A rewarding view on the mouse visual cortex: Effects of associative learning and cortical state on early visual processing in the brain

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Discussion
Chapter 5. Discussion

The central goal of this thesis is to contribute to a better understanding of how the daily routines of seeing, experiencing and learning affect the neural circuits of the very senses that are used to perform these functions in the first place. As has been described in the Introduction, neurons in the visual cortex can be modulated by, for instance, internal state and attention, but can also incorporate long-term changes as a result of learning. The work in this thesis aimed to provide evidence for reward related plasticity in V1, and insight into the mechanisms that the visual cortex employs to improve stimulus representations, either as a consequence of cortical state or learning.

Summary of chapter 2: Selective reward effects in V1 assemblies

The first experimental chapter focused on the question of how learning a visual stimulus-reward association changes stimulus specific circuits in the visual cortex. In a video-screen equipped conditioning chamber, mice learned that the presentation of an oriented moving grating predicted the delivery of a food-pellet, while presentation of gratings having an orthogonal orientation was never followed by reward. After these animals reliably expressed the learned association in their behavior, orientation tuning of large sets of cells in the primary visual cortex was assessed using in vivo two-photon calcium imaging under anesthetized conditions. Neurons in V1 that had a preferred orientation similar to the conditioned orientation, showed overall broader tuning curves as well as increased selectivity for stimulus direction. These effects were, at least in part, related to increased amplitudes of responses to the rewarded stimulus orientation, as compared to the unrewarded stimulus. In addition, cells with a tuning preference for the reward-associated orientation were more likely to be neighbors in the two-dimensional imaging field of view, suggesting a non-uniform effect of reward-related plasticity in the visual cortex. The primary conclusion of this study was that stimulus-reward learning selectively changes response properties in stimulus specific sets of neurons, already at the level of the primary visual cortex.

Summary of chapter 3: Conditioning refines spatial coding in V1

The next chapter addressed the question of whether primary sensory cortical representations optimize their response properties and organization to support detection and/or discrimination of behaviorally relevant stimuli. Mice were exposed to a behavioral setting in which the location (up or down) of a small square patch of moving grating was indicative of reward delivery. The orientation and other features of the moving grating were held constant for the two different outcome conditions. The spatial mapping of the visual field in V1 restricted the reward association to a single region of cortex, while the non-rewarded region and non-trained stimulus orienta-
tions could be used to study effects of visual conditioning and exposure. Mesoscale intrinsic optical signal imaging under light anesthesia showed that cortical representations for trained stimuli in the rewarded and non-rewarded locations were spatially more segregated after conditioning, as compared to before. Small populations of calcium-imaged V1 neurons located at the border between the two stimulus representations discriminated the conditioned stimuli better, compared to untrained control orientations. The improved population coding for the conditioned stimuli was reflected in specific differences in tuning curves as well as in the correlation structure of responses to the trained stimuli. This study indicated that learning a stimulus-reward association improves the discriminability of the rewarded from non-rewarded stimuli, affecting cortical representations at the level of single-cell tuning curves, population correlation structure and mesoscopic spatial organizations.

Summary of chapter 4: Anesthesia impairs direction coding in mouse V1

The last chapter revolved around the question of how a change in brain state, i.e. the difference between wakefulness and being under anesthesia, alters activity patterns of neurons in the primary visual cortex that emerge spontaneously and in response to visual stimuli. Using two-photon calcium imaging, a set of V1 neurons was imaged while the mouse was awake and subsequently revisited after the mouse was anesthetized (or in reversed order). Activity patterns of randomly selected pairs of cells were much more strongly correlated under anesthesia. This was especially apparent in the low frequency domain, suggesting that cells entrained their activity patterns to a single dominant rhythm or input rather than responding in a more individualistic fashion. Interestingly, the way neurons were tuned to stimulus orientation was not affected, but selectivity for stimulus direction was reduced. This loss in selectivity could not be attributed to overall reductions in response amplitudes, or to increased correlations in activity patterns of pairs of cells. The reduction in selectivity was rather explained by an increase in response amplitude to the null direction of the cells. The overall conclusion was that spontaneous and stimulus-selective activity patterns of primary visual cortex neurons are shaped by processes that can change between brain states and do not only reflect a straightforward linear integration of converging synaptic connectivity.

The data that were presented in these experimental chapters have certainly not provided a complete picture of how cortical state and learning affect the function of the visual system. In the next sections of this discussion I will therefore attempt to combine and supplement the experimental results of this thesis with other
5.1 Learning related plasticity in the primary visual cortex

5.1.1 Changes in response properties

How do changes in response properties of primary visual cortex neurons contribute to learning of a visual stimulus reward association? To fully answer this question, it is preferable to have a complete understanding of the computations that are performed in the primary visual cortex. Because my knowledge of visual cortex function is, to say the least, incomplete, I propose to discuss visual cortex cells primarily in their role of feature detection. From this point of view the answer to the question must at least include that plasticity, as observed in the visual cortex of a mouse in the experiments of chapter 2 and 3, besides supporting the memory of a learned association, could improve the mouse’s ability to detect, recognize and discriminate the learned stimuli. The next paragraphs will discuss the results of chapters 2 and 3 in the context of these functions.

Detection

Increasing the chance of seeing a behaviorally relevant stimulus is a useful capacity. Association of a stimulus with reward can increase its saliency and therefore the likelihood of the stimulus to draw the attention of the organism (Anderson et al., 2011). The probability of a visual stimulus to be detected likely depends on the amount of activity it generates in primary visual cortex (Histed and Maunsell, 2014) and indeed, the population response amplitude to a rewarded stimulus increases with learning (Poort et al., 2015). The main results of chapter 2 and 3 support this finding of increased response amplitudes to conditioned, and especially reward-associated, stimuli. Considering that most inhibitory neurons in mouse cortical layer 2/3 are weakly orientation-tuned (Liu et al., 2009; Kerlin et al., 2010; Runyan et al., 2010), the highly orientation-selective effect of conditioning is unlikely solely mediated by changes in local inhibitory circuits, although there is no direct evidence excluding this possibility. It is also not very likely that the increased responses reflect acute effects of top-down or neuromodulatory inputs because the recordings in chapter 2 and 3 were made under anesthesia, which generally disrupts such influences (Lamme et al., 1998b; Van Dort et al., 2008; Poort et al., 2015; Makino and Komiyama, 2015).
5.1. Learning related plasticity in the primary visual cortex

Therefore, by exclusion, it is most likely that the increased response amplitudes reflect a potentiation of bottom-up excitatory synapses (Cooke and Bear, 2010) or lateral excitatory connectivity (Ko et al., 2011; Cossell et al., 2015).

