates on all Conditions;
B) Raster plot; C) PSTH; D-E) Tuning curves;
F) Circular Variance for Orientation;
G) Average Firing Rates Peaks on Visual and Visuo-Tactile Conditions;
H) Average Firing Rates on Tactile Conditions.

Figure 3.5 Examples of visually responsive and tuned neurons that are suppressed by the tactile stimulation



A) Matrix of Average Firing Rates on all Conditions;
B) Raster plot; C) PSTH; D-E) Tuning curves;
F) Circular Variance for Orientation;
G) Average Firing Rates Peaks on Visual and Visuo-Tactile Conditions;
H) Average Firing Rates on Tactile Conditions.

Figure 3.6 Examples of visually responsive and tuned neurons that are enhanced by the tactile stimulation



A) Matrix of Average Firing Rates on all Conditions;
B) Raster plot; C) PSTH; D-E) Tuning curves;
F) Circular Variance for Orientation;
G) Average Firing Rates Peaks on Visual and Visuo-Tactile Conditions;
H) Average Firing Rates on Tactile Conditions.

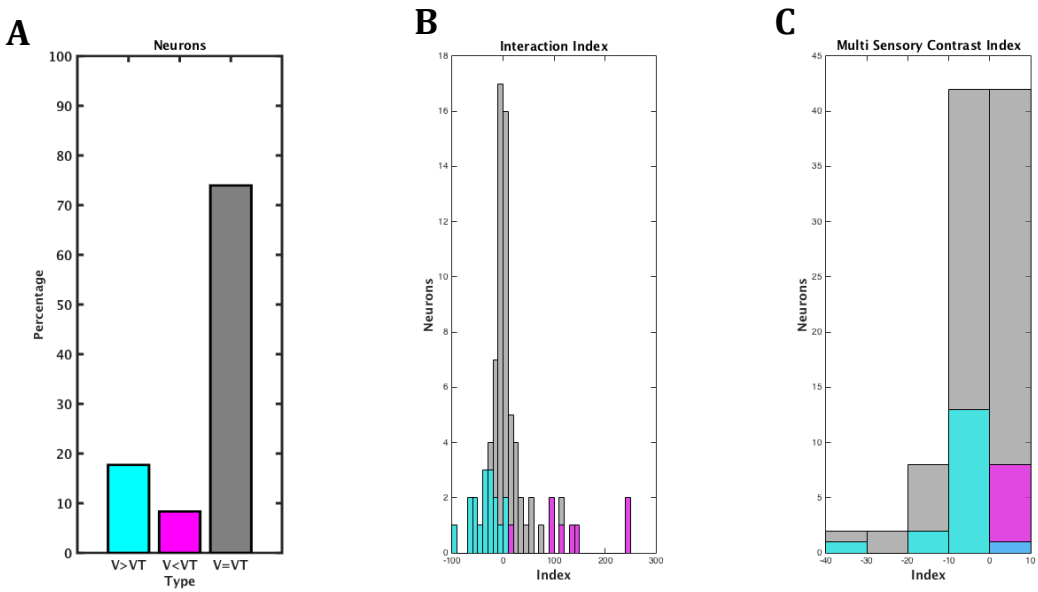
3.4.1 Unisensory, Crossmodal and Multimodal Response Analysis

Following the visual inspection of figures like the ones described in the previous section, I conducted a population analysis on all the units recorded and extracted so far, trying to better asses the effect of the multisensory integration on neuronal response.

First, I looked at the neurons in terms of afore mentioned categories (see 3.3.4), characterizing them as units: A) unaffected by the concurrent tactile stimulation (shown in gray in figures 3.7-3.9); B) affected, with a depressed response (shown in cyan in figures 3.7-3.9); C) affected, with an enhanced response (shown in magenta in figures 3.7-3.9). My analyses indicate that only a fraction of the recorded neurons showed a significant modulation ($p < 0.05$; unpaired t-test) of their response to the most effective visual stimuli, as a consequence of the concomitant tactile stimulation (26 out of 96, 37% of the total). Out of these, 18 showed a depression of the response to the best visual stimulus, while 8 showed an enhancement (see Fig. 3.7 A).

