# Noradrenergic modulation of human visual cortex excitability assessed by paired-pulse visual-evoked potentials

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**Paired-pulse paradigms are common tools to explore excitability in the human cortex. Although the underlying mechanisms of intracortical inhibition and facilitation in the motor system assessed by paired transcranial magnetic stimulation are well understood, little is known about the physiology of excitability in the human cortex measured by paired-pulse visual-evoked potentials (VEPs). We therefore aimed to explore the noradrenergic influence on paired-pulse VEPs. We recorded and analysed VEPs following a single and paired-pulse stimulation in healthy individuals before and after they received single doses of 60 mg atomoxetine and in a control group. Paired-pulse suppression was expressed as a ratio of the amplitudes of the second and the first peaks. We found that the selective norepinephrine reuptake inhibitor atomoxetine reduced paired-pulse suppression significantly, indicating a facilitatory effect on visual cortex excitability, whereas in the control group, no significant effects were found. Single-pulse VEPs were unaffected. We conclude that single-dose atomoxetine is able to increase excitability in the visual cortex, indicating an involvement of the noradrenergic system. *NeuroReport* 23:707–711 © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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## Introduction

Paired-pulse paradigms are common tools to explore cortical excitability in the motor, visual and somatosensory system. By measuring the suppressive effect of a stimulus on a subsequent second stimulus expressed as paired-pulse suppression, cortical excitability can be estimated. Low paired-pulse suppression indicative of high cortical excitability is indicated by high-amplitude ratios and vice versa. Although the underlying mechanism of paired-pulse suppression is not completely understood, using pharmacological approaches, the potential mechanisms mediating paired-pulse behaviour were explored in a somatosensory and motor system [1–4]. Similar to paired-pulse techniques in the somatosensory and motor system, we recently introduced a paired-pulse stimulation protocol to examine cortical excitability in the visual system [5]. These data showed similar paired-pulse suppression at short intervals between successive stimuli as described for the primary somatosensory cortex and the primary motor cortex [3,6]. In patients with migraine, we found reduced paired-pulse suppression indicative of enhanced excitability of the visual cortex [7]. Furthermore, in a recent study, we compared paired-pulse visual-evoked potentials (VEPs) with phosphene thresholds after the transcranial stimulation of the occipital lobe. We found a correlation of both excitability parameters and suggested that both approaches may reflect the common characteristics of visual cortex excitability, but each method most likely targets different mechanisms [8]. Many studies of paired-pulse stimulation in the sensorimotor system during various pharmacological interventions have yielded further insights into the underlying physiological mechanisms. In the motor system, the development of a paired-stimulation protocol using transcranial magnetic stimulation provided an understanding of intracortical excitability on the basis of *N*-methyl-D-aspartate-dependent excitatory and γ-aminobutyric acid inhibitory interneuronal activity (for an overview, see Kobayashi *et al.* [9]). Furthermore, there is evidence of a fundamental role for norepinephrine in enhancing plasticity in the motor cortex. In a study using transcranial magnetic stimulation of the motor cortex, significantly reduced short-interval intracortical inhibition and increased intracortical facilitation were found after the administration of a single dose of atomoxetine [10]. Atomoxetine is a drug approved for the treatment of attention deficit hyperactivity disorder. It is a potent and selective inhibitor of presynaptic norepinephrine transporter and lacks affinity for dopaminergic and serotoninergic receptors [11].

However, little is known about the underlying mechanism of paired-pulse behaviour in the human visual system. Therefore, the aim of our study was to examine the effect of noradrenergic stimulation on excitability in the human visual cortex assessed by paired-pulse VEPs. Given the above-described pharmacological actions of atomoxetine, we hypothesized that noradrenergic stimulation should increase visual cortex excitability.

