The neurochemical correlate of consciousness: exploring neurotransmitter systems underlying conscious vision
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Chapter 8. SUMMARY & DISCUSSION

Traditionally, many studies investigating the neural correlate of consciousness manipulated either the content of consciousness or the state of consciousness. In this thesis, I have tried to investigate how these are related by changing the state of consciousness while looking at the effect it has on the content of consciousness. I used different content manipulation paradigms such as backward masking, bistable perception and object recognition and combined it with manipulations and measures of neurotransmission, specifically the gamma-aminobutyric acid (GABA) and N-methyl-D-aspartate (NMDA) receptors, while measuring neural activity with EEG and fMRI. In the following sections I will summarize the obtained results.

Summary of the results

In Chapter 2, I sought for a common pathway for the effect on consciousness of two very different manipulations of consciousness: masking and pharmacological intervention. The behavioral results show that detection performance reduced with increasing masking strength and that of all the drugs, only Lorazepam (a GABA<sub>A</sub> receptor agonist) induced a further decrease in detection performance. Surprisingly – given the very different nature of the two manipulations – we find that with respect to neural activity the effects of both Lorazepam and masking appeared very similar in timing and scalp topography. For both manipulations, the early-evoked neural activity (< 120 ms) was relatively intact, while the neural activity after ~150 ms was decreased, as assessed with an EEG component recorded from the visual cortex of human participants. The effect of masking is in line with several previous studies that also demonstrated that masking disrupts late activity, while leaving early activity intact (Boehler, et al., 2008; Del Cul, et al., 2007; Fahrenfort, et al., 2007, 2008; Koivisto & Revonsuo, 2010; Lamme & Roelfsema, 2000; Lamme, et al., 2002). Effects of increasing GABAergic inhibition, by means of pharmacology, on visual processing have also been observed previously (Beckers, et al., 2001; Giersch & Herzog, 2004; Giersch & Lorenceau, 1999; Van Den Boomen, de Graaff, de Jong, Kalkman, & Kemner, 2013). Interestingly, we observed an interaction between the effects of masking and Lorazepam on the correlation between this late component and behavior. Therefore, this chapter provided the first evidence for a common mechanism for these two distinct ways of manipulating consciousness, possibly by affecting a network of recurrent excitation that is balanced by GABAergic interneurons (Roelfsema, et al., 2002). Additional study on GABA is likely to provide important insights into the neural mechanisms and pharmacological underpinnings of conscious vision.

Therefore, in Chapter 3 and Chapter 4, I further explored the effect of GABA on conscious vision. In Chapter 3, we used the novel possibility that the concentration of GABA can be measured non-invasively in the human brain with magnetic resonance spectroscopy (MRS) (Waddell, et al., 2007). We investigated whether variations in the concentrations of GABA in visual cortex could predict individual differences in bistable perception. Bistable perception is a prominent tool for the study of the neural correlates of consciousness. In bistable perceptual phenomena, conscious perception fluctuates spontaneously between two distinct interpretations of a constant sensory input (Blake, 1989; Blake & Logothetis, 2002;
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Hering, 1964; Lehky, 1988; Noest, et al., 2007). Computational models of bistable visual phenomena such as binocular rivalry have posited that mutual inhibition between different populations of neurons in the visual cortex plays a key role in the spontaneous dynamics of bistable perception (Noest, et al., 2007). We combined the MRS measurements with simulations of a neural computational model of bistable perception to make specific predictions about the way in which inhibition between sensory representations should affect the perceptual dynamics. In line with these predictions from modeling, we find that participants with higher GABA concentrations in their visual cortex experience slower spontaneous perceptual dynamics. We observed this to be the case for three distinct visual bistable illusions of which the mutual relationship was currently largely unknown: binocular rivalry, motion-induced blindness and structure from motion. Furthermore, we show that the MRS results exhibit chemical specificity (no/opposite effect for Glx (combined signal of glutamate and glutamine)), anatomical specificity (no effect for frontal cortex), and task-specificity (there was no effect of a visual cortex GABA on a simple RT task). These striking results imply that a person’s GABA concentration in visual cortex reliably predicts how fast endogenous changes of conscious perception occur for that person, irrespective of the specific type of bistable phenomenon experienced.

