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The role of the locus coeruleus-noradrenaline system in temporal attention and uncertainty processing

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The role of the locus coeruleus-noradrenaline system in temporal attention and uncertainty processing

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Promotores

Prof.dr. S.T. Nieuwenhuis
Prof.dr. B. Hommel

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- Joni Mitchell, *Both Sides Now*

1. General introduction

1.1 Introduction

Noradrenaline (also known as norepinephrine, commonly abbreviated to NE) is a neuromodulator synthesized from the amino acid tyrosine, that plays a crucial role in cognition. Noradrenergic neurons are concentrated mainly in the locus coeruleus (LC), a small pontine nucleus in the brainstem, which innervates a large number of cortical and subcortical brain areas (Berridge & Waterhouse, 2003; Stahl, 2008).

Due to the neuromodulatory nature of noradrenaline, and the wide-spread projections of the locus coeruleus, the locus coeruleus-noradrenaline (LC-NE) system is of crucial importance in many cognitive processes (Chamberlain & Robbins, 2013). In this introductory chapter, the LC-NE system is described, and an outline of this dissertation is presented.

1.2 The locus coeruleus: neuroanatomy

The locus coeruleus ("dark blue spot") is a bilateral nucleus located in the rostral pontine tegmentum (Figure 1.1) that gets its distinctive blue color from neuromelanin deposits in noradrenergic neurons. Each LC nucleus contains about 10,000-15,000 noradrenaline-containing neurons in humans (Berridge & Waterhouse, 2003). These neurons project broadly throughout the cortex, and innervate many brain areas, including the neocortex, hippocampus, thalamus, basal forebrain, hypothalamus (Nieuwenhuys, Voogd, & Van Huijzen, 2008), amygdala (Mason & Fibiger, 1974), and cerebellum (Moises & Woodward, 1980). These wide-spread projections supply the brain with the neuromodulator NE, making the LC an important brain area to study (but see also Robertson, Plummer, de

Marchena, & Jensen, 2013, who show that the LC is not the only noradrenergic nucleus that innervates the cortex.

NE receptors can be broadly divided into three classes: α1, α2, and β (Stahl, 2009). In the context of this dissertation, α2 receptors are particularly relevant. Being the only receptors that can occur presynaptically, they form an interesting target for psychopharmacological challenge studies in which these receptors are agonized in order to reduce NE signaling. We have used such a strategy in chapters 3, 4 and 5.

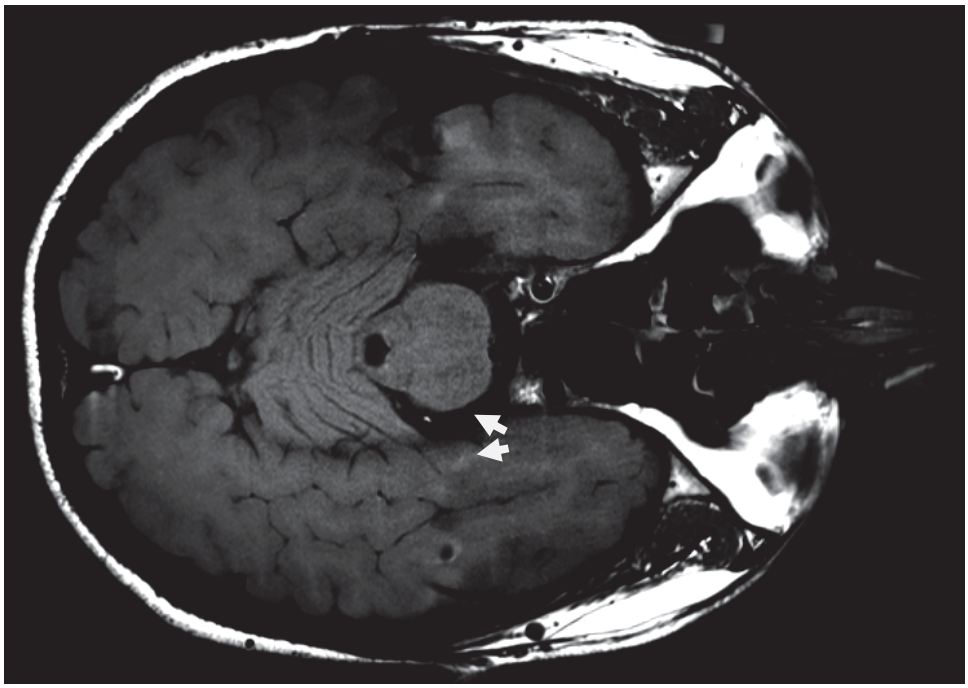

Figure 1.1. My loci coerulei (white arrows). Neuromelanin-sensitive MRI scanning sequence described by Sasaki et al. (2006) and Shibata et al. (2008).

1.3 The LC-NE system

Animal studies suggest that the LC has two modes of neuronal firing. When an animal is engaged in a cognitive task, single-cell recordings demonstrate that target stimuli evoke short, *phasic* bursts of LC activity, which are associated with accurate performance. Distracter stimuli do not evoke phasic firing. Temporally, the phasic response seems to be locked to a behavioral response and not to the presentation of a stimulus or a reward; furthermore, incorrect behavioral responses are associated with a less pronounced phasic response than correct trials (Aston-Jones, Rajkowski, Kubiak, & Alexinsky, 1994; Clayton, Rajkowski, Cohen, and Aston-Jones, 2004).

In addition to the phasic firing mode, LC neurons can fire *tonically*: a mode of increased baseline firing. Cell recordings in monkeys performing cognitive tasks demonstrate that during the tonic firing mode, false alarm rates were increased (Aston-Jones, Rajkowski, Kubiak, & Alexinsky, 1994), and monkeys showed less foveations to the center of a computer screen to initiate a new trial (Aston-Jones, Rajkowski, Kubiak, Valentino, & Shipley, 1996). Typically, phasic responses are less pronounced or even completely absent during tonic firing (Aston-Jones & Cohen, 2005). These results suggest a state of increased distractibility during tonic firing of the LC.

These two modes of firing can influence cognition in different manners. We will now turn to theories of LC functioning.

1.4 The influence of NE on cognition

Over the years, a number of theories have been formed about the function of the LC and NE. In the 1960s, the LC was mainly associated with vigilance and rhythms of wakefulness and sleep, because the LC fires at a regular, slow rate during quiet wakeful activity, its firing rate is increased when arousing stimuli are

encountered, and decreased during non-REM sleep (for a review see Sara, 2009). For years, NE was conceptualized to have a relatively simple function in cognition and to be merely associated with arousal. As technology improved, we have learned that NE is a much more complicated chemical: as a neuromodulator, it does not just affect postsynaptic neurons per se, but it can modulate the postsynaptic effects of neurotransmitters like glutamate and gamma aminobutyric acid (GABA) (reviewed in Aston-Jones & Cohen, 2005). A number of theories on the influence of NE on cognition are based on these neuromodulatory premises.

1.4.1. A gain-based account of NE functioning

As discussed earlier, the LC's phasic mode of firing is associated with accurate task performance. In the phasic mode, tonic firing is generally reduced, and phasic bursts of firing are selectively elicited by motivationally significant stimuli, leading to a decreased effect of irrelevant distracting stimuli on the agent's performance. The result of this is relatively optimal task performance, and exploiting task rewards.

The tonic mode of firing is the exact opposite of the phasic mode: it is characterized by increased baseline activity and reduced phasic bursts, and it is associated with task disengagement, which leads to poorer task performance due to increased distractibility. Although the tonic firing mode may not seem to be adaptive, it can actually be beneficial to disengage from a task or course of action so as to explore the environment to search for more rewarding endeavors.

This fundamental distinction between exploitation and exploration makes sense at an intuitive level, and forms the basis of the *adaptive-gain theory* (Aston-Jones & Cohen, 2005), but the question that arises is how the tonic and phasic modes of LC firing actually affect cognitive processing on a neural level. Every neuron has an activation function, which can be conceptualized as a sigmoidal

function that relates the neuron's activity to its net input. Once the net input to the neuron exceeds a certain threshold, the cell fires.

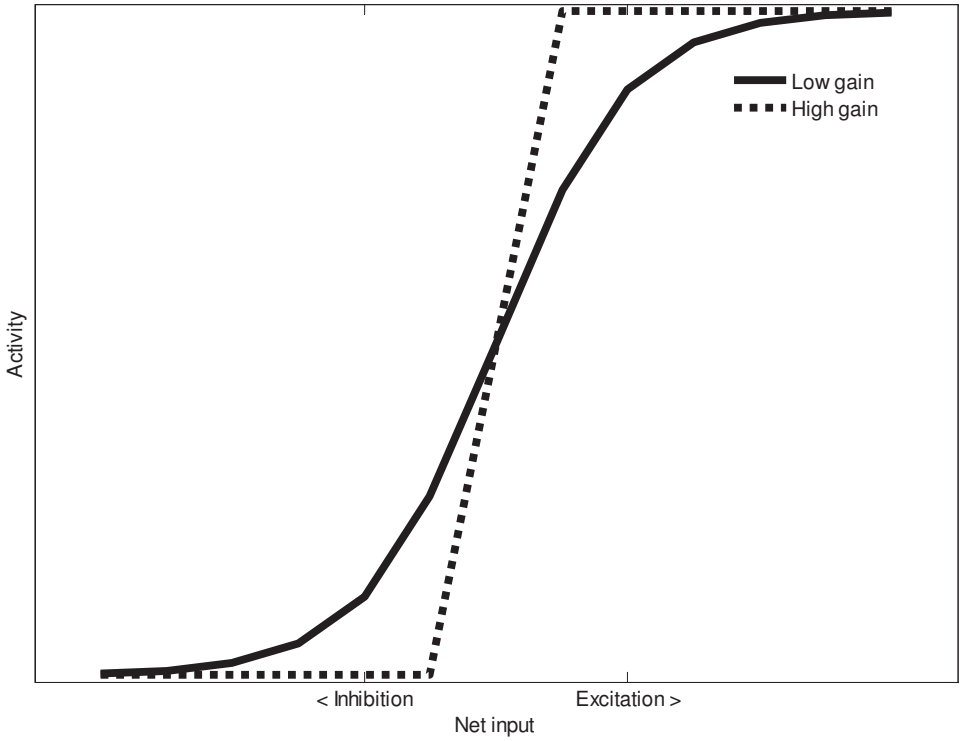

Figure 1.2. A neuron's activation function. The release of catecholamine neuromodulators like noradrenaline is thought to make the activation function steeper. This facilitates signal processing at the level of the network. Figure based on Aston-Jones and Cohen (2005).

The shape (i.e. steepness) of the activation function can be altered by adjusting the value of its gain parameter, where higher gain values make the function steeper (see Figure 1.2). It is thought that when catecholamine neuromodulators like NE are released, they adjust the shape of the activation function in this manner (Servan-Schreiber, Printz, & Cohen, 1990). Although a steeper activation function does not increase the neuron's ability to detect a signal per se, it does improve the ability to do so at the level of the network. Servan-Schreiber et al. (1990) demonstrated that increasing the gain of the units in a

network model improved signal detection in a simulated continuous performance task.

According to the adaptive gain theory, the phasic NE response can act as a temporal filter of attention: during periods of moderate increases in tonic levels of NE and robust phasic firing to targets, task performance is at an optimal level; baseline levels of NE are low, and phasic bursts are temporally correlated with target presentation, thus facilitating and improving performance by increasing gain at a crucial time in the decision process. During periods of increased tonic levels of NE and an absence of phasic bursts, gain is increased throughout the brain in a temporally nonspecific manner, which induces a state of easy distractibility and decreased task performance (Nieuwenhuis, Aston-Jones, & Cohen, 2005a).

1.4.2. A learning account of NE functioning

Yu and Dayan (2005) have proposed an alternative theory of NE functioning that emphasizes learning and uncertainty. According to this Bayesian theory, an agent tries to make causal inferences about his environment by minimizing the effect of sources of uncertainty. To this end, an agent has to process two types of uncertainty: expected and unexpected uncertainty. Yu and Dayan give an informative illustration of the difference between the two concepts (2005, p. 681): imagine a person who listens to the weather forecast on the radio every day. She will know that there are always small differences between the forecast and the actual weather (predicted uncertainty), but occasionally, there may also be sudden, considerable errors in those predictions, as caused by the onset of *el niño*, for example (unexpected uncertainty). Both types of errors can motivate one to search for another, more reliable (or rewarding) source of information. In more abstract terms, expected uncertainty refers to known irregularities in the relationships

between events and objects in a given context[1], while unexpected uncertainty reflects profound and sudden changes in the context. According to this theory, an agent's goal is to determine whether a given cue's invalidity is caused by expected uncertainty (suggesting that it might be optimal to maintain the current associative context) or by unexpected uncertainty (in which case it might be optimal to abandon the current context).

Pharmacological evidence, reviewed by Yu and Dayan (2005), suggests that NE might be important for the signaling of unexpected uncertainty, while the neuromodulator acetylcholine (abbreviated ACh, cf. Section 1.5) appears to underlie signaling of expected uncertainty. Yu and Dayan suggest a complicated relationship between NE and ACh: the neuromodulators are antagonistic when learning associations and classifying uncertainty as being expected or unexpected, while the relationship between NE and ACh is synergistic when predicting targets on the basis of cues.

Yu and Dayan devised a cognitive task in which both expected and unexpected uncertainty are manipulated, then simulated a model's performance on that task. The results of their simulations demonstrated that depleting levels of NE led their model to be overconfident and commit errors of perseveration, while depleting levels of ACh led to hyperdistractibility. Depleting levels of both NE and ACh led to decreased performance as well and supports Yu and Dayan's hypothesis that NE and ACh may operate antagonistically: performance was not as bad as when the levels of either NE or ACh were depleted, which suggests that if the level of one of these neuromodulators is depleted, the level of the other is increased to compensate.

[1] Context is a formal Bayesian term that refers to "a set of stable statistical regularities that relate the myriad environmental entities, such as objects and events, to each other and to our sensory and motor systems" (Yu & Dayan, 2005, p. 681).

1.4.3. A network reset account of NE functioning

Bouret and Sara (2005) proposed a network reset theory of theory of LC-NE functioning that, like the theory of Yu and Dayan (2005), relates the release of neuromodulators to making rapid adaptations to environmental circumstances. This network reset theory is not tested in this dissertation, so it will not be described in great detail here (for a review, see Sara, 2009). The theory is based on the premise that neurons form parts of networks and that these networks can be adapted flexibly by reconfiguring circuits to meet changes in task demands. Firing of noradrenergic neurons is suggested to interrupt the activity of functional networks and facilitate the reorganization of those networks to adapt to changing task demands (Bouret & Sara, 2005; Sara & Bouret, 2012).

This theory implies that the distractibility that is associated with tonic firing of the LC can be caused by several (counterproductive) network resets. During accurate task performance, associated with phasic firing of the LC, network resets are functional because they facilitate adaptation to changing task demands; furthermore, because the phasic firing mode of the LC is associated with lowered baseline firing (Aston-Jones & Cohen, 2005), the agent is less distractible and network resets are therefore more functional and task-related than the repeated resets in tonic firing mode.

1.5 Acetylcholine

Noradrenaline is one of the major neurotransmitters of the sympathetic nervous system, while acetylcholine is the major neurotransmitter of the parasympathetic nervous system (Baynes & Dominiczak, 2009). Although acetylcholine does not play a central role in this dissertation, Briand, Gritton, Howe, Young, and Sarter (2007) review evidence that suggests that the NE system can influence the cholinergic system, and therefore ACh is studied in chapters 3, 4 and 5, and it will be briefly described here. Acetylcholine (ACh) is synthesized

from choline and acetyl coenzyme A by choline acetyl transferase (Stahl, 2008). Cholinergic neurons are located mainly in the nucleus basalis of Meynert and the septal nuclei and project widely throughout the cortex from there (Felten & Shetty, 2010).

ACh can bind to two types of receptors, nicotinic and muscarinic; both receptor types have several subtypes. The muscarinic receptor type is most relevant in the context of this dissertation, because these receptors are the target site for many typical anticholinergics such as atropine and scopolamine (Stahl, 2008). The latter drug is used in studies reported in this dissertation (chapters 3, 4 and 5).

Functionally, acetylcholine is related strongly to learning and memory, and deterioration of this neurotransmitter system is linked to pathologies like Alzheimer's disease and even schizophrenia (Yakel, 2013). Gil, Connors, and Amitai (1997) showed that a nicotinic agonist can enhance thalamocortical synapses in rats. Ragozzino et al. (2012) showed that administration of a partial muscarinic agonist improved both working memory and facilitated strategy shifts in rats. Hasselmo and Sarter (2011) review evidence from animal studies that corroborates these findings and suggests that lesions of the cholinergic system impair attention.

Newman, Gupta, Climer, Monaghan, and Hasselmo (2012) describe the relationship between attention and ACh in terms that are reminiscent of the adaptive-gain theory of NE: these authors propose that ACh may affect neuronal signal-to-noise ratio. Newman et al. review evidence from modeling studies that suggests that ACh release enhances the processing of specific stimuli, thereby increasing the signal-to-noise ratio of perceptual processing. Hasselmo and Sarter (2011) describe a possible mechanism in more detail: glutamatergic pathways import information into the prefrontal cortex from the mediodorsal thalamic nucleus; this information stream can evoke a cholinergic response that biases attention.

An alternative view of the relationship between ACh and attention has already been mentioned: Yu and Dayan (2005) suggested that uncertainty signals suppress top-down information while enhancing bottom-up information. Based on reviewed evidence and simulation work, Yu and Dayan propose that ACh signals expected uncertainty and its release will therefore reduce top-down relative to bottom-up processing.

1.6 An overview of this dissertation

The adaptive-gain theory of Aston-Jones and Cohen (2005) and the learning account of Yu and Dayan (2005) form the basis of this dissertation, and the various empirical chapters can be related to these theories. As described before, the LC has two firing modes, which are related to exploitative (phasic firing) and explorative (tonic firing) behavior. Phasic firing is associated with high levels of attention and accurate task performance, while tonic firing is associated with distraction and deteriorated task performance. Therefore, it stands to reason that firing of the LC influences the way attention is exerted.

According to Yu and Dayan (2005), the NE signal modulates learning by signaling unexpected uncertainty. Following this theory, it seems plausible that NE influences attention by influencing the way in which uncertainty is processed.

In the next sections, more detailed information will be presented on the relation between NE, temporal attention, and uncertainty learning, and the various chapters of the dissertation will be presented.

1.6.1. NE and temporal attention

The first three empirical chapters of this dissertation are devoted to the topic of temporal attention, that is, the dynamic changes in attention on a fast time scale. Phasic LC responses are associated with an increase in the gain of neuronal populations, as shown in simulation work by Usher, Cohen, Servan-Schreiber,

Rajkowski, and Aston-Jones (1999). This increase in gain facilitates focusing attention on (task-relevant) stimuli that are already actively represented, while it impedes focusing attention on stimuli that are not actively represented and that might compete for attention with task-relevant stimuli. Due to the temporal dynamics of the phasic LC response, Aston-Jones and Cohen (2005) suggest that the phasic response can serve as "an attention filter that selects for the occurrence of (i.e. timing) of task-relevant events and facilitates responses to these events" (p. 416). The tonic firing mode, which is not locked to behavioral responses, does not serve such a filtering function.

An outline of the dissertation chapters that are devoted to the relationship between NE and temporal attention is presented below.

1.6.1.1. Functional significance of the emotion-related late positive potential

In Chapter 2 of this dissertation, we report a study on the function of a specific event-related potential (ERP) in the EEG signal, referred to as the late positive potential (LPP). This ERP component is strongly modulated by the emotional intensity of a stimulus: emotional stimuli of either a positive or negative valence elicit a larger (i.e. more positive) LPP than neutral stimuli (Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000; Hajcak, MacNamara, & Olvet, 2010; Keil et al., 2002).

We do not explicitly relate the LPP to the LC-NE system in this chapter, but work from our lab has suggested a relationship between noradrenergic functioning and this ERP component, by showing that propranolol, an antagonist of β-adrenoceptors, attenuates the amplitude of the LPP (de Rover et al., 2012). Furthermore, the LC has been suggested to be related to emotional processing (for a review, see Aston-Jones, Rajkowski, Kubiak, Valentino, & Shipley, 1996). For example, Sterpenich et al. (2006) demonstrated that the LC was active when

retrieving pictures of neutral faces, but only when an emotional response had been shown while encoding those faces (as indexed by a pupillary dilation response). These authors also showed an increase in functional connectivity between the LC and the amygdala (a brain area that is generally associated with emotional processing) when the faces were encoded in an emotional rather than a neutral context. Puglisi-Allegra and Ventura (2012) review evidence that corroborates these findings and argue that NE is necessary to attribute emotional salience to salient stimuli by modulating dopamine in the nucleus accumbens. Finally, Bradley, Miccoli, Escrig, and Lang (2008) demonstrated that the pupil dilates when a participant sees emotional stimuli; although unequivocal evidence that links pupil dilation to LC activity is lacking as of yet (cf. Section 1.6.2.2), there is much indirect evidence for the existence of such a relationship, making it all the more relevant to study the LPP in the light of the LC-NE system.

In this dissertation **[Chapter 2]**, we argue, on the basis of two experiments, that the LPP may well be related to temporal attention, because our results tentatively suggest that the LPP may reflect a global inhibition of potentially competing representations in the visual cortex, which may allow more selective processing of the emotional stimulus (Brown, van Steenbergen, Band, de Rover, & Nieuwenhuis, 2012).

1.6.1.2. The effect of clonidine and scopolamine on temporal attention as measured in the attentional blink paradigm

The next chapter of the dissertation focuses on a cognitive task that is representative of the temporal attention literature: the attentional blink task. In this task, participants have to identify two targets embedded within a stream of distractors, e.g. identify two numbers embedded in a stream of distracter letters. Ample research demonstrates that participants often cannot report the second target (T2) when it follows the first (T1) within 200-400 ms (Raymond, Shapiro, &

Arnell, 1992; Chun & Potter, 1995): this phenomenon is referred to as the attentional blink (AB).

A number of theories have been proposed to account for the AB phenomenon (for reviews, see Dux & Marois, 2009; Martens & Wyble, 2010). In the context of this dissertation, the theory proposed by Nieuwenhuis, Gilzenrat, Holmes, and Cohen (2005b) is particularly relevant, because these authors relate the AB to the LC-NE system. According to this theory, identification of a salient T1 is associated with arousal and hence a phasic peak in LC neuronal firing. The ensuing increase in NE release facilitates the processing of T1, increasing the probability of its correct identification. After firing phasically, LC neurons enter a refractory period of reduced firing. If T2 follows T1 within 200-400 ms, it coincides with this refractory period, and therefore a participant fails to notice T2 because T2 processing lacks the benefit of NE-mediated potentiation. This also explains so-called lag-1 sparing, that is, the lack of an AB when T1 and T2 immediately follow each other without intervening distracters (Raymond et al., 1992; Hommel & Akyürek, 2005), because the LC neurons have not yet entered their refractory period when T2 is presented. Typically, the AB disappears gradually when there is more than about 400 ms between T1 and T2: this seems plausible in the light of the theory of Nieuwenhuis et al. (2005b) as well, because the refractory period of the LC neurons has ended after that time.

Empirical work also suggests a strong involvement of the LC-NE system with AB performance. For example, De Martino, Strange, & Dolan (2008) showed that administration of the β-blocker propranolol reduced T2 identification accuracy. Furthermore, Jepma et al. (2011) found that a group of patients that lacks the enzyme dopamine-β-hydroxylase and therefore completely lacks NE and epinephrine had both a larger AB than healthy controls, as well as a smaller AB when on versus off medication that normalized their NE levels.

We sought to test the theory of Nieuwenhuis et al. (2005b) by administering the centrally acting α_2 agonist clonidine to a group of healthy volunteers **[Chapter 3[2]]**. In doing so, we followed up on work by Nieuwenhuis, Van Nieuwpoort, Veltman, and Drent (2007), in which participants also performed an AB task after ingesting clonidine. Nieuwenhuis et al. (2007) did not find a robust effect of clonidine on the AB, but the authors note that their study may have been slightly underpowered and had a between-subjects design. We expanded group size and used a within-subjects design and added a second drug (scopolamine, a muscarinic antagonist) to study the possible involvement of the cholinergic neuromodulator system in the AB.

1.6.1.3. The effect of clonidine and scopolamine on temporal attention as measured in the accessory stimulus paradigm

A large body of literature suggests that it is possible to influence temporal attention exogenously. Niemi and Näätänen (1981) review ample evidence that suggests that a warning cue that precedes an imperative stimulus can shorten reaction times to that stimulus. Bernstein, Clark, and Edelstein (1969a/1969b) ran experiments in which an auditory cue was presented almost simultaneously with a visual imperative stimulus, and showed that reaction times to the imperative stimulus decreased as a result of this auditory "accessory stimulus" (AS). Extensive work by Hackley and Valle-Inclán (1998; 1999; 2003) suggests that this reaction time effect is not caused by a mere facilitation of motor processes by the AS, but that the AS actually influences pre-motor processes. Jepma, Wagenmakers, Band, and Nieuwenhuis (2009) fit a drift-diffusion model to AS data and concluded that the AS only influences non-decision time, not the actual decision-making process itself. Jepma et al. argue that an AS therefore facilitates stimulus encoding (see Chapter 4 for a more detailed discussion of this phenomenon).

[2] Please note that the data for chapters 4, 5, and 6 were acquired in one experi-ment and the results presented in these chapters are based on the same sample.

In the context of this dissertation, it is important to note that the AS effect has been related to the LC-NE system. Coull, Nobre, and Frith (2001) showed that administration of the α_2 agonist clonidine reduces the behavioral benefits of a warning cue. These authors also demonstrate that clonidine is associated with reduced BOLD activity in the left temporo-parietal junction during cue presentation as compared to placebo. Similarly, it was shown that clonidine eliminates the beneficial effects of cues in rhesus monkeys (Witte, Gordon-Lickey, & Marrocco, 1992; Witte & Marrocco, 1997). Jepma et al. (2009) suggest that the involvement of the LC-NE system may be based on a phasic burst of NE that is elicited by the AS, a salient and arousing stimulus; as a result of this, NE may be released in the motor cortex, thereby increasing the responsivity of neurons in the motor cortex. This suggestion is in line with work by Stafford and Jacobs (1990) that showed that the release of NE can facilitate simple behaviors, as demonstrated by augmenting the masseretic reflex in cats by exposing them to arousing, NE-releasing stimuli. Attenuation of the NE system by administering an NE antagonist removed this augmentation.

We sought to test the hypothesis that the AS effect has a perceptual locus, by using a psychophysical task based on work by Rolke and Hofmann (2007) **[Chapter 4]**. These authors demonstrated that when the time period between a warning cue and an imperative stimulus decreases, visual perception improves. Rolke and Hofmann therefore suggest that temporal uncertainty influences perceptual processing as opposed to motor processes. This effect might depend on a participant's ability to start evidence accumulation for a given response decision earlier because temporal uncertainty about when to expect a stimulus is reduced. We had healthy volunteers perform a psychophysical accessory stimulus task after ingesting either clonidine (to attenuate the NE system), scopolamine (to study the possible involvement of the cholinergic system in the AS effect), or a placebo.

1.6.2. NE and learning

The second part of this dissertation is related to the relationship between NE and learning. The role of NE in learning is studied widely and has been implied for the first time by Kety (1970), who suggested that arousal that is evoked by novel or salient stimuli can modulate the synapses of neurons that are activated by those stimuli, thereby influencing learning. Devauges and Sara (1990) showed that stimulating the NE system facilitated attentional shifts in rats. Nieuwenhuis (2011) reviews evidence that plausibly suggests the involvement of the LC-NE system in learning. For example, when an agent updates his current context, that is, learns, this is often accompanied by a P3 (see section 1.6.2.1). Furthermore, NE is known to be able to influence long-term potentiation in the hippocampus (Berridge & Waterhouse, 2003), a brain area commonly associated with memory.

A number of theories have been proposed to account for the relationship between NE and learning. One of these theories, which suggests that phasic NE bursts increase Hebbian learning for all representations that are active at the time of the phasic burst, will be discussed in Section 1.5.2.2, because a dissertation chapter has been devoted to testing hypotheses derived from this theory.

As discussed previously, Yu and Dayan (2005) theorized that NE may be crucial for signaling unexpected change, while ACh may be crucial for reporting expected change. Therefore, according to their theory, both neuromodulator systems are intimately involved in learning. Dayan and Yu (2006) have elaborated on their theory by suggesting, on the basis of physiological and simulation data, that phasic NE release may serve as an interrupt signal. These authors propose that unexpected stimuli will evoke a phasic NE burst and that this burst interrupts an agent's default state. In their simulations, this meant that an agent would stop pressing a bar when an unexpected target stimulus was presented, which was associated with phasic NE release. The authors propose that the current context (cf. Section 1.3.2) is maintained by cortical areas, to which the LC projects widely.

Furthermore, the concept of phasic NE release as an interruption signal is in accordance with the network reset theory by Bouret and Sara (2005), which proposes that NE bursts serve to reset neuronal networks to flexibly adapt to task demands (cf. Section 1.3.2.).

1.6.2.1. The effect of clonidine and scopolamine on learning as reflected by the P3 ERP

Of all the ERP components studied, P300 (henceforth P3) is one of the most popular (Nieuwenhuis, Aston-Jones, & Cohen, 2005a). Being a large and robust ERP component, the P3 is relatively easy to study and since its identification in 1965 (Sutton, Braren, Zubin, & John, 1965), both the P3 and the specific antecedent conditions that evoke it have been studied extensively. The LC-P3 theory (Nieuwenhuis et al., 2005a) relates the P3 to NE signaling. This theory is based on three salient observations: firstly, there is a strong overlap in the antecedent conditions that evoke the P3 and the phasic LC response. For example, infrequent and motivationally salient stimuli evoke both a P3 and a phasic LC response. Secondly, there is a strong temporal overlap between the P3 and the phasic LC response: the P3 typically has an onset of about 150-200 ms poststimulus, which overlaps with NE release in the cortex. Finally, there is a strong spatial overlap between the brain areas that are involved with or demonstrate a P3 (Soltani & Knight, 2000) and LC projection areas. Furthermore, it is relevant to note that Swick, Pineda, and Foote (1994) showed that clonidine administration attenuated the P3, while Glover, Ghilardi, Bodis-Wollner, and Onofrj (1988) demonstrated that NE depletion eliminated the P3 completely. According to the LC-P3 theory, the P3 is therefore a reflection of phasic NE release and the increase in cortical gain that is associated with this mode of firing (cf. Section 1.3.1.; Nieuwenhuis et al., 2005a).

Recently, the P3 has been related to learning (for a review, see Nieuwenhuis, 2011). Several studies have reported a so-called difference due to memory (Dm)

effect, that is, a broad positive ERP deflection to stimuli that are later remembered as opposed to forgotten (for a review, see Wagner, Koutstaal, & Schacter, 1999). The Dm effect typically starts around 400 ms poststimulus and is sustained until about 800-900 ms poststimulus; the broad positive wave is probably a complex of a number of ERP components, but the P3 probably "makes up an important part of the effects that are reported." The Dm effect is manifested irrespective of the level of stimulus encoding: both shallowly and more elaborately encoded stimuli elicit a Dm effect (Friedmann, Ritter, & Snodgrass, 1996). Not only is the Dm effect found in tasks where participants are not aware that their memory will be tested later, but it is also observed in intentional encoding tasks. In these studies, the Dm effect is only found when participants engage in rote learning (Fabiani, Karis, & Donchin, 1986; Karis, Fabiani, & Donchin, 1984). Participants who used elaborate encoding strategies demonstrated normal P3s during the study phase, but this P3 did not correlate with memory performance, suggesting that the P3 is more strongly related to simple learning processes than to elaborate learning processes. Taken together, these findings suggest that phasic NE signals influence learning in a bottom-up manner; when participants utilize top-down mediated learning strategies, other neurophysiological processes are activated and the relationship between the P3 and learning becomes considerably less reliable.

The evidence reviewed above suggests a general relationship between (rote) learning and the P3, but the question that remains is what specific learning processes are reflected by the P3. The so-called context-updating hypothesis (Donchin, 1981; Donchin & Coles, 1988) suggests that a P3 is elicited whenever we update our schema of the environment on the basis of novel incoming information. As described previously, Yu and Dayan (2006) suggested that phasic NE bursts signal unexpected uncertainty. This idea seems in line with Donchin's context-updating hypothesis: if the expectations generated by our internal model are violated, a phasic NE response is generated and the model will be updated. The LC-P3 theory would suggest that this phasic NE response is reflected by a P3.

In the context of this dissertation, it is imperative to note that, in addition to involvement of the NE system in P3 generation, there is also substantial evidence for an influence of the ACh system on P3 generation (Wang et al., 1997; Hammond, Kimford, Aung-Din, & Wilder, 1987). However, the relative contributions of these two neuromodulator systems to the generation of the P3 are not well understood. We therefore sought to test the involvement of the NE system as well as the ACh system in P3 generation **[Chapter 5]**. The goal of this experiment was to test a hypothesis proposed by Ranganath and Rainer (2003). Their hypothesis is that the two neuromodulator systems contribute to separate subcomponents of the P3: the P3a (or novelty-P3) and the P3b. The P3b (or classic P300) has a parietocentral scalp distribution and is mainly sensitive to infrequent task-relevant stimuli. The P3a has a prominent frontocentral scalp distribution and is mainly sensitive to novel and highly deviant or salient task-irrelevant stimuli. Most P3s are a mixture of these two subcomponents (Spencer, Dien, & Donchin, 2001). Ranganath and Rainer discuss one study that found that administration of a NE antagonist in monkeys selectively abolished the posterior P3b, but left the frontal P3a unaffected. By contrast, in another study, administration of an ACh antagonist in humans attenuated the P3a but not the P3b. These findings suggest that the NE system may primarily affect the P3b, whereas the ACh system may primarily affect the P3a. Indeed, the topography of cortical generators of the P3a and P3b seems consistent with the primary projection areas of the basal forebrain (i.e., ACh: frontal and anterior medial) and locus coeruleus (i.e., NE: parietal). However, note the apparent contradiction between this hypothesis and the model by Yu and Dayan (2005) that we discussed above: novel and other unexpected stimuli (ACh: P3a) seem a source of unexpected uncertainty, whereas infrequent task-relevant stimuli (NE: P3b) seem a source of expected uncertainty. Our intended experiment allows us to contrast these two hypotheses by pharmacologically manipulating NE and ACh activity in participants.

1.6.2.2. The relationship between NE, arousal, pupil diameter, and Hebbian learning

There are other conceptualizations of the role of the LC-NE system in learning besides that of Yu and Dayan (2005): another theory is presented by Verguts and Notebaert (2008/2009), who modelled the role of NE in conflict-related learning. The Stroop task is commonly used to study conflict (MacLeod, 1992): participants are instructed to name the color in which a color word is printed (e.g., when the stimulus is *blue*, the correct answer is black). Typically, the amount of conflict a participant experiences, referred to as the Stroop effect, is quantified by subtracting the mean reaction time to congruent trials from the mean reaction time to incongruent trials; higher values indicate larger amounts of experienced conflict.

One particular and robust finding in this task is that the proportion of congruent stimuli (e.g. *black*) in a task block influences the magnitude of the Stroop effect: in blocks with mostly congruent trials, participants generally display larger Stroop effects than in blocks with mostly incongruent trials. One theory of conflict processing proposes that conflict is monitored continuously (i.e. on a trial-by-trial basis) by the anterior cingulate cortex (ACC), which recuits frontal control areas when conflict is detected, so as to exert control and optimize task performance (Botvinick, Braver, Barch, Carter & Cohen, 2001; Botvinick, Cohen, & Carter, 2004). According to this theory, task blocks with mainly congruent trials lull the conflict monitoring system into complacency, which exacerbates the conflicting impact of incongruent trials and therefore increases the magnitude of the Stroop effect. Blocks with mainly incongruent trials, however, require continuously high levels of control, which helps to reduce the impact of conflict on a participant's performance and therefore diminishes the size of the Stroop effect.

Recently, Verguts and Notebaert (2008/2009) formulated an alternative model of cognitive control, that resembles the model by Botvinick et al. (2001) to the extent that the ACC monitors conflict. In this model, however, when conflict is

registered, the LC is recruited and fires phasically in response to the conflict evoked by incongruent trials (connectivity between ACC and LC is reviewed in Berridge and Waterhouse, 2003). This phasic NE burst increases Hebbian learning (Berridge & Waterhouse, 2003), which strengthens the synaptic connections between stimulus and task demand representations, thereby facilitating responding to a given incongruent stimulus type the next time it is presented.

We provided the first empirical test of this conflict-modulated Hebbian learning hypothesis, as formulated by Verguts and Notebaert (2008/2009), by capitalizing on the hypothesized (and intuitively plausible) relationship between phasic arousal and pupil dilation (Bradley, Miccoli, Escrig, & Lang, 2008; Bradshaw, 1967; Kahneman, 1973; Nieuwenhuis, de Geus, & Aston-Jones, 2011) **[Chapter 6]**. In two experiments, we find only tentative evidence for the conflict-modulated Hebbian learning hypothesis: In one experiment the behavioral results from a Stroop task supported the predictions derived from this hypothesis, but the participants' pupil dilations only partially supported those predictions. In a second experiment, in which participants performed a Simon task with an accessory stimulus manipulation (cf. Section 1.5.1.2) to influence the amount of arousal directly, we found no robust relationship between arousal and learning rate, which also does not provide evidence for the conflict-modulated Hebbian learning hypothesis.

2. Functional significance of the emotion-related late positive potential

Abstract

The late positive potential (LPP) is an event-related potential component over visual cortical areas that is modulated by the emotional intensity of a stimulus. However, the functional significance of this neural modulation remains elusive. We conducted two experiments in which we studied the relation between LPP amplitude, subsequent perceptual sensitivity to a non-emotional stimulus (Experiment 1) and visual cortical excitability, as reflected by P1/N1 components evoked by this stimulus (Experiment 2). During the LPP modulation elicited by unpleasant stimuli, perceptual sensitivity was not affected. In contrast, we found some evidence for a decreased N1 amplitude during the LPP modulation, a decreased P1 amplitude on trials with a relatively large LPP, and consistent negative (but nonsignificant) across-subject correlations between the magnitudes of the LPP modulation and corresponding changes in d-prime or P1/N1 amplitude. The results provide preliminary evidence that the LPP reflects a global inhibition of activity in visual cortex, resulting in the selective survival of activity associated with the processing of the emotional stimulus.

This chapter is based on:

Brown, S. B. R. E., van Steenbergen, H., Band, G. P. H., de Rover, M., & Nieuwenhuis, S. (2012). Functional significance of the emotion-related late positive potential. *Frontiers in Human Neuroscience, 6:*33.