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## Materials and methods

### Participants

We studied cortical excitability separately in 12 healthy individuals receiving atomoxetine (mean age ± SD: 25.6 ± 2.2) and in a control group (28.7 ± 5.0). All participants were not taking any regular medication and did not have neurological diseases, and were especially free from headache. In addition, participants were instructed to abstain from alcohol before the experiment. All individuals participating in the study gave their informed consent. The study was approved by the Ethics Committee of the Ruhr-University Bochum and was performed in accordance with the Declaration of Helsinki.

### Stimulation

The experimental set-up and the stimulation were the same as those described in our previous studies [5,7]. The stimuli were displayed on a cathode ray tube spanning $23^\circ \times 17^\circ$ of visual angle at an observation distance of 60 cm. The cathode ray tube was set to a frame rate of 75 Hz and a pixel resolution was $800 \times 600$. The experimental paired-pulse paradigm consisted of checkerboard patterns with 36% contrast and a check size of $0.5^\circ$ with a mean luminance of 16 cd/m$^2$, which were presented at three different stimulus onset asynchronies (SOAs). The first stimulus appeared for one frame (13.33 ms), followed by presentations of frames containing a homogenous grey background without a change in the mean luminance. The second stimulus appeared after variable SOAs in multiples of the frame interval of 13.33 ms to avoid temporal aliasing [12]. We used three different SOAs between 107 ms (seven frames), 133 (nine frames) and 160 ms (11 frames) in which the highest paired-pulse suppression was found in healthy individuals [5]. All three SOA values were presented successively, with 10 presentations per SOA value. After the last SOA value, the entire cycle was repeated for a total of 40 sweeps per SOA step. In a second session, after recording paired-pulse stimulation, single VEPs with a sequence of 100 checkerboard patterns, at the same contrast and luminance as that used in the paired-pulse paradigm, were presented for one frame (13.33 ms), followed by frames containing a homogenous grey background (intertrial interval 1000 ms, resulting in a stimulation frequency about 1 Hz) without a change in the mean luminance. The stimuli were produced by the EP2000 system [13]. VEPs were recorded and stored for offline analysis with a 32-channel-amplifier (Brain Amp; Brain Products, Munich, Germany), with a sampling rate of 5 kHz and band-pass filtering between 2 and 1000 Hz).

Evoked potentials after single and paired-pulse stimulation were recorded in epochs from 200 ms before and 400 ms after the stimulus and averaged. Peak-to-peak amplitudes of the C1/C2-response components were analysed (C1 is characterized as the negative peak 60 ms after stimulus onset and C2 as the subsequent positive peak). After paired-pulse stimulation, the response to the second pulse adds to the response to the first pulse, leading to a superposition of both evoked potentials. Therefore, the amplitude of the response to the second pulse may appear misleadingly higher or lower. To assess the 'true' paired-pulse interaction, confounds from superposition were removed by subtracting the response to a single-pulse stimulation from the paired-pulse stimulation trace. We analysed the amplitude of the response to the second stimulus of the paired-pulse stimulation after subtraction of the response to single-pulse stimulation (second amplitude after subtraction = A2s) and compared it with the response to the first stimulus of the paired-pulse stimulation before subtraction (A1). Paired-pulse suppression was expressed as a ratio (A2s/A1) of the amplitudes of the second (A2s) and the first (A1) peaks.

### Procedure

During the recording sessions, which were performed in a darkened room, participants sat in a comfortable chair at a distance of 60 cm from the stimulus screen. The active electrode was placed on the scalp over the visual cortex at Oz with the reference electrode at Cz according to the International 10–20 system. A reference electrode was placed over the Fpz position. Participants were instructed to relax and to keep their eyes focused on the centre of the display marked by a small dim cross, which was displayed during the entire course of the measurements. The VEP testing paradigm consisted of two sessions: paired-pulse VEPs with three different SOAs and single-pulse VEPs. Atomoxetine was administered perorally as a single dose of 60 mg after the first VEP testing. According to the pharmacokinetics of atomoxetine, the paradigm was retested 2 h after drug administration respectively at the expected maximal plasma concentration. The individuals in the control group received no medication and the paradigm was retested after 2 h. Electrodes were removed after the presession. The positions of recording electrodes were marked at the head to allow accurate repositioning in the postsession.