To complement these findings, we performed an additional pharmacological experiment in Chapter 4 that demonstrated a causal relationship between GABA and bistable perception. Crucially, we observed that stimulating the GABA<sub>A</sub> receptor with Lorazepam slowed down the perceptual dynamics. Summarizing, the combination of MRS, pharmacology and modeling in these chapters provide a set of complementary lines of evidence for the role of GABA in the dynamics of conscious vision.

In the previous chapters the role of GABA in conscious vision has been stressed. However, modeling studies have demonstrated that recurrent processing consists of both excitatory glutamatergic feedback connections and inhibitory horizontal connections (Dehaene, et al., 2003; Lumer, et al., 1997). More specifically, the feedback connections in RP are thought to be mediated by the NMDA receptor. However, no studies to date have directly tested the contribution of NMDA receptor functioning to these processes in humans. In Chapter 5 and Chapter 6, we intravenously administered ketamine, a NMDA receptor antagonist, to focus on the role of the NMDA receptor in consciousness and feedback activity.

Blocking NMDA receptors in monkeys abolished neural signals related to figure-ground segregation and feature integration, but did not yield any behavioral effects on figure-ground perception, probably because the NMDA receptor blocker was administered very locally in a small patch of V1 (Self, et al., 2012). Therefore, in Chapter 5, we tested whether ketamine would reduce visual feature integration at the perceptual level, and in humans. In a placebo-controlled within-subject design participants performed a texture discrimination task (Karni & Sagi, 1991). We indeed observed that under ketamine administration participants performed worse as compared to placebo suggesting that manipulating NMDA receptor functioning interferes with perceptual integration by means of reducing feedback activity. However, in this chapter we used relative simple textures, which were presented in the periphery of the visual field and did not include any neural measure.
Therefore in Chapter 6, we recorded fMRI and used more complex stimuli to assess the effects of our ketamine manipulation. We used two-tone ambiguous Mooney images of animate and inanimate objects (Mooney, 1957; Moore & Cavanagh, 1998) and the greyscale photographic version of the same images. Mooney images are typically hard to recognize when never seen before, yet are recognized once participants are exposed to the greyscale version of it. We studied the effect of such disambiguation on the neural representation of Mooney images, which gave us the opportunity to dissociate feedforward processing from top-down processing since the sensory bottom-up input was constant (for non recognized and recognized Mooney images) but perception changed. We compared the neural representations using multi-voxel pattern analysis (MVPA). Our results demonstrate that the pattern of brain activations changed with recognition. But, we found that ketamine administration distorted this recognition effect only in early visual cortex and not in higher-level area lateral occipital complex (LOC). Thus, by influencing the NMDA receptor with ketamine feedback activity was specifically reduced.

Finally, we were interested in the role of recognition in shaping category selective responses in LOC (Chapter 7). Therefore, we used the same data as in Chapter 6 but now compared the category-selective responses to the animate and inanimate images in LOC. We observed that category-selective responses to animate and inanimate Mooney image in LOC could occur even without recognition. But recognition of the Mooney images did increase the animate / inanimate distinction. More fine-grained object basic-level categorization, for example a cat being more similar to another cat, did only occur for the greyscale images. We speculated that categorization for unrecognized Mooney images can arise based on the bottom-up visual input since we observed difference in the physical properties of the animate and inanimate images as assessed with low-level image statistics, whereas perceptual categorization arises, using recognition-related top-down and experience dependent processing.

Discussion

In the following section, I will interpret the obtained results and argue that conscious vision requires integration of information via recurrent interactions that are modulated via the NMDA receptor and GABAergic inhibition. This will first be discussed in the light of influential models concerning the neural correlates of consciousness.