A second observation in chapter 2 and 3 was that cells, preferentially responding to the reward-associated oriented grating, showed increased selectivity for movement direction. This could be achieved through two different mechanisms. First, increased direction selectivity could have directly resulted from a potentiation of excitatory inputs selectively driving the responses to stimuli of the preferred direction. Alternatively, local inhibitory networks may have mediated this effect. The observed overall increase in response amplitude to the conditioned orientation likely recruited increased network-wide inhibition (Shu et al., 2003; Katzner et al., 2011; Taub et al., 2013; Xue et al., 2014), which has been shown to sharpen direction selectivity (Lee et al., 2012). Therefore, the effect on direction selectivity could reflect direction-specific reward-related plasticity, but also emerge as a mere consequence of increased network activity for the conditioned orientation.

Recognition

It can be beneficial to an organism to have the ability to recognize behaviorally relevant visual patterns under poor circumstances, like when an image falling onto the retina is distorted. Contextual pattern completion has been associated with the function of the hippocampal circuit CA3 (Mizumori et al., 1989; Rennó-Costa et al., 2014; Rolls, 2015), which is proposed to operate using its recurrent circuitry on suggested pattern-separated inputs from dentate gyrus (Rolls and Kesner, 2006; Leutgeb et al., 2007; Clelland et al., 2009; Nakashiba et al., 2012). The cortical visual system may as well employ its strong lateral and recurrent connectivity (Lamme and Roelfsema, 2000) to perform the task of compensating for incomplete visual inputs (Lerner et al., 2004; Chen et al., 2010; Tang et al., 2014). Indeed, conditioning has been found to increase stimulus generalization, which could be attributed to strengthened abilities for pattern completion (e.g. Hovland, 1937; Hoffman and Fleshler, 1961; Siegel et al., 1968; Lissek et al., 2008). As shown in chapter 2 and 3, the response amplitude to the conditioned orientation was increased in cells that were tuned to stimuli that slightly differed from this orientation. This may directly reflect bottom-up stimulus generalization through converging inputs onto single cells, or rather be the consequence of increased lateral connectivity between layer 2/3 cells that are roughly tuned to the conditioned orientation (Ko et al., 2011; Cossell et al., 2015) and support pattern completion or stimulus generalization at the microcircuit level.
Chapter 5. Discussion

Discrimination

Last, when exposed to an environment containing multiple visual patterns, an organism will benefit from the ability to differentially represent behaviorally relevant stimuli. Orientation-tuned cells can increase their ability of discriminating two very similar orientations by sharpening their tuning curve, if the steepest part of their tuning curves falls between the to be discriminated stimuli, and thereby increases the difference in their response amplitude to those two stimuli (Schoups et al., 2001; Murray and Wojciulik, 2004; Yang and Maunsell, 2004). No such effect on orientation tuning was observed in the studies of chapter 2 and 3; tuning curves were actually broadened, potentially to amplify the summed V1 output for the conditioned stimuli. It has to be emphasized here that the orientation discrimination in chapter 2 was between stimuli differing 90 degrees in orientation, which already leads to very different response patterns in the visual cortex in untrained animals and leaves hardly room, nor need, for improvement.

In chapter 3, orientation was altogether irrelevant for differentiating the reward predicting from the non-reward predicting stimulus. Location in visual space rather predicted reward or its absence, and indeed, the spatial representation in V1 showed a clear signature of sharpening; a stronger differentiation in response amplitude to the two locations and a steeper retinotopic gradient for the conditioned orientation compared to other orientations. Local inhibitory circuits are spatially restricted and can be differentially activated by top-down inputs during learning (Zhang et al., 2014), but are not likely to have mediated these mesoscale effects of conditioning because the spatial selectivity of inhibition is affected by anesthesia (Adesnik et al., 2012; Haider et al., 2012). Alternatively, the increased mesoscopic spatial selectivity could arise from selective potentiation of lateral excitatory connectivity. Neurons could preferentially strengthen connectivity with other local neurons, tuned to the same orientation. Cells, that were tuned to one location, but embedded in the cortical region that was predominantly responding to the other location (Smith and Häusser, 2010; Bonin et al., 2011), may through such a mechanism become tuned to the location of the majority of surrounding cells. This situation would increase the local homogeneity of the cortical representation for location of conditioned stimuli and lead to a sharpening of the location-representation boundary.

In summary, the conditioning related changes in orientation selective receptive fields of primary visual cortex neurons may have served to facilitate multiple aspects of the visual computation. Increased response amplitudes for conditioned and similar orientations likely improved the detection of potentially degraded versions of the behaviorally relevant stimuli. The sharper organization of the retinotopic map may have supported discriminability of the conditioned stimuli. In each of these cases,
5.1. Learning related plasticity in the primary visual cortex

Multiple mechanisms can be hypothesized to underlie learning effects, both through changes in inhibitory circuits or changes in excitatory connectivity. Knowledge of tuning changes in the different identified subtypes of inhibitory neurons, simultaneously with neighboring excitatory cells, may reveal to what extent plasticity in function of inhibitory cells is involved in learning. Additionally, one could measure these learning-related changes in tuning curves, while selectively interfering with feedback from higher visual areas and/or neuromodulatory inputs, to reveal their contributions (e.g. Makino and Komiyama, 2015). To answer the question of whether changes in excitatory bottom-up and lateral connectivity contribute to learning effects will require, for instance, an in vivo/in vitro approach to study connectivity between functionally identified cells (Volker Scheuss and Simon Weiler, personal communication; Ko et al., 2011; Cossell et al., 2015; Okun et al., 2015) or in vivo circuit mapping (Packer et al., 2014; Wertz et al., 2015).

