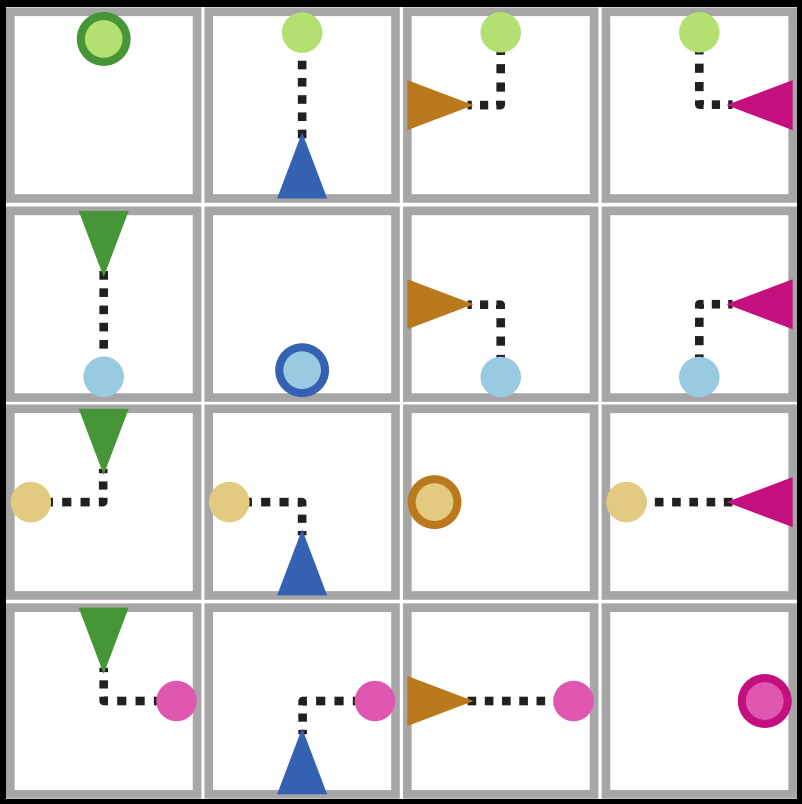
Spatial goals and actions in the orbitofrontal cortex

Niccolò Bonacchi



Dissertation presented to obtain the
Ph.D degree in Biology | Neuroscience

Instituto de Tecnologia Química e Biológica António Xavier | Universidade Nova de Lisboa

Oeiras,
June, 2017

UNIVERSIDADE NOVA DE LISBOA

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per Olivia

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Resumo

Acredita-se que o córtex orbitofrontal (OFC) esteja envolvido na representação antecipada de objectivos/'outcomes' comportamentais que dirigem o comportamento 'goal-directed'. Entre as propriedades destes 'outcomes', representados no OFC, está a sua localização espacial, uma característica fundamental especialmente importante para animais que dependem da sua capacidade de locomoção para foragear. Estudos prévios descreveram correlatos neuronais de escolhas e localização de 'outcomes' em ratos enquanto realizavam tarefas espaciais binárias, *i.e.*, em que podiam escolher entre duas alternativas. No entanto, relativamente pouco se sabe sobre as propriedades espaciais destas representações neuronais no OFC.

As tarefas comportamentais usadas anteriormente, apresentam uma extensão espacial da arena comportamental relativamente restrita que não permite a caracterização detalhada das propriedades espaciais destas representações. Para além disso, devido à ausência de um contexto de navegação explícito, factores como a localização da recompensa, a ação que leva à recompensa e a direção em que a recompensa se encontra, estão completamente correlacionados. Consequentemente, os dados obtidos até ao momento não permitem desambiguar os contributos das variadas representações para o funcionamento do OFC, nomeadamente: representações associadas à ação, à direção ou à localização espacial dos 'oucomes'.

Neste estudo mostramos que o OFC é necessário para manter a performance numa tarefa comportamental, espacialmente estendida, de escolha alternada livre. Este facto é verdadeiro apenas se o sujeito é obrigado a visitar o lado oposto da caixa antes de recolher uma

recompensa, e é falsificado se retirarmos esta contingência ao testarmos uma simples tarefa de inversão espacial. A introdução desta componente espacial, e possivelmente de navegação, parece ser a variável fulcral e explicativa destes resultados.

Para melhor investigar estas propriedades espaciais, desenvolvemos uma tarefa de navegação espacial guiada por odores, usando uma regra allocentrica, onde os estímulos olfactivos são mapeados para localizações espaciais de onde os sujeitos podem recolher uma recompensa. Resolver esta tarefa requer um mapa cognitivo do espaço e uma representação da localização espacial do 'outcome'. Ao realizarmos uma análise de viés baseada historial de escolhas dos sujeitos, concluímos que esta tarefa comportamental evidencia as localizações espaciais enquanto variáveis de decisão mas não as ações ou trajectórias que os sujeitos executam. Neste estudo, utilizamos registros extracelulares com tetrodos no OFC de ratos para revelar o papel destes neurónios na codificação de localizações espaciais dos 'outcomes', das ações, bem como o seu envolvimento na navegação espacial. Encontramos populações neuronais distintas que codificam ações e localizações, e que, dado o design da nossa tarefa, podem ser diferenciadas claramente. Finalmente, propomos o OFC como o local de integração da informação espacial com outras expectativas do 'outcome' quando o sujeito age num contexto 'goal-directed'.

Abstract

The orbitofrontal cortex (OFC) is thought to be involved in the representation of anticipated behavioral outcomes that drive goal-directed behavior. Among the properties of goals or outcomes that may be represented in the OFC is their spatial location, a fundamental feature of goals for animals that rely heavily on locomotion for foraging. Previous studies have described neural correlates of choice and goal location in rats performing spatial two-alternative choice tasks. However, relatively little is known about the spatial properties of these OFC neural representations.

In previous tasks, the constrained spatial extent of the behavioral arena did not allow characterization of the detailed spatial properties of representations. Furthermore, because of the absence of an explicit navigational context, the location of the reward and the choice side were always correlated. Consequently, the data could not disambiguate between representations of the nature of the action, of the direction or of the spatial location of the goal.

Here we show that the OFC is necessary to maintain performance in a spatially extended 2 alternative free choice task only if the subject is required to initiate a trial by visiting the opposite side of the box but not in a simple spatial reversal task. The introduction of this spatial and possibly navigational component seems to be the key variable behind our results.

In order to better investigate these spatial properties we developed an odor guided spatial navigation task where odor stimuli are mapped to outcome locations using an allocentric rule. Solving such task requires a cognitive map of space and a representation of the cued outcome spatial

location. A bias analysis show that rats in this task seem to care about locations more than actions. We use extracellular tetrode recordings in the rat's OFC to reveal its role in coding for outcome locations, actions and spatial navigation. We find that distinct neuronal populations in OFC respond to actions, or locations, and that we are able to clearly differenciate between the two by the nature of the task we developed. We propose the OFC as the site of integration of location information with other outcome expectations in goal-directed behavior.

Abbreviations list

AUC	Area under the curve
AFC	Alternative forced choice
AP	Anterio-posterior
CCTV	Closed circuit television
dPCA	Demixed principal component analysis
DLS	Dorso-lateral striatum
DMS	Dorso-medial striatum
DV	Dorso-ventral
IL	Infra limbic cortex
INDP	International Neuroscience Doctoral Programme
IR	Infra red
LED	Light emitting diode
ML	Medial-lateral
OFC	Orbitofrontal cortex
OSD	Odor sampling duration
PFC	Prefrontal cortex
PL	Prelimbic cortex
PSTH	Peri-stimulus time histogram
RL	Reinforcement learning
ROC	Receiver operator characteristic
RTLFSM	Real time linux finite state machine
SVM	Support vector machine
VLO	Ventro-lateral orbital cortex

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1 General Introduction

Author contributions: Bonacchi N. wrote the manuscript.

1.1 Chapter summary

General notes on the manuscript:

This chapter features the general introduction to the relevant theoretical and conceptual framework that oriented our exploration of behavior and the orbitofrontal cortex (OFC). **Chapter 2** revolves around an inactivation experiment in a simple free choice spatial task and its results which led us to further explore and develop a new task. The odor guided spatial navigation task and its behavioral results is the focus of **Chapter 3**. **Chapter 4** will report the results of the neurophysiological recordings we performed in this task. Finally, the thesis will wrap up with a general discussion chapter and the bibliography.

With the exception of the present and last chapters, all other chapters will feature a Chapter summary that, just like this paragraph, will summarize and comment on the chapter. This summary will be followed by an Introduction where we will set up the different themes of each chapter followed by the Methods section that will begin with a description of the behavioral task. The subsequent Results section will exemplify the main findings of the experiment and finally, the Discussion section will summarize the results and bridge to the following chapter. For readability purposes, all bibliography will be presented in a final Reference Chapter. Overall the organization of the thesis revolves around behavior, not only conceptually but also practically. Consequently, **Chapter 2**, **Chapter 3** and **Chapter 4** are organized around the 2 behavioral paradigms developed during the thesis work.

1.2 Introduction

Imagine you just arrived at your grandmother's house. As you walk in, the smell of her famous apple pie greets you, your stomach echoes a gurgle and your mouth salivates slightly. You say hello to everyone, embrace your grandparents and start talking, you haven't seen each other for a while and you really want to spend some time with them and catch up. As you are talking however, in the back of your mind, this persistent image of apple pie grows to the point you're trying to find a polite way of asking for some pie. Reading your mind (as they do), your grandma asks if you want some pie. "Of course! …", you say smiling, and almost as to attone for the fact that you haven't been listening for the last 30 seconds, you utter: "...don't bother getting up I'll fix myself a plate! Do you want some?". Sure says grandpa, who's sitting on the couch. "None for me." answers grandma while she gets up anyway to set the table for dinner. As you enthusiastically stand up with the image of your grandma's apple pie etched in your brain, your stomach growls again, and you go... but where? You know there is pie in the house, you smelled it, you have some prior of where pies 'live' in general and some prior about where your grandma sets her pies to cool down. Maybe unsurprisingly, you decide to check the toilet seat in the bathroom, find the pie and fix a piece for you and grandpa.

The reader might, up to a point, have a similar story (details about relatives notwithstanding), and I'd also venture the guess that the last sentence was found to be somewhat strange. The only strange thing about the last sentence was the substitution of the location of the apple pie from 'kitchen counter' to 'toilet seat in the bathroom'. This substitution is received as surprising only because an expectation about the location of the apple pie exists. Objects in the world have properties that usually

correspond to our sensory experience of them, they have a color, a shape, a size, a texture, a resistance, a temperature, a weight, an odor, and a taste. What objects also have, is a context. Context, unfortunately, is quite a generic word used by people with multiple backgrounds. From the arts and social sciences, to biology and physics, the definition of context can refer to very different things.

Journalism, that is arguably tasked with the accurate report of events, has used the 5 'W' rule to write a news story, meaning: who, what, when, where and why. This is thought to be the best way to get an unbiased and accurate depiction of some event. A scientific experiment, is also a report of an event, and experimentalists, also worried with biases and concerned with accuracy, usually think of their experiments in similar terms: subjects (who), objects (what) and context (when, where and why). Temporal/rithmic information, spatial/locational information, as well as motivational context are critical criteria that should be considered when developing tasks and interpreting behavioral results.

As the reader might have gathered already, this thesis will focus on locations and although locations are not exactly a primary sensory experience, we hope our little story has demonstrated that they are an integral part of the description of an object when this object becomes a behavioral goal. So, while for the identity of an object its physical location might be superfluous, it becomes critical if this object needs to be acted upon in some way. During the decision and implementation of a goal-directed behavior, the spatial information about an object, as well as other 'secondary properties' like timing, are probably integrated with the sensory information that defines this object, somewhere in the brain (effectively 'contextualizing' the object). We propose, based on our own

results as well as previous studies, the orbitofrontal cortex (OFC) as the region that serves this function.

Our hope is that this thesis will contribute to the understanding of goal-directed behavior, spatial processing and the function of OFC in a spatial context and help pave the way for the emerging functional theory of OFC in the brain.

We will start by briefly introducing the OFC at the anatomical and functional levels introducing the relevant concepts, previous works and ideas about OFC that gave rise to this project.

Orbitofrontal cortex

The orbitofrontal cortex (OFC) in primates and orbital cortex in rodents refers to the ventral surface of the frontal lobe, it is called this way because of its close proximity to the eyes. It receives projections from a considerable number of other brain areas including visual, olfactory, somatosensory and visceral/gustatory cortices. Besides its many other functions that we will introduce in this section, OFC is interestingly considered the secondary gustatory cortex and around 8% of neurons respond to different gustatory stimuli and are sensitive to devaluation protocols (Thorpe et al., 1983; Nakano et al., 1984; Rolls et al., 1989, 1990).

OFC Anatomy

The OFC is the target of many different areas both directly and indirectly through the medial dorsal nucleus of the thalamus (Carmichael and Price, 1995a, 1995b). These projections that include but are not limited to: striatal, somatosensory, olfactory, and viscera inputs, carry sensory

and reward related information that can be integrated in the OFC. OFC also possesses medial-prefrontal and limbic reciprocal connections two major areas in decision-making, implicating it in related functions (Ongür and Price, 2000; Carmichael and Price, 1995a). While medial OFC shares reciprocal connection with the ventro-medial prefrontal cortex, central and lateral sections of the OFC receive reciprocal projections mainly from visceral afferents (Carmichael and Price, 1996; Ongur and Price, 2000). OFC's connectivity pattern is largely consistent between species from rodents to primates (Krettek and Price, 1977a, 1977b, 1978; Ferry et al., 2000; Ongür and Price, 2000; Kondo et al., 2003, 2005; Price, 2007; Kondo and Witter, 2014). In contrast to primates that have granular and agranular prefrontal cortices (PFC), PFC in rats, and consequently OFC, is exclusively agranular (Ongür and Price, 2000). This fact poses as a limitation to the use of morphology to support comparisons of brain areas in different species. Homology between species can be thus asserted at the connective and functional level. Both of these criteria are currently under debate, in fact some go as far as questioning if rodents have a prefrontal cortex all together (Preuss, 1995; Uylings et al., 2003).

Nonetheless, connection similarities, of both inputs and outputs, have been reported especially pertaining to the caudal agranular OFC in primates and rodents (Croxson et al., 2005; Price, 2007). Furthermore, thalamic, amygdalar complex (especially baso-lateral amygdala), anterior hippocampus, hypothalamus and nucleus accumbens reciprocal projections show remarkable similarities (Deacon et al., 1983; Groenewegen, 1988; Carmichael and Price, 1995a, 1996; Haber et al., 1995; Cavada et al., 2000; Ongür and Price, 2000; Ramus et al., 2007; Mailly et al., 2013). Similar impairments are also observed in lesions studies to the amygdala and OFC (Jones and Mishkin, 1972; Gaffan and

Murray, 1990; Schoenbaum et al., 1999, 2000, 2002, 2003b; Schoenbaum and Setlow, 2001; Fellows and Farah, 2003; Pears et al., 2003; Wallis and Miller, 2003; Mariano et al., 2009). In fact, the strong reciprocal connectivity between baso-lateral amygdala and OFC has been hypothesized as contributing to the emotional and motivational aspects of learning (Davis, 1992; Holland and Gallagher, 1999; Schoenbaum et al., 2000; Baxter and Murray, 2002).

OFC Function

It is known that OFC lesions or inactivations during contingency reversals (reversal learning), strongly affect performance (Teitelbaum, 1964; Jones and Mishkin, 1972; Schoenbaum et al., 2002, 2003a; Bohn et al., 2003; Izquierdo et al., 2004). At the same time however, learning new stimulus-action associations is thought to be independent of OFC as the acquisition of new associations is not affected by these lesions (Schoenbaum et al., 2002; Chudasama and Robbins, 2003). This means that although OFC is not important for the initial stimulus-action associations *per se*, it becomes necessary if these previously learned associations need to be updated.

The Iowa gambling task (Bechara et al., 1994) has been used in humans to assess impairments in evaluating risk and future rewards. Subjects are asked to pick cards from a number of decks that can yield gains and losses. Losses are distributed in different amounts and probability in such a manner that, over time, some decks will be ‘good’ decks and some will be ‘bad’ decks yielding more losses than gains. Humans with OFC lesions performing this task choose decks with higher losses over time and demonstrate, what the authors called: “impairments in future consequences”. However, a later study (Fellows and Farah, 2005)

demonstrated that a slight modification in the experimental design was sufficient to remove the impairment previously observed. While the original task only presented rewards for the first 10 trials for each deck, this modification involved shuffling randomly the gains and losses since the beginning. Having only rewarded trials at the beginning was supposed to help subjects assess the statistics of the gains quickly. However, this practice resulted in the subjects learning the gains and then having to 'reverse' or update that learning once the losses started to appear. Moreover, in a series of studies using an analogue of the shuffled version of the Iowa gambling task for rodents (Zeeb et al., 2009; Zeeb and Winstanley, 2011, 2013), the authors also report that inactivating OFC causes no impairment in selecting the best option overall. These results suggest that the hypothesized lack of ability to evaluate future losses resulting from OFC lesions can be better explained as a deficit in the ability to update previously learned associations as assessed by reversal learning paradigms.

From reversal learning to outcome expectancies

Behaviorally, the inability to reverse or update previously learned associations, could be explained if OFC is required for inhibiting a learned response. Presumably, in order to learn something new, mapped to the same behavioral output, subjects need the ability to, first and foremost, inhibit the previously learned response. Indeed, OFC is necessary for animals to be sensitive to devaluation protocols (Critchley and Rolls, 1996; Gallagher et al., 1999; Izquierdo et al., 2004; Pickens et al., 2005; Plassmann et al., 2007; Roesch et al., 2009). However, multiple studies have reported OFC as not necessary for response inhibition (Schoenbaum et al., 2002, 2003a; Pickens et al., 2003).

Several results however, have suggested that the association between reversal learning and OFC was not the full story. Some of these results suggested a bigger picture for OFC beyond reversal learning. OFC has been shown to be embedded in a hierarchical network that is responsible for outcome identity (Keiflin et al., 2013) and outcome location reversals (Young and Shapiro, 2009) but not strategy switches (which are attributed to prelimbic/infralimbic cortex – PL/IL). Meaning that OFC would not be necessary to learn reversals, but sufficient to overrule other brain areas that had learned them.

The actual involvement of OFC in reversal learning altogether has also been questioned, at least in primates by Rudebeck and colleagues (Rudebeck and Murray, 2011; Rudebeck et al., 2013). These authors suggest that, in primates, the reversal effects previously observed were due to the removal of fibers of passage. In fact, instead of using the usual aspiration method, in their study they performed lesions to OFC using excitotoxic methods, which target specifically cell bodies, and fail to observe the reversal effects previously described. Temporal lobe and limbic system damage seem to reproduce reversal learning impairments in primates (Murray et al., 1998; Izquierdo et al., 2005; Chudasama et al., 2009) and, although one can also find the same projections from temporal and limbic areas to mainly the ventral and medial orbital areas, in rats (Carmichael and Price, 1995a, 1995b; Schmahmann et al., 2007; Kondo and Witter, 2014; Timbie and Barbas, 2014), the same observation made by Rudebeck and colleagues has, to our knowledge, yet to be reported.

Furthermore, if OFC neurons were to be responsible for the reversal impairments observed, one would predict that neurons that are sensitive to a particular reward would, upon reversal, either stop firing or reverse

their tuning to now represent the new reward. However, studies that compared OFC neurons with amygdala neurons in both rats and primates, reveal a much higher change in preference for amygdala neurons, whilst OFC neurons tend to maintain their preferred responses (Thorpe et al., 1983; Schoenbaum et al., 1999; Paton et al., 2006; Stalnaker et al., 2006).

Several more studies, show OFC to be important for more than reversal effects and together they contribute to paint a picture of a broader functional scope of OFC. Specifically, a considerable number of reports have surfaced implicating OFC in the representation of outcome properties and the representation of cues associated with specific outcomes. These properties include diverse features, amongst which: identity and taste (McDannald et al., 2011; Jones et al., 2012; Keiflin et al., 2013), size and economic value (Tremblay and Schultz, 1999; Schultz, 2000; Hikosaka and Watanabe, 2004; Padoa-Schioppa and Assad, 2006; Jones et al., 2012), uncertainty (Kepecs et al., 2008; Kepecs and Mainen, 2012; Lak et al., 2014; Zariwala et al., 2013), regret (Steiner and Redish, 2012, 2014), and spatial location (Corwin et al., 1994; Feierstein et al., 2006; Roesch et al., 2006). Reward prediction errors (Sutton and Barto, 1998) have also been shown to require OFC for proper computation (Takahashi et al., 2011) and the additivity and transitivity (or inferred values) properties in an economic value framework seem to be important factors that explain the modulation of firing rates of OFC neurons (Jones et al., 2012; Takahashi et al., 2013).

