Discussion
DISCUSSION

At the outset of the work covered in this dissertation, I decided to look for answers to the question “How do cortical neurons encode cognitive output?” Now, some years, a lot of experiments, and even more analyses later, it is time to look back and take stock of the answers I found and which questions still remain. For this discussion, I will take apart my main question in the same way that we started the introduction: Below I will discuss our findings with respect to cortical neurons, how these encode the sensory and behavioral world, and how neuronal encoding in turn is linked to cognitive output. In addition, I will draw conclusions from our findings and, where necessary, propose experimental steps that could provide a more thorough answer to questions regarding cortical encoding of cognition.

Cortical neurons

The cortex can be subdivided in so-called columns, a few thousand spatially clustered neurons forming a fully functional subunit of cortex (Mountcastle VB 1957; Woolsey TA and H Van der Loos 1970; Douglas RJ et al. 1989; Mountcastle V 1997; Katzel D et al. 2011). Through network interactions, cortical columns should be able to perform computational operations on afferent inputs and integrate information to consequently drive cognition. Cortical columns are highly laminar and each layer contains specific neuronal subtypes (Harris KD and GM Shepherd 2015; Tremblay R et al. 2016). It is assumed that distinct layers and cell types each play their own role in the columnar circuitry; however, it has remained unclear how each cell type fits into the circuitry to perform computations. Most of what we know about neuronal subtypes rather concerns projection target (Molyneaux BJ et al. 2007; Dembrow NC et al. 2010; Oberlaender M et al. 2012; Harris KD and GM Shepherd 2015; Lur G et al. 2016; Yamashita T et al. 2018) or genetic makeup. In addition, we were not able to discern between subtypes of putative pyramidal neurons within the layers and we only recorded the spiking output of cortical neurons. Therefore, I will speculate about the function of layers as output centers, rather than discussing them as part of the computationally enigmatic cortical column.

In Chapter 3 we show that, under anesthetic conditions, in PPC L5 is the only layer that shows a relatively reliable spiking response after whisker stimulation, while L2-4 and L6 neurons usually do not spike. Under awake conditions this is switched, so that L5 does not spike in response to whisking or touch, but L2-4 does and L6 spikes only in response to whisking. L5 is the layer containing pyramidal tract neurons (Oberlaender M et al. 2012; Harris KD and GM Shepherd 2015), projecting to subcortical regions (Morishima M and Y Kawaguchi 2006; Dembrow NC et al. 2010; Kiritani T et al. 2012), often brainstem.

When the rest of the cortical network is silent, it may be that L5 is allowed to send the most salient information to movement centers in pons or brainstem to generate a behavioral response, but that under awake conditions top-down inputs gate vibrissal information through different layers to other regions. This could, for example, be top-down input from motor cortex, which has been shown to also modulate spiking in S1 (Lee S et al. 2013), or from mPFC (Pi HJ et al. 2013; Zhang S et al. 2014). In addition, high burstiness of L5 neurons (Chapter 4; de Haan et al., in preparation; Boudewijns ZS et al. (2013)) may be a universal feature across cortex and may keep synapses onto L5 dendrites plastic, allowing flexible integration and scalable output to brainstem centers.

Neurons in ventral posteromedial nucleus (VPM) of thalamus encode input from single whiskers, including motion and touch, while posteromedial nucleus (POm) neurons have broader receptive fields and do not strongly encode whisker motion (Moore JD et al. 2015; Urbain N et al. 2015). The role of POm is heavily debated, however, its strong connectivity with motor and somatosensory cortex suggest a role in somatosensory processing, while its activity correlating with high-frequency cortical oscillations associated with alertness suggests a role in attentional processing (Urbain N et al. 2015). It can be assumed that whisker self-motion is important information for interpretation of somatosensory processing. The absence of whisker self-motion in POm spiking rates is therefore surprising (Moore JD et al. 2015; Urbain N et al. 2015). However, self-motion may still be encoded in POm using coding schemes that do not rely on spiking rates, e.g. temporal codes. Somatosensory corticothalamic communication is thought to consist of driver inputs from L5 and modulatory inputs derived from L6 (Sherman SM and RW Guillery 1998; Groh A et al. 2008). PPC L6 neurons encode whisker self-motion (Chapter 3) and it may be modulation of spike timing or burstiness in POm by PPC L6 axons provides sufficient information on whisker self-motion. For this hypothesis to be feasible, presence of axons from PPC L6 to POm would first need to be verified, for example using retrograde tracers injected in POm. If the axons are found, the function of this projection could be tested by expression of inhibitory opsins in PPC L6 neurons and inhibiting their terminals in POm during whisk start, while POm activity and the behavior are monitored. In case this manipulation changes the behavior, it is clear that L6-to-POm projections play a direct role in informing behavior, while changes in POm spiking during whisking indicate that PPC L6-to-POm axons play a role in thalamic interpretation of feedforward vibrissal afferent input.