### Statistical analysis

The statistical analysis was performed separately for each group. The data were analysed using a mixed effects (repeated measures) analysis of variance (ANOVA) in each group. Factors were Time (pre/post) and SOA (107, 133 and 160 ms). For all statistical tests, the SPSS 17.0 software package (SPSS Software, Munich, Germany) was used with subsequent sequential Bonferroni adjustment, and significance was assumed at the 0.05 level. Paired, two-tailed *t*-tests were used to compare the amplitudes of single VEP in premeasurement and postmeasurement and for post-hoc analysis if the ANOVA indicated a significant interaction. For these *t*-tests, the significance level was adjusted by dividing it by the number of comparisons (Bonferroni correction).

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**Table 1 Means and SDs of cortical C1/C2 amplitudes after single and paired pulse and single visual stimulation in the atomoxetine group and the control group**

| | Pre | | | Post | | |
|---|---|---|---|---|---|---|
| | SOA 107 | SOA 133 | SOA 160 | SOA 107 | SOA 133 | SOA 160 |
| Atomoxetine | | | | | | |
| A1 | 32.9±15.5 | 34.0±17.1 | 34.0±17.3 | 28.3±13.5 | 30.7±13.5 | 30.7±12.2 |
| A2 | 19.7±11.7 | 23.1±13.3 | 27.8±18.3 | 19.4±9.2 | 23.5±9.6 | 28.4±13.1 |
| A2s | 26.4±12.8 | 27.1±14.2 | 29.0±14.3 | 27.9±12.6 | 29.0±9.6 | 28.9±10.9 |
| Ratio | 0.8±0.2 | 0.8±0.3 | 0.9±0.4 | 1.0±0.3 | 1.0±0.4 | 1.0±0.3 |
| Single | 34.6±18.5 | | | 32.1±11.7 | | |
| Control | | | | | | |
| A1 | 37.6±13.4 | 36.8±12.9 | 37.6±13.8 | 37.2±13.7 | 37.1±14.9 | 37.3±15.5 |
| A2 | 28.8±12.1 | 32.2±16.2 | 36.7±15.8 | 24.9±12.4 | 33.9±14.2 | 39.4±14.5 |
| A2s | 34.9±17.0 | 35.5±16.6 | 37.5±16.7 | 34.2±15.2 | 35.6±15.3 | 37.8±16.5 |
| Ratio | 0.9±0.3 | 0.9±0.3 | 1.0±0.2 | 0.9±0.2 | 0.9±0.2 | 1.0±0.2 |
| Single | 39.9±13.3 | | | 39.3±13.0 | | |

Amplitudes in μV of the atomoxetine group and the control group in premeasurement and postmeasurement. A1 response to the first stimulus of the paired-pulse stimulation, A2 response to the second stimulus of the paired-pulse stimulation, A2s response to the second stimulus of the paired-pulse stimulation after subtraction of the response to a single-pulse stimulation (single). Ratios are calculated as A2s/A1 for each individual participant and then averaged.
SOA, stimulus onset asynchrony.