The role of NMDA and GABA in recurrent processing

In this thesis, I proposed that the effects observed in Chapter 5 and Chapter 6, were caused by reduced recurrent processing due to the NMDA blocker ketamine. Since the gating of the NMDA receptor is voltage and ligand depending, the opening depends on prior depolarization of the postsynaptic neuron. When information is processed in the brain, initially, a brief wave of excitation progresses through fast AMPA-mediated feedforward connections. Due to this depolarization via AMPA the magnesium block gets removed from the NMDA receptor, which enables the opening of NMDA receptor to pass current that may be mediated by feedback connections (Daw, Stein, & Fox, 1993). In this way, recurrent connections only impact cells that have been activated by the feedforward connections and this
explains why these modulatory effects are most pronounced for neurons that are well driven by a visual stimulus (Ekstrom, et al., 2008). Additionally, modeling studies (Dehaene, et al., 2003; Lumer, et al., 1997) and physiological recordings in monkeys (Herrero, et al., 2013; Self, et al., 2012) showed that RP is mainly driven by NMDA receptors, whereas the feedforward sweep is mediated by fast AMPA receptors.

But in this thesis I also show that GABA plays a crucial role in conscious vision as well (Chapter 2, 3, and 4). What is the role of GABA in recurrent processing and how do excitatory glutamatergic pyramidal cells and inhibitory GABAergic interneurons interact? Research initially focused on the pyramidal cells since they comprise 80% of the cortical neurons. But the last decade the focus has shifted to the role of interneurons in information processing. GABAergic interneurons are now considered to have a crucial role in controlling the activity of excitatory neuronal networks and to functionally segregate competing cell assemblies. As a result, in response to the same input, a given network can produce different output patterns at different times, depending on the state of inhibition (Buzsáki, Geisler, Henze, & Wang, 2004; Mody & Pearce, 2004).

Interestingly, this can be applied to theories on the neural correlate of consciousness and could even be the underlying neural mechanism. Although different neural correlates of consciousness have been proposed, depending on the type of manipulation used, the kind of neural signals recorded and the interpretation of behavioral results (see for reviews Crick & Koch, 2003; Dehaene, et al., 2006; Lamme, 2006; Seth, 2007; Tononi & Koch, 2008), they all seem to agree that consciousness requires integration of information. For example, Tononi and Koch (2008) suggest that consciousness can only arise when an assembly of neurons achieves integration (where the sum carries more information than the separate parts alone) and differentiation (the selection of one representation out of many potential representations). Interestingly, this theory is very much in line with neural computational models for binocular rivalry (Noest, et al., 2007; Tong, et al., 2006) also see Chapter 3). To account for spontaneous rivalry alternations, these models have proposed reciprocal inhibition between competing visual neurons, with inhibitory influences adapting over time. Tong and Blake (2006) propose that feedback projections from higher areas modulate the activity of neurons in earlier areas, in order to establish perceptual grouping or integration of information.

When combining these models, one could suggest that integration requires the NMDA receptor whereas differentiation depends on GABAergic inhibition. Interestingly, in a neural network model of the global workspace theory, the role of the NMDA receptor and GABA were explicitly modeled (Dehaene, et al., 2003). The global workspace theory proposes that conscious access results from a “global ignition” formed by sustained activity of a subset of pyramidal cells (Dehaene, et al., 2006). First, incoming sensory information activates a subset of pyramidal cells. Since these pyramidal cells have long-distance excitatory axons they can then interconnect sensory and high level areas. When the activation between the interconnected areas is sustained by means of recurrent processing it creates a global workspace or “global ignition”. Therefore, conscious content is assumed to be encoded by this sustained activity (e.g. recurrent processing) of a fraction of the global workspace neurons. But inhibition comes into play when these activated
neurons need to temporarily inhibit other neurons in the global workspace to ensure exclusivity or selection for conscious access.

**Summary & Discussion**

Figure 8.1 Neural network model
(A) Neural network model of Global Workspace Theory which models only the neural assemblies evoked by two stimuli (T1 and T2) (Dehaene, et al., 2003). The visual input can be presented at the lower level of a hierarchy of four successive areas, linked by inhibitory interneurons (GABA), feedforward nearest-neighbor connections (AMPA) and by global feedback (NMDA connections). (B) Overview of how the different chapters of this thesis could be related to the neural network model.