2.1 Introduction

In recent years, emotion has become an important and well-respected topic of neuroscientific research. One of the most powerful neuroscientific methods for assessing emotional processing in the human brain is the study of event-related potentials (ERPs). In the past decade, researchers have identified an ERP component, the late positive potential (LPP), that is strongly modulated by the emotional intensity of a stimulus: Emotional stimuli of either a positive or negative valence elicit a larger (i.e. more positive) LPP than neutral stimuli (Hajcak, MacNamara, & Olvet, 2010; Keil et al., 2002; Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000) and more arousing neutral pictures (e.g. scenes that include people) elicit a larger LPP than less arousing neutral pictures (such as scenes without people; Weinberg & Hajcak, 2010). This modulation is most pronounced around 400-600 ms following the stimulus (Schupp, Flaisch, Stockburger, & Junghöfer, 2006), but can continue for up to a second after the offset of the stimulus (Hajcak & Olvet, 2008). The LPP has been used in a range of applied fields. For example, it has been used as an index of abnormal emotional responding in both adults and children (Dennis & Hajcak, 2009; Horan, Wynn, Kring, Simons, & Green, 2010; Marissen, Meuleman, & Franken, 2010), to study social biases in ingroup/outgroup classification (Crites jr., Mojica, Corral, & Taylor, 2010; Hurtado, Haye, Gonzáles, Mares, & Ibáñez, 2009), and as a tool in criminological lie detection (Matsuda, Nittono, Hirota, Ogawa, & Takasawa, 2009).

The conditions that modulate the amplitude of the LPP have been charted extensively (Hajcak et al., 2010; Schupp et al., 2006; Olofsson, Nordin, Sequeira, & Polich, 2008). For example, stimuli that are rated as more arousing elicit a larger (i.e. more positive) LPP (Cuthbert et al., 2000; de Rover et al., in press). Most LPP studies have used pictures from the International Affective Picture System (IAPS; Lang, Bradley, & Cuthbert, 1999), but the emotion-related LPP modulation can

also be evoked by other stimulus types, such as threatening faces or emotional gestures (Schupp et al., 2004b; Flaisch, Häcker, Renner, & Schupp, 2011). Although most studies present emotional and neutral pictures for five to six seconds, an LPP modulation has also been observed with much shorter presentation times, even down to 120 ms (Schupp, Junghöfer, Weike, & Haum, 2004a). Furthermore, the LPP is relatively robust to multiple presentations of the same stimulus material (Codispoti, Ferrari, & Bradley, 2007).

Although the antecedent conditions of the LPP have been thoroughly studied, the functional significance of the LPP remains rather elusive. As Donchin (1981) argued, in the context of the P3: "I want to emphasize the distinction between an enumeration of the antecedent conditions and a process theory of a phenomenon. […] The theory I seek is to be a description of the functional significance of this process. The theory should elucidate the specific processing activities undertaken by the neuronal population whose activity is manifested on the scalp by a component." (p. 498/500). As we will discuss below, emotional stimuli appear to be able to both enhance and impair visual perception (Bocanegra & Zeelenberg, 2009b). This allows us to formulate two contrasting hypotheses about the functional significance of the LPP.

The implicit or (sometimes) explicit assumption in many LPP studies is that the LPP reflects a spatially nonspecific (i.e. global), temporary increase in attention, that serves to facilitate the processing of the affective stimulus that elicited the LPP. This *enhanced perception hypothesis* of the LPP is consistent with the neural generators of the LPP in ventral and dorsal visual areas (Sabatinelli, Lang, Keil, & Bradley, 2007; Baetens, Van der Cruyssen, Achtziger, Vandekerckhove, & Van Overwalle, 2011; Frühholz, Fehr, & Herrmann, 2009; Moratti, Saugar, & Strange, 2011) and with the typical finding that emotional stimuli automatically attract attention (Vuilleumier, 2005; Fox et al., 2000) and are processed more efficiently (Zeelenberg, Wagenmakers, & Rotteveel, 2006).

Comparable increases in temporal attention have been reported in the accessory stimulus paradigm, in which a task-irrelevant auditory accessory stimulus accompanying a visual target shortens reaction times by decreasing the time needed to encode the target (e.g., Jepma, Wagenmakers, Band, & Nieuwenhuis, 2009); and in the temporal cuing paradigm in which cues that predict the moment of target presentation are used by subjects to optimize reaction times, in part by speeding up the encoding of the target (Correa, Lupiáñez, & Tudela, 2005).

Although the enhanced perception hypothesis is plausible, it is not supported by any direct evidence. The fact that emotional stimuli modulate the LPP *and* are processed efficiently reflects a correlation and does not imply a causal relationship. The efficient processing of emotional stimuli may be due not to the process underlying the LPP but to other, earlier processes, some of which may be visible in the EEG signal. For example, emotional stimuli elicit an early posterior negativity (EPN), an ERP component that like the LPP is modulated by the emotional intensity of a stimulus, but that peaks earlier than the LPP (Schupp et al., 2006). Improved perception after emotional stimuli has also been related to early interactions between the amygdala and the magnocellular processing channel (Bocanegra & Zeelenberg, 2009a).

However, while emotional stimuli are generally processed more efficiently, they often impair the perception of concurrently presented neutral stimuli when they are in spatial competition (e.g., Pourtois, Grandjean, Sander, & Vuilleumier, 2005). In addition, Bocanegra and Zeelenberg (2009b) review evidence that, under some circumstances, the presentation of emotional stimuli can impair the perception of a *subsequent* neutral stimulus if the two stimuli are presented in close temporal proximity. Thus, the capturing of attention by emotional stimuli may be accompanied by and/or followed by a global inhibition of other representations in the visual cortex, and the LPP may reflect this global inhibition. This *global inhibition hypothesis* of the LPP is consistent with work by Birbaumer and

colleagues, who have argued, on the basis of biophysical arguments, that slow cortical positivities, like the LPP, must reflect decreased cortical excitability (Birbaumer, Elbert, Canavan, & Rockstroh, 1990).

To contrast these two hypotheses of the LPP, we require a paradigm that allows us to differentiate between the effects on perception of the LPP process and other, earlier brain processes like the EPN by separating the emotional stimulus (that elicits the LPP) and a subsequent target (that is used to probe the perceptual system). The critical question that can then be asked is whether the presentation of a *non-emotional* target *during* the LPP but *after* other processes like the EPN, will lead to improved or impaired perception of that target. Recent behavioral studies have used this paradigm to demonstrate that early perception of non-emotional stimuli is modulated after seeing emotional faces (Bocanegra & Zeelenberg, 2009a; Phelps, Ling, & Carrasco, 2006; for a similar paradigm, see Bradley, Codispoti, & Lang, 2006). However, these results do not inform the current research question because of the short stimulus onset asynchronies (SOAs: around 100 ms) used: the modulation of the LPP by emotional faces starts considerably later.

We conducted two experiments to contrast the enhanced perception hypothesis and global inhibition hypothesis of the LPP. In Experiment 1, we examined the relationship between LPP magnitude and perceptual sensitivity to non-emotional stimuli presented during the LPP. In Experiment 2, we examined the relationship between LPP magnitude and neural signatures of visual cortical excitability: the P1 and N1 components of the ERP waveform evoked by non-emotional stimuli.

2.2 Experiment 1

In Experiment 1, we examined the relationship between LPP magnitude and a behavioral index of perception. We manipulated LPP magnitude by varying the emotional valence of a series of IAPS pictures (negative or neutral valence; each presented for 200 ms) and measured the effect of the pictures on participants'

sensitivity to the orientation of a subsequent Gabor patch. The low spatial frequency of our Gabor patch was based on Bocanegra and Zeelenberg (2009a), who reported a beneficial effect on orientation sensitivity of an immediately preceding fearful face picture (SOA = 100 ms). The SOA between the IAPS picture and the Gabor target in our experiment was systematically varied between 570, 1070 (both during the LPP modulation) and 1570 ms (presumably after the LPP modulation). We used IAPS pictures because they are commonly used in psychophysiological research on emotion and elicit reliable and pronounced LPPs. We used an orientation discrimination task because orientation is processed in ventral and dorsal visual areas where the LPP is generated (Faillenot, Suneart, Van Hecke, & Orban, 2001). To avoid competition for temporal attention between the IAPS picture and the target (cf. Bocanegra & Zeelenberg, 2009b), we chose the shortest SOA to be longer than the typical attentional blink (Shapiro, Arnell, & Raymond, 1997). We also took various measures to avoid competition for spatial attention between the two stimuli (see Methods).

If the emotion-induced LPP modulation reflects increased global perceptual sensitivity, then orientation sensitivity should be enhanced following negative pictures, but only for the short and medium SOA (i.e., during the LPP modulation). Furthermore, participants with a larger LPP modulation should show a larger increase in orientation sensitivity following negative pictures. In contrast, the global inhibition hypothesis predicts the opposite pattern of results: orientation sensitivity should be decreased following negative pictures, and participants with a larger LPP modulation should show a larger decrease in orientation sensitivity following negative pictures. We used *d'* as an unbiased measure of perceptual sensitivity.

2.3 Materials and Methods

2.3.1. Participants

Twenty healthy young females, aged 19–28 years, took part in a single two-hour experimental session in return for course credit or €15. Participants were informed on the research procedures before their inclusion in the study.

2.3.2. Task

Participants performed a computerized decision-making task, based on that used by Bocanegra and Zeelenberg (2009a). Each trial started with a 500-ms fixation point (a white plus sign on a grey background), followed by the presentation, for 200 ms, of a picture from the International Affective Picture System (IAPS; Lang, Bradley, & Cuthbert, 1999) of either negative (e.g., mutilations, frightening animals) or neutral (e.g., faces with a neutral expression, clouds) valence (Figure 2.1). These pictures[3] (166 neutral and 167 negative) were repeated randomly to create a total of 252 trials of either valence. The negative and neutral pictures differed in mean normative valence rating (2.59 vs. 5.36, $t_{165} = 33.29$; $p < .001$) and mean normative

[3] The following IAPS pictures were used. Neutral valence: 1121 1313 1333 1450 1610 1616 1670 1675 1910 2038 2102 2104 2190 2191 2200 2210 2214 2220 2221 2235 2270 2305 2320 2357 2370 2372 2381 2385 2393 2396 2397 2440 2441 2445 2480 2487 2493 2495 2499 2501 2512 2513 2516 2560 2570 2575 2579 2580 2593 2620 2745.1 2840 2850 2870 2880 2890 2980 4000 4100 5000 5020 5030 5120 5130 5200 5390 5410 5471 5500 5510 5520 5530 5532 5533 5534 5535 5551 5711 5731 5740 5750 5760 5779 5800 5870 5875 5890 5891 5900 5982 5990 5991 5994 6150 6900 6930 7000 7002 7004 7006 7009 7010 7020 7025 7030 7031 7034 7035 7036 7037 7038 7039 7040 7041 7042 7043 7044 7050 7052 7053 7055 7056 7059 7060 7080 7090 7100 7110 7130 7140 7150 7160 7161 7170 7175 7179 7180 7185 7187 7190 7205 7217 7224 7233 7234 7235 7490 7491 7500 7545 7547 7550 7595 7640 7700 7705 7900 7950 8475 9070 9080 9171 9210 9360 9700 9913. Negative valence: 1050 1052 1070 1111 1114 1120 1200 1201 1205 1220 1240 1270 1300 1310 1321 1525 1930 1931 1932 2053 2095 2110 2120 2200 2276 2352.2 2455 2490 2683 2688 2703 2710 2730 2800 2811 2981 3000 3010 3016 3017 3030 3051 3053 3060 3061 3063 3064 3068 3069 3071 3080 3100 3101 3102 3110 3120 3130 3140 3150 3160 3168 3170 3180 3181 3191 3215 3220 3225 3230 3261 3266 3300 3350 3400 3500 3530 3550.1 5971 5972 6020 6021 6022 6190 6200 6210 6212 6213 6230 6242 6243 6244 6250 6260 6300 6311 6312 6313 6314 6315 6350 6360 6370 6410 6415 6510 6530 6540 6550 6555 6560 6570 6571 6821 6830 6834 6836 6838 6840 7359 7380 8230 8480 8485 9040 9042 9050 9140 9160 9180 9250 9252 9253 9254 9300 9301 9400 9402 9405 9409 9410 9419 9423 9424 9425 9426 9427 9428 9429 9433 9500 9520 9570 9571 9600 9611 9620 9621 9622 9630 9635.1 9800 9810 9900 9902 9910 9920 9921.

arousal rating (6.04 vs. 3.24, t_{165} = 37.58; p < .001; Lang, Bradley, & Cuthbert, 1999).

The entire task consisted of 504 trials, split up in eight blocks that each lasted about 5 minutes. In half of these trials neutral IAPS pictures were shown; in the other half, negative pictures. Pictures were shown in a random order. A yellow rectangle (visual angle 13.7° x 8.2°) was overlaid on the IAPS pictures and participants were instructed not to move their eyes outside of this rectangle.

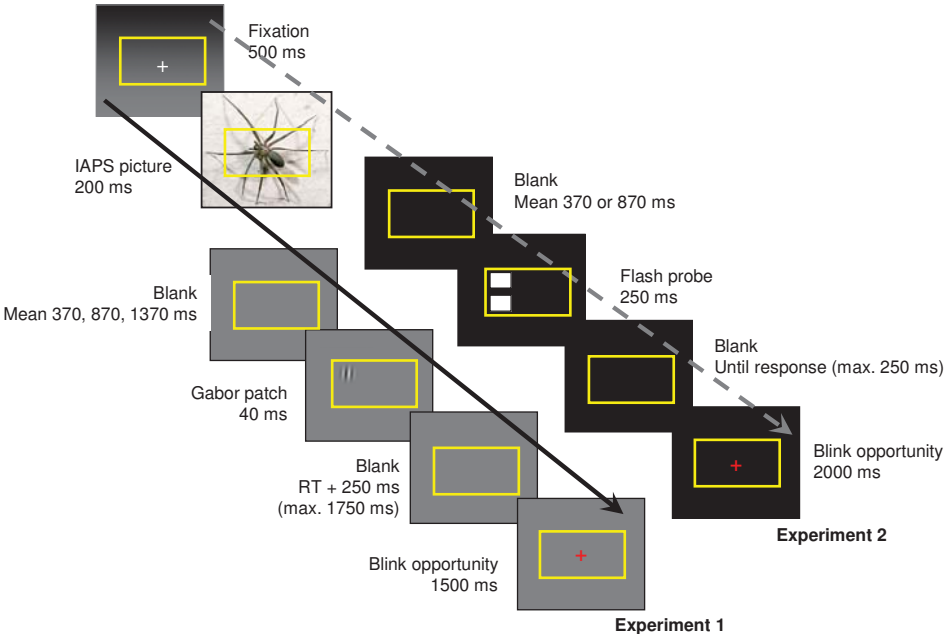

Figure 2.1. Sequence of trial events in Experiment 1 (continuous arrow) and Experiment 2 (discontinuous arrow). The participants' goal was to indicate whether a Gabor patch was tilted (Experiment 1), or to make a spatially compatible button press in case two flashes were presented to the left or right of fixation (Experiment 2).

This measure was taken to ensure that participants' attention stayed focused on the center of the screen, which contained the most salient part of the IAPS picture and the target stimulus, and to preclude eye movements as much as possible. The IAPS picture was followed by a blank screen of 350-390, 850-890 or 1350-1390 ms

(equiprobable within each valence, all jittered in steps of 20 ms, with means of 370, 870, and 1370 ms). Then, a Gabor stimulus (visual angle 1.8° x 1.8°) was presented in one of the four corners of the yellow rectangle for 40 ms. Gabor patches (2° Gaussian enveloped sinusoidal gratings) were created in MATLAB (The MathWorks Benelux), with a Michelson luminance contrast of 20% and a spatial frequency of 1.98 cycles per degree. The stimulus onset asynchrony (SOA) between the IAPS picture and the Gabor stimulus was, on average, 570 (short SOA), 1070 (medium SOA), or 1570 ms (long SOA). The task of the participant was to decide whether a Gabor patch was either tilted or not and to respond to straight Gabor patches by pressing a response button with their left hand, and to tilted Gabor patches by pressing a response button with their right hand. After the Gabor stimulus, a blank screen was presented until the participant's response, with a maximum duration of 1500 ms. This screen was followed by a short blank screen, which was presented for 250 ms. Finally, a red plus sign was presented for 1500 ms; the participant was instructed to try to blink only during the presentation of this screen.

Prior to the start of the task, participants first performed a short practice block (20 trials), in which the tilt of the Gabor patches was very pronounced (8°), so as to familiarize the participants with the purpose of the task. After these trials, the participants performed a practice block of 10 trials, in which the tilt of the Gabor patches was 4°. This block was repeated until the participants reached an accuracy of 70% or greater. Once this level of accuracy was established, the participants then performed 4 blocks of 10 trials each, in which the tilt of the Gabor patch varied per block (2°, 3°, 4°, or 5°). After finishing the fourth of these blocks, the participants' accuracy was evaluated per block, and the tilt that was associated with a performance of approximately 70% correct responses was selected to be used in the actual task. No IAPS stimuli were presented during these practice and tilt-adaptation blocks.

2.3.3. EEG recording and analyses

We recorded EEG from 30 Ag/AgCl scalp electrodes (Fpz, Fz, FC1, FCz, FC2, C3, C1, Cz, C2, C4, CP3, CP1, CPz, CP2, CP4, P7, P5, P3, P1, Pz, P2, P4, P6, P8, PO7, PO3, POz, PO4, PO8, Oz), and from the left and right mastoids. We measured the horizontal and vertical electro-oculogram (EOG) using bipolar recordings from electrodes placed approximately 1 cm lateral of the outer canthi of the two eyes and from electrodes placed approximately 1 cm above and below the participant's right eye. The EEG signal was pre-amplified at the electrode to improve the signal-to-noise ratio and amplified with a gain of 16x by a BioSemi ActiveTwo system (BioSemi B.V., Amsterdam). The data were digitized at 24-bit resolution with a sampling rate of 512 Hz using a low-pass fifth order sinc filter with a half-power cutoff of 102.4 Hz. Each active electrode was measured online with respect to a common mode sense (CMS) active electrode producing a monopolar (non-differential) channel, and was referenced offline to the average of the left and right mastoids. EEG and EOG were high-pass filtered at 0.01 Hz (24 dB/octave) and low-pass filtered at 15 Hz (24 dB/octave). Both of these filters are fourth-order Butterworth zero-phase filters.

Ocular and eyeblink artifacts were corrected using the method of Gratton, Coles, and Donchin (1983). Epochs with other artifacts (a gradient greater than 30 μV, spike artifacts [50 μV/2 ms] and slow drifts [300 μV/200 ms]) were also discarded. We extracted single-trial epochs for a period from 100 ms before until 1800 ms after picture onset. Then, for each participant and stimulus type, we averaged the EEG epochs to create stimulus-locked event-related potentials (ERPs). The average signal during the 100 ms pre-stimulus baseline was subtracted from each ERP. In the main analyses, the LPP was defined as the mean signal amplitude in medium- and long-SOA trials (i.e. unconfounded by target-evoked ERPs) over electrodes CP1, CPz, and CP2 during the interval 400-1200 ms, where the LPP modulation was maximal. In follow-up analyses, we computed "local" LPP modulations corresponding to each SOA, by creating negative – neutral

difference scores for three time windows (600-700 ms, 1100-1200 ms, and 1600-1700 ms, corresponding to the short, medium, and long SOAs). We conducted 2 (valence) × 3 (SOA) repeated-measures analyses of variance (ANOVAs) on *d'* and mean LPP amplitudes. A Greenhouse-Geisser correction was applied when appropriate. To examine the participants' perceptual sensitivity, we calculated *d*' as *z*(number of hits) – *z*(number of false alarms; Stanislaw & Todorov, 1999).

2.4 Results

2.4.1. Late positive potential

Figure 2.2 displays the ERPs elicited by the negative and neutral IAPS pictures. For each SOA there is a clear emotion-induced LPP modulation, starting around 400 ms post-stimulus and ending roughly 1 second later. As expected, the mean amplitude of the LPP was more positive following negative pictures (3.1 μV) than following neutral pictures (0.9 μV), $F(1, 19) = 81.89$, $p < .0005$, $\eta_p^2 = .81$. There was no interaction between valence and SOA, $F(2, 38) < 1$, $p = .57$, $\eta_p^2 = .03$. As expected, the local LPP difference between negative and neutral pictures was significant for the short (3.9 vs. 0.7 μV, $t_{19} = 7.27$, $p < .0005$) and medium SOAs (1.5 vs. -0.6 μV, $t_{19} = 4.22$, $p = .0005$). Contrary to our expectations, the local LPP difference for the long SOA was also significant (-0.1 vs. -1.4 μV, $t_{19} = 2.27$, $p = .04$).

2.4.2.Behavioral results

In line with our methods, error rates were close to 30%: 26% for negative pictures and 25% for neutral pictures. The *d'* values in each condition are reported in Figure 2.2. *d'* was neither influenced by valence, $F(1, 19) < 1$, $p = .36$, $\eta_p^2 = .05$, nor by SOA duration, $F(2, 38) = 1.60$, $p = .22$, $\eta_p^2 = .08$. Furthermore, although we expected to find increased or decreased perceptual sensitivity during the time of

maximal LPP modulation (i.e. for the short and medium SOAs), the interaction between valence and SOA was not significant, $F(2, 38) < 1$, $p = .50$, $\eta_p^2 = .04$. These results indicate that visual perception was not improved or impaired during the LPP modulation.

2.4.3. LPP-behavior correlations

We calculated negative – neutral *d'* difference scores for each SOA, and calculated cross-subject Pearson correlations between these difference scores and the mean magnitude of the LPP modulation (in the time window 400-1200 ms). In contrast with our expectations, we found no robust relationship between the magnitude of the LPP modulation and *d*' difference scores: correlations varied between -.35 and .03 (Figure 2.3, A). In addition, we calculated, for each SOA, the correlation between the *d'* difference score and the corresponding local LPP modulation (Figure 2.3, B). Again, we found no significant correlations, which indicates that larger LPP modulations following negative pictures were not accompanied by commensurate improvements or impairments in visual perception. However, it is noteworthy that most of the reported correlations were negative.

2.4.4. Within-subject comparisons between LPP amplitude quartiles

In addition to the inter-individual correlations described above, we also exploited intra-individual, trial-to-trial differences in LPP amplitude. We computed single-trial LPP values, using the same "global LPP" definition as in previous analyses. We then binned the trials in four quartiles, based on single-trial LPP value, separately for the negative and neutral IAPS trials, but collapsed across the three SOAs to collect enough trials per bin. Finally, we computed the average *d'* value for the trials in the first and fourth quartiles, and submitted these to a repeated-measures ANOVA with quartile (1 vs. 4) and valence as within-subjects factors. Small-LPP trials (quartile 1: *d'* = 1.4) and large-LPP trials (quartile 4: *d'* = 1.4)

were associated with a similar *d'* value, $F < 1$, and quartile did not interact with valence.

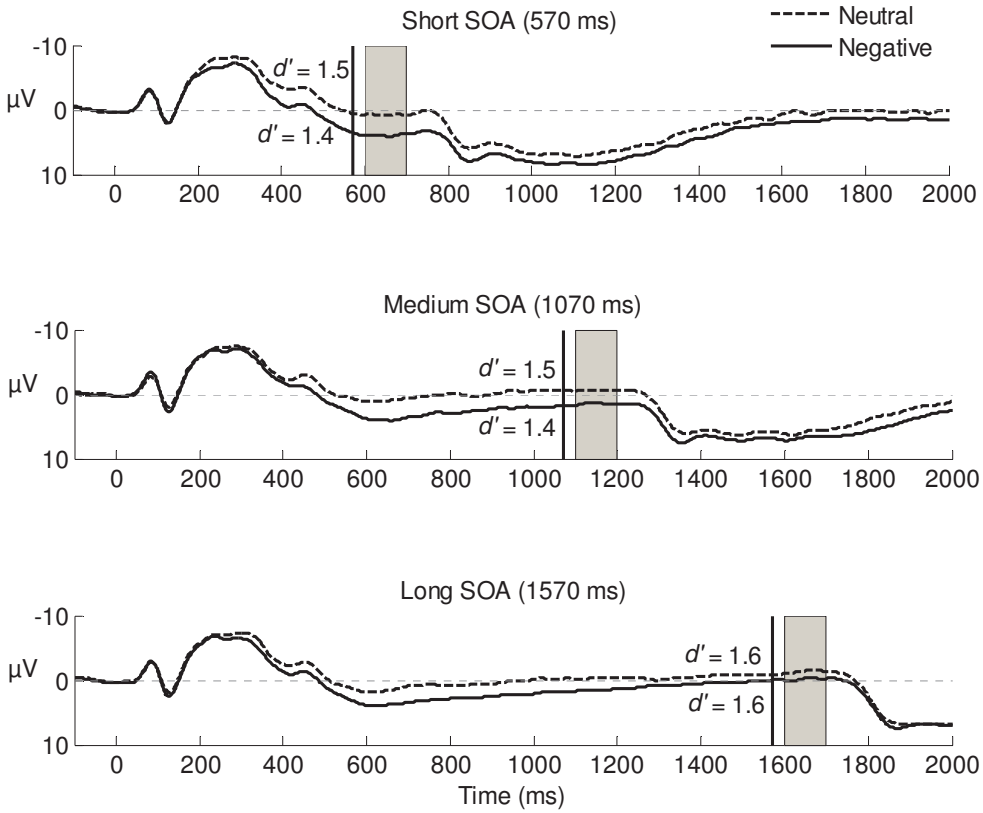

Figure 2.2 Stimulus-locked grand-average ERP waveforms averaged across electrodes CP1, CPz and CP2 following the presentation of negative or neutral IAPS pictures. Vertical black lines indicate the onset of presentation of the Gabor patch; gray rectangles represent the time window used for the analysis of the local LPP modulations1. d' averages for each SOA and valence are printed near the corresponding ERPs.

2.5 Discussion

In Experiment 1, we briefly presented vertically oriented and slightly tilted Gabor patches during and after a robust emotion-induced modulation of the LPP. The

results did not provide unequivocal evidence in favor of either the enhanced perception hypothesis or the global competition hypothesis: perceptual sensitivity to the orientation of the Gabor stimuli was neither improved nor impaired during the LPP modulation. Furthermore, individuals with larger LPP modulations did not show more improvement or impairment in *d'* after negative pictures.

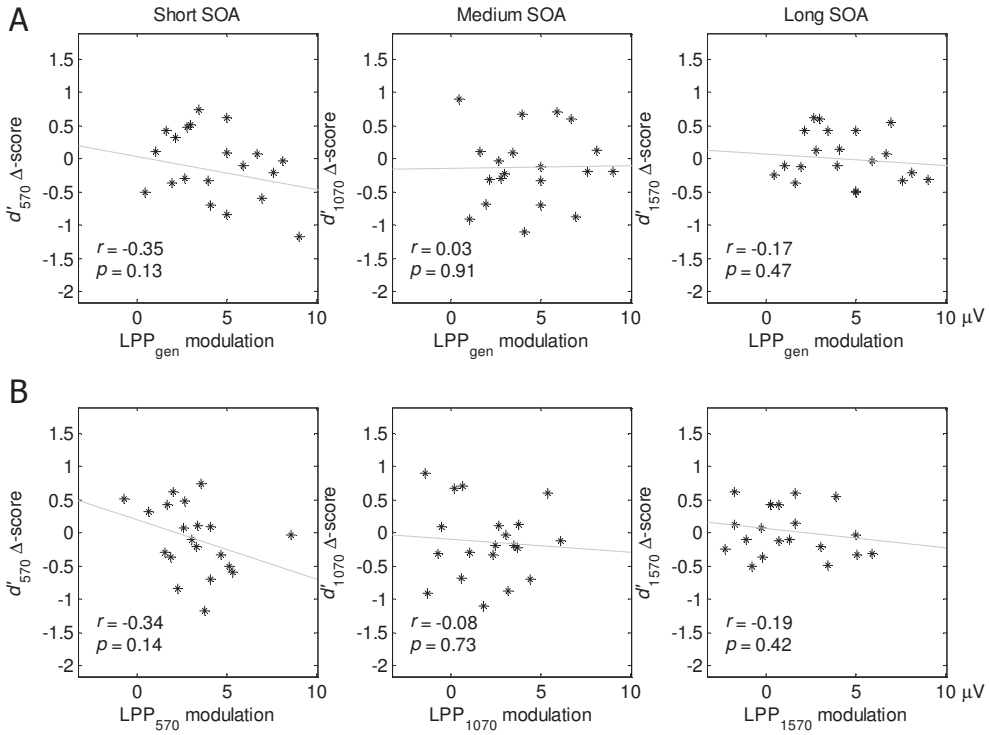

Figure 2.3. A: cross-subject correlations between a general LPP measure (LPP_{gen}: averaged over a 400-1200 ms time window) and the *d'* negative - neutral difference scores for each SOA. Subscripts indicate SOA length. B: correlations between local LPP modulations for each SOA (see Methods and Figure 2.2) and the corresponding *d'* negative - neutral difference scores.

If anything, the results seem tentatively compatible with the global inhibition hypothesis, because all but one of the reported correlations were negative: Individuals with larger LPP modulations showed a decline in orientation sensitivity

after negative pictures. To examine if these findings were robust across tasks and measures, we conducted a second experiment.

Our behavioral results seem at odds with Bocanegra and Zeelenberg (2009b), who found that a negative visual stimulus (a briefly presented word, like 'rape') improved perception of a neutral target word presented ~1000 ms later. An interesting question for future research is whether this discrepancy reflects the similarity between the emotional cue and the target stimulus, which was low in our experiment and high in Bocanegra and Zeelenberg (2009b). These authors did not collect ERP data, so it is unclear whether their emotional cue modulated the LPP.

2.6 Experiment 2

In Experiment 2, we investigated the relation between the LPP and direct electrophysiological correlates of perceptual sensitivity. As in the previous experiment, we manipulated LPP amplitude by varying the emotional valence of a series of briefly presented IAPS pictures. After one of two SOAs (570 or 1070 ms; both during the LPP modulation), the IAPS picture was followed by a non-emotional stimulus—a brief high-intensity flash known to elicit sizeable P1 and N1 components. The P1 and N1 reflect the early processing of stimuli in the visual cortex and are sensitive to spatially nonspecific increases in attention. For example, increases in temporal attention after accessory stimuli and temporal cues are often associated with increased target-evoked P1 and/or N1 amplitudes (Böckler, Alpay, & Stürmer, 2011; Correa, Lupiáñez, Madrid, & Tudela, 2006; Jepma et al., 2009). If the emotion-induced LPP modulation reflects increased global visual-cortex excitability, then the P1 and N1 to the flash probes should be larger after negative pictures (during the LPP modulation). Furthermore, participants with larger LPP modulations should show larger emotion-related increases in P1 and N1 amplitude. In contrast, if the LPP modulation reflects global inhibition of activity in visual cortex, the P1 and N1 amplitudes should be reduced after negative pictures, and especially so in participants with larger LPP modulations.

2.7 Materials and Methods

2.7.1. Participants

Twenty-five healthy young adults (twenty-two women; no overlap with Experiment 1), aged 18–27 years, took part in a single two-hour experimental session in return for course credit or €15. Participants were informed on the experimental procedures before their inclusion in the study.

2.7.2. Task

Participants performed a straightforward reaction time (RT) task (Figure 2.2). On each trial of this task, participants saw an IAPS picture depicting either a neutral event (158 individual pictures), or a negative event (158 individual pictures)[4]. The negative and neutral pictures differed in mean normative valence rating (2.54 vs. 5.30, $t_{319} = 51.69$; $p < .0001$) and mean normative arousal rating (6.09 vs. 3.19, $t_{319} = 54.16$; $p < .0001$). Each picture was presented twice during the course of the task, in a randomized order. After a 500-ms fixation stimulus (a white plus sign on a black background) the IAPS picture was presented for 200 ms. As in Experiment 1, a yellow rectangle was overlaid on the IAPS pictures and participants were instructed not to move their eyes outside of this rectangle. The IAPS picture was followed by a blank screen for either 350-390 ms (jittered in steps of 20 ms; mean 370 ms) or 850-890 ms (mean 870 ms). This blank screen was followed by one of four probe events: two white rectangles ('flashes'; visual angle 6.9° x 5.7°) were presented (i) in the upper and lower left corners of the screen; or (ii) in the upper and lower right corners of the screen (lateral flashes); (iii) in all four corners of the screen (quadruple flashes); or (iv) no white rectangles were presented at all (no

[4] We used the same IAPS pictures as in Experiment 1, except for the following: 1240 1270 1310 3230 3220 6311 6314 9140 9402 (negative valence) and 2516 2560 2575 5890 5982 5990 5991 5994 (neutral valence).

flashes). The probe stimulus lasted 250 ms, resulting in SOAs between picture onset and flash onset of, on average, 570 ms (short SOA) or 1070 ms (medium SOA). These SOAs are identical to the short and medium SOAs used in Experiment 1. A blank screen followed the stimulus until the participant's response, with a maximum of 250 ms. If participants did not respond before termination of this screen (which happened rarely), the trial was considered incorrect. Finally, a red plus sign was presented for 2000 ms; participants were asked to blink only during the presentation of this screen. Participants were instructed to respond as quickly as possible to lateral flashes by pressing a spatially compatible button. The purpose of this task was to actively engage the participants during the task and to be able to investigate effects of valence on RT (cf. Weinberg & Hajcak, 2011). Participants were instructed to refrain from responding to quadruple flashes or when no flashes were presented. The quadruple-flash trials were used to measure the flash-elicited P1 and N1, uncontaminated by response-related ERP components. The no-flash trials were used to measure the LPP, in a way that is not contaminated by visual ERP components evoked by the flashes. The task consisted of 640 trials in total: 160 with lateral flashes, 320 with quadruple flashes, and 160 with no flashes. There was a short break after every 80 trials. The total trial duration was between 3450 and 3990 ms.

2.7.3. EEG recording and analyses

The EEG recording and analysis methods were the same as in Experiment 1, with the following exceptions. For the P1 and N1 components, EEG and EOG were high-pass filtered at 3 Hz (24 dB/octave) and low-pass filtered at 15 Hz (24 dB/octave). Both of these filters are fourth-order Butterworth zero-phase filters. We used a different high-pass filter for the LPP and the P1 and N1 components to make sure that the mean-amplitude measurements of the latter high-frequency components were not distorted by simultaneous, unrelated low-frequency shifts.

The P1 was defined as the mean amplitude over electrodes PO7 and PO8, during the interval 80-120 ms. The N1 was defined as the mean amplitude measured over PO3 and PO4 during the interval 140-180 ms. Both were measured relative to the 100-ms pre-stimulus baseline. As in Experiment 1, the local LPPs were defined as the average signal in the 600-700 and the 1100-1200 ms windows, corresponding to the short and medium SOAs, respectively. We performed 2 (valence) × 2 (SOA) repeated-measures ANOVAs on mean RTs and mean P1/N1 amplitudes.

2.8 Results

2.8.1. Late positive potential (LPP)

Figure 2.4 displays the stimulus-locked ERPs for the no-flash trials. As expected, the mean amplitude of the LPP was more positive following negative pictures (0.42 μV) than following neutral pictures (-1.3 μV), $F(1, 24) = 18.02$, $p < .0005$, $\eta_p^2 = .43$. Furthermore, the local LPP difference between negative and neutral pictures was significant for both the short (1.1 vs. -0.7 μV, $t_{24} = 3.93$, $p = .001$) and the medium SOA (0.9 vs. -0.6 μV, $t_{24} = 3.52$, $p = .002$).

2.8.2. P1/N1 complex

Figure 2.5 displays the ERPs elicited by the flash probes on quadruple-flash trials. Contrary to the predictions of the two examined hypotheses, P1 amplitudes (reported in Figure 2.5) were not affected by the valence of the IAPS picture, $F(1, 24) < 1$, $p = .64$, $\eta_p^2 = .01$. Importantly, however, N1 amplitudes were lower after negative pictures (-3.3 μV) than after neutral pictures (-3.8 μV), $F(1, 24) = 12.97$; $p = .001$, $\eta_p^2 = .35$: an effect consistent with the global inhibition hypothesis. This significant main effect of valence was qualified by a significant interaction with SOA, $F(1, 24) = 40.17$, $p < .0005$, $\eta_p^2 = .63$. Post-hoc pairwise comparisons

showed that the difference between N1 amplitudes was significant for the short SOA, $t_{24} = 6.05$, $p < .0005$, but not for the medium SOA, $t_{24} = 0.32$, $p = .76$.

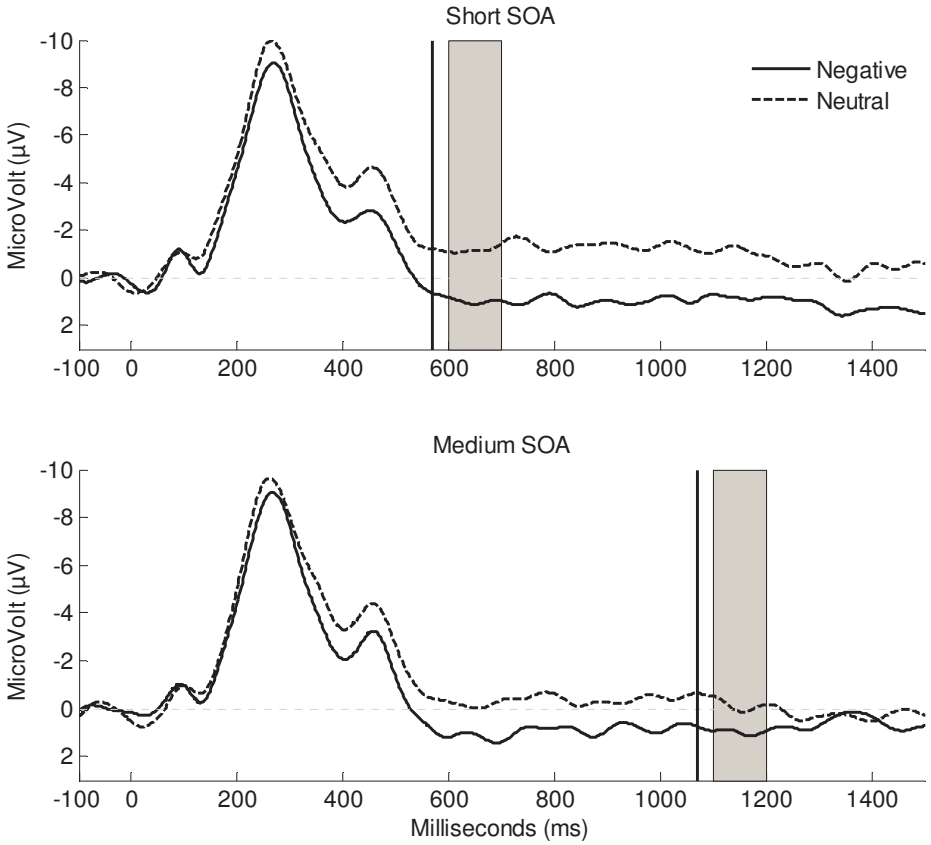

Figure 2.4. Stimulus-locked grand-average ERP waveforms averaged across electrodes CP1, CPz and CP2 following the presentation of negative or neutral IAPS pictures. Vertical black lines indicate the onset of presentation of the flashes; gray rectangles represent the time window used for the analysis of the local LPP modulations.