## Results

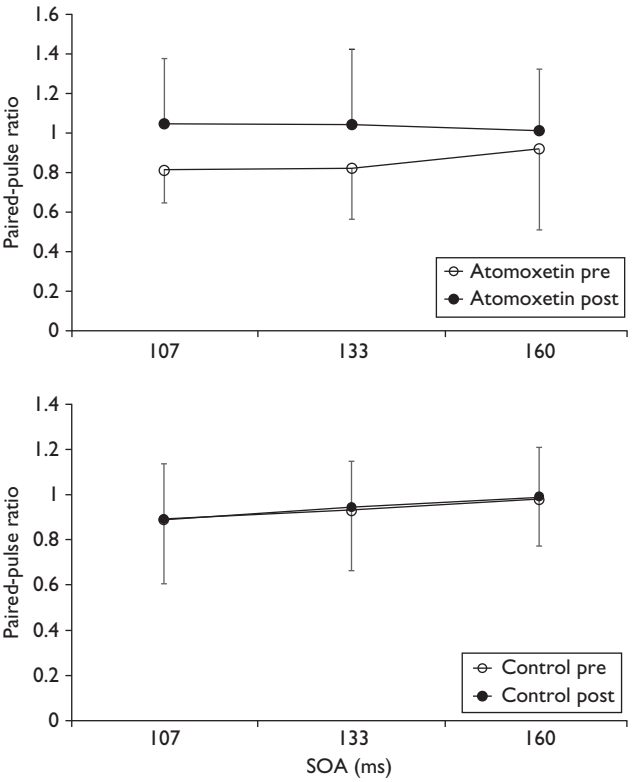
### Atomoxetine

To study the effect of atomoxetine on single-pulse VEP, we compared the precondition and postcondition using a two-tailed, paired Student's *t*-test. We found no statistical difference between premeasurement and postmeasurement of the single-pulse VEP amplitude (C1/C2 amplitude, $P = 0.372$). We analysed the C1/C2 amplitude of the response to the second stimulus of the paired-pulse stimulation after linear subtraction of the response to a single-pulse stimulation (second amplitude after subtraction = A2s) (Table 1). Paired-pulse suppression was expressed as a ratio (A2s/A1) of the amplitudes of the second (A2s) to the first (A1) peaks (Fig. 1). According to ANOVA, there was a significant influence of Time (preatomoxetine and postatomoxetine administration) with $P$ value less than 0.001 and $F_{1,33} = 18.209$. We found no significant effects of interaction between factors SOA and Time ($P = 0.31$, $F_{2,33} = 1.215$). In principle, an increased amplitude ratio and thus a reduced paired-pulse suppression as found in the atomoxetine group can be achieved by two different types of alterations of the response behaviour: either by an increase in the second response or by a reduction in the first response. We therefore analysed A1 and A2 separately. ANOVA indicated that there was a significant effect of atomoxetine on A1 on comparing premeasurement and postmeasurement ($P = 0.002$, $F_{1,33} = 11.896$), but there were no significant effects of interaction between the factors SOA and Time ($P = 0.862$, $F_{2,33} = 0.149$). We found no effects on A2 ($P = 0.878$, $F_{1,33} = 0.024$) and A2s ($P = 0.326$, $F_{1,33} = 0.996$).

### Control

In the control group, we found no difference in the amplitudes ($P = 0.753$) of single-pulse VEP ($P = 0.823$). There was no significant influence of Time with $P = 0.8$, $F_{1,33} = 0.065$ and no significant interaction between the factors SOA and Time ($P = 0.95$, $F_{2,22} = 0.051$) (Fig. 1).

**Fig. 1**




Amplitude ratios of the atomoxetine and the control group before (pre, open symbols) and in the retest (post, filled symbols) as a function of stimulus onset asynchronies (SOAs), grand mean±SD.

## Discussion

The present results provide the first evidence that the excitability of the visual cortex in humans assessed by paired-pulse VEPs can be increased by noradrenergic

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stimulation. Here, we applied a recently developed paired-pulse paradigm in which checkerboard patterns appeared interleaved with a homogenous grey background without a change in the mean luminance [5]. According to our findings, the norepinephrine reuptake inhibitor atomoxetine significantly reduced paired-pulse suppression in the visual cortex, whereas paired-pulse suppression remained unchanged in a control condition without drug administration. After the administration of atomoxetine, the magnitude of the first response component was significantly smaller at the lowest SOA of 107 ms. In the case of unchanged paired-pulse suppression, the second response amplitude should be reduced as well, which was, however, not the case. Instead, the ratio between the first and the second response was higher than that in the control group, indicative of a reduction in paired-pulse suppression caused by an atomoxetine-induced reduction in the first response amplitude.