5.1.2 Population coding

Considering the tasks that the mice performed, as described in chapter 2 and 3, another important function of the primary visual cortex would be to ‘inform’ subsequent brain areas of which visual features, potentially belonging to the rewarded/non-rewarded stimulus patterns, were projected onto the retina. These higher brain areas will thus have a mechanism to ‘interpret’ the joint responses, or population code, of V1 neurons. As described above, the primary visual cortex may improve this code for relevant stimuli by adjusting response amplitudes and selectivity of tuning curves of specific subsets of cells. As in any concert, however, the quality of a performance does not only hinge on the tuning of the individual instruments, but also on the way the artists, or neurons, hit their tuned strings together. Some specific aspects of this population code (or neuronal concert), signal correlations and noise correlations and their mutual interaction, were investigated in chapter 3.

Signal correlations

When considering a pair of neurons that have a similar preferred orientation, the most obvious correlation between responses of these two cells would likely be the result of the cells having a preference for responding to the same stimuli. The quantification of this correlation, the signal correlation ($r_{\text{sig}}$ or $r_{\text{signal}}$), reflects the similarity of the tuning curves or receptive fields of these two cells (Fig. 5.1A) and is determined by inputs and connectivity that leads the cell to be selective for a specific set of stimuli (Oram et al., 1998; Averbeck et al., 2006; Cossell et al., 2015). Low signal correlations could reflect sparse, efficient population coding for learned stimuli (Atick, 2011;
Pitkow and Meister, 2012; Gdalyahu et al., 2012) and have a positive effect on the amount of stimulus information that can be represented (Chelaru and Dragoi, 2008). Cells having redundant tuning curves with high signal correlations will under most circumstances lead to a less than optimal amount of information being represented in their population activity patterns (Atick, 2011).

High signal correlations, however, can also reflect the existence of a specialized interconnected group of cells, “choristers”, that transmit stimulus information more reliably (Kenet et al., 2005; Osborne et al., 2008; Hung et al., 2014; Lin et al., 2014; Okun et al., 2015). In chapter 3, we observed overall higher signal correlations in groups of cells that were tuned to the conditioned stimulus, as compared to stimuli having other orientations. This could be explained by increased interconnectivity of cells tuned to the conditioned stimuli, reflecting the existence of stimulus specific assemblies consisting of neurons that co-excite each other and thus have more similar tuning curves (see also Chapter 2; Hebb, 1949). Such connectivity may impose an attractor state of neuronal activity patterns, and can thus increase the likelihood of the network to converge to representing behaviorally relevant visual information. Alternatively, increased functional connectivity between cells tuned to conditioned stimuli might simply emerge as a consequence of rigorous simultaneous activity patterns during behavior, without serving a computational purpose or perceptual advantage.

Noise correlations

The noise correlation (or spike count correlation; $r_{\text{noise}}$ or $r_{\text{sc}}$) reflects all correlations between responses of a pair of neurons that cannot be explained by the signal correlation. The noise correlation is quantified using fluctuations of neuronal responses to repeated presentations of the same stimulus (Fig. 5.1B; Oram et al., 1998; Shadlen and Newsome, 1998; Averbeck et al., 2006) and can reflect any shared modulating factor of the activity of two or more neurons. These factors include physiological influences like activity patterns of connected cells, impact of feedback or lateral projections from other cortical areas and neuromodulation, but also factors like anesthesia-depth, image movement and other technical artifacts can affect these correlations. Positive physiological (noise) correlations have been implicated in setting an upper bound on the amount of information that a system can represent, by reducing the number of independent degrees of freedom of the population code (Sompolinsky et al., 2001). In chapter 3, cells that were tuned to the conditioned stimuli had overall lower noise correlations, which can help increasing the amount of stimulus information being represented, but this ultimately depends on the tuning preferences of the cells between which the noise-correlations are considered (see next section; Oram et al., 1998; Averbeck et al., 2006; Poort and Roelfsema, 2008).
5.1. Learning related plasticity in the primary visual cortex

**Figure 5.1: Signal correlations, noise correlations and the sign-rule.**

**A** An example pair of cells with a high signal correlation (up) and a low signal correlation (down). Red and blue curves represent orientation tuning curves.

**B** Each dot represents the joined response of two cells. The amplitude of cell 1’s response maps to the x-axis, cell 2’s response maps to the y-axis. Left panel: In absence of noise correlations, the response amplitude of two cells varies independently. Middle panel: When $r_{\text{noise}}$ is positive, the variability in response amplitude of the two cells is positively correlated. Right panel: A negative $r_{\text{noise}}$ results in the opposite effect. When one cell responds strongly, the other responds weakly (anti-correlation).

**C** Joined responses of two cells (on x and y axis) to two different stimuli (purple and green dots). Upper panel: $r_{\text{signal}}$ is positive, both cells respond more strongly to stimulus 1 compared to stimulus 2. Lower panel: $r_{\text{signal}}$ is negative, cell 1 responds more strongly to one stimulus, while cell 2 responds more strongly to the other stimulus.

**D** When $r_{\text{signal}}$ has an opposite sign compared to $r_{\text{noise}}$, the joined response distributions of the two stimuli are stretched as in B (middle or right panel) but such that the joined population response shows less overlap.

**E** If $r_{\text{signal}}$ has the same sign as $r_{\text{noise}}$, the response space stretches again, but now increases the overlap between joined response distributions of the two stimuli and reduces the ability to discriminate them. In general, the sign-rule states that signal and noise correlations of identical signs hamper the discriminability of the population code, while opposite signs of signal and noise correlations improve the discriminability of joined response patterns (see also e.g. Oram et al., 1998; Averbeck et al., 2006).
Interactions between signal and noise correlations

Noise and signal correlations can interact to increase or decrease the discriminability of population responses (Fig. 5.1C–E; Oram et al., 1998; Averbeck et al., 2006; Poort and Roelfsema, 2008) and such interactions were indeed observed in the forebrain of birds that had learned to discriminate songs and in the visual cortex of monkeys discriminating curves (Poort and Roelfsema, 2008; Jeanne et al., 2013). One of the main findings in chapter 3 is of a similar nature; groups of cells that preferentially responded to conditioned stimuli and that had low signal correlations were more strongly driven by common fluctuations, while the opposite was the case for high signal correlation cell-groups. As extensively described in the discussion of Chapter 3; when two cells have negative signal correlations, noise correlations improve the discriminability of their joined activity patterns (Fig. 5.1D). The opposite is true for pairs of cells that have largely overlapping stimulus preferences (strong positive noise correlations), in that case noise correlations lead to more overlap of their joined response patterns and hampers the discriminability of their ‘population code’ (sign-rule; Fig. 5.1E; Hu et al., 2014).