Neurons in the superficial layers project intra-telencephalically and these neurons are the only ones that project to contralateral cortex (Harris KD and GM Shepherd 2015). In Chapter 4 we show that superficial neurons have low spiking rates, are relatively hyperpolarized and are not very likely to show action potential bursts. We confirm low
spiking rates in Chapter 3 and we find that in PPC superficial neurons spike during free whisking, as well as touch. Knowledge on touch as well as whisking activity is relevant for both the local network and the contralateral hemisphere. PPC representations of the dorsal whiskers overlap with the representation of the dorsal visual field (Zhuang J et al. 2017). Therefore, it is very likely that PPC integrates somatotopic representations of whisker activity with retinotopic visual cues (Zhuang J et al. 2017) and other relevant information. Informing the contralateral hemisphere about integrated somatosensory-visual cues may also be very useful, for example when the width of an object or passage needs to be estimated.

PPC receives little input from somatosensory thalamus (Wilber AA et al. 2014), but does receive many afferents from S1 (Lee T et al. 2011; Wilber AA et al. 2014), thus it was hypothesized that somatosensory-triggered signals in PPC arise from cortico-cortical inputs. This should be confirmed and could be tested using optogenetic inhibition of S1 axon terminals in PPC during awake free whisking (and/or naïve touch). If silencing these terminals abolishes somatosensory signals in PPC, it is clear that S1 was the driver. However, if PPC would still show similar spiking rate increases during whisking and touch, we will have to consider other sources of somatosensory input. Cortical spiking rates are very low during anesthesia (see e.g. Chapter 3 and 4), and in barrel cortex L5 neurons can be driven by thalamic input alone (Constantinople CM and RM Bruno 2013). PPC receives small direct input from the thalamic POm nucleus (Wilber AA et al. 2014), which can by itself encode vibrissa stimulation under urethane anesthesia (Diamond ME et al. 1992). L5 evoked spiking in PPC during anesthesia could therefore also be due to input from the POm. Furthermore, if we do find S1 to PPC projections carrying somatosensory information, it remains unclear what layers/neurons send somatosensory signals from S1 and what PPC layers and cell types are postsynaptic to S1 cortico-cortical axons (Chapter 3). This case could be elucidated further by analyzing latencies of response of PPC units and local field potential (LFP). The first layer with spiking units after whisk or touch onset can be expected to receive the most direct input. In addition, the input layer could be determined using current source density analysis or by looking at the depth profile of early LFP deflections after whisker somatosensory events.

Classically the cortical column in sensory cortex was assumed to be a relatively sequential information processor. Feedforward thalamic inputs were thought to arrive in L4, be broadcast to L2/3 and subsequently L5 and L6, which form the strongest outputs from the cortical circuit (Douglas RJ and KA Martin 2004). However, this canonical cortical circuit has been shown to be only part of the truth. For example, L5 and L6 receive strong thalamo-cortical input (Constantinople CM and RM Bruno 2013), regions without L4 also receive strong thalamic input (Hoover WB and RP Vertes 2007), and L2/3 neurons have axons projecting over long-ranges (Gabbott PL et al. 2005; Yamashita T et al. 2013; Yamashita T et al. 2018). I propose that, while the cortical column has obvious value as a high-level information integrator, it seems that we do not yet sufficiently understand columnar neuronal subnetworks and interactions between them, and that we can thus not completely comprehend the information processing at the level of the column.

