The neurochemical correlate of consciousness: exploring neurotransmitter systems underlying conscious vision

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Chapter 2. GABA\textsubscript{A} agonist reduces visual awareness: a masking-EEG experiment

Consciousness can be manipulated in many ways. Here, we seek to understand whether two such ways, visual masking and pharmacological intervention, share a common pathway in manipulating visual consciousness. We recorded electroencephalography (EEG) from human participants who performed a backward masking task in which they had to detect a masked figure form its background (masking strength was varied across trials). In a within-subject design, participants received Dextromethorphan (NMDA receptor antagonist), Lorazepam (GABA\textsubscript{A} receptor agonist), Scopolamine (muscarinic receptor antagonist), or placebo. The behavioral results show that detection rate decreased with increasing masking strength and that of all the drugs, only Lorazepam induced a further decrease in detection rate. Figure-related event-related potential (ERP) signals showed three neural events of interest: (1) an early posterior occipital and temporal generator (94-121 ms) that was not influenced by any pharmacological manipulation nor by masking, (2) a later bilateral peri-occipital generator (156-211 ms), which was reduced by masking as well as Lorazepam (but not by any other drugs) and (3) a late bilateral occipital temporal generator (293-387 ms) that was mainly affected by masking. Crucially, only the intermediate neural event correlated with detection performance. In combination with previous findings, these results suggest that Lorazepam and masking both reduce visual awareness by means of modulating late activity in the visual cortex but leave early activation intact. These findings provide the first evidence for a common mechanism for these two distinct ways of manipulating consciousness.

**Introduction**

Consciousness can be manipulated in many ways. A visual stimulus can be rendered invisible through physical manipulations such as masking or rivalry, by physiological manipulations like TMS, or via pharmacological interventions such as anesthesia. Different as these methods may seem, they all share that consciousness sensations are - partly or fully - abolished. Does that mean that these manipulations all have a common neural pathway?

A problem with research on consciousness is that different neural correlates of consciousness are proposed, depending on the type of manipulation used, the kind of neural signals recorded and the interpretation of behavioral results (for reviews see Crick & Koch, 2003; Dehaene, et al., 2006; Lamme, 2006; Seth, 2007; Tononi & Koch, 2008), a fruitful way out of this conundrum may be to seek for the common factor between these proposals. More specifically, the search for a final common pathway for all types of manipulations of consciousness may lead the way towards understanding what consciousness actually is. Here, we seek to understand whether a common pathway can be found for two very different ways of manipulating visual consciousness: pharmacological intervention that is considered to affect conscious *state* and backward masking, a technique that manipulates conscious *content*.

With a pharmacological intervention it is possible to specifically manipulate neurotransmitters by antagonizing or agonizing their receptors. Different theories are proposed on which neurotransmitter or receptor is important for consciousness and several candidates have been suggested, including acetylcholine (Perry, Walker, Grace, & Perry, 1999) and N-methyl-D-aspartate (NMDA) receptor (Flohr, Glade, & Motzko, 1998). However, to induce anesthesia, the most common way to pharmacologically reduce consciousness state, various other receptors such as receptors for gamma-aminobutyric (GABA), glycine and muscarinic (Alkire, et al., 2008) can be targeted. How can such a variety of pharmacological interventions produce the same effect: loss of consciousness?

The answer may be found in the effects on neural activity when these receptors are manipulated. One suggestion is that anesthesia disrupts cortical communication, causing a loss of integration of information (Alkire, et al., 2008; Tononi, 2004). Crucially, the same might be true for visual masking. A stimulus becomes less perceptible or even invisible through the presentation of a second stimulus - the “mask” - shortly after the first. Accumulating evidence suggests that when stimuli are effectively masked, recurrent processing in visual cortex is reduced, whereas feedforward activity remains relatively unaffected (Boehler, et al., 2008; Di Lollo, et al., 2000; Fahrenfort, et al., 2007; Lamme, et al., 2002). Therefore, a pharmacological intervention (known to affect conscious state) and masking (known to affect conscious content), might both disrupt cortical communication, although a direct link between both manipulations of consciousness has so far not been demonstrated. Here, we try to find out whether a sub-anesthetic pharmacological intervention and backward masking have similar effects.