Attempting to integrate these ever growing and incredibly varied results, several functional hypotheses of OFC have arisen. One such hypothesis postulates its role in facilitating behavioral and associative flexibility of downstream areas by encoding “outcome expectancies” (Schoenbaum

and Eichenbaum, 1995; Schoenbaum et al., 1998, 1999, 2003a, 2007). OFC's involvement in reversal learning loss of fuction experiments would thus be a consequence of the inability to represent outcome expectancies or properties. This suggests that a more generic function for OFC should be considered as, possibly, an integrating information hub for outcomes, cues and context (Wallis, 2006, 2007; Mainen and Kepecs, 2009; Schoenbaum and Esber, 2010).

More recently, a more general role of OFC in decision-making and learning has been proposed. This proposal implicates OFC in the representation of a cognitive map (Tolman, 1949) of 'task space' (Wilson et al., 2014), and OFC would be responsible for learning and representing hidden states. The authors of this study used a series of model-free and model-based reinforcement learning (RL) models to revisit some of the classical results known from the loss of function OFC literature. Their hypothesis was that OFC would represent hidden states. Hidden states or, non-stimulus-bound states are posited in opposition to states that can be differentiated by some sensory stimulus that would act like a cue that informs the animal of the current state. In this view, inactivating OFC would result in an impoverished state space over which the RL agent had to learn. As it turns out, this simple manipulation was able to recapitulate a great number of classical OFC inactivation results.

This result is particularly interesting because it links RL, specifically model-based RL (Sutton, 2012) to goal-directed behavior through a particular brain structure. A goal-directed action is defined in opposition to a habitual action and is an action performed on the basis of the consequences the action will cause rather than in response to a stimulus (Adams and Dickinson, 1981; Colwill and Rescorla, 1985, 1986). Goal directedness can be assessed experimentally by outcome devaluation

and contingency degradation (Dickinson, 1985; Dickinson and Balleine, 1994; Balleine and Dickinson, 1998). When planning a goal-directed action, the goal, or outcome desired, is not present and needs to be imagined in order to accurately implement a decision. Furthermore, because this outcome is a desired future state that is not immediately present it is effectively a hidden state. Outcomes, or goals, can be represented in the brain either as a categorical variable, where a population of neurons represent this category specifically, or as a vector of sensations, where the categorical property could be considered only if one knows the precise combination of sensations the animal is sensitive to. In this context, representing goals, consequences, outcome properties or hidden states is arguably equivalent.

Tolman's cognitive map is thus reinterpreted from an actual spatial map to a more abstract state map, that might or might not have a particular relationship with physical space. Interestingly, however, the brain area that is essential for spatial navigation, the hippocampus (O'Keefe and Conway, 1978; Wilson and McNaughton, 1993) is also known to affect memory formation (Scoville and Milner, 1957; Squire, 2009), suggesting an intimate relationship between spatial variables, memory and goal-directed behavior.

OFC and space

This thesis will expand on previous results (Corwin et al., 1994; Feierstein et al., 2006; Roesch et al., 2006; Young and Shapiro, 2009) that implicate OFC in the coding of spatial, or spatial-like features in the context of navigation. A more detailed description of these studies can be found in **Chapter 2** and **Chapter 3** in the introduction and discussion sections.

So why would space be important? Kant, in the Critique of Pure Reason, described time as an a priori notion that, together with other a priori notions such as space, allows us to comprehend sense experience. It would follow that spatial variables, not only must be represented somewhere in the brain but should also be used as a fundamental cognitive anchor for our not-only-sensory experience. The most obvious case where spatial variables would be involved in decisions is in the case of spatial navigation.

Spatial processing is important for animals as they move about in the world, moreso for rodents that rely on foraging for survival. Rats, for example, live in intricate underground burrow systems (Pisano and Storer, 1948; Calhoun, 1963), and rely on exploration of their surroundings for food (Barnett, 2007). A delicate exploration-exploitation balance is important as rats will lower their probability of predation the less they explore but increase the probability of running out of resources if no exploration attempt is made (Charnov, 1976). Knowing where the food is and how to get there becomes paramount to properly allocate the correct amount of time and resources to exploiting one particular patch of resources, or exploring the environment to find another. Furthermore, the location, direction or just general area exploration efforts should focus on, should be informed by a cognitive spatial map of the animal's surroundings. Lastly, in case of danger, the relative location of the animal's home is fundamental in order to rapidly plan an escape route.

Navigation can be accomplished using different types of cognitive strategies, the 2 extreme cases of which are called egocentric and allocentric navigation. The egocentric reference frame is centered on the subject and defines positions and orientations as a sequence of actions

relative to a single localizing cue, usually visual, that resets the initiation. Allocentric reference frames are centered on an area map and are built using a configuration of different cues where the subject is one of these cues (Dolins and Mitchell, 2010; Lihoreau, 2010). Different brain areas have been involved with one or the other type of navigation and because these are conceptual, extreme cognitive strategies, perhaps unsurprisingly, the neuronal substrates that enable them, have been found to have complex interactions and a somewhat mixed strategy (Iaria et al., 2003; Ekstrom et al., 2014). Nonetheless, several studies have found striatum, caudate nucleus and putamen to be important for egocentric navigation (Maguire et al., 1998; Rubio et al., 2012; Chersi and Burgess, 2015) whereas hippocampus and para-hippocampal regions have shown involvement in allocentric navigation (Hartley et al., 2003; Rubio et al., 2012; Chersi and Burgess, 2015).

Final remarks

Several pieces of evidence seem to implicate OFC in spatial processing, among them we find: OFC's reciprocal projections to hippocampus (Carmichael and Price, 1995a); hippocampal involvement in allocentric navigation (Dolins and Mitchell, 2010; Lihoreau, 2010); OFC's involvement in goal-directed behavior; the presence of spatial like features in OFC (Feierstein et al., 2006; Roesch et al., 2006); and the fact that OFC is required for allocentric navigation (Corwin et al., 1994). Furthermore, if the hippocampus is responsible for providing 'contextual' information to the rest of the brain (Moser et al., 2008) and, at the beginning of the chapter, we defined context as the where, the when, and the why, looking at location correlates in OFC would support the hypothesis that the hippocampus 'contextualizes' prospective sensory objects, at least in terms of location. In any case, whether OFC is

involved in spatial navigation, or the hippocampus is involved in providing contextual information to the representation of expected outcomes, the involvement of OFC in the representation of outcome expectancies would still hold as long as we consider locations as just another outcome expectancy (i.e. a property of a sought outcome).

Examining OFC's spatial properties becomes important especially considering the limitations of previous studies. While some studies that look at spatial properties of OFC (Corwin et al., 1994; Young & Shapiro, 2009) have an explicit navigational context, they are framed in terms of reversal learning and not of location representation in the context of outcome expectancies. Contrary to this, studies that have focused on outcome expectancies and spatial features (Feierstein et al., 2006; Roesch et al., 2006), used small behavioral boxes, with no navigational contextor demands, and don't separate locations, direction, or actions.

If OFC is involved in integrating spatial information with other outcomes expectations, effectively representing location as one of the outcome expectancies referred to above, then this representation, while relevant for learning, should be persistent even after learning.
Our proposal is thus, to investigate the representation of outcome locations in the OFC in overtrained animals performing a task that has a precise navigational context.

The following chapters report our attempts of teasing apart these issues.

1.3 Bonsai

While thinking about the implementation of a navigational task, we rapidly decided that such an experiment would require a fast and customizable tracking system. While tools to this purpose are commercially available, their implementation was highly optimized for particular physical setups and didn't allow low level control of parameters. Rapid and flexible prototyping of experimental designs is paramount to any exploratory endeavour at the basis of the development of a new behavioral paradigm. Considering this, in collaboration with Gonçalo Lopes, another PhD student, we started to develop our own video tracking system which rapidly evolved into a full fledged generic framework that processes data streams: Bonsai (Lopes, **Bonacchi**, et al., 2015). Bonsai has been published in Frontiers of Neuroinformatics and has been adopted by several labs around the world for, among other things, the integration of behavioral protocols, electrophysiological recordings and real-time video processing.

Bonsai: an event-based framework for processing and controlling data streams
The design of modern scientific experiments requires the control and monitoring of many parallel data streams. However, the serial execution of programming instructions in a computer makes it a challenge to develop software that can deal with the asynchronous, parallel nature of scientific data. Here we present Bonsai, a modular, high-performance, open-source visual programming framework for the acquisition and online processing of data streams. We describe Bonsai's core principles and architecture and demonstrate how it allows for flexible and rapid prototyping of integrated experimental designs in neuroscience. We specifically highlight different possible applications which require the combination of many different hardware and software components, including behavior video tracking, electrophysiology and closed-loop control of stimulation parameters.

2 Free Choice Spatial Task

Unpublished data

Author contributions: Bonacchi N. and Mainen Z.F. designed the studies. Bonacchi N. built the apparatus, ran the experiments, analyzed the data and wrote the manuscript.

2.1 Chapter summary

This chapter reports the rationale, implementation and results of the free choice spatial task inactivation experiment. This was our first attempt of introducing spatial locations as relevant decision variables for animals performing a decision-making task. We will start by introducing the historical and conceptual rationale behind the development of this task, present the results, and discuss the implications for the rest of the thesis.

2.2 Introduction

Behavioral tasks used to study OFC function never focused on spatial components, with few notable exceptions (Corwin et al., 1994; Feierstein et al., 2006; Roesch et al., 2006; Young and Shapiro, 2009). Even these exceptions were arguably not designed specifically to examine spatial representations in the context of navigation and location. For example, behavioral tasks in these studies generally did not explicitly parse out action versus direction.

At the time I joined the Mainen Lab, the task that was used was no exception. The *two-alternative choice odor discrimination task* (Uchida and Mainen, 2003; Kepecs et al., 2008) was designed in a relatively small behavior box and the task entailed the animals to remain mostly stationary when interacting with the apparatus. This task is a particular case of a 2 AFC (Alternative Forced Choice) task that uses as guiding stimuli a mixture of 2 odors where the relative concentration of the individual odors is used as a way of changing the difficulty of the choice on a trial by trial basis. This task is one particular example of a category

of tasks one might call sensory decision making tasks under uncertainty. These type of tasks are designed to look at sensory processing and usually add a source of uncertainty to the stimulus in order to manipulate its difficulty parametrically. This emphasis on the stimulus as the relevant decision variable as well as the trial by trial difficulty manipulations, allows the experimenter to build classical psychometric functions by measuring behavioral output variables like accuracy.

Although this task, as previously mentioned, is not optimized to study spatial features, a 2006 paper (Feierstein et al., 2006) used a pure odor variation of this behavioral paradigm, and was one of the first to describe spatial-like variables in OFC. The authors found OFC cells, appropriately called goal cells, that significantly changed their firing rate for particular goal locations. These cells fired both in the presence or absence of rewards, and to some extent independently from the action just performed. Finally, these cells fired for the same goal location even independently of stimulus identity when multiple stimuli were associated with the same reward location or direction. In other words, these cells seem to care about the goal location/direction but not: the presence of reward, the action performed or the stimulus that led the animal there. Furthermore, by looking at the choice moment and at the trial reinitiation moment, the authors were able to describe a set of cells that were selective for particular left or right actions. Nonetheless, we know hippocampal place cells and grid cells in the entorhinal cortex, demonstrate an increase in size and spacing of the associated place fields as one navigates dorso-ventrally (Sargolini et al., 2006; Brun et al., 2008; Stensola et al., 2012). If a place field like response in OFC is to be found – one could speculate that there could be an increase in place field and most probably a conjunctive aspect of place and other goal expectancies that are characteristic of OFC already. Given the reduced size of the behavior box and the proximity between pokes it

could very well be the case that the 'action' cells found by Feierstein and colleagues (2006) were not correlated with a left or right action *per se* but might have been representing locations of a wider place field. There is the possibility that the location/direction selective cells reported and the action cells were one and the same population sensitive to different size place fields.

Another study (Lak et al., 2014) using the same task, performed inactivations and found that the absence of a functioning OFC affected the time animals are willing to wait for a reward, both depending on trial difficulty and expected outcome. Most importantly for our purpose, rats could perform the task with no impairment in accuracy regardless of stimulus difficulty. This means that to "solve" the task, or more accurately, for implementing the initial decision of where to go given a particular stimulus, OFC was not being used. So, while OFC cells were found to be causally involved in the decision to stay or wait for a reward depending on the trial difficulty, they didn't seem to be involved in the initial decision of where to go.

From these two studies we can conclude that OFC is not necessary to solve the two-alternative choice odor discrimination task. At the same, however, and in an apparent contradictory fashion, OFC seems to represent some spatial variables i.e., the location or direction of the goal.

On these basis, we set out to explore a behavioral task, with a higher spatial component, where animals have to implement decisions that necessarily require OFC activity, i.e., we tried to find a task where OFC would be 'used' and therefore required to solve the task. If successful, and by inactivating OFC we find a behavioral effect, this alone would falsify the claim made by Feierstein and colleagues that OFC is

monitoring task variables but not involved in the decision *per se*. In any case, given all the above, some characterization of the spatial properties of OFC neurons seemed to be an interesting direction.

Considering that the goal cells Feierstein et al. (2006) reported could be OFC cells sensitive to outcome locations, we decided to introduce an obvious spatial component to the outcome. The easiest way of making space a relevant feature, was actually inspired from the discovery of grid cells (Fyhn et al., 2004; Hafting et al., 2005) where a simple increase in size of the recording arena was the key change from previous work that allowed for such discovery. This increase in space would also, possibly help, in teasing apart action selective cells reported previously, from location selective cells that cover more than one port.
Finally, considering the reversal learning literature, we decided that a change in contingencies would probably be helpful in engaging OFC especially if the reversal was in the spatial dimension.

With these things in mind we modified the two-alternative choice odor discrimination task in a number of significant ways:

1. We increased the size of the box to 1 m^2
2. We located the initiation port on the opposing wall of the reward ports
3. We removed stimuli
4. We made the reward change places (spatial reversal)

With these modifications we hoped that goal location would become a more relevant feature. Firstly, by making the box's footprint bigger and separating the pokes further apart; and secondly, by making the animal move from initiation port to reward port on every trial. Lastly, by removing stimuli, we hoped to make the animals focus on 'where' the

reward would be rather than on ‘what’ odor was delivered. Removing stimuli also had the added benefit of not needing special stimulus training and thus hopefully reduce training times. Finally, the introduction of a spatial reversal component of the reward would hopefully engage OFC and also contribute to highlight the reward location property.

2.3 Materials and methods

All experiments and procedures were approved by the Champalimaud Foundation Bioethics Committee and the Portuguese National Authority for Animal Health, Direcção-Geral de Alimentação Veterinária (DGAV).
After having optimized a training protocol, we designed the testing phase to ascertain necessity by pharmacologically inactivating the OFC in 2 conditions: in the presence of an initiation port, and in its absence. The order of events was: cannula implantation surgery; water restriction; testing with no initiation port; and testing with initiation port.

Behavioral Task

The task was initiated by poking in the lit initiation port located on one side of the box; rats could subsequently retrieve a drop of water from either the left or right reward ports located on the other side of the box as shown in **Figure 1**. No stimulus was delivered and water rewards switched location every fifty trials starting from a random side. A poke in the currently rewarded port was scored as correct and contrarily a poke in a non rewarded port was scored as an error (**Figure 2** top panel).

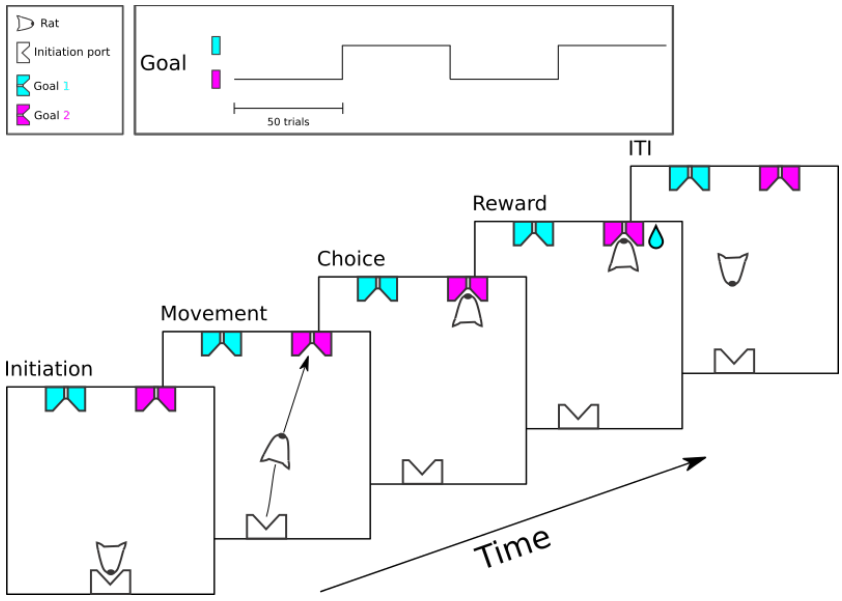

Figure 1 - Free choice spatial task
Task timeline and structure

Rats were exposed to two different task conditions: with or without initiation port. When the initiation port was not present, the task structure remained exactly the same but rats did not need to initiate a trial from the (now absent) initiation port. In fact, after a uniformly distributed random inter trial interval of 2 to 4 seconds a new trial was automatically initiated allowing them to stay at the rewarded port and just collect the rewards. In either condition, the best that any animal could do is one mistake per block-switch plus or minus one mistake if they happen to start from the wrong port. This is because there was no way to predict when the block would switch unless, of course, rats could count all the trials. From our data we concluded that rats don't seem to be able to count to 50. As soon as they make the first mistake however, they should be able to know the location of the reward given only 2 reward ports were available.

Animal Subjects

A total of 15 Long-Evans male rats were used for the experiment. Data from all rats was used to optimize training protocol. 6 rats were submitted to the surgical implantation of guide cannulas, 4 of these rats were used for investigating OFC inactivation in the Free Choice Spatial Task. During both training and testing rats had *ad libitum* food and motivation was obtained by water restriction. Body weight was kept higher than 85%, other health indicators were also monitored daily for the duration of the experiment.

Pre-handling

In order to reduce the stress on the animal during surgery and during subsequent behavioral tasks, each animal is handled for 3-5 days before surgery. During this familiarization procedure the animals are placed for ~20 minutes in the behavioral box in which they will later undergo behavioral training and testing, in addition each animal is handled by the experimenter for ~10 minutes.

Surgery

All surgical procedures for cannulae implantation were carried out under aseptic conditions. Anesthesia was initiated and maintained with isoflurane inhalation at ~2% (1.5-3%) in O2, at a flow rate of 0.5 lpm. Isofluorane adjustments were made according to paw withdrawal reflex. After craniotomy, guide cannulae (24-gauge Plastics One, Roanoke, VA) were stereotaxically implanted in each hemisphere and targeted using a rat brain atlas (Paxinos and Watson, 2006), 2 mm above OFC (AP: +3.72, ML: +/-2.5, DV: +4.2 from skull surface). Stainless steel stylets

were inserted into the guide cannulae to ensure patency (protruding 0.5 mm below the tip of the guide cannulae).

Recovery

Postoperative analgesia was administered, ketoprofen (5 mg/kg, IP) or Buprenorphine (0.05-0.1 mg/kg, SQ) and lidocaine was applied topically to the surgical site. To prevent infection, an antibiotic (0.3% gentamicin sulfate) is applied (once daily for 2-3 days) to the surgical site. To assist in rehydration, a prewarmed isotonic Lactated Ringer's solution may be given (15 ml/kg, SC). During the postoperative recovery (2-4 hrs), the animal is placed in an absorbent blanket on a microwavable heating pad. Body temperature and breathing rate are monitored during this period. The animal is then returned to its home cage and allowed to recover for at least 5 days. DietGel® Boost and Recovery purified high calorie dietary supplement from ClearH_2O® is administered for 2 days and water consumption is closely monitored, activity and appearance are used to assess postoperative recovery and as a warning sign for postoperative pain. Conditions such as non-healing of skin margins, wound infection, seizures or abnormal behavior (e.g. hyperactivity, stereotypy) were considered parameters indicating early endpoint.

Water restriction

For behavioral training and testing, the animal was placed on a water restriction schedule. Water restriction is always ceased at least 2 days before surgery and 5 days post surgery. During water restriction, food is continuously available and hydration is monitored by the CR animal facility staff, that checked water consumption and skin elasticity. Animals received water (>10 ml) during the behavioral session and 15-30 min of free water access at a variable time after the behavioral session.