A purely excitatory neuronal network would have problems with runaway excitation and could not perform any computations (Buzsáki Gr 2006). While in this dissertation I mostly studied the excitatory output population of the cortex, I think it important to devote some time to the inhibitory counterparts of pyramidal neurons. Computations rely heavily on inhibition and it could be that currently, small scale circuit motifs provide a more comprehensive/comprehensible representation of neuronal interaction and subsequent information processing than a purely output-focused view. In small scale circuit motif frameworks, inhibitory interneurons play a major role in information processing. Three main forms of circuit motifs involving inhibition are recognized: 1) feed-forward inhibition, 2) feedback inhibition, and 3) disinhibition (Tremblay R et al. 2016). First, feed-forward inhibition refers to afferent inputs arriving both on excitatory neurons and on inhibitory interneurons, with these interneurons disinhibiting the excitatory neuron (Fig 7.1A; Toyama K et al. (1974)). This limits afferent inputs in their temporal extent (Buzsaki G 1984), high-pass filtering input, limiting over-excitation after strong afferent inputs, and increasing the dynamic range of the network (Pouille F et al. 2009). By modifying the strength and delay between feedforward excitation and inhibition, the gain of the network and the window for temporal integration can be adjusted, allowing flexible gain control and coincidence detection windows. Some PPC L5 neurons in Chapter 3 show sharp on- and offset of action potential spiking in PPC L5 neurons after whisker stimulation, this could be due to feedforward inhibition suppressing excitation immediately after initial supra-threshold input. We could test this hypothesis by recording from a sharp-spiking L5 neuron in PPC and briefly optogenetically inhibiting GABAergic interneurons (Olcence U et al. 2013) with short latency (10-50 ms) after whisker stimulation under anesthetic conditions. In case this manipulation leads to increased bursting and prolonged spiking, we can conclude that feedforward inhibition is indeed responsible for the found short-duration spiking activity. In addition, bursting of nPFC neurons reported in Chapter 4 could be gated by feedforward inhibition (or disinhibition, see below) when prolonged afferent input depresses the inhibitory synapse more than the excitatory one (Gabernet L et al. 2005), providing a window for temporal summation, potentially reaching bursting threshold.

Secondly, feedback inhibition is local inhibition, driven by excitatory output of local excitatory neurons (Fig. 7.1B; Silverberg G 2008; Tremblay R et al. 2016). Feedback
inhibition can be recurrent or lateral, serving distinct functions. During recurrent feedback inhibition the neuron(s) that excited the interneuron is inhibited itself, while lateral feedback inhibition provides inhibition onto other (excitatory) neurons in the local circuit. Recurrent feedback inhibition is thought to track the output of the network and lowers the excitation/inhibition balance in the cortex, preventing runaway excitation. Lateral inhibition, on the other hand, allows for small populations of neurons to (temporarily) inhibit the rest of the network. This allows on one hand for winner-takes-all excitation, the neurons that are sufficiently excited first will inhibit the other neurons in the local network and will dominate the circuit output (Kvitsiani D et al. 2013). On the other hand it can synchronize the network. If interneurons are excited by synchronous input and subsequently provide short ‘blanket’ inhibition, they open up a post-inhibition excitatory window where many neurons can be simultaneously active and in this way provide rhythmic periods in which output excitation is gated. It is thought that this type of local feedback inhibition underlies many oscillatory patterns in the brain (Kvitsiani D et al. 2013). Another potential role of lateral inhibition is gating of connectivity between dendrite and soma. When the network sufficiently excites a dendrite-targeting interneuron, this may preferentially turn off somatic integration of some dendrites (Fig. 7.1C), by shunting inhibition of excitatory potentials. Several key findings of this dissertation could be attributed to (lateral) feedback inhibition, of which competing neuronal populations for (not) licking in Chapter 6 is most notable. Neurons that show increased spiking when the rat withholds a licking response decrease spiking when the rat does lick and vice versa, which is what would be expected from two neuronal populations competing through lateral inhibition for output dominance. The best way to test this would be to selectively express excitatory opsins in one of the two populations and using intracelluar recordings or fluorescent chloride recordings (Kuner T and GJ Augustine 2000) to see if the other population receives stronger inhibitory inputs when the opsin is switched on. A technically less challenging method to test lateral inhibition could be to chemogenetically increase the resting potential of interneurons that provide lateral inhibition (Boldog E et al. 2018; Obermayer J et al. 2018) and record whether the spiking rate difference between the two states becomes larger. If it does not, another mechanism (such as distinct afferent input) must underlie opposite spiking rate modulations between the two populations. Integration of feedforward information with recent outputs through coincidence detection of action potentials and synaptic inputs, such as proposed in Chapter 4, could also be strongly influenced by feedback inhibition.