We recorded EEG while participants discriminated between trials containing a figure or no figure under various masking strengths (ranging from fully “seen” figures to fully “unseen” figures). This task was performed on separate days, on each of which we either administered Dextromethorphan (a NMDA receptor antagonist),
Lorazepam (a GABA_A receptor agonist), Scopolamine (a muscarinic receptor antagonist), or a placebo. This experimental set-up allowed us to explore the existence of a common neural pathway for two different ways of manipulating visual consciousness.

**Methods**

**Participants**

Twenty-two participants (all females, age $M = 21.54$ years of age, $SD = 2.65$ years) with no relevant psychiatric or neurological history participated in the experiment. They all had normal or corrected-to-normal vision. Written informed consent was obtained from each participant during a screening session. The ethical committee of the Psychology Department of the University of Amsterdam approved the experiment. Two participants were excluded from analysis because they were experiencing extreme side effects of the Lorazepam. Therefore, all reported analyses are based on the remaining twenty participants.

**Backward Masking Task**

In this experiment visual awareness was manipulated using a backward masking paradigm in combination with a pharmacological manipulation. In separate sessions, different drugs that operate on different synaptic mechanisms were administered to study their effects on subjective stimulus visibility. In the backward masking task, each trial started with a fixation (300 ms) after which the target stimulus was presented for 16.7 ms. Participants had to detect whether the target contained oriented line elements that all had the same orientation (“no-figure trials”) or contained an orientation-defined square (“figure trials”). To manipulate the visibility of the target, a texture-defined pattern mask (of varying strengths) or an isoluminant grey screen with no texture (“no-mask”) was presented for 500 ms immediately after the target. Participants had to indicate within 1500 ms after the target stimulus whether they had perceived a figure or no figure (see Figure 2.1).

The presentation of the stimuli was rendered unpredictable by using a jitter for the fixation duration (added random 300-550 ms). Participants were instructed to fixate throughout the task. Participants indicated their response through pressing a button with their left hand for “figure present” or a button with their right hand for “no-figure trial” (buttons were counterbalanced across participants). Participants were instructed to guess if they did not see the target.

**Stimuli**

The target stimuli consisted of textures with oriented line elements. The oriented line elements had either the same direction (“no-figure trials”) or were oriented in a 45° angle relative to the background to form an orientation-defined square (“figure trials”). In total, background and figure line segments could appear in four different orientations (22.5°, 67.5°, 112.5°, and 157.5°). The orientations were counterbalanced across trials so that local stimulation of the visual cortex was on average identical for no-figure and figure trials (for a similar procedure see Caputo & Casco, 1999; Fahrenfort, et al., 2007; Fahrenfort, Scholte, & Lamme, 2008; Lamme, et al., 2002; Scholte, Witteveen, Spekreijse, & Lamme, 2006).
Figure 2.1. Schematic description of a trial
Participants indicated whether a figure was present or not. The target visibility was manipulated, spanning the range from weakly masked to strongly masked.

The orientations of the line elements in the mask differed from the line elements in the preceding target stimuli. We created multiple levels of masking strength (eleven in total) by changing the color of the line elements in the mask from black to light grey while keeping the background color constant. The lighter the line elements, the lower the contrast of the mask and hence the easier it was to detect the target. These multiple levels of masking strength spanned the range from invisible, where the mask had the same color (black) and luminance as the target and performance was at chance level (“full-mask”), to visible, when there was no mask presented (“no-mask”). This also allowed us to discover whether increasing masking strength lead to a linear reduction of the neural activity or whether masking had a more an “all-or-none” effect. In addition, a subjective 75% mask condition (“subjective-mask”) was selected out of the intermediate masks, based on performance on the backward masking task in the screening session. Stimuli were presented using Presentation (Neurobehavioral Systems, Inc, Albany, CA, USA).

Experimental Procedure
The experiment consisted of a screening session and four morning test sessions (10-14h), which occurred on separate days with a minimum interval between each session of 1 week.

In the screening session, participants were screened on the contraindications for the different drugs and practiced the backward masking task. During the practice they received feedback on their detection after each trial. After the practice, the participants performed 5 blocks of 176 trials of the backward masking task. Based on their detection performance on this task, the “subjective-mask” was set to a performance of 75% correct.