Drinking time was adjusted to maintain 85-90% of free-drinking weight. During the weekend, animals are given free water. If the weight after the weekend exceeds the 'free drinking weight', the standard is adjusted to the weight of the day of beginning of the week. For immature animals, this is calculated by comparison to a cage of age-matched non water-restricted controls.

Training

The training protocol had two phases corresponding to the two testing conditions explained above. After at least one week of recovery from surgery, rats were placed on a water restriction schedule and behavioral sessions started. Rats were initially exposed to the behavior box for a short period of time ~15 minutes with no pokes lights or sounds in the box in order to recall their pre-handling experience. After all rats have gone through this recall the actual training started. A port was selected pseudo-randomly to be the first rewarded port and from there on every 50 trials the reward would switch to the other goal port. Each drop of water rewarded was preceded with an 80ms 3 KHz tone in order to cement a strong association between the tone and the reward. Poking in the non rewarded port was initially ignored. After animals had reached training criterion (>200 trials per session or 3-4 block switches, block size = 50 trials) pharmacological inactivation protocol of the first condition could begin. After this testing phase an initiation port was introduced in the opposing wall. Poking this port would yield the previously associated tone that would now work as a bridging stimulus. Rats were trained to poke in the initiation port before going to either reward port. After the correct port was found error trials were introduced, meaning an 80ms white noise burst was played if the animal chose the wrong port and no water reward. Animals remained in this new

configuration until they reached the same criterion with an acceptable performance (>80% correct). Once this criterion was obtained, the pharmacological inactivation protocol in this second condition started.

Pharmacological Inactivation

Animal subject were tested in two different conditions, both in the presence of only the goal ports and in the presence of an initiating port located on the opposite wall. The goal was to get one session a day interleaving inactivated sessions with vehicle sessions for 6 days yielding 3 vehicle and 3 muscimol sessions per subject, per condition. Inactivation and control sessions were counterbalanced. Temporary inactivation was achieved via localized injections of γ-aminobutyric acid (GABAA) receptor agonist muscimol (Sigma Alderich) under light anesthesia induced by 1-2% isoflurane (for about 6 min during which hind leg reflex never disappeared over the course of infusion). On each testing day the stylets were replaced with 33-gauge (Plastics One) injector cannulae protruding 2.0 mm below the tip of guide cannulae. One minute after proper bilateral placement of the injectors, muscimol (0.4µl of 0.125 µg/µl solution or 0.05 µg of muscimol) or sterile saline (0.9%; 0.4 µl) was injected over a 4 minute period at the rate of 0.1 µl/min on each side. Fluid was infused via 0.38 mm diameter polyethylene tubing (Intramedic, New York, NY) attached to the injector on one end and to two 2 ml Hamilton syringe (Hamilton, Reno, NV) on the other end. The syringes were driven with a syringe pump (Harvard Apparatus, MA). Injections were confirmed by monitoring the movement of mineral oil fluid in the tubing via a small saline bubble. After infusions were complete, the injector cannulae were left in place for 4 minutes and then replaced with stylets. Behavioral testing began about 45 minutes after infusion. (Martin and Ghez, 1999) showed that the maximal extent

of muscimol spread, using this procedure, was 1.5 to 2 mm within 10-20 minutes of injection.

Histology

Upon completion of behavioral tests, rats were injected with 0.4µl of evans blue solution to mark both the location as well as to give an indication of the spread of the muscimol injection. After 24 hours all animals were subsequently deeply anesthetized and then transcardially perfused with PBS and a saline 4% paraformaldehyde solution. Brains were removed, postfixed, and sectioned in 50 µm coronal slices using a fixed-tissue vibratome (VT1000S, Leica Instruments, Germany). Standard Cresyl Violet staining (Nissl staining) immunohistochemistry was performed in order to better visualize brain areas for cannula placement estimations.

Testing apparatus

The testing apparatus consisted of a custom built box with a footprint of ~1 m^2 built with 20 mm aluminum rails and M4 screws with pre and post assembly nuts from MISUMI Group Inc. 6mm thick white, high density polyethylene (HDPE) modules were used as 'tiles' to construct and apply the box's surface. Sensors and actuators from IslandMotion™ were assembled using HDPE single modules of 120x120x6mm, which ensured the possibility of fastly and flexibly adapt the behavioral box to most possible configurations.

A Point Gray camera, Flea3 1.3 MP Color USB3 Vision (Sony IMX035) was used to monitor and track subject's behavior. The Bonsai framework (Lopes et al., 2015) was used to interface with the camera. A real time linux finite state machine (RTLFSM) and Bcontrol (behavioral control system) were used to program the task.

Data analysis

All data were analyzed using custom scripts developed with the Python programming language and relevant libraries (Python Software Foundation. Python Language Reference, version 2.7 and 3.5. Available at http://www.python.org).

2.4 Results

We found that the inactivation of OFC during this task yielded an impairment in the recovery of performance after a block switch as compared with vehicle sessions. This effect was visible at the single session level where rats tended to make more errors after a block switch in inactivated sessions as shown in **Figure 2**.

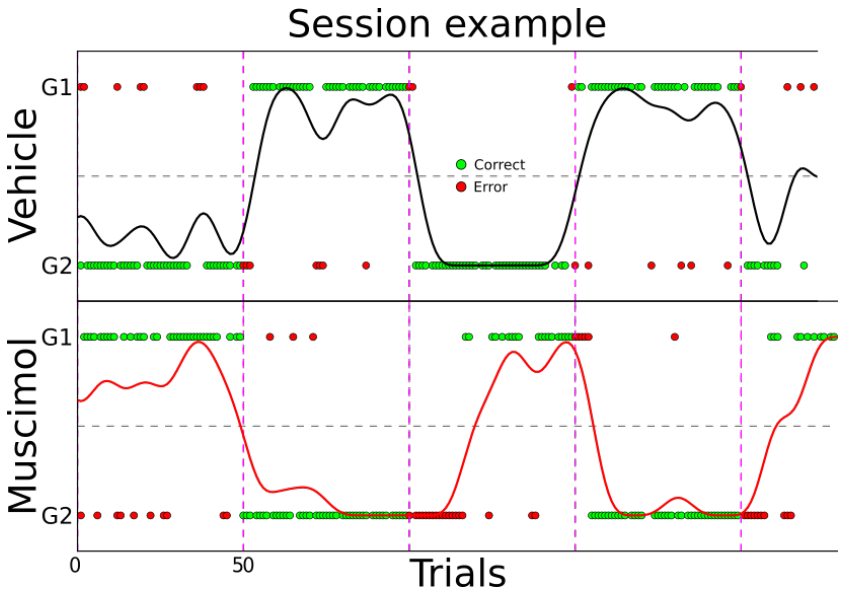

Figure 2 - Free choice spatial task raw
Raw data example session for one muscimol (bottom panel) and one vehicle (top panel) session. Red and green dots represent error and correct single

trials; black and red curves are the smoothed local averages using a Gaussian kernel.

The average performance aligned at a block switch (**Figure 3** left panel) for the comparison of the first vehicle session with the first muscimol session of an example rat also shows this difference as a slower recovery of performance after a block switch. This effect, although smaller, was still present in the average across sessions (**Figure 3** right panel).

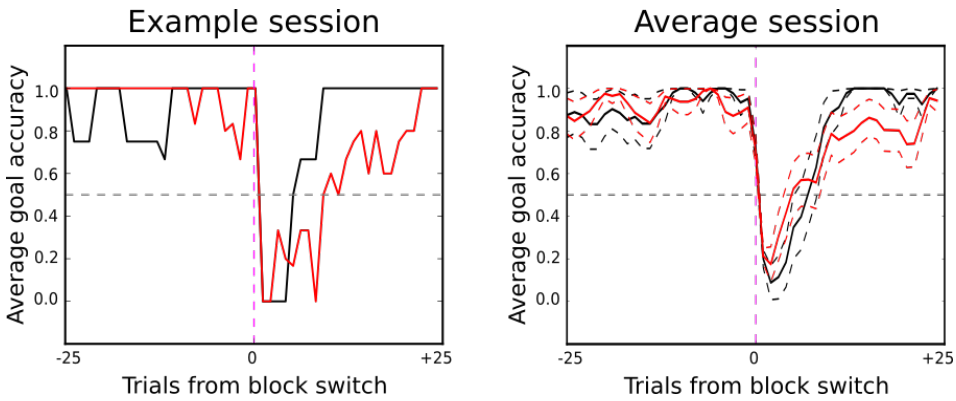

Figure 3 - Goal accuracy aligned at block switch
Average goal accuracy aligned at block switch for example session (left) and average session (right); black and red curves represent vehicle and muscimol sessions respectively; dashed lines are standard error of the mean.

This effect was only present in the initiation port condition. Comparing **Figure 3** its equivalent in the condition where the initiation port was not present (**Figure 4**) we find no effect of OFC inactivation if rats are allowed to stay at the rewarded port and switch whenever the water stops coming.

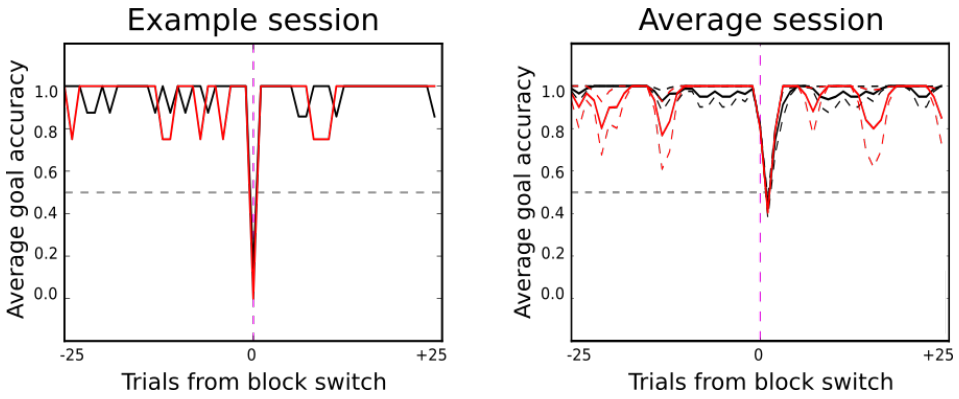

Figure 4 - Goal accuracy aligned at block switch - no initiation port
Average goal accuracy aligned at block switch for example session (left) and average session (right); black and red curves represent vehicle and muscimol sessions respectively; dashed lines are standard error.

To quantify this delay in recovery of performance we performed exponential fits (example in **Figure 5**) for all rats and all sessions using:

$$f(x) = -e^{-bx} + c$$

Where **b** was the free parameter and **c** was fixed to be 90% of the mean performance pre block switch. These fits yielded a consistent difference in rate between muscimol and vehicle conditions. Inactivated sessions almost always had a lower rate than vehicle sessions (**Figure 6** right panel).

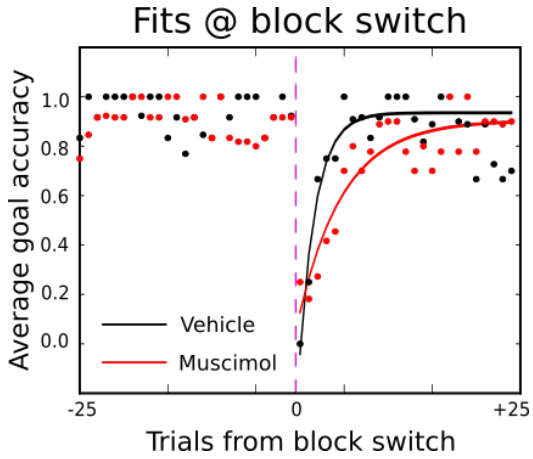

Figure 5 - Example fit
Example of exponential fits of accuracy data; muscimol sessions in red and vehicle sessions in red

This effect is greatest if one compares the first inactivated session with the first vehicle one (colored lines in **Figure 6** left panel) and diminishes with following comparisons. The only exception was in the case of one particular subject (cyan line in **Figure 6** left panel) which upon histological verification was found to have had an error in targeting mostly in D/V positioning of one of the cannulas (**Figure 7** left hemisphere). **Figure 6** right panel shows all the fitted rate values for all muscimol sessions plotted against the vehicle sessions. Most rats fall beneath the unity line indicating a lower fitted rate for muscimol sessions than for vehicle ones.

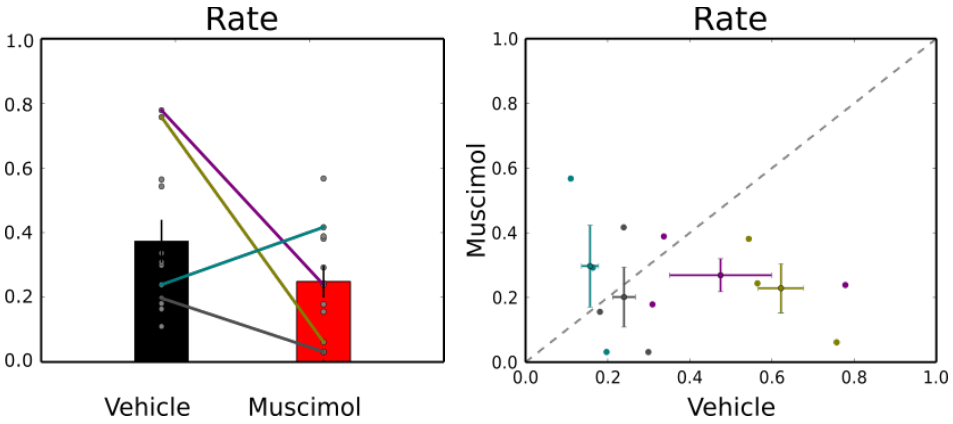

Figure 6 - Rate parameter comparisons
Left panel: Comparison of vehicle and muscimol values for the rate of the fitted exponential. Vehicle sessions in black, muscimol sessions in red. Colored lines underline the first session comparison for every subject. Right panel: Muscimol sessions fitted rate parameter as a function of Vehicle sessions, Colors represent individual subjects. Error bars are standard deviation; Markers with error bars are the average parameter value per subject.

Figure 7 shows the placement of the tip of the cannulas after histological examination. All anterio-posterior measurements were estimated from the ubiquitous Paxinos and Watson's The Rat Brain in Stereotaxic Coordinates, which unfortunately describes the average Wistar-Kyoto rat brain and not the Long-Evans strain. As a result of the slight differences between these two species although the aimed A/P (Anterior / Posterior) target was 3.72 mm after histological examination of the subject's brains we found that we consistently hit A/P 4.2 mm. Because this atlas shows the average brain, it is understandable that the further away from the center (interaural zero) one targets, the bigger the error. These coordinates were kept throughout the study.

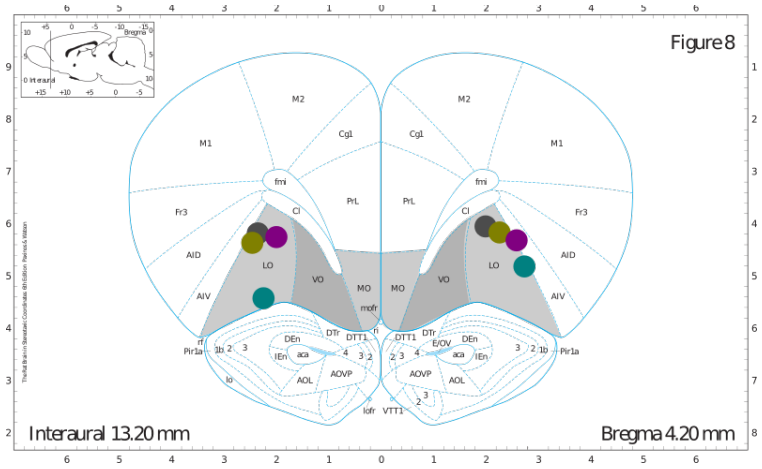

Figure 7 - Cannulae placement
Diagram of cannulae placement after histological examination, OFC target areas in gray; different colors represent different subjects.

2.5 Discussion

Two main conclusions can be drawn from this set of experiments:
We can conclude that inactivating OFC in these task conditions causes a decrease in performance, leading us to believe that OFC is necessary to implement the choice of where to go. The fact that this impairment is aligned to the block switches can be interpreted as evidence of an impairment in selecting between two different and opposing actions based on reward history and not a result of general apathy, confusion or other motor effects caused by the inactivation. This by itself would point to the reversal of reward locations as being a significant behavioral factor. However, although a reversal component is present and probably a factor, *a priori* we would expect to be outside of what is classically considered reversal learning as the reward's spatial reversals have been pre-trained and rats have extensive exposure to the task's statistics. The learning experiment we did not do, would have been to compare how

long animals take to switch location with or without OFC function. In this case however, the number of animals tested would have to be much larger in order to compare between rats and moreover, if this hypothetical experiment would have worked we wouldn't have learned anything new and if it failed we would have had no way of knowing why.

Secondly, comparing the inactivations in the two different conditions (with and without initiation port) we conclude that one of the relevant behavioral changes seems to be the movement from initiation port to reward port. The difference between the left panels of **Figure 3** and **Figure 4** is striking and seem to imply some change in the nature of the task. Just by making the animals move through space, by making them 'go' to the reward port ~1m away, animals seem to be entering a different state, maybe engaging the navigational system that cares about locations and trajectories and specifically goal locations.

Corwin et al. (1994) in fact, reported that electrolytic lesions to VLO (ventrolateral orbital cortex) impact learning allocentric but not egocentric navigation tasks. In this 23 year old study, animals were tested in two different tasks: the cheeseboard task and the adjacent arm maze task (Kesner et al., 1989); these tasks accentuate the importance of allocentric spatial localization and egocentric spatial lateralization respectively. Latencies to reach reward were significantly higher in VLO lesioned animals when compared with sham controls only in the cheesboard task and not in the adjacent arm maze task, leading the authors to conclude that OFC is necessary for allocentric navigation.

One possible explanation of OFC's involvement in allocentric navigation could be related to its involvement in the representation of spatial locations. In fact, to plan an allocentric action it is necessary to represent

the current location and the goal location (in world coordinates). In this sense the location of the reward could be construed as one of the outcome expectancies suggested by Schoenbaum (Schoenbaum et al., 2007) and consequently, inactivating OFC would prevent the goal location from being represented and thus impact any attempt of planning a trajectory in an allocentric reference frame.

Our results would make sense in light of this evidence if animals were somehow using an allocentric representation of the goal to reach the reward. Unfortunately, there is no way, using this task, of claiming that the results we observe are because of the animal's engagement in some type of allocentric strategy to reach the reward. If that were the case we could maybe conclude something about reward locations, trajectories or spatial representations in OFC. The best we can do to explain the observed impairment is speculate that maybe the reward ports were far enough from the animals' initiation position for them to use a representation of the box to guide their behavior. More concretely though, despite the LEDs at the reward ports lighting up to indicate the presence of a reward, the rest of the task was done in darkness which could bias for the use of a cognitive map (Tolman, 1948).

This task however, can be solved easily enough by an egocentric reference frame by just going to the left ot to the right of the box. This means that there is no behavioral way of distinguishing between reward locations and actions associated with rewards, i.e., between the use of an allocentric or an egocentric strategy from the rat, to guide its behavior. Going somewhere and doing the action that leads you somewhere are completely confounded. It is possible that rats don't use locations at all and if that were the case, the present task would not be

very helpful to help characterize goal location representations in the OFC as we set out to do.

At this point, our decision was to either increase the number of subject tested in this task or, alternatively, in order to be able to say something about locations, allocentric reference frames and actions, further modify the task in a way that would address our main concern resulting from this experiment i.e., have a clear behavioral distinction between actions and locations.

So, why did the rat cross the box? Well, to get to the water on the other side, obviously! But how did it get to the other side? Well this is, arguably, a somewhat more interesting question and the type of question science should focus on and be well equipped to answer. In an effort to try to explain *how* the rat crossed the box, we decided, maybe unsurprisingly, to continue our investigation by changing the behavioral task once more.

In the next chapter we'll introduce the results of the development of such task.

3 Odor Guided Spatial Navigation Task

In preparation

Author contributions: Bonacchi N., Poo C. and Mainen Z.F. designed the studies. Bonacchi N., Poo C. and Cruz A.S. ran the experiments. Bonacchi N. built the apparatus, analyzed the data and wrote the manuscript.

3.1. Chapter Summary

This chapter will introduce the odor guided spatial navigation task and characterize the behavior of rodents in this task.

3.2 Introduction

We concluded from the previous chapter that by making animals move through space to select a reward location on a trial-by-trial basis we are able to observe a requirement of a fully functional OFC in order to maintain performance after the reward changes place. We also formulated the hypothesis that these results would be a consequence of engaging a different brain mode, and because of Corwin et al. (1994) we think that, whatever this bdifferent brain mode might be, it might be related to the allocentric reference frame in which the rat is performing an action. In order to explore this further we should be able to design a behavioral task that is able to clearly distinguish between allocentric (based on reward locations) and egocentric (based on actions) reference frames.