When an interneuron inhibits interneurons more strongly than excitatory neurons in the network, the interneuron is disinhibiting the network (Fig. 7.1C). Using disinhibition, the strength of other network motives (i.e. feedforward or feedback inhibition) can be tuned with respect to afferent excitation and with respect to each other. Disinhibitory neurons usually extend over a small area of cortex (Pronneke A et al. 2015), thus disinhibition may serve to limit the spatial extent of cortical activation. Somatopy found throughout the somatosensory pathway and specifically in S1 and PPC (Chapter 3) could thus be governed in part by disinhibitory circuits, in addition to spatial mapping of feedforward cortico-cortical inputs onto pyramidal neurons (Lee T et al. 2011). This could be tested by optogenetic or chemogenetic disruption of (disinhibitory) VIP neurons in PPC and identifying changes to somatotopic responses of pyramidal neurons. Inputs onto disinhibitory interneurons often have a long-range and top-down character (Lee S et al. 2013; Pi HJ et al. 2013; Zhang S et al. 2014) and may serve to inform the network of the behavioral state (Fu Y et al. 2014) and adjust computational properties to current requirements (Fu Y et al. 2014; Zhang S et al. 2014). It can be hypothesized that long-range inputs from motor systems onto disinhibitory mPFC interneurons drive spiking rate increases during motor output and that a separate (potentially neuromodulatory (Pi HJ et al. 2013; Fu Y et al. 2014) input on a distinct subset of disinhibitory interneurons drives contextual spiking rate modulation (Chapter 6). This could be tested by using silicon probe recordings in mPFC in the ‘Go’/’No-go’ tactile discrimination task, while at the same time performing closed-loop optogenetic inhibition (Siegle JH and MA Wilson 2014) of disinhibitory neurons (Pronneke A et al. 2015) during detected motor output. If negatively modulated neurons are no longer negatively modulated when disinhibitory neurons are inhibited, we can attribute our findings to a disinhibitory circuit. Furthermore, it is thought that a major mode of neuromodulatory influence is through disinhibitory circuits (Krulikov I and B Rudy 2008; Letzkus JJ et al. 2011; Fu Y et al. 2014). It is very possible that shifts in neuromodulator-driven disinhibition underlie changes in spiking rates (Chapter 3-4) and neuronal encoding of stimulus (Chapter 4) between anaesthetized and

**FIGURE 7.1** Common circuit motifs in cortex. (A) In feedforward inhibition motifs afferent input synapses on a pyramidal and an interneuron, if this brings the interneuron to spike it will inhibit the pyramidal neuron. (B) Feedback inhibition occurs in the local circuit, one pyramidal neuron spikes and if it brings a connected interneuron to spike it will in turn inhibit either the excited pyramidal (recurrent inhibition) or other pyramidal neurons in the local circuit [lateral inhibition]. (C) In the disinhibitory circuit motif one interneuron inhibits other interneurons, reducing inhibitory drive onto pyramidal neurons. Figure adapted from Tremblay R et al. (2016).
The brain is the computing entity of the body, it drives behavior and thus it needs to contain relevant information to perform computations on. Neurons are the elements containing or encoding information in the brain. In this section I will take a look at two frameworks for the interpretation of single neuron spiking for information encoding, being rate coding and temporal coding. For the duration of this section, I will use the word information for the interpretation of single neuron spiking for information encoding, being rate coding or encoding information in the brain. In this section I will take a look at two frameworks relevant information to perform computations on. Neurons are the elements containing spatiotemporal differences in attention encoding between mPFC areas.

The experimental work presented in this dissertation mostly describes findings from excitatory pyramidal neurons, and not interneurons. This decision was a conscious one and has two main reasons. The strongest argument for the consideration of pyramidal neurons, and not interneurons, for the study of neuronal encoding of information is as follows: The cortical regions that I used as model systems for cortical functioning (PPC and mPFC) are not in direct control of motor behavior. This means that any information encoded in these regions must necessarily be projected to other (output) regions before it can influence cognitive output. The pyramidal neurons are the output of cortex and the results of any computations in PPC and mPFC must therefore be encoded in the pyramidal population. It is for the same reason that disturbing spiking of pyramidal neurons, as done in Chapter 5, will disturb cortical output and thus all computations that originate in the targeted stretch of cortex. The second, more practical reason is that pyramidal neurons are by far the largest class of neurons in cortex, making up 80-90% of neurons. This means that, without targeting methods (Kvitsiani D et al. 2013; Pi HJ et al. 2013; Kim D et al. 2016; Lagler M et al. 2016; Munoz W et al. 2017), it is very difficult to electrophysiologically record from interneurons. When we record cortical spiking activity using juxtasomal (Chapters 2-4) or multi-electrode recording techniques (Chapters 3 and 6), less than 10% of units is identified as putative interneurons based on their waveform (Baeg EH et al. 2001; Bartho P et al. 2004) and these represent a heterogeneous class of neurons.