In each test session a different drug was administered at a dosage selected to have mild psychogenic and sedating effects (Boroojerdi, Battaglia, Muellbacher, & Cohen, 2001). The following agents were used (within-subject): (i) Dextromethorphan (120 mg, 40 ml of Dampo syrup), a potent noncompetitive
NMDA receptor antagonist (Wong, Coulter, Choi, & Prince, 1988); (ii) Lorazepam (1.5 mg, pill), a short-acting benzodiazepine that at this dose produces functional potentiation in specifically the GABA_A receptors (Sybirska, et al., 1993); and (iii) Scopolamine (1.5 mg, dermal patch behind the ear), a muscarinic receptor antagonist (Frey, et al., 1992). Since the intake differed for all three drugs, the participants received a pill, syrup and a patch behind the ear in each session. As a result, two placebos and one condition-dependent active drug were administered per visit. In the placebo condition, all three substances were placebos. The order of the drug conditions was counterbalanced across sessions and participants were blind to this order.

Participants were instructed to have a good night’s sleep, abstain from alcohol and caffeine containing products 24 hours prior to and following testing. At 10:00 am, the drug was administered. Immediately after drug intake, participants practiced a block of 216 trials. The task and EEG recording commenced ~120 minutes after ingestion of the medication to maximize the levels of drug during the task (Borojjerdi, et al., 2001). During the task, participants performed 2160 trials (with a short break after each block of 216 trials) with a breakdown of 480 no-mask trials, 480 full-mask trials, 480 subjective-mask trials, and an additional 80 trials for the intermediate masking strengths. For the analyses of the intermediate masks, we binned three levels of the intermediate masks, since each condition only contained 80 trials. As a result, three masking strength conditions were created (easy, medium and strong), which all contained 240 trials. In each condition, 50% of the trials contained a figure. The order of the trials was random; each block contained a similar distribution of the conditions.

During each session, participants filled in a set of five visual analogue scales (Bond & Lader, 1974) that assessed their subjective state before medication ingestion, after 2h, and 3h into testing, and on completion of testing. The mean score of these scales (length 100 mm) assessed complementary aspects of sedation (alert / drowsy, excited / calm, clear-headed / muzzy, energetic / lethargic, and quick / slow), where a high value indicates that participants feel subjectively more sedated (Danion, Zimmermann, Willard-Schroeder, Grange, & Singer, 1989).

**Behavioral Analysis**

Based on the individual ability to discriminate at 75% accuracy between figure and no-figure trials, we created the participant-specific subjective mask. Here, we fitted the individual performance for the different intermediate masks with a Weibull psychometric function, using the the psignifit toolbox (http://bootstrap-software.org/psignifit/) version 2.5.6 for Matlab that implemented the maximum-likelihood method for curve fitting (Wichmann & Hill, 2001a, 2001b).

Forced-choice detection performances expressed in d-prime were tested for significance using paired sample t-tests and repeated measures ANOVA with Drugs (placebo vs. Dextromethorphan or Lorazepam, or Scopolamine) and Masking (no-, subjective-, full-, weak-, medium-, strong mask) as within-subject factors. The ratings on the visual analogue scales were analyzed with repeated measures ANOVA with Drug (placebo vs. Dextromethorphan or Lorazepam or Scopolamine) and Time (drug intake, 2h, 3h, end of experiment) as within subject factors. Since we were
interested in the effects of drugs in comparison with placebo, we compared each drug with placebo separately.

**EEG Analysis**

We recorded the EEG data from the scalp using a BioSemi ActiTwo 64-channel active EEG system (BioSemi, Amsterdam, the Netherlands) sampled at 2048 Hz and referenced to two ear electrodes. Four external electrodes were placed around the eyes (two horizontal and two vertical, referenced to their counterpart) in order to record eye movements. We filtered the data with a high pass filter of 0.1 Hz, a low pass filter of 30 Hz and a notch filter of 50 Hz. After filtering, the data was downsampled to 256Hz. Ocular correction was performed with an independent component analysis (Hyvärinen, Karhunen, & Oja, 2001). The EEG data was segmented based on the target position. A segment consisted of 100 ms before target presentation (baseline) and 400 ms after target presentation. If a segment contained EEG activity outside the -75 μV and the +75 μV range, or contained voltage steps larger than 50 μV, or had an activity lower than 0.5 μV, and an amplitude larger than 2.5*standard deviation of the maximum amplitude it was removed from further analysis. Baseline correction was applied in the -100 ms to 0 ms interval before stimulus presentation. Spherical interpolation was used to create a signal for the removed channels. To estimate current source densities (CSD), Spline Laplacian distributions were calculated (Nunez & Srinivasan, 2006; Perrin, Pernier, Bertrand, & Echallier, 1989). With CSD, deep sources are filtered out and greater weight is put onto local sources in the superficial cortex (Nunez & Srinivasan, 2006; Perrin, et al., 1989).