We set out, once more, to modify the task in order to integrate an allocentric (based on locations) and an egocentric (based on actions) component that could be separable.

We decided that rats would need multiple initiation points, like in the cheeseboard task mentioned previously, but with less degrees of freedom in terms of possible trajectories. Our idea was to develop a task that would be compatible with the electrophysiological recordings of

neuronal activity, so restricting space in a way that made animals use a particular paths to get to point A to point B, seemed like an important feature to have.

A classical set of studies on response and place learning (Tolman et al., 1946, 1947a, 1947b, 1992; Tolman and Gleitman, 1949) as well as a more recent one by Young & Shapiro (2009) served as inspiration for the task. The task's apparatus would be similar to the apparatus used in these papers and the navigational context maintained. Importantly, however, by over training the animals, the learning component would be removed.

3.3 Materials and methods

All experiments and procedures were approved by the Champalimaud Foundation Bioethics Committee and the Portuguese National Authority for Animal Health, Direcção-Geral de Alimentação Veterinária (DGAV). Pre-handling, water restriction protocols used are identical to the previously described experiment in **Chapter 2**.

Behavioral Task

The odor-guided spatial navigation task uses an elevated plus maze that contrary to the classical elevated plus maze task has no closed arms as its arena (**Figure 8** at the end of this section). As all arms are open arms, the corridors have a small 25mm ledge around the rims in order to discourage rats from jumping to the ground. Located at the end of each arm there are 4 ports (one for each arm). Each port has a light emitting diode (LED) that upon poking by the rat, can yield an odor stimulus or a water reward. A trial begins when one of the LEDs turns on indicating to

the rat the location of the initiation port for that trial (**Figure 8a**). After poking for a uniformly distributed random delay of 0.1 to 0.25 seconds, one of 4 odors was delivered for a minimum of 150ms after which a tone would play indicating the trial was valid. The 4 odors (1-Hexanol, Caproic Acid, R-Limonene and Amyl Acetate) were associated with the 4 different possible reward locations (North, South, West and East). After poke out a 1 second dead time period existed. During this period nothing happened and only after this one second had elapsed one of two things could happen: Either all LED's would turn on (question trial) where animals had to make a decision based on odor information, or only one LED would turn on at the correct location of the reward (answer trial) and rats could presumably ignore the odor and just follow the light to get to the goal port. In either case, there was a delay of 0.4 to 0.6 seconds (uniformly distributed random draw). A poke in the correct location would yield a tone (80ms 3KHz) that coterminated with a 40µl water reward while a poke in an incorrect port would yield an error tone (80ms white noise burst). At this point a 4 to 6 seconds ITI would be enforced before restarting a new trial. A trial had to be completed in a maximum of 10 seconds to be considered valid otherwise the trial had to be restarted. In order to prevent involuntary initiation of trials the subsequent initiation port was never the port that had just been assigned as the reward port.

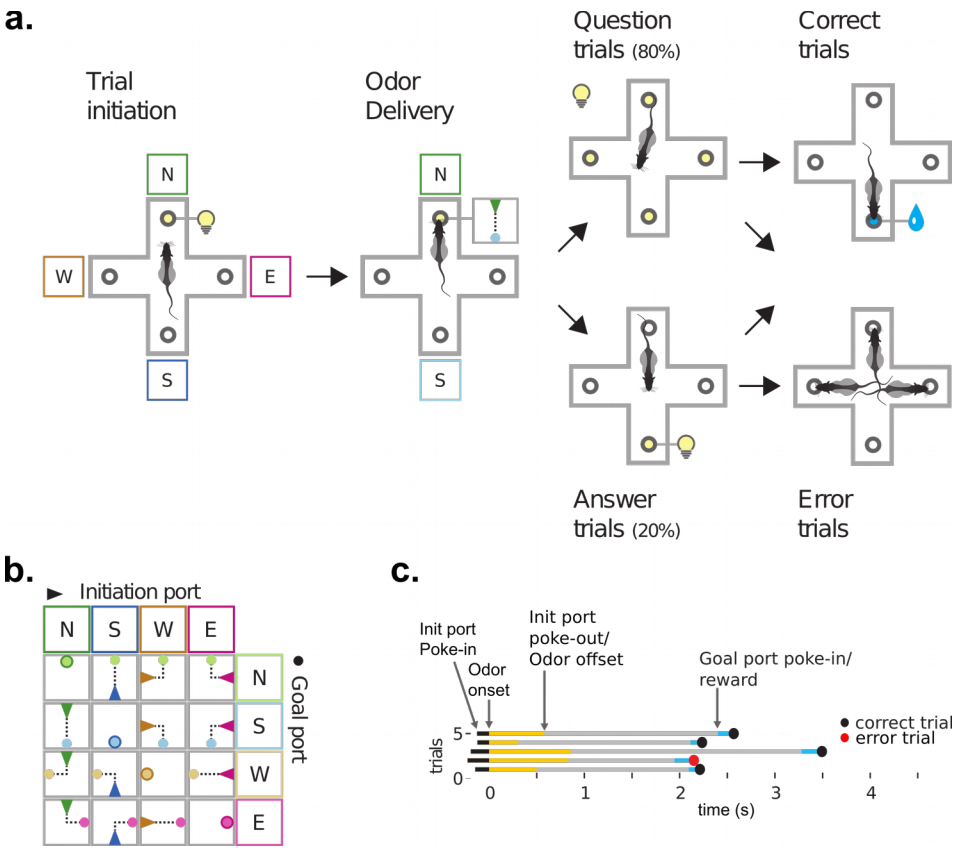

Figure 8 - Odor guided spatial navigation task
a. Task structure and timeline. **b.** Trial characterization matrix, all initiation/goal combinations. The plus maze is not represented in each square only the trajectory; arrows signify initiation port and circles represent goal ports. Colors represent different locations, lighter colors represent the goal locations/stimuli association. **c.** Time course example of trial structure aligned on odor onset.

Figure 8b depicts the full matrix of different trajectories or trial types by initiation port and goal port. The following analyses will respect color coding and be performed on columns or lines of this matrix, further grouping of trials consider the egocentric reference frame and will be explained further on. **Figure 8c** shows an example of five trials aligned on odor onset of one of the sessions.

Animal Subjects

A total of 38 Long-Evans male rats were used for the experiment. Data from all rats was used to optimize training protocol. 3 rats were submitted to the surgical implantation of an 8 Tetrode VersaDrive™ from Neuralynx© (results shown in **Chapter 3**). During both training and testing rats had *ad libitum* food and motivation was obtained by water restriction. Body weight was kept higher than 85% as well as other health indicators were monitored daily for the duration of the experiment.

Training Protocol

The maze was kept in dim light to prevent rats from jumping to the floor, but illuminated enough to allow the usage of wall queues located in the north (blue and white stripes) and south wall (red and white triangle).
After handling procedures the training protocol follows 6 main steps:

Exploration of the maze

Subjects were allowed to explore the maze. Water rewards were delivered manually as they approached any of the 4 ports. All port's LED's were turned on. These pre-sessions were not longer than 10-15 minutes and occured after having started the water deprivation protocol.

"Follow the light"

This stage introduced a 3KHz, 80ms tone that coincided with a water reward being delivered at the goal port. Only one of the goal ports would yield a water reward in each trial and was signaled by the turning on of the corresponding LED. The yielding port was pseudo-randomly assigned at the beginning of each trial. This step usually only lasted for one session, 30 to 40 minutes and had a water intake per trial of 140 µL. Criterion to next step ~100 completed trials.

"Wait for it"

The same protocol as the previous step was used, but gradually increasing the delay between poke in and reward delivery up to ~U(0.4, 0.6) seconds. If rats poked out before the delay elapsed the trial was considered invalid and had to be repeated after an ITI of ~U(2, 4) seconds. Rats underwent one, 1 hour, or two 30 to 40 minutes sessions per day. Water intake per trial was maintained at 100 µL. The criterion to be promoted to the next step was, ~160 valid trials and an invalid trial ratio under 0.3.

Introduction of initiation port

Reward delivery was now contingent on an 'init' poke, i.e., animals had to poke in an odor yielding port before collecting a reward. This step introduced the full trial structure and although the odors were already present the initiation poke time was kept lower and odor sampling duration (OSD) was not enforced. All pokes were signaled to the animals by turning on the LED present at the pokes. The odor was still not relevant for the decision. Initiation ports were never in the same location of goal ports so animals had to always move toward int just like they moved towards goals. This step effectively diminished the strength of the light-reward association by ~½. Water intake per trial was also maintained at 100 µL. The criterion to the next step was ~160 valid trials and an invalid trial ratio under 0.2. ITI's were increased to ~U(4, 6) seconds to reflect the increase in trial duration.

Introduction of minimum odor sampling duration (OSD)

Same as previous step but OSD is slowly increased to 200ms. Average learning period around 2 to 3 sessions. One hour sessions per day with

water intake per trial of 100 µL. Criterion to next step, ~160 valid trials and invalid trial ratio under 0.2.

Introduction of "Question Trials"

At this stage the animal should be doing the full trial structure, getting all the odors, but still the LED will always tell the animal what port has the water reward. Because the odor predicts which LED will turn on, rats should already know what odor maps to what location. This step introduces the solution probability and errors. Until this point animals could make invalid trials but not errors so the number of trials was informative of the span of the rat's experience with odors locations and rewards. The introduction of question trials can be seen as a test to the odor location association. With some (decreasing) probability all LED's would turn on after a successful initiation poke. Rats had to consequently make a decision based on the odor information and could not use the location of the LED as indicative of which one was the rewarded port. A poke in the correct location would yield a water reward, while a poke in one of the other 3 locations would yield an error tone (80ms white noise burst) and no reward. Errors for particular odors were monitored carefully and a correction loop would guarantee that no odor was 'unlearned'. If an animal had more than a threshold value of 3 errors for the same odor, the protocol switched modes and would only present that odor from all different locations until the error count would be back under the threshold level. Once solution probability was at 0.2 (80% probability of having to use odors to direct the location choice) and the performance was above ~75% water intake per trial was decreased to 60 µl. Rat was considered trained at this point. The whole training protocol lasts for ~2-3 months depending on subjects.

Testing apparatus

The testing apparatus consisted of a custom built cube with a footprint of ~1.7 m^2 and a custom built elevated plus maze at ~700mm from the ground (full measurements in **Figure 8**) The whole project was designed in Google Sketchup (now owned by Trimble™) and was built with 20mm aluminum rails and M4 screws with pre and post assembly nuts from MISUMI Group Inc. 5mm thick black acrylic custom laser cutted modules were used as 'tiles' to construct and apply the maze's surface. Sensors and actuators from IslandMotion™ were assembled using the acrylic 90x90x5mm single modules. The modular design of the maze allowed for fast customization and modification as seen fit during the development of the training protocol. A Point Gray camera, Flea3 1.3 MP Color USB3 Vision (Sony IMX035) was used to track the subject's behavior and a 800x600 CCTV IR camera was mounted at an angle to monitor the animals. The Bonsai framework (Lopes et al., 2015) was used to interface with the cameras. The task was designed and implemented using a RTLFSM and Bcontrol as previously.

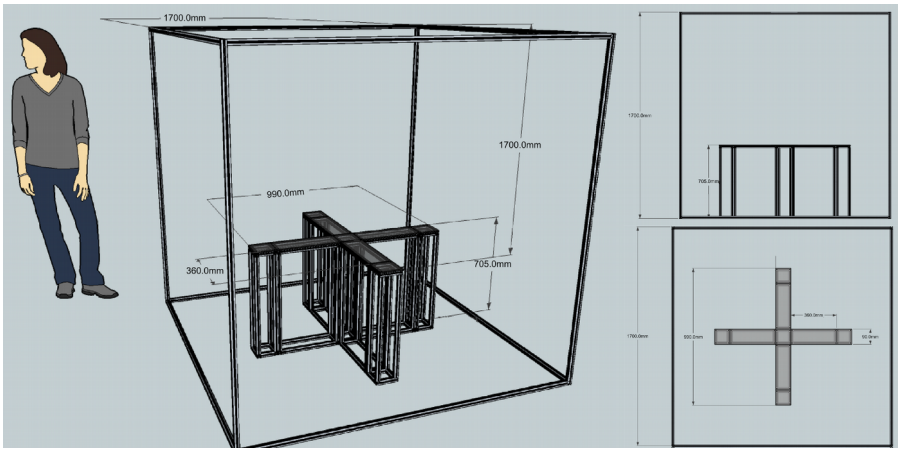

Figure 9 - Testing apparatus
3D representation of behavioral apparatus in left panel. Right panels represent a side view and a top view of the maze.

Data analysis

All data were analised using custom scripts developed with the Python programming language and relevant libraries (Python Software Foundation. Python Language Reference, version 3.5. Available at http://www.python.org).

3.4 Results

The main result of any new behavioral paradigm is always binary, either animals are able to perform the proposed task or not. Fortunately we find ourselves in the former category and not the latter. After this, the question becomes how they are doing what they are doing, if and how it may deviate from what was expected and finally, if it is interesting.

Developing and automating any behavioral task is usually a tortuous path often paved with an extensive chain of tweaking of parameters, changes in approach, tests, mostly failures and overall general frustration, the bigger the complexity the bigger the probability of failure. It is easy to fall in an optimizing spiral trying to avoid all the possible failing points, prematurely optimizing what later one discovers to be unneeded parameters and options.

What follows is the characterization of the behavior of rats in the odor guided spatial navigation task, all of the analyses except when specifically mentioned, show the behavioral profile from data of the 3 rats that underwent tetrode drive implantation that will be shown in **Chapter 3**.

The first thing we looked at was performance. Performance was calculated as the proportion of rewarded trials over valid trials. After training the animals (training protocol in the Methods section) the performance of all animals was found to be stable across all the recording sessions as shown in **Figure 10a**. Although one of the rats seem to have plateaued at a slightly lower performance than the other 2 subjects, all rats performed well above chance level.

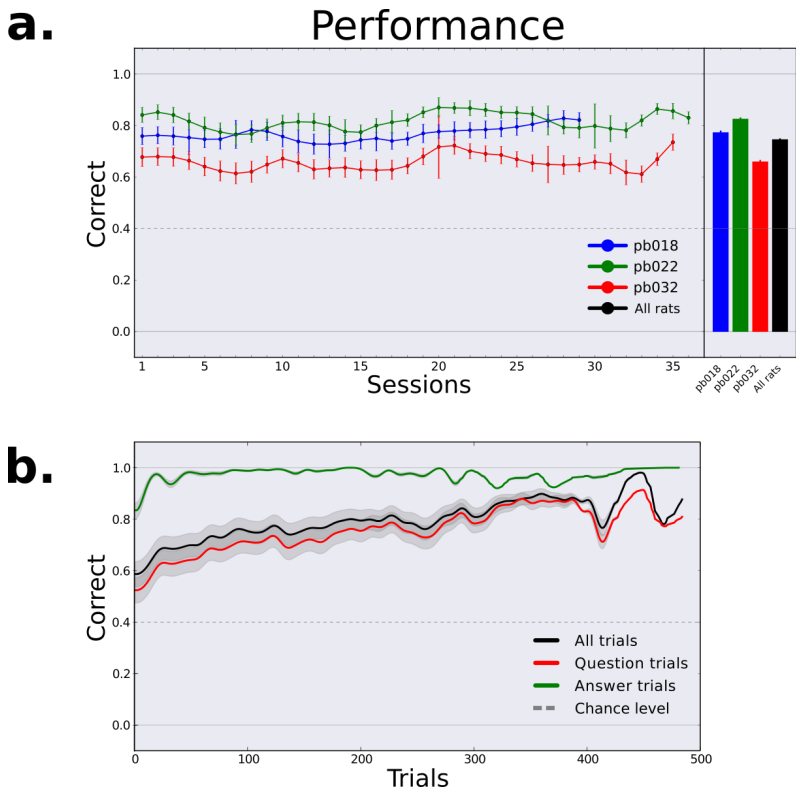

Figure 10 - Performance
a. Average performance across sessions (left panel) and global averages (right panel); error bars are standard error of the mean. **b.** Performance of the average session of an example rat as a function of trials. Black, red and green curves are all, question and answer trials respectively; standard error of the mean is represented as a shaded gray area around the curves. dashed line represents chance level.

Chance level for all the sessions was 0.4 as indicated by the dashed line in **Figure 10**. Normally, a 4AFC would have a 0.25 chance level, however on each trial the rat had a 20% probability of getting an answer trial. We conservatively considered the answer trials as always being correct, which is approximately what we observe in the green curve in **Figure 10b**.
The chance level (**cl**) was calculated using:

$$cl = sp + (1 - sp)/4$$

where **sp** is the solution probability (or the probability of getting an answer trial).

We decided next to look at performance in different subsets of trials. As this task has 4 initiation ports, 4 odors and 4 goal ports the obvious first step would be to compare performances considering these groupings of trials. These groupings are summarized in **Figure 8b** where initiations correspond to the columns and odors (or goal requests) correspond to the lines. Analysis of performance from initiation port, odor or goal choice did not reveal any bias, indicating that animals treat all of these equally (**Supplementary figures 1 and 2**).

Egocentric analysis of performance in **Figure 11** however, revealed a strong bias toward back actions. This analysis, groups trials according to the 4 actions rats could perform: Left, Right, Front and Back, so a trial that, for example, starts in the **S**outh port and ends in the **N**orth (**SN**) will be considered a Front action trial and grouped with all its corresponding trials that started in all the different locations. The complete set of Front trials would be: **NS**, **SN**, **W**est **E**ast and **EW**. Back trials are trials that initiate and terminate in the same location (e.g. **NN** or the diagonal in

Figure 8b.), while an example of a Left and Right trial would be **SW** and **SE** respectively.

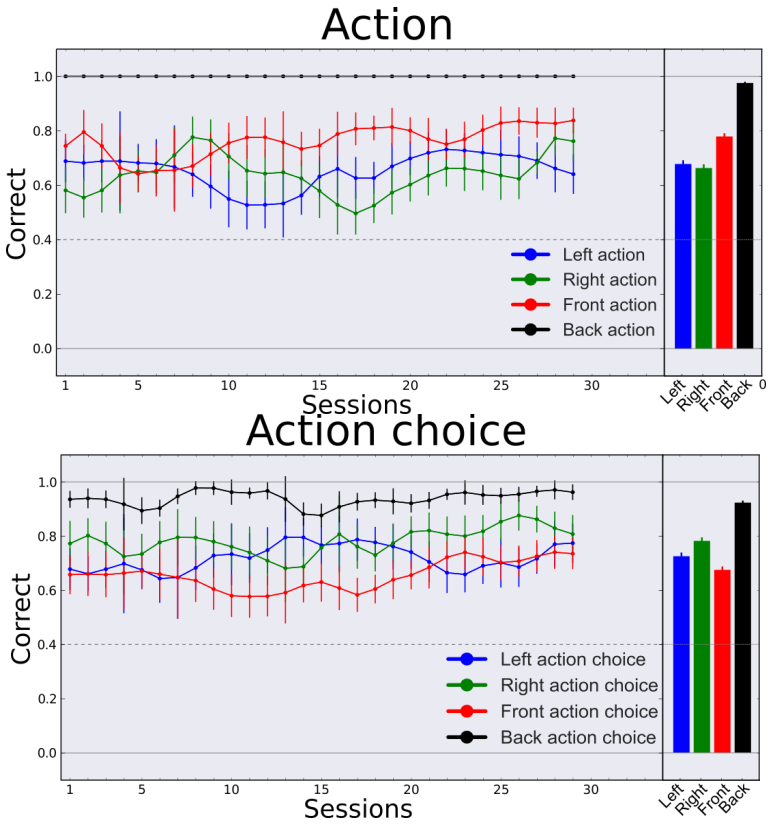

Figure 11 - Action performance
Average performance for an example rat across sessions split by action. Vertical bars are standard deviation. Top panel action performance (split by trial type), bottom panel action choice proportion of correct trials (split by animal's action choice).

All rats demonstrated an almost perfect performance in Back trials (**Figure 11**, top panel) and also a slightly higher performance for front trials although not in all animals. Looking at the animal's choice behavior (**Figure 11**, bottom panel) this bias, although smaller, still persists. The difference between action trials (top) and action choice trials (bottom) is that the former classifies trials according to the initiation port and odor

delivered while the latter groups the trials according to the choice behavior animals performed. This distinction becomes more important when more than a binary option is available to the animal. In this particular case, correct and error trials need not be symmetrical and the probability of correct given an action is not the same as the probability of an action given correct trials. An error trial has now become not an opposing choice but just one of a set of available choices to the animal. Consequently, it becomes important to look more closely at the error trials that, although much less in number, they can now reveal another source of bias or choice preference.