The model has implemented both the fast AMPA-mediated feedforward connections, and the slower NMDA-mediated feedback connections leading to a reverberating activation of the network or “global ignition” and conscious access (see Figure 8.1A). Lateral inhibition via GABAergic interneurons serves to suppress the neurons and thus define what the current conscious content is not. In the attentional blink model described in Figure 8.1, separate neural assemblies initially process T1 and T2 in two separate perceptual areas (A1 and A2 respectively). When activation reaches higher association areas (area C and D), they compete for conscious access via reciprocal inhibitory interactions. In this illustration, propagation of the activation of T2 to higher areas is prevented by T1-elicited inhibition. Consequently, top-down activation via feedback connections only
occurred for T1 and not for T2. As a result, the winning T1 representation is broadcasted by additional long-distance connections to yet other cortical regions (this was not explicitly modeled) to create a global ignition and thus conscious access.

Taken together, our results add behavioral and neural evidence to this neural network model (see Figure 8.2B). We directly showed that measurements and manipulations of GABA transmission influenced reciprocal inhibition in backward masking (Chapter 2) and bistable perception (Chapter 3 and Chapter 4). Additionally, our ketamine results provide both neural and behavioral evidence for the role of NMDA in feedback activity from higher to lower visual areas (Chapter 5 and Chapter 6). Although the results presented in this thesis fit very nicely within these neural network models, the present research also revealed some issues related to the use of pharmacological interventions and some contradictory results that will be discussed in the following section.

**Sedation**

The results presented in this thesis have been based on pharmacological interventions; we either increased inhibition with a GABA_A receptor agonist (Chapter 2 and 3) or decreased excitation by blocking the NMDA receptor with an antagonist (Chapter 2, 5 and 6). We ensured that participants were still able to perform the tasks by using sub-anesthetic doses in all experiments. However, in all experiments, the pharmacological manipulations did induce sedation compared to placebo.

We assessed sedation with subjective visual analogue scales. One could argue that our sedation measures could function as an indication of the effectiveness of our pharmacological manipulations. It even seemed that the drugs manipulations that displayed the strongest sedation also showed the strongest behavioral and neural effects.

![Figure 8.2. Sedation](image)

**Figure 8.2. Sedation**

Subjective sedation for Dextromethorphan (Chapter 2) and ketamine (Chapter 5 and 6) and placebo (Chapter 2, 5 and 6). Using independent t-test, we found that right after drug administration ketamine was significantly more sedative than Dextromethorphan both when we compared Dextromethorphan with the ketamine sedation levels in Chapter 5 ($t_{(1,32)} = 3.13, p = .004$) and in Chapter 6 ($t_{(1,32)} = 3.63, p = .001$). Error bars indicate SEs.
For example, Lorazepam was the most sedative compared to Scopolamine and Dextromethorphan in Chapter 2, and we only observed effects of Lorazepam (see Figure 2.8). Moreover, even if we directly compare the sedative effects of Dextromethorphan and ketamine (see Figure 8.2) - both NMDA receptor antagonists - sedation was significantly higher for ketamine. This could maybe even explain why ketamine did have a behavioral and neural effect and Dextromethorphan did not.

However, one needs to exclude the possibility that the observed effects can be purely attributed to these sedative effects, instead of to more specific pharmacological effects of the drugs. The problem with inducing sedation is that maybe participants were just not able to perform the task properly. To exclude this possibility of a more general effect of sedation, the observed results need to be specific.

Importantly, all our reported results did show specificity. In Chapter 2, both masking and the GABA<sub>A</sub> receptor agonist only reduced late activity while the early activity was not affected. In Chapter 3 and Chapter 4, the observed results displayed chemical specificity (no/opposite effect for Glx), anatomical specificity (no effect for frontal cortex), and task-specificity (there was no effect of a visual cortex GABA on a simple RT task) cortex. In Chapter 5, we specifically observed effects on the texture discrimination task and not on a central fixation task. However, here, one could argue that this was due to a difference in task difficulty, the texture discrimination task being more difficult than the fixation task. But the reduced discrimination performance that we found in the ketamine condition was constant over various levels of task difficulty; we observed no effect of decreasing stimulus onset asynchrony. The ketamine effects that we reported in Chapter 6 were specific as well. We observed that ketamine distorted the recognition effects in early visual areas and not in higher visual areas like V3, V4 and LOC. Moreover, there was no difference in mean BOLD between placebo and ketamine. In behavior, we only found reduced effects of ketamine in categorization of the Mooney images after the greyscale phase and no effect on the categorization of the greyscale images themselves or on reaction times.