These neurons were highlighted in the Interaction Index and Multisensory Contrast distribution plots, according to the color convention explained above (see Fig. 3.7 B and C). As expected, the units that were depressed by the tactile stimuli are mostly located in the negative side of the distributions, both for the Interaction Index and the Multisensory Contrast Index (cyan bars), while the neurons that were enhanced by the tactile stimuli are located on the positive side (magenta bars). Most of the other units are distributed around 0 in both plots (gray bars), indicating that these neurons were not affected at all by the tactile stimulation. Overall, the consistency between the sign and significance of the comparison between the responses to the visual and visuo-tactile stimuli and the location of the neurons along the Interaction Index and Multisensory Contrast Index axes confirms the solidity of my categorization approach.

Figure 3.7 Neurons Categorization, Interaction Index and Multisensory Contrast Index

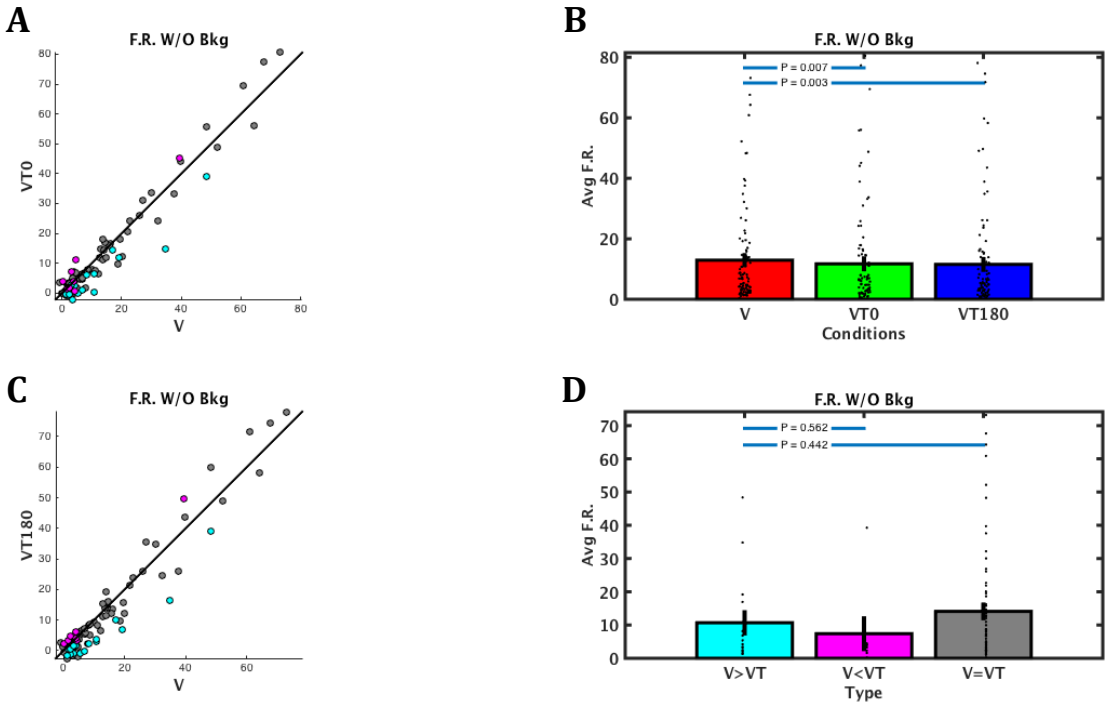


To further investigate the magnitude of the cross-modal modulation at the population level, I plotted the response of each unit to the best stimulus condition, when presented in the visual modality, against its responses to the same visual stimulus, but when paired to either the 0º direction or the 180º direction tactile stimulus (see Fig. 3.8 A and C). In both cases, the resulting scatter plots had most of the units placed near the diagonal, thus showing only moderate differences of firing rate, between the visual and visuo-tactile conditions.