After the CSD transform, event related potentials (ERP) averages were calculated per drug and masking strength (no-, subjective-, full- mask and binned intermediate masks). Analyses were conducted on difference waves, obtained by subtracting the ERP average of no-figure trials from the ERP average of figure trials. Since a mask followed both trials, any direct influence of the mask was subtracted out, ensuring that the two brain signals only differed in the presence or absence of a figure (see also Caputo & Casco, 1999; Fahrenfort, et al., 2007, 2008; Lamme, et al., 2002; Scholte, et al., 2006).

To increase the independence of data, we performed a split half procedure, which randomly divided the data. This split half procedure boosts the reliability of the effects that are found by precluding coincidental significance of sources that are identified visually (Poldrack & Mumford, 2009). Half of the trials were used to select those electrodes that showed a difference between figure and no-figure trials in the no-mask placebo condition using the subtraction method described above. The selected electrodes were then pooled to increase the signal to noise ratio. This procedure results in the following regions of interest (ROIs): an occipito-temporal ROI (P5, P7, PO7, P6, P8, PO8), an occipital ROI (Iz, Oz, POz), a peri-occipital ROI (O1, PO7, PO3, Oz, O2, PO4, PO8), a central ROI (P1, P2, Pz, CPz) and an occipital temporal parietal ROI (O1, P5, P7, PO7, PO3, O2, P6, P8, PO4, PO8). The other half of the trials was then used for statistical analyses and for selecting the moments of significant deflections per ROI. The moments of significant deflections per ROI (referred to as a neural event) were ascertained by sample-by-sample paired t-tests between figure
and no-figure trials. Multiple comparison correction with respect to the number of
time points being tested was performed to limit the false discovery rate (FDR).

To explore the effect of drugs and masking on the neural events, we
contrasted the mean amplitude of the difference wave per condition at each neural
event. If a neural event consisted of two ROIS with both a positive and a negative
deflection, we took the average of the absolute value of both. For statistical analyses
we used a repeated measures ANOVA on the mean activity values for each neural
event, with drugs (placebo vs. Dextromethorphan or Lorazepam or Scopolamine)
and Masking Strength (weak, medium, strong) as within-subject factors. Post-hoc
paired sampled t-tests were used to compare conditions more directly. Preprocessing was performed using Brain Vision Analyzer (Brain Products, Munich,
Germany). Statistical analyses and visualization of the time courses were done using
Matlab (The MathWorks, Natick, MA, USA).

Results

Behavioral Results: Lorazepam reduces stimulus visibility

Figure trials were easily discriminated from no-figure trials in the no-mask condition
(see Figure 2.2A), as evidenced by detection performance (expressed as d-prime)
close to 7. Crucially, masking strongly reduced stimulus visibility. When the
subjective mask was presented, the d-prime dropped to approximately 2, while it
was at chance-level with the full-mask (d-prime 0; main effect for masking, all Fs(2,38)
> 354.8, ps < .001). When looking at the effect of drug on stimulus visibility, only
Lorazepam differed significantly from placebo (main effect for drugs, F(1,19) = 35.56, p
< .001). Lorazepam reduced the detection performance in the no-mask and
subjective-mask conditions (assessed with paired sample t-tests (t(1,19) = 4.40, p <
.001 and t(1,19) = 6.60, p < .001, respectively), but not in the full-mask condition since
the full-mask already reduced stimulus visibility to chance-level for all conditions. In
contrast, the other two drugs had no significant effect on stimulus visibility
(Dextromethorphan, F(1,19) = .49, p = .492, Scopolamine, F(1,19) = .23, p = .636).