Recent work indicates that, at least in mouse visual cortex, joined modulations of activity patterns take effect partly through inhibitory circuits (Fu et al., 2014) and can be specifically modulated by learning visual associations (Makino and Komiyama, 2015). The changed amount and structure of noise correlations may have been imposed by altered top-down modulation of local inhibitory circuits, although for instance direct top-down modulation from retrosplenial cortex is abolished under anesthesia (Makino and Komiyama, 2015). Correlations in activity patterns could also reflect the common entrainment by specific oscillations in the local field potential (Nase et al., 2003). Non slow-wave oscillations, like theta and gamma band oscillations in rat and cat primary and higher visual areas, have been associated with reward and learning (Salazar et al., 2004; Zold and Hussain Shuler, 2015). Although such oscillations, or the way neurons entrain their activity to them, may be altered under anesthesia (Ruiz-Meijias et al., 2011), repeated conditioning- or reward-related synchronous activity may have left a lasting trace in the synaptic connectivity underlying the structure of signal and noise correlations in the experiments of chapter 3. And as observed in Chapter 4, anesthesia may increase overall correlations in the brain, but does not necessarily abolish all structure from these correlated activity patterns. Learning imposed traces could therefore, even in the absence of a rhythm that supported its formation, be observed as changes in the correlation structure under anesthesia.

Eventually, this all brings us back to the question of how higher visual (or other) brain areas interpret the V1 population code. The analyses used in chapter 3 were
suitable for linear decoding of correlated population codes, but may have ignored higher-order interactions (Graf et al., 2011). It has been proposed that the unknown mechanism by which higher visual areas integrate V1 population responses ignores these correlations altogether (Shadlen and Newsome, 1998; Shadlen and Movshon, 1999; Reid, 2001; London et al., 2010; Eyherabide and Samengo, 2013; Histed and Maunsell, 2014). These statements, however, are mainly concluded from experimental and theoretical studies investigating precise temporally coincident action potential activity versus average firing rate responses. Based on this work, it seems indeed likely that the visual cortex transmits the identity of a stimulus using a code that is primarily dependent on mean firing rates, rather than exact spike timing. Average single-trial stimulus-induced firing rates of multiple cells, however, can still be better distinguished when signal and noise correlations are structured according to the sign-rule (Fig. 5.1C–E). Therefore, although the overall amount of correlations in the visual cortex is rather low (Chapter 4; Ecker et al., 2010), the effect of correlations on population coding may be embedded in its specific structure (Poort and Roelfsema, 2008; Jeanne et al., 2013).

Techniques for functional and structural circuit tracing have by now the ability to discover how receptive fields of neurons in higher visual areas are constructed by mapping activity patterns of input neurons to a post-synaptic neuron (Cossell et al., 2015; Wertz et al., 2015). Future experiments may pursue how the response of a post-synaptic cell (potentially in a higher cortical area) varies as a function of activity of presynaptic cells in the primary visual cortex that provide its input. Such an approach can be used to reveal how simultaneous activity patterns of multiple neurons can facilitate each other. Moreover, studies performing specific manipulations of spatio-temporal activity patterns in visual cortex will be able to provide more insight into whether learned behaviors or discriminations depend on the correlation structure of the population response (Histed and Maunsell, 2014; Packer et al., 2014). A next step would be to specifically manipulate the correlation structure of a population of cells in accordance, or disagreement, with the sign-rule. Subsequently, one may attempt to synchronize activity patterns of V1 cells to internally generated rhythms of higher visual areas while animals perform a discrimination task as in Histed and Maunsell (2014) and Clancy et al. (2014). Similar efforts can be undertaken in the context brain machine interface development, all of which will certainly contribute to the general understanding of how the brain encodes and transmits information.

5.1.3 Evaluative feedback

Learning evidently alters stimulus processing in the primary visual cortex, but the mechanism that drives this plasticity is far more elusive. Learning may have occurred
through a reinforcement signal that was fed back into V1 and selectively altered processing of rewarded stimuli. Neuromodulators are strong candidates to perform this task, either through individual actions of e.g. dopamine or acetylcholine, or as a result of an interaction between multiple neuromodulators. Dopamine release has been associated with reward (Schultz, 1986) or error in prediction in reward (Schultz et al., 1997; Schultz, 2013), can selectively modulate plasticity in the striatum (Yagishita et al., 2014) and auditory cortex (Bao et al., 2001) and dopamine signals may be sufficient for learning to occur (Steinberg et al., 2013). There is some evidence supporting that dopamine signals make it to V1 (Febvret et al., 1991; Müller and Huston, 2007), but the techniques these studies employed to detect dopamine or dopamine fibers may have been very unspecific. Besides doubts that there is substantial dopamine release in V1, its role as reinforcement signal in general is strongly competed by the view that dopamine release signals unexpected events, novelty and/or a behavioral switching signal (Pennartz, 1995; Redgrave et al., 1999).