As described in previous findings, paired-pulse suppression showed a severe interindividual variability, both in terms of the magnitude of inhibition and facilitation, and for the SOA at which it develops [5]. In this study, we analysed participants each before and after drug administration, therefore reducing interindividual differences.

The underlying mechanism mediating paired-pulse suppression remains controversial. In fact, different laboratories use different terms such as excitability, preactivation level, habituation, gating, hyper-responsitivity, hypersensitivity, hyper-reactivity or cortical dysbalance [14]. We use the term paired-pulse suppression to refer to a reduction in the neuronal response to the second of a pair of two successive stimuli, a phenomenon often also called forward suppression or short-term plasticity [15].

In principle, paired-pulse suppression can be altered in at least three qualitatively different ways: first, by changing the response to the first stimulus or by changing the response magnitude of the second stimulus. A third possibility arises from changes in the effectiveness of the inhibitory influence of the first response on the second response. Although paired-pulse behaviour most presumably reflects intracortical processing, the observation of reduced first amplitudes after atomoxetine likely reflects alterations in thalamocortical transmission and/or changes occurring along the entire visual pathway. In many studies describing changes in paired-pulse suppression, the magnitude of the first peak remained unaffected, but the response to the second stimulus was substantially enhanced [2,16,17]. Our findings are in contrast to these observations and therefore imply that the alterations in paired-pulse suppression measured after cortical lesions or tactile coactivation are controlled by mechanisms other than those involved here.

Cellular in-vitro studies have reported that noradrenalin seems to produce an increase in neuronal excitability through β-adrenoceptor activation, a decrease in synaptic excitatory transmission through α1-adrenoceptors and a long-lasting hyperpolarization mediated through α-adrenoceptors. These effects on membrane properties and synaptic transmission described could provide the basis for an increase in the signal-to-noise ratio generally attributed to noradrenalin in sensory cortices in-vivo studies [18]. In animal models, it has been shown that norepinephrine seems to play a crucial role as a mediator of cortical plasticity [19]. *In vivo*, a single dose of reboxetine and atomoxetine, drugs selectively blocking norepinephrine reuptake, enhanced excitability in the human motor cortex, assessed by paired-pulse transcranial magnetic stimulation [10,20,21]. Although any comparison of the physiological processes in an afferent somatosensory and an efferent motor system must be carried out with caution, according to the findings in the human motor cortex, we found a noradrenergic effect on excitability in the visual cortex assessed by analysing paired-pulse suppression without affecting VEPs after a single-pulse stimulation. In the visual cortex, the modulatory effect of norepinephrine seems to be mediated by α-receptors of the α1 type [22]. In an animal study using paired-pulse stimulation on rat visual cortex, norepinephrine induced induction and expression of synaptic plasticity [22]. These studies suggested that norepinephrine serves as an 'enabling factor' for activity-dependent cortical plasticity by the facilitation of *N*-methyl-D-aspartate receptor-dependent homosynaptic long-term depression in the visual cortex.

Our findings of reduced first VEP amplitudes after the administration of atomoxetine may be attributed to a reduced preactivation level in the visual cortex. In patients with migraine, it has been argued that a low preactivation level would allow a wide range of supra-threshold activation before reaching the 'ceiling' and initiating a 'reducing' response [23]. The preactivation level of cortical excitability seems to depend on 'state-setting, chemically addressed connections' that originate in the brainstem and involve serotonin and norepinephrine as transmitters [23]. Despite substantial experimental and theoretical work, the mechanisms mediating paired-pulse behaviour are not fully understood. Further studies are required to examine the modulatory effect of atomoxetine on learning performance and task-dependent excitability in the visual cortex.

## Conclusion

The excitability of the human visual cortex assessed by paired-pulse stimulation can be modulated by noradrenergic stimulation. The selective norepinephrine reuptake inhibitor atomoxetine is able to reduce suppression of excitability in the visual cortex in a single dose. Atomoxetine is a promising agent for a selective and controlled modulation of cortical excitability.

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### Conflicts of interest

There are no conflicts of interest.

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