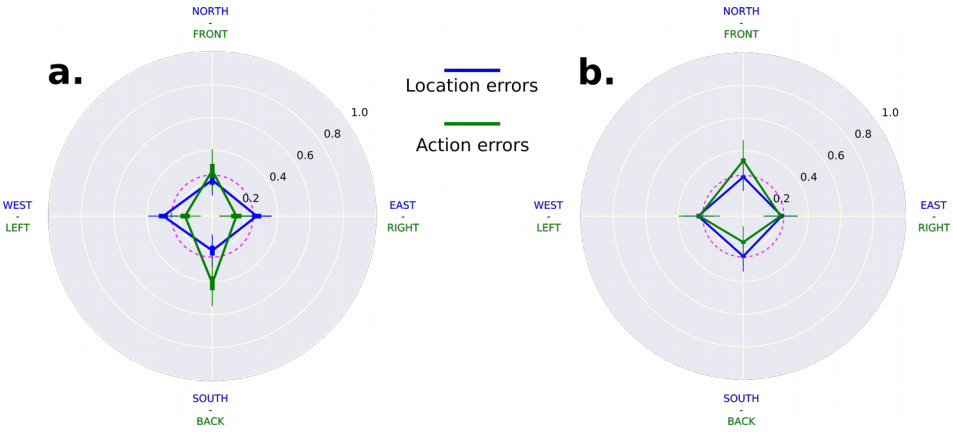

Figure 12 - Error trials
a., **b.** Error proportions for different locations (blue) and actions (green) for example rat before de-biasing (**a.**) and after (**b.**). Thick error bars represent standard error of mean; thin error bars are standard deviation; chance level shown by purple dashed line.

Figure 12 shows error trials for one rat after having normally completed training in panel **a**. and for the same number of sessions after undergoing a de-biasing regime in panel **b**. De-biasing training consisted in the removal of Back trials for 13 sessions (about 2 weeks of training).

Errors towards the 4 different cardinal locations (blue lines) was close to chance level (purple dashed line) and not affected by the action de-biasing. Action errors however (green lines), show a reduction of Back errors and a slight increase in Front, Left and Right errors. While Left and Right errors settle at chance level, Front errors seem to have accumulated the decrease in back errors probably indicating a secondary preference towards Front actions.

Further investigations into Back trials revealed them to be somehow different from the other trial types. For one they are right there, meaning that the animal need not move to make a decision which would impact movement time.

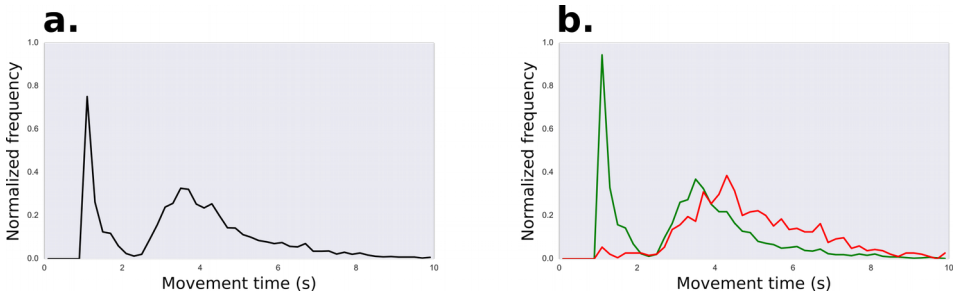

Figure 13 - Movement time distributions
a. Movement time histogram for example rat, all sessions; **b.** Same as a. split by correct (green) and error (red).

Movement time histograms show a two peak distribution for all rats. **Figure 13** shows movement time histograms of an example rat that had a 1 second delay period between poking out of the initiation port and being able to poke in the goal port. Panel **b.** splits trials in correct and error trials. The first peak appears to be present only in the correct trials, furthermore, average movement time for error trials appears to be

slightly slower than correct ones although this difference is not consistent across rats.

The pronounced peak around 1 second reflects the fact that in Back trials animals do not have to move toward the reward port. This can be clearly seen in **Figure 14**, specifically in the top panel.

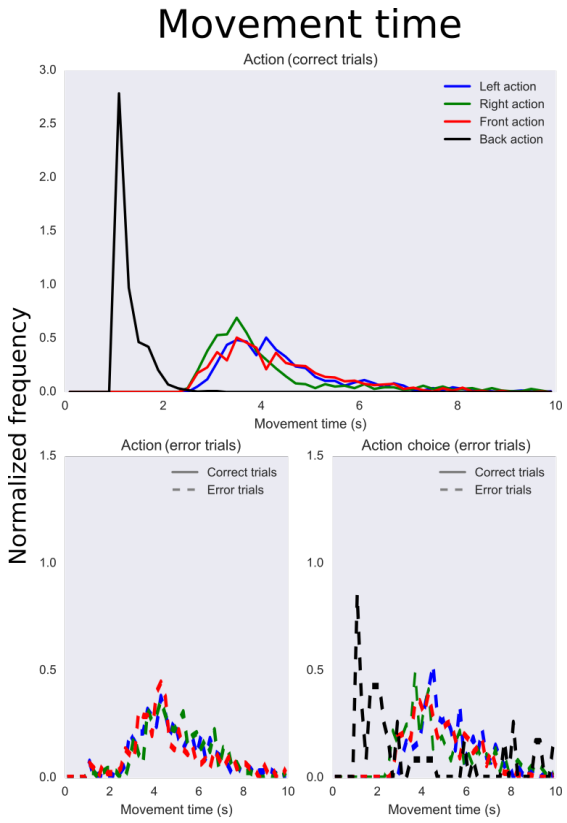

Figure 14 - Movement time distributions by action
Normalized movement time histogram for example rat split by different actions. Correct trials in top panel; error trials in bottom panels for action trials (left) and actions choices (right).

Distributions for action trials and action choice trials are exactly the same in correct trials, hence the presence of only one top panel. Bottom panels of **Figure 14** show movement time distributions for error trials for

both action trials and action choice trials. The absence of a black curve in the bottom left panel is due to the total absence of errors in trials where the back action was required, the full figure with all movement times can be found in **Supplementary figure 3**.

Furthermore, as also expected, Back trials where the rat does not have to move to reach the goal port, have a lower velocity profile than other trials as shown in **Figure 15**'s green line.

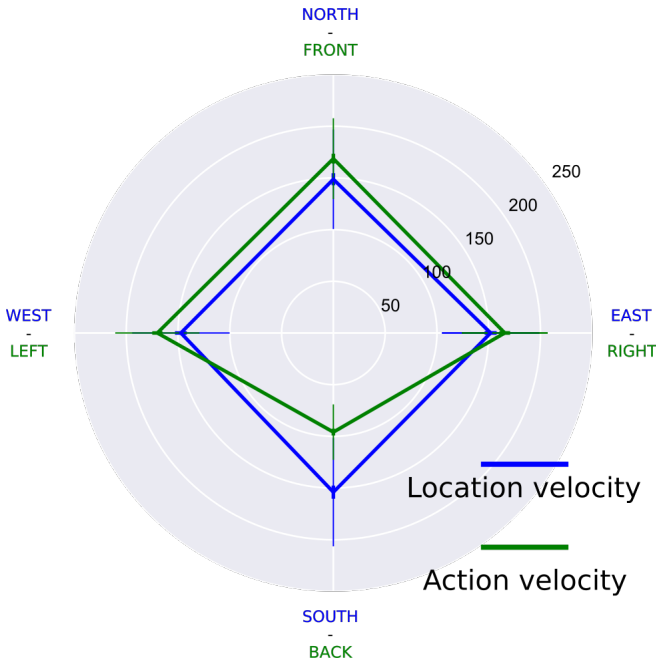

Figure 15 - Velocity
Average velocity in pixels per second for trials towards the different goal locations (blue) and trials that required the different actions (green); thick and slim error bars are respectively, standard error and standard deviation.

Velocity towards all other actions and goal ports was found to be similar for each location or action.

Finally we performed a bias analysis to ascertain the impact of receiving or not receiving a reward at a particular location or after having performed a particular action. We find that receiving a reward at a particular location biases the choice probability of animals towards the same location, however not so for actions. Furthermore, unrewarded locations (errors, where reward is omitted) do the inverse biasing the animal against the unrewarded location but not for unrewarded actions.

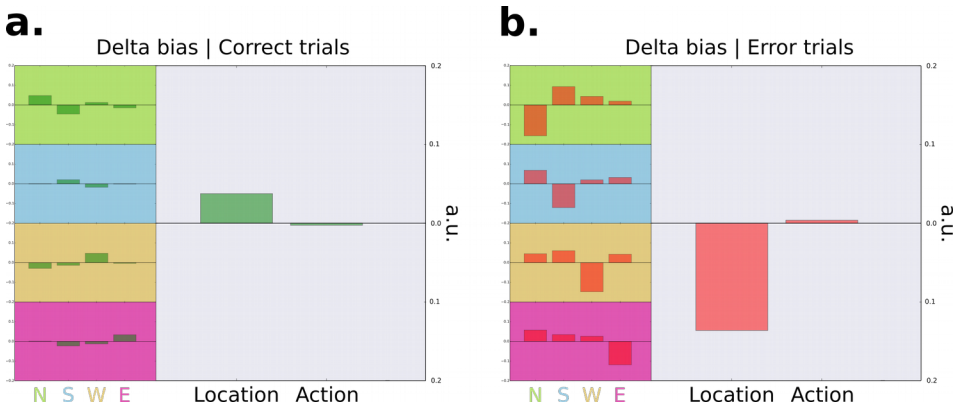

Figure 16 - Trial history bias
a., **b.** Trial history bias analysis of actions and locations conditioned on current trial outcome, location and action. Left column shows only location analysis and current trial location is indicated by background colors. Main panels in both figures show delta-bias towards the same location or same action conditioned on current correct (**a.**) or error (**b.**) trials.

This analysis used ~40,000 trials from 6 different rats in equal task conditions. For location bias, rewarded (**Figure 16a**) or unrewarded (**Figure 16b**) trials were selected by goal choice location and trial outcome. The proportion of trials towards the 4 locations in the previous and subsequent trial was calculated and subtracted. The result, is the change in probability of choosing a particular location in the next trial as

a consequence of the outcome of the current trial. We can express this as:

$$P\left(Loc_{(t+1)}|Loc_t, R_t\right)=P\left(Loc_{(t+1)}|Loc_t, R_t\right)-P\left(Loc_{(t-1)}|Loc_t, R_t\right)$$

Where **t** is current trial; location $Loc\in\{N, S, W, E\}$ and reward $R\in\{0,1\}$.

The main panels of **Figure 16** show the change in probability of choosing the same current location, conditioned on reward:

$$P\left(S_{t+1}|R_t\right)=\sum_{i}^{Loc}\Delta P\left(Loc_{it+1}|Loc_{it}, R_t\right)$$

Where $S_{t+1}=1\ if\ Loc_t=Loc_{t+1}$ and $S\in\{0,1\}$ show the probability of visiting the same location if this location was rewarded or not. The same analysis was performed for actions by substituting Loc for $Act\in\{L, R, F, B\}$ in the adjacent bar for each plot.

3.5 Discussion

The version of the odor guided spatial navigation task here presented was but one of a number of different configuration of parameters and rules that were tried over time. Initially, rats were trained both with an allocentric rule, like in the presented results, and also using an egocentric rule where odors were mapped to the 4 different actions that led to reward. Incipient versions of the task also managed to train rats to switch between egocentric and allocentric strategies during the same session albeit only two different options were available (data not shown),

and because of only these two options the strategy used was not discriminable.

Rats are amazing bug finding agents, both at the conceptual level when this manifests itself in some sort of game or task with which they can interact, as well as actually being very good code debuggers, if something is amiss in your task structure, they will eventually find it.

Tolman, had already reported in the 40's that response learning was slower than place learning (Tolman et al., 1946) and our own experience also confirmed this. The egocentric version of the task, alone took approximately 3 months to train and to further train animals to switch between strategies took another extra month to two months.

Several non optimal conditions made us reassess the plan of recording using the two different reference frames. Firstly, the elevated temporal cost of training an animal was less than optimal. Secondly, the behavior with the 2 different strategies at the same time also seemed to have morphed into some sort of hybrid strategy, and further troubleshooting would have been required in order to be able to claim that animals were actually using both strategies. The only caveat at this point would be that cells that would fire prospectively for location at odor delivery would only be separable from cells that responded to the odor themselves by looking at error trials, significantly reducing the statistical power of the task. In light of these issues, we decided that the drastic reduction in training time was reason enough to drop the egocentric version of the task. Optimizing the training protocol was also a never ending continuous activity. Although I'm positive the task could yet be improved in terms of training time, the 'final' training time of two to three months, seemed comparatively a major improvement.

We presented here a new operant task where olfactory stimuli are mapped to outcome locations effectively defining an allocentric navigational context in which animals are able to achieve good performance profiles (**Figure 10**). The average number of ~200 trials presents a low proportion of errors and trial trajectories are comparable because of the restricted corridor of the maze. In light of this, we concluded the task was adequate for electrophysiological recordings of neuronal activity.

Nonetheless, the task space is quite big if one considers the 16 different trajectories (**Figure 8**), especially given the fact that this space can further be doubled if one considers "question" and "answer" trials and doubled again when considering correct and error trials – resulting in a whooping 64 different trial types. One possible solution to this ever expanding strategy would be to reduce the number of trial types. In fact, in light of the strong bias towards Back action trials found (**Figure 11**), it could be possible to remove these trials altogether, effectively reducing the trial space. Only trials where the animal has to go to a different location to collect the reward would thus be considered. If this were the case this would reduce the trial space to a worst case scenario of a more manageable 48 different trial types.

Another argument towards the removal of Back trials is that, they seem altogether a different type of trial. The overall strategy rats seem to be using is a 2-step strategy, i.e., when first initiating a trial, they 'look' for the odor that would correspond to a Back trial at that location, next, if another odor is delivered, they turn around and decide where to go. This is reflected in the fact that errors seem to be biased towards these trials as we saw in the error analysis of **Figure 12** that demonstrates a prevalence of Back action errors. Moreover, the performance profile for

Back trials is almost perfect. This means that these trials should have an experienced value that is higher than other trial types. Moreover, they also should have a negligible movement cost, and be less temporally discounted, as they are immediate, proximal options. This proximity variable could actually help explain why rats make mistakes in our task given that the uncertainty about the stimulus should be low.
In fact, the stimuli we used are pure, well distinguishable odors at reasonably high concentrations (10^{-1}), the reason why rats make mistakes in this task is probably related to this asymmetry between distal and proximal options. If animals take into account the relative cost of each option, there might be a conflict between optimizing reward intake overall and reducing the cost of particular trials; this might result in rats making Back errors more often. If this were to be the case, we would be able to observe a hierarchy of errors that should be correlated with less "expensive" action errors, which is exactly what we see in **Figure 12**, where the relative preference for Back, Front, and Left and Right errors reflects the cost of the four different actions. The cost of performing different actions could be calculated using both temporal and spatial criteria and would presumably correlate with the error preference showed. Back actions are both temporally (**Figure 14**) and spatially faster, and should consequently sit at the top of this hierarchy.

So although we see locations and not actions as the target of inter-trial updating from our trial history bias analysis shown in **Figure 16**, and despite our allocentric reference frame, actions seem to still be a relevant behavioral dimension within trials. What is not clear is why the comparatively cheap cognitive cost of remembering the stimulus (or, more precisely, the location the stimulus points at) does not seem to be taken into account. Maybe there is a slower modulation on the basis of a memory cost (Fagan et al., 2013) that depletes over multiple trials and

not only in our task but in all behavioral tasks a proportion of error is just a function of 'task engagement' or memory depletion.

The inactivations in VLO performed by Young and Shapiro's 2009 study suggest a special role for location/allocentric coding rather than action/egocentric reference frames. This study had some difference to ours:

1. It had less options, only 2 outcome locations were available, North and South. East and West were used as initiation arms.
2. The authors were interested in learning, specifically focusing on reversals (of actions and locations) and strategy switches.

The task was manual and not automated, implying a low number of trials as they had to physically remove the rats from the maze to a platform in order to reset the trial.

Their results show that VLO neurons are important for location reversals but not for response/place strategy switches. Meaning that animals trained to go North for a reward and presented with a South reward, would have an impairment when OFC was inactivated as compared to control animals; whilst animals trained to go North to collect a reward and presented with a strategy switch (meaning the reward was always present after a Left turn) were indistinguishable from controls. Unfortunately, no results were reported about the action reversal side of the equation. The only thing stated in this study about action reversals is consistent both with the Tolman observation mentioned previously, and with our previous statement about response learning:

"*Only spatial reversals – changing between North to South or South to North Goals – were used because animals would not reliably perform response reversals in a single testing day*"

In **Chapter 2** we reported an effect of OFC inactivation in our free choice spatial task. We proposed, based on previous reports from Corwin et al. (1994), that rats might be engaging the allocentric navigation reference frame as an explanation of the observed impairment. Unfortunately, we did not perform inactivations in the task here presented. The clear navigational context as well as the hypothesis proposed would force us to predict a big impairment in all but Back trials. This would be due to the action bias which drives Back actions in contrast to the location bias that we believe drives the behavior of rats in other trials.

Finally, we believe this task addresses the main problems of previous location coding studies in the OFC; i.e., It has a bigger physical footprint that increases the salience of different locations; it is performed in the context of a particular reference frame that further biases the requirement of locational information; it can clearly distinguish between actions and locations (multiple locations can be reached performing the same action and multiple actions lead to the same location); and most importantly we've shown evidence that rats seem to 'care' more about location (rather than actions) when we compare biases after rewards and omissions (**Figure 16**).

We decided to record for OFC neurons in this version of the task. In **Chapter 4** we will report the results from the recording of OFC neurons during the odor guided spatial navigation task.

4 Odor Guided Spatial Navigation Task OFC recordings

In preparation

Author contributions: Bonacchi N., Poo C. and Mainen Z.F. designed the studies. Bonacchi N. and Poo C. ran the experiments. Bonacchi N. built the apparatus, analyzed the data and wrote the manuscript.

4.1 Chapter Summary

This chapter will report the results from the recordings of neurons in the OFC during the odor guided spatial navigation task.

4.2 Introduction

Now that we have a task where outcome locations are important decision variables, we set out to record from neurons in OFC to identify units that could carry information about the location of an outcome but not the action, and *vice-versa*. We mentioned already, in the introduction, that the main problem of previous studies by Feierstein and Roesch (Feierstein et al., 2006; Roech et al., 2006) was, besides the reduced size of the behavioral box, the absence of a properly defined navigational context that would allow to separate egocentric representations of actions from allocentric representations of spatial locations, consequently, in both studies, these two features were unfortunately confounded. Moreover, the action selective cells found while animals were moving towards a particular location could also be, in principle, location selective cells (see introduction of **Chapter 2**) if one takes into account the relative distance of the pokes in the behavioral apparatus used in this study. One orthogonalization that was carefully made by Feierstein and colleagues, however, was the separation of OFC cells that represented stimuli or stimulus properties from goal cells, that were selective for location / direction.

Our odor guided spatial navigation task addressed both the size and the navigational context issues by design; egocentric directions or actions

and allocentric locations are separable thus allowing to assess the contribution of neurons to one or the other. If indeed we do find global overall independent modulation of location, i.e. cells that during response fire selectively for different locations (NSWE) independently of the egocentric actions that is being performed, we should be able to asses the relative importance of outcome locations (as well as actions) for the firing rate profile of neurons in OFC.

Considering previous results, we had several *a priori* expectations about what we could find in terms of cell selectivity:
Odor and reward selective neurons that would fire differentially to different stimuli and rewards independently of the location where these stimuli are presented;
Location selective units, whenever a rat is at a particular location;
Action selectivity whenever an animal is performing a particular action;
Prospective action or location selectivity, i.e., cells that would fire in a selective way prior to the arrival at a particular location or prior to the enactment of a particular action (resolving the location/direction confound of previous studies);
Allocentric direction, i.e., cells that could divide the allocentric map in two or more parts, independent from animals location or facing direction.

On the other hand, considering our behavioral results presented in the previous chapter, we would expect to find an over representation of action selective neurons that responded to Back trials when compared to the remaining actions and, because rats seem to care more about locations than actions, we would expect to find comparatively more location than action selective cells.