Cholinergic modulation is another potential candidate and it has been shown that it has the ability to modulate and facilitate experience dependent plasticity in the visual cortex (Bear and Singer, 1986; Kilgard and Merzenich, 1998). More recent studies strongly implicate acetylcholine in driving reward related activity patterns of primary visual cortex cells in the rat (Shuler and Bear, 2006; Gavornik et al., 2009; Chubykin et al., 2013). But in general, the effects of acetylcholine have been linked to attentional modulation and state dependent changes in cortical function rather than reward or reinforcement feedback (e.g. Hagan and Morris, 1988; Muir et al., 1994; Pennartz, 1995; Deco and Thiele, 2011; Polack et al., 2013; Pinto et al., 2013; Han et al., 2014). Selective plasticity in stimulus specific assemblies, however, does not necessarily have to be driven by reward selective circuits, but may have been mediated by effects of attentional modulation (Stănişor et al., 2013). Moreover, a very recent study strongly suggests a patchy distribution of cholinergic modulation in layer I of the visual cortex (Ji et al., 2015), which may explain the non-uniform spatial organization of cells preferring the rewarded conditioned stimulus in the experiments of chapter 2.

A final set of candidate circuits for mediating reward effects in V1 is the direct glutamatergic feedback projections from higher cortical and limbic areas of the brain (Pennartz, 1997). Anatomically, the existence of such projections to V1 from e.g. medial prefrontal cortex (in monkey and mouse; Freese and Amaral, 2005; Nguyen et al., 2015) and amygdala (in cat; Chen et al., 2009) has been verified using retrograde tracing. Providing glutamatergic feedback inputs directly onto dendrites of V1 pyramidal cells may strengthen or weaken specific synaptic connectivity through inducing NMDA receptor mediated currents, modulating intracellular calcium concentrations (Pennartz, 1997; Lamme and Roelfsema, 2000; Self et al., 2012). But these excitatory
projections may alternatively activate local interneuron circuits to bias V1 activity patterns (Fu et al., 2014; Petro et al., 2014; Zhang et al., 2014). The glutamatergic modulation of local V1 interneuron circuits has spatially restricted properties and can also provide an explanation for non-uniformly distributed reward effects observed in chapter 2 (Adesnik et al., 2012; Zhang et al., 2014; Chen et al., 2015), although direct glutamatergic feedback may be non-uniformly organized as well.

Future experiments studying the origin of learning related feedback in V1 could commence with directly blocking local involvement of neuromodulators or glutamatergic feedback connections during learning using local and selective inhibition of axonal projections or neuromodulator action through either disconnection lesions, optogenetic inhibition, pharmacology or a combinations of these (e.g. Zhang et al., 2014; Makino and Komiyama, 2015). A second, interesting lead is the non-uniform distribution of cells expressing cholinergic receptors in V1 (Ji et al., 2015), which offers the option to spatially match learning effects to the potential local presence of cholinergic modulation (although this has to be verified). Last, layer specificity of plasticity and learning in V1 has not been studied very extensively (but see e.g. Kreile et al., 2011). Connectivity of layer 2/3 is, however, more focused on local and distal corticocortical connections, such as those targeting higher visual areas, while layer 5 cells rather project to subcortical (like striatum and colliculus) and transcallosal targets. Stimulus tuning of visual cortex cells reflects the preferential tuning in the neighboring cortical regions where they project to (Glickfeld et al., 2013). The extent to which V1 cells show learning related plasticity may depend as well on the layer in which the cells reside and the brain areas they project to.

Although the experiments in this thesis do not directly single out any of the possible candidates providing feedback information to the visual cortex, the results agree best with the proposal that either acetylcholine or glutamatergic feedback projections mediate changes in local V1 connectivity. It needs to be stated, however, that the learning related differences in the experiments of chapter 2 and 3, were measured under anesthesia. The observed changes in neuronal response properties therefore likely reflect changes in the backbone of synaptic connectivity rather than acute effects of neuromodulation (Poort et al., 2015).

5.1.4 Memory

While studying the effects of stimulus-reward learning on cells in the primary visual cortex, one is inevitably confronted with the question to which extent V1 partakes in the neural representation of the association. Much of our everyday declarative memory is dependent on plasticity in the hippocampus, although the memory trace may subsequently transfer to cortical regions like entorhinal and perirhinal cortex.
(Marr, 1971; Squire and Zola-Morgan, 1991; Dash et al., 2004; Tse et al., 2007). During sleep, the hippocampus can also reactivate earlier experienced patterns in the visual cortex, which can be an indication of transfer of a memory trace or a strengthening of earlier learned patterns (Ji and Wilson, 2007). But even if plasticity is observed in a primary sensory brain area, it does not necessarily mean that this learning-related change in function of a V1 cell reflects its relevance for the memory of, for instance, a stimulus-reward association.

In his book ‘The organization of behavior’, Hebb (1949) proposed that cells specialized in a certain function or behavior would form strong recurrent connections through mechanisms now known as synaptic plasticity. These interconnected groups of cells, termed assemblies, can extend across brain areas and function as paved paths for sensory information to translate to behavior. In the decades before and around Hebb’s publication, Karl Lashey’s work focused on localizing cortically stored stimulus-reward associations, of which the cumulated results were discussed in the paper ‘In search of the engram’ (Lashley, 1950). Combining results of a wealth of experiments, Lashley found that lesions or fiber disconnections in selective cortical association areas had very little effect on learned associations; only widespread lesions of these areas or lesions of primary sensory cortical regions resulted in strong deficits. Lashley reached the conclusion that the memory trace is likely stored in a distributed fashion across primary sensory and multiple association areas of the neocortex. According to both Hebb’s and Lashley’s views, the inception of these distributed memory traces or assemblies should thus lead to observable forms of learning related plasticity, also in the primary sensory cortex.

The modern view on memory localization in the brain acknowledges these initial findings, but proposes that the reason why these memories are stored in a distributed fashion across multiple systems is that each contribute a specific aspect to the memory (O’keefe and Nadel, 1978). Since Lashley (1950), experimental studies have found links between several aspects of memories like emotional significance, motivation, contextual setting, and ability to generalize; and brain regions including amygdala, striatum, and entorhinal, retrosplenial, perirhinal, cingulate and (medial) prefrontal cortex, but also indicate a critical link with the hippocampus and highlight a role for sleep in memory-consolidation (see e.g. McDonald and White, 1993; Muller et al., 1997; Sakai and Miyashita, 1991; Miyashita et al., 1998; Bast et al., 2001; Tronel and Sara, 2003; Mavel et al., 2004; Lansink et al., 2009; Sterpenich et al., 2014; Tanaka et al., 2014). The degree to which primary visual cortex is involved in the consolidation and storage of (part of) the memory trace has, however, remained relatively unexplored (but see e.g. Nieuwenhuis et al., 2008; van de Ven et al., 2012).