It is possible that we did not find increased P1 and N1 amplitudes after negative pictures because these components already reached a maximum amplitude in the neutral condition, perhaps because of the high intensity of the flash probe. To address the possibility of a ceiling effect, we computed the ipsilateral and contralateral P1 and N1 on neutral lateral-flash trials. As expected, given the

topographical organization of the visual cortex, the ipsilateral P1 (1.9 μV) was smaller than the contralateral P1 (3.5 μV), $F(1, 24) = 15.21$; $p = .001$, $\eta_p^2 = .39$. This indicates that the ipsilateral P1 in the neutral condition was not at ceiling and could be modulated upwards. However, the corresponding ipsilateral P1 after negative pictures (1.7 μV) was numerically smaller instead of larger, $F(1, 24) = 3.51$, $p = .07$, $\eta_p^2 = .13$. We conducted a comparable analysis for the N1 amplitudes. The ipsilateral N1 after neutral pictures was smaller (i.e. less negative; 1.2 μV) than the contralateral N1 (-0.9 μV), $F(1, 24) = 27.05$, $p < .0005$, $\eta_p^2 = .53$. And yet, the ipsilateral N1 was not modulated after negative pictures (1.3 μV), $F(1, 24) < 1$, $p = .58$, $\eta_p^2 = .01$. These findings indicate that the absence of a P1/N1 amplitude increase after negative pictures cannot be due to a ceiling effect.

2.8.3. LPP-P1/N1 correlations

For each SOA we calculated cross-subject correlations between the negative – neutral LPP difference scores and the negative – neutral P1/N1 difference scores (Figure 2.6, A). To circumvent confusion about negative and positive effect sizes, we reversed the signs of the N1 difference scores, such that the enhanced perception hypothesis predicted positive correlations and the global inhibition hypothesis predicted negative correlations in all of the analyses reported here.

To further examine the relationship between the picture-related LPP and flash-related P1/N1, we correlated local LPP modulations with corresponding P1 and (sign-reversed) N1 difference scores (Figure 2.6, B). As shown in Figure 6, all of the eight correlations were negative but none of them were significant.

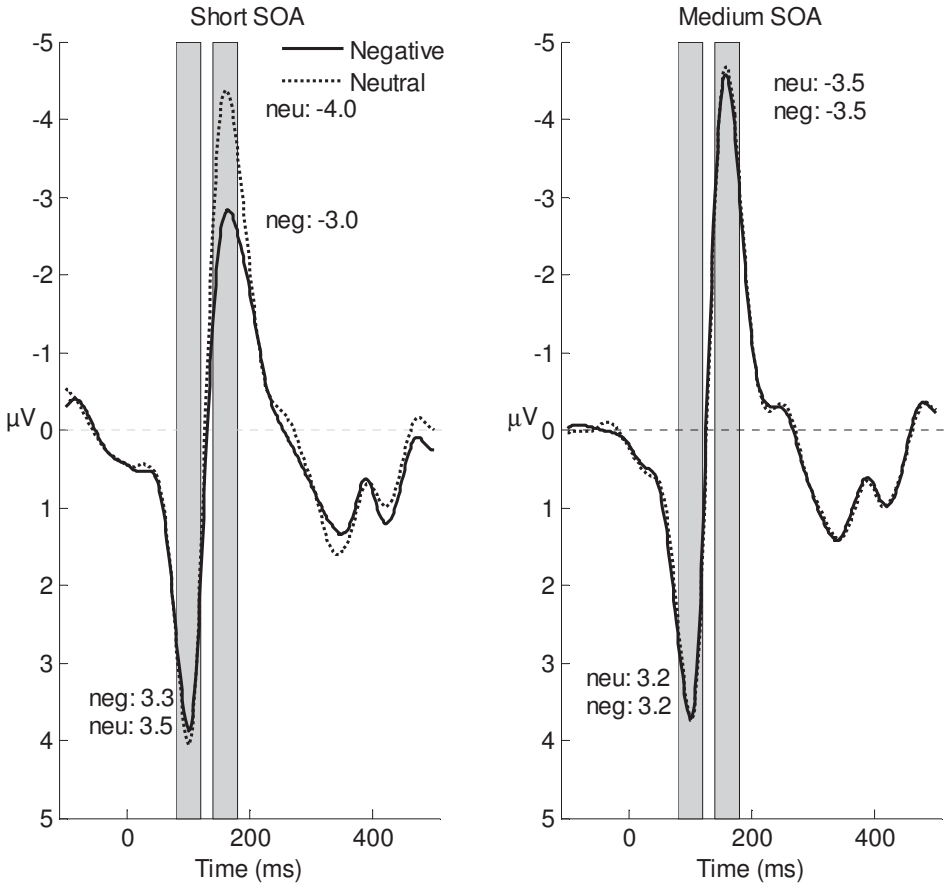

Figure 2.5. Stimulus-locked grand-average ERP waveforms averaged across electrodes PO7 and PO8 following the presentation of quadruple flashes. Gray rectangles represent the time windows used for the analysis of the P1/N1 effects. Average P1 and N1 amplitudes are printed near the corresponding waveform components.

2.8.4. Within-subject comparisons between LPP amplitude quartiles

As in Experiment 1, we also exploited intra-individual, trial-to-trial variability in LPP magnitude to test our main predictions. Because this required a comparison between the LPP and P1/N1 from the same trials, the analysis was necessarily based on the quadruple-flash trials, such that the LPP measures were contaminated by flash-elicited ERP components, including P1 and N1.

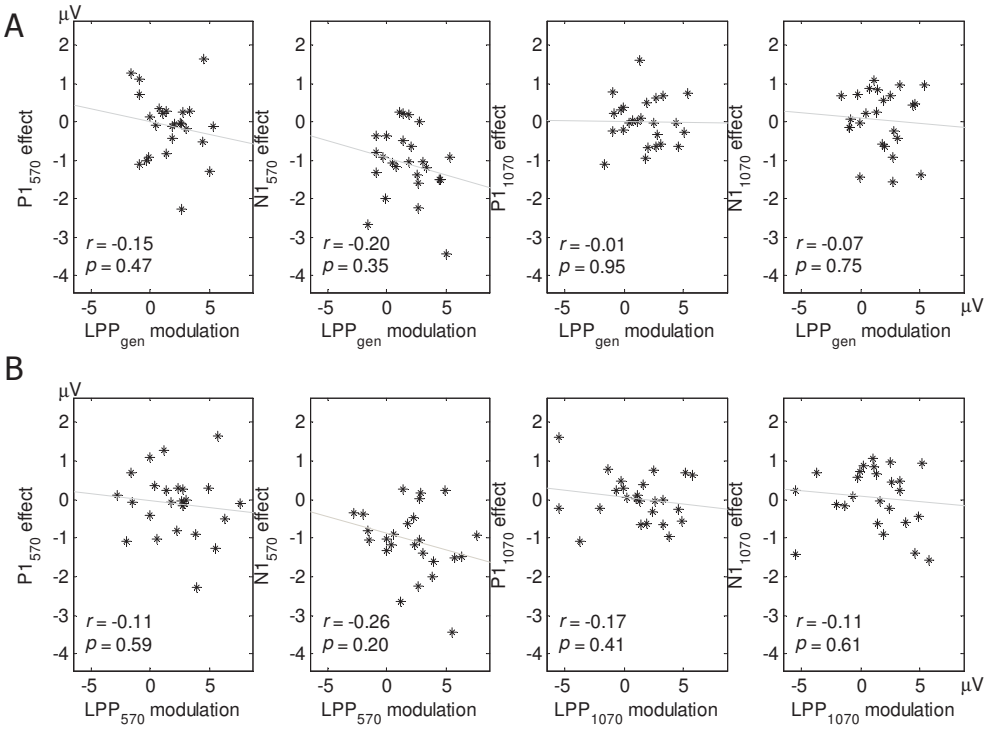

Figure 2.6. A: cross-subject correlations between a general LPP measure (LPP_{gen}: averaged over a 400-1200 ms time window) and P1 and N1 effects for each SOA. Subscripts indicate SOA length. B: correlations between local LPP modulations for each SOA (see Methods and Figure 2.4) and the corresponding P1/N1 effects. Note that N1 effects have been sign-reversed to facilitate visual comparisons between the graphs (see Methods).

We binned the trials in four quartiles, based on single-trial LPP value, separately for each task condition. Finally, we computed the average P1 and N1 amplitudes for the trials in the first and fourth quartiles (to compare the most extreme LPP amplitudes within participants), and submitted these to a repeated-measures ANOVA with quartile (1 vs. 4), valence, and SOA as within-subjects factors.

P1 amplitudes were lower for large-LPP trials (quartile 4: 3.1 μV) than for small-LPP trials (quartile 1: 3.3 μV), $F(1, 24) = 6.3$, $p = .02$. Note that this effect, predicted by the global inhibition hypothesis, is in the opposite direction of any effect caused by the confound described above. N1 amplitudes were also lower for

large-LPP trials (-3.2 μV) than for small-LPP trials (-3.4 μV), but this difference was not significant, $F(1, 24) = 1.3$, $p = .27$. Quartile did not interact with SOA or valence.

2.8.5. Behavioral results

Mean correct RTs on lateral-flash trials were similar in the negative (422 ms) and neutral condition (421 ms), $F(1, 24) < 1$, $p = .76$, $\eta_p^2 = 0.0$. Because mean error rates were very low and did not differ much between the negative (1.9%) and neutral condition (1.5%), we did not analyze them further.

2.9 Discussion

In Experiment 2 we presented high-intensity flashes during a robust emotion-induced modulation of the LPP. The amplitude of the P1 to these flashes was not affected by the emotional intensity of the preceding IAPS pictures. In contrast, N1 amplitudes were significantly smaller following negative pictures, but only if the interval between the picture and the flash was relatively short (570 ms). Furthermore, in line with Experiment 1, all cross-subject correlations between LPP modulations and corresponding changes in P1/N1 amplitudes were negative, although (again) none were statistically significant. Within-subject comparisons demonstrated that large-LPP trials were associated with lower P1 amplitudes. Taken together, these findings provide some support for the global inhibition hypothesis, but any truly existing effect is likely to be modest.

We found no difference in RTs and accuracy after negative pictures. These findings appear to contrast with results of Weinberg and Hajcak (2011), who reported small but reliable increases in RT following positive and negative IAPS pictures. This discrepancy is probably due to differences in stimulus duration. Weinberg and Hajcak presented the IAPS pictures for 1000 ms immediately before,

and for 400 ms immediately after the target stimulus, which was presented for 150 ms. That is, direct competition between the IAPS picture and the target for spatial and/or temporal attention was probably larger than in our study, and this competition may have been increased for arousing stimuli. Furthermore, subjects were planning and executing their response while the IAPS picture was back on the screen, allowing a direct effect of picture valence on RTs. We note that these response-time effects are of secondary interest to the current purposes, because RTs include the duration of several processes other than perception.

2.10 General Discussion

We conducted two experiments to gain insight into the functional significance of the LPP. In particular, we contrasted the enhanced perception hypothesis with the global inhibition hypothesis. The enhanced perception hypothesis proposes that the LPP reflects a broadly enhanced perceptual sensitivity throughout the visual field, a potentially adaptive attentional response to emotional stimuli. Conversely, the global inhibition hypothesis assumes that the LPP reflects a global inhibition of potentially competing representations in the visual cortex, which may allow more selective processing of the emotional stimulus. Given that the LPP modulation outlasts the presentation duration of the emotional stimulus itself, we assumed that the effect of the underlying process would carry over onto the visual processing of subsequently presented neutral stimuli (cf. Bocanegra & Zeelenberg, 2009b). However, we found no conclusive evidence for either an improved or an impaired relationship between LPP amplitude and a behavioral measure of perceptual sensitivity (Experiment 1) or more direct neural signatures of visual cortical excitability (Experiment 2).

Nevertheless, the results seemed to reveal a pattern. In Experiment 1, the behavioral index of perceptual sensitivity (*d'*) was unaffected by the valence of IAPS stimuli. More specifically, the robust LPP modulation to negative pictures

was not accompanied by enhanced or impaired perception of non-emotional targets presented during the LPP modulation. In Experiment 2, however, the N1 (but not P1) elicited by non-emotional stimuli presented during the LPP modulation was smaller following negative than following neutral IAPS stimuli, specifically when the interval between the IAPS picture and the flash probe was relatively short. Furthermore, the P1 (but not N1) was smaller on trials with a large LPP than on trials with a small LPP. Finally, participants with a larger LPP modulation tended to show an impairment in perceptual sensitivity (Experiment 1) and a (larger) decrease in P1 and N1 amplitude (Experiment 2). Although these correlations were of small to modest size, and not statistically significant, 13 out of 14 of the reported correlations were negative—a striking proportion. Thus, if anything, the pattern of results provides tentative support for the global inhibition hypothesis.

Let us, for the moment, assume that the LPP reflects global inhibition of activity in visual cortex. What might be the mechanism underlying this global inhibition? An interesting possibility is that the global inhibition reflects the threshold control of cortical excitability (Elbert, 1990; Elbert & Rockstroh, 1987). According to this account, the presentation of a motivationally significant stimulus is immediately followed by an increase in neuronal firing threshold, such that ongoing (pre-stimulus) neural activity will instantaneously drop to a low level, and activity will survive only in cell assemblies processing the just arrived stimulus. This interrupt function, while preventing overactivation in a network with primarily excitatory connections, will facilitate the processing of the motivationally significant stimulus and inhibit cortical excitability elsewhere in the visual cortex. This threshold regulation may be achieved by a local population of inhibitory interneurons or, more likely, by non-specific thalamo-cortical afferents (Elbert, 1990). Importantly, Birbaumer, Elbert and colleagues have argued that the reflexive up-regulation of firing threshold after an important stimulus, and corresponding dampening of competing neural activity, should manifest at the scalp as a slow positive potential, such as the LPP (Birbaumer et al., 1990). Future

studies could test a prediction of this account that larger LPPs should be accompanied by an increased threshold for effects of transcranial magnetic stimulation of the visual cortex.

A perhaps closely related possibility is that the LPP reflects a phase resetting of low-frequency delta oscillations, caused by the presentation of the emotionally arousing stimulus (cf. Schroeder & Lakatos, 2009). Lakatos and colleagues have found that the smallest visual-evoked responses and the slowest reaction times occur when stimuli are presented around the positive peak of delta oscillations, which they refer to as *low-excitability phase* (as opposed to the negative peak / high-excitability phase; Lakatos, Karmos, Mehta, Ulbert, & Schroeder, 2008). Thus, we propose that the indications that we found for a global inhibition of visual processing during the LPP may reflect the consequences of a positive-amplitude low-excitability phase of delta oscillations, reset by the presentation of emotional stimuli. In this case, the evidence for a reduction of P1/N1 amplitudes during the LPP reflects a case of cross-frequency coupling, with the power of faster oscillations (reflected in P1/N1 amplitude) modulated by the phase of slower (delta) oscillations. An interesting possibility is that phase resetting of delta oscillations is a result of the threshold-regulation process discussed above.

Although we found very little effects of our manipulations, we are confident that our dependent measures were sufficiently sensitive. Bocanegra and Zeelenberg (2009a) have reported emotion-related improvements in orientation sensitivity for the same Gabor stimuli that we used in Experiment 1. P1 and N1 amplitude, used in Experiment 2, are broadly accepted measures of perceptual sensitivity, and we demonstrated that the results could not be attributed to ceiling effects on these component amplitudes. In theory, it is possible that the manipulation of LPP amplitude was not sufficiently strong to be accompanied by robust effects on behavior. Our IAPS pictures, presented for only 200 ms, yielded LPP modulations of 2.2 μV (Experiment 1) and 1.7 μV (Experiment 2). These

modulations would probably have been larger if the picture duration had been longer. However, our paradigm required that the IAPS pictures disappeared well before the onset of the neutral target stimuli.

We do not understand why negative pictures were followed by a reduction of the probe-related N1 amplitude but not P1 amplitude. Studies using other temporal-attention tasks, like the accessory-stimulus task and the temporal-cuing task, have reported similar variable results: at times the P1 is modulated while the N1 is not, and sometimes the reverse is found (Böckler et al., 2011; Correa et al., 2006; Jepma et al., 2009). However, in these tasks the P1 and/or N1 increased in amplitude under conditions of enhanced temporal attention, whereas in our experiment the probe-related N1 decreased in amplitude. In contrast, Rockstroh, Müller, Cohen, and Elbert (1992) found similar results, using a paradigm that is much more comparable to our paradigm. These authors presented a probe (auditory click) during the P3 evoked by an oddball stimulus, and found decreased probe-related N1 amplitudes compared to probes presented during the small P3 evoked by standard stimuli. In contrast, the probe-related P1 was relatively unaffected by the size of the P3. A tentative hypothesis is that the emotionally arousing effects induced by the IAPS pictures in our study (and oddball stimuli in Rockstroh et al.'s study) only affect later stages of perceptual processing (reflected by the N1), which might account for the unmodulated P1. However, this seems incompatible with findings that emotion can affect very early stages of perceptual processing (e.g., Bocanegra & Zeelenberg, 2009b). Indeed, the within-subject comparison between trials with small and large LPP amplitudes (controlled for valence) revealed a reduction of the P1 but not N1 amplitude for large-LPP trials.

In sum, our results do not allow us to unequivocally reject or confirm either the enhanced perception hypothesis or the global inhibition hypotheses. However, while we found no evidence whatsoever for the enhanced perception hypothesis, some aspects of the results are consistent with the global inhibition hypothesis—

the notion that the LPP reflects a dampening of activity in visual cortex, perhaps as a result of reflexive threshold regulation after an emotionally arousing stimulus. In any case, we believe that our study provides significant clues for future studies that will try to link the LPP to cognitive and behavioral functions; we hope that our study will encourage others to study not just *when* the LPP occurs, but also *what function* it reflects.

3. Effects of clonidine and scopolamine on multiple target detection in rapid serial visual presentation

Abstract

In this study we examined the effects of clonidine and scopolamine on multiple-target detection in a rapid serial visual presentation task to assess the role of the central noradrenergic and cholinergic systems in temporal attention. Eighteen healthy volunteers took part in a crossover double-dummy study in which they received clonidine (150/175 μg), scopolamine (1.2 mg) and placebo by mouth in counterbalanced order. A dual-target attentional blink task was administered at 120 min after scopolamine intake and 180 min after clonidine intake. The electroencephalogram was measured during task performance. Clonidine and scopolamine both impaired detection of the first target (T1). For clonidine, this impairment was accompanied by decreased amplitudes of the P2 and P3 components of the event-related potential. The drugs did not impair second-target (T2) detection, except if T2 was presented immediately after T1. The attentional blink for T2 was not affected. These and other results suggest that clonidine and scopolamine may impair temporal attention through a decrease in tonic alertness, and that this decrease in alertness can be temporarily compensated by a phasic alerting response to a salient stimulus. The comparable behavioral effects of clonidine and scopolamine are consistent with animal studies indicating close interactions between the noradrenergic and cholinergic neuromodulator systems.

This chapter is based on:

Brown, S. B. R. E., Slagter, H. A., van Noorden, M. S., Giltay, E., van der Wee, N. J. A., & Nieuwenhuis, S. (submitted). Effects of clonidine and scopolamine on multiple target detection in rapid serial visual presentation.

3.1 Introduction

Temporal attention—the dynamic changes in attention on a fast timescale—is widely studied because it is crucial for organisms to be able to prioritize and accurately identify incoming information, for example to make successful decisions. In recent years, the neuromodulatory basis of temporal attention has attracted considerable scientific interest. One theory relates temporal attention to the noradrenergic neuromodulator system. This theory is founded on the idea that the release of noradrenaline by the locus coeruleus (LC) adjusts the gain of postsynaptic neurons, thereby modulating these neurons' responsivity (Servan-Schreiber, Printz, & Cohen, 1990). The LC has been demonstrated to fire phasically following the presentation of task-relevant or salient stimuli (Aston-Jones, Rajkowski, & Cohen, 2000) and these phasic bursts are temporally closely related to behavioral responses (Bouret & Sara, 2004). These findings suggest that the phasic LC response acts as a temporal attention filter that selectively facilitates the processing of motivationally significantly stimuli (Aston-Jones & Cohen, 2005; Nieuwenhuis, Aston-Jones, & Cohen, 2005a).

Rapid changes in temporal attention are commonly studied with the attentional blink task, in which participants have to identify two targets that are embedded in a rapid serial visual stream (RSVP) of distractor stimuli. Participants are usually able to accurately identify the first of those targets (T1). The crucial finding in this task is that when the second target (T2) follows the first target within 200-400 ms, participants are often unable to report T2 accurately (Raymond, Shapiro, & Arnell, 1992; Chun & Potter, 1995). This phenomenon is referred to as the attentional blink.

Nieuwenhuis and colleagues have proposed a theory in which the attentional blink reflects the temporal dynamics of the noradrenergic system

(Nieuwenhuis, Gilzenrat, Holmes, & Cohen, 2005b; see also Warren et al., 2009). This theory assumes that identification of T1 is associated with a transient burst of arousal and concomitant phasic firing of the LC. Following this T1-related phasic burst, LC neurons enter a refractory period of reduced firing. During this period, no noradrenaline-mediated facilitation of stimulus processing can occur. The characteristic attentional blink window of 200-400 ms corresponds to the duration of this refractory period, which would explain why participants often fail to detect T2 if it is presented within this time window. This theory also accounts for the phenomenon of lag-1 sparing (Raymond et al., 1992; Hommel & Akyürek, 2005): participants generally do detect T2 if it directly follows T1 (i.e. at "lag 1"). When there is such a close temporal proximity of T1 and T2, detection of T2 is proposed to benefit from the phasic noradrenaline burst elicited by T1 (Usher, Cohen, Servan-Schreiber, Rajkowski, & Aston-Jones, 1999).

There is direct empirical evidence for the involvement of the noradrenergic system in target detection under rapid serial visual presentation conditions. For example, antagonization of noradrenergic β-receptors by propranolol led to impaired T2 identification in humans (de Martino, Strange, & Dolan, 2008). Furthermore, patients with dopamine-β-hydroxylase deficiency, a rare genetic syndrome characterized by the complete absence of noradrenaline, were shown to have a larger attentional blink than healthy controls, and this impairment was restored by treatment with a synthetic precursor of noradrenaline (Jepma, et al., 2011). However, when Nieuwenhuis, van Nieuwpoort, Veltman, and Drent (2007) used the noradrenergic α_2 agonist clonidine to attenuate noradrenergic baseline activity, they did not find a reliable decrease in T2 identification accuracy, a finding that seems at odds with the theory of Nieuwenhuis et al. (2005b).

The goal of the present experiment was to replicate the study by Nieuwenhuis et al. (2007), but with two improvements: a crossover design instead of a between-subjects design to increase statistical power; and an increased dose of

clonidine to induce a more pronounced attenuation of the noradrenergic system. Furthermore, we recorded the electroencephalogram (EEG) to acquire more insight into the electrophysiological correlates of treatment effects on RSVP performance.

We tested 18 healthy adult participants in a double-blind placebo-controlled randomized crossover design. Participants received, in different test sessions, a single dose of clonidine, scopolamine and placebo. Clonidine is a centrally-acting α_2 agonist that attenuates baseline noradrenergic activity by agonizing pre-synaptic α_2 receptors, and decreases the amplitude of the human P3 component, a putative electrophysiological correlate of phasic noradrenaline release (Nieuwenhuis, Aston-Jones, & Cohen, 2005a; Pineda, Foote, & Neville, 1989). If the attentional blink is mediated by a phasic noradrenergic burst following presentation of T1, and clonidine decreases this phasic burst, then clonidine may be expected to reduce the attentional blink. Notably, previous event-related potential (ERP) studies have associated the attentional blink with a larger or a delayed T1-elicited P3 (Martens, Munneke, Smid, & Johnson, 2006; Sergent, Baillet, & DeHaene, 2005; Slagter et al., 2007). We were thus specifically interested in possible effects of clonidine on the T1-elicited P3.

The involvement of the cholinergic system in temporal attention has been investigated less extensively than that of noradrenaline. Previous empirical work has focused on the nicotinic cholinergic system and has not studied RSVP performance, but other temporal attention tasks like temporal cuing (e.g., Beane & Marrocco, 2004; Stewart, Burke & Marrocco, 2001). Therefore, we also administered the muscarinic antagonist scopolamine, a drug with a sedation profile comparable to that of clonidine. The use of scopolamine allowed us to gain insight into the role of the cholinergic muscarinic system in temporal attention and to test whether any treatment effects are specific to the noradrenergic system.

3.2 Methods and Materials

3.2.1. Participants

Eighteen healthy young adults (15 women), aged 18-26 years (mean age 21 years), drafted through Leiden University's participant recruitment system, took part in three 4.5-hour experimental sessions in return for €140. Only participants with a systolic blood pressure above 100 mmHg, a diastolic blood pressure above 70 mmHg and a heart frequency over 65 beats per minute in rest were included in the study (cf. Nieuwenhuis et al., 2007). All participants underwent a medical screening which included a routine physical examination prior to being included in the experiment: only healthy individuals without a history of neurological or psychiatric disorders were allowed to participate. Participants took no prescribed medication and did not smoke; participants were instructed to abstain from using recreational drugs, caffeine, or alcohol 15 hours prior to the study. Participants received a single oral dose of clonidine, a single oral dose of scopolamine (1.2 mg), and a placebo in a randomized, double-blind, counterbalanced double-dummy crossover design. The first 11 participants received a clonidine dose of 175 µg. As the eleventh participant showed an unexpected large drop in blood pressure of 35 mmHg systolic, but without clinical consequences, 60 minutes after the ingestion of clonidine 175 µg (blind was broken by the supervising physician), we decided to reduce the dose of clonidine to 150 µg for the final 7 participants. Preliminary repeated-measures ANOVAs with dose as between-subject factor revealed no reliable main effect of dose or interactions including this factor, so in the analyses reported below the 18 participants are pooled. Clonidine, scopolamine, and placebo were administered during three separate test sessions, spaced one week apart. The study was approved by the medical ethics committee of the Leiden University Medical Center. Written informed consent was obtained from all participants prior to inclusion in the study.

3.2.2. Task

Participants performed an attentional blink task. Each trial started with a 500-ms fixation point (black plus-sign on light grey background, visual angle 0.6 x 0.6°), followed by a 2-s blank, after which a RSVP stream of 22 uppercase letters was presented centrally (visual angle of each letter approximately 0.7 x 0.7°). Each letter was randomly drawn without replacement from the alphabet and presented for 74 ms, followed by a blank of 24 ms. The letters *I*, *O*, *Q*, and *S* were left out, as they resemble digits too much. On each trial, two letters were replaced by digits (range 2-9, chosen randomly without replacement): targets 1 and 2 (T1 and T2). T2 was presented three to six temporal positions from the end of the stream. The temporal distance between T1 and T2 was either one (12.5% of trials), two (37.5% of trials), three (37.5% of trials), or seven items (12.5% of trials), corresponding to lags of 98, 196, 294, and 658 ms. Immediately after the end of the RSVP stream, participants were asked to identify T1 and T2 by typing them, in order, on a standard keyboard. The task consisted of 6 blocks of 40 trials each, and was preceded by a practice block of 12 trials, in which feedback on the participants' performance was given on every trial (e.g., a display of "+ –" indicated that a participant had entered T1 correctly and T2 incorrectly).

3.2.3. Procedure

Each participant was tested at approximately the same time of day. During every test session participants received a capsule of clonidine or placebo at 09.35 AM and a capsule of scopolamine or placebo at 10.35 AM. The different kinetic profiles of clonidine and scopolamine necessitated administrations at different times prior to testing. This double-dummy design resulted in one clonidine session (i.e. clonidine verum plus scopolamine placebo), one scopolamine session (clonidine placebo plus scopolamine verum), and one placebo session (clonidine plus scopolamine placebos). To eliminate any possible confound of drug order, we

stratified this factor by distributing the six possible drug orders evenly across participants.

At the start of each session (t = –20 min), a peripheral intravenous cannula was placed and connected to an intravenous 0.9% NaCl (saline) drip to be able to increase blood pressure through volume expansion and to have an entryway to administer escape medication in the case of a severe drop in tension and/or heart frequency. Furthermore, three cardio electrodes were applied to the participant's chest and connected to an electrocardiography (ECG) monitor. To measure the sedative, alertness-reducing properties of clonidine and scopolamine, we administered a 40-trial simple reaction time task (SRT) task upon a participant's arrival in the lab, as well as right before and after the participant performed the attentional blink task. Participants had to respond as quickly as possible whenever a white circle appeared on the computer screen. Stimulus onset asynchrony was jittered between 500-1250 ms, with a mean of 1000 ms; this task lasted less than two minutes.

At t = 0 min, participants ingested a microcrystalline cellulose-filled capsule with either clonidine or placebo. Clonidine has well-established antihypertensive properties: therefore, blood pressure and heart rate were monitored four times an hour from t = 0 onwards for participant safety with an Omron M10-IT automatic sphygmomanometer. At t = 60 min, participants ingested a microcrystalline cellulose-filled capsule with either scopolamine or placebo.

At t = 180, participants performed the attentional blink task which lasted approximately 30 minutes; during the 90 minutes prior to this time point, participants performed three unrelated cognitive tasks (Brown et al., 2015; Brown, van der Wee, van Noorden, Giltay, & Nieuwenhuis, submitted). Participant fitness was checked at t = 240, and participants were sent home via public transportation if their blood pressure and heart rate were close to the values measured at t = –20. At the end of the third test session, participants received their financial compensation.

3.2.4. EEG recording and analyses

We recorded EEG from 64 Ag/AgCl scalp electrodes and from the left and right mastoids. We measured the horizontal and vertical electro-oculogram (EOG) using bipolar recordings from electrodes placed approximately 1 cm lateral of the outer canthi of the two eyes and from electrodes placed approximately 1 cm above and below the participant's right eye. The EEG signal was pre-amplified at the electrode to improve the signal-to-noise ratio and amplified with a gain of 16x by a BioSemi ActiveTwo system (BioSemi B.V., Amsterdam). The data were digitized at 24-bit resolution with a sampling rate of 512 Hz using a low-pass fifth-order sinc filter with a half-power cutoff of 102.4 Hz. Each active electrode was measured online with respect to a common mode sense (CMS) active electrode producing a monopolar (non-differential) channel, and was referenced offline to the average of the left and right mastoids. Data were high-pass filtered at 0.1 Hz and low-pass filtered at 30 Hz. Ocular and eyeblink artifacts were corrected using the method of Gratton, Coles, and Donchin (1983). Epochs with other artifacts (a gradient greater than 30 μV, slow drifts [>300 μV/200 ms], and low activity [<0.50 μV/100 ms]) were discarded (placebo: 1.2%, clonidine: 1.3%, scopolamine: 2.5%). Data were epoched from -100 to 600 ms relative to the onset of T1 and then averaged. A baseline, computed as the average signal activity across the 100 ms prior to T1, was subtracted for each ERP.

As the active compounds only reliably influenced T1 accuracy (regardless of lag), and given previous studies linking the T1-evoked P3 to the attentional blink (e.g., Martens et al., 2006; Sergent et al., 2005; Slagter et al., 2007), we focused our electrophysiological analyses on T1-evoked potentials. We analyzed the ERP elicited by T1 with a sliding-window approach to examine the main effect of treatment on T1 processing and its interaction with T1 accuracy, focusing in particular on electrodes Cz, CPz and Pz, where the P3 was largest in amplitude. We

collapsed T1-locked ERPs across lags, then split the ERPs for each treatment and each participant into 19.5-ms windows, starting at t = 0 (i.e. 0-19.5 ms, 19.6-39 ms, etc.), averaged the amplitudes in each window across participants, and then submitted these averages to a repeated-measures ANOVA with treatment as a within-subjects factor. We considered the difference between the three treatments significant if at least two neighboring windows were significant at $p < .05$. To examine the interaction between treatment and T1 accuracy, we took the same approach but distinguished between correct and incorrect T1 epochs. In this analysis, we only included participants who had 15 or more trials in each cell of the design, which led to the inclusion of 11 participants. The other 7 participants made too few T1 errors.

3.3 Results

3.3.1. Physiological and alertness data

Figure 3.1A shows that, as expected, clonidine lowered systolic (mean tension 101 mmHg) and diastolic (65 mmHg) blood pressure relative to placebo (mean tension 112/73 mmHg), also during performance of the attentional blink task (t = 180-210), *p*s < .0005. The difference in systolic and diastolic blood pressure between placebo and scopolamine was not significant. Figure 3.1B shows that scopolamine (61/min), as expected, lowered heart frequency relative to placebo (71/min) and clonidine (69/min), also during performance of the attentional blink task, *p*s < .004.

Data from the SRT task (Figure 3.1C), administered at baseline (arrival of participant), right before, and right after performing the attentional blink task, yielded a main effect of treatment, $F(2, 34) = 4.7$, $p < .03$, partial $\eta^2 = .22$. Treatment did not interact with time, $F(4, 68) = 3.2$, $p = .07$, partial $\eta^2 = .16$. Pairwise comparisons indicated that clonidine and scopolamine reliably slowed down SRT compared to placebo during both the pre-test ($t_{17} = 3.0$, $p = .009$ and $t_{17} = 2.3$, $p = .03$, respectively) and the post-test ($t_{17} = 4.2$, $p = .001$ and $t_{17} = 3.2$, $p =$

.006, respectively). The differences between clonidine and scopolamine were not significant.

3.3.2. Behavioral data

Trials on which T1 and T2 were accurately identified but in the wrong order were treated as correct (cf. Nieuwenhuis et al., 2007). Greenhouse-Geisser corrections were applied whenever the assumption of sphericity was violated; in such cases, uncorrected degrees of freedom are reported.

Figure 3.2 (left panel) shows average T1 accuracy as a function of treatment and lag. The main effect of treatment was significant, $F(2, 34) = 4.4$, $p = .02$, partial $\eta^2 = .21$. Both clonidine (79.3%, $t_{17} = 2.5$, $p = .02$) and scopolamine (79.4%, $t_{17} = 2.4$, $p = .03$) decreased T1 identification accuracy relative to placebo (85.6%). T1 identification accuracy increased with lag, $F(3, 51) = 6.6$, $p = .001$, partial $\eta^2 = .28$. Treatment and lag did not interact, $F(6, 102) = 0.2$, $p = .96$. To determine if the two drugs also decreased T2 accuracy, we examined average T2 accuracy, non-contingent on T1 identification, as a function of treatment and lag (Figure 3.2, middle panel). Treatment did not reliably influence T2 identification accuracy, $F(2, 34) = 1.6$, $p = .21$, partial $\eta^2 = .09$. T2 identification performance showed the characteristic pattern of lag-1 sparing, a subsequent decrease of accuracy for lags 2 and 3 (i.e. the attentional blink), and a recovery of performance for lag 7, $F(3, 51) = 12.1$, $p < .0005$, partial $\eta^2 = .42$. Treatment and lag did not interact ($p = .051$). We found it remarkable that the treatment effects for lag 1 were of a similar magnitude as those on T1 accuracy.

Indeed, although there was no overall effect of treatment, analysis of individual lags yielded a reliable effect of treatment for lag 1, $F(2, 34) = 5.8$, $p = .007$, partial $\eta^2 = .25$, but not for the other three lags (all ps $> .44$). Pairwise comparisons for lag 1 revealed that accuracy in the clonidine (76.6%; $t_{17} = 3.1$, $p =$

.007) and scopolamine (79.1%; $t_{17} = 2.9$, $p = .009$) conditions was lower than in the placebo condition (87.7%), indicating that the treatment effects for T1 extended into lag 1 but not further. There was no reliable difference in accuracy between the clonidine and scopolamine conditions ($p = .51$).

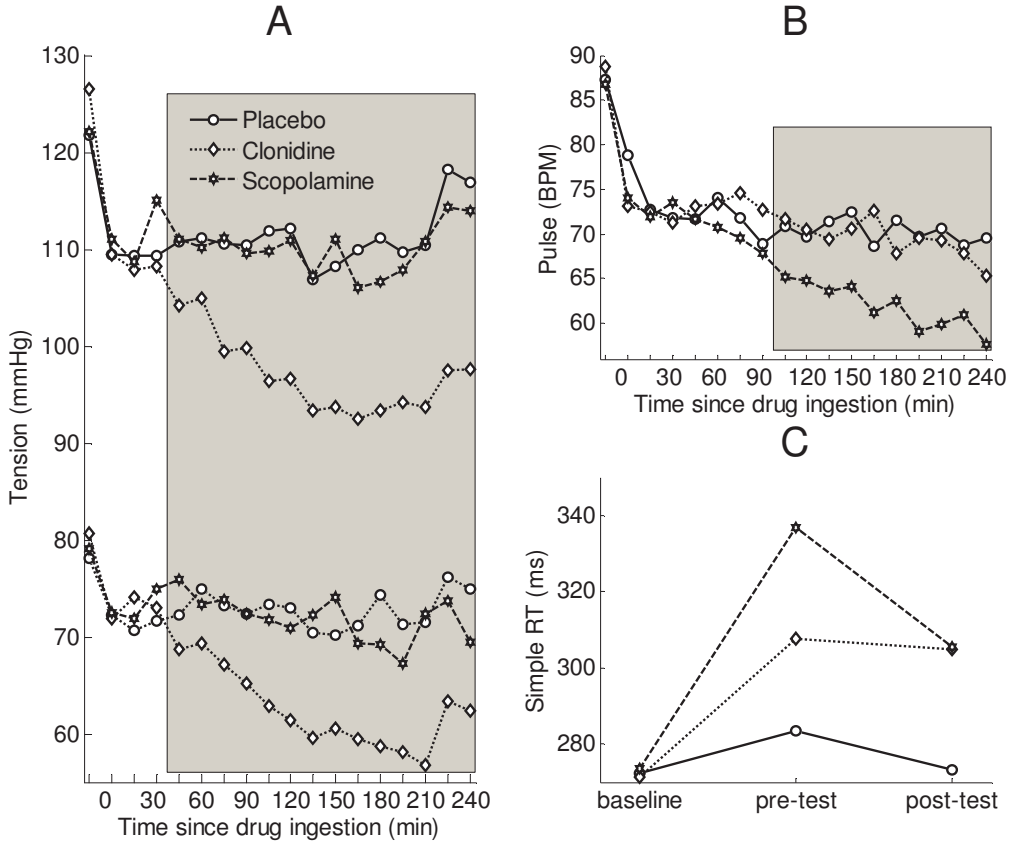

Figure 3.1A. Blood pressure data for the three treatments. The shaded grey area indicates significant pairwise comparisons between clonidine and placebo ($p < .05$). B. Heart frequency for the three treatments. The shaded grey area indicates significant pairwise comparisons between scopolamine and placebo ($p < .05$). C. Results from a simple reaction-time task, administered at the start of the test session (baseline) and right before (pre-test) and after (post-test) participants performed the attentional blink task.