Next, we were interested in how the variation in masking strength influenced
stimulus visibility. To test whether there was a linear or all-or-none relationship
between masking and detection performance, we analyzed the effect of the
clustered intermediate masks (weak, medium, strong) on performance. As can be
seen in Figure 2.2B, for all drugs, increased masking strength reduced the detection
performance (main effect for Masking Strength F(2,38) > 55.50, p < .001; linear
contrast, F(2,38) > 51.92, p < .001). However, only Lorazepam significantly affected
detection behavior, as demonstrated by better performance for the intermediate
masks with placebo than with Lorazepam (main effect of drugs, F(1,19) = 13.83, p <
.001). For Dextromethorphan and Scopolamine, no significant differences were
observed from the placebo (all Fs < 0.49, ps > .49). Overall, these findings indicate
that increasing the strength of the mask gradually decreases stimulus visibility and
that administering Lorazepam further increases the difficulty of perceiving the
targets at all levels of masking strength.
**Figure 2.2. Behavioral results**

(A) Detection performance as expressed in mean d-prime for no-mask, subjective-mask and full-mask stimuli per drug. Only with Lorazepam the targets were more difficult to detect compared to placebo. (B) With increasing masking strength detection performance also decreased and, again, participants’ performance decreased significantly more with Lorazepam. Error bars indicate SEs.

**EEG Results**

Since detection rates were impaired only with Lorazepam, follow-up EEG analyses were first performed for the placebo and Lorazepam conditions. In order to capture the neural processes involved during the detection of the figure, the event related potentials (ERPs) for no-figure trials were subtracted from the ERPs for figure trials (see Methods). Figure 2.3 shows the course of neural processing of the subtracted ERPs (indicated as the difference) for the no-mask trials in the placebo condition only. Based on visual inspection of the data (see Methods) and results from previous studies (Fahrenfort, et al., 2007, 2008), the following three neural events were distinguished: (1) an early neural event observed at occipito-temporal and occipital electrodes at ~94-121 ms, (2) a somewhat later event at ~156-211 ms at occipital and central electrodes, and (3) a late large positive deflection at occipital temporal-parietal electrodes at ~293-386 ms. These three events will be referred to as early (94-121 ms), middle (~156-211 ms) and late event (~293-386 ms) from now on.
Interestingly, Lorazepam had similar effects on figure-related neural processing since it reduced activity for the two later neural events, while leaving activity on the early
event relatively unaffected. The reduction in neural activity due to Lorazepam was most pronounced at the middle neural event compared to placebo (no-mask: \( t_{(1,19)} = 3.09, p = .006 \), subjective-mask: \( t_{(1,19)} = 2.09, p = .05 \)). We observed a trend in the no-mask condition for the late neural event (\( t_{(1,19)} = 1.91, p = .071 \)).

**Figure 2.5. Effects of masking and Lorazepam on the neural events**

**(A)** Difference waves (figure – no-figure trials) for placebo and Lorazepam (no-mask and subjective-mask). **(B)** Mean activity per neural event, Lorazepam and masking only affect the later two events; the early event remained relatively unaffected. Error bars indicate SEs.

*Increasing masking strength influences activity at the middle event linearly*

Additionally, we compared the effects of the binned intermediate masks (weak, medium, strong) per neural event (see Methods and Figure 2.6A). A linear effect for masking strength was found only at the middle event (main effect linear contrast for Masking Strength, \( F_{(1,19)} = 5.74, p = .03 \)), reflecting that increasing masking strength led to decreased neural activity in the 156-211 ms range. This effect was reduced with Lorazepam compared to placebo (main effect for Drug, \( F_{(1,19)} = 6.14, p = .02 \)). The neural activity at the middle event seems therefore important for stimulus visibility as it was this activity that reduced with increasing masking strength and reduced even more with Lorazepam, similar to what we observed in our behavioral results. At the other two neural events there were no effects of Lorazepam or masking strength (all \( F_{S,(1,19)} < .99, ps > .347 \)).
GABA reduces visual awareness

**Figure 2.6. Effect of masking strength on the middle event for Lorazepam and placebo**

(A) For placebo increasing masking strength reduced the neural activity in a linear manner. Error bars indicate SEs. (B) The scatter plot for the middle event shows only a positive correlation between the size of the deflection and detection performance in the placebo condition.

**Correlating the reduced neural activity at the events with stimulus detection behavior**

To test the role of the middle event in stimulus detection, we calculated Spearman’s rank correlations between the mean amplitude per neural event for masking strength (weak, medium, strong) and the detection rate. As depicted in Figure 2.6B, a significant positive correlation was found only for the middle event with placebo (Rho = .262, p < .044), and no correlation with Lorazepam (Rho = .139, p > .290). This suggests that participants with higher detection rates had with larger ERP deflections at this time-window. No correlations were observed in the other events (all Rho’s < -.150, ps > .261).