Causally testing whether plasticity in the primary sensory cortices reflects the creation of a new memory trace is notoriously difficult because one has to differentially
target the function of the primary sensory area in e.g. visual processing, from local plasticity leading to long-term changes. Pharmacological or optogenetic inactivation of the primary visual cortex will block visual processing (Newsome et al., 1985) as well as the expression of a learned visual association (Glickfeld et al., 2013). NMDA receptor antagonists have been used in attempts to block plasticity without interfering with visually driven responses (Cooke and Bear, 2010). Inhibition of NMDA channel function, however, can disrupt feed-back inputs to V1 and therefore alter visual processing (Self et al., 2012). A promising tool is ZIP, an inhibitor of protein kinase M zeta, which is required for long-term potentiation. Local infusion of ZIP in the hippocampus erases memories and in V1 it reverses effects of plasticity and learned familiarity, but its temporal specificity is poor, affecting memories acquired over many days (Shema et al., 2007; Cooke et al., 2015). Another approach to revert a local network to its original state is to chemically or optically remove newly formed synaptic connections (Patricio Opazo, Personal communication; Hübener and Bonhoeffer, 2010) or return potentiated synaptic spines to their original strength (Hayashi-Takagi et al., 2015). The latter approach has recently been shown to revert learned motor skills when applied to motor cortex. Benefiting from these novel techniques that selectively and locally revert plasticity and improved techniques for tracing inputs to specific cells (Wertz et al., 2015), in combination with the recently developed rapid and reliable behavioral essays for rodent (visual) learning (Bussey et al., 2001; Bartko et al., 2011; Horner et al., 2013), discovery of the full distributed network of an associative memory is within reach.

5.2 Does anesthesia reduce the complexity of stimulus representations in the brain?

Does the function of circuits in the brain change when the studied organism is under anesthesia? The answer may seem obviously ‘yes’, because anesthesia results in the loss of consciousness and a complete suppression of behavioral responses to incoming stimuli other than some reflexive responses (Mashour, 2010). When one looks under the hood of the brain, however, this question becomes more difficult to answer. Neurons in, for instance, the primary visual cortex still respond to stimuli, albeit with a lower response amplitude, suggesting that anesthesia primarily reduces the gain of external inputs to the brain (e.g. Ikeda and Wright, 1974). Moreover, experiments in anesthetized animals report neurons to respond in a similar, although not identical, fashion to visual stimuli as different neurons do in these primary sensory regions in awake animals (e.g. Hubel and Wiesel, 1968; De Valois et al., 1982; Andermann et al., 2011; Marshel et al., 2011). A straightforward comparison of how the exact same
visual cortex cells function in awake animals and in the animal under anesthesia was missing so far and subtle changes in, for instance, orientation tuning under light anesthesia could easily have been overlooked.

### 5.2.1 Direction selectivity

In chapter 4, we lift a tip of the veil by reporting that light anesthesia reduces direction selectivity of orientation tuned neurons. Besides it being a cautionary message for colleagues studying brain function under anesthesia, this specific result may provide some insight into the mechanisms that underlie direction selectivity of cortical layer 2/3 cells. There are two overall models for the generation of direction selectivity in the cortex. The first theory proposes asymmetric inhibition (Fig. 5.2A), which may be spatially targeted to inhibit one side of an orientation selective receptive field
5.2. Does anesthesia reduce the complexity of stimulus representations in the brain?

stronger than the other side. A stimulus moving over the receptive field, starting at the inhibitory side, will induce strong inhibition of the response of the cell. Moreover, because of the intrinsically longer time-constant of inhibitory currents, the inhibition will reduce the response of the cell when the stimulus proceeds and drives the other side of the receptive field. Moving a bar in the opposite direction, activates excitation before inhibition and thereby generates a larger response, resulting in direction selectivity (Barlow and Levick, 1965; Torre and Poggio, 1978).

Isoflurane anesthesia acts through multiple mechanisms, but has a strong potentiating effect on the function of GABAA neurotransmission (Rudolph and Antkowiak, 2004; Franks, 2008). According to the asymmetric inhibition model, potentiated inhibition will likely suppress the null response even more, and direction selectivity would therefore be expected to be even stronger under anesthesia. Effects of anesthesia in the brain, however, are not very predictable. For instance, Haider et al. (2012) found that surround suppression, which is thought to be mediated by SOM+ interneurons (Adesnik et al., 2012), is reduced under isoflurane anesthesia, while isoflurane mediated potentiation of inhibition would predict stronger surround suppression. One explanation could be that these effects depend on the specific way inhibitory neurons inhibit other inhibitory neurons (Kameda et al., 2012; Zhang et al., 2014), but a more detailed model of inhibitory interneuron circuitry is needed to fully address such a mechanism.

The second model explains direction selectivity using the principle of delayed excitation. Orientation-tuned V1 neurons can have varying response onsets to visual stimulation of their receptive field. Direction selectivity can be achieved by connecting neurons with different delays to a postsynaptic cell, such that e.g. the inputs with the longest delays respond to the left part of the visual field, while cells with the shortest delays respond to the right visual field. The input cells would, as a consequence of the intrinsic delayed response in some of them, simultaneously drive the postsynaptic cell if a bar moves from left to right (preferred direction) across the input-cells’ receptive fields. Moving the bar in the opposite (null) direction, will lead to a spread of synaptic inputs and drive the cell to a lesser extent (Fig. 2B; see Adelson and Bergen, 1983; Reid et al., 1987). Under anesthesia, the delayed excitation model might fail because of the slower timing of cortical responses (Pisauo et al., 2013), but see also (Luczak et al., 2007). If action potential responses of individual cortical neurons are indeed sufficiently delayed and slackened, their inputs may overlap more in time, also for a bar moving in the direction that normally does not drive the cell, and therefore increase the response to the null direction as was observed in chapter 4.