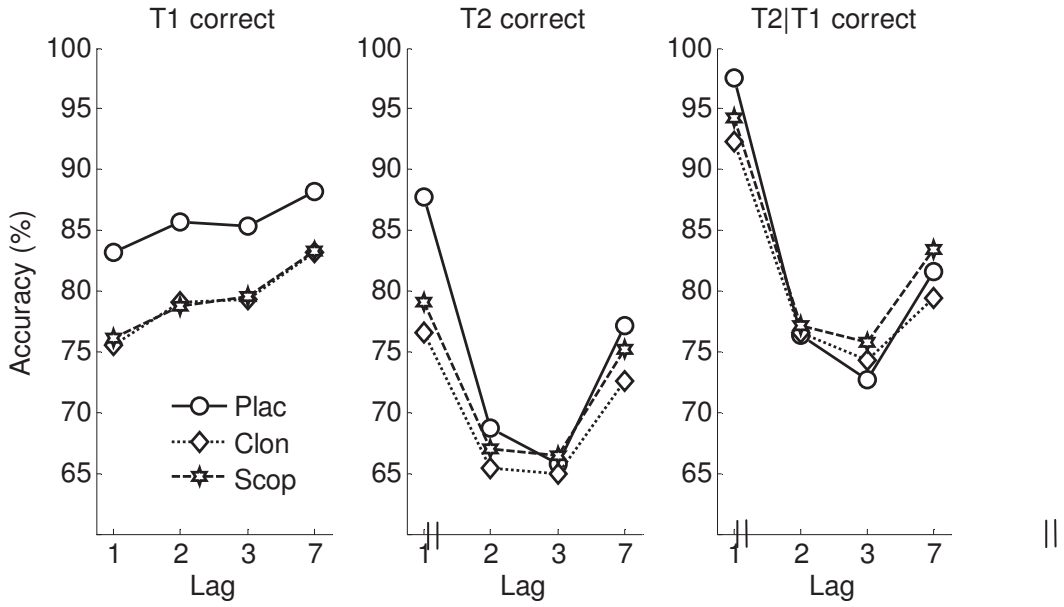

Figure 3.2. T1 identification accuracy (left panel), T2 identification accuracy (middle panel), and T2 identification accuracy (conditional upon T1 correct) as a function of treatment and lag.

Figure 3.2 (right panel) shows the results of a similar T2 analysis, but constrained to T1-correct trials, as is common in attentional blink research. This analysis yielded similar statistical results. Treatment did not reliably influence T2 identification accuracy, $F < 1$. There was a main effect of lag, $F(3, 51) = 18.6$, $p < .0005$, partial $\eta^2 = .52$, but no interaction between treatment and lag ($p = .33$). Again, separate analyses of the four lags yielded a reliable effect of treatment for lag 1 only, $F(2, 34) = 3.3$, $p = .05$, partial $\eta^2 = .16$. Pairwise comparisons for lag 1 revealed that T2 accuracy was lower in the clonidine (92.3%, $t_{17} = 2.3$, $p = .04$) and scopolamine ($t_{17} = 2.1$, $p = .05$) conditions than in the placebo condition (97.5%). There was no reliable difference in accuracy between the clonidine and scopolamine conditions ($p = .40$).

3.3.3. Electrophysiological data

As the active components only reliably affected T1 accuracy, we next examined the effects of drugs on T1-evoked ERPs, including both correct and incorrect trials. As can be seen in Figure 3.3 (horizontal bars), the sliding-window approach led to the identification of two significant windows for electrode Cz: 197-236 ms (corresponding to the P2 component) and 373-412 ms (corresponding to the P3). The P2 effect was also significant for electrode CPz, suggesting a centroparietal locus of this effect. In the P2 window, clonidine was associated with a smaller mean amplitude (1.6 μV) than placebo (2.6 μV, t_{17} = 3.9, p = .001) and scopolamine (2.1 μV, t_{17} = 2.2, p = .046); the difference between placebo and scopolamine was not statistically significant (p = .19). In the P3 window, clonidine was also associated with a smaller mean amplitude (2.2 μV) than placebo (3.5 μV, t_{17} = 4.1, p = .001); scopolamine (3.0 μV) did not differ reliably from placebo (p = .30) and clonidine (p = .12). Thus, relative to placebo, clonidine attenuated the amplitude of the P2 and P3 components evoked by T1. Relative to scopolamine, clonidine attenuated the P2; the numerical difference in P3 amplitude was not significant.

Control analyses further clarified the nature of the treatment effects on P2 and P3 amplitude. First, the P2 and P3 effects were also clearly visible in T1-evoked ERPs that included only correct trials, suggesting that these treatment effects were not primarily driven by treatment effects on (number of included) incorrect trials. Second, examination of lag-specific T1-evoked ERPs indicated that the P2 and P3 effects were also present for longer lags, excluding the possibility that the effects constituted treatment effects on T2-related potentials that confounded the T1-evoked waveforms.

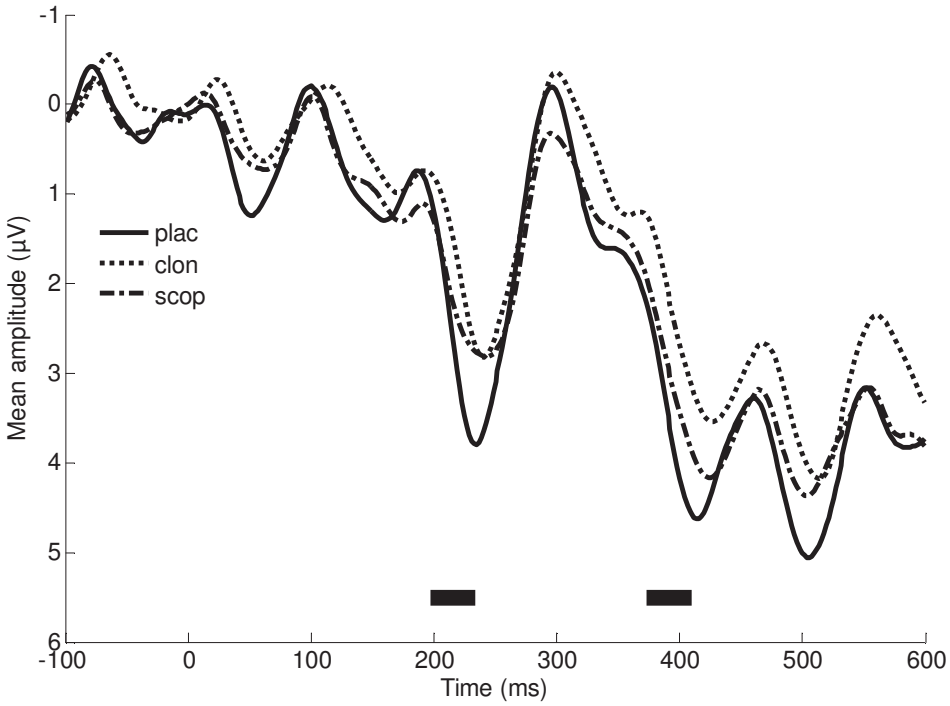

Figure 3.3. T1-locked grand average ERP waveforms for electrode Cz, plotted separately for each treatment. The horizontal black bars indicate windows where the main effect of treatment was significant (see Methods and Materials).

Perhaps due to the lower number of included participants and lower trial numbers in some conditions, and hence less precise estimates, a subsequent sliding-window analysis did not yield any windows with a significant interaction between treatment and T1 accuracy. Figure 3.4 shows mean amplitudes in the P2 and P3 windows identified in the previous analysis as a function of T1 accuracy and treatment. P3 amplitude is somewhat reduced on T1-incorrect trials, consistent with findings of reduced P3 amplitudes on T2-incorrect (i.e. attentional-blink) trials (e.g., Rolke, Heil, Streb, & Hennighausen, 2001; dell'Acqua, Jolicoeur, Pesciarelli, Job, & Palomba, 2003). Accordingly, the P3 window yielded a marginally significant main effect of T1 accuracy, $t_{10} = 2.2$, $p = .051$; there was no reliable main effect in the P2 window ($p = .41$).

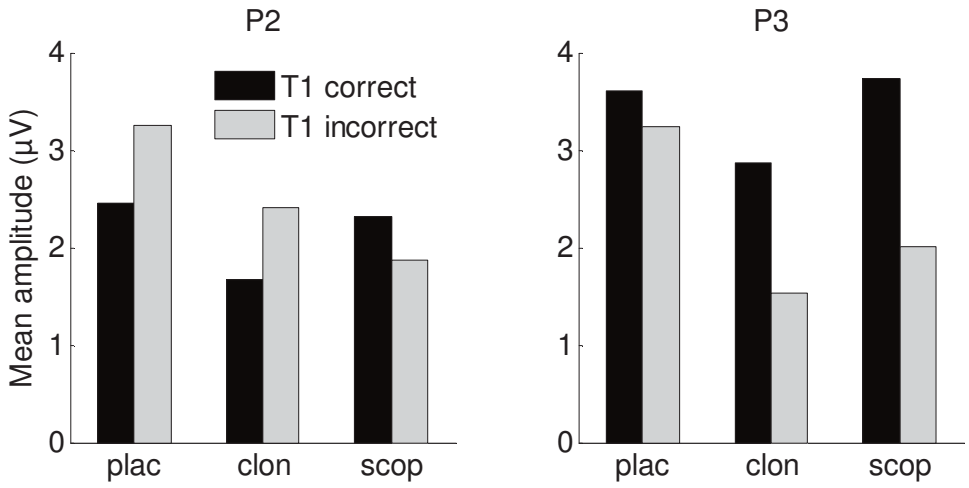

Figure 3.4. Mean average amplitude in the P2 and P3 windows as a function of treatment and T1 accuracy.

3.4 Discussion

In the present research we investigated the effects of clonidine and scopolamine on multiple target detection in an RSVP context. In line with Nieuwenhuis et al. (2007), we found no effect of treatment on the attentional blink for T2. In contrast, we found that both clonidine and scopolamine impaired T1 accuracy. For clonidine, this effect was accompanied by a significant reduction in T1-evoked P2 and P3 amplitude.

The current study replicated Nieuwenhuis et al. (2007) with two design improvements: a crossover design instead of a between-subject design; and a higher dose of clonidine, although, after a clinically relevant drop in blood pressure in one participant, the final 7 participants were given the clonidine dose that was used by Nieuwenhuis et al. (2007). Like Nieuwenhuis et al. (2007), we found no effect of clonidine on the attentional blink, which poses a challenge for

the attentional blink theory of Nieuwenhuis et al. (2005b) under the assumption that clonidine affects the phasic LC response. However, the evidence that addresses this assumption is limited. As in the present study, clonidine has been found to attenuate the amplitude of the P3 (Joseph & Sitaram, 1989), which has been proposed to reflect phasic LC activity (Nieuwenhuis et al., 2005a). Furthermore, the suppressive effect of clonidine on tonic LC activity (e.g., Abercrombie & Jacobs, 1987; Adams & Foote, 1988), along with the reported interaction between tonic and phasic activity of the LC (Aston-Jones & Cohen, 2005), suggests that clonidine may also affect sensory-evoked LC responses. However, the only study that we are aware of that directly examined this issue found mixed results: Adams and Foote (1988) found that during the onset of clonidine-induced suppressed LC firing, LC responses to sensory (footshock) stimuli were relatively preserved, although later during the experiment the reliability of sensory-evoked LC responses was greatly reduced. Furthermore, previous work from our lab has failed to find evidence that clonidine modulated the phasic alerting response to a task-irrelevant, auditory ("accessory") stimulus, despite it having an effect on general alertness (Brown et al., 2015). Thus, we may not have found an effect of clonidine on the attentional blink because it is possible that phasic LC responses to RSVP targets were preserved.

Alternatively, if clonidine affects the phasic LC response, as the observed reduction in T1-evoked P3 amplitude might indicate, the theory of Nieuwenhuis et al. (2005b) could be incorrect. For example, the size and the duration of the LC refractory period, which is purportedly mirrored in the attentional blink, may not be proportional to the size of the phasic LC response, as the theory suggests. Or noradrenaline may not be involved in the attentional blink at all. At first sight, the study by de Martino et al. (2008) suggests a role of the noradrenergic system in the attentional blink: these authors found that administration of the β-adrenoceptor antagonist propranolol decreased T2 accuracy, while administration of the noradrenergic reuptake inhibitor reboxetine increased accuracy for emotional T2

stimuli. However, the authors found no interactions between lag and treatment, and in one of their experiments propranolol impaired T1 accuracy as well. Taken together, these findings suggest that propranolol and reboxetine do not specifically modulate the attentional blink, but target detection under RSVP conditions in general. More convincing evidence for noradrenergic modulation of the attentional blink was provided by Jepma et al. (2011), who studied patients with dopamine-β-hydroxylase deficiency, a rare genetic syndrome characterized by the complete lack of noradrenaline. They found that these patients had a larger attentional blink than healthy controls, and that this impairment was restored by treatment with a synthetic precursor for noradrenaline. Although these findings pose a challenge for the theory of Nieuwenhuis et al. (2005a), which explains the attentional blink as a byproduct of phasic noradrenaline release in the LC, they are generally consistent with a role for noradrenaline in the attentional blink. Recent studies have also reported evidence that decreased levels of dopamine in the striatum are associated with a larger attentional blink (Colzato, Slagter, de Rover, & Hommel, 2011; Colzato, Slagter, Spapé, & Hommel, 2008; Slagter et al., 2012). Other neurotransmitters may hence also play a role in the attentional blink.

In our study, clonidine had a clear detrimental effect on T1 identification accuracy. Presumably, clonidine impaired performance by reducing general alertness (e.g., Brown et al., in press; Coull, Frith, Dolan, Frackowiak, & Grasby, 1997; Coull, 2001; Smith & Nutt, 1996), a possibility that is supported by the negative effects of clonidine on SRT, and the fact that scopolamine, which also increased SRT, similarly reduced T1 identification accuracy. Nieuwenhuis et al. (2007) did not find a significant effect of clonidine on T1 accuracy. However, in their study, all participants took the lower clonidine dose, and their effect was in the same direction and of a similar magnitude (5%) as the effect we observed here (6%). To further understand the effect of clonidine on T1 accuracy, we examined T1-related ERP waveforms (Kenemans & Kähkönen, 2011).

Clonidine attenuated the amplitude of the T1-evoked P2 and P3 components. The functional significance of the P2 is relatively ill-defined, but it has been related to some aspect of stimulus classification (reviewed in Key, Dove, & Maguire, 2005). The opposite seems to apply for the P3: since its discovery in 1965, a number of theories have been proposed to account for its functional significance. In the context of the current paper, the work by Nieuwenhuis et al. (2005a) is particularly relevant, as these authors conceptualized the P3 as reflecting phasic noradrenergic activity and the concomitant increase in neural gain. As noted above, the clonidine-related decrease in P3 amplitude is consistent with several previous studies (Nieuwenhuis et al., 2005a; Joseph & Sitaram, 1989). In contrast, previous studies have reported no effect of clonidine on the amplitude of the P2 (Abuljawad, Langley, Bradshaw, & Szabadi, 2001; Turetsky & Fein, 2002). We propose that the effects of clonidine on T1-evoked P2 and P3 amplitudes and corresponding behaviors were mediated by a general decrease in alertness.

If clonidine reduced general alertness, why is that not manifested in reduced overall T2 accuracy? Relatedly, why did clonidine reduce lag-1 sparing? We propose that the perception of T1 caused a phasic alerting response that temporarily compensated for the drug-induced decrease in tonic alertness. As we have shown in other work, drug-related reductions in alertness yield room for compensatory accessory stimulus-induced performance improvements (Brown et al., in press). In a similar vein, Smith and Nutt (1996) found that arousal evoked by white noise can reduce the frequency of attentional lapses induced by clonidine intake. Furthermore, we propose that this phasic alerting response takes some time to unfold. This is suggested by our finding that the drug-related impairments in T1 accuracy extended to T2 accuracy if T2 was presented immediately after T1 (i.e. at lag 1). Only after that, from lag 2 onward, did accuracy return to placebo levels.

The scopolamine findings show a remarkable similarity to the clonidine findings. Like clonidine, scopolamine reduced T1 accuracy without having a clear

effect on T2 accuracy. The reduction in T1 accuracy is generally consistent with a number of studies that have reported scopolamine-induced attentional impairments, as indicated by impaired performance in sustained attention tasks (Hasselmo & Sarter, 2011). Scopolamine also led to reduced amplitudes of the P2 and P3 relative to placebo, although these reductions were not statistically significant.

It is possible that the effects of clonidine and scopolamine on behavior and ERP waveforms, though similar, were achieved via largely independent neural pathways, that both affect general alertness. However, we believe it is more plausible that the similar effects of these two drugs in the current study and another recent study in our lab (Brown et al., 2015) reflect interactions between the two neuromodulator systems involved (Briand, Gritton, Howe, Young, & Sarter, 2007). On the one hand, acetylcholine has been demonstrated to activate LC neurons in rats and co-administration of scopolamine reduces this effect (Egan & North, 1985; Adams & Foote, 1988). Egan and North proposed that scopolamine antagonizes muscarinic receptors in the LC, leading to reduced noradrenergic baseline activation. On that assumption, both clonidine and scopolamine may have reduced noradrenergic baseline activity, leading to a similar pattern of results for both treatments. On the other hand, there is solid evidence that clonidine inhibits cortical ACh release (Acquas, Wilson, & Fibiger, 1998), probably via α_2 receptors in the basal forebrain (cf. Dringenberg & Vanderwolf, 1998). This suggests that both clonidine and scopolamine may have reduced basal forebrain activity and consequent release of acetylcholine, thus leading to a similar pattern of results. The current data underline the importance of studying interactions between the noradrenergic and cholinergic neuromodulator systems in regulating temporal fluctuations in attention.

4. Noradrenergic and cholinergic effects on speed and ensitivity measures of phasic alerting

Abstract

An intense but task-irrelevant auditory accessory stimulus that is presented almost simultaneously with a visual imperative stimulus can reduce reaction times to that stimulus. The information-processing locus and neural underpinnings underlying this phasic alerting effect are still poorly understood. We investigated a possible noradrenergic or cholinergic basis of the accessory stimulus effect in a double-blind pharmacological study (N=18), in which healthy participants received a single dose of clonidine (an α_2–adrenergic agonist), scopolamine (a muscarinic antagonist) and placebo in separate test sessions. A backward-masking procedure was employed to examine, for the first time, the effect of accessory stimuli on perceptual sensitivity. We found that accessory stimuli enhanced perceptual sensitivity and decreased reaction times to target stimuli, consistent with a recent hypothesis that phasic alerting speeds up stimulus encoding. In contrast to our expectations, clonidine increased the accessory stimulus effect, a finding that seems at odds with earlier proposals that phasic alerting effects are mediated by a phasic noradrenergic response. Furthermore, the accessory stimulus effect was modulated to a similar extent by clonidine and scopolamine, suggesting that the effect of clonidine was not specific to the noradrenergic system. Our results instead suggest that clonidine and scopolamine decrease general alertness, and that these drug-related reductions in alertness yield room for compensatory performance improvements by phasic alerting.

This chapter is based on:

Brown, S. B. R. E., Tona, K.-D., van Noorden, M. S., Giltay, E., van der Wee, N. J. A., & Nieuwenhuis, S. (2014). Noradrenergic and cholinergic effects on speed and sensitivity measures of phasic alerting. *Behavioral Neuroscience, 129,* 42-49.

4.1 Introduction

An auditory *accessory* stimulus (AS), an intense but task-irrelevant sound that is presented almost simultaneously with a visual imperative stimulus, can reduce choice reaction times to that stimulus even though it contains no information about the correct response (Bernstein, Clark, and Edelstein, 1969a; 1969b). It has been demonstrated that an auditory AS can even speed up a response when it immediately *follows* a visual imperative stimulus (Morrell, 1968). Studies of the AS effect can significantly contribute to our understanding of temporal fluctuations in attention. However, although several studies have begun to unravel the mechanisms underlying the AS effect, it remains uncertain which stage of the information processing stream is precipitated by an AS, and the neural basis of this effect is poorly understood. We performed an experiment to ascertain the information processing locus of the AS effect, as well as to study the neuromodulatory basis of the AS effect.

Various theories have been proposed to account for the information processing locus of the AS effect. For example, it has been suggested that the presentation of an AS speeds up the decision-making process. Decision-making has been conceptualized as the accumulation of noisy data until the threshold for a given response is reached and the concomitant response is executed (Gold & Shadlen, 2007; Smith & Ratcliff, 2004). It has been suggested that an AS might speed up responses either by lowering the decision threshold (Posner, 1978) or by increasing the rate at which evidence is accumulated (Hackley & Valle-Inclán, 1999). An alternative account suggests that the presentation of an AS affects motor execution, a later stage of information processing. This hypothesis is based on findings that response force increases (Stahl & Rammsayer, 2005; Miller, Franz, & Ulrich, 1999) and reflexes are sped up on AS trials (Low, Larson, Burke, & Hackley, 1996; Stafford & Jacobs, 1990). Yet another theory suggests that an AS influences stimulus encoding. This account is based on the assumption of

multisensory integration: presenting an auditory AS is thought to increase the subjective intensity of a visual imperative stimulus (Bernstein, Rose, & Ashe, 1970), thereby facilitating encoding, and thus resulting in a faster response.

Recently, Jepma, Wagenmakers, Band, and Nieuwenhuis (2009) demonstrated that the early visual P1 component of the event-related potential that is evoked by imperative stimuli is larger on AS trials, providing evidence for this so-called energy integration hypothesis. Furthermore, in a diffusion-model analysis, these authors demonstrated that the parameter that reflects the duration of nondecision processes was smaller on AS trials, whereas parameters that reflect evidence accumulation and response threshold levels were not affected. These findings, together with electrophysiological evidence that an AS speeds up processes prior to motor preparation (Hackley & Valle-Inclán, 1998), provide important evidence that an AS may influence early encoding instead of motor execution or decision-making processes. Converging evidence for an effect of temporal attention on the duration of stimulus encoding has been obtained in foreperiod paradigms: shorter or validly cued foreperiods, conditions of relatively low uncertainty about the timing of the upcoming imperative stimulus, were associated with a decreased nondecision time, but low temporal uncertainty did not affect either evidence accumulation rate or the decision threshold (Jepma, Wagenmakers, and Nieuwenhuis, 2012). These findings suggest that both exogenous (AS effect) and endogenous (foreperiod effect) changes in temporal attention influence the stimulus encoding stage of information processing.

In the present study, we performed a psychophysical experiment to test the hypothesis that the AS effect influences the encoding stage of information processing. Our cognitive task was based on that used by Rolke and Hofmann (2007), who found that reducing temporal uncertainty about the onset of an imperative stimulus led to increased perceptual sensitivity. This finding corroborates the hypothesis that accessory stimuli speed up the encoding stage of information processing. Following Rolke and Hofmann (2007), our participants

had to detect a small opening on either side of a backward-masked square stimulus. On half of the trials the visual imperative stimulus was accompanied by an auditory AS. Although this design does not allow us to distinguish between encoding and rate of evidence accumulation as loci of the AS effect (cf. Rolke & Hofmann, 2007), it can nevertheless provide a first demonstration of an AS effect on perceptual sensitivity.

Another important question that remains, concerns the neuromodulatory underpinnings of the AS effect. Witte and Marrocco (1997) investigated the effect of pharmacological modulation of noradrenergic activity on the alerting effect in rhesus monkeys. Monkeys were trained to respond as quickly as possible to a visual target stimulus that was occasionally preceded by a visual alerting stimulus that provided no information about the correct response. Cue-target interval was systematically varied (100, 400, 700 ms), with the shortest interval being similar to AS-target intervals in typical AS studies. Attenuation of noradrenergic activity by administration of clonidine (see below) and to a lesser extent guanfacine, significantly reduced the size of the alerting effect in a dose-dependent fashion. This effect of drug was similar across cue-target intervals. These findings suggest that the AS effect may be mediated by the noradrenergic system. Converging evidence for this view is provided by studies in humans that have linked noradrenergic functioning to other measures of temporal attention such as the temporal-cuing effect (Coull, Nobre, & Frith, 2001), vigilant attention (Langner & Eickhoff, 2013) and the attentional blink (Jepma, Deinum, Asplund, Rombouts, Tamsma, Tjeerdema, Spape, Garland, Robertson, Lenders, & Nieuwenhuis, 2011; De Martino, Strange, & Dolan, 2008); and by a study showing that AS-related facilitation of a monosynaptic reflex in cats can be diminished or blocked by antagonism or destruction of the noradrenergic input to the motor system (Stafford & Jacobs, 1990).

To gain further insight in the involvement of the noradrenergic system in the AS effect, we tested healthy adult participants in a placebo-controlled

randomized crossover design with clonidine. Clonidine is a centrally acting α_2 agonist that attenuates baseline noradrenergic activity by agonizing pre-synaptic α_2 autoreceptors, and decreases the amplitude of the human P3 component, an electrophysiological correlate of phasic noradrenaline release (Nieuwenhuis, Aston-Jones, & Cohen, 2005; Pineda, Foote, & Neville, 1989). If the AS effect is subserved by a phasic response of the noradrenergic system, attenuating activity of that system ought to reduce or even abolish the AS effect.

While several studies have investigated the effects of cholinergic nicotinic agents on temporal alerting (Beane & Marrocco, 2004; Stewart, Burke, & Marrocco, 2001), little is known about the involvement of the cholinergic muscarinic system in temporal attention. In a third condition, we administered scopolamine, a muscarinic antagonist that has a similar sedative profile as clonidine, to gain more insight in the role of the cholinergic system in temporal attention, and to test whether modulation of the AS effect by clonidine reflects involvement of the noradrenergic system or reflects iatrogenic sedation.

4.2 Methods

4.2.1.Participants

Eighteen healthy young adult students (15 women), aged 18-26 years (mean age 21 years), drafted through Leiden University's participant recruitment system, took part in three 4.5-hour experimental sessions in return for €140. Only participants with a systolic blood pressure above 100 mmHg and a diastolic blood pressure above 70 mmHg and a heart frequency over 65 beats per minute in rest were included in the study. All participants underwent a medical screening which included a routine physical examination; only healthy persons were allowed to participate. Participants took no prescribed medication and did not smoke. Participants received a single oral dose of clonidine, a single oral dose of scopolamine (1.2 mg), and a placebo in a randomized, double-blind,

counterbalanced double-dummy crossover design. The first 11 participants received a clonidine dose of 175 µg. For safety reasons, the dose of clonidine was reduced to 150 µg for the final seven participants. Preliminary analyses revealed comparable effects for these dosages, so in the analyses reported below they are pooled. Clonidine, scopolamine, and placebo were administered during three separate test sessions, spaced one week apart. The study was approved by the medical ethics committee of the Leiden University Medical Center. Informed consent was obtained from all participants prior to inclusion in the study.

4.2.2. Task

Participants performed a psychophysical version of the accessory stimulus (AS) task, modeled after Rolke and Hofmann (2007). Each trial started with a 500-ms fixation point (black plus sign on a white background, visual angle 0.4 x 0.4°), followed by a target, a black square (0.18 x 0.18°) with a small opening (0.4 x 0.4°) on either the left or the right side, presented for 32, 48, or 64 ms. The square was masked by a visual patch of random noise, which remained on-screen until the response (with a maximum of 4 s). Participants were instructed to respond with a button press ipsilateral to the side of the opening in the square. The mask stimulus was followed by a blank screen that lasted 2, 3, or 4 seconds. One of three different random noise patches was randomly presented during each trial. To keep participants' attention focused on the center of the screen, all stimuli were presented within a square frame (3.9 x 3.9°). On a random 50% of all trials, a loud noise ('accessory stimulus', 800 Hz, 72 dB(A)) was presented for 150 ms: the sound started 30 ms prior to the onset of the target stimulus.

The task consisted of 384 trials, divided over 8 blocks of 48 trials each. In the first test session, the difficulty of the task was adjusted on-line to keep participants' performance away from ceiling and chance levels of performance: at the end of each block, if the participants' accuracy was below 60%, easier target stimuli (i.e. squares with larger openings) were used in the next block; if accuracy

was above 75%, more difficult target stimuli (i.e. with smaller openings) were used in the next block. In total, three stimulus sets of varying difficulty were available, and the first block always started with the easiest target stimuli (i.e. largest openings). For every participant, the difficulty settings were kept constant over the three test sessions. The task was preceded by a practice block of 12 trials, in which feedback on performance was given after every response.

4.2.3 Procedure

Participants were instructed to abstain from caffeine, alcohol, and all psycho-active substances from 15h prior to the start of each session. Each participant was tested at approximately the same time of day. During every test session participants received a capsule of clonidine or placebo at 09.35 AM and a capsule of scopolamine or placebo at 10.35 AM. The different kinetic profiles of clonidine and scopolamine necessitated administration at different times prior to testing. This double-dummy design resulted in one clonidine session (i.e. clonidine verum and scopolamine placebo), one scopolamine session (clonidine placebo and scopolamine verum), and one placebo session (clonidine and scopolamine placebos). To eliminate the confound of treatment order, we stratified this factor by distributing the six possible treatment orders evenly across participants.

At the start of each session (t = -20), a peripheral intravenous cannula was placed and connected to an IV normal saline drip to be able to increase blood pressure through volume expansion and to have an entryway to administer escape medication in the case of a severe drop in tension and/or heart frequency. Furthermore, three cardio electrodes were applied to the participant's chest and connected to an ECG monitor. Blood pressure and heart rate were then measured, and measures of participant alertness were obtained: participants completed a simple reaction time (SRT) task, in which they had to respond as quickly as possible whenever a white circle appeared on the computer screen. Stimulus onset asynchrony was jittered between 500-1250 ms, with a mean of 1000 ms. To

measure the sedative properties of clonidine and scopolamine, we administered the SRT task upon a participant's arrival in the lab, as well as right before and after the participant performed the AS task.

At t = 0, participants ingested a microcrystalline cellulose-filled capsule with either clonidine or placebo. Clonidine has well-established antihypertensive properties: therefore, blood pressure and heart rate were monitored four times an hour from t = 0 onwards for participant safety with an Omron M10-IT automatic sphygmomanometer. At t = 60, participants ingested a microcrystalline cellulose-filled capsule with either scopolamine or placebo.

At t = 90, participants performed the AS task, as part of a larger test battery of which the results are not reported here. The task lasted approximately 30 mins. Participant fitness was checked at t = 240, and participants were sent home via public transportation if their blood pressure and heart rate were close to the values measured at t = -20. At the end of the third test session, participants received their financial compensation.

4.2.4. Analyses

To test for AS effects on perceptual sensitivity and response speed, we submitted *d'* and reaction time (RT) data to 3 (treatment) × 3 (target presentation duration) × 2 (AS presence) repeated-measures analyses of variance (ANOVAs). *d'* was computed as z(proportion of hits)—z(proportion of false alarms; Stanislaw & Todorov,1999). Greenhouse-Geisser corrections were applied whenever the assumption of sphericity was violated; in such cases, uncorrected degrees of freedom are reported. To examine noradrenergic and cholinergic modulations of the AS effect, we submitted *d'* and RT data to a 3 (treatment) × 3 (imperative stimulus presentation duration) × 2 (AS presence) repeated-measures multivariate analysis of variance (MANOVA). Trials were excluded from analysis if an RT fell below or above 2 standard deviations of a given participant's standardized mean RT.

4.3 Results

4.3.1. Physiological and alertness data

Figure 4.1A shows that clonidine lowered systolic (mean tension 101 mmHg) and diastolic (65 mmHg) blood pressure relative to placebo (mean tension 112/73 mmHg), also during performance of the AS task (t = 90-120). The difference in systolic and diastolic blood pressure between placebo and scopolamine was not significant. Figure 4.1B shows that scopolamine (67/min) lowered heart frequency relative to placebo (72/min) and clonidine (72/min), also during (t = 105) and right after (t = 120) task performance.

Results from the SRT task, administered at baseline (arrival of participant), right before, and right after performing the AS task, suggest that clonidine increased SRT (306 ms) relative to placebo (275 ms) and scopolamine (291 ms), $F(2, 34) = 10.4$, $p < .0005$, partial $\eta^2 = .38$. Furthermore, mean SRT increased as the test session progressed, $F(2, 34) = 17.8$, $p < .0005$, partial $\eta^2 = .51$. As depicted in Figure 4.1C, clonidine increased SRT more strongly as the test session progressed than scopolamine and placebo, $F(4, 68) = 5.3$, $p = .007$, partial $\eta^2 = .24$. Pairwise comparisons for pre-test and post-test indicated that clonidine reliably differed from placebo and scopolamine during the pre-test, and that both clonidine and scopolamine reliably differed from placebo during the post-test.

4.3.2. Effect of AS on reaction times and perceptual sensitivity

As expected, trials that were accompanied by an AS were associated with shorter RTs (576 ms) than trials that were not accompanied by an accessory stimulus (noAS trials; 621 ms), $F(1, 17) = 54.5$, $p < .0005$, partial $\eta^2 = .76$ (see Figure 4.2, left panel). RTs decreased with increasing target presentation duration, $F(2, 34) = 37.1$, $p < .0005$, partial $\eta^2 = .69$. There was no interaction between AS presence and target presentation duration ($p = .55$).

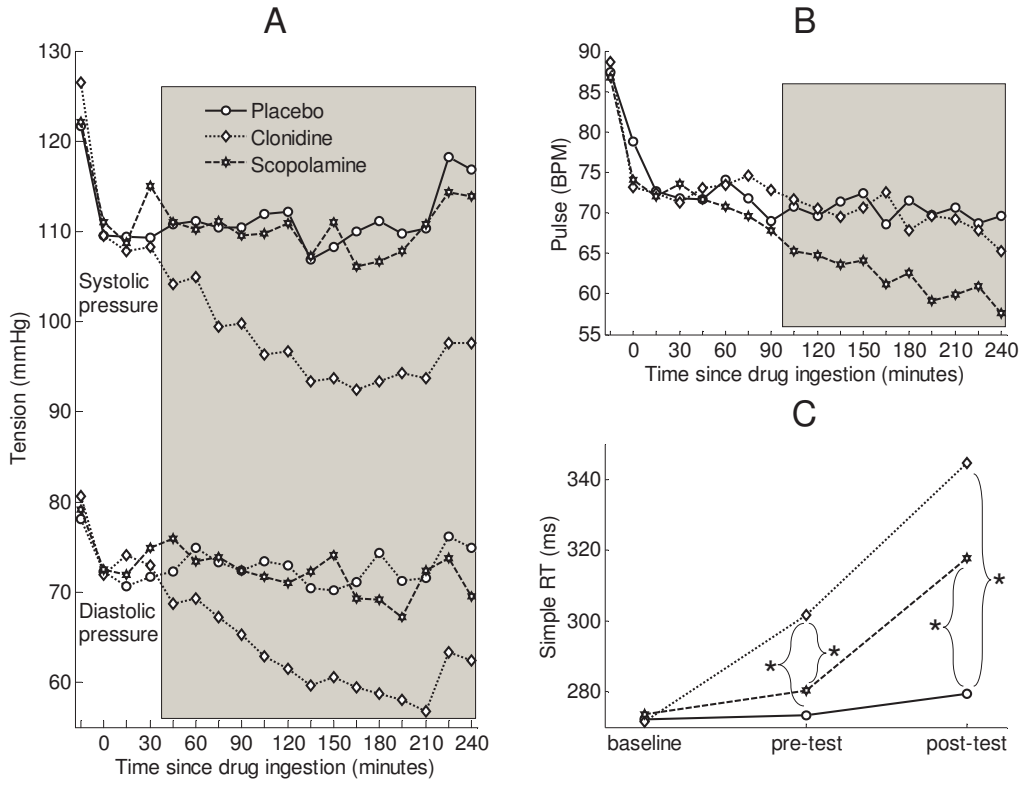

Figure 4.1A. Blood pressure data for the three treatments. The shaded grey area indicates significant pairwise comparisons between clonidine and placebo ($p < .05$). B. Heart frequency for the three treatments. The shaded grey area indicates significant pairwise comparisons between scopolamine and placebo ($p < .05$). C. Results from a simple reaction-time task, administered at the start of the test session (baseline) and right before (pre-test) and after (post-test) participants performed the AS task. All pairwise comparisons accompanied by an asterisk were significant ($p < .05$).

Importantly, we obtained similar results for perceptual sensitivity (Figure 4.2, right panel). AS trials were associated with increased perceptual sensitivity (d' = 1.61) relative to noAS trials (d' = 1.51), $F(1, 17) = 11.2$, $p = .004$, partial $\eta^2 = .40$. Furthermore, perceptual sensitivity increased with target presentation duration, $F(2, 34) = 80.3$, $p < .0005$, partial $\eta^2 = .83$. There was no interaction between AS presence and target presentation duration ($p = .22$).

4.3.3. Effect of treatment on AS effect

As can be seen in Figure 4.3, clonidine was associated with the lowest perceptual sensitivity (*d'* = 1.31) and longest RTs (639 ms), followed by scopolamine (*d'* = 1.50; RT = 601 ms), and placebo (*d'* = 1.86; RT = 557 ms). This pattern was expressed in a significant main effect of treatment in the repeated-measures MANOVA, Wilks' lambda = .51, $F(4, 14) = 3.3$, $p = .04$, partial $\eta^2 = .49$.

Crucially, we found an interaction between treatment and AS presence, Wilks' lambda = .42, $F(4, 14) = 4.8$, $p = .01$, partial $\eta^2 = .58$. Follow-up pairwise comparisons between the treatments indicated that clonidine was associated with a greater AS benefit (RT AS – noAS = -65 ms, *d'*AS – noAS = 0.18) than placebo (RT difference = -29 ms, *d'* difference = 0.02; Wilks' lambda = .44, $p = .001$). Scopolamine also increased the AS effect compared to placebo (RT difference = -43 ms, *d'* difference = 0.13), but not reliably so, Wilks' lambda = .89, $p = .41$). The AS effects for scopolamine and clonidine also did not reliably differ, Wilks' lambda = .86, $p = .31$.

Figure 4.3 also shows that there was no AS effect on *d'* in the placebo condition; the significant main effect of AS presence in the *d'* ANOVA reflected the AS effects observed in the two drug conditions.

4.4 Discussion

4.4.1. Accessory stimuli enhance perceptual sensitivity

We have provided a first demonstration, using psychophysics, of an AS effect on perceptual sensitivity. Accessory stimuli in our location-discrimination task with backward-masking not only speeded up RTs—the typical finding in AS studies—but also increased *d'*, a signal-detection measure of perceptual sensitivity. These *d'* findings can be explained by two different hypotheses (cf. Rolke & Hofmann, 2007). According to one hypothesis, an AS reduces the time needed for target

encoding so that evidence accumulation can start earlier and accumulated evidence can increase to a higher level before the target is masked.

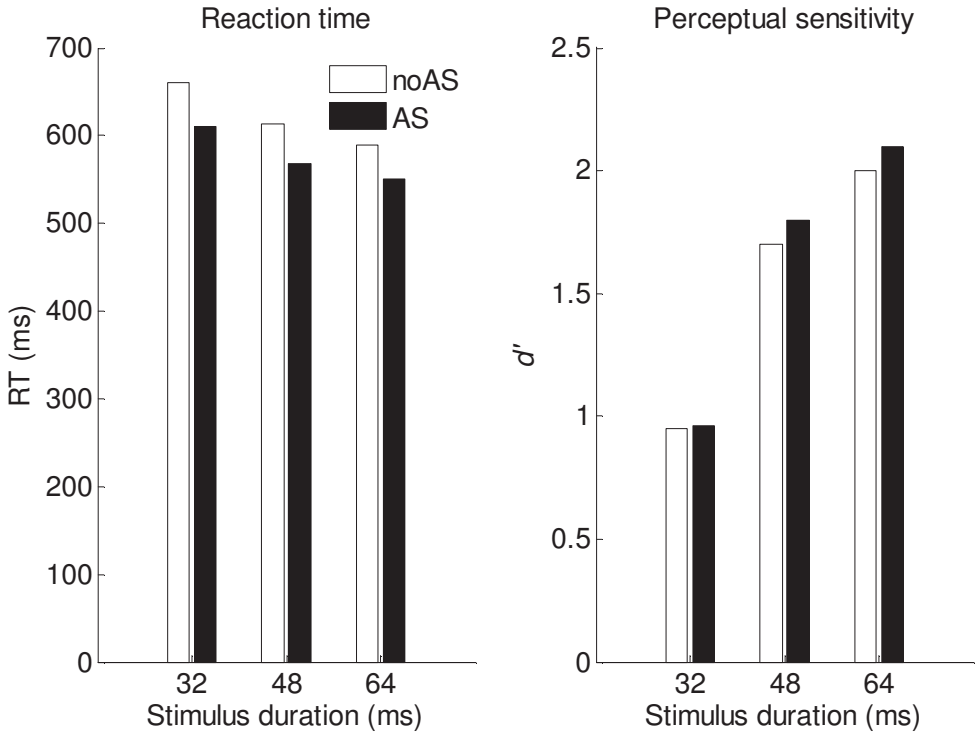

Figure 4.2A. AS effect on RT for every target presentation duration. B. AS effect on *d'* for every target presentation duration.

We will refer to this model as the early onset hypothesis (Nieuwenhuis & de Kleijn, 2013; Seifried, Ulrich, Bausenhart, Rolke, & Osman, 2010). According to the other hypothesis, an AS increases the rate (as opposed to the onset) of evidence accumulation, so that more evidence can be accumulated before the target is masked. Although the present study cannot arbitrate between these two hypotheses, other literature strongly favors the early onset hypothesis (Jepma et al., 2009). Thus, our study supports previous work that suggests that the AS effect is rooted in the encoding stage of information processing.

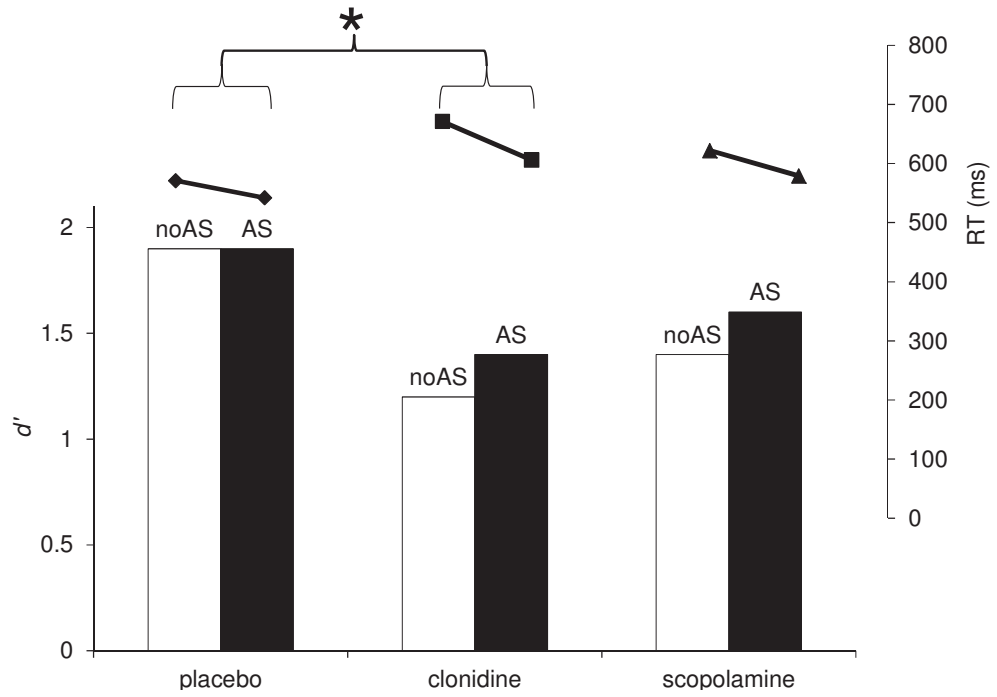

Figure 4.3. Effect of treatment and AS presence on *d'* (bars) and RT (lines). Asterisk indicates significantly larger AS effect after clonidine than after placebo treatment (p = .01).

The fact that we only found an AS effect on perceptual sensitivity in the two drug conditions begs the question why this effect was not present in the placebo condition. Indeed, our findings in the placebo condition do not replicate Rolke and Hofmann (2007), who found that increased temporal attention enhanced perceptual sensitivity in the same task. Admittedly, the AS paradigm differs considerably from the constant-foreperiod paradigm used by Rolke and Hofmann (2007). In the AS paradigm, temporal attention is increased mainly by phasic alerting, whereas in the foreperiod paradigm, improvements in performance are caused by controlled temporal attention shifts and/or associative learning between the warning signal and the imperative stimuli (Capizzi, Sanabria, & Correa, 2012; Steinborn, Rolke, Bratzke, & Ulrich, 2010). However, in earlier work (Jepma et al., 2009, 2012), we found that these modulations of temporal attention influence RT

and accuracy in the same manner: by reducing the time required for stimulus encoding. So why did we not replicate the findings of Rolke and Hofmann?

We hypothesize that the AS effect on perceptual sensitivity is only manifested in conditions that are associated with suboptimal alertness. This notion is consistent with literature that suggests that the AS effect on RT is relatively small under conditions of low temporal uncertainty (Hackley et al., 2009; Sanders, 1980). Our placebo condition was probably associated with a state of relatively high alertness, especially around the time of the AS, because the onset of the AS could be predicted using a fixation cross that preceded the AS by a fixed time interval. In contrast, in Rolke and Hofmann (2007), the onset of the warning cue was not predictable, and therefore subjects were presumably less alert when the warning cue was presented. In the current study, the clonidine and scopolamine conditions were clearly associated with reduced general alertness, as indicated by increased SRTs and impaired overall performance in the location-discrimination task. We hypothesize that AS-triggered phasic alerting temporarily compensated for this reduction in alertness, resulting in a pronounced AS effect in the drug conditions. In line with this view, it has been demonstrated that an auditory warning cue enhances performance to visual stimuli in patients with right hemisphere neglect, a condition characterized by decreased tonic alertness (Robertson, Mattingly, Rorden, & Driver, 1998).

In conclusion, we found a reliable AS effect on perceptual sensitivity, in line with the hypothesis that the AS effect has a locus in the encoding stage of information processing (Jepma et al., 2009). Although the literature suggests that accessory stimuli also affect motor processes (Hackley & Valle-Inclán, 2003), as manifested in such parameters as response force, this effect does not contribute to the speeding up of RTs (Jepma et al., 2009; Hackley & Valle-Inclán, 1998).

4.4.2. The AS effect is not mediated by a phasic noradrenergic response

We found a clear effect of clonidine on the AS effect, consistent with the general notion of an important role for noradrenaline in temporal attention. However, in contrast to our expectations, clonidine increased the AS effect, a finding that is incompatible with the hypothesis that phasic alerting effects such as the AS effect are mediated by a phasic noradrenergic response (Fernandez-Duque & Posner, 1997; Hackley & Valle-Inclán, 2003). Furthermore, the AS effect was modulated to a similar extent by clonidine and scopolamine, suggesting that the effect of clonidine was not specific to the noradrenergic system. As proposed above, our observations instead suggest a general alertness explanation of our findings: drug-related reductions in alertness yield room for compensatory AS-induced performance improvements. In a related vein, arousal evoked by white noise (Smith & Nutt, 1996) and caffeine consumption (Smith, Brice, Nash, Rich, & Nutt, 2003) has been found to remove many of the cognitive performance impairments caused by clonidine intake. This suggests that other, tonic alerting manipulations can also counteract the effects of clonidine on general alertness. This effect might be mediated by increased crosstalk between attention-related brain areas during periods of high arousal, which might counteract the deteriorating effects of clonidine (Coull et al., 2001).

Clonidine, the "prototype α_2 agonist" (Wecker, Crespo, Dunaway, Faingold, & Watts, 2010), has been around for almost 50 years (Stähle, 2000). It is still widely prescribed; current indications include hypertension, attention-deficit/hyperactivity disorder, and menopausal hot flushes. The drug is also used as an adjuvant in opiate withdrawal treatment. Therefore, the above observations that exogenous auditory cues and tonic alerting manipulations can compensate clonidine-induced attentional impairments seem particularly relevant. More in general, our findings have significant implications for research in both the neuropsychological domain and the ergonomic and human-factors domain, since

they demonstrate that exogenous stimulation may be capable to compensate impairments in endogenous alertness.

Acetylcholine has been suggested to play a key role in attention, but to date most studies have focused on the nicotinic cholinergic receptor class (for a review, see Beane & Marrocco, 2001). The nicotinic system appears to be involved in regulating spatial attention but seems to have no role in alerting (e.g., Stewart, Burke, & Marrocco, 2001; Thiel, Zilles, & Fink, 2005; Witte, Davidson, & Marrocco, 1997). Our study is among the first to study the involvement of the muscarinic cholinergic receptor class in temporal attention. We found no difference between AS effects in the scopolamine and placebo conditions, suggesting that muscarinic receptors do not play an important role in phasic alerting.

Our data do not support the hypothesis that phasic noradrenaline responses mediate the AS effect, so we briefly consider two alternative hypotheses. As discussed in the introduction, the energy integration account explains the AS effect in terms of energy integration of the auditory AS and visual imperative stimulus across sensory modalities. This increases perceived intensity of the imperative stimulus and reduces RTs. The mechanism responsible for translating the additional energy into enhanced performance may be stochastic resonance. Stochastic resonance refers to the phenomenon that the addition of noise to a nonlinear system can enhance its response to a weak input signal (Benzi, Sutera, & Vulpiani, 1981). The AS effect could be a manifestation of stochastic resonance: by adding noise (the AS) to a subthreshold imperative stimulus, the intensity of that stimulus is boosted to a supra-threshold level, which facilitates its encoding and reduces ensuing RTs (cf. Moss, Ward, & Sannita, 2004). The neural substrate of this effect might be increased responsiveness of multisensory neurons to the combined energy of the AS and imperative stimulus (Manjarrez, Mendez, Martinez, Flores, & Mirasso, 2007).

Another explanation of the AS effect is provided by the phase reset hypothesis, which assumes that the AS disrupts ongoing neural oscillations so as to

synchronize their phase (Diederich, Schumburg, & Colonius, 2012). The presentation of an AS is hypothesized to reset neural oscillations to their ideal phase; stimuli following the AS, presented during this ideal phase, evoke amplified responses, while stimuli presented outside this phase are suppressed (Lakatos, Chen, O'Connell, Mills, & Schroeder, 2007; Kayser, Petkov, & Logothetis, 2008). Work with saccadic RTs to visual stimuli preceded by an auditory AS provides evidence for this hypothesis (Diederich et al., 2012). More in general, this evidence is consistent with other studies that claim an important role for phase entrainment in temporal expectation effects (Stefanics et al., 2010; Cravo, Rohenkohl, Wyart, & Nobre, 2013).

It is important to note that α_2 agents like clonidine can have both pre- and postsynaptic effects (Samuels & Szabadi, 2008). Predominantly presynaptic stimulation of α_2 receptors leads to attenuation of noradrenergic activity and decreased arousal, while predominantly postsynaptic stimulation of α_2 receptors leads to increased noradrenergic activity and increased arousal (Samuels & Szabadi, 2008). Indeed, clonidine can both enhance and deteriorate task performance in monkeys, an effect that has been suggested to depend on the dose of clonidine that was administered (Witte & Marrocco, 1997). We found an enhanced AS effect following clonidine administration, which at first blush seems to suggest a predominance of postsynaptic α_2 stimulation and concomitant increase in arousal. However, low doses of clonidine as used in our study are generally assumed to act predominantly presynaptically (Frith, Dowdy, Ferrier, & Crow, 1985; Coull, Middleton, Robbins, & Sahakian, 1995a/1995b; Coull et al., 1995c; Jäkälä et al., 1999). Furthermore, our participants exhibited clear signs of sedation (as reflected by SRTs), which is a common side effect of presynaptic α_2 stimulation, an effect that is subserved by inhibition of wakefulness-inducing histaminergic pathways due to "switching off" of LC neurons (Samuels & Szabadi, 2008).

Our study is the first to demonstrate an AS effect on perceptual sensitivity, and we have provided evidence that argues against a phasic noradrenergic mechanism mediating the AS effect in humans. Further work, including a replication study with larger sample size, will be necessary to better understand the neural underpinnings of the AS effect.

5. Noradrenergic and cholinergic modulation of late ERP responses to deviant stimuli

Abstract

Researchers have proposed several hypotheses about the neuromodulator systems involved in generating P3 components of the event-related potential (ERP). To test some of these hypotheses, we conducted a randomized placebo-controlled crossover study in which we investigated how the late positive ERP response to deviant stimuli is modulated by (i) clonidine, an α2 agonist that attenuates baseline noradrenergic activity; and (ii) scopolamine, a muscarinic antagonist of acetylcholine receptors. We collected EEG data from 18 healthy volunteers during the performance of a series of active and passive oddball tasks. We then used spatiotemporal principal component analysis (PCA) to decompose the ERP waveforms. The PCA revealed three distinct late positive ERP components: the classic parietal P300, the frontal novelty P3, and a biphasic response that we identified as the N2-P3a. Statistical analysis of the temporal factor scores indicated that the amplitude of the classic P300 was increased by clonidine and scopolamine in some conditions, and not modulated in other conditions. In contrast, the amplitude of the novelty P3 was decreased by both drugs. Scopolamine also decreased the amplitude of the N2-P3a. The similar pattern of results for clonidine and scopolamine probably reflects the strong interactions between the noradrenergic and cholinergic systems. The results, in combination with previous pharmacological studies, suggest a critical role for both neuromodulator systems in the generation of several distinct P3 components.

This chapter is based on:

Brown, S. B. R. E., van der Wee, N. J. A., van Noorden, M. S., Giltay, E., & Nieuwenhuis, S. (submitted). Noradrenergic and cholinergic modulation of late ERP responses to deviant stimuli.

5.1 Introduction

The P300 to task-relevant stimuli has undoubtedly been the most intensively studied component of the electroencephalogram (EEG). It is ubiquitous in stimulus-related scalp-recorded and intracranial EEG activity, it is sensitive to a wide range of variables and states, including attention, expectation and value (Johnson, 1986), and an abnormal P300 has been proposed as a biomarker of a large array of brain disorders such as ADHD (Barry, Johnstone, & Clarke, 2003) and post-traumatic stress disorder (Duncan, 2003). Furthermore, the P300 has been proposed to reflect key aspects of cognitive function, including evidence accumulation for perceptual decisions (O'Connell, & Dockree, & Kelly, 2012; Verleger, Jaskowski, & Wascher, 2005), memory updating (Donchin & Coles, 1988; Nieuwenhuis, 2011), and potentiation of responses to motivationally significant stimuli (Nieuwenhuis, Aston-Jones, & Cohen, 2005). The classic P300 can be distinguished from two potentially related late event-related potential components, with a more frontal scalp distribution and somewhat earlier latency, both of which have been described as a central nervous system component of the orienting response: the P3a, which is elicited by simple task-irrelevant deviants (Polich, 2007; Squires, Squires, & Hillyard, 1975) and the novelty P3, which is elicited by attended highly salient deviants or novel stimuli (Friedman, Cycowicz, & Gaeta, 2001; Yamaguchi & Knight, 1991). However, it is still unclear whether the P300 (or P3b), novelty P3 and P3a only happen to share some characteristics (e.g., latency range, polarity) but do in fact reflect independent underlying neural processes, or whether they reflect the same underlying process operating in different brain areas under different circumstances.

A powerful way to address this question would be to investigate the neurochemical basis of these ERP components. Examining whether these late ERP responses reflect distinct or common neuromodulatory actions in the cortex can be

informative both about the relationship between the components and about the functional significance of underlying neural activity. Researchers have proposed several theories about the neuromodulator systems involved in generating the P300 and related components. Although there is some concensus that the noradrenergic system plays a critical role in generating the classic P300, there are several competing views about the origin of the novelty P3 and/or P3a, with different theories proposing a key role for noradrenaline (Nieuwenhuis et al., 2005), dopamine (Polich, 2007) and acetylcholine (Ranganath & Rainer, 2003). Although these theories are based on a variety of neuroscientific findings, they are primarily based on acute pharmacological challenge studies in humans and non-primate animals. Indeed, there is a substantial pharmacological literature that documents the effect of neurotransmitters and neuromodulators on P3 components (Frodl-Bauch, Bottlender, & Hegerl, 1999), including various studies using noradrenergic, dopaminergic and cholinergic agents. Unfortunately, these studies generally suffer from several limitations. Many studies are based on a limited sample size (e.g., 10 or fewer subjects), resulting in low statistical power for detecting treatment effects. Furthermore, most studies have used only one type of task: an active oddball task, meant to elicit a large P300; effects of task-irrelevant deviant stimuli were not studied. Also, only very few studies have attemped to extract separate late ERP components, despite their overlap in latency and scalp distribution. Indeed, many papers only report results from one electrode (e.g., the peak amplitude between 200 and 500 ms at electrode Pz). Finally, direct comparisons between two or more pharmacological challenges have been exceedingly rare.

Here we report an important first step in addressing these shortcomings and toward more definitive conclusions about the neurochemical basis of late ERP responses. First, we administered several tasks specifically designed to elicit clear P300, novelty P3 and P3a components. Second, to deal with the issue of spatial and temporal overlap between components we performed a rigorous spatiotemporal principal components analysis (PCA) of the event-related potential waveforms.

These methods for eliciting and disentangling components of the P3 complex closely followed those used by Spencer and colleagues (Spencer, Dien, & Donchin, 2001). And finally, we directly compared pharmacological challenges of the noradrenergic and cholinergic system. Specifically, we administered clonidine, scopolamine and placebo in separate sessions of a double-blind randomized crossover design.

Clonidine is a centrally-acting α_2 agonist that attenuates baseline noradrenergic activity by agonizing pre-synaptic α_2 receptors. Scopolamine is a cholinergic antagonist of the muscarinic receptors, a drug with a sedation profile comparable to that of clonidine.

5.2 Methods

5.2.1. Participants

Eighteen healthy young adults (15 women), aged 18-26 years (mean age 21 years), drafted through Leiden University's participant recruitment system, took part in three 4.5-hour experimental sessions in return for €140. For safety reasons, only participants with a systolic blood pressure above 100 mmHg and a diastolic blood pressure above 70 mmHg and a heart frequency over 65 beats per minute in rest were included in the study. All participants underwent a medical screening which included a routine physical examination prior to being included in the experiment: only healthy individuals without a history of neurological or psychiatric disorders were allowed to participate. Participants took no prescribed medication and did not smoke. Participants received a single oral dose of clonidine, a single oral dose of scopolamine (1.2 mg), and a placebo in a randomized, double-blind, counterbalanced double-dummy crossover design. The first 11 participants received a clonidine dose of 175 µg. As the eleventh participant showed an unexpected large drop in systolic blood pressure of 35 mmHg, but without clinical consequences, 60 minutes after the ingestion of clonidine 175 µg (blind was broken

by the supervising physician), we decided to reduce the dose of clonidine to 150 µg for the final seven participants. Clonidine, scopolamine, and placebo were administered during three separate test sessions, spaced one week apart. The study was approved by the medical ethics committee of the Leiden University Medical Center. Informed consent was obtained from all participants prior to inclusion in the study.

5.2.2. Task

The auditory oddball task consisted of four blocks of 300 trials each: during the first two blocks, participants were instructed to solve word puzzles (block 1) or read either a book or a magazine, based on their preference (block 2), while ignoring the sound stimuli (ignore blocks). During the other two (attend) blocks, participants were instructed to respond as quickly and accurately as possible to the rare stimuli with their left or right index finger (counterbalanced across participants, but kept constant within participants). The stimuli used were auditory tones of either high (500 Hz) or low pitch (350 Hz). Every tone lasted 336 ms and was presented at 70 dB(A) with an interstimulus interval of 850 ms. Tonal pitch of frequent and rare stimuli (12% of trials) was counterbalanced across participants. In all blocks, a fixation point (black plus-sign on a white background, visual angle 0.2 x 0.2°) was presented on the screen continuously, while auditory tones were presented. In the first three blocks, 88% of the stimuli were frequents and 12% were rares. The fourth block was comparable to the third block, but in addition to standard frequent (76%) and rare stimuli (12%), 36 sounds were used as infrequent novel stimuli (12%). We used six sounds each from the following categories: animal sounds (but not birds), birds, video game bleeps, human bodily noises, and random noises (e.g., phone, hammer). These novel sounds were the same as those used by Fabiani and Friedman (1995) and Spencer et al. (2001). Participants were not informed about the presence of novel stimuli, but they were explicitly instructed to respond to rare stimuli only.

5.2.3.Procedure

Participants were instructed to abstain from coffee, alcohol, and all psycho-active substances from 15h prior to the start of each session. Each participant was tested at approximately the same time of day. During every test session participants received a capsule of clonidine or placebo at 09.35 AM and a capsule of scopolamine or placebo at 10.35 AM. The different pharmacokinetic profiles of clonidine and scopolamine necessitated administrations at different times prior to testing. This double-dummy design resulted in one clonidine session (i.e. clonidine verum and scopolamine placebo), one scopolamine session (clonidine placebo and scopolamine verum), and one placebo session (clonidine and scopolamine placebos). To eliminate the confound of drug order, we stratified this factor by distributing the six possible drug orders evenly across participants.

At the start of each session (t = –20 min), a peripheral intravenous cannula was placed and connected to an intravenous normal saline drip to be able to increase blood pressure through volume expansion and to have an entryway to administer escape medication in the case of a severe drop in tension and/or heart frequency. Furthermore, three cardio electrodes were applied to the participant's chest and connected to an electrocardiogram monitor. Blood pressure and heart rate were then measured, and measures of participant alertness were obtained: participants completed a simple reaction time task (SRT), in which they had to respond as quickly as possible whenever a white circle appeared on the computer screen. Stimulus onset asynchrony was jittered between 500-1250 ms, with a mean of 1000 ms. To measure the sedative properties of clonidine and scopolamine, we administered the SRT task upon a participant's arrival in the lab, as well as right before and after the participant performed the auditory oddball task.

At t = 0 min, participants ingested a microcrystalline cellulose-filled capsule with either clonidine or placebo. Clonidine has well-established antihypertensive properties: therefore, for participant safety, blood pressure and heart rate were monitored four times an hour from t = 0 min onwards with an

Omron M10-IT automatic sphygmomanometer. At t = 60 min, participants ingested a microcrystalline cellulose-filled capsule with either scopolamine or placebo.

At t = 150 min, participants performed the auditory oddball task which lasted approximately 30 minutes; during the 60 minutes prior to this time point, participants performed two unrelated cognitive tasks (reported elsewhere). Participant fitness was checked at t = 240 min, and participants were sent home via public transportation if their blood pressure and heart rate were close to the baseline values measured at t = –20 min. At the end of the third test session, participants received their financial compensation.

5.2.4. EEG recording and analyses

We recorded EEG from 64 Ag/AgCl scalp electrodes and from the left and right mastoids. We measured the horizontal and vertical electro-oculogram (EOG) using bipolar recordings from electrodes placed approximately 1 cm lateral of the outer canthi of the two eyes and from electrodes placed approximately 1 cm above and below the participant's right eye. The EEG signal was pre-amplified at the electrode to improve the signal-to-noise ratio and amplified with a gain of 16x by a BioSemi ActiveTwo system (BioSemi B.V., Amsterdam). The data were digitized at 24-bit resolution with a sampling rate of 512 Hz using a low-pass fifth-order sinc filter with a half-power cutoff of 102.4 Hz. Each active electrode was measured online with respect to a common mode sense (CMS) active electrode producing a monopolar (non-differential) channel, and was referenced offline to the average of the left and right mastoids. Data were high-pass filtered at 0.1 Hz and low-pass filtered at 30 Hz in Brain Vision Analyzer 2 (Brain Products GmbH, Gilching, Germany). Ocular and eyeblink artefacts were corrected using the method of Gratton, Coles, and Donchin (1983). Epochs with other artefacts (a gradient greater than 30 µV, slow drifts [>300 µV/200 ms], and low activity [<0.50 µV/100 ms) were also discarded. Data were epoched from -200 to 800 ms relative to stimulus

onset and then averaged. A 200-ms prestimulus baseline was subtracted for each ERP.

Preprocessed, segmented, averaged, and baseline-corrected data were then exported from Brain Vision Analyzer and submitted to a spatiotemporal principal components analysis (PCA) in the ERP PCA Toolkit (Dien, 2010a). Following Spencer et al. (2001), we analyzed a 0-752 ms subwindow from the exported epochs. We first reduced ERP data dimensionality in the spatial domain, by submitting a 64 (electrodes) × 186,624 (observations: 384 timepoints × 9 stimulus types × 18 participants × 3 treatments) data matrix to spatial PCA. Use of a parallel test (Horn, 1965) suggested data truncation to 7 spatial factors, which were then rotated using the Varimax procedure. Then, to reduce the temporal dimensionality of the data, we carried out a separate temporal PCA for each of the individual spatial factors (Dien, 2010a; cf. Spencer et al., 2001). Observations were the virtual electrodes (i.e. the spatial factor loadings, or correlations between the original electrode sites and the retained spatial factors), the participants, and the experimental conditions. Use of a parallel test suggested 11 factors for retention; these factors were submitted to Varimax rotation.

5.3 Results

5.3.1. Physiological and alertness data

Figure 5.1A shows that clonidine lowered systolic (mean tension 101 mmHg) and diastolic (65 mmHg) blood pressure relative to placebo (112/73 mmHg), also during performance of the oddball task (t = 150-180 min). The difference in systolic and diastolic blood pressure between placebo and scopolamine was not significant. Figure 5.1B shows that scopolamine (67/min) lowered heart frequency relative to placebo (72/min) and clonidine (72/min), also during (t = 150 min) and right after (t = 180 min) task performance. The difference in heart frequency between placebo and clonidine was not significant.

Results from the SRT task (Figure 5.1C), administered at baseline (arrival of participant), right before, and right after performance of the oddball task, show that clonidine (303 ms) and scopolamine (309 ms) increased SRT relative to placebo (278 ms), $F(2, 34) = 5.3$, $p = .02$, partial $\eta^2 = .24$. Furthermore, mean SRT increased as the test session progressed, $F(2, 34) = 14.0$, $p < .0005$, partial $\eta^2 = .45$. The interaction between treatment and time point just reached significance, $F(4, 68) = 3.2$, $p = .05$, partial $\eta^2 = .16$. Pairwise comparisons for pre-test and post-test indicated that clonidine and scopolamine reliably differed from placebo, but not from each other.

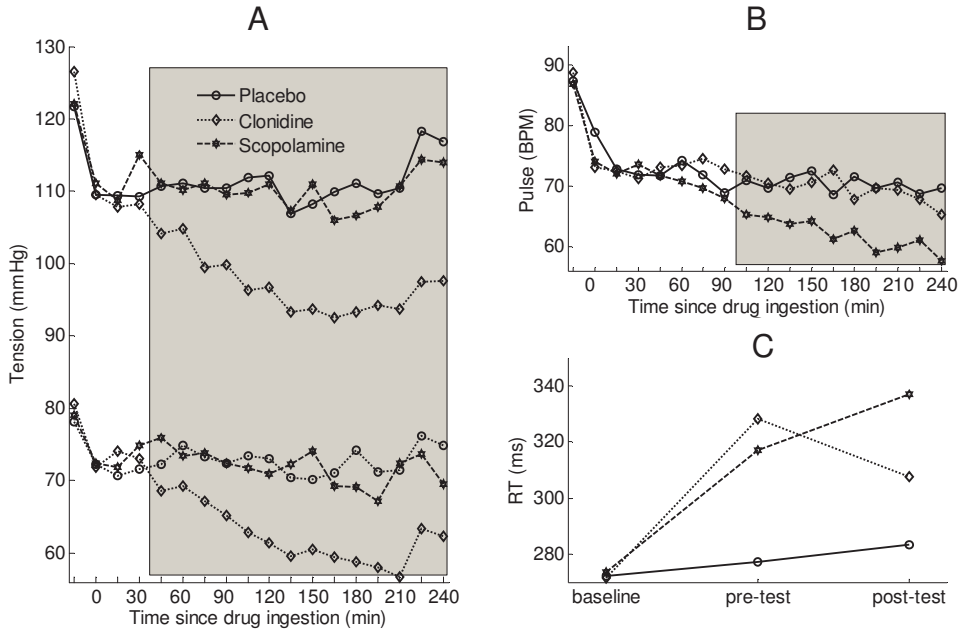

Figure 5.1A. Blood pressure data for the three treatments. The shaded grey area indicates significant pairwise comparisons between clonidine and placebo ($p < .05$). B. Heart frequency for the three treatments. The shaded grey area indicates significant pairwise comparisons between scopolamine and placebo ($p < .05$). C. Results from a simple reaction-time task, administered at the start of the test session (baseline) and right before (pre-test) and after (post-test) participants performed the auditory oddball task.

5.3.2. Behavioral Results

Table 6.1 presents average reaction times (RTs), accuracy, and *d'* values computed according to Stanislaw and Todorov (1999). In the classic oddball task, treatment did not reliably influence RT, $F(2, 34) = 1.4$, $p = .26$, partial $\eta^2 = .08$. Treatment influenced perceptual sensitivity as reflected by *d'*, $F(2, 34) = 6.0$, $p = .006$, partial $\eta^2 = .26$. *d'* values were lower for clonidine and scopolamine than for placebo, both *p*s <.005. In the novelty oddball task, treatment reliably influenced RT, $F(2, 34) = 3.7$, $p = .03$, partial $\eta^2 = .18$. Pairwise-comparisons indicated that RTs for clonidine were faster than RTs for placebo, $p < .005$. However, this decrease in RTs was accompanied by decreased *d'*, suggesting a speed-accuracy trade-off. There was a significant main effect of treatment on *d'*, $F(2, 34) = 6.4$, $p = .004$, partial $\eta^2 = .27$, and pairwise-comparisons indicated that *d'* was decreased relative to placebo for both clonidine and scopolamine, *p*s < .005.

Table 5.1. Behavioral results: average correct reaction time (RT), number of false alarms (FA) and misses, and *d'* in the classic and novelty oddball tasks

	Classic Oddball				Novelty Oddball			
	RT (ms)	FA	Misses	*d'*	RT (ms)	FA	Misses	*d'*
Plac	407	0.4	1.1	5.11	483	0.8	1.3	4.69
	(50)	(0.9)	(0.5)	(0.25)	(76)	(1.2)	(1.6)	(0.46)
Clon	421	1.6	3.3	4.38	427	1.3	6.4	3.92
	(49)	(0.9)	(4.5)	(0.91)	(92)	(1.8)	(6.8)	(0.99)
Scop	433	1.8	2.1	4.51	448	1.8	4.4	4.07
	(81)	(2.8)	(3.7)	(0.81)	(79)	(1.7)	(5.9)	(1.00)

Note: Standard deviations are provided between parentheses.

5.3.3. Standard ERP analyses

Figure 5.2 shows P3 scalp distributions and stimulus-locked ERP waveforms in the placebo condition. The rares in both the classic and novelty oddball tasks elicited a centroparietal P300. This impression was confirmed by a 2 (classic/novelty task) × 3 (Fz/Cz/Pz site) ANOVA on rare-locked P3 peak amplitude defined in a 200-400 ms window. This analysis indicated that the P3 peak was greater for centroparietal sites (Cz = 6.39 μV; Pz = 7.83 μV) than for the frontal site Fz (2.80 μV), as indicated by a main effect of site, $F(2, 34) = 22.7$, $p < .0005$, *partial* $\eta^2 = .57$. There was no main effect of task, nor an interaction between task and site.As expected, novel stimuli elicited a positivity that was maximal at frontocentral electrode sites, so shifted frontally relative to the rare-related P3. A 2 (rare/novel stimulus) × 3 (Fz/Cz/Pz site) ANOVA on stimulus-locked P3 peak amplitudes evoked in the novelty oddball task confirmed that novels evoked a different P3 scalp distribution than rares, as indicated by a significant interaction between stimulus type and site, $F(2, 34) = 22.1$, $p < .0005$, *partial* $\eta^2 = .57$ (see Figure 5.2). To quantify this effect, we computed difference scores as novel peak amplitude minus target peak amplitude: this revealed a larger effect at frontocentral sites (Fz = 9.3 μV, Cz = 12.6 μV) than at parietal electrode Pz (4.8 μV). Overall, P3s were larger at centroparietal sites than at frontal site Fz, $F(2, 34) = 16.4$, $p < .0005$, *partial* $\eta^2 = .88$ and novels evoked a larger P3 than targets, $F(1, 17) = 130.1$, $p < .0005$, *partial* $\eta^2 = .88$. Furthermore, a repeated-measures ANOVA on P3 peak latency suggests that the peak latency (averaged across Fz, Cz, and Pz) is earlier for novels (277 ms) than for rares (317 ms), $F(1, 17) = 6.4$, $p = .02$, *partial* $\eta^2 = .27$. The properties described above correspond with what is usually referred to as the novelty P3.

Taken together, the pattern of results for the attend tasks in the placebo condition is very similar to the data described in previous classic and novelty oddball studies. In the two ignore task blocks (Figure 5.2), it was difficult to visually identify part of the ERP as the P3a. Therefore, we did not statistically analyze the ERP data from these two task blocks.

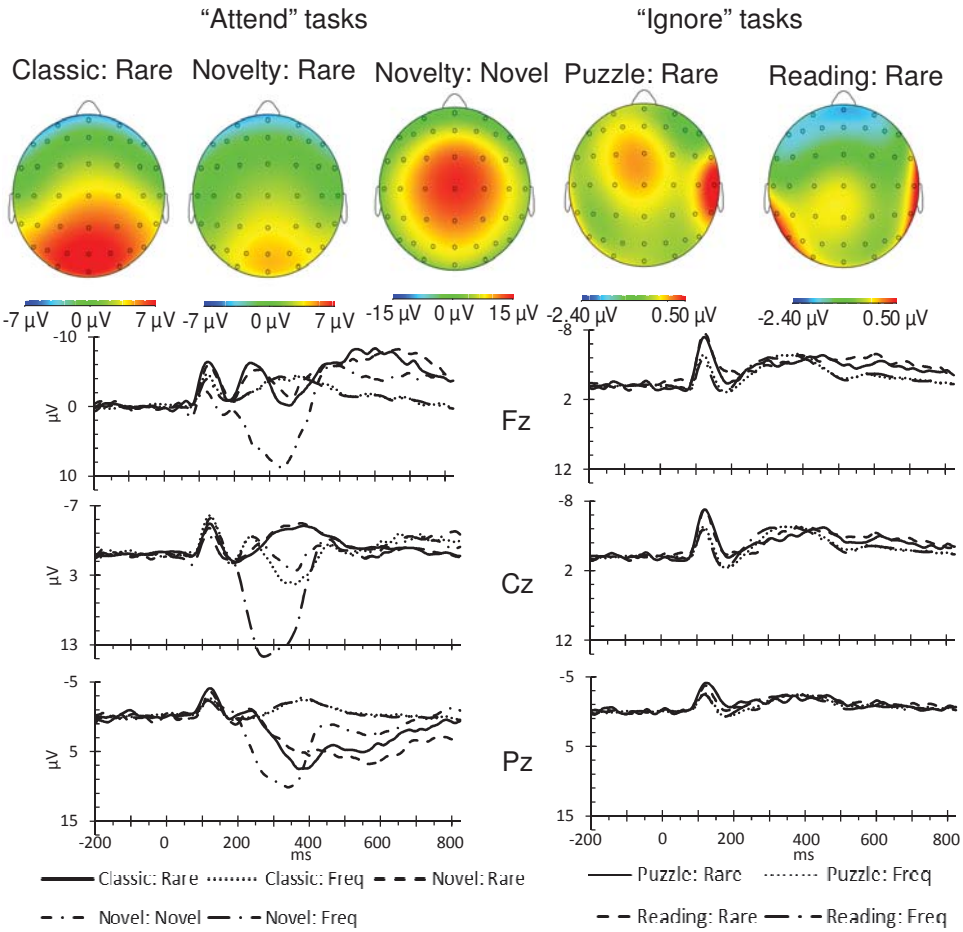

Figure 5.2. Scalp distributions (left panel) and grand-average stimulus-locked ERPs from electrodes Fz, Cz and Pz (right panel) for the placebo condition. Scalp distributions reflect the timepoint at which the response to stimuli was most positive.

Figure 5.3 presents stimulus-locked ERP waveforms elicited by rares and novels in the attend tasks as a function of treatment. Clonidine and scopolamine appeared to modulate the amplitude of the rare-related and especially novelty-related P3s. We confirmed that the drug-related modulations of the large rare-related positivity in the classic oddball task were also clearly present in response-locked averages, suggesting that they do not reflect temporal smearing of a

response-related component. Having established that our data contained the expected P3 patterns in the classic and novelty oddball tasks, we subjected the ERP data to a spatiotemporal PCA before statistically testing for treatment effects on P3 components.

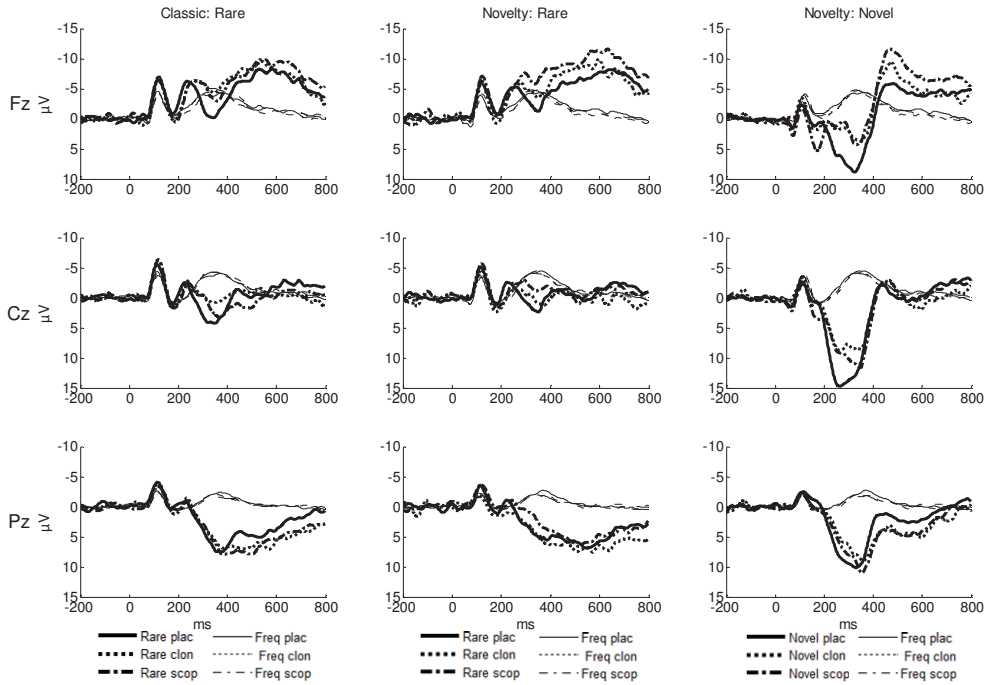

Figure 5.3. Grand-average stimulus-locked ERPs from the attend tasks (classic and novelty oddball) as a function of treatment.

5.3.4. Spatiotemporal principal components analysis

As described under Methods, we first reduced spatial dimensionality of the data with a spatial PCA, which yielded 7 spatial factors, that is, 7 virtual electrodes that captured the relevant spatial variance of the original data. Figure 5.4 shows topographic maps of spatial factor loadings (i.e. correlations between the original electrodes and a given spatial factor). As can be seen in this figure, spatial factors 1

and 2 jointly account for the largest proportion of variance in the original data (70.2%); frontal electrodes load strongly on spatial factor (SF) 1, while centroparietal electrodes load strongly on SF2. Figures 5.5 and 5.6 show spatial factor scores (i.e. the contribution of every spatial factor to every original observation) plotted as virtual ERP time series, averaged over participants and collapsed across treatments, for the attend and ignore tasks. As can be seen in these plots, SF1 and SF2 are most responsive to target and novel stimuli, especially in the attend tasks, so we decided to focus on these two spatial factors in subsequent analyses. SF5 is also responsive to novel stimuli and has the frontocentral scalp distribution that is associated with the novelty P3, but this factor accounts for only 3.9% of the variance of the original dataset.

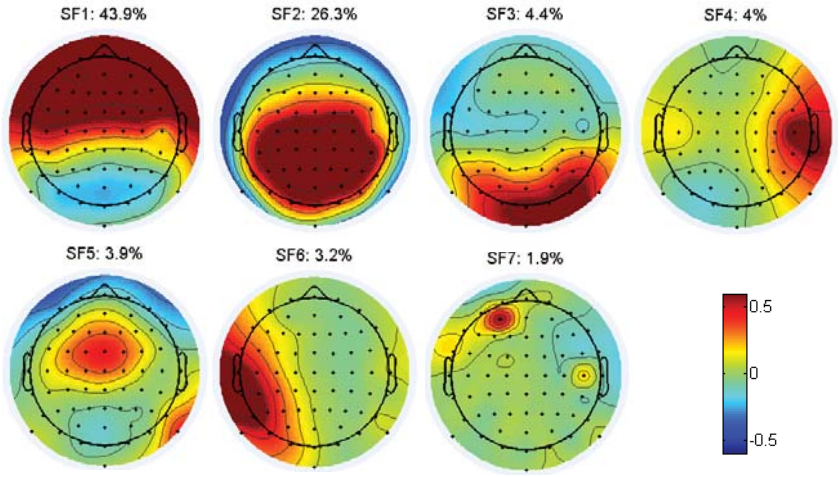

Figure 5.4. Spatial factor loadings, presented as topographic maps (virtual electrodes). Spatial factors 1 (anterior scalp distribution) and 2 (posterior distribution) collectively account for 70.2% of the variance in the original data set.

Our spatial PCA was followed up with a separate temporal PCA for SF1 and SF2 to reduce the temporal dimensionality of the data. Figure 5.7 presents temporal factor loadings, that is, virtual epochs that represent temporal patterns in the data. The contribution of each temporal factor to the original data is represented

by spatiotemporal factor scores (see Figure 5.8). These spatiotemporal factor scores are available for every cell of the design, and formed the basis for statistical analysis.

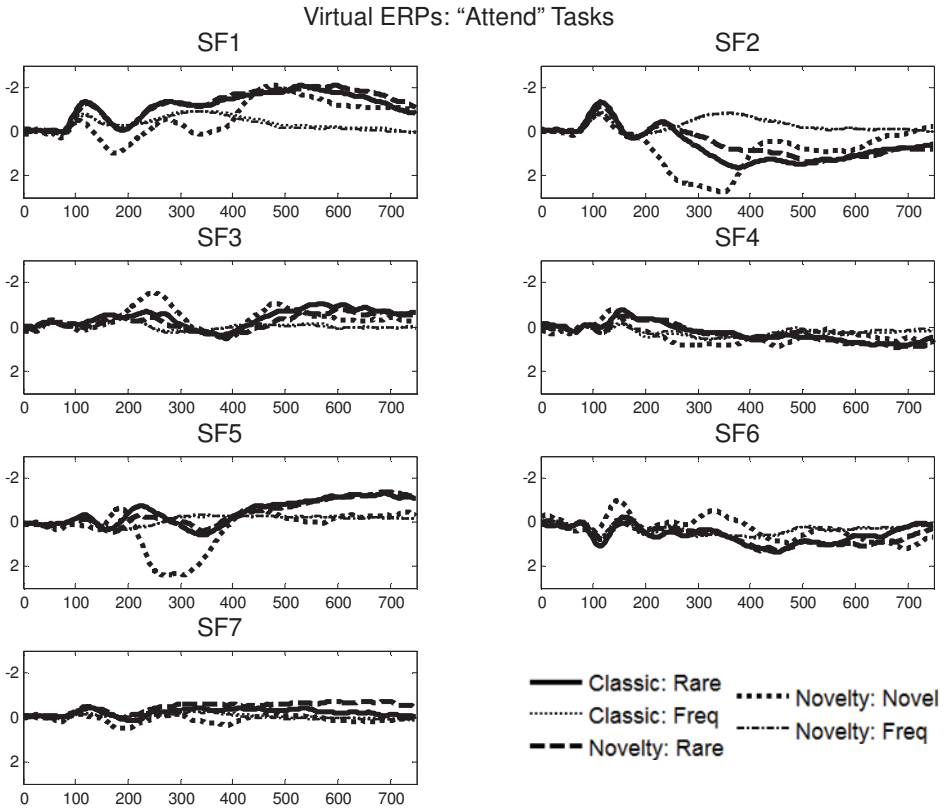

Figure 5.5. Spatial factor scores, presented as virtual ERPs, for the various stimulus types in the two attended task blocks. Note that the values on the Y-axis are arbitrary units.

The combination of spatial factor 2 and temporal factor 3 (SF2TF3; Figure 5.8, top panel) appears to represent the classic P300, given its posterior scalp distribution and time course (~300-600 ms). In line with this interpretation, statistical testing of the factor scores associated with this spatiotemporal component indicated that rares elicited a larger P300 than frequents, both in the classic oddball task $F(1, 17) = 28.9$, $p < .0005$, *partial* $\eta^2 = .63$, and in the novelty oddball task, $F(1, 17) = 17.7$, $p = .001$, *partial* $\eta^2 = .51$. Novel stimuli also elicited

a P300, $F(1, 17) = 5.2$, $p = .04$, *partial* $\eta^2 = .24$, but smaller than that elicited by rares, $F(1, 17) = 8.0$, $p = .01$, *partial* $\eta^2 = .32$. A similar result was reported by Spencer et al. (2001). Rares did not elicit a significant P300 in the ignore tasks.

The combination of spatial factor 1 and temporal factor 2 (SF1TF2; Figure 5.8, middle panel) appears to represent the novelty P3, given its frontal scalp distribution and time course (~250-400 ms). Accordingly, statistical testing of the factor scores associated with SF1TF2 indicated that novels elicited a larger novelty P3 than frequents, $F(1, 17) = 64.6$, $p < .0005$, *partial* $\eta^2 = .79$. In contrast to the results reported by Spencer et al. (2001), rares did not elicit a significant novelty P3 in the classic and novelty oddball tasks ($p = .61$ and $p = .41$, respectively). Ignored rares elicited a small novelty P3 relative to ignored frequents in both the puzzle block, $F(1, 17) = 19.0$, $p < .0005$, *partial* $\eta^2 = .53$, and the reading block, $F(1, 17) = 5.7$, $p = .03$, *partial* $\eta^2 = .25$.

Ignored rares have been shown to elicit both an N2 and a subsequent positivity, often called the P3a (e.g., Snyder & Hillyard, 1976; Squires, Donchin, Herning, & McCarthy, 1977), that some have argued to be distinct from the P300 and novelty P3. Our data seem to corroborate that observation (Figure 5.2): ignored rares elicited a clear N2 followed by a small positive dip. This pattern in the ignored ERPs was captured by the combination of spatial factor 1 and temporal factor 4 (SF1TF4; Figure 5.8, lower panel), which has the frontocentral scalp distribution and temporal characteristics (~200-350 ms) that are often associated with the N2-P3a complex. We therefore tentatively classify SF1TF4 as the N2-P3a complex described by Snyder and Hillyard (1976). Rares in the attend tasks did not reliably elicit this complex. Ignored rares elicited a larger N2-P3a than frequents in the reading block, $F(1, 17) = 9.9$, $p = 006$, *partial* $\eta^2 = .37$, but not in the puzzle block[5]. Ignored rares also elicited a larger N2-P3a than attended rares, $F(1, 17) = 5.0$, $p = .04$, *partial* $\eta^2 = .23$.

[5] Note that block order was not counterbalanced and that the puzzle block was always presented first, at which point participants had not established a "frequent" template yet. Furthermore, anecdotal observations suggest that many participants in our study were very

We will now present the effects of treatment on these spatiotemporal factor scores. Figure 5.8 contains all relevant averages; to aid legibility, we have tabulated test statistics for each spatiotemporal component (Tables 6.2-6.4). In almost all cases, treatment effects on P3 amplitude (e.g., in the classic oddball task) were accompanied by similar effects of treatment on P3 amplitude modulation (i.e. rares > frequents). Therefore, for the sake of simplicity, when we state below that a drug modulated P3 amplitude, this refers to both the main effect of treatment and the interaction of treatment and stimulus type. Relative to placebo, clonidine did not reliably influence classic P300 amplitude as evoked by rares in the classic and the novelty oddball tasks, but clonidine *increased* P300 amplitude evoked by novels (Table 6.2). In contrast, clonidine *decreased* the amplitude of the novelty P3 as evoked by both novels and classic rares (but not novelty rares; Table 6.3).

Finally, clonidine had no reliable effect on the amplitude of the N2-P3a component evoked by ignored rares (Table 6.4). Thus, clonidine decreased novelty P3 amplitude whereas, if anything, it increased P300 amplitude (i.e., to novels).

Scopolamine showed a similar pattern of results as clonidine but more pronounced (Figure 5.8; Tables 5.2-5.4). Relative to placebo, scopolamine increased the P300 evoked by rares in the classic oddball task and by novels in the novelty oddball task. In contrast, scopolamine decreased the amplitude of the novelty P3 evoked by classic rares, novelty rares, and novels. Scopolamine also reduced N2-P3a amplitude relative to placebo in the reading task; a similar but nonsignificant effect was manifested in the puzzle task. With one exception (see Table 6.2), direct comparisons between scopolamine and clonidine yielded no significant differences.

As we will discuss below, our finding that scopolamine and clonidine increased P300 amplitude, at least in some conditions, has not been reported before in the literature. To examine if a convential analysis of the late positivity observed

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engaged by their crossword puzzles, and therefore may have ignored auditory stimuli more strongly than was intended.

at Pz would have revealed these treatment effects, we computed the mean amplitude of the signal at Pz in a 200-600 ms window for rares and frequents in the classic oddball task, where the treatment effect of scopolamine on the P300 was most pronounced. We chose a mean-amplitude measure in a relatively broad time window, because Figure 5.3 suggests that the treatment effect of scopolamine was specifically evident for the trailing slope of the P3, and hence would not be detected by a peak-amplitude analysis.

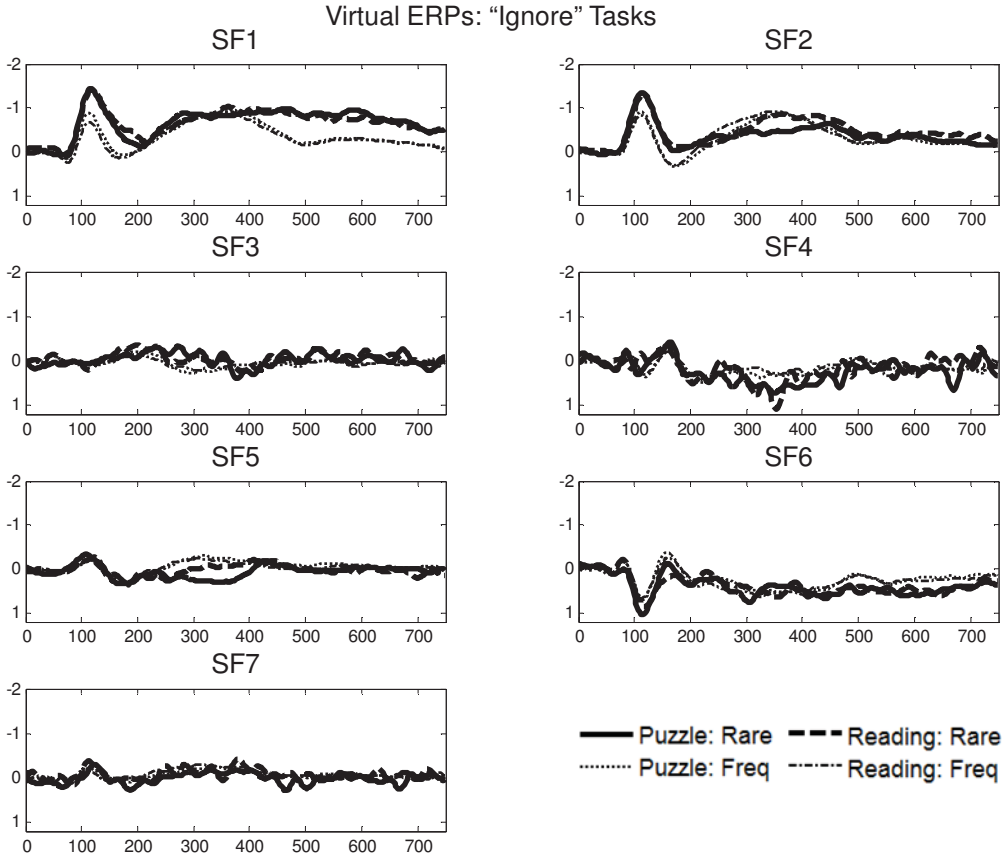


Figure 5.6. Spatial factor scores, presented as virtual ERPs, for the various stimulus types in the two ignore task blocks. Note that the values on the Y-axis are in arbitrary units.

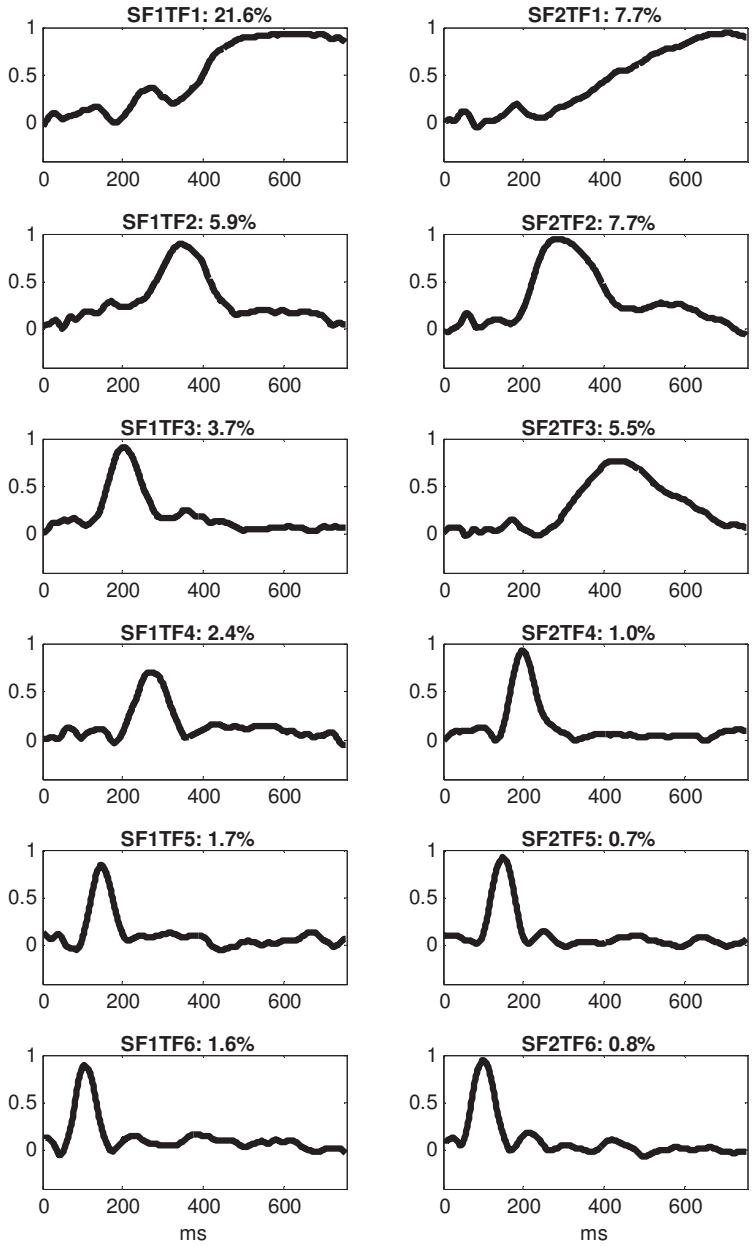


Figure 5.7. Temporal factor loadings, plotted as virtual epochs, for spatial factors 1 (left) and 2 (right). For every temporal factor, the proportion of explained variance is indicated: note that this is the percentage of explained variance in the original set of ERPs, not in the set of virtual ERPs that was submitted to the temporal PCA.

A 2 (scopolamine vs. placebo) x 2 (rares vs. frequents) repeated-measures ANOVA showed a non-significant main effect of treatment, $F(1, 17) = 1.7$, $p = .21$, and a non-significant interaction with stimulus type, $F(1, 17) < 1$, $p = .52$. Similar results were obtained for a comparison between clonidine and placebo. Even comparisons limited to the rare-related ERPs, where differences between treatments were most evident, yielded no reliable effects of drug. We conclude that a conventional analysis would not have revealed the treatment effects on the P300 that were identified by the PCA.

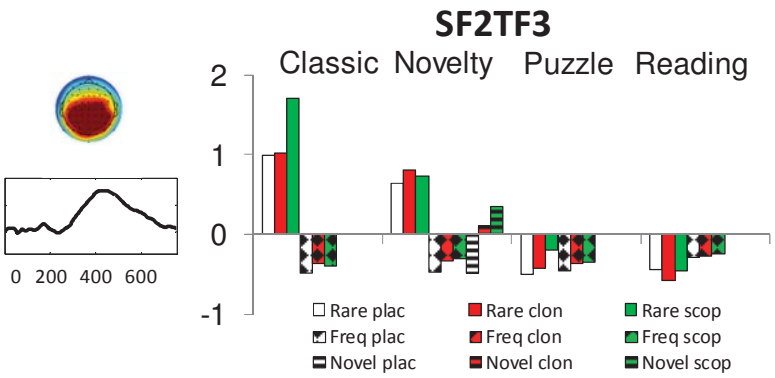
5.4 Discussion

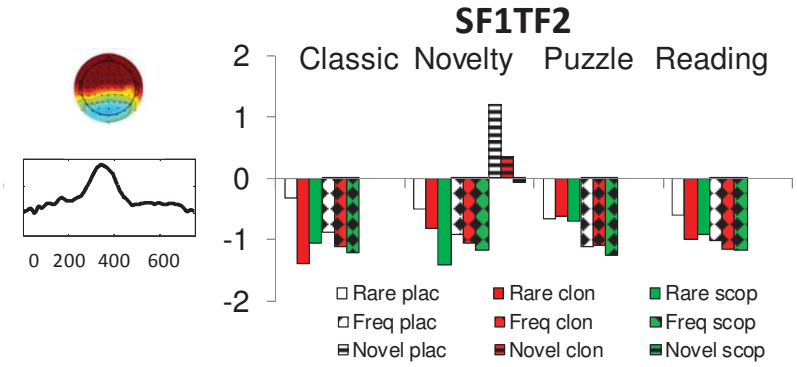
To examine the neurochemical basis of late ERP responses to deviant stimuli, we collected EEG data while participants performed a series of active and passive oddball tasks. We then used spatiotemporal PCA to extract three distinct late positive ERP components: (i) the P300, which was elicited by attended rares and, to a lesser extent, by novels; (ii) the novelty P3, which was elicited by novels and, to a limited (nonsignificant) extent, by attended rares. These results largely replicate the findings of Spencer, Dien, and Donchin (2001), whose methods formed the basis for our pharmacological study. In addition, and in contrast to Spencer et al., our PCA isolated a component that we identified as (iii) the N2-P3a, a biphasic response that was only elicited by ignored rares in the reading block. Several previous PCA studies have failed to find evidence for a distinction between the novelty P3 and P3a, suggesting instead that they reflect the same component (Dien, Spencer, & Donchin, 2004; Simons, Graham, Miles, & Chen, 2001; Spencer et al., 2001). Remarkably, in two of those previous studies the PCA did not include conditions in which participants were presented with ignored deviants, the condition that elicited the N2-P3a component in our study (Dien et al., 2004; Simons et al., 2001). A potentially important difference between our study and Spencer et al. (2001) is that we applied separate temporal PCAs to each spatial

factor, which may help distinguish components with subtle time course differences within a given spatial factor (see Dien, 2010a; Dien et al., 2004), such as the novelty P3 and N2-P3a for our frontal factor.

Our specific goal was to examine how the P300, novelty P3 and N2-P3a components were modulated by clonidine and scopolamine. Clonidine, at the moderate dose used here, reduces activity in the noradrenergic nucleus locus coeruleus and decreases noradrenaline release in projection areas throughout the brain. The effects of scopolamine are somewhat more complicated (Hasselmo & Sarter, 2011). Scopolamine blocks postsynaptic muscarinic receptors, but also presynaptic muscarinic autoreceptors in cholinergic basal forebrain neurons, which increases overall acetylcholine release.This, in turn, leads to increased stimulation of nicotinergic acetylcholine receptors, which, like muscarinic receptors, are widely distributed across the brain. Despite these fundamental differences in their principal modes of action, clonidine and scopolamine had surprisingly similar effects on the examined P3 components. Although the effects of scopolamine were generally more pronounced, we cannot conclude that the cholinergic system is more heavily involved in the generation of these components, because this pattern of results may simply reflect the relative doses of the two drugs that we used. We will now discuss the drug effects in turn, first focusing on the P300 and then on the novelty P3 and N2-P3a.

Figure 5.8 (next page). TF3 scores associated with SF2 (posterior) and TF2 and TF4 scores associated with SF1 (frontal) for each treatment, task and stimulus type. The value of the factor scores is a unitless dimension.


SF2TF3
Classic Novelty Puzzle Reading
2
1
0
-1
0 200 400 600
Rare plac
Rare clon
Rare scop
Freq plac
Freq clon
Freq scop
Novel plac
Novel clon
Novel scop


SF1TF2
Classic Novelty Puzzle Reading
2
1
0
-1
-2
0 200 400 600
Rare plac
Rare clon
Rare scop
Freq plac
Freq clon
Freq scop
Novel plac
Novel clon
Novel scop

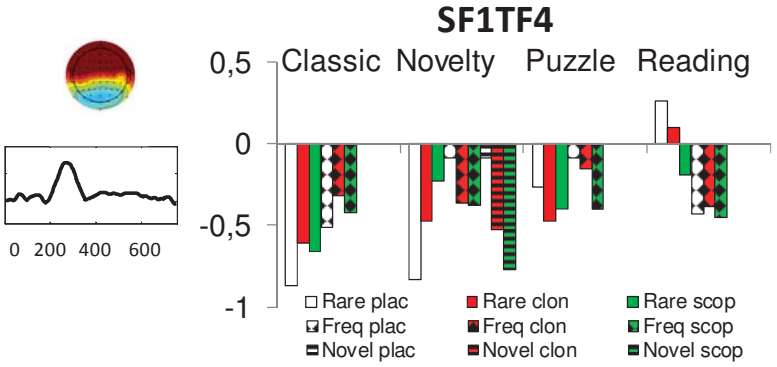

SF1TF4
Classic Novelty Puzzle Reading
0,5
0
-0,5
-1
0 200 400 600
Rare plac
Rare clon
Rare scop
Freq plac
Freq clon
Freq scop
Novel plac
Novel clon
Novel scop

Table 5.2. SF2TF3: statistical effects of treatment on spatiotemporal factor scores

Statistical term		*F* value	*p* value
Classic rare vs. classic frequent			
Treatment		$F(2, 34) = 3.6$	$p = .04$
	clo = pla		$p = .69$
	sco > pla	$F(1, 17) = 5.8$	$p = .03$
	sco > clo	$F(1, 17) = 6.5$	$p = .02$
Treatment x Stimulus Type		$F(2, 34) = 3.4$	$p = .03$
	clo = pla		$p = .74$
	sco > pla	$F(1, 17) = 4.7$	$p = .045$
	sco > clo	$F(1, 17) = 10.1$	$p = .005$
Novelty rare vs. novelty frequent			
Treatment			$p = .57$
Treatment x Stimulus Type			$p = .96$
Novelty novel vs. novelty frequent			
Treatment		$F(2, 34) = 7.3$	$p = .002$
	clo > pla	$F(1, 17) = 8.2$	$p = .01$
	sco > pla	$F(1, 17) = 9.5$	$p = .007$
	sco = clo		$p = .24$
Treatment x Stimulus Type		$F(2, 34) = 4.0$	$p = .03$
	clo > pla	$F(1, 17) = 3.8$	$p = .07$
	sco > pla	$F(1, 17) = 5.0$	$p = .04$
	sco = clo		$p = .26$

Note: Significant main and interaction effects are followed by pairwise comparisons between the treatments. The direction of the effect is indicated.

Table 5.3. SF1TF2: statistical effects of treatment on spatiotemporal factor scores

Statistical term		*F* value	*p* value
Classic rare vs. classic frequent			
Treatment		$F(2, 34) = 14.8$	$p < .0005$
	clo < pla	$F(1, 17) = 17.9$	$p = .001$
	sco < pla	$F(1, 17) = 18.7$	$p = .001$
	sco = clo		$p = .24$
Treatment x Stimulus Type		$F(2, 34) = 8.2$	$p = .001$
	clo < pla	$F(1, 17) = 16.1$	$p = .001$
	sco < pla	$F(1, 17) = 4.5$	$p = .048$
	sco = clo		$p = .07$
Novelty rare vs. novelty frequent			
Treatment		$F(2, 34) = 5.7$	$p = .008$
	clo = pla		$p = .19$
	sco < pla	$F(1, 17) = 14.5$	$p = .001$
	sco = clo		$p = .96$
Treatment x Stimulus Type			$p = .43$
Novelty novel vs. novelty frequent			
Treatment		$F(2, 34) = 64.4$	$p < .0005$
	clo < pla	$F(1, 17) = 7.1$	$p = .02$
	sco < pla	$F(1, 17) = 23.8$	$p < .0005$
	sco = clo		$p = .22$
Treatment x Stimulus Type		$F(2, 34) = 3.9$	$p = .03$
	clo < pla	$F(1, 17) = 6.1$	$p = .03$
	sco < pla	$F(1, 17) = 7.4$	$p = .02$
	sco = clo		$p = .50$

Note: Significant main and interaction effects are followed by pairwise comparisons between the treatments. The direction of the effect is indicated.

Table 5.4. SF1TF4: statistical effects of treatment on spatiotemporal factor scores

Statistical term		*F* value	*p* value
Reading rare vs. reading frequent			
Treatment			$p = .36$
Treatment x Stimulus Type		$F(2, 34) = 3.4$	$p = .047$
	clo = pla		$p = .22$
	sco < pla	$F(1, 17) = 8.1$	$p = .01$
	sco = clo		$p = .24$

Note: Significant main and interaction effects are followed by pairwise comparisons between the treatments. The direction of the effect is indicated.

Scopolamine increased the amplitude of the P300 to rares in the classic oddball task and to novels. Clonidine only augmented the P300 to novels. Neither drug modulated the P300 to rares in the novelty oddball task. The difference in results for the attended rares in the two active oddball tasks was also clearly apparent in the ERP waveforms (Figure 5.2). Previous studies of the effects of scopolamine and clonidine on P300 all used active oddball tasks or other discrimination tasks for eliciting the P300. No previous studies have reported that scopolamine enhances the P300, although it should be emphasized that previous studies usually reported results for only one electrode and did not attempt to decompose their ERP data. Several studies reported no significant effect of scopolamine on the amplitude of the late positive ERP response at Pz (Callaway, Halliday, Naylor, Schlechter, 1985; Potter, Pickles, Roberts, & Rugg, 2000a, 2002b). One study reported an amplitude reduction at Pz (Brandeis, Naylor, Halliday, Callaway, & Yano, 1992) and a final study reported an amplitude reduction by scopolamine and no interaction with electrode site (Fz, Cz, Pz) (Curran, Pooviboonsuk, Dalton, & Lader, 1998). We do not know why our pattern of results diverges somewhat from previous literature. The scopolamine-induced amplitude increase for attended rares was evident only for the trailing tail of the P300, not for the peak, and we found that a convential mean-amplitude analysis of the Pz data would not have detected this treatment effect. However, in those previous articles that plotted the Pz waveforms, we noticed no hint of a scopolamine-induced amplitude increase of the trailing tail.

Previous work is also generally at odds with our findings that clonidine did not modulate or, if anything, slightly increased the P300. Two studies found no reliable effect of clonidine on the amplitude of the late positive response over posterior electrodes (Shelley et al., 1997; Turetsky & Fein, 2002). Three other studies found a reliable amplitude reduction after clonidine (Duncan & Kaye, 1987; Halliday et al., 1994; Joseph & Sitaram, 1989). Most studies used a dose similar to that used here. In general we have noticed no systematic relationship between

scopolamine and clonidine dose in previous studies and whether or not the results matched ours. Altogether, the general discrepancy between our P300 findings and those in previous scopolamine and clonidine studies is puzzling. Some of the discrepancy may be due to the small sample sizes used in previous research in combination with the possibility that individuals may show highly diverse, or even opposite, effects of the same pharmacological agent on P300 amplitude, depending on tonic neuromodulator levels or personality traits (de Rover et al., submitted). In any case, the discrepancy emphasizes the need for more solid research, using PCA or other decomposition methods to isolate the P300.

Whereas scopolamine tended to increase the posterior P300, it decreased the novelty P3 and N2-P3a, the two frontal components. Likewise, clonidine decreased the novelty P3 and the N2-P3a, although the latter effect was not reliable. Although the novelty P3 did not reliably differ between attended rares and frequents (as indicated by a nonsignificant main effect of trial type), the two drugs significantly modulated this component even for these trial types (as indicated by significant main effects of treatment and/or treatment x trial-type interactions). To our knowledge, there have been no previous studies that examined the effects of scopolamine or clonidine on the late frontal response to novel stimuli, the stimulus class that probably elicits the most pronounced novelty P3. However, some information may be gleaned from reported drug effects on the late frontal response to attended rares. Here, previous literature is more consistent with our findings. Two scopolamine studies reported a reduced P3 amplitude at Fz (Potter, Pickles, Roberts, & Rugg, 2000b) and Cz (Meador et al., 1989). One clonidine study reported a reduced frontal P3 (Turetsky & Fein, 2002), while another study reported no effect of clonidine over frontal electrodes (Joseph & Sitaram, 1989). Together these results present fairly strong evidence that scopolamine and clonidine decrease the amplitude of the novelty P3; more research is needed to establish their effects on the N2-P3a, if this is indeed a separate component. A possibly related finding is that clonidine reduces the amplitude of the stop P3

(Logemann, Böcker, Deschamps, Kemner, & Kenemans, 2013), a frontocentral component associated with successful inhibitions, which requires a systematic comparison with other frontal P3 components.

Why did clonidine and scopolamine show such similar effects on the late positive components? A plausible reason is that the central noradrenergic and cholinergic systems strongly interact with each other. On the one hand, there is solid evidence that stimulation of the locus coeruleus inhibits cortical acetylcholine release (Acquas, Wilson, & Fibiger, 1998; Bianchi, Spidalieri, Guandalini, Tanganelli, & Beani, 1979), probably through the activation of presynaptic α_2 receptors in the basal forebrain and on cortical cholinergic nerve endings (Beani, Bianchi, Giacomelli, & Tamberi, 1978; Buccafusco, 1982). In addition, there is some in-vitro evidence that clonidine may also directly block muscarinic receptors (Buccafusco & Aronstam, 1986). On the other hand, acetylcholine has been demonstrated to activate locus coeruleus neurons in rats and co-administration of scopolamine reduces this effect (Adams & Foote, 1988; Egan & North, 1985; Engberg & Svensson, 1980), suggesting that the effect of acetylcholine on locus coeruleus neurons is mediated by muscarinic receptors. So the locus coeruleus and basal forebrain strongly interact with each other, and clonidine and scopolamine may each have reduced this interaction, leading to a similar pattern of results for the two drugs. This view is further supported by findings that lesions to both the locus coeruleus (Pineda, Foote, & Neville, 1989) and to the nucleus basalis of Meynert (Wang et al., 1997), the primary source of cortical acetylcholine, result in a significant amplitude reduction of the late positivity to infrequent passive rares.

A question for future research is why clonidine and scopolamine have differential or even opposite effects on the amplitudes of the posterior P300 and frontal P3 components. An interesting possibility is that this pattern reflects the important role of noradrenaline and acetylcholine in
modulating the balance between (“bottom-up”) thalamocortical input and (“top-down”) intracortical activity (Hasselmo & Sarter, 2011; Yu & Dayan, 2005).

However, in light of the complicated effects of clonidine and scopolamine, interactions between the neuromodulator systems, and the fact that very little is known about the cellular basis of P3 components (cf. Nieuwenhuis et al., 2005), this hypothesis remains purely speculative. One way to address the hypothesis in humans would be to examine the effects of clonidine and scopolamine on functional connectivity patterns in EEG and neuroimaging data (Coull, Büchel, Friston, & Frith, 1999), and relate these to drug effects on P3 components.

Following Spencer et al. (2001), we used spatiotemporal PCA with Varimax rotations to decompose the ERP responses to deviant events in space and time. Recently, arguments have been presented that suggest that temporospatial PCA might lead to more optimal decomposition of ERP data than spatiotemporal PCA and that Varimax rotations might not be the optimal choice to decompose the variance of ERPs (see, e.g, Dien, 2010b; Dien, Khoe, & Mangun, 2007). From this point of view, our choice of specific PCA methods presents a limitation. The reason why we chose these methods is that we wanted to stay as close as possible to the methods of Spencer et al. (2001), because of its rigorously detailed description (including many interim results) of the decomposition of P3 components, which allowed a step-by-step comparison with (and validation of) our own results.

To summarize, the presents results provide clear evidence for a role of the noradrenergic system in the generation of the frontal novelty P3, while only tenuously supporting previous evidence for a role in generation of the posterior P300 (de Taeye et al., 2014; Nieuwenhuis et al., 2005; Nieuwenhuis, 2011). In addition, the results provide unequivocal evidence for a role of the cholinergic system in generation of not only frontal P3 components (Ranganath & Rainer, 2003), but also the posterior P300. Future research in animals, for example using optogenetic methods, needs to examine the role of interactions between the two neuromodulator systems in generating P3 components.

Another goal for future research will be to test the hypothesis that dopamine is involved in generating the P3a/novelty P3 (Polich, 2007). While specific dopaminergic agents have little or no effect on the late positive response to attended rares (Luthringer et al., 1999; Oranje et al., 2006; Takeshita & Ogura, 1994), there is some evidence that they affect the late positive response to ignored rares and novel stimuli (Kähkönen et al., 2002; Rangel-Gomez, Hickey, van Amelsvoort, Bet, & Meeter, 2013). However, these effects were observed at centroparietal rather than frontal electrodes, and no attempt was made to distinguish specific components contributing to the late positive response. Therefore, methods such as those used here are needed to examine the relationship between dopamine and P3 components.

6. Effects of arousal on cognitive control: Empirical tests of the conflict-modulated Hebbian-learning hypothesis

Abstract

An increasing number of empirical phenomena that were previously interpreted as a result of cognitive control, turn out to reflect (in part) simple associative-learning effects. A prime example is the proportion congruency effect, the finding that interference effects (such as the Stroop effect) decrease as the proportion of incongruent stimuli increases. While this was previously regarded as strong evidence for a global conflict monitoring-cognitive control loop, recent evidence has shown that the proportion congruency effect is largely item-specific and hence must be due to associative learning. The goal of our research was to test a recent hypothesis about the mechanism underlying such associative-learning effects, the conflict-modulated Hebbian-learning hypothesis, which proposes that the effect of conflict on associative learning is mediated by phasic arousal responses. In Experiment 1, we examined in detail the relationship between the item-specific proportion congruency effect and an autonomic measure of phasic arousal: task-evoked pupillary responses. In Experiment 2, we used a task-irrelevant phasic arousal manipulation and examined the effect on item-specific learning of incongruent stimulus-response associations. The results provide little evidence for the conflict-modulated Hebbian-learning hypothesis, which requires additional empirical support to remain tenable.

This chapter is based on:

Brown, S. B. R. E., van Steenbergen, H., Kedar, T., & Nieuwenhuis, S. (2014). Effects of arousal on cognitive control: empirical tests of ffthe conflict-modulated Hebbian-learning hypothesis. *Frontiers in Human Neuroscience, 8:*23.

6.1 Introduction

Cognitive control is required to flexibly adapt our behavior to situational demands. It refers to the human capability to obtain a desired outcome given conflicting options. Stopping for a red traffic light, choosing an apple over chocolate, or finishing a paper rather than sitting in the sun, are all examples of cognitive control. In laboratory settings cognitive control is often measured using congruency tasks such as the Stroop task (MacLeod, 1992). Participants in the Stroop task are required to name the printed color of a color word (e.g., the word *blue* written in black ink). To do so they need to suppress their habitual tendency to respond to the color word (blue) and instead respond to the demanded ink color (black). Participants lacking cognitive control would respond habitually to the stimulus, which is demonstrated by many patients with damage to their prefrontal cortex (Vendrell et al., 1995).

Botvinick and colleagues proposed the conflict-monitoring hypothesis to explain how our cognitive system detects situations in which cognitive control is required (Botvinick, Braver, Barch, Carter, & Cohen, 2001). They suggested that the anterior cingulate cortex (ACC) monitors the occurrence of conflict in information processing. When conflict is detected, compensatory adjustments in control are made by passing on information to brain systems responsible for the exertion of cognitive control. Numerous neuroimaging studies have provided support for the idea that the ACC responds to the occurrence of conflict and then recruits areas responsible for cognitive control, such as the prefrontal cortex (Botvinick, Cohen, & Carter, 2004).

Botvinick et al. (2001) suggested that the conflict-monitoring hypothesis can also explain a number of important behavioral phenomena, including the conflict-adaptation effect and the proportion congruency effect. The conflict-adaptation effect refers to the finding that the magnitude of behavioral interference

effects in congruency tasks is influenced by the congruency of the previous trial (Gratton, Coles, & Donchin, 1992). When two consecutive incongruent trials are presented, the degree of interference is smaller for the second trial. For example, in a Stroop task, responses to incongruent stimuli are faster and more accurate when those stimuli are preceded by another incongruent stimulus rather than a congruent stimulus. According to the conflict-monitoring hypothesis, this conflict-adaptation effect reflects an adjustment of cognitive control, signaled on a trial-by-trial basis by the ACC. Conflict on the preceding trial thus leads to higher levels of control on the subsequent trial.

The proportion congruency effect refers to the finding that the proportion of incongruent stimuli influences the magnitude of the interference effect that is measured in congruency tasks. For example, in a Stroop task, blocks of trials with a high proportion of incongruent stimuli will be associated with a smaller Stroop effect than blocks of trials with a small proportion of incongruent stimuli (Jacoby, Lindsay, & Hessels, 2003; Logan & Zbrodoff, 1979). The conflict-monitoring hypothesis explains this reduced interference effect as the result of a general increase in cognitive control, brought about by the frequent occurrence of conflict-inducing incongruent trials. A comparable hypothesis has been developed by Jacoby and colleagues (Jacoby et al., 2003; Bugg, Jacoby, & Toth, 2008; Blais, Robidoux, Risko, & Besner, 2007; Bugg, Jacoby, & Chanani, 2001), who suggest that control is exerted at the item-level by attenuating word reading for items that are presented mostly incongruently, while boosting word reading for items that are presented mostly congruently.

However, recent evidence appears to contradict the notion that the behavioral phenomena discussed above can be fully explained by a global, pro-active control mechanism. Increasing evidence suggests that both the conflict-adaptation effect (e.g., Nieuwenhuis et al., 2006; Mayr, Awh & Laurey, 2003) and the proportion congruency effect (Blais & Bunge, 2010; Bugg et al., 2008; Jacoby

et al., 2003; Notebaert & Verguts, 2007; Schmidt & Besner, 2008) can be explained, at least in part, as a result of simple associative learning. For example, Blais and Bunge (2010) used a modified Stroop task, in which the global (or *list-level*) proportion congruency in a block of trials was either 30%, 50%, or 70%. Importantly, embedded within each block was an item-level proportion-congruent manipulation. The 30% block contained two items (i.e. color names) that were congruent on 10% of the trials and two items that were congruent on 50% of the trials. In the 50% block, all items were congruent on 50% of the trials. And in the 70% block, two items were congruent on 50% of the trials and two items were congruent on 90% of the trials. When comparing the 50% conditions from each block, Blais and Bunge found no proportion congruency effect. That is, when item-specific proportion congruency (ISPC) was held constant, list-level proportion congruency did not modulate the Stroop effect. In contrast, there were clear ISPC effects within the 30% and 70% blocks: items that occurred in incongruent form more often, were associated with a smaller Stroop effect. Thus, in Blais and Bunge's study, the proportion congruency effect seemed to be driven entirely by ISPC effects, thus undermining explanations in terms of global changes in control (but see Bugg, McDaniel, Scullin, & Braver, 2011, for a demonstration of list-level control effects). A straightforward explanation of the ISPC effect is that it reflects the strengthening of incongruent stimulus-response associations as a function of the number of encounters with a particular item: the stronger the learned association between stimulus and response, the faster the RTs (Schmidt & Besner, 2008).

The goal of the current research was to test a recent hypothesis about the mechanism underlying associative-learning effects in conflict paradigms like the Stroop task: the conflict-modulated Hebbian-learning hypothesis, proposed by Verguts and Notebaert (2008, 2009). They proposed that conflict, such as experienced on an incongruent Stroop trial, triggers a phasic arousal response. This increase in arousal increases the rate of Hebbian learning of all representations that are active at the same time, thus enhancing learning of the association between the

stimulus and relevant task (i.e. attentional control) representation. The strengthening of these associations then allows faster responses the next time they are activated. More formally, Verguts and Notebaert propose that the ISPC effect follows from a Hebbian-learning rule with a variable learning-rate parameter that is proportional to the degree of conflict (and consequent arousal) experienced on each trial. Thus, in a Stroop task a color word, say "red" printed in blue ink, may be presented (Figure 6.1). In accordance with the conflict-monitoring hypothesis (Botvinick et al., 2001), a conflict-monitoring system detects the conflict evoked by this incongruent stimulus. Contrary to the conflict-monitoring theory, however, conflict-mediated arousal then increases Hebbian learning, updating the weights of the connections between stimulus and task-demand representations. The next time the word red is presented in blue, the corresponding connections are strengthened and Stroop interference decreases. The more frequent a particular item, the more pronounced the improvement in performance associated with that item.

Verguts and Notebaert (2009) have also proposed a neural mechanism for conflict modulated Hebbian-learning (Figure 6.1). Similar to the conflict-monitoring hypothesis, they suggest that conflict is detected by the ACC. The ACC then triggers a phasic response of the locus coeruleus (LC), a small noradrenergic brainstem nucleus with a major role in regulating arousal, through its widespread ascending projections throughout the brain (Sara, 2009). LC activation results in the global release of the neuromodulator noradrenaline (NE), which is known to strengthen Hebbian learning (reviewed in Berridge & Waterhouse, 2003; Bouret & Sara, 2005; Nieuwenhuis, 2011).

Although the conflict-modulated Hebbian-learning hypothesis is in line with neurophysiological and anatomical findings (Verguts & Notebaert, 2009), there is very little empirical evidence that item-specific associative learning in cognitive control tasks is indeed mediated by phasic arousal (van Bochove, van der Haeghen, Notebaert, & Verguts, 2013). In the current study, we investigated this

hypothesis in two experiments. In Experiment 1, we took a correlational approach and examined in detail the relationship between the ISPC effect and an autonomic measure of phasic arousal: task-evoked pupillary responses. In Experiment 2, we used a phasic arousal manipulation and examined the effect of arousal on item-specific learning of stimulus-response associations in a cognitive control task.

6.2 Experiment 1

We adapted the Stroop task experiment conducted by Blais and Bunge (2010, Figure 6.1). Participants performed two blocks of Stroop trials with 240 trials each. List-level and item-level proportion congruency were manipulated: one block consisted of item types that were congruent on 10% or 50% of the trials (list-level congruency = 30%) and the other block consisted of item types that were congruent on 50% or 90% of the trials (list-level congruency = 70%).

Throughout the experiment we measured task-evoked pupil dilation, a broadly accepted measure of phasic autonomic arousal (Bradley, Miccoli, Escrig, & Lang, 2008; Bradshaw, 1967; Kahneman, 1973; Nieuwenhuis, de Geus, & Aston-Jones, 2011). Previous research has found increased pupil dilations on incongruent trials compared to congruent trials in the Stroop task (Laeng, Orbo, Holmlund, Miozzo, 2011; Siegle, Steinhauer, Thase, 2004) and similar paradigms (van Steenbergen & Band, 2013), suggesting that pupil diameter is sensitive to conflict. The conflict-modulated Hebbian learning hypothesis suggests that participants with a larger pupillary arousal response to conflict, as indexed by the modulation of pupil dilation by congruency, should show a larger ISPC effect.

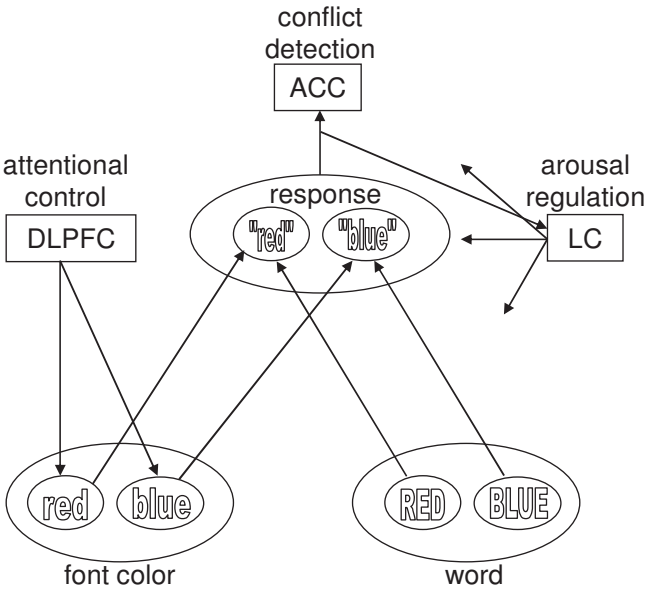


Figure 6.1. The presentation of an incongruent Stroop stimulus (e.g., the word BLUE printed in red) causes conflict, which is detected by a conflict-monitoring system (anterior cingulate cortex, ACC). The ACC activates the locus coeruleus (LC), which releases noradrenaline throughout the cortex. Noradrenaline strengthens Hebbian learning for active representations. During subsequent presentations of the specific stimulus type, conflict is reduced and reaction time is shorter. The DLPFC influences the input layers, based on task demands: in this example, font color is the relevant stimulus dimension, and the task demand unit therefore biases that input layer. For the sake of simplicity, only two colors and response options are plotted here. The arrows from the LC module in this model exemplify the widespread projections of the LC. However, in the model of Verguts and Notebaert (2008, 2009) conflict-induced release of noradrenaline only modulates the connections between input and task demand units—an assumption that was modified in later work by these authors (e.g., Braem et al., 2011).

6.3 Methods

6.3.1. Participants

Twenty-four non-color blind participants (2 males), aged 18-27 took part in a single 1.5-h experimental session in return for course credit or €10. Participants signed informed consent prior to their inclusion in the study.

6.3.2. Stimuli and Task

Participants performed a version of the Stroop task adapted from Blais and Bunge (2010), implemented in E-Prime (Psychology Software Tools, Sharpsburg, PA). Each trial started with a fixation stimulus that was presented for 2.5, 3.0, or 3.5 seconds.

To preclude luminance differences between the fixation stimulus and the subsequent target stimulus, we created fixation stimuli by scrambling pixels of all four target colors used in a task block. A Stroop stimulus, presented for 1,500 ms, followed the fixation, after which the next trial started. Participants were instructed to respond to the color of this stimulus, not to the color word presented on the screen. To help participants maintain the stimulus-response mappings, throughout the experiment 4 color patches were located at the bottom of the screen. These color patches corresponded spatially with the d, f, j, and k keys on a standard QWERTY keyboard, and represented the stimulus colors presented in that block. We used a subset of eight colors from the twelve used by Blais and Bunge (2010). In one block, a color set of pink (RGB values 255,192,203), green (000,176,080), brown (139,069,019) and yellow (255,255,000) was used; in the other block blue (000,112,192), red (255,000,000), white (250,250,250) and purple (112,048,160) were used. Within a block, two sets of stimuli were grouped together; for example, green was presented in either green or pink, but never in yellow or brown.

Participants were instructed verbally to fixate the center of the screen throughout each trial.

6.3.3. Design and Procedure

Following Blais and Bunge (2010), we manipulated the item-specific proportion congruency within task blocks and the list-level proportion congruency between task blocks. In one block, two color items were congruent in 50% of the trials, while the other items were congruent in 10% of the trials, resulting in a list-level proportion congruency of 30%. In the other block, two color items were congruent in 50% of the trials, while the other items were congruent in 90% of the trials, resulting in a list-level proportion congruency of 70%. For the sake of brevity, we will use codes like 30/10 in the description of the results to indicate list-level proportion congruency (30%) and then item-level proportion congruency (10%). Both color sets and proportion congruency order were counterbalanced across participants. Each block consisted of 240 trials, yielding a total of 480 trials; participants could take a short break halfway through a block.

Prior to each experimental block, participants received on-screen instructions and performed 24 practice trials to familiarize themselves with the stimulus-response mappings. Each color was presented six times in the form of a large rectangle in the middle of the screen. Participants had to respond to the color of the rectangle. If following the practice trials participants indicated that they had not correctly learned the stimulus-response mappings, they received another practice block of 24 trials. Participants then proceeded to the experimental condition.

During the experiment, pupil diameter was measured continuously. The experiment was conducted in a slightly dimmed room.

6.3.4. Pupil data acquisition and analysis

We recorded pupil diameter at 60 Hz using a Tobii T120 eye tracker monitor (Tobii Technology, Stockholm, Sweden), integrated into a 17" TFT monitor. Participants were seated at about 60 cm from the screen. Pupil measurements were made without the use of a head rest, because the Tobii T120 eyetracker is not sensitive to head movements (user manual; Tobii, Danderyd, Sweden). We analyzed the pupil data in Brain Vision Analyzer with custom-made macros. Artifacts and blinks were adjusted by linear interpolation. Extremely unreliable interpolated data points (i.e. less than 30% valid data points in the interval of interest) were excluded from analyses. Pupil dilation was defined in the averaged waveform as the peak pupil diameter during the period from 550 - 2500 ms following stimulus onset, relative to a 200-ms prestimulus baseline.

6.4 Results

6.4.1. Behavior

Table 3.1 displays the mean correct reaction times (RTs) for each task condition. Mean Stroop effects are plotted in Figure 6.2A. Because item and list proportion congruency were not varied in an orthogonal fashion, a traditional factorial analysis is difficult to interpret. Following the approach by Blais and Bunge (2010), we analyzed the ISPC effect independently of list-level proportion congruency by running two separate 2 (congruency) × 2 (item proportion congruency) analyses of variance (ANOVAs).

Table 6.1. Mean correct reaction times (standard deviation) for each task condition.

	30/10	30/50	70/50	70/90
Congruent	626 (56)	608 (60)	618 (59)	607 (51)
Incongruent	630 (63)	645 (71)	664 (77)	691 (109)

Incongruent trials were associated with longer RTs than congruent trials, both in the 30% block, $F(1, 23) = 11.8$, $p = .002$, and in the 70% block, $F(1, 23) = 32.8$, $p < .0005$. Importantly, the Stroop effect was larger in the 30/50 condition (37 ms) than in the 30/10 condition (4 ms), $F(1, 23) = 11.8$, $p = .002$. Similarly, the Stroop effect was larger in the 70/90 condition (84 ms) than in the 70/50 condition (46 ms), $F(1, 23) = 8.3$, $p = .009$. Thus, participants showed robust ISPC effects. In contrast, the difference in Stroop effects between the 30/50 (37 ms) and 70/50 (46 ms) conditions was not significant, $t_{23} = 1.2$, $p = .25$, indicating that RTs were not substantially influenced by list-level proportion congruency.

Because mean error rates were very low and did not differ much between congruent (2%) and incongruent trials (3%), we did not analyze them further.

6.4.2. Pupillometry

Grand-average pupil waveforms are plotted in Figure 6.3. If the ISPC effect described above is driven by conflict-induced arousal, then pupil dilations should demonstrate a similar sensitivity to task conditions as RT. Mean pupil Stroop effects are plotted in Figure 6.2B; as is clear from that graph, the pupil data do not match the RT data in Figure 6.2A. We analyzed the pupil data in the same manner as the behavioral data, by running two separate 2 (congruency) × 2 (item proportion congruency) ANOVAs. In the 30% block, there was no reliable difference in pupil dilation between congruent (.094 mm) and incongruent trials (.090 mm), $F(1, 23) = .43$, $p = .52$. In the 70% block, we found a trend in the expected direction: incongruent stimuli elicited larger dilations (.106 mm) than congruent stimuli (.084 mm), $F(1, 23) = 3.69$, $p = .07$.

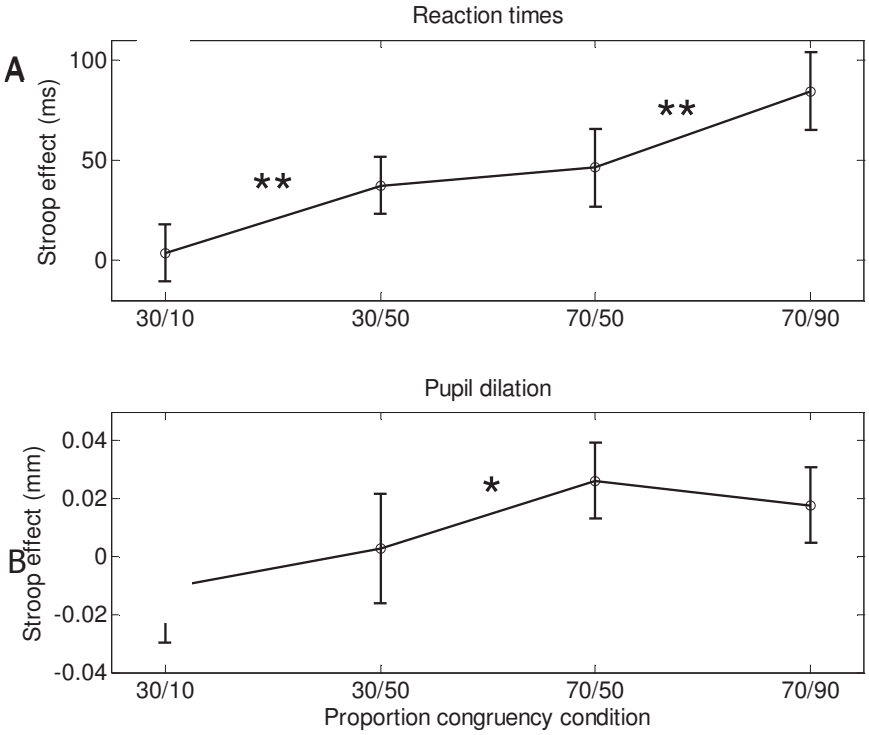


Figure 6.2. Congruency (Stroop) effect on RT (A) and pupil dilation (B) for each list-level/item-specific proportion congruency. Asterisks indicate significant differences: **, $p < .01$; *, $p < .05$. Error bars are based on within-subjects error (Masson & Loftus, 2003).

Importantly, there was no reliable difference in pupil Stroop effects between the 30/50 (-0.011 mm) and the 30/10 (0.002 mm) conditions, $F(1, 23) = 1.1$, $p = .52$; and also no reliable difference between the 70/50 (0.026 mm) and the 70/90 (0.018 mm) conditions, $F(1, 23) = 0.7$, $p = .69$. So, unlike the behavioral data, the pupil-dilation data did not show evidence of a robust ISPC effect. Furthermore, the difference in pupil Stroop effect between the 30/50 (0.003 mm) and 70/50 (0.026 mm) conditions was significant, $t_{23} = 2.3$, $p = .03$, indicating a list-level proportion congruency effect, again unlike in the behavioral data.

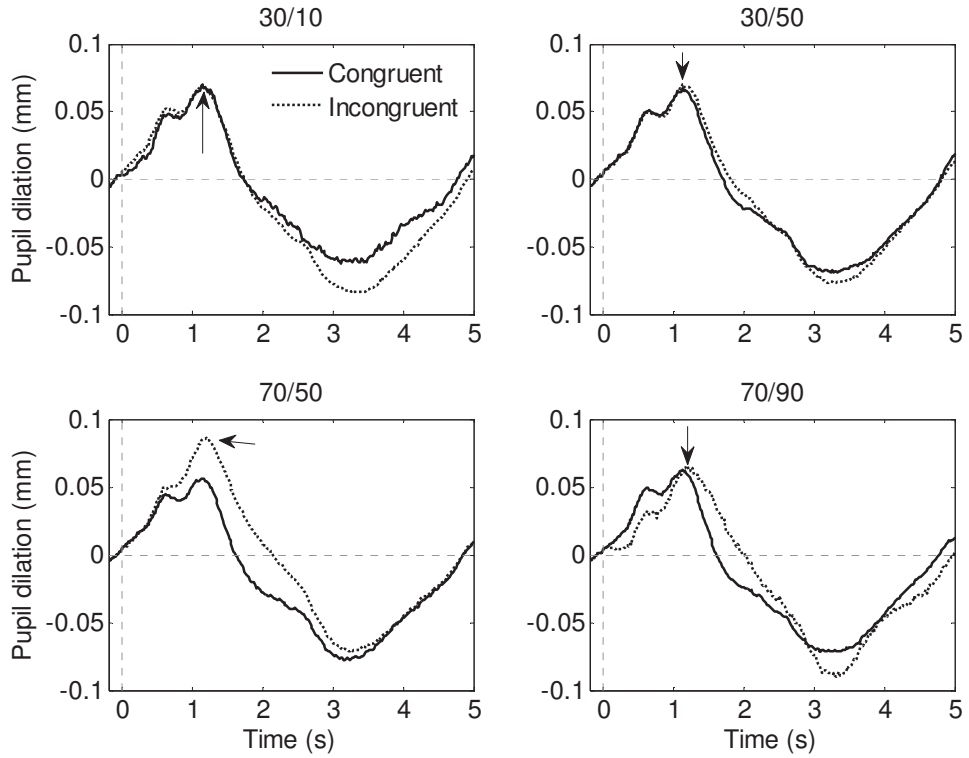


Figure 6.3 Grand-average pupil waveforms in each task condition. Time = 0 ms indicates the onset of the stimulus. The arrows indicate the dilatory peaks the analysis was based on.

To examine the robustness of these results, we carried out some additional analyses, in which we focused on the frequently observed coupling between baseline pupil diameter and pupil dilations. First, because pupil dilations (averaged across all conditions) were modulated by individual differences in baseline pupil diameter (dilations on incongruent trials: r = .42, p = .04; on congruent trials r = .25, p = .23), we analyzed the data separately for the 12 participants with the smallest and the 12 with the largest baseline pupil: in both groups, we found a pattern similar to the grand average in Figure 6.2B. Indeed, only 3 out of 24 participants showed a pattern of pupil Stroop effects that was monotonically increasing like the RT Stroop effects.

Second, we checked if the list-level effect in the pupil-dilation data was accompanied by and perhaps caused by a difference between blocks in baseline pupil diameter. Such a difference in baseline pupil might reflect the difference in task difficulty associated with different proportions of incongruent trials. However, a *t*-test showed no reliable difference in baseline diameters between the 30% (3.21 mm) and the 70% (3.22 mm) blocks, $t_{23} = .39$, $p = .70$. Together, these control analyses suggest that the pattern of pupil-dilation Stroop effects cannot be explained by differences in baseline pupil.

6.4.3. Behavior-pupil correlations

Although the pupil data showed no robust ISPC effect, there were substantial individual differences. To gain insight in these individual differences, we computed a number of correlations. First, we quantified the behavioral ISPC effect for every participant by averaging the item-related difference in Stroop effects in each block, that is ((Stroop3050 – Stroop3010) + (Stroop7090 – Stroop7050))/2. This ISPC effect reflects the sensitivity of each participant to the item-level proportion congruency, with large ISPC scores indicating high sensitivity. We also calculated for each participant the average pupil Stroop effect to index the effect of conflict on the arousal system, the process hypothesized by Verguts and Notebaert (2008) to underlie the ISPC effect. We then investigated whether participants with a larger pupil Stroop effect also showed a larger ISPC effect. The Pearson correlation was $r = .47$, $p = .020$. However, Figure 6.4A suggests that this significant correlation may have been driven by a few outliers. Spearman's rank correlation, which is less sensitive to (univariate) outliers than Pearson's coefficient, was marginally significant, $\rho = .38$, $p = .07$, suggesting some evidence that people whose pupil diameter is more sensitive to Stroop conflict tend to have a larger ISPC effect.

Next, we examined if differences in overall baseline pupil diameter were also predictive of a participant's behavioral ISPC effect. Indeed, we found a significant Pearson correlation, $r = .60$, $p = .002$, and Spearman rank correlation, $\rho = .41$, $p < .05$ (Figure 6.4B). The Pearson correlation remained significant after partialling out the contribution of the pupil-dilation Stroop effect: $r = .51$, $p = .01$, suggesting that baseline pupil diameter explains unique variance in the ISPC effect. Indeed, step-wise regression analysis indicated that a model with both pupil-dilation Stroop effect and baseline pupil diameter as predictors explained the individual differences in the size of the ISPC effect better than a model with only the pupil-dilation Stroop effect as predictor, $F_{change} = .013$.

Finally, because pupil dilation showed only a modest Stroop effect (in the 70% block), we further investigated the sensitivity of pupil diameter to response conflict by correlating the Stroop effects in the pupil-dilation and behavioral data. Pooled across conditions these measures showed a large positive correlation ($r = .62$, $p = .001$). Significant positive correlations (ps $< .05$) were also found within the 30/50, 70/50 and 70/90 conditions, but not in the 30/10 condition, presumably because the Stroop effects in that condition were virtually absent (Figure 6.2). Thus, altogether the data indicate that pupil dilation reliably scaled with response conflict.

6.5 Discussion

The results of Experiment 1 provide mixed evidence for the conflict-modulated Hebbian learning hypothesis. Partici-pants showed a strong ISPC effect: the observed list-level proportion congruency effect on Stroop interference was almost entirely due to differences in proportion congruency at the item-level, suggesting an important role for associative learning.

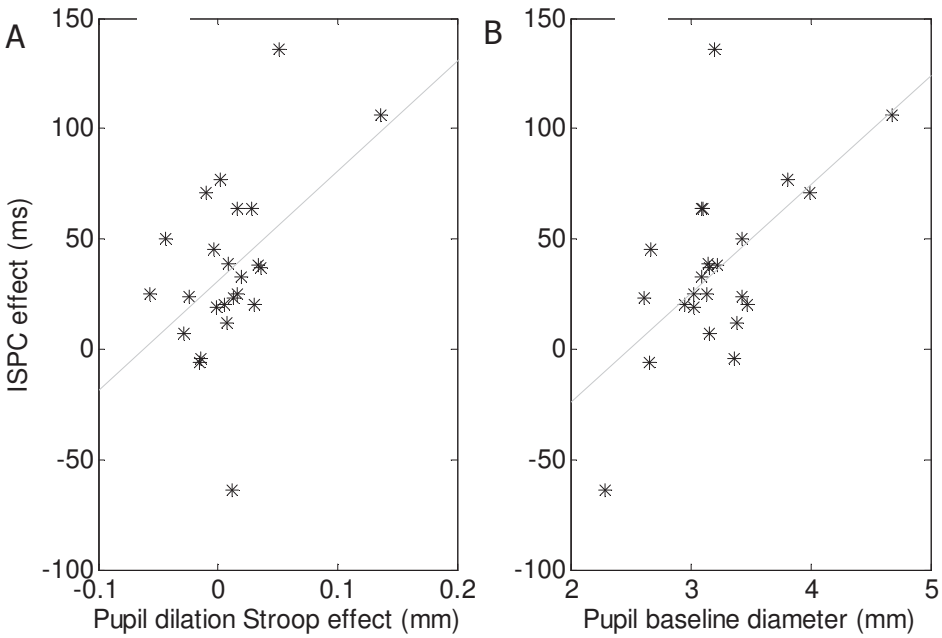


Figure 6.4. Correlation between the pupil-dilation Stroop effect and the behavioral ISPC effect (A). Correlation between pupil baseline diameter and the ISPC effect (B). Each star represents one subject.

However, the differences in Stroop effect between items were not mirrored by corresponding changes in pupil dilation; in contrast, pupil dilation showed a list-level effect and no ISPC effect. This is inconsistent with the hypothesis that associative learning effects in the Stroop task are modulated by conflict-induced arousal. Conversely, the behavior-pupil correlations did show some evidence for another key prediction of the conflict-modulated Hebbian learning hypothesis, namely that people whose pupil diameter is more sensitive to Stroop conflict (i.e. who exhibit more conflict-induced arousal) should have a larger ISPC effect. Finally, baseline pupil diameter, measured across the whole experiment, also predicted the behavioral ISPC effect, accounting for significant variance over and above that explained by conflict-induced pupil dilation.

These results are broadly consistent with a recent study in which pupil dilation and baseline pupil together predicted learning rate in a predictive-inference task (Nassar et al., 2012). In that study, the amplitudes of baseline pupil and pupil dilation correlated with distinct measures of uncertainty (as defined by a normative model) that together indicated the influence that new data should have on existing beliefs. Although most of the results concerned relationships across single trials within participants, Nassar and colleagues also found evidence that participants with a larger average pupil size attributed more weight to incoming data, i.e. exhibited larger learning rates. That result, albeit in a different context, mirrors our finding that participants with a larger baseline pupil showed enhanced learning of stimulus-response associations. Furthermore, Silvetti, Seurinck, van Bochove, and Verguts (2013) reported a correlation between pupil diameter and learning rate in a probabilistic learning task. The observed relationship between individual differences in pupil metrics and the ISPC effect also seems broadly consistent with the hypothesized role of the LC-NE system in associative learning in cognitive control contexts (Verguts & Notebaert, 2009). Although the evidence is still preliminary, neurophysiological (Aston-Jones & Cohen, 2005), neuroimaging (Murphy, Robertson, Balsters, & O'Connell, 2011;Murphy, O'Connor, O'Sullivan, Robertson, & Balsters, in press), anatomical (Nieuwenhuis et al., 2011), pharmacological (Phillips, Szabadi, & Bradshaw, 2000) and behavioral evidence (Gilzenrat, Nieuwenhuis, Jepma & Cohen, 2010; Jepma & Nieuwenhuis, 2011) suggests that pupil diameter is a correlate of LC-NE activity: baseline pupil diameter of tonic LC activity and task-evoked pupil dilations of phasic LC activity. On this assumption our results are consistent with empirical evidence and models that posit an important role for both tonic and phasic LC activity in learning (Bouret & Sara, 2005; Yu & Dayan, 2005; Nieuwenhuis, 2011).

6.6 Experiment 2

According to Verguts and Notebaert (2009), the conflict-modulated Hebbian-learning hypothesis predicts that arousal-inducing but task-irrelevant stimuli should lead to enhanced learning of the association between accompanying task-relevant stimuli and responses. Experiment 2 was designed as a first test of this important prediction, using a task-independent manipulation of phasic arousal. We used a conflict task in which specific incongruent stimuli were frequently accompanied by a task-irrelevant loud auditory tone. Such an accessory stimulus (AS) is known to decrease RTs to the task-relevant stimulus (e.g. Bernstein, 1970), and increase the weight of new data (Nassar et al., 2012), presumably through a phasic burst of arousal (Hackley & Valle-Inclán, 2003; Jepma, Wagenmakers, Band, & Nieuwenhuis, 2009; Sanders, 1983). In Experiment 2 we were primarily interested in the progression of RTs on incongruent trials without an AS: The conflict-modulated Hebbian-learning hypothesis predicts a steeper learning rate (i.e. a faster decrease in RT) for stimulus-response associations that were frequently paired with an AS, compared to associations that were never paired with an AS.

We expected any existing arousal effect on learning to be small in size. To be able to detect such a small effect we designed a task in which RT differences between incongruent stimulus-response associations were minimal. The task was a four-choice Simon task, in which participants were required to classify stimulus identity by pressing 1 of 4 spatially arranged buttons, while trying to suppress the urge to respond according to the task-irrelevant stimulus location. Previous research has reported a conflict-based arousal effect (van Steenbergen & Band, 2013) and a typical proportion congruency effect (Borgmann, Risko, Stolz, & Besner, 2007) in this type of task, suggesting that performance in this task is sensitive to the same type of learning as performance in the Stroop task.

6.7 Methods

6.7.1. Participants

Twenty participants (5 males), aged 19-28, took part in a single 30-min experimental session in return for course credit or €3.50. Participants signed informed consent prior to inclusion in the study.

6.7.2. Stimuli and Task

Participants performed a Simon task, implemented in E-Prime (Psychology Software Tools, Sharpsburg, PA). Each trial started with a fixation stimulus that was presented for 500 ms. The fixation stimulus was followed by an imperative stimulus, presented for 1,000 ms, and a blank screen, presented for 750 ms. The imperative stimulus, one of 4 Glagolitic characters, could appear in 4 positions on the screen, indicated by black frames (see Figure 6.5). The participant's task was to classify the stimulus identity by pressing one of 4 keys on a QWERTY keyboard (a, z, k, m). To make stimulus location, the task-irrelevant stimulus dimension, more salient, the 4 keys had a similar spatial configuration as the 4 screen locations where stimuli could appear.

To learn the stimulus-response mappings, participants first performed 80 practice trials in which stimuli were presented in the center of the screen, and feedback on response accuracy was presented after every trial. Following this block, participants performed 16 additional practice trials in which stimuli appeared in one of the 4 positions on the screen, as in the experimental block. Participants' accuracy was presented at the end of this block. After ensuring the participant fully comprehended the task, the experimental block was commenced. In total, 720 experimental trials were presented in four blocks of 180 trials: 360 trials were congruent (i.e. stimulus and response locations matched) and 360 trials were incongruent (nonmatching stimulus and response locations). Every stimulus

was presented 180 times: in incongruent trials, it appeared with equiprobability in one of the three incongruent locations.

Two of the 4 stimuli were frequently accompanied by a loud AS tone (800 Hz, 77 dB(A), 150 ms) that started 30 ms prior to the onset of the imperative stimulus. The AS accompanied these stimuli on 50% of the incongruent trials, never on congruent trials. The other two stimuli were never accompanied by an AS. The stimuli that could be accompanied by an AS were counterbalanced across participants, so that either the stimuli that were congruent in the lower left and upper right, or those that were congruent in the lower right and upper left locations could be associated with an AS (cf. Figure 6.5A). Participants were told that the tones were unrelated to the task and that they should try to ignore the sounds.

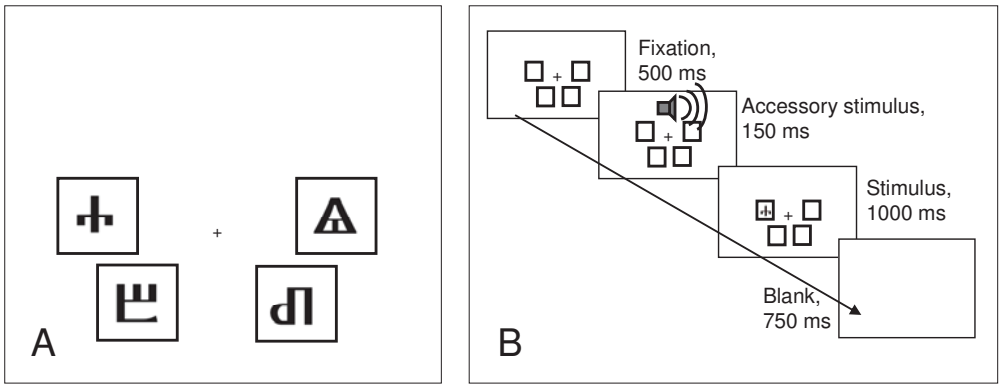


Figure 6.5A. An overview of the four Glagolitic stimuli and their congruent locations on the screen. B. Order of task events in Experiment 2.

6.8 Results

As expected, congruent trials were associated with shorter RTs (562 ms) than incongruent no-AS trials (588 ms), $F(1, 19) = 441$, $p < .0005$, as well as lower error rates (3.9%) than incongruent no-AS trials (11.2%), $F(1, 19) = 81$, $p < .0005$.

6.8.1. Manipulation check

To test whether our manipulation of arousal was successful, we computed the AS effect: the difference in correct RT between AS trials and no-AS trials. RTs on incongruent AS trials (620 ms) were significantly shorter than RTs on incongruent no-AS trials (635 ms), $F(1, 19) = 7.43$, $p = .01$, yielding a typical AS effect of 15 ms. Incongruent AS trials were associated with a marginally higher error rate (13.8%) than incongruent no-AS trials (12.2%), but this difference was not reliable $F(1, 19) = 1.20$, $p = .29$.

6.8.2. AS effect on learning rate

To examine the effect of arousal on learning rate, we compared the progression of RTs on incongruent no-AS trials that were frequently paired with an AS with the progression of RTs on incongruent no-AS trials that were never paired with an AS. We refer to these categories of trials as AS^+ and AS^-. For each participant, the 90 AS^+ trials and 180 AS^- trials were equally divided in 5 chronological bins. Before averaging the RTs in each bin, RT outliers and incorrect trials were replaced by an RT that was interpolated by computing the average RT of trials n -1 and n + 1 of the corresponding trial type (AS^+ or AS^-). The resulting time series were averaged across participants

As shown in Figure 6.6, incongruent RTs monotonically decreased with time on task (bin), reflecting (at least in part) the gradual strengthening of learned stimulus-response associations in the face of conflict.

Importantly, there was no systematic difference in learning rate between AS^+ and AS^- trials. To test this, we quantified the learning rate for each trial type as the average RT of bin 1 minus the average RT of bin 5. Indeed, learning rate on AS^+ trials (37 ms) did not differ significantly from the learning rate on AS^- trials

(35 ms), $t_{19} = .19$, $p = .86$[6]. A similar analysis with learning rate quantified as the difference between bin 1 and bin 3 also yielded a nonsignificant difference between AS^+ and AS^- trials, $p = .32$.

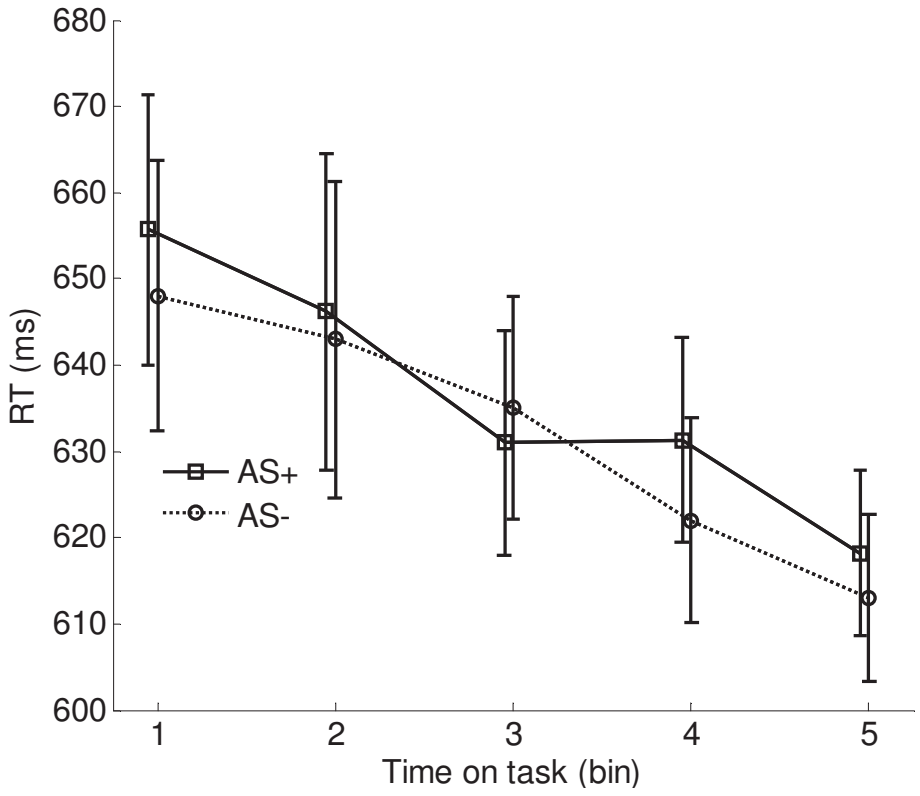


Figure 6.6. Binned RTs on no-AS trials for stimuli that were frequently accompanied by an accessory stimulus (AS^+) vs. stimuli that were never accompanied by an AS (AS^-). Error bars are based on within-subjects error terms associated with each of the five pairwise comparisons (Masson & Loftus, 2003)

[6] A Bayesian t-test comparing these learning rates yielded a JZS Bayes factor (Rouder, Speckmnan, Sun, Morey, & Iverson, 2009) of 5.76, indicating that the data are 5.76 times more likely under the null hypothesis (i.e. no difference) than under the hypothesis suggested by the model of Verguts and Notebaert (2008, 2009).

Furthermore, comparisons, for each bin, between AS^+ and AS^- trials yielded no significant differences in RT, all *p*s > .26. These findings suggest that there was no robust effect of arousal on learning of task-relevant stimulus-response associations.