Neuronal encoding

The brain is the computing entity of the body, it drives behavior and thus it needs to contain relevant information to perform computations on. Neurons are the elements containing or encoding information in the brain. In this section I will take a look at two frameworks for the interpretation of single neuron spiking for information encoding, being rate coding and temporal coding. For the duration of this section, I will use the word information for an abstract and relatively loosely defined concept, it can take many shapes and forms depending on the brain regions, computations and behaviors under consideration. Below in the section Cognitive output, I will put the concept information in perspective and will provide some examples, such as sensory input, decisions, and behavioral output, and I will try to put these in perspective with the encoding frameworks covered here.

The rate coding perspective to neuronal encoding, assumes that the spiking rate (number of spikes per unit of time) contains all information that can be decoded from the spike train. As we will see below, there are other, more efficient codes. However, I will first discuss our findings with a rate code in mind, taking sensory systems as an example. In sensory systems with a rate code, each neuron has a preferred stimulus and a range of spiking rates that it can assume. Higher spiking rates reflect a closer match to the preferred stimulus and downstream neurons will sample the spiking rate of the neuron to determine how close to preferred the stimulus is. For this code to be precise it needs to be faithful and every time the same stimulus is shown, the neuron should produce the same spiking rate. Experiments have shown that this is not the case, therefore we have to assume some trial-to-trial noise, which may be attributable to internal processes. For most studies in this dissertation I took a rate coding perspective and I used multiple repetitions of the same stimulus/trial type to find mean neuronal spiking responses, taking out most of the independent noise. In Chapter 3 we look at how tactile stimuli are encoded by PPC neurons. Taking a rate coding perspective, we find unidirectional spiking rate increases after tactile events. Spiking rates increase when whiskers are stimulated (L5), when the rat starts whisking (L2-4 and L6) and when the rat touches an object (L2-4), while none of these neurons or layers show spiking rate decreases. This is similar to sensory brain regions. Sensory regions are tuned to a narrow band of stimuli and respond to changes in the sensory environment, i.e. to appearance and presence of a stimulus, but rarely to its absence and lack of change (however, see de Haan R et al. (2013)). In mPFC, however, approximately equal numbers of neurons increase and decrease their spiking during tactile decision making (see Chapter 6). This difference may be due to our bias during recording of deep layer mPFC neurons; these usually have higher spiking rates and therefore have a larger bandwidth to decrease spiking. As I have shown in the section on cortical neurons, decreased spiking rates in response to stimuli may reflect lateral inhibition. It is possible that populations of PPC neurons encoding tactile stimuli do not have opposing ‘no stimulus’ populations, but rather that competing populations have more abstract coding rules. This could mean that populations integrate a wider range of inputs and that tactile stimulation by itself is present throughout, or that tactile input is not sufficient to drive lateral inhibition. The results in Chapter 4 do not make a lot of sense when seen through a rate coding lens. Neurons in mPFC L5 preferentially spike in short bursts of two or more action potentials. If during analysis we would use temporal windows of 50-250 ms, as is common in mPFC research, there would be no difference between spikes with inter-spike intervals of 10 ms and those with 25 ms. Physiologically, however, high-frequency action potential bursts
may be very distinct from long-interval spikes, and therefore the information encoded in them could be very different. We show that mPFC L5 has relatively low threshold for calcium electrogenesis, meaning that relatively low frequencies could drive intracellular calcium-dependent processes, such as plasticity. This in turn could mean that mPFC L5 changes its encoding with relative ease and that these neurons may integrate into new ensembles during learning and fine-tuning of behavior. This cannot happen if spiking rates remain below electrogenesis threshold, therefore rate coding may not in all cases be the most relevant framework to study cortical encoding.

Attention behavior can be disrupted using reduction of spiking rates in mPFC pyramidal neurons (Chapter 5). At first glance, this seems to point towards rate coding of attention in mPFC, but changes in spiking rates could automatically also lead to disruption of other (temporal) codes. Temporal codes, in contrast to rate codes, assume that information is not purely encoded in the number of spikes per unit of time, but rather that information can also be contained in temporal measures, such as inter-spike intervals and ongoing oscillations. A large reduction of spiking rates, such as is (probably) the result of long-lasting optogenetic inhibition (Chapter 5), would also disrupt information encoded in inter-spike intervals, while any remaining spikes may still be phase-locked to ongoing oscillations, but the remaining output may not be strong enough to drive downstream circuits. I can draw conclusions on cortical encoding using a rate coding framework (Chapter 3 and 5), but I also find strong evidence for a temporal code (Chapter 3), while our manipulation experiments (Chapter 4) do not shed light on either temporal or rate coding. Cortex may not always utilize either a rate or temporal code, but rather it may switch between coding schemes or the codes may be complementary (Melzer P et al. 2006; Zuo Y et al. 2015).