The direction selectivity in mouse visual cortex, however, is becoming a bit of a special case and there may be multiple simultaneous mechanisms acting in parallel. Other than in higher mammals (Wiesel and Hubel, 1966; Cleland and Levick, 1974),
direction selectivity in mouse visual cortex may be to some extent inherited from direction selectivity initially computed in the retina. In early development, direction selective neurons in mouse V1 have a bias for responding to the cardinal directions, which are preferred by retinal ganglion cells (Rocheport et al., 2011). Indeed, relay cells in the dorsal LGN show direction selectivity (Marshel et al., 2012; Piscopo et al., 2013) and project these activity patterns to cortical layer 1, while they generally project less to layer 4 (Cruz-Martín et al., 2014). Still, direction selective neurons in layer 4 do receive excitatory inputs with a direction bias, although their origin is unknown. Direction selectivity in these cells is additionally sharpened by suppression of the null direction with delayed but un-tuned inhibition, arguing against the asymmetric inhibition model (Li et al., 2015). To what extent direction selectivity in layer 2/3 is dependent on retino-thalamic-cortical direction selective inputs remains unclear, but because inhibitory and excitatory receptive fields of mouse V1 layer 2/3 cells also generally overlap in space, the delayed excitation and/or retinal inheritance models have the strongest case at the moment (Liu et al., 2009).

Besides a difference in direction selectivity, the data in Chapter 4 showed a tendency of orientation selectivity to be increased under anesthesia (in tuning curves and population decoding analysis). The mean orientation selectivity index (OSI) was, however, not significantly different between states (Fig. 4.3). OSI (and also DSI, direction selectivity index) are tuning curve quantifications that are sensitive to noise (Mazurek et al., 2014). To address this potential methodological issue, we implemented an analysis to estimate the extent to which noise on top of the tuning curves contributed to the observed awake-anesthesia differences. This analysis showed that the DSI difference between awake and anesthetized recordings remained stable with varying signal-to-noise levels, while the OSI difference was strongly influenced by higher signal-to-noise responses of cells under anesthesia (Fig. 4.8). Decoding performance can also depend on the signal-to-noise ratio of the to be decoded activity patterns, which could explain the improved population coding for orthogonal orientations under anesthesia (Fig. 4.9). Together, these results indicate that orientation tuning under anesthesia may sharpen as a result of increased signal-to-noise ratio, while the effect of anesthesia on direction selectivity was observed independent of the noisiness of tuning curves.

Effects of anesthesia are fundamentally difficult to interpret because anesthetics have multiple molecular targets and affect many neural circuits simultaneously (Alkire et al., 2008). I, for one, do not feel comfortable excluding any of the possible models/circuits purely based on the findings of chapter 4. Future experiments, interfering with direction selectivity either at the level of the retina or the cortex, will likely demonstrate to which extent cortically observed direction selectivity is dependent on the retina (Daniel Hillier, personal communication; Yoshida et al., 2001).
5.2. Does anesthesia reduce the complexity of stimulus representations in the brain?

Clever optical control of single cell activation patterns in the cortex (Packer et al., 2014) can subsequently be used to play exact patterns of excitatory and inhibitory neuron activations and distill from these the patterns that drive direction selective cells the best, in order to identify mechanisms that underlie their computation (Allesandro LaChioma and Mark Huebener, personal communication). Rabies virus mediated tracing, however, may provide an alternative way of identifying presynaptic circuits and in combination with targeted in vivo electrophysiology could reveal the exact spatio-temporal input structure to a postsynaptic direction selective cell (Wertz et al., 2015).

5.2.2 Correlations

The observation of reduced direction selectivity is an example of how anesthesia changes the complexity and dimensionality of a single-cell tuning curve. Effects of anesthesia, however, extend far beyond just changes in stimulus selectivity and are thought to disrupt dynamic properties of cortical networks by interfering with local and long-range functional connectivity (Collins et al., 2007; Lee et al., 2009b, a; Schröter et al., 2012; Untergehrer et al., 2014). A number of neuroscientific theories of consciousness include a role for such widespread recurrent cortical activity in supporting awareness by promoting availability and binding of information (Tononi and Edelman, 1998; Baars, 2002; Lamme, 2003; Dehaene et al., 2006). Although activity in the primary visual cortex has been dismissed to strongly correlate with conscious perception (Crick and Koch, 1998), studying how anesthesia disrupts activity patterns in V1 may reveal changes in network dynamics that can be generalized to other brain regions.

In a series of papers, Giulio Tononi proposed the information integration theory of consciousness, which defines consciousness to reflect the capacity of a brain (or other system) to integrate information (Tononi and Edelman, 1998; Tononi, 2001, 2004; Tononi and Koch, 2008; Oizumi et al., 2014). The concept of integrated information refers to the principle that numerous different (conscious) brain states can be assumed by the same network of neurons, reflecting unique experiences without a possibility of dividing the network into two or more sub networks that can each assume part of the repertoire of states (or experiences) in an unaltered fashion. Although there is a serious lack of evidence supporting the information integration theory of consciousness (Cerullo, 2015), it outlines an interesting framework of how brain function could relate to conscious experience. Other (earlier developed) theories rely on similarly and simultaneously recruited (recurrent) activity patterns across many brain areas, like parietal cingulate and frontal regions, which may support information to be available for longer durations and promote integrative processing (Crick and
Koch, 1998; Dehaene and Naccache, 2001; Baars, 2002; Lamme, 2003; Dehaene et al., 2006; Dehaene and Changeux, 2011). Nearly all proposed theories have in common that they acknowledge a purpose for consciousness in the ability of being able to have a single coherent representation of the outside world ‘in mind’, potentially to reduce ambiguity in subsequent action selection (Ramachandran and Hirstein, 1997; Crick and Koch, 1998). One way of binding our sensory inputs into such integrated experiences and holding them available for longer durations could be through the action of brain-wide continuous-attractor states, shaped by short and long-range recurrent projections between neuronal networks, and dynamically propagating as our conscious experiences proceed (Pennartz, 2009).