**No eye movement differences between masking and drugs conditions**

To ascertain that the observed effects were not due to different patterns of eye movements across conditions, we performed exactly the same analysis as for the EEG data on the raw electro-oculography (EOG) signal for the horizontal EOG (HEOG) and vertical EOG (VEOG) channels over time. For both the HEOG and VEOG, we did not observe any significant differences between the relevant drugs and masking conditions, which highlights that blinks or eye movements did not affect our EEG results.

**Dextromethorphan and Scopolamine did not affect the neural activity**

As a control, all EEG analysis were also performed for the two other drugs (Dextromethorphan and Scopolamine), however, just as for the behavioral data, no significant differences were observed when compared to placebo (see Figure 2.7).
No correlation between sedation and reduced visual awareness

To assess the influence of the pharmacological intervention on changes in subjective mood ratings of sedation we analyzed the visual analogue scales (Bond & Lader, 1974) (see Methods). Overall, participants felt more sedated in the drug conditions compared to placebo and the sedation increased over time (interaction drug and time, all $F_s > 3.11$, $ps < .03$, Figure 2.8). In the Lorazepam condition the sedative effect was strongest (main effect drug $F_{(1,16)} = 24.01$, $p < .001$). To assess whether this may have contributed to the effects we reported, the Spearman's rank correlations were calculated between the difference in sedation (Lorazepam minus placebo: an average of the second and third time point) and the difference in EEG activity for both the no-mask and subjective-mask condition. Moreover, the specificity of the reduction in neural activity, being that only the later neural events were affected, already suggests that there was not an overall reduction in neural activity due to sedation only (see Figure 2.5). No positive correlations were observed (all Rho's $< .16$, $ps > .54$). The absence of reliable and consistent positive correlations between sedation scores and neural measures suggests that the sedative effects of Lorazepam cannot explain the reported neural results.

**Figure 2.8. Sedation**

Ratings of sedation per drug over time in hours (2h -begin of task-, 3h -end of task-, 3.5h -end of session-) as assessed with visual analogue scales. Participants felt most sedated with Lorazepam and the sedation increased over time. Error bars indicate SEs.
Discussion

In this study, we combined a masking paradigm with a pharmacological intervention to study the neural mechanisms underlying visual awareness. Behaviorally, both Lorazepam (GABA\_A receptor agonist) and masking reduced visual awareness as evidenced by decreased detection performance. With respect to neural activity, the effects for these two distinct ways of manipulating visual consciousness appeared very similar with respect to 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.

This effect of masking is in line with several previous studies in humans 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). Additionally, our results seem similar to average response times in early visual areas with macaque intracranial recordings with very comparable stimuli (Lamme & Roelfsema, 2000). Therefore the early figure – no figure difference might reflect sustained activity resulting from feedforward processing, although it cannot be ruled out that some feedback is already incorporated at this interval (Foxe & Simpson, 2002).

The observed reduced neural activity in the 156-211 ms range seems similar to the Visual Awareness Negativity (VAN) (Koivisto & Revonsuo, 2010). VAN is described as a posterior negativity around 200 ms that emerges across different manipulations of visual awareness, including masking, change blindness, and the attentional blink (Koivisto & Revonsuo, 2010). In addition, we found that in the 156-211 ms range the amount of disruption depended on the strength of the mask: the stronger the mask the more the neural activity was reduced (see Del Cul, et al., 2007 for comparable findings). This reduction was correlated with a decrease in detection behavior and was mostly effected with Lorazepam, stressing the importance of this activity in visual awareness. Previous studies have also demonstrated that this activity correlated with perception and detection performance (Fahrenfort, et al., 2008; Koivisto & Revonsuo, 2010). Furthermore, it seems in line with studies showing contextual modulation in this time frame in V1 and higher areas possible due to recurrent processing (Lamme, 1995; Lamme, Van Dijk, & Spekreijse, 1992; Lamme, et al., 1998; Lamme, et al., 2002). These recurrent processing loops in the visual cortex seem to serve integration of information from distant receptive fields and provide perceptual organization.

The observed late positive neural activity (297-387 ms) has been found in other studies on visual awareness as well and might be involved in the transition of visual perception to a reportable stage (Del Cul, et al., 2007; Lamy, Salti, & Bar-Haim, 2009; Sergent, Baillet, & Dehaene, 2005). However, in this study it did not correlate with stimulus visibility and was less affected by Lorazepam. Therefore, it might reflect more higher-level cognitive processes (Donchin & Coles, 1988), either decision related processes (Eimer & Mazza, 2005; Koivisto & Revonsuo, 2010; Nieuwenhuis, Aston-Jones, & Cohen, 2005) or processes related to non-spatial attention (Boehler, et al., 2008; Koivisto & Revonsuo, 2010).