4.3 Materials and methods

Surgery

All drive implantation surgical procedures were carried out under aseptic conditions. Anesthesia was initiated and maintained with isoflurane inhalation at ~2% (1.5-3%) in O2, at a flow rate of 0.5 lpm. Adjustments in isoflurane percentage were made according to paw withdrawal reflex during the surgical procedure. After craniotomy, 32 channel, 8 tetrode drive Versa drive 8, (Neuralynx Inc.) was stereotactically implanted in the left hemisphere targeting OFC (AP: +3.72, ML: +/-2.5, DV: +4.2 from skull surface. The Rat Brain in Stereotaxic Coordinates 6th Edition (Paxinos and Watson, 2006) was used for targeting. Twelve stainless steel bone screws (PlasticsOne) and dental acrylic (Kerr, TAB 2000) were used to hold the implant in place. Recovery procedures were the same as in **Chapter 2**.

Histology

In order to verify the ultimate location of the tetrodes, electrolytic lesions were produced after the final recording session (30 μA cathodal current for 3 sec per channel). The next day, rats were then deeply anesthetized with pentobarbital and perfused transcardially with 4% paraformaldehyde. The brain was removed from a skull, stored in 4% paraformaldehyde, sectioned at 50 μm. Every slice was stained with Cresyl violet solution with a standard Nissl staining protocol to observe the sites of electrolytic lesions. Drive implants and tetrode placements were assessed and **Figure 17** shows the tetrode placement for each rat maintaining the color code for individual animals shown in **Chapter 3**.

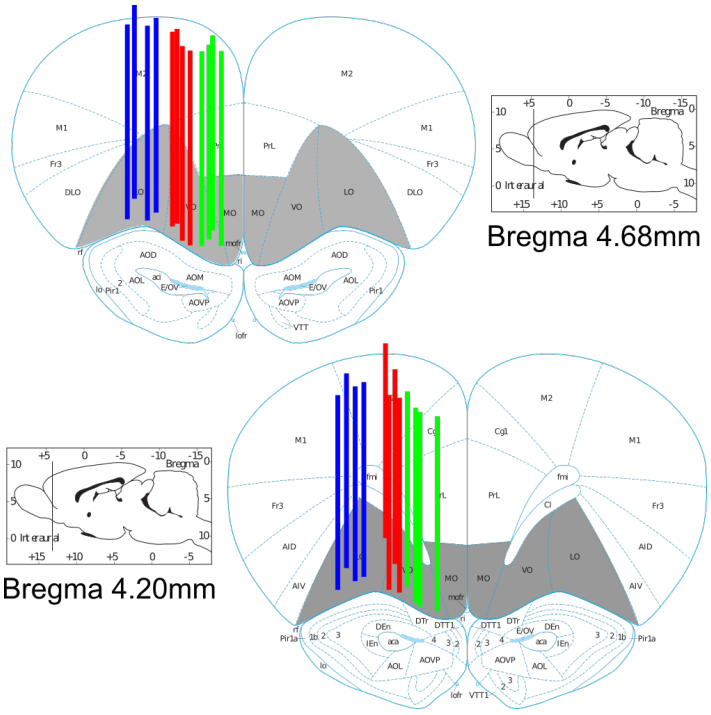

Figure 17 - Tetrode placement
Coronal slices from rat brain atlas (Paxinos and Watson, 2006). Different colors represent different rats.

Drive, Gold plating and Recording system

The 32 channel, 8 tetrode drive used was a modified commercial microdrive (Versa drive 8, Neuralynx Inc.) built with 25µm nichrome coated wire from California Fine Wire Co. Gold plating and impedance test were made with a Nano-Z (Neuralynx Inc.) to 0.2-0.5 MΩ impedance at 1KHz. Tetrode depths were adjusted before or after each recording session in order to sample an independent population of neurons across sessions. The locations of tetrode tips during each recording session were estimated based on their depth and histological examination based on electrolytic lesions and the visible tetrode tracks. Electrophysiological recordings were performed with a Cerebus™ System by Blackrock

Microsystems®. Neural and behavioral data were synchronized by acquiring time-stamps from the behavioral system along with the electrophysiological signals.

Event detection & Clustering

Custom software packages for event detection, semi-automated and manual clustering done using SpikeDetekt, KlustaKwik2, KlustaViewa and phy (Rossant and Harris, 2013; Pachitariu et al., 2016).

Data analysis

All data were analised using custom scripts developed with the Python programming language and relevant libraries (Python Software Foundation. Python Language Reference, version 3.5. Available at http://www.python.org). The only piece of commercial software (MATLAB R2014b, The MathWorks Inc., Natick, MA, 2000) involved in the analysis was used solely to strip the header and footer of the proprietary format (*.nsx) raw data files that the Blackrock Microsystems® Cerebus™ recording system yielded.

4.4 Results

The following analysis includes 132 processed units (of ~230) recorded from 3 rats. Most of the data shown here belongs to pb018 (~75 units) and the rest equally distributed between the remaining two rats.

During the description of the results as well as the discussion we will refer to ‘epochs’ and ‘features’. epochs are the 6 specific moments or epochs during the trial we focused our analysis on, these are shown in **Figure 18g**. Briefly: ‘init_in’ refers to initiation port entry; ‘odor_on’ to

odor onset; ‘init_out’ is initiation port exit and end of odor presentation; ‘lights_on’ is the end of the delay period after initiation poke out before the animal knows if the trial is a question or an answer trial (as defined in **Chapter 3**); ‘goal_in’ refers to goal port entry and ‘tone_on’ corresponds to the reward delivery or omission (in case of a mistake) that is always accompanied by a tone.

In terms of features, we considered task features to be the different trial and behavioral variables presented or performed by the rats: ‘init’ initiation location of the trial; ‘odor’ the odor delivered to the animal which is completely correlated with the requested goal location; ‘question’ is the type of trial (question or action) presented to the rat; ‘action_choice’ is the egocentric action performed by the rat; ‘goal’ refers to the goal location chosen by the animal and finally ‘correct’ refers to the outcome of the trial, i.e., rewarded or unrewarded (error) trials.

General modulation

Global firing rate was assessed by z-scoring the average firing rate of every neuron aligned on each of the 6 epochs and plotting the population PSTH. **Figure 18** shows heat plots for all recorded neurons and respective population PSTHs.

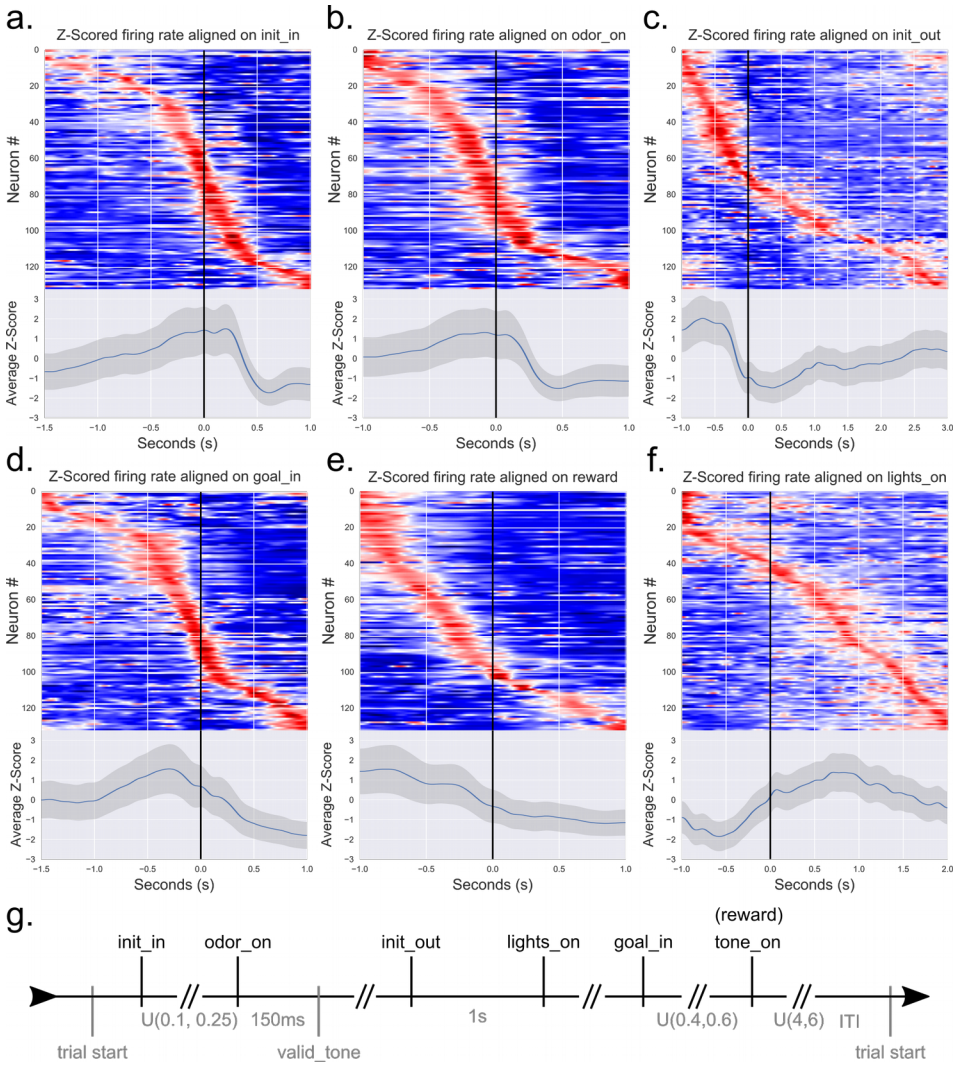

Figure 18 - Average population responses

Average z-scored responses for all neurons aligned at initiation port entry (**a.**), odor onset (**b.**), initiation port exit (**c.**), goal port entry (**d.**), reward onset (**e.**) end of dead time period (**f.**). **g.** Task timeline: Initiation port entry (init_in); Odor onset (odor_on); Initiation port exit (init_out); End of dead time period (lights_on); Goal port entry (goal_in); Reward delivery (tone_on). All heat plots are sorted by peak firing rate of neuron. Bottom panel are the average population peri-stimulus time histogram (PSTH). Error bars are standard error of the mean (s.e.m.)

Receiver operator characteristic (ROC) analysis was performed comparing the distributions of average firing rates in a 500ms bin pre and post alignment. Significant neurons were selected at a 95% confidence interval by comparing the area under the curve (AUC) to the distribution of AUCs generated by shuffling the labels of the neurons in the dataset 500 times.

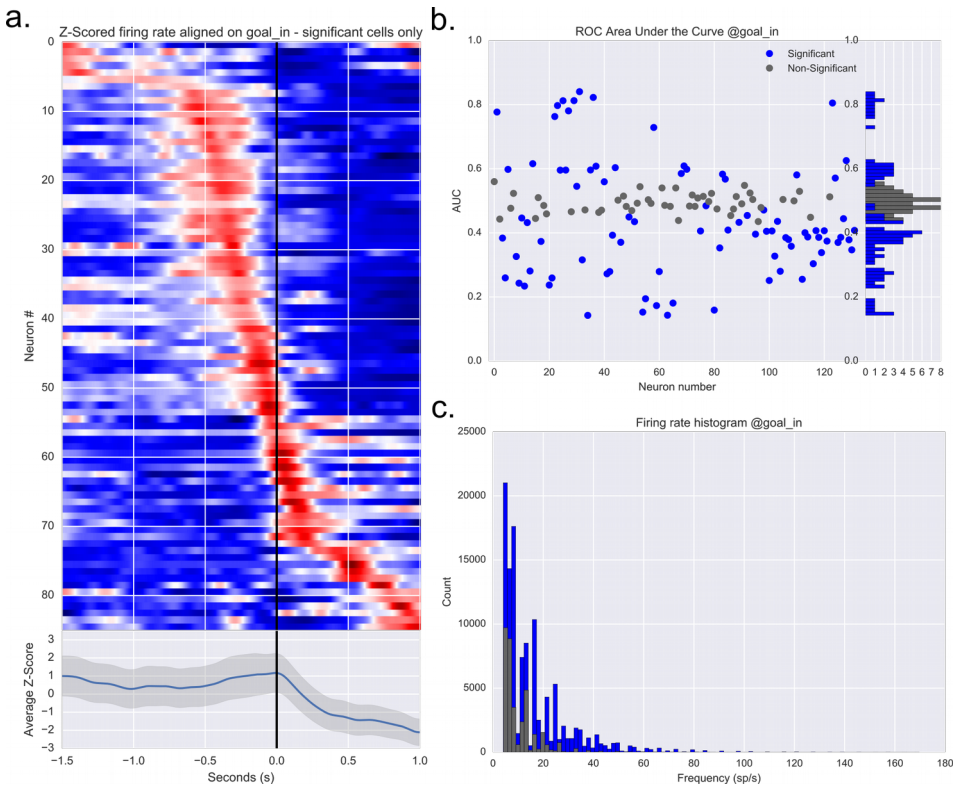

Figure 19 - ROC @ Goal port in
Significant cells after ROC analysis at goal port in (example event). **a.** Z-scored firing rate and population PSTH (bottom panel) of significantly modulated cells aligned at goal port entry sorted by peak firing rate. **b.** AUC values and distribution of all cells; gray values are rejected cells. **c.** Firing rate histogram for selected and non-selected cells, colors are same as in **b.**

Figure 19 shows an example event of the ROC analysis output. The same process was used to select cells aligned at all the relevant task epochs (**Figure 20**).

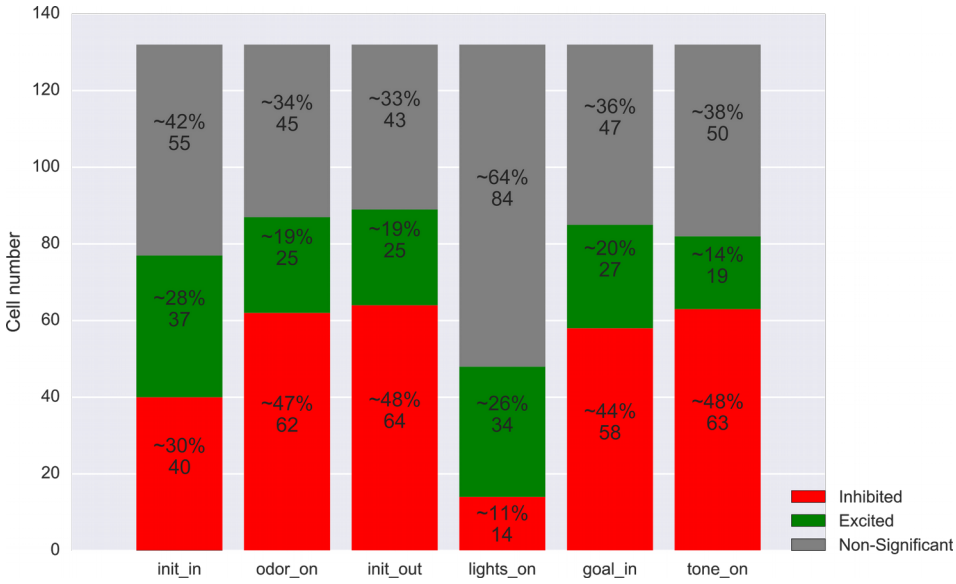

Figure 20 - Significantly modulated cells
Number and proportion of of cells with a significant firing rate change between the 500ms pre and post alignment. Approximate proportion and corresponding number of cells are displayed; green, red and gray denote increase, decrease and not significantly modulated cells. X-axis is the 6 epochs in chronological order, from left to right as seen in **Figure 18g**.

Feature selectivity

Having analyzed average firing rate of cells in a feature independent manner, we performed multiple one-way ANOVAs with Tukey-HSV post-hoc and Bonferroni correction for multiple comparisons over all the different epochs and features. This process allowed us to extract different cell populations that had a significant difference in firing rate depending on the different features of the task. These cells are referred

to as being ‘selective’ for the different features meaning that they can distinguish between e.g. goal locations. This distinction is however agnostic to which options of each feature is distinguishable. The post-hoc analysis will be later used to assess tuning preference.

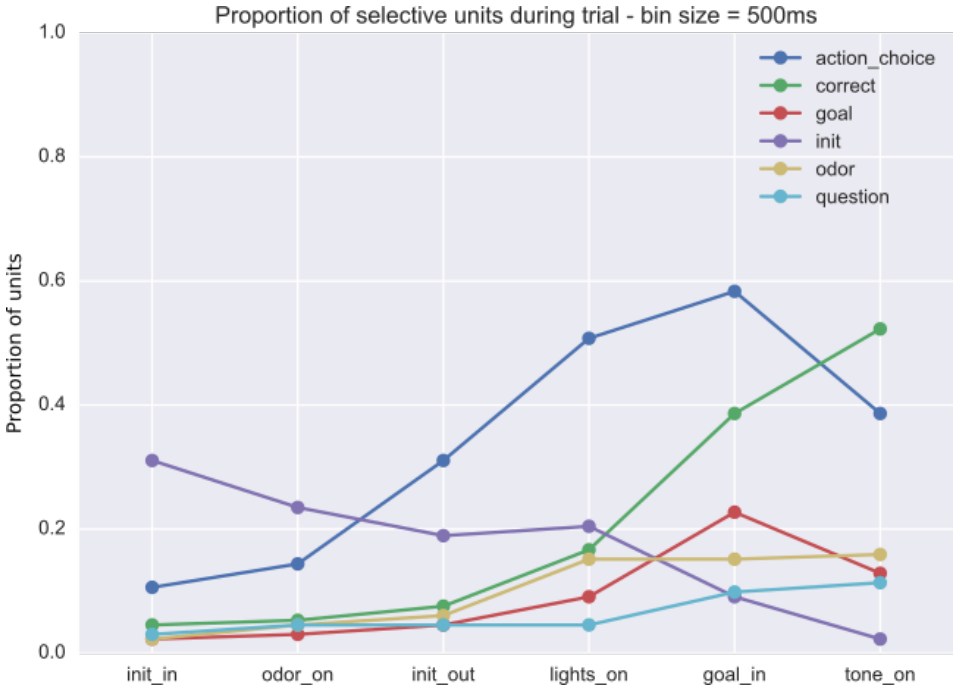

Figure 21 - Proportion of selective cells
Proportion of cells selective for the different features as a function of epochs.

Feierstein et al., (2006) found outcome expectancy cells that fired for the goal of the animal, during the execution of the action towards a particular choice. In their case however, direction (or action) and location would be confounded.

In our data, as seen in **Figure 21**, actions are the most represented feature reaching ~60% around goal port entry, followed by outcome or reward selective cells (correct vs error) at reward onset that reach ~50%.

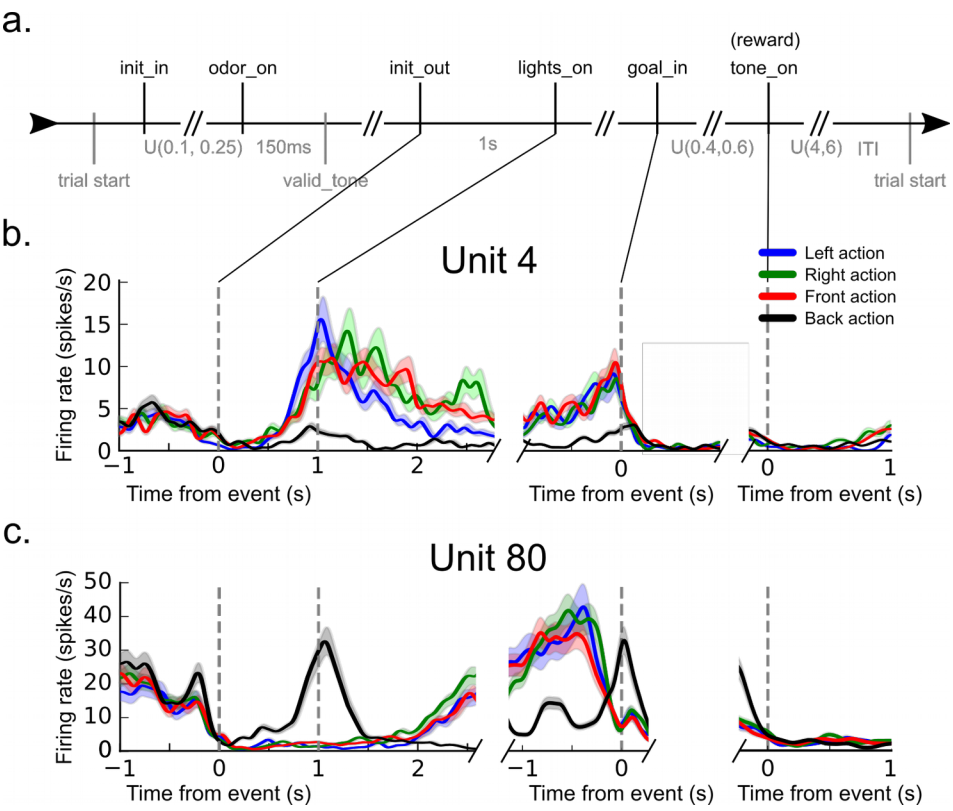

Figure 22 - Action selective cells
a. Task timeline, extended lines signal the selected epochs and corresponding alignment of the PSTHs. **b.** and **c.** Example units aligned on the 4 different epochs. Colors represent different action choices performed by rats.