Several approaches could be taken to further elucidate the rate or temporal code in any cortex during behavior. For example, viral expression of chemogenetic proteins (excitatory or inhibitory designer receptors exclusively activated by designer drugs (DREADDs)) can slightly shift spiking rates of cortical neurons in a small segment of relevant cortex up or down. Activation of these receptors leads to membrane potential shifts of a few millivolts and should not strongly disrupt spike timing, while it will affect the spiking rate. Changes of behavior or downstream encoding would be due to rate codes, while unchanged behavior/downstream encoding would point towards temporal coding. These experiments of course need a control to show that the segment of cortex is involved in the behavior that is being studied. Additionally, closed-loop optogenetic manipulations of identified bursting neuronal populations during behavior could be used. Using electrophysiology and optogenetic tagging (with excitatory opsins) behaviors that reliably lead to bursting in the population of interest can be identified. Spikes detected during burst-linked behavior could then trigger a laser (closed-loop) to activate an inhibitory opsin expressed in the same population. These experiments, though extremely challenging, would be very informative on what coding schemes are used to encode which types of information.
the black box inward. In this way, like peeling an onion, we can peel back layer by layer of neuronal processing. This means exactly understanding the features represented at every level of processing and understanding the code that represents them. I feel strongly that once we understand exactly what information enters and leaves the mPFC or PPC, we can fully understand the cognitive processes that are represented.

**Cognitive output**

In this dissertation I used the term cognitive output to describe the performance of an animal in a behavioral paradigm. Behavioral paradigms have been designed to probe a host of cognitive processes, but these can seldom be probed in isolation. Cognitive output usually is a composite of many cognitive processes, such as sensation, working memory, attention, and motivation. Without motivation an animal may not perform a task and without well-directed attention it may not extract the right information from sensation. In the previous chapters I have described three behavioral paradigms, these are directed at the study of tactile sensation in Chapter 3, sustained attention in Chapter 5 and decision making, reward and punishment processing, and behavioral updating in Chapter 6. These paradigms have their strong points and their weaknesses, which I will discuss below.

Of the used paradigms, the 5-choice serial reaction time task (5-CSRTT) in Chapter 5 has been validated most thoroughly (Robbins TW 2002). It was developed as a rodent version of the continuous performance test for humans (Rosvold HE et al. 1956). The trials of this paradigm are highly sequential. Trials are initiated by nose pokes in the reward port, followed by a 5 s ‘preparatory attention’ period after which a cue hole is illuminated for 1 s and the rat gets an additional 1 s to respond to the cue with a nose poke in the illuminated hole. This paradigm has several read-outs and provides rich information on several cognitive or behavioral parameters and it is sensitive to pharmacological disturbance. The major read-out is response accuracy, the percentage of correct trials over correct + incorrect trials, which should reflect attention directed towards the task; when the rat is more attentive it will choose the correct aperture more often. Further, omission of responding can be counted and reflects (lack of) motivation and is also a measure related to attention. Finally, premature responses, i.e. when the rat responds before the cue, reflect impulsivity and lack of behavioral inhibition. As described above under Neuronal encoding, attention is not well-defined and may consist of several processes.

![Figure 7.2](image-url)