Regardless of this, the population averages of the firing rates obtained for the visual and the two visuo-tactile conditions were significantly different from each other ($p < 0.001$ for both comparisons with paired t-test; see Fig. 3.8 B; each dot represent a single unit's average firing rate to its best condition; colors are the same defined in the Figs. 3.3-6).

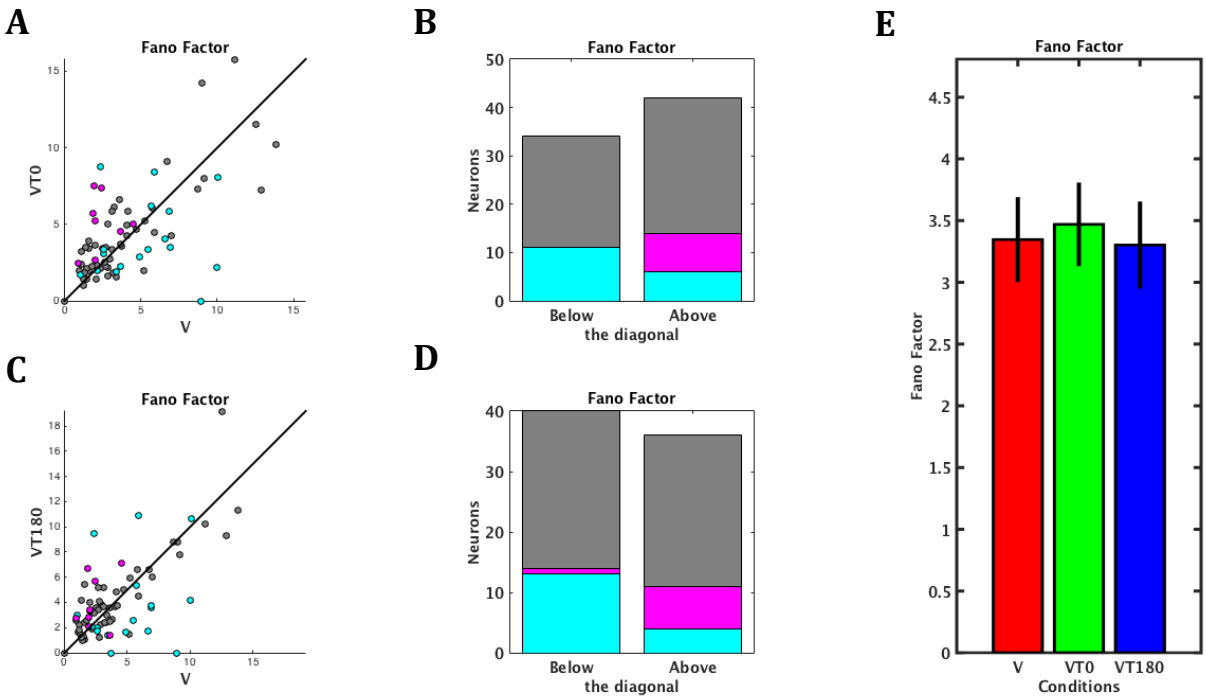
To better characterize the units' firing rate profile, in terms of the previous classification, I highlighted the units with significant cross-modal modulation in the scatter plots of Fig. 3.8 A and C (see the magenta and cyan dots, respectively). As expected, the tactile-depressed units were placed in the lower part of the plot (cyan dots), under the diagonal, and, vice versa, the tactile enhanced units were located above the diagonal (magenta dots). An interesting observation, anyway, was that most of these significantly modulated units were placed in the lower range of the firing rates axes. To better assess this trend, I computed the population average responses in the visual modality only, separately for the three categories of neurons: unaffected (gray), enhanced (magenta) and suppressed (cyan). The resulting bar plot (see Fig. 3.8 D) shows that, indeed, the tactile depressed and enhanced units were those neurons that, on average, responded less strongly to the visual stimuli.