Most (~80%) of action selective cells seem to differentiate between back trials and the remaining actions. In back trials the time between initiation port exit and goal port entry is compressed. Mostly as a consequence of the absence of movement and the vast majority of these trials are rewarded. We decided to remove back trials and run the analysis of **Figure 21** again. This way, although we might loose some power by reducing the number of trials by approximately ¼, we would be able to

assess the influence of back trials in the selectivity to the different features at all the different epochs.

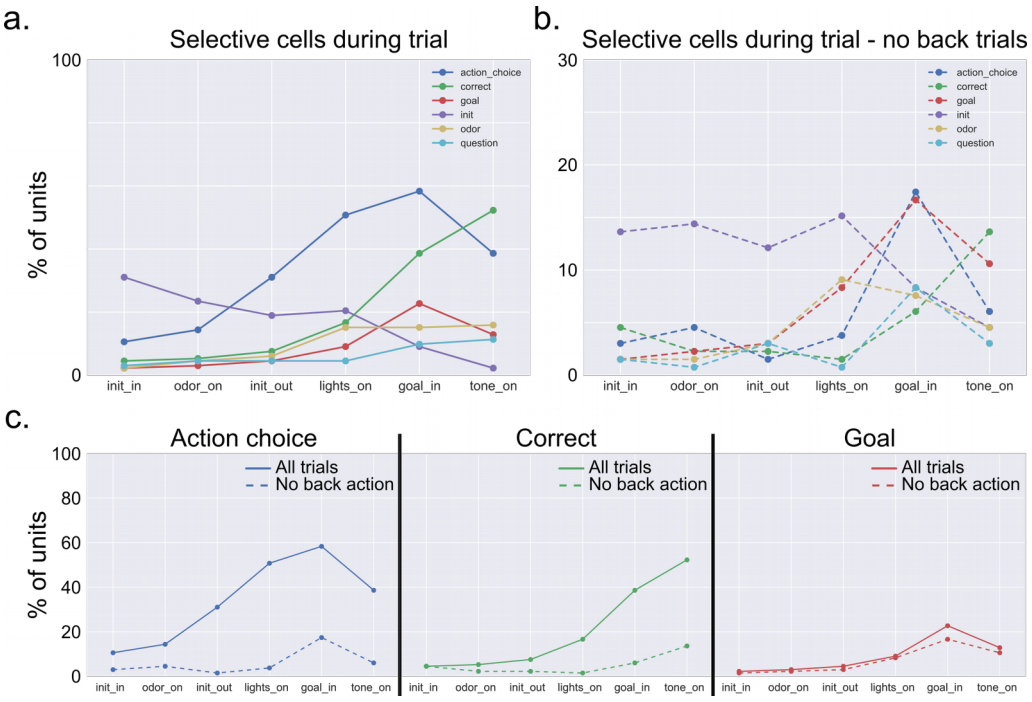

Figure 23 - Proportion of selective cells - no back action trials
a. Same as **Figure 21** for comparison. **b.** Selectivity of cells without back trials. **c.** Example of 3 different features and relative decline in proportion of selective cells after removing back trials.

Figure 23b shows the proportion of selective cells after removing back trials. Overall, the proportions of significant cells in all features is reduced (note the axis in panel b. only goes up to 30%) this might be in part because of the loss of ~25% of trials. Notably, we cease to see actions and correct as the dominant features, but most importantly the relative decrease of the number of significant cells gives us a measure of influence of the back trials in the cells' selectivity to features. Indeed, as we can see in **Figure 23c** the 2 main features affected are action choice

and correct. In contrast, the change in the number of (e.g.) goal location selective cells is minimal as it was for the remaining features.

Next we looked for location selectivity. Cells that distinguish between different locations are the ones that are selective for different initiation ports around initiation port entry and cells that are selective for goal locations at goal port entry. **Figure 24** shows two example location cells. We find that ~42% (56) of cells are selective for either goal or initiation location. A chi-square analysis $(\chi^2 = 8.9599, pvalue = 0.0028)$ indicated that the overlap between these two populations was significantly higher than chance. Indeed 15 of these cells belong to both categories and ~33% (5) maintain their tuning preference.

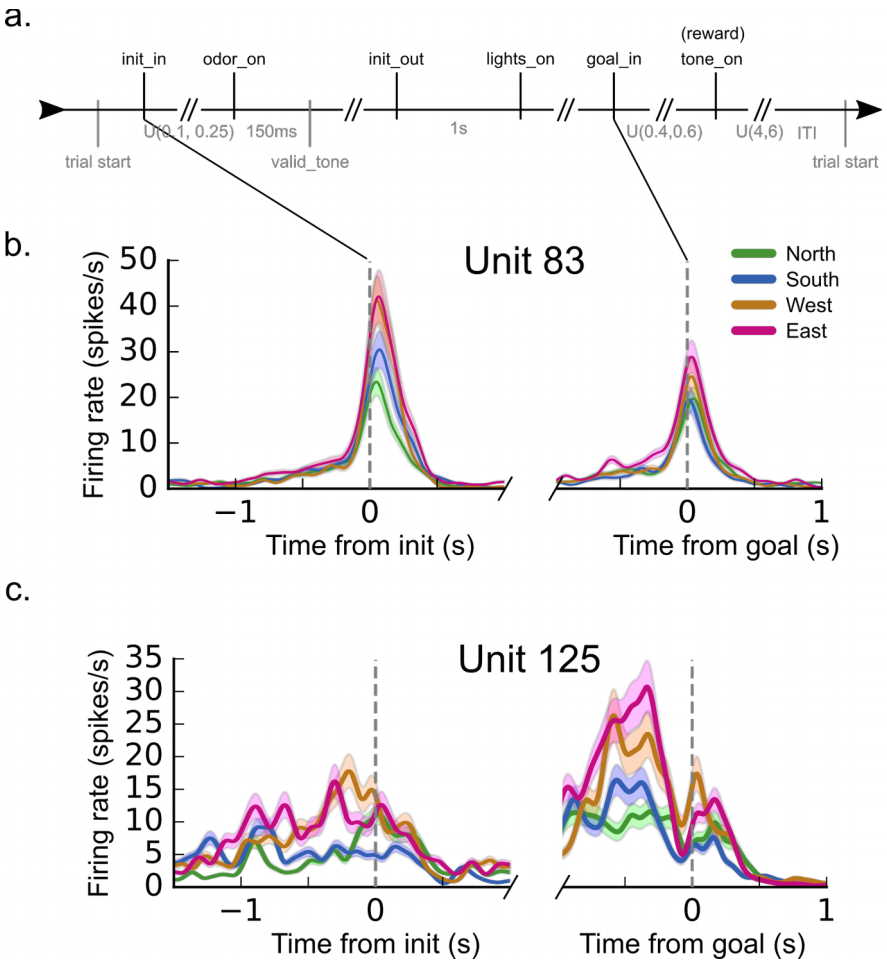

Figure 24 - Location selective cells
a. Task timeline. **b.** and **c.** show 2 example cells aligned on both initiation and goal port entry. Colors represent the 4 different locations.

Similarity of tuning was assessed by correlating the average firing rate of neurons around initiation and goal port location for the 4 different locations and significance at 95% confidence interval, was assessed by comparing the correlation coefficients of these neurons with a population of coefficients obtained by shuffling the labels of the data 500 times. **Figure 25** shows two example cells that maintain their tuning from initiation to goal port entry. Overall, ~30% (17) of location selective cells

maintain their tuning from initiation to goal port (even if the selectivity analysis doesn't pick them up in both epochs).

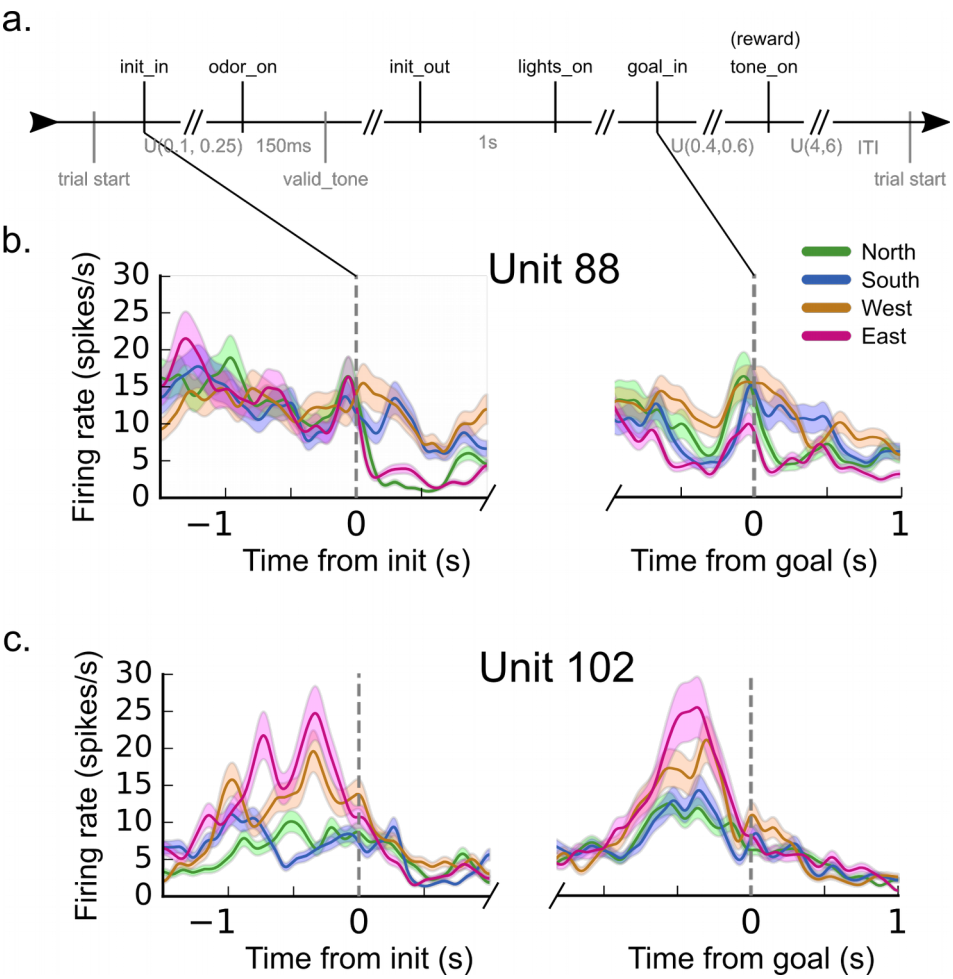

Figure 25 - Location selective cells tuning
a. Task timeline. **b.** and **c.** show two example cells that maintain selectivity from initiation to goal locations either pre (**c.**) or post (**b.**) poke in.

Furthermore, out of the 41 cells that have been found to be selective for initiation location at initiation ~76% are exclusively selective for location and no other feature. This is not surprising, as the only information the

rat has at this point is the location of the initiation port that is signaled by a lit LED at the appropriate location. At goal, however out of the 30 cells that show location selectivity only 2 (~6.7%) show selectivity only for location. 15 cells (or 50%) show selectivity for location and another feature and the remaining 13 cells showed some selectivity for 3 or more features.

4.5 Discussion

As noted in the beginning of the results section, analysis of this dataset is still ongoing, nonetheless we consider these results encouraging and can already draw some conclusions from this first order analysis of our recordings.

Odor

Although we expected odor selectivity at odor onset, as studies implicated LO in representing this type of information (Schoenbaum and Eichenbaum, 1995), in our recordings only very few neurons were found to be selective to odor stimuli. Feierstein and colleagues while showing that ‘goal’ cells are stimulus independent also reported a relatively low odor selective cells in their recordings. One reason for this could be the location of our recordings that cover the medial part of OFC and not only the lateral portion, but also the AP region that we recorded from (AP 4.2-2.6mm) has been found to be quite rostral comparing to all previous studies that tend to have the 4.2mm mark as the most anterior portion of the OFC, e.g., Feierstein and colleagues’ coordinates spanned from 3.6 to 4.2mm.

Another reason for the lack of odor selectivity could be because of the details of our task, i.e., if OFC is really a cognitive map of task space / encoding outcome expectancies or response categories (Wilson et al., 2014) the details of the task should be important and in our task odors are not uncertain or informative in any explicit way, except their link to the outcome location. This means that our task is ultimately a categorical task and odor identity should not matter except as a trigger for the relevant decision variable, the location of the reward. The number of odor selective cells shown **Figure 21** and **Figure 23** is higher at 'lights_on' and 'goal_in' epochs, i.e., around the initiation and termination of movement, the exact moment when location and action information should be important to execute a trajectory. This indicates that the labeled odor selective cells could very well be location selective ones. In fact odor and goal location are almost identical in that, odor is the goal location requested and goal is the actual choice of the rat. Because rat's performance in the task is high, it might be that these two features become indistinguishable. If we hadn't dropped the egocentric version of the task, this situation would not pose a problem, we could just compare these cells to cells that fire to the same odor in the egocentric task where the locations are different. However, not having this possibility, one way we could look at this would be by looking at error trials. In error trials, a cell that fires for an odor when the rat has made a mistake cannot be representing prospective locations and could thus be called an odor selective cell. Unfortunately, because of both the low number of odor selective cells at odor onset and the low number of errors of rats this analysis has been found hard to do. One thing we can do in the near future is at least increase the number of cells that take part in this analysis, as soon as we integrate the remaining unsorted cells we recorded from.

Reward

Regarding reward selectivity and because of OFC's previous involvement with reward, value and expected value (Tremblay and Schultz, 1999; Schultz, 2000; Hikosaka and Watanabe, 2004; Padoa-Schioppa and Assad, 2006; Jones et al., 2012) we expected to find a robust response on approach to the goal port indicating the expected value of the reward about to be consumed. We found that overall cells tended to be inhibited by a factor of 4 rather than excited as shown in **Figure 20**. Cells that selectively responded to reward increase in number as animals approach the rewarded port (green curve in **Figure 21**) and we noticed that reward selective cells tended to respond more for errors (when reward was not delivered) than for correct trials. However, there is no way for us to claim that these cells are representing reward, value or expected value as rewards were kept equal in type (only water was delivered) and equal in size, the only variable that should be different is their location.

Action & Location

Location cells are defined as cells that show selectivity for init location around initiation and goal selectivity around goal location. Action cells on the other hand are cells that are selective for actions between the exit of the initiation port and entry into the goal port.

Although we only recorded from the left OFC, no particular lateralization biases were found regarding location or action selectivity. Rats in our task span all directions and locations uniformly, so it might be the case that OFC's function does not require this feature. No other study, to our knowledge, has reported any type of lateralization bias in OFC.

Removal of Back trials

As noted in **Chapter 3**, 'Back' trials (i.e. trials where the odor delivered corresponded to the current location of the animal) were characterized by, low velocity, fast response time and almost perfect accuracy. We found a significant proportion of cells that in some way proved to be selective for actions (**Figure 21**). Looking at this population of cells we noticed that most cells differentiated Back trials from the rest, but not necessarily between the remaining actions. An example of this can be seen in **Figure 22** - Unit 80. As a consequence of this observation we decided to re-run the selectivity analysis only on Front, Left and Right trials. The results are shown in **Figure 23** and we found a big decrease in action selective cells and reward selective cells while most of the other features were almost unaffected. One reason for this could be because the average movement time plus the delay to reward delivery was smaller than the bin size used to perform the selectivity analysis effectively confounding actions, reward expectation, reward delivery and any possible representations of current or future location. In total we find ~23% of cells to be action selective after removing back trials.

Locations

Of the 2 studies (Feierstein et al., 2006; Roesch et al., 2006) that reported direction selectivity, Feierstein and colleagues reported 41% of goal cells (location/direction selective), while Roesch and colleagues reported (in supplemental) 36% of direction selective cells.

Location selective cells, now independent of egocentric direction (or actions) are found mostly when the rats are approaching one of the poke locations or while they remain at that location. Consequently location cells at initiation are the most numerous population and remain so until the rat starts moving towards the goal (**Figure 23b**). Selectivity for init

location decreases and selectivity for goal location increases as animals approach the goal port. We found locations to be the most represented feature in terms of cell selectivity. This is consistent with both the idea that OFC represents task space and the results in **Chapter 3** where we show locations rather than actions to be relevant decision variables. Furthermore, we have been calling actions to the egocentric direction reported in previous studies, however, having an allocentric reference frame one could ask if there is some preference for particular allocentric directions, e.g., cells selective for North and South. We find no significant over-representation of cells that code for allocentric direction, however we notice a trend where the two directions of the arms of the maze, i.e., North / South and East / West locations seem more easily distinguishable.

Unfortunately, possibly because of our 4 options, selectivity for any of the features does not imply a clean uniform representations of e.g., locations one by one. Instead, we find than any individual neuron might differentiate one, two, three or all options. This is true for all non binary features. These results suggest that a more complicated conjunctive code, both within feature and across features (where a single neuron is selective for multiple features) exists within the OFC.

Conjunctive coding

Conjunctive coding is the property of cells to code for 2 or more features of a behavioral task or 2 or more options within a feature (if and when more than two options are available to the animal). In our task we find that the proportion of selective neurons that fall in this category increases as the trial develops, from ~0.26 at initiation port in, to ~0.67 at goal port in. While it's true that there is no information available to the rat

at initiation except the location of the port where the stimulus will come from, cells that display some conjunctive representation of features at this moment could be instrumental in updating values and weights of associations of the statistics of the task. In contrast, once at the goal port and after having received the reward these cells might be providing the substrate over which the rat learns about stimuli, actions and locations.
Overall, ~47% of selective cells show conjunctive properties for 2 or more features. The reason why cells are selective for multiple features could be either because they code for different features or if there is a behavioral correlation between factors. As we've just seen, the odor and goal features are basically the same feature, one in terms of the trial that was selected by the experimenter and the other in terms of the behavior of the animal; or in more simple terms, 'odor' is the goal location requested and 'goal' is the goal location visited, and if performance were to be 100% would be identical. Even when removing the odor feature this percentage did not change significantly. For example at goal port, only ~15% of cells were selective for goal and odor, but only 2 of these units were selective for odor and goal exclusively. Meaning that even removing the odor/goal confound the same cells would have been picked up by the analysis not changing the proportion of cells that conjunctively code multiple features. Another reason for this is the low odor selectivity already reported.

We seem to have a lot of complex interactions of features that are present in single cells in the OFC. We think this observation is not inconsistent with the hypothesis that implicate OFC in representing hidden states or outcome expectancies, it could actually provide a richer 'playground' that would allow OFC neurons to represent an infinitude of expectancies. A more detailed analysis of the particular contribution of each cell to the selected features and option within those features seems

to be in order. Unfortunately this is not a simple problem. One possible solution could be dPCA (Kobak et al., 2016), a refinement of principal component analysis that allows us to use feature labels to identify principal components and assess their contribution to the firing rate of a particular neuron.

Finally, analysis of tracked trajectories and head direction is also on our to-do list and should yield interesting results. Specifically, we have been using the end of the delay period after initiation (lights_on) as a proxy for the initiation of the movement, however, rat's reaction times may vary and having access to the precise moment when the movement is initiated as well as the moment when the rats have committed to a decision (after they passed the center of the maze) would help clarify the tuning properties of the neurons recorded. Lastly, head direction information would also constitute a whole new dimension that might be relevant for OFC neurons.

5 General discussion

Author contributions: Bonacchi N. wrote the manuscript.

Understanding the brain can only be achieved if we have a good understanding of behavior. Behavior in a general sense, is a description of the interaction of an agent with everything else, the context of behavior is thus our world. Consequently, observing behavior has to necessarily tell us something about our reality and, because behavior is a direct observable consequence of neural processing, it has also the ability of contextualizing brain function in the most relevant way possible. A focus on behavior thus ought to be paramount to neuro-scientific endeavours as it is impossible to ascribe function if one does not consider the context in which a particular brain area operates.

Although we agree with this general principle, we find ourselves doing experimental science, which implies putting rats in bigger or smaller boxes in a room and under very particular conditions. In fact, one of the common criticism made to experimental science, is often about its relevance for understanding naturalistic behavior.

Experimental and systems level neuroscience allow us to answer a set of questions about what brain areas do, by asking what they *can* do in particular tightly controlled behavioral settings. The behaviors we observe in the lab are limited, often repetitive and sometimes, when compared with more naturalistic behaviors, might even seem far fetched. Nonetheless, experiments in a laboratory setting have been and continue to be extremely successful in understanding causes and effects in the world. These type of experiments might actually be the only way we can ask questions that yield interpretable and consistent results as well as test specific hypotheses about how the brain works.