**Figure 7.2. The problem of looking for information encoding in higher order cortices.** *(A)* Using a black box approach we can start thinking about how the brain transforms information from in- to output and what this means with respect to computations in the brain. A problem with this model is that it views the brain as a one-step transformational unit, which it is not. *(B)* Top: A more faithful representation of information transduction and processing would be a multi-layer heavily interconnected system of black boxes. For simplicity only feedforward excitation is visualized. Red boxes indicate abstracted location of PPC (1) and mPFC (2) in the network. Bottom: Complexity of encoding is low ‘close to the outside world’, where it represents sensation or action, but it is much higher at intermediate stages during more abstract processing. *(C)* As processes become more abstract they generally become less quantifiable and therefore harder to interpret.
in theory be sufficient if the animal faces the wall with the apertures. Adding visual or auditory distractors could increase the validity of the 5-CSRTT as an attentional model. A different aspect of attention that is said to be probed using the 5-CSRTT is sustained attention, i.e. keeping focus or attention at the task at hand for a prolonged period. This is a valid claim if we assume that the rat is focused for the full preparatory period. In the current form, however, trials have fixed intervals and rats could adopt simple timing strategies (i.e. turn towards the choice wall right before the cue is expected), meaning that they do not need sustained attention during the trial. Evidence in favor of the 5-CSRTT measuring sustained attention is that rats start ‘scanning’ the cue holes well in advance of cue appearance, and that they thus pay attention for a prolonged period of time. The task could be geared more towards sustained attention by taking out predictability, for example by varying the duration of the preparatory attention period. One final major downside at the 5-CSRTT in its current form is the immense time investments necessary for training. It takes up to 3 months to properly train rats as is and adding distractors or variable intervals will likely increase the training period even further. Fortunately, recent developments have made the development of home cage training possible (Remmelink E et al. 2016; Bruinsma et al., accepted). This means that the 5-CSRTT arena is connected with a tube to the home cage and the rat is allowed to freely walk back and forth between the two. Depending on the research questions, the rat can then either start trials throughout the day or during a fixed time interval. Training time under this regime is reduced to 1-2 weeks (Bruinsma et al., under review). Nonetheless, strict validation of this training method is needed for large scale adoption.

Free whisking and naïve touch, the paradigms used in Chapter 3, are very relevant for the study of neuronal encoding of voluntary sensation. Rats in these paradigms are implanted with a head post to get head-fixed and they are habituated to head-fixation for several days prior to the experiment. On the day that physiology is recorded, rats are filmed using high-speed videography to determine their behavior and they are presented with an object to touch that they have never encountered before. In many other paradigms, animals are trained to report a stimulus or distinguish between several stimuli. This means that they have seen the same stimulus repeatedly and that they are rewarded or punished according to their response. Repeated exposure and valence-predictability of stimuli may change neuronal encoding of the stimulus and therefore may influence the code that represents it. Most situations/stimuli that we encounter in daily life do not carry value and have not been encountered many times before. If we want to learn about neuronal encoding of regular real-life stimuli, we need paradigms that simulate the real-life encounter of stimuli. However, the naïve touch paradigm has its own downsides. Head-fixation does not allow for free movement and, even after habituation, sometimes leads to nervous behavior. Stress and the neuromodulation it involves have the potential to change the coding schemes used by neurons, depriving us of a clear view of natural, naïve neuronal encoding. A potential solution could be to review blood-cortisol levels (or heart rate) during habituation and performing recordings only after the rat has been quantitatively confirmed to be stress-free. Head-fixation is used to stabilize the head for two different, but related reasons: 1) To have a stable, non-moving brain for outside approach with electrodes, and 2) to standardize the sensory environment for averaging purposes. The first of these points brings with it another problem, the necessity for pre-recording surgery and related potential lasting effects of anesthesia. These issues could easily be solved by using chronically implanted electrodes, as has become routine in recent years (Csicsvari J et al. 2003). Implantation can be done with or without using a ‘drive’ to move the array from site to site, and the first papers have even appeared showing juxtasmal and whole cell recordings in freely moving rodents (Lee AK et al. 2006; Diamantaki M et al. 2016; Lagler M et al. 2016; Munoz W et al. 2017; Diamantaki M et al. 2018), allowing for morphological identification and reconstruction of the recorded neuron. Standardization of the tactile environment is harder to accomplish without head-fixation, especially when low depth of field high-speed videography is needed to quantify behavior, as was the case in our experimental design. A potential solution is using larger depth of field videography from two orthogonal directions while providing the rat with a head-mounted tactile ‘object’ to ensure fixed location along the whiskers. Using 3D reconstruction of the whiskers and object in space (hence two-direction videography) one could quantify whisking episodes, touch events together with curvature changes and potential forces on the whisker follicle. Reconstruction in 3D should be relatively straightforward with modern GPU hardware and open source libraries. The final methodological problem of the free whisking and naïve touch paradigms is the lack of control over behavior. Rats will (almost) never repeat the exact same behavior, i.e. their whiskers will not follow the same trajectory multiple times at the same angular velocity and touches will seldom have the exact same duration, curvature change and impact velocity. This makes it very hard to judge what behavioral parameters are encoded in S1 neuronal spiking and whether this signal is clean or noisy. And even if these parameters could be duplicated using behavioral manipulation (e.g. using optogenetic manipulation of facial muscles), there would still be learning and expectation effects (the rat knows where to expect the object after first touch), potentially influencing encoding at the cortical level. It is very hard to tackle this final problem and I have not been able to think of a realistic solution.