Figure 3.8 Firing Rates



Finally, I replicated the previous analysis, also for the Fano factor values. As before, I plotted the Fano factor value obtained for each neuron on the best visual stimulus, for the visual sensory condition and both the visuo-tactile sensory conditions (see Fig. 3.9 A and C). Compared to the previous analysis, the values were much more scattered around the diagonal. More interestingly, the units that had been previously categorized as depressed by the tactile stimulation had, on average, a higher Fano factor value for the visual sensory condition, when compared to both visuo-tactile ones (see Fig. 3.9 B and D). The opposite was true for the units that were enhanced by the tactile stimulation (see Fig. 3.9 B and D). Therefore, the mechanisms that are responsible of slightly inhibiting the responses of V1 neurons through the tactile stimulation, also tend to make these responses more reproducible. These differences were not due to any major difference between the population average Fano factor across the three sensory conditions, as it is shown in Fig. 3.9E.

Figure 3.9 Fano Factor



3.4.2 Discussion

The goal of my experiment was to assess whether tactile sensory inputs would affect the representation of visual stimuli in rat primary visual cortex, when both the visual and tactile stimuli contained complex spatio-temporal patterns. In fact, previous work has already shown the existence of tactile-modulated responses in rat V1, but all the previous studies have used very simple stimuli, such as flashes of light and abrupt whisker deflection through an air puff. In my experiments, I used, instead, oriented drifting gratings, with many different orientations/directions and spatial frequencies for the visual stimuli and two directions for the tactile stimuli.

My analysis revealed that, for a substantial fraction of the recorded V1 neurons, the response to the most effective visual grating was modulated by one or both the concurrent tactile stimuli. Such a modulation was small in terms of magnitude, but significantly larger than expected by chance according to a t-test comparing the response to the visual stimulus alone with the response to the visual stimulus presented along with the tactile one. In most cases, this modulation had a suppressive effect on the peak response of the V1 neurons, thus suggesting that the tactile input somehow reduced the tuning of the cells for the visual stimuli. In a few cases, however, the modulation enhanced the neuronal response to its best visual stimulus, suggesting an increase of the tuning along the orientation/direction axis.

These results are consistent with previous studies, in which it was found that the tactile stimulation could have both a suppressive and excitatory effect on V1 neurons, depending on the cells type (i.e., inhibitory vs. pyramidal) and location (i.e., deep layers vs. superficial; Iurilli et al., 2012). In my experiments, I have not attempted yet a precise laminar localization of the recorded neurons, but I plan to do it for the next recording sessions, using a combination of histological procedures and Current Source Density (CSD) analysis. I also plan to estimate whether a unit is a putative excitatory or inhibitory one, by measuring the width of the spike waveform. These approaches will allow me to achieve a more precise comparison with earlier studies.

Interestingly, my analysis has revealed that the neurons that were more affected by the tactile stimuli were those with lower response for the best visual stimulus. This suggests that the tactile input may be particularly effective in modulating visually-drive responses in V1, when the firing rate is low. In my next experiments, to better test this hypothesis, I will include visual drifting gratings at lower contrast. My prediction is that, with such less salient stimuli, the tactile input will be more effective at modulating V1 neuronal responses, thus possibly playing an important role in the coding of the visual gratings.

My experiments also show a tendency for the neurons that were suppressed by the tactile stimuli to increase the reproducibility of their firing. Again, it should be interesting to test this in the context of lower contrast stimuli, in order to understand whether a trade-off exists between the decrease of peak firing rate and the noise reduction produced by the tactile input in terms of coding for the features of the gratings.

Overall, I believe that these pilot experiments have already provided an original contribution to the study of cross-modal interaction between visual and tactile stimuli in V1. More importantly, they will serve as a solid basis for the development of a more refined experimental protocol and analysis.

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Bartko, S. J., Winters, B. D., Cowell, R. a, Saksida, L. M., & Bussey, T. J. (2007b). Perirhinal cortex resolves feature ambiguity in configural object recognition and perceptual oddity tasks. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *14*(12), 821–32.

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