The major limitation of experimental neuroscience is arguably technical, usually posited in terms temporal and spatial limitations of one technique

when compared with another. The ideal experiment would be able to look and/or manipulate many more neurons, from multiple brain areas simultaneously and in an 'interesting enough' behavior, this way we could start answering questions not only about brain area X in behavior Y but about the dynamics of whole brains.
On the other hand, behavioral tasks used to probe the brain have traditionally been reductions or simplifications of more general behaviors, designed to extract specific features of brain function. This fact usually results in task designs that posit one option in comparison with another reducing the decisions animals make to binary options. While useful and successful, this tendency might bias our explanations of brain function by exploring a specific subset of behavioral tasks, leaving out dynamics and explanations that could only be revealed if one was to choose to implement a task which is slightly more complicated.

Our odor guided spatial navigation task presented in **Chapter 3** and **Chapter 4** is a non-binary categorical behavioral task that increases the number of choices available from the usual two (Go-No go, left-right, etc.) to four options. Indeed, by increasing complexity we hoped to be able to reveal more complex dynamics, however, we found some difficulties both in training and data analysis, all the rules of thumb one might have used previously in binary tasks, were found to not necessarily apply when more options are available. For example, in a 2 AFC errors tend to be 'symmetrical' with corrects trials, i.e., an error trial just by the fact of being an error is informative of the choice of the animal. In a 4 AFC tagging a trial as an 'error trial', tells us nothing about the particular choice the animal made. So whereas performance measures could be used (conditioned on choice) to look at biases, when multiple options are available choice behavior becomes more important than performance. Looking at choices in errors for example can reveal

particular strategies animals employ to solve the specific problems we pose them. In **Chapter 3** we show one such example where we find a specific hierarchy of preference in terms of errors that reflects a specific strategy.

In **Chapter 2** we show that increasing the spatial footprint of the behavior box and making animals move in this extended space, was sufficient to engage OFC in such a way that it becomes necessary for maintaining performance in a simple free choice spatial alternation task. We hypothesized that the reason for these results might be the possible engagement of an allocentric strategy used to reach the reward location. However this task did not possess an explicit navigational strategy that allowed us to draw any conclusions about strategies.
We then developed an odor guided spatial navigation task in **Chapter 3**, with an explicit allocentric rule. We claim, based on our behavioral analyses that rats use the location of the reward as the relevant decision variable to solve the task. Furthermore we described the properties of a 'special' trial type (Back action trials), that had different properties and where rats possibly used a different strategy. We concluded, based on our behavioral analysis that rats were using a two-step strategy in each trial first dividing trials in go-nogo, where Back action trials were the nogo trials and the rest were go trials where the location of the reward would be the relevant variable driving their behavior. Finally, in **Chapter 4** we performed extracellular electrophysiological recordings of single cells where we could assess the distinct independent contributions of action representations and location representations in OFC. Furthermore we reported a high level of conjunctive coding, i.e., neuron sensitive to multiple features, that increase as animals reach the goal port.

Although tracking analysis has not been performed yet, we have no reason to believe that a topographic organization of location selective cells, like one can find in hippocampus, is to be found (O'Keefe and Conway, 1978; Wilson and McNaughton, 1993). One reason we might think this is patent in our introduction where locations are presented as properties attached to outcomes (and not vice-versa) and we have no idea about the possible shape of an outcome cognitive map, nor of the correlation, or lack thereof, to cognitive maps of space. Furthermore, although absence of evidence is not evidence of absence, some studies that were looking at planning and reward in OFC and VS, did not report any location or hippocampal like 'OFC-place-cell' (Steiner and Redish, 2012, 2014). Although from a strong hippocampus lab, in these studies, reward identity, egocentric and allocentric direction and reward location are not separable, hence reward coding neurons might be confounded with reward/outcome location selective ones.
The relationship between memory and space, or, more specifically, between object representation and space, is unfortunately still mysterious.

We already saw, from the reversal learning literature, that OFC is believed to not be important for acquiring new associations but only for the updating of an existing ones (Schoenbaum et al., 2002; Chudasama and Robbins, 2003). We also saw that, OFC has been hypothesized as one of the main brain areas where expected outcomes are represented – more specifically as a vector of different properties (**Chapter 1**). Finally, OFC has also been implicated in the representation of hidden states (Keiflin et al., 2013), which include everything that is not stimulus bound, like imagining a future outcome or remembering a past one.
Rats, in our task, are presumably aware of the existence of water rewards, the information they lack however, is the location of this reward

on a trial-by-trial basis. Consequently, whatever the nature of the representation of the desired outcome might be, this representation would need to be updated in order to reflect the new information provided by the odor delivered at the beginning of each trial.
This interpretation is consistent with both the reversal learning literature, the goal expectancies and hidden states hypothesis. OFC has been shown to be atop of a putative hierarchical network responsible for goal-directed behavior (Keiflin et al., 2013). Considering this, our results, are consistent with OFC being the main brain area where location information, presumably from the hippocampus, is integrated with other sensory information about the expected outcome. This integration would only be revealed in the context of a goal-directed behavior, where the representation of the desired outcome, has to be updated with other relevant information necessary to perform an action (in our case, location).

A connection between locations, allocentrism and goal-directedness has already been proposed involving OFC, hippocampus, and PL/IL cortices. In a similar task (Young and Shapiro, 2011), although with no queues and in the context of a learning paradigm, this study presents some evidence of OFC involvement in the representation of goal-directed paths and a high theta band coherence with hippocampus LFP. The authors propose a specific role for OFC: In conjunction with IL/PL, and hippocampus, OFC would be responsible for associating spatial paths, recent memory, and integrate reward history.

Furthermore, allocentric navigation is, by its very nature, a goal-directed behavior. A cognitive map of spatial locations containing subject and objects needs to exist in order to plan and execute an action. Most importantly, the the primary characteristic of allocentrism is its

independence from egocentric action-based strategies (Dolins and Mitchell, 2010; Lihoreau, 2010). Nonetheless, and despite the fact that we are somewhat guilty if doing so, actions should not be used as a shorthand for habitual behavior or egocentrism. In fact, the relationship between actions and goal-directed behavior has also already been shown to depend on DMS and OFC (Gremel and Costa, 2013), in contrast to DLS's involvement with habitual or action-based strategies.

Final remarks

As scientist we often use analogies (some might say that's what information actually is), and more often than not, just like in our behavioral tasks, we reduce concepts to dichotomies, in order to understand the world. This tendency is probably a consequence of a structural ontological dualism present in both science and philosophy and also probably a consequence of our natural inclination to reduce cognitive dissonance. Over the course of this thesis we've seen several of these dichotomies: goal-directed vs. habitual behavior; model-based vs. model-free RL; allocentric vs. egocentric navigation; locations vs. actions. We have attempted to show a link between goal-directedness, model-based RL, allocentrism, locations as a way to reveal this, and OFC. Consequently, we've also, by omission, related habitual behavior, egocentric strategies, model-free RL, and actions. Specifically, we showed, in the context of goal-directed behavior, independent populations of cells in OFC are found to represent both egocentric actions and spatial locations. Moreover, we reported that the number of cells that code conjunctively for multiple features increase as the animals reach the end of the trial. We believe these data support our initial hypothesis by proposing a 'new' role for OFC as the site of integration of

contextual information, specifically outcome locations, with the representation of desired outcomes in goal-directed behavior.

Finally, I would like to propose a thought experiment that can hopefully help re-frame the concepts addressed in this thesis and perhaps promote some new insights on the matter. I call it the processing vs. memory hypothesis and it's another dichotomy, this time from the realm of computer science.

Imagine you have infinite memory capacity, all possible states of the universe precomputed, all possible decision trees explored and stored in an infinite SSD (solid state drive). In such a situation 'you' are just a pointer somewhere in this infinite memory space that moves on, each action, each decision is simply the readout of the information at that particular pointer, followed by the next, and the next. There is nothing to decide, everything is set and automatic, fast and efficient, the next action as certain as the present one.
In opposition to this, imagine you have no memory, but you possess infinite processing capability, you have no need for memory because you can just recompute everything at each time-step and calculate the next action to be implemented. Each action is the natural consequence of the recapitulation of all of the history of the universe plus one time-step and once this is done you restart from scratch to calculate the next action. Everything is re-evaluated always and as time goes by the processing steps grow exponentially, if it where not for your infinite processing capability the decisions you make would take longer and longer.

So, what would happen if these agents have partial information? The main difference we would probably see is that the processing agent would eventually make a 'mistake' due to the non complete information

received, it would have to add a rule or a variable to account for partial information but would rapidly recover in the next time step. The memory agent however, would have a much harder time, having partial information means that the pointer that blindly moves forward can be in the wrong location thus every action following can be a mistake or would possibly be nonsensical. This agent would have to have computed a best possible model given the information it had and upon new information it would have to recompute... Alas, not having any processing power, it would be stuck in the reading out of a sequence of actions not optimized for the environment.

These two extremes rapidly break down as one makes the world more similar to our own. Besides partial information we could now add limits, to memory capacity or processing power and the shortcomings of these two extremes would be painfully evident. In no time however, it would become obvious that a good system would be some sort of hybrid between these two extremes. How much memory and how much processing power would depend on the precise details of the variability of the environment, the speed of reproduction, the cost of being less than optimal, etc… Brains have arguably to solve this same problem, some things can be optimized, processed and stored in memory to be readout when needed, other things have to be processed. Of course, as a consequence of its limited (at least not infinite) memory and processing capacity, decisions have to be made: what to store, how permanently, what and how much to process. While the memory system is more efficient and would allow a better exploitation of something that was already encountered, its rigidity and lack of adaptability would not be well suited to deal with changes in the environment. The processing system, on the other hand, would adapt extremely fast but would require

time and energy to operate that might be suited for exploration but counterproductive or even dangerous in some instances.

Animal behavior could be seen as a consequence of the delicate balance between these two extremes. Brain areas responsible for the implementation of the behaviors we observe in the lab would consequently be better understood under the lens of their involvement with one or the other system. In this, flawed, simplistic, and not complete analogy, the OFC would be part of the processing, goal-directed, model-based, and allocentric system, while other areas (e.g., DLS) would support more memory based, habitual, model-free, egocentric implementations.

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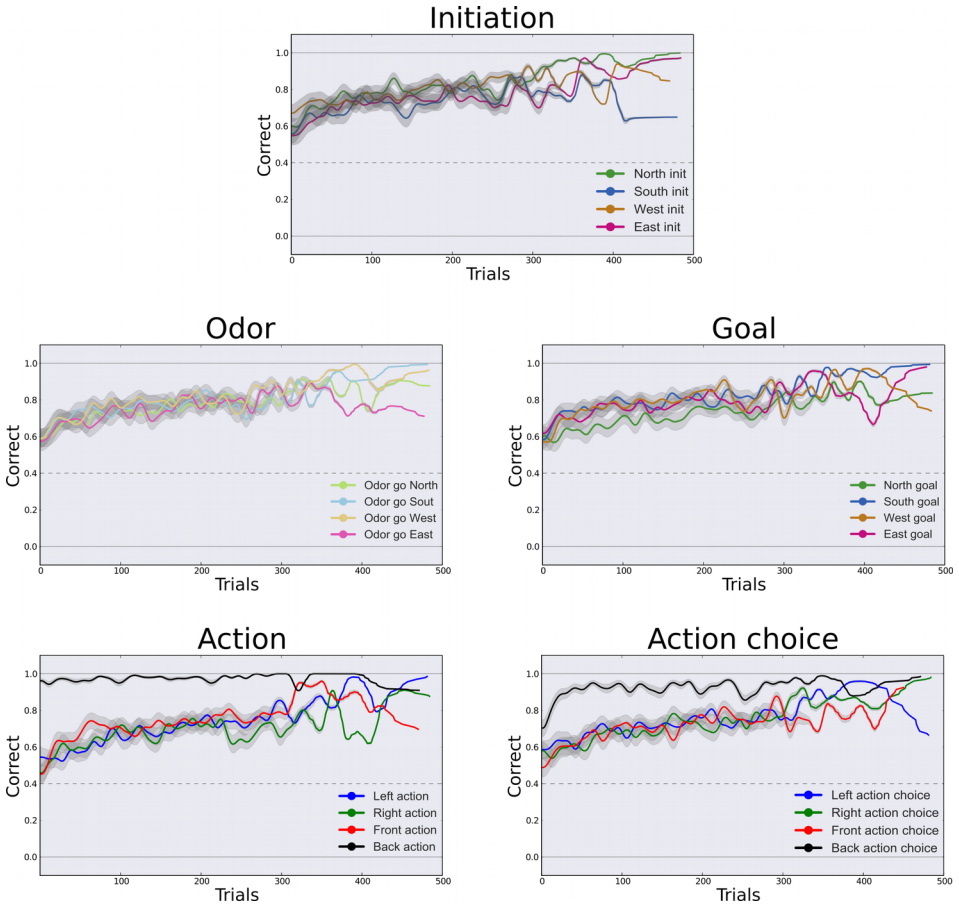
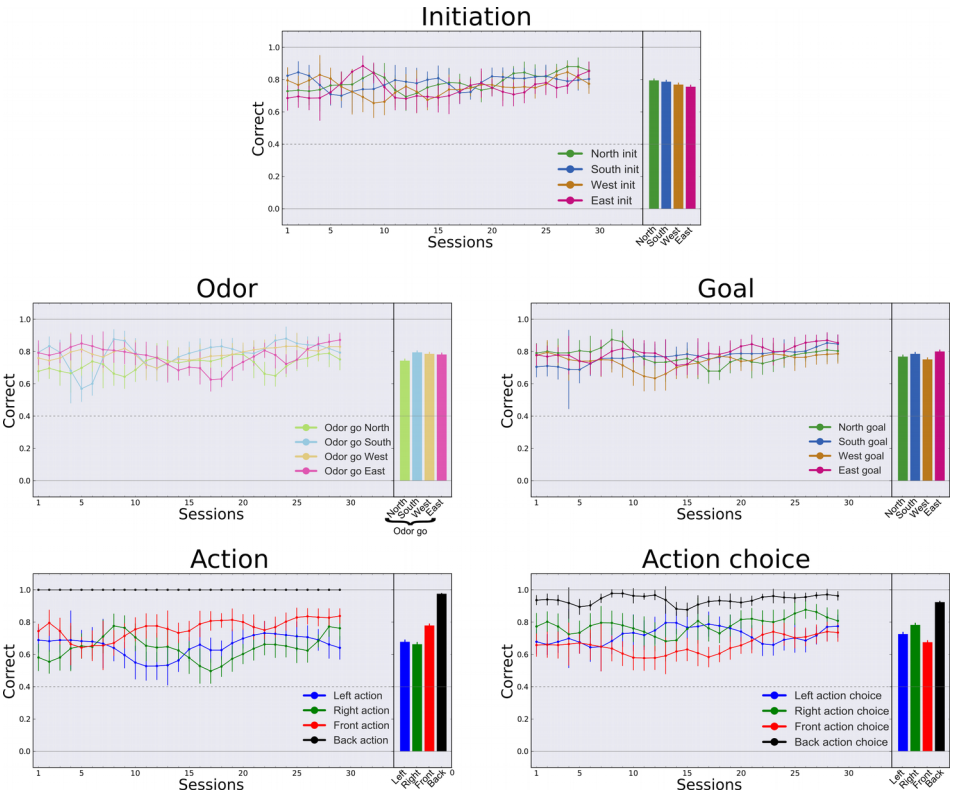
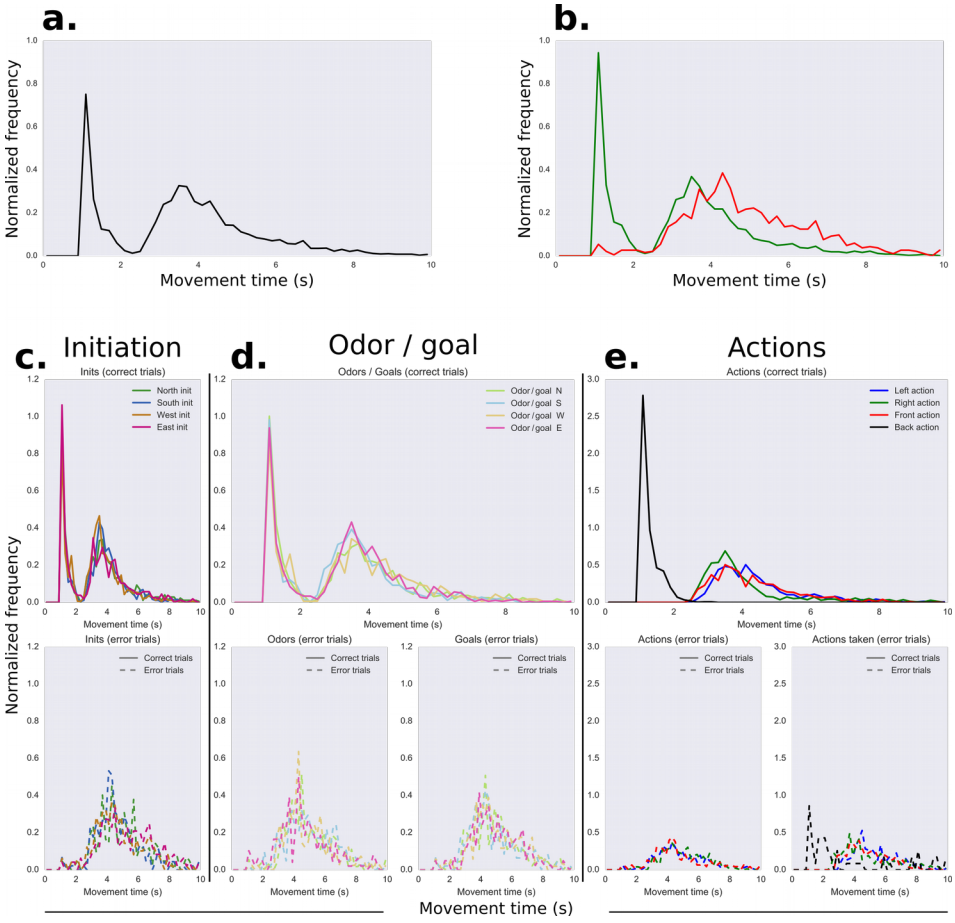
Supplementary figures



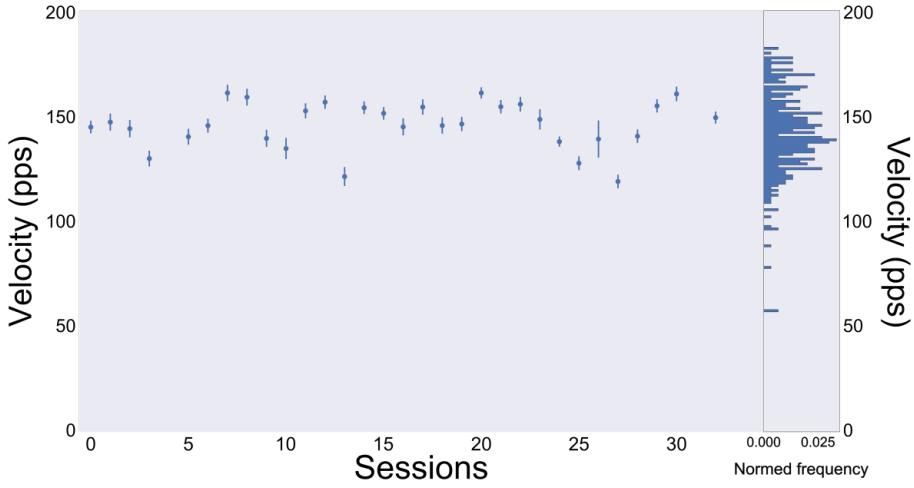
Supplementary figure 1
Average performance as a function of trials for all rats in both question and answer trials by: initiation port, odor delivered, goal choice, action, and action choice. Different colors in each panel represent the 4 different locations, odors or actions; dashed line represents chance level.



Supplementary figure 2
Average performance as a function of sessions for all rats in both question and answer trials by initiation port, odor delivered, goal choice, action, and action choice. Different colors in each panel represent the 4 different locations, odors or actions; dashed line represents chance level. Bar plots on the right side of each panel are averages across sessions for each option; error bars represent standard error of the mean.



Supplementary figure 3
Movement time histograms: **a.** for all rats; **b.** split by correct (green) and error (red) trials; **c.**, **d.**, and **e.** top panels are correct trials by initiation, odor or goal choice (correct odor trials and correct goal choice trials are equivalent), and actions (same logic applies for correct action and action choice trials). Bottom panels are error trials movement time histograms for all condidtions.



Supplementary figure 4
Average velocity as a function of sessions for an example rat. Error bars are standard error of the mean. Right panel velocity histogram of all trials, all sessions.

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