6.9 Discussion

In Experiment 2 we found no evidence that phasic arousal enhances learning of incongruent stimulus-response associations. Although the manipulation of phasic arousal was successful, as indicated by a robust AS effect on RT, the monotonic decrease in RTs on no-AS trials across the experiment was virtually identical for AS^+ and AS^- trials. This is inconsistent with the conflict-modulated Hebbian-learning hypothesis (Verguts & Notebaert, 2008, 2009), which suggests that arousal should precipitate the gradual strengthening of stimulus-response associations. Jacoby and colleagues (2003) reported evidence that the ISPC effect can emerge rapidly, suggesting that in our experiment associative learning might already have occurred within the course of our first bin. However, we also found no evidence for a learning effect in the first couple of bins: if anything, RTs on AS^+ trials were slower than RTs on AS^- trials.

A limitation of Experiment 2 is that we did not collect a physiological measure of tonic arousal level, such as baseline pupil size. An interesting question for future research is whether the predicted learning effect might be present for a subgroup of participants with lower baseline arousal. This question is inspired by the study of Nassar et al. (2012), in which the direction of AS-induced learning effects was dependent on *trial-specific* changes in baseline pupil size.

6.10 General Discussion

An increasing number of empirical phenomena that were previously interpreted as a result of cognitive control, turn out to reflect (in part) simple

memory and learning mechanisms (Jacoby et al., 2003; Mayr et al., 2003; Schneider & Logan, 2005). A prime example is the proportion congruency effect, the finding that interference effects, such as the Stroop effect, decrease as the proportion of incongruent stimuli increases. While this was previously regarded as strong evidence for a global conflict monitoring-cognitive control loop (Botvinick et al., 2001), recent evidence has shown that the proportion congruency effect is largely item-specific and must be due to cumulative associative learning. The goal of our research was to test a recent hypothesis about the mechanism underlying such associative-learning effects: the conflict-modulated Hebbian-learning hypothesis (Verguts & Notebaert, 2008, 2009), a computationally and neurobiologically grounded account which proposes that the effect of proportion congruency on associative learning is mediated by conflict-triggered phasic arousal responses. Our study provided the first direct empirical tests of the conflict-modulated Hebbian-learning hypothesis. In general, the results present some positive but mainly negative evidence for this account. We conclude that although Verguts and Notebaert's hypothesis presents an elegant integrative account of conflict-related associative learning effects, it requires additional empirical support to remain tenable.

In Experiment 1, we found that participants who exhibited more conflict-induced arousal, as indexed by task-evoked pupillary responses, had a larger ISPC effect. This finding provides compelling support for the conflict-modulated Hebbian-learning hypothesis. However, a different analysis showed that the behavioral Stroop effect is sensitive to item-level proportion congruency, while pupil dilation showed a list-level effect and no ISPC effect. This is hard to reconcile with the hypothesis that associative learning effects reflected in item-specific Stroop effects were driven by conflict-induced arousal. Furthermore, baseline pupil diameter was an even stronger predictor of the behavioral ISPC effect than conflict-induced pupil dilation, suggesting that the ISPC effect reflects tonic more than phasic arousal. In Experiment 2, we found that a task-irrelevant

phasic arousal manipulation did not affect item-specific learning of stimulus-response associations, even though the manipulation was clearly successful in modulating response speed. This finding refutes an important prediction of the conflict-modulated Hebbian-learning hypothesis.

The fact that we do not find unequivocal evidence for the conflict-modulated Hebbian learning hypothesis in two experiments suggests that certain assumptions of the model may have to be revised. One possibility implied by our findings is that the relationship between conflict and associative learning is not mediated by LC-induced arousal. However, this assumption (Verguts & Notebaert, 2009) is supported by various lines of evidence: the anatomical connection between the ACC and the LC is well-established (Aston-Jones & Cohen, 2005), and the noradrenergic system is known to be important for learning (Eldar, Cohen, & Niv, 2013; Nieuwenhuis, 2011; Yu & Dayan, 2005). Furthermore, it is known that conflict leads to arousal (Berlyne, Borsa, Hamacher, & Koenig, 1966; Laeng et al., 2011; van Steenbergen & Band, 2013) and that arousal is important for learning (Berlyne, 1957; Nassar et al., 2012). Nonetheless, there are indications that the relationship between conflict and learning rate may be (in part) mediated by other neuromodulator systems, such as the cholinergic system (Doya, 2002) and the dopaminergic system (Van Bochove et al., 2013).

Alternatively, the model of Verguts and Notebaert (2008) could be misspecified at a more fundamental level. For example, the ISPC effect may not be related to conflict. However, that seems unlikely in the face of data (e.g., Crump, Vaquero, & Milliken, 2008; for reviews, see Bugg & Crump, 2012 and Schmidt, 2013) that conflict does seem to be crucial for the ISPC effect. Furthermore, if conflict would not be relevant, then arguably the effect of item frequency on RT should be similar on congruent and incongruent trials, which is often not the case (see, e.g., Table 1; Blais & Bunge, 2010; Crump et al., 2008). Accordingly, if conflict is kept constant in the Hebbian-learning model of Verguts and Notebaert

(2008), the model does not show an ISPC effect. These arguments suggest that conflict detection is essential for the ISPC effect.

It is also possible that conflict-modulated associative learning occurs not just between stimulus and task-demand representations, as in the model of Verguts and Notebaert (2008), but also between stimulus and response representations. Incorporating that assumption in the conflict-modulated Hebbian-learning account would unify the contingency account of the ISPC effect (Schmidt & Besner, 2008), which emphasizes learning of stimulus-response associations, and the item-specific control account (Blais & Bunge, 2010; Bugg et al., 2008), which assumes a major role for learning of stimulus-attention associations in causing the ISPC effect. A recent review reports evidence supporting both of these types of learning (Bugg & Crump, 2012). Indeed, in more recent work Verguts and Notebaert have proposed that conflicts also modulates stimulus-response associations (Braem, Verguts, & Notebaert, 2011; cf. Hommel, Proctor & Vu, 2004). However, it is unlikely that this additional assumption can account for the current results.

To conclude, although important progress has been made in understanding the constituent components of proportion congruency effects (Bugg & Crump, 2012), much work remains to be done to elucidate the neurocognitive mechanisms underlying these effects. An important advantage of the model by Verguts and Notebaert (2008) is that it is computationally explicit, unlike some other models of the ISPC effect (but see Blais et al., 2007). This should allow validation of the current predictions, as well as facilitate the generation of new predictions, to be tested in future empirical research.

7. General discussion and future directions

7.1 Introduction

The chapters of the current dissertation are of a relatively heterogeneous nature. Therefore, it seems difficult to draw very detailed conclusions about specific details of the theories that form the foundation of this dissertation (i.e. the adaptive gain and uncertainty-processing theories). Instead, I have attempted to provide a number of general conclusions about the two themes that are central to this dissertation: the role of the locus coeruleus-noradrenergic (LC-NE) system in temporal attention and in learning. To aid the legibility of this closing chapter, and given its reflective and interpretative nature, no literature references are presented: the reader is kindly referred to the General Introduction of the dissertation for relevant literature suggestions.

7.2 The role of the LC-NE system in temporal attention

Although this dissertation contains some unexpected and null findings, the first three empirical chapters clearly suggest a role for the LC-NE system in temporal attention. Regarding the late positive potential (LPP), our findings suggest that this ERP may reflect the inhibition of irrelevant stimulus representations in the visual cortex, allowing for more selective processing of the emotional stimulus that evoked it. Given the proposed relationship between the LPP and the noradrenergic system, we have tentatively demonstrated the LC-NE system to be involved in temporal fluctuations in attention.

Although we found no reliable effect of the noradrenergic drug clonidine on the attentional blink, we found clear effects of this drug on T1 identification accuracy and related ERP components. While this finding may have implications for the theory that related the attentional blink to phasic noradrenergic activity, these findings do suggest a general role for the LC-NE system in temporal

attention, as reflected in behavioral (reduction of noradrenergic baseline activity reduces target identification accuracy) and electrophysiological results (reduction of noradrenergic baseline activity reduces the amplitude of two information-processing related ERPs).

The final chapter devoted to temporal attention suggests that phasic noradrenergic bursts do not mediate the accessory stimulus effect, contrary to our expectations. However, administration of clonidine did *increase* the size of the accessory stimulus effect. We provide an explanation in terms of compensatory mechanisms (drug-related decreases in cognitive performance can be compensated for by arousing stimuli). Again, even though we did not provide evidence for the theory that phasic noradrenergic bursts subserve the accessory stimulus effect, we did show that reducing noradrenergic baseline activity influenced temporal attention.

In sum, although we have found some counterintuitive effects that do not necessarily provide evidence for popular theories that propose a role for noradrenaline in temporal attention, our work does show that the LC-NE system influences temporal attention.

7.3 The role of the LC-NE system in learning

The two dissertation chapters that are devoted to the role of the LC-NE system in learning present a more complicated conclusion than the results on temporal attention. Regarding our P3 study, we did not confirm the hypothesis that the P3b to rare target stimuli is subserved by the noradrenergic system while the P3a to novel stimuli is subserved by the cholinergic system. Clonidine attenuated baseline noradrenergic activity, but it actually increased the amplitude of the target-related P3b (while it decreased the amplitude of the P3a). Scopolamine decreased the amplitude of the novel-related P3a, but, like clonidine, it increased the

amplitude of the target-related P3b. These effects are difficult to interpret: although scopolamine antagonizes muscarinic receptors, it does so both pre- and postsynaptically, which can actually lead to increased extracellular levels of acetylcholine. Given our relatively high dose of scopolamine, presynaptic stimulation is to be expected, but contrary to the hypothesis that we tested, the purportedly increased levels of acetylcholine were not accompanied by an increased P3a amplitude.

The question that arises, is what these results imply functionally. Our findings seem in line with a theory on uncertainty processing that relates noradrenergic activity to the signaling of unexpected uncertainty and cholinergic activity to the signaling of expected uncertainty (cf. Chapter 1 of this dissertation). Indeed, administration of a drug that reduces noradrenergic baseline activity reduces the P3 amplitude to (unexpected) novel-related targets, while a drug that increases extracellular levels acetylcholine levels increases the P3 amplitude to (expected) target stimuli. Another theory may functionally account for these observations: P3 amplitude is related to context updating, so our findings may imply that a drug that reduces noradrenergic activity leads to less updating of an agent's current context (reflected by a smaller P3 amplitude), while a drug that increases availability of acetylcholine leads to increased context updating (reflected by a larger P3 amplitude). The behavioral effects of modulated context updating are unclear: in this and other work we have shown that ingestion of clonidine and scopolamine is associated with increased reaction times, and decreased accuracy and perceptual sensitivity.

The final chapter of the dissertation reveals a complicated image of the role of the LC-NE system in learning as well. We find mainly counter-evidence for the conflict-modulated Hebbian learning hypothesis that suggests that registration of conflict is associated with arousal and leads to LC activation, phasic noradrenergic release, and concomitant increased Hebbian learning. Although behavioral results

from a Stroop task are in accordance with this hypothesis and therefore suggest a role of the LC-NE system in this type of learning, a purported physiological marker of noradrenergic activity (i.e. pupil dilation) suggests no such relationship. Furthermore, we found no correlation between arousal and learning rate, which again provides no support for this hypothesis.

In sum, we find mixed evidence for a role of the LC-NE system in learning. It is possible that the LC is involved in other types of learning that we have not studied here. Our only manipulation of unexpected uncertainty was the inclusion of novel stimuli in one oddball block – but participants did not have to respond to these stimuli, which does not allow us to study whether presentation of these stimuli led to altered behavioral performance.

7.4 On the possible interactions between the noradrenergic and cholinergic neuromodulator systems

One remarkable finding in this dissertation is that clonidine and scopolamine had very similar effects on two measures of temporal attention (the attentional blink and accessory stimulus effect) and one electrophysiological reflection of learning (the P3). These results were unexpected but highly consistent, supporting their reliability. We offer a number of explanations for these results in the relevant dissertation chapters: one important option is that the noradrenergic and cholinergic neuromodulators interact during the execution of specific cognitive processes. For example, scopolamine has been shown to antagonize muscarinic receptors in the rat LC, while clonidine has been demonstrated to inhibit acetylcholine release in the forebrain.

In sum, three studies presented in this dissertation present a powerful demonstration of the importance of studying the *interactions between neuromodulator systems* as opposed to isolated neuromodulator systems.

Studying isolated neuromodu-lator systems is associated with the risk of creating chemical homunculi.

7.5 Future directions

The human brain is an extremely complicated organ, with about half as many cells as there are stars in the Milky Way galaxy (give or take a few billion neurons – estimates vary quite a bit). This observation complicates studying the brain considerably. Even with modern technology—a host of neuroimaging methods with increasingly fine-grained spatial and temporal resolution—studying the brain remains complex, all the more so given the impressive amount of interactions between various brain areas and neuromodulator systems. As a result, this dissertation has not proven, once and for all, the functional significance of the LC-NE system. Then again, attempting to provide such evidence in the span of five years would be exceedingly ambitious.

Instead, the work in this dissertation has provided a few more pieces of the puzzle: we have provided evidence that the LC-NE system seems to be involved in temporal attention, albeit not necessarily in the way popular theories predict, and we have studied the involvement of the LC-NE system in learning; although those findings are relatively complicated, they do suggest that the LC-NE system is involved in specific forms of learning.

Much more work is needed to fully understand the functions of the LC-NE system. A few suggestions are listed below, following the chronological order of the dissertation chapters.

1. Our work on the LPP provides tentative evidence for what we termed the *global inhibition hypothesis*. It would be interesting to further test predictions made by this hypothesis. Because other work from our lab suggests that attenuation of noradrenergic baseline activity reduces LPP

amplitude, it would be interesting to test whether pharmacological attenuation of baseline noradrenergic activity would be associated with reduced global inhibition in the visual cortex, as reflected by a reduced-amplitude LPPs to arousing stimuli and attenuated P1/N1 components.

2. Based on our work, the role of the LC-NE system in the attentional blink remains elusive. It is crucial to shed more light on this problem, especially given the intuitive appeal of the phasic noradrenaline-attentional blink theory. As we describe in Chapter 3 of this dissertation, clonidine attenuates noradrenergic baseline activity, but it may leave phasic noradrenergic bursts intact. If a pharmacological agent can be found that reduces phasic noradrenergic responses in addition to tonic LC responses, it would be interesting to administer this drug and then have participants perform an attentional blink task. If the theory on the role of the LC-NE system in the attentional blink is correct, the blink should be strongly modulated after ingestion of this drug.
3. We found that clonidine increases the size of the accessory stimulus effect. It would be interesting to see if other pharmacological agents that attenuate noradrenergic baseline activity (e.g. propranolol) evoke similar effects, and whether agents that increase noradrenergic baseline activity (e.g. yohimbine, atomoxetine) reduce the size of the accessory stimulus effect. Such findings would corroborate our finding that phasic noradrenergic responses do not mediate the accessory stimulus effect in the manner that is often proposed.
4. We did not provide evidence that suggests that the posterior target-related P3b is subserved by the noradrenergic system and that the anterior novel-related P3a is subserved by the cholinergic system. Given the body of evidence that suggests that this is the case, our pharmacological agents may not have been chosen optimally. Another possibility is that in other empirical work, the distinction between P3a and P3b was not made by a

quantitative method as was implemented in our work (i.e. principal components analysis), but by other methods (e.g. “eyeballing” the data). The latter observation might have far-fetched implications for our conceptualization of the subcomponents of the P3. It would be interesting to replicate our findings with other pharmacological manipulations, to gain more insight in the robustness of our findings and the role of the noradrenergic and cholinergic neuromodulator systems in the generation of the different subcomponents of the P3.

5. Our results regarding the conflict-modulated Hebbian learning hypothesis appear relatively clear-cut: we find little empirical evidence for this hypothesis in two experiments. Therefore, the model that generated this hypothesis requires more empirical evidence to remain tenable, or be revised. In this context, the tenet discussed in section 8.4 might be particularly relevant: perhaps the model is correctly specified, but the noradrenergic system might not be the only neuromodulator system involved in conflict processing and learning rate; perhaps other neuromodulators like dopamine and/or acetylcholine are involved in these processes. It would therefore be interesting to conduct a study in which activity of these systems is attenuated systematically (through pharmacological manipulations or perhaps, in the case of dopamine, transcranial magnetic stimulation or transcranial direct current stimulation) and in which specific predictions generated by the conflict-modulated Hebbian learning model are tested.

7.6 Concluding remarks

“If you wish to make an apple pie from scratch, you must first invent the universe.” These words of cosmologist Carl Sagan apply to cognitive neuroscience as well as to patisserie. We are trying to understand the LC-NE system, but given

the many projection areas of the LC, the various noradrenergic receptor types, the LC's different modes of firing, the many functions of the neuromodulator noradrenaline, not to mention its interactions with other neuromodulators, there are many challenges to be overcome. This leads to a paradoxical situation: to study the functions of the LC-NE system, we must completely comprehend (rather than invent, as Sagan proposes) all of these constituents of the LC-NE system, but to adequately comprehend the functions of the LC-NE system, we must study them. We clearly cannot take all variables (receptor types, interactions with other brain areas and neuromodulator systems) into account in isolated experiments, and much work remains to be done in this field. However, we have provided some pieces of the grand LC-NE puzzle with the work that is presented in this dissertation.

8. Nederlandse samenvatting

8.1 De locus coeruleus en noradrenaline

Dit proefschrift beschrijft een aantal uiteenlopende studies. Al deze studies worden echter met elkaar verbonden door een klein hersengebied in de hersenstam: de locus coeruleus (letterlijk: donkerblauwe plek, afgekort tot LC). Deze kleine kernen (één aan elke zijde van het vierde ventrikel, een met vloeistof gevulde holte in het brein) bevatten zenuwcellen (neuronen) die de signaalstof noradrenaline afscheiden en verbonden zijn met een groot aantal hersengebieden. Aangezien de loci coerulei verbonden zijn met zoveel andere hersengebieden en noradrenaline (NE) een signaalstof is die effect kan hebben op veel verschillende groepen neuronen (een zogenaamde neuromodulator), wordt het locus-coeruleus-noradrenerge (LC-NE) systeem beschouwd als een cruciale neurologische factor in een groot aantal cognitieve processen.

Dieronderzoek suggereert dat de LC-neuronen op twee manieren actief kunnen zijn: tonisch en fasisch. Tonische activiteit houdt in dat LC-cellen vuren (dus signalen geleiden) met een verhoogde intensiteit ten opzichte van hun normale vuurpatroon. Deze staat van vuren wordt geassocieerd met een verhoogde mate van afleidbaarheid en het maken van meer fouten op cognitieve taken. Fasische activiteit betekent dat de LC-neuronen kortdurend vuren met een zeer hoge intensiteit. Fasisch vuren wordt geassocieerd met een hoge mate van concentratie en accurate prestaties op cognitieve taken. De wijze waarop de LC vuurt, beïnvloedt onze cognitie. In het kader van deze dissertatie zijn twee theorieën over het functioneren van de LC bijzonder relevant.

8.2 De invloed van NE op cognitie

Verschillende theorieën zijn geponeerd om de rol die de LC en NE in cognitie spelen te beschrijven. Voorheen werd de LC vooral geassocieerd met

slaapritmiek en waakzaamheid, maar tegenwoordig wordt een aanzienlijk grotere cognitieve rol toegedicht aan dit hersengebied. In dit samenvattende hoofdstuk zullen twee theorieën worden besproken die de rol van de LC in cognitie in kaart proberen te brengen.

De zogenaamde *adaptive-gain*-theorie is gebaseerd op het eerder besproken onderscheid tussen tonische en fasische activiteit van de LC. Het is relevant om op te merken dat wanneer de LC tonisch vuurt, fasische uitbarstingen sterk zijn gereduceerd, maar het omgekeerde is ook waar: tijdens fasisch vuren treedt juist minder tonisch vuren op. Tijdens optimale cognitieve prestaties vuurt de LC fasisch, terwijl tonisch vuren juist wordt gekenmerkt door cognitieve afleidbaarheid en suboptimale prestaties. Aston-Jones en Cohen (2005) formuleerden op basis van deze observaties de *adaptive-gain*-theorie, die suggereert dat tonisch vuren van de LC is geassocieerd met een staat van exploratie (het actief verkennen van de omgeving op zoek naar beloning), terwijl fasisch vuren is geassocieerd met exploitatie (hoge concentratie en daardoor goede taakprestaties om zo beloningen binnen te halen).

Maar hoe beïnvloeden deze twee manieren van neuronaal vuren onze cognitie nou precies? De *adaptive-gain*-theorie stelt dat noradrenaline de activatiefunctie van neuronen kan moduleren. Dit houdt in dat wanneer noradrenaline fasisch wordt afgescheiden, een neuron gevoeliger wordt en minder input nodig heeft om geactiveerd te worden (dit wordt een toename in *gain* genoemd). Hoewel dit weinig gevolgen heeft op het niveau van individuele neuronen, wordt het voor de netwerken waar die neuronen zich in bevinden eenvoudiger om signalen in de omgeving te detecteren. Dit effect is het grootst wanneer de tonische vuurniveaus laag zijn en de LC stelselmatig vuurt in reactie op belangrijke stimuli: de activatiefunctie van neuronen wordt dan op een gunstig moment aangepast, zodat we cognitieve taken efficiënt en relatief foutloos kunnen uitvoeren. Wanneer echter sprake is van verhoogd tonisch vuren, worden de

activatiefuncties van neuronen in het gehele brein aangepast, maar de timing daarvan loopt niet synchroon aan de presentatie van relevante taakstimuli.

Een andere theorie over de rol van noradrenaline in cognitie werd geponeerd door Yu en Dayan (2005), die suggereren dat personen continu proberen om causale verbanden te ontdekken, om zo te leren wat zinvolle, belonende handelingen zijn. Het is echter niet alitjd makkelijk om die verbanden te leren, omdat onze omgeving wordt gekenmerkt door veel onzekerheden; bij het voorspellen van het weer, kan het bijvoorbeeld 's ochtends zonnig ogen, maar 's middags toch ineens gaan regenen. Dat soort onzekerheden zorgt voor ruis op de lijn tijdens het leren. We proberen bronnen van onzekerheid—dus ruis—daarom zoveel mogelijk te vermijden. Yu en Dayan onderscheiden daarbij twee verwachte en onverwachte onzekerheid. Ze geven daarvan een goed voorbeeld: stel dat iemand dagelijks naar het weerbericht op de radio luistert. Deze persoon kan ervan uitgaan dat er altijd kleine afwijkingen zullen zijn tussen de weersvoorspelling en het daadwerkelijke weer: verwachte onzekerheid. Soms kunnen er echter grote verschillen ontstaan tussen de weersvoorspelling en het daadwerkelijke weer, bijvoorbeeld door een fenomeen als *el niño*: onverwachte onzekerheid. De beide vormen van onzekerheid kunnen ertoe leiden dat iemand op zoek gaat naar een betere, meer betrouwbare informatiebron. Wanneer de bron van fouten is veroorzaakt door verwachte onzekerheid, kan het verstandig zijn om bij de huidige bron van informatie te blijven; worden de fouten echter veroorzaakt door onverwachte onzekerheid, dan zou het beter kunnen zijn om op zoek te gaan naar een andere informatiebron. Yu en Dayan bespreken verschillende onderzoeken, die suggereren dat noradrenaline een belangrijke rol speelt bij het verwerken van onverwachte onzekerheid, terwijl acetylcholine (een andere neuromodulator) een vergelijkbare rol speelt bij de verwerking van verwachte onzekerheid.

De *adaptive-gain*-theorie en de veronderstelde rol van noradrenaline in leren vormen de basis van deze dissertatie. Het proefschrift kan daartoe in twee

delen worden opgesplitst: in het eerste deel worden drie experimenten gepresenteerd waarin de rol van de LC en noradrenaline in temporele aandacht werd bestudeerd; in het tweede deel worden twee studies beschreven die de rol van de LC en noradrenaline in leren bestudeerden.

8.3 De rol van noradrenaline in temporele aandacht

De term 'temporele aandacht' verwijst naar snelle veranderingen in aandacht. Zoals hierboven besproken, leiden fasische LC-responsen tot aanpassingen in de activatiefunctie van netwerken van neuronen. Deze veranderingen in activatiefuncties zorgen ervoor dat de aandacht wordt gericht op taakrelevante stimuli, maar juist niet op irrelevante stimuli. Er is dan ook gesuggereerd dat de fasische LC-respons zorgt voor betere verwerking van taakrelevante stimuli, maar tonische LC-responsen doen dat juist niet. Er zal nu een samenvatting worden gegeven van de dissertatiehoofdstukken die zijn gewijd aan de rol van het LC-NE-systeem in temporele aandacht.

8.3.1. Hoofdstuk 2: Functionele significantie van de late positive potential (LPP)

De late positive potential (LPP) is een onderdeel van het EEG-signaal dat sterk wordt gemoduleerd door de emotionele intensiteit van een stimulus: emotionele stimuli wekken een meer positieve LPP op dan neutrale stimuli. De LPP wordt op allerlei manieren gebruikt, bijvoorbeeld om te kijken hoe effectief therapie was, of zelfs als leugendetectiemaat. In dit hoofdstuk hebben wij geprobeerd een theorie te vormen over de functionele significantie van de LPP; in andere woorden: welke cognitieve processen liggen ten grondslag aan de LPP?

We relateren de LPP in dit hoofdstuk niet expliciet aan het LC-NE-systeem, maar ander onderzoek uit ons lab suggereert wel een relatie tussen de LPP en het LC-NE-systeem: propranolol (een β-blokker die de activiteit van het noradrenerge systeem vermindert) maakt de LPP kleiner. In dit hoofdstuk

presenteren wij data uit twee experimenten die suggereren dat de LPP gerelateerd kan worden aan temporele aandacht. Onze resultaten leveren voorlopig bewijs dat suggereert dat de LPP wellicht een globale inhibitie reflecteert van representaties die mogelijk interfereren met de verwerking van de emotionele stimulus die de LPP opwekte—een soort beschermende functie dus.

8.3.2. Hoofdstuk 3: De effecten van clonidine en scopolamine op temporele aandacht zoals gemeten met de attentional blink-taak

Temporele aandacht wordt vaak gemeten met de zogeheten attentional blink-taak, waarin proefpersonen worden geconfronteerd met een snelle stroom van afleidende stimuli (bijvoorbeeld letters), waarin twee targetstimuli (bijvoorbeeld cijfers) zijn ingebed. De proefpersoon wordt gevraagd deze twee targets (respectievelijk T1 en T2 genoemd) te rapporteren. Wat deze taak zo interessant maakt, is dat wanneer T2 binnen ongeveer 200-400 milliseconden volgt op T1, proefpersonen vaak niet in staat zijn T2 accuraat te rapporteren. Dit fenomeen wordt de attentional blink genoemd.

Er zijn verschillende theorieën geformuleerd om het attentional blink-fenomeen te verklaren. In het kader van deze dissertatie, is de theorie van Nieuwenhuis, Gilzenrat, Holmes, en Cohen (2005b) bijzonder relevant. Deze theorie suggereert dat de LC fasisch vuurt wanneer T1 wordt gezien, wat ervoor zorgt dat T1 makkelijker verwerkt wordt. Na het fasisch vuren, komt de LC echter in een periode waarin hij verminderd vuurt. Als T2 precies in dit interval wordt gepresenteerd, dan vindt voor deze target niet de verwerkingsverbetering plaats die plaatsvond voor T1, met als gevolg dat de proefpersoon T2 “mist” en er een attentional blink optreedt.

In dit hoofdstuk presenteren we data van een psychofarmacologische studie, waarin we gezonde proefpersonen de middelen clonidine en scopolamine

toedienden en ze vervolgens een attentional blink-taak lieten doen. Clonidine is een bloeddrukverlager die de activiteit van het noradrenerge systeem vermindert; scopolamine is een middel tegen misselijkheid dat de activiteit van het cholinerge systeem vermindert. Onze resultaten suggereren dat clonidine geen effect heeft op de attentional blink, maar dat dit middel wel een effect heeft op de het gemak waarmee een proefpersoon T1 identificeert. Tevens vonden we veranderingen in het EEG-signaal. Enigszins onverwacht bleek scopolamine zeer vergelijkbare effecten op zowel gedrag als EEG te hebben. Hoewel deze studie geen onomstotelijk bewijs heeft geleverd voor een rol van noradrenaline of acetylcholine in de attentional blink, suggereren onze resultaten wel een duidelijke rol voor deze twee neuromodulatoren in temporele aandacht.

8.3.3. Hoofdstuk 4: De effecten van clonidine en scopolamine op het accessory stimulus-effect

Een grote hoeveelheid literatuur suggereert dat temporele aandacht ook van buitenaf kan worden beïnvloed. Verschillende experimenten hebben aangetoond dat het presenteren van een geluid dat vrijwel tegelijkertijd wordt aangeboden met een visuele targetstimulus, leidt tot kortere reactietijden op die visuele target. Het accessory stimulus-effect wordt toegeschreven aan het LC-NE-systeem. Niet alleen is gerapporteerd dat toediening van clonidine (zie vorige paragraaf) leidde tot verminderd effect van een waarschuwingscue in zowel rhesusapen als mensen, maar Jepma, Wagenmakers, Band, en Nieuwenhuis (2009) suggereerden dat een accessory stimulus fasisch vuren in de LC opwekt, wat vervolgens leidt tot de afgifte van noradrenaline in de motorcortex, met verhoogde prikkelbaarheid van dat hersengebied tot gevolg.

Wij hebben in dit hoofdstuk getracht om de hypothese van Jepma et al. (2009), dat de accessory stimulus waarneming versnelt, te toetsen. In het experiment dat in dit hoofdstuk wordt beschreven, werd clonidine en scopolamine

(zie vorige paragraaf) toegediend aan gezonde proefpersonen, waarna zij een accessory stimulus-taak uitvoerden. Het experiment noopt tot twee interessante conclusies: het presenteren van een accessory stimulus leidde niet alleen tot kortere reactietijden, maar verhoogde ook het gemak waarmee proefpersonen de targetstimuli konden beoordelen. Daarnaast *vergrootte* clonidine het accessory stimulus-effect; wederom gaf scopolamine zeer vergelijkbare resultaten als clonidine. Het is mogelijk dat zowel clonidine als scopolamine algemene alertheid verlagen en dat schenkt een accessory stimulus de ruimte om te compenseren voor cognitieve beperkingen die worden veroorzaakt door die middelen.

8.4 De rol van noradrenaline in leren

De rol van noradrenaline in leren werd al gesuggereerd door Kety (1980), die aangaf dat nieuwe of saillante stimuli een *arousal*-respons kunnen opwekken die de connecties tussen neuronen die worden geactiveerd door die stimuli kan moduleren, waardoor het leren beïnvloed wordt. Meer recente theorieën impliceren ook een rol voor het LC-NE-systeem in leren. Zo werd aangetoond dat stimulatie van het noradrenerge systeem leidt tot het verschuiven van aandacht in ratten (Devauges & Sara, 1990). Nieuwenhuis (2011) gaf een overzicht van dierliteratuur die suggereert dat fasisch vuren van de LC als reactie op nieuwe of onverwachte stimuli leidt tot het updaten van het mentale schema dat we maken van onze omgeving. Al deze evidentie suggereert dat het LC-NE-systeem een rol speelt in leren, maar verklaart niet *hoe* precies.

Er zal nu een samenvatting worden gegeven van de dissertatiehoofd-stukken die zijn gewijd aan de rol van het LC-NE-systeem in leren.

8.4.1. Hoofdstuk 5: Het effect van clonidine en scopolamine op leren, zoals gereflecteerd door de P3-ERP

De P3 is een van de meest bestudeerde onderdelen van het EEG: aangezien hij een grote amplitude heeft, valt hij eenvoudig te bestuderen. De theorie van Nieuwenhuis, Aston-Jones, en Cohen (2005a) relateert de P3 overtuigend aan het noradrenerge systeem. De toediening van clonidine (zie paragraaf 8.3.2.) verkleint de grootte van de P3 en het elimineren van noradrenaline leidde zelfs tot een totale verdwijning van de P3.

Een groot aantal geheugenonderzoeken heeft laten zien dat proefpersonen een grotere P3 laten zien als reactie op stimuli die ze later onthouden, in vergelijking met stimuli die ze later vergeten. Een belangrijke theorie op dit gebied suggereert dat we een intern model van onze omgeving hebben, en dat de P3 wordt gemanifesteerd wanneer we dat model bijstellen op basis van nieuwe informatie. De LC-NE-theorie veronderstelt dat die P3 een fasische LC-respons reflecteert.

Hoewel een grote hoeveelheid bewijs dus een relatie suggereert tussen de P3 en het noradrenerge systeem, is er ook bewijs voor een rol van het cholinerge systeem in de totstandkoming van de P3. Het doel van dit proefschrifthoofdstuk was dan ook de relatieve bijdrages van de beide neuromodulatorsystemen in kaart te brengen, op basis van een recente theorie van Ranganath en Rainer (2003). Deze theorie suggereert dat de twee neuromodulatorsystemen bijdragen aan verschillende subcomponenten van de P3: de P3a en de P3b. De P3a wordt voor op het hoofd gemeten en wordt vooral opgewekt door nieuwe, sterk afwijkende, taak-irrelevante stimuli, terwijl de P3b een meer achter op het hoofd wordt gemeten en juist wordt opgewekt door weinig-voorkomende maar taakrelevante stimuli. Doorgaans bestaat de P3-component uit een mengeling van P3a- en P3b-subcomponenten. Ranganath en Rainer opperden op basis van eerdere farmacologisch onderzoeken dat het noradrenerge systeem ten grondslag ligt aan

de P3b, terwijl de P3a juist tot stand komt door toedoen van het cholinerge systeem.

In dit dissertatiehoofdstuk werden clonidine en scopolamine (zie paragraaf 8.3.2.) toegediend aan gezonde proefpersonen, die vervolgens een auditieve oddball-taak uitvoerden. Tijdens deze oddball-taak werden de proefpersonen geconfronteerd met geluiden die op elkaar leken: sommige kwamen vaak voor, andere weinig. Proefpersonen moesten op een knop drukken wanneer ze een geluid hoorden dat niet vaak voorkwam; daarnaast werden soms ook vreemde, onverwachte geluiden aangeboden. Niet-veelvoorkomende geluiden wekken doorgaans duidelijke P3b's op, terwijl vreemde geluiden juist een duidelijke P3a opwekken. Onze resultaten suggereerden dat clonidine en scopolamine vergelijkbare effecten op de P3 hadden: beide middelen verkleinden de grootte van de P3a, maar deden de grootte van de P3b juist iets toenemen. Op grond van deze resultaten kunnen wij de hypothese van Ranganath en Rainer over de rol van noradrenaline en acetylcholine in de opwekking van de P3 dus niet bevestigen.

8.4.2. Hoofdstuk 6:De relatie tussen noradrenaline, arousal, pupildiameter, en Hebbiaans leren

De rol van het LC-NE-systeem in leren kan ook op een andere manier worden bekeken dan Yu en Dayan deden: Verguts en Notebaert (2008/2009) bestudeerden de rol van noradrenaline in leren. Hiertoe gebruikten deze auteurs de Stroop-taak, waarin proefpersonen de inktkleur moeten benoemen waarin kleurnamen zijn gedrukt. Doorgaans kost het proefpersonen meer moeite om de inktkleur te benoemen wanneer deze niet correspondeert met de kleurnaam: het is dus moeilijker om bij de incongruent stimulus *blauw* het correcte antwoord ('zwart') te geven dan bij de congruente stimulus *zwart* ('zwart').

Verguts en Notebeart suggereerden dat een bepaald hersengebied, de *anterior cingulate cortex*, conflict monitort; wanneer conflict wordt gedetecteerd, wordt de LC gerekruteerd. De LC vuurt vervolgens fasisch in reactie op het cognitieve conflict dat incongruente stimuli opwekken. Deze fasische respons versterkt de connecties tussen neuronen die bij de taak betrokken zijn. Deze versterkte connecties vergemakkelijken de verwerking van die specifieke incongruente stimulus wanneer hij weer wordt gepresenteerd.

In dit proefschrifthoofdstuk presenteren wij de eerste empirische test van de hypothese van het model van Verguts en Notebaert. Hierbij maken wij gebruik van de veronderstelde relatie tussen fasische *arousal* en pupildilatie. In twee experimenten vonden wij slechts voorlopig bewijs voor deze hypothese: de gedragsresultaten die voortkwamen uit een Stroop-taak ondersteunden de voorspellingen van het model van Verguts en Notebaert, maar de bijbehorende pupildilaties ondersteunen deze voorspellingen slechts ten dele. In een tweede experiment, waarin proefpersonen een ander type taak uitvoerden, gebruikten we een accessory stimulus-manipulatie (zie paragraaf 8.3.3.) om de mate van *arousal* die ze ervoeren direct te manipuleren. In dit experiment vonden we geen betrouwbare relatie tussen *arousal* en leersnelheid: ook dit biedt geen ondersteuning voor de hypothese van Verguts en Notebaert.

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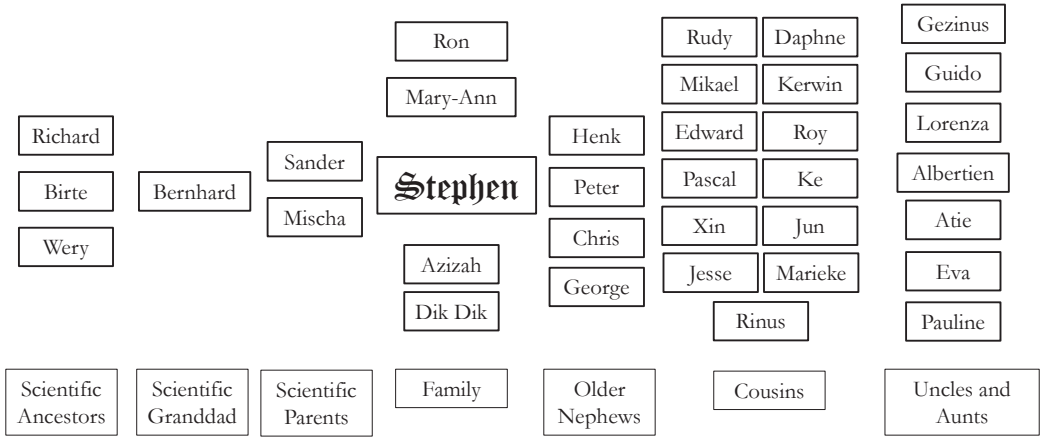
Scientific Genealogy (Acknowledgements)



Curriculum Vitae

tephen B.R.E. Brown was born in Tilburg on February 23rd, 1985. He completed secondary school in Hoorn, at the Werenfridus, Scholengemeenschap Tabor, in 2003. He obtained a Bachelor of Science (*cum laude*/with honours), in Developmental Psychology in 2006, followed by a Master of Science (*cum laude*/with honours) in Developmental Psychology and Cognitive Psychology in 2008, both from the University of Amsterdam. During and following graduation, Stephen was involved in various teaching activities at the University of Amsterdam and ran the secretariat for the national cognitive psychology graduate school EPOS. In September 2009, he started to work on a PhD project under supervision of Sander Nieuwenhuis at Leiden University, which led to the current dissertation on the effects of noradrenaline on temporal attention and uncertainty process-sing. Following a short position as a programmer in the technical and research support department of Leiden University, Stephen now works as a part-time lecturer at the Institute for Interdisciplinary Studies of the University of Amsterdam and as a part-time post-doctoral fellow at the Health, Medical, and Neuropsychology unit of Leiden University.