Based on this principle, one can make the prediction that loss of consciousness during anesthesia and sleep could be reflected in the way activity patterns of populations of cells entrain to stereotyped patterns of slow wave activity in these states (Steriade et al., 1993; Fellin et al., 2009; Fucke et al., 2011; Ruiz-Mejas et al., 2011; Chauvette et al., 2011). In chapter 4, we were able to confirm the emergence of low frequency fluctuations in visual cortex input signals in anesthetized mice using two-photon calcium imaging. In addition, we found that activity patterns of neurons in the primary visual cortex were more strongly entrained by these low frequency fluctuations, as those of the same neurons when the animal was awake. This modulation was visible as an increase in pairwise correlations between the activity patterns of neurons (see also Greenberg et al., 2008; Golshani et al., 2009) and their increased coherence was again restricted to slow wave frequencies. This pattern of altered functional interactions within local groups of cells may reflect the merging of dynamic attractor states into an indiscriminate, non-differentiated pattern of coherence, and possibly reduced integration of information.

Slow wave oscillations, however, are not specific to the anesthetized state and have also been observed in the awake mouse brain (Mohajerani et al., 2010). Indeed, the data in chapter 4 showed no strong increase in strength of slow wave components of individual activity patterns of V1 cells under anesthesia. A possible explanation could be that anesthesia changed the way neurons entrained their activity in concert with neighboring cells and common inputs, rather than changing the total amount of intrinsic rhythmic activity in their activity patterns. Although the assumed mechanism of complexity in information processing relating to phenomenal experience is appealing, there is still a large conceptual gap between dividing the altered correlation structure of neuronal activity patterns and the reduced capacity for information integration and consciousness. Future experiments may test theories of consciousness and awareness by interfering with selective long-range recurrent circuits, explore whether consciousness emerges as a gradient or all-or-none phenomenon in the brain and may also benefit from the continued development of biologically plausible artificial intelligence.
models.

5.3 Imaging into the future

The introduction of this PhD thesis outlined some basics in two-photon imaging, but new developments in imaging and optogenetic techniques have arrived in rapid succession. For instance, techniques for imaging of neuronal activity patterns in living, behaving and learning mice have significantly improved (see e.g. Dombeck et al., 2007; Andermann et al., 2010; Dombeck et al., 2010; Margolis et al., 2012; Lütcke et al., 2010; Heys et al., 2014; Montijn et al., 2015). Using the most recently developed genetically encoded calcium indicators (Chen et al., 2013), the obvious next step is to now follow the same groups of neurons over periods of weeks or months and study how their function in the circuit changes during development or with learning (Poort et al., 2015; Makino and Komiyama, 2015). Using such an approach of time-lapse imaging during a learning paradigm, one can subsequently pursue simultaneous monitoring of multiple brain regions (Lecoq et al., 2014) in order to study how the flow of activity between subsequent cortical areas changes with learning, or as a result of anesthesia.

Continued development of various genetically encoded indicators or fluorescently labeled neuromodulators will make it possible to perform long term imaging of spatial aspects of neuromodulator release together with activity patterns of large groups of neurons. Such indicators could be employed to unravel the role of different neuromodulators and their interactions in plasticity, learning, attention and reinforcement signaling (Inagaki et al., 2012). In addition, the incorporation of genetic techniques for achieving cell-type identification and selective labeling of projection pathways (Chen et al., 2015) can help single out specific functions of cells and pathways. For this, it will be crucial to have indicators and optogenetic tools with the same specifications, but operating at different wavelengths, so that optical selectivity can be achieved and multiple spatially intermingled pathways can be monitored and manipulated independently.

By now, it is possible to optogenetically manipulate specific (online selected) groups of cells, while simultaneously recording the activity patterns of those cells and all other neurons in the nearby surrounding tissue in living and behaving animals (Packer et al., 2014). This approach can be combined or improved with holographic photoactivation (Anselmi et al., 2011) and ultrafast random access laser scanning techniques (Duemani Reddy et al., 2008; Grewe et al., 2010), and potentially be performed in freely moving animals (Helmchen et al., 2001; Ghosh et al., 2011; Szabo et al., 2014). By activating or disrupting specific patterns of cortical activity during
learning one could try to extract the code by which information is communicated, or learn how receptive fields of post-synaptic neurons are constructed from specific patterns of presynaptic inputs.

A second promising technique is tracing of neuronal circuits using modified rabies viruses (Ghanem and Conzelmann, 2015). Glycoprotein depleted rabies viruses can be targeted to a population or single cells expressing a specific receptor that allows the virus to infect the cell and subsequently its monosynaptic inputs (Wickersham et al., 2007). The rabies virus can express a genetically encoded fluorescent protein, photoactivatable ionchannel, calcium indicator or other protein in the starter-cell and also in its presynaptic partners. Using this approach one can trace largely complete input populations of cells that have specific tuning properties (Cruz-Martín et al., 2014) and a specific anatomical/genetic identity or location in the cortex (Vélez-Fort et al., 2014; Wertz et al., 2015), or explore the input to neurons that have changed their response properties as a consequence of learning. These techniques may be combined to be able to identify subnetworks in the brain that are relevant for, for instance, learning and to manipulate them as described above. My outlook is that optical techniques will continue to revolutionize neuroscience for many years.
5.4 Conclusion

As has been described in the introduction, and demonstrated in the experimental chapters, neurons in the visual cortex can be acutely modulated by internal state but can also incorporate long-term changes as a result of learning. It is fair to assume that the ability to adapt the function of the neural circuits of vision has the aim of improving the representation of visual stimuli in such a way that it benefits the organism. The changes observed in chapter 2 and 3 suggest that learning of a stimulus reward association can lead to changes in the visual cortex that may improve detection, salience, generalization, pattern completion and/or discrimination of these stimuli. Visual cortex activity between awake and anesthetized animals differs with respect to the amount of visual detail that the response patterns represent, and suggests that awake visual processing incorporates more complex network interactions as compared to anesthesia. If one needs to remember one single message from reading this thesis, it would have to be that the functioning of the primary visual cortex in the mouse is dynamic, depending on the state of the animal, and adapts, if needed, to optimally process the specific environment that is being experienced.