To our knowledge, this is the first study to show that Lorazepam affects neural activity in a specific way in that it has a selective effect on late activity and not
on the early activity of the ERP, i.e. on figure-ground selective signals and mainly in the 156-211 ms range and thereby reducing conscious content. Similar results have been observed in studies that affected conscious state using anesthesia, for example, in a study where monkeys were anesthetized with Isoflurane, an anesthetic that binds to NMDA, GABA and glycine receptors, while activity in the primary visual cortex (V1) was simultaneously recorded. Anesthesia did not reduce early feedforward activation of V1 receptive fields; orientation selectivity of cells was not affected. What was abolished, were the later recurrent interactions between V1 and higher-level visual areas (Lamme, et al., 1998). In another study with anesthetized rats, a selective reduction of synchronization in the long-range anterior-posterior coherence was observed while the local anterior coherence was not affected (Imas, Ropella, Wood, & Hudetz, 2006). Furthermore, a breakdown of cortical effective connectivity was found in humans when loss of consciousness was induced with Midazolam, also a GABA<sub>A</sub> receptor agonist (Ferrarelli, et al., 2010). Our results thus add that apart from manipulating conscious state, a pharmacological intervention can also influence conscious content.

Behavioral effects of Lorazepam on visual processing have been observed previously (Beckers, Wagemans, Boucart, & Giersch, 2001; Giersch, Boucart, Speeg-Schatz, Muller-Kaufmann, & Danion, 1996; Giersch & Lorenceau, 1999; Lorenceau, Giersch, & Seriès, 2005). For example, in a “shine-through” backward masking paradigm, the time between the presentation of the target and the mask - stimulus onset asynchrony (SOA) - was varied. The SOA needed to be much longer with Lorazepam than in the control condition in order to reach a similar detection performance (Giersch & Herzog, 2004). In addition, other behavioral research showed that Lorazepam affected perceptual integration and segmentation processes, which are both important for visual awareness (Lorenceau, et al., 2005; Pompéia, Pradella-Hallinan, Manzano, & Bueno, 2008). Lorazepam reduced the detection of objects when the spacing and alignment of local contour elements of these objects was incomplete (Giersch, et al., 1996) or when participants had to detect discontinuities in random-shaped outlines (Beckers, et al., 2001; Giersch & Lorenceau, 1999; Lorenceau, et al., 2005; Pompéia, et al., 2008). Performance was thus impaired when the physical properties involved in the computation of contour information (e.g. spacing and alignment of contour elements) was manipulated. Lorazepam distorted the integration of the contour information, which in turn disturbs perceptual organization.

This constitutes a possible link with our data. Perceptual organization has been shown to rely on recurrent processing between visual areas (Lamme, 1995; Roelfsema, 2006) and is reflected in the human EEG by the very same signals that we have recorded here (Bach & Meigen, 1992; Caputo & Casco, 1999; Fahrenfort, et al., 2007, 2008; Lamme, et al., 1992; Lamme, et al., 2002) and that were selectively affected by Lorazepam and masking.

We observed no effects for Dextromethorphan and Scopolamine on behavior or neural signals. Previous research did, however, show that these agents do play a role in visual processing. For example, a study that recorded the activity of single V1 neurons in macaque monkeys demonstrated that Scopolamine suppressed top-down attentional modulation of the primary visual cortex (Herrero, et al., 2008). Furthermore, research by Boroojerdi and colleagues (2001) showed that experience-
dependent plasticity in the human visual cortex was blocked with all three the agents (Lorazepam, Scopolamine and Dextromethorphan). A combination of task and measurement differences between the reports and our experiments may have contributed to the conflicting results.

In summary, we found reduced EEG activity in the visual cortex with Lorazepam and masking at relatively late latencies (especially between 156-211 ms), which is related to visual awareness. This suggests that there is a common pathway for these distinct methods of manipulating consciousness. Both masking and Lorazepam affect the late activity while leaving early activity relatively intact, possibly by affecting a network of recurrent excitation that is balanced by GABAergic interneurons. Additional study on GABA is likely to provide important insights into the neural mechanisms and pharmacological underpinnings of visual awareness.