The ‘Go’/’No-go’ tactile object localization task used in Chapter 6 is an adaptation of a paradigm frequently used for the study of S1 in mice (O’Connor et al. 2010a,b). We adapted this task for use in rats and standardized it as much as possible, placing the ‘Go’ and ‘No-go’ locations on the same azimuth from the whisker follicle (Pammer L et al. 2013). In the task, water restricted, head-fixed rats are trained to use their whiskers to distinguish
two locations of an object. If the object is proximal, the rat should lick to receive a water reward, while they should refrain from licking to avoid a time out punishment signaled with a tone. The paradigm has proven very useful to study tactile encoding in S1, S2 and motor cortex in mice. This is the first time the task is adapted to rats and used to study higher level cortex, in our case mPFC. My aim was to study the neural correlates of decision making, reward processing and behavioral updating during learned behavior in mPFC of rats performing the ‘Go’/’No-go’ task. I was able to draw a lot of conclusions on reward processing and behavioral updating, but at the same time a lot of questions remain and new questions have been raised. First and foremost, causal involvement of mPFC in the tactile object localization task should be established. The most elegant method would be to express inhibitory opsins in mPFC pyramidal neurons (as in Chapter 5) and activate these in some trials during the grace period, in some trials during the response window and in some trials not at all. One of several things can happen, the simplest being no change with or without mPFC manipulation, discarding mPFC as essential during decision making in this task. If rats improve performance for both ‘Go’ and ‘No-go’ trials, mPFC function is counteracting correct decision making. If rats reduce performance for both ‘Go’ and ‘No-go’ trials, mPFC is likely involved in the task, however, an alternative explanation is that mPFC inhibition distracts the rat from the task. If the rat starts licking more when mPFC is inhibited in both trial types, we can assume that mPFC is involved in impulse control, as is known from literature (Gourley SL and JR Taylor 2016; Luchicchi A et al. 2016; Kamigaki T and Y Dan 2017), this could be verified if rats start licking more often during ‘No-go’ trials when mPFC inhibition occurs only during the response window, but not the grace period. Finally, if rats lick in fewer trials when mPFC is inhibited, it may be that mPFC is necessary to generate motor commands or maintain motor behavior (Gourley SL and JR Taylor 2016), in the case of maintaining motor behavior this could be verified if rats stop licking after mPFC inhibition is started.

Furthermore, in the current paradigm it was impossible to disentangle neural correlates of decision making from those of perception and motor output, while motor output and reward processing are also strongly related in the current paradigm. Motor output, sensation, and decision are highly intertwined, since motor output is generated only if the rat decides it has sensed that the object was proximal. This problem could be partially solved by using an adaptation of the task, using a two-alternative forced choice paradigm (Mayrhofer JM et al. 2013). In this adaptation, one of two (active) responses have to be given for each trial, in our task that would mean two lick-ports both generating rewards, one for the decision ‘proximal’ and one for the decision ‘distal’. In this way, trials will always have a motor component, whichever decision the rat makes, averaging out most confounding neuronal correlates of motor behavior. Additional improvements involve temporally separating the sensory phase from the response and the outcome (reward/punishment) phases of the task. In this way, neural correlates of task phases or parameters are separated in time and will be easier to distinguish. However, this adaptation will integrate a working memory component into the paradigm, changing interpretation of task outcomes. It will probably also increase training times needed to get to stable performance.

At the conclusion of this dissertation we come back to the question “How do cortical neurons encode cognitive output?” The short answer based on my work is that several similar, but distinct codes are identified in cortex. For example, single neurons in PPC can encode vibrissal sensation and whisker motion in their spiking rate (Chapter 3), while mPFC L5 neurons use bursts in addition to single spikes for their encoding scheme (Chapter 4). Furthermore, when the rate code in dorsal or ventral mPFC is disrupted by strongly reducing spiking, encoding of attention and subsequent attention performance is disrupted (Chapter 5). Finally, distinct subpopulations of mPFC neurons encode behavioral adaptation, motor output, and its context with increases and decreases of spiking rates (Chapter 6). But as often in science, the above conclusions are likely only a part of the answer. At the same time, the insights obtained from this work generate more questions that will hopefully guide future research.
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