Additionally, we also checked the influence of sedation by correlating the subjective sedation scores with our behavioral and neural measures. In none of the pharmacological chapters did we observe a significant positive correlation. Thus, more sedation did not seem to result in larger behavioral or neural effects. We can therefore conclude that although participants felt more sedated in the drug conditions compared to placebo, the observed results cannot be attributed solely to the sedative effects of our pharmacological manipulations.

Other neurotransmitter systems
Although we have now focused only on the involvement of GABA and NMDA, there are more receptors involved in the balance between excitation and inhibition underlying recurrent interactions (Hemmings, et al., 2005). We know that in anesthesia more neurotransmitter systems apart from NMDA and GABA are involved such as glycine and muscarinic receptors (Alkire, et al., 2008; Franks, 2008; Rudolph & Antkowiak, 2004). Additionally, neurotransmitter systems do not operate as single systems but influence and modulate each other. Therefore, it is very likely that the pharmacological intervention used in this thesis have indirect effects on other
neurotransmitter systems that might be of importance in creating visual awareness as well. Further research is necessary to elucidate the role of these and other neurotransmitter systems and how they interact.

For example, the neurotransmitter acetylcholine has been suggested to be the neurotransmitter correlate for consciousness, since acetylcholine is thought to control activities that depend on selective attention (Perry, et al., 1999). Acetylcholine operates on the muscarinic and nicotinic receptors. Although we did not observe an effect of a muscarinic receptor antagonist, scopolamine, on recurrent processing (Chapter 2) others have found effects of acetylcholine manipulations on feedback activity related to attention (Bauer, et al., 2012; Herrero, et al., 2008). For example, Herrero et al, (2008) found reduced spatial attentional feedback in V1 with a muscarinic antagonist scopolamine in monkeys. In humans, the cholinergic agonist physostigmine enhanced spatial attention by influencing low-frequency alpha and beta oscillations in visual cortex (Bauer, et al., 2012). Synchrony of these low-frequency oscillations has been related to feedback pathways (Buffalo, Fries, Landman, Liang, & Desimone, 2010) and changes in the low-frequency alpha oscillations are thought to reflect up- and down regulation of the excitability of relevant neuronal populations thereby gating sensory processing (Jensen & Mazaheri, 2010). Interestingly, the phase of pre-stimulus alpha oscillations predicted whether a target reached awareness (Mathewson, Gratton, Fabiani, Beck, & Ro, 2009). Thus, acetylcholine might especially influence attention (Bauer, et al., 2012; Herrero, et al., 2008; Perry, et al., 1999; Worden, Foxe, Wang, & Simpson, 2000), and the gating of sensory processing, thereby influencing the depth of processing and reportability.

But some regard attention as being orthogonal to consciousness (Lamme, 2010). Moreover, many have now demonstrated that attention and consciousness can be manipulated independently from each other (for reviews see (Koch & Tsuchiya, 2007, 2012; Lamme, 2010; Van Boxtel, Tsuchiya, & Koch, 2010). Based on the results presented in this thesis, one could speculate that a similar orthogonality might be present at the neurotransmitter level, in the sense that consciousness depends on the NMDA and GABA mediated interactions whereas attention operates via acetylcholine. Further research, could specifically test this idea, for example by investigating the role of acetylcholine on consciousness and recurrent processing using paradigms that independently manipulate attention and consciousness.

Conclusion
Taking together this thesis provided more insights on the role of neurotransmitters in the neural correlate of consciousness. The NMDA receptor is important for recurrent activity thereby integrating information of different areas. But, inhibition guides this process and is required for the selection process to determine the outcome of the competition for conscious access. Additionally, we show that combining manipulations of conscious state and content may give more direct insights into the neural and pharmacological underpinnings of consciousness. This may open the way for a molecular biological understanding of consciousness and towards a neurochemical correlate of consciousness.