Spatial Cognition of Emotional Faces

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How fast is our brain?

PSY-3900

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SPATIAL COGNITION OF EMOTIONAL FACES – HOW FAST IS OUR BRAIN?

Abstract

There are two different routes in the brain that deal with the processing of sensory information and initiate a response. One fast route, the dorsal magnocellular pathway, involves structures like the amygdala and starts a rapid and automatic fear reaction, necessary in so-called fight-or-flight situations. The other route comprises the neocortex, initiating a slower response via the parvocellular ventral pathway. Previous studies have shown that different features of pictures, such as spatial frequency, orientation or amplitude, influence different pathways. In general, neurons in the brain get more activated to higher amplitude. Based on that, this study - consisting of two experiments - investigated the influence of amplitude in low spatial frequency (LSF) pictures on emotional reactions in the brain. In experiment 1, eighty-four volunteers participated in a startle eyeblink response study. Responses were enhanced to LSF facial pictures of high amplitude in comparison to low amplitude. In addition, a stronger eyeblink response was observed when measured at longer latencies (after 3,000 ms) in comparison to short latencies (250 ms), which could be explained by prepulse inhibition at shorter latencies. Moreover, a subjective test revealed a significant difference between the different emotional valences on a positivity scale. Experiment 2 further investigated whether reaction times were enhanced when watching high amplitude LSF facial pictures. Contrary to predictions, higher amplitude LSF pictures led to significantly longer reaction times, while low amplitude pictures initiated a faster response.

Keywords: Amplitude, low spatial frequency (LSF), startle eyeblink response, reaction time, facial stimuli

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Abstract (norsk)

I hjernen er det to forskjellige ruter som sender sensorisk informasjon og utløser en reaksjon. En rask rute, den dorsale magnocellulare ruten, som inkluderer strukturer som amygdala- Denne setter i gang en rask automatisk fryktreaksjon, noe som er nødvendig i en såkalt fight-or-flight situasjon. Den andre ruten involverer neocortex og setter i gang en tregere respons via den parvocellulare ventrale ruten. Tidligere studier har vist at ulike trekk i bilder, slik som *spatial frequency*, orientering eller amplitude, påvirker de forskjellige rutene. Generelt sett blir nevronene i hjernen mer aktivert til bilder med høy amplitude. Basert på det, er denne studien - bestående av to eksperimenter - rettet mot å undersøke hvilken påvirkning amplituden har i bilder med *low spatial frequency* (LSF), mot emosjonelle reaksjoner i hjernen. I forsøk 1, deltok 84 frivillige i en *startle eyeblink response* studie. Responsen var forsterket ved LSF ansiktsbilder med høy amplitude i forhold til de med lav amplitude. I tillegg ble det observert en sterkere øyeblink respons når den ble målt over en lengre periode (etter 3,000 ms) i forhold til ved en kortere (etter 250 ms), som kan forklares av *prepulse inhibiton* effekten ved kortere intervaller. Videre viste en subjektiv test at det er en signifikant forskjell mellom de ulike emosjoner, målt ved hjelp av en positivitetsskala. I forsøk 2 ble det undersøkt om reaksjonstiden ble raskere som reaksjon til LSF ansiktsbilder med høy amplitude. Overraskende ble det oppdaget at en høyere amplitude førte til en signifikant tregere respons enn LSF bilder med en lav amplitude.

Nøkkelord: Amplitude, low spatial frequency (LSF), startle eyeblink response, reaksjonstid, ansiktsbilder

SPATIAL COGNITION OF EMOTIONAL FACES – HOW FAST IS OUR BRAIN?

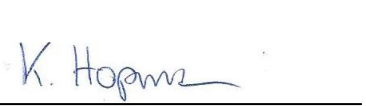
Preface

This master's thesis is the result of my study at the University of Tromsø, which I started in 2011. Previous successful work together with my supervisor Ole Åsli has led me to decide to work with him again. Together with Morten Øvervoll he suggested a possible project of study for my thesis. The given project was similar to the one at hand, using fMRI as a method, and I was immediately interested in taking part in this project. After a short while we figured out that the time scope of the project would be too big for my master's thesis and we decided to change the methods and conduct an EMG study. A pilot study conducted by Ole and Morten had shown that startle eyeblink response should be enhanced to pictures with higher amplitude and we decided to confirm these results by conducting this experiment on a bigger scale. This was a great opportunity for me as well to contribute with previous knowledge and be part of a larger and engaging project.

Additionally, I came up with the idea of conducting a second study to confirm the time component of the hypotheses. I was inspired by an article I read during literature research and together we decided to set up a software, similar to the one in the given article, to test whether reaction times are faster to high amplitude pictures. Here, my special thanks go once more to Morten Øvervoll, who managed to do a tremendous job, installing the software given the short time window at hand.

I was given free rein in the laboratory work, but the process of carrying out the whole project is a result of great teamwork and supervision. Moreover, this project has given me further insight in conducting a study and given me vast knowledge of the current field of study.

Tromsø, 02.05.2013



Karen Hopmann (student)

Ole Åsli (supervisor)

SPATIAL COGNITION OF EMOTIONAL FACES – HOW FAST IS OUR BRAIN?

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First and foremost, I wish to thank my supervisor at Tromsø University, Ole Åsli, whose encouragement, guidance and support from the initial to the final level enabled me to develop an understanding of the subject. He has shown his support in a number of ways, starting with laboratory instructions, guidance with writing, and help whenever needed during the data collection and writing process. When the project and methods had to be changed he came up with suggestions and alternatives as well as encouraging me to bring in ideas myself at all levels. Furthermore, giving me free rein to execute all laboratory work helped me a lot to develop independence in my work.

My thanks go to Morten Øvervoll at Tromsø University, for his technical support and help regarding methodological and statistical questions. Without his effort, a smooth delivery of the thesis would not have been possible.

Finally, I offer my regards to all of those who supported me in any respect during the completion of the project, most of all to my family and friends.

Karen Hopmann

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Emotional Processing of Facial Images

People react to fear, not love – they don't teach that in Sunday school, but it's true.

- Richard M. Nixon

Probably every healthy human being has been experiencing the physiological expressions of fear: the heart rate increases, breathing becomes more rapid, cold sweat, and increased blood pressure. The body prepares for a fight-or-flight reaction automatically and fast. Sometimes we experience these sensations even before knowing what makes us feel this way and where the threatening situation comes from, such as when we get frightened by a loud noise before identifying direction and nature of the origin. As emotions take a big part of our lives, it has long been of interest for research.

Being one of our most basic emotions, fear can be experienced in a wide range of severity degrees. While some enjoy having snakes or spiders as pets in their homes, others avoid seeing those animals even from a far distance. The identification of fear can happen easily through facial expressions as there is little cultural variety (Gazzaniga, Ivry, & Mangun, 2009). The DSM-IV states a lifetime prevalence of 28.8% for anxiety disorders, which means that almost every third person experiences some form of anxiety symptoms at least once during the lifespan (American Psychiatric Association, 1994). This illustrates that phobias and other forms of anxiety disorders belong to the most common psychological disorders in our society.

Accordingly, Öhman and Mineka (2003) explain that certain kinds of phobias, such as abnormal fear of snakes, have an evolutionary basis in all mammals. Even if no phobia is present, fear can be conditioned much faster for snakes than less commonly feared objects,

such as flowers. This effect is present in both humans and primates. Moreover, conditioning effects are still present for masked objects, indicating that no cortical areas of the brain need to be involved in the fear reaction. Hence, a faster, subcortical route is believed to be involved in rapid fear reactions. Evolutionarily, this rather primitive neural circuit is recognized already in the early development of the brain before higher brain functions evolve, such as the neocortex. Based on that, Öhman and Mineka (2003) proposed a concept of an independent fear module, which is "selectively sensitive to, and automatically activated by […] threats" (Öhman & Mineka, 2003, p. 7). This module is based on fairly simple cognition relying on a specific neural route known as the fast, subcortical route. It involves the amygdala, an almond-shaped cluster of neural nuclei in the medial temporal region of the brain. Generally, the amygdala responds to facial threats and takes part in initiating the fight-or-flight response. It receives sensory information and is, as a structure of the limbic system, involved in emotional responses (Gazzaniga et al., 2009; Öhman, 2002). The subcortical route is activated automatically and rapidly when facing potential threats. Danger can therefore be detected fast enough to initiate a spontaneous reaction. Correspondingly, it has been shown that angry faces, as a potential threat, are detected faster and more accurately than happy or sad faces in a crowd of people (Öhman, Lundqvist, & Esteves, 2001).

Fear Reactions in the Brain

Emotional processing takes place virtually at all times. People can be chronically frightened or react with fear as a reflex to a frightening situation. Sometimes we do not even know why we are scared and just feel the consequences of it. The entire body is involved when we react with fear. Physiological consequences such as cold sweat or increased heart rate can easily be observed. However, mechanisms in the brain elicited by fear can only be observed using imaging techniques. Much of the current knowledge about fear processing in

the brain is based on findings made by Joseph LeDoux (1996). One important finding he made is that fear conditioning can take place in rats even after removal of the cortex. LeDoux concluded that the amygdala can process sensory information without involvement of the cortex, hence acting independently of higher cortical areas (Gazzaniga et al., 2009; LeDoux, 1996; Oatley, Keltner, & Jenkins, 2006). Consequently, subcortical areas, comprising the amygdala, are seen as the center for emotional processing, especially for appraisal of emotions and fear.

Largely, immediate fear reactions are unconscious and rapid, as the body has to be set in an alarming state right away. On the other hand, conscious processing takes more time and in dangerous situations humans and other animals are dependent on a rapid and autonomic reaction. However, after the initial fear reaction, conscious processing takes place when the threatening stimulus is recognized. LeDoux suggested two different channels in the brain, which are activated for unconscious and conscious processing, respectively. The subcortical route, sometimes called the low road, processes information fast - though sometimes inaccurate. Sensory information reaches the thalamus, which relays information further to the amygdala without a thorough analysis but as a rough signal regarding a potentially threatening situation. Consequently, a rapid fear reaction can be initiated before information reaches consciousness. At the same time, information is processed via a slower route – also called the high road. Here, information is sent further from the thalamus to the sensory cortex for more thorough analysis. After reaching the cortex and hence consciousness, the processed information is sent to the amygdala, and a reaction is initiated (see figure 1). In a threatening situation, the fast road is anticipating the amygdala for a reaction, so that the right reaction can be initiated immediately when information from the slow road confirms the threat. In this way, the two routes are working together to ensure a fast and accurate response in a potentially dangerous situation, accomplishing object recognition in a simultaneous and bidirectional way (Bar, 2003; Gazzaniga et al., 2009).

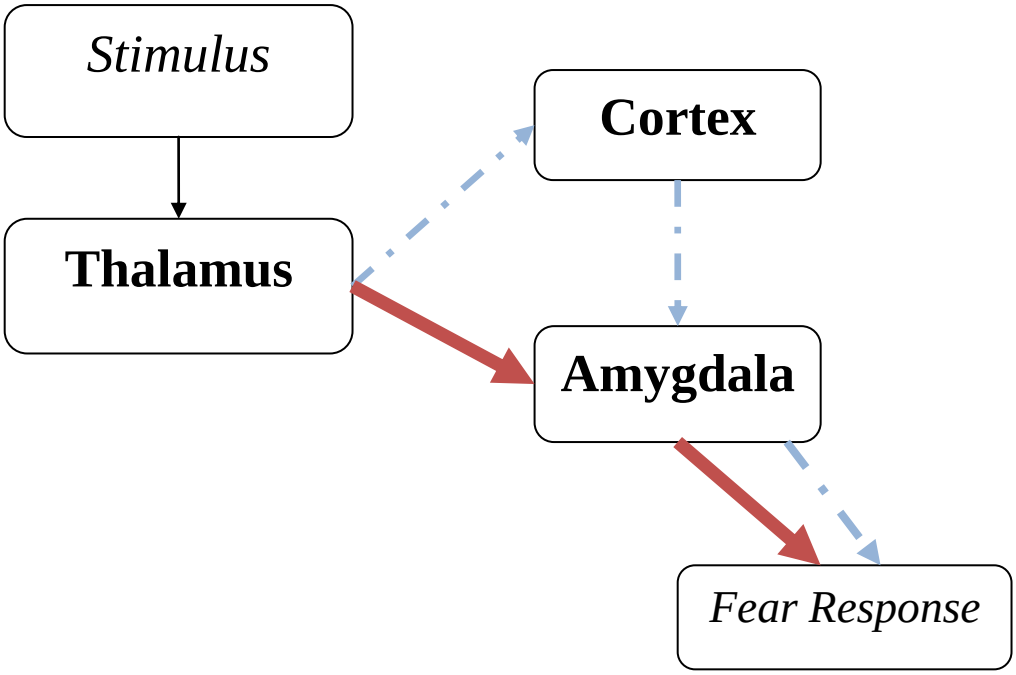

Figure 1: Slow cortical (blue/dashed) and fast subcortical (red/bold) route

Nevertheless, the two different pathways do not only differ in the way they process sensory information, but also comprise different brain structures. Moreover, the two routes process information via divergent cells. Consisting of mainly larger cells, the subcortical route is also called the magnocellular pathway. Moreover, it is especially involved in top-down object recognition. This dorsal pathway is projecting visual information from the early visual cortex to prefrontal areas (PFC), which are further directly connected to the inferior temporal cortex (IT) and the amygdala (Bar, 2003). This supports the theory that the amygdala and connected brain areas are involved in rapid processing of fear. On the other hand, smaller cells forming the parvocellular pathway, process more fine-grained object features in a slower manner via the ventral stream. This process is also identified as bottom-up processing, integrating the information into complete object recognition (Bar, 2003).

In fact, cognition and emotion were historically often thought to be separated in the brain. Certain functions are assigned to specialized parts of the brain, dividing cognitive and affective sites. Pessoa (2008) claims that interaction and integration is much more common

than originally and still assumed by for example lesion studies. The amygdala is the main structure involved in affective processes and automatic reactions to fear stimuli. However, the amygdala is not the only structure involved in automatic fear processing. The fact that an automatic activation takes place without involvement of the neocortex seems to be proven for rapid fear reactions (Öhman, 2000), but in what manner the different structures are involved has to be further investigated. Morris, Öhman, and Dolan (1999) do not only point towards a lateralization of the amygdala regarding conscious and unconscious fear reaction to a conditioned stimulus, but additionally describe two further structures involved in the automatic processing of fear – the superior colliculus of the midbrain and the right pulvinar nucleus of the thalamus. These structures might act as connections or routes to the amygdala without passing through the cortex and conscious awareness.

Interestingly, all structures involved in rapid, automatic fear processing are known to be the more primitive and earlier developing sites of the brain (Pessoa, 2008; Pessoa, 2010). Yet, it does not mean that an automatic fear reaction is solely affective, but interaction with cognitive sites of the brain most probably occurs. Processing of visual information takes place and features of objects are recognized (Pessoa, 2010). However, involvement in only one certain brain function, or emotion in this case, is unlikely and interconnections between the given areas of the brain are most probably present (Rolls, 2005). Therefore, interpretation of results stating one specialized function for one brain area has to be done with caution.

Low (LSF) and High (HSF) Spatial Frequencies

The difference between LSF and HSF cues

When identifying faces and their emotional valences, different parts of the brain get activated. Especially when seeing an angry face an automatic fear reaction is initiated by the body to escape or fight potential danger. This reaction is often referred to as the fight-or-flight

reaction. Which parts of the brain get activated is not merely dependent on the emotional valence of the face but also on its spatial frequency. In general, spatial frequency is defined as "the number of cycles per degree of visual angle and/or number of cycles per image" (Park, Moon, Kim, & Lee, 2012, p. 778). There are three types of spatial frequency cues in pictures: an intact image with broad spatial frequency (BSF), pictures with high spatial frequency (HSF), and images with low spatial frequency (LSF); where both HSF and LSF information are combined in a BSF picture. LSF images have a small number of around 2-8 cycles per image, showing more coarse features of an expression, important for recognition of the position of the eyes or mouth. HSF pictures, with 8-16 cycles per image, are more detailed and can provide information about age or expression more easily (Vuilleumier, Armony, Driver, & Dolan, 2003). Figure 2 gives an example of the different spatial frequencies.

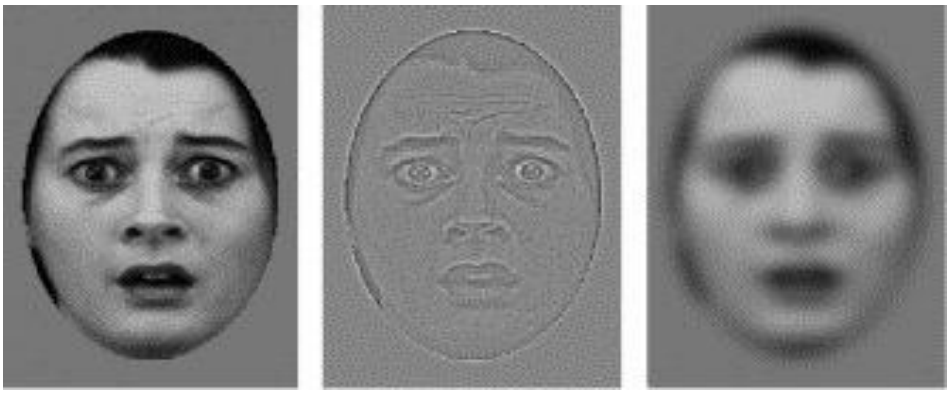

Figure 2: Broad spatial frequency, high spatial frequency, and low spatial frequency images (from left to right)

Emotional responses to LSF and HSF stimuli

A number of studies have investigated the influence of spatial frequencies on fear reaction (Bar et al., 2006; Holmes, Green, & Vuilleumier, 2005; Vlamings, Goffaux, & Kemner, 2009; Vuilleumier et al., 2003). Even the effect of cardiac vagal tone has been shown to influence the reaction to different spatial frequencies (Park et al., 2012).

Additionally, Holmes et al. (2005) found that fearful LSF images led to faster reaction times in comparison to neutral LSF stimuli. This difference was not present for HSF pictures. Further testing revealed that this effect could not be explained by better emotion recognition in LSF pictures, as accuracy was higher for HSF than LSF stimuli. However, faster reactions were only observed for upright LSF pictures. The effect disappeared when inverted pictures were presented, indicating that not solely the spatial frequency features account for the previously found effect. Moreover, when exposure time of stimuli presentation was extended, the preliminary effect of automatic reaction to LSF stimuli disappeared. This supports the theory that after longer exposure conscious processing can take place via the cortical route and the automatic processing via the subcortical route - initiated by LSF stimuli - is not present any longer.

Despite different methods, most studies in this field agree on the fact that LSF pictures elicit stronger fear reactions than HSF images seen in, for instance, more amygdala activation, higher startle response or higher Event Related Potential (ERP) amplitude to LSF pictures. While the magnocellular system is mainly responding to coarse information and thereby low spatial frequency, the parvocellular system is responsive to high spatial frequency (Murav'eva, Deshkovich, & Shelepin, 2009). As mentioned before, especially the amygdala is involved in rapid fear reactions, such as the fight-or-flight response. Certain features of a facial expression trigger a fear reaction either automatically or consciously. Vlamings et al. (2009) found a stronger activation of the right hemisphere to LSF stimuli. The authors explain this lateralization by a focus of the right hemisphere on global instead of local processing. Hence, the entire situation is recognized and interpreted faster and a fear response is set off. In an fMRI study, Vuilleumier et al. (2003) investigated activation patterns to LSF, HSF, and BSF pictures. They found that the amygdala showed a stronger activation in response to fear for LSF in comparison to HSF pictures. On the other hand, HSF cues activated the fusiform cortex. An additional fMRI study by Bar et al. (2006) showed that dorsal areas are activated

earlier than ventral areas, supporting the view of rapid processing via the dorsal magnocellular stream before reaching more ventral areas like the fusiform gyrus via the parvocellular route. This pattern is consistent with findings from other imaging studies (Vuilleumier et al., 2003). Based on these studies, we can conclude that coarse LSF cues are influencing the fast subcortical route involving the amygdala, pulvinar, and superior colliculus. The magnocellular channels send information rapidly to the amygdala and the prefrontal cortex (Bar et al., 2006). On the other hand, HSF cues are activating the slower cortical route with input from parvocellular cells reaching the fusiform cortex and neocortex. More detailed information can be processed here, reaching into consciousness. Responses are therefore slower.

Taken as a whole, different features of pictures, such as spatial frequency, influence dissimilar sites of the brain. Previous studies have shown that especially the amygdala is involved in rapid processing of coarse LSF stimuli. The structure is virtually unresponsive to fearful HSF expressions, in contrast activating the cortical route. However, it is important to explore why and what features of LSF cues are important in object recognition and subsequent fear reactions. As found by Holmes et al. (2005), faster responses for LSF pictures only take place when presented in an upright position. Therefore, the special features of LSF cues have to be further investigated to understand the full nature of the given effects. Moreover, Awasthi, Friedman, and Williams (2011) state that faces are prioritized for early processing of LSF features. Likewise, it is claimed that facial images elicit stronger attentional effects, important for the investigations of the present study (Fox, Russo, Bowles, & Dutton, 2001).

Amplitude of Low Spatial Frequency Pictures

Low spatial frequency pictures contain coarse information with only 2-8 cycles/degree (Vuilleumier et al., 2003). To the common viewer pictures seem blurry, but an overall structure or expression of the stimulus can generally be recognized. More fine-grained information is hardly identifiable in LSF pictures. Nevertheless, stimuli do not only differ in spatial frequency, but have other features influencing object recognition. For example, a stimulus can differ in orientation or contrast. In particular, contrast, also called amplitude, seems to influence object recognition and affect (see figure 3). Contrast is a fundamental element in vision science. Moreover, problems with contrast sensitivity are accompanying many visual and neurological problems such as glaucoma, Parkinson's disease, or multiple sclerosis (MS) (Baek, 2012). Up to 75 % of MS patients experience problems with contrast sensitivity, mostly due to problems with either the magno- or parvocellular system (Murav'eva et al., 2009). Generally, neurons in the brain are more activated to higher contrasts, and information processing can take place more rapidly. As it has been demonstrated previously in cats, neurons of the visual cortex increased their responses with increasing contrast (Maffei & Fiorentini, 1973).

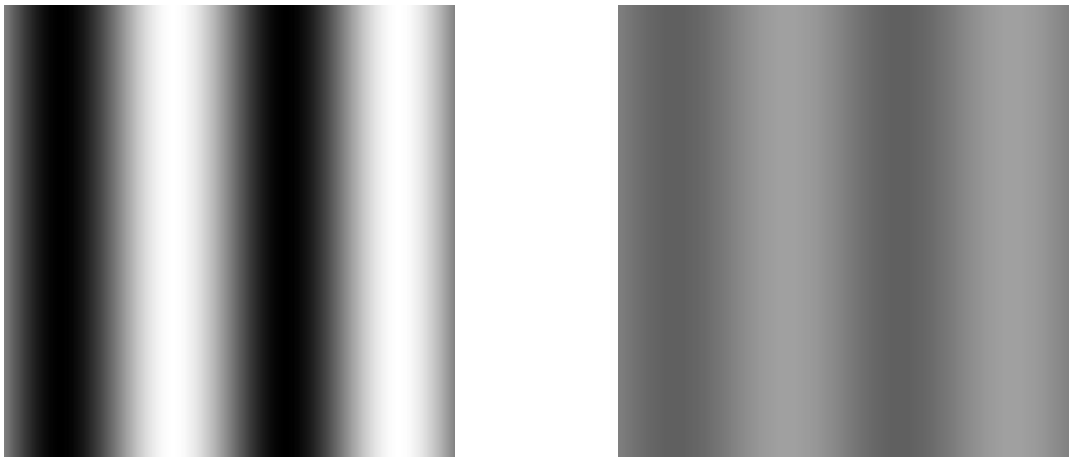

Figure 3: Low spatial frequency pictures with high (left) and low (right) amplitude

Already almost 40 years ago, researchers investigated the effect of amplitude in low and high spatial frequency pictures. Reed, Marx, and May (1984) conducted a study, in which they tested visual evoked potentials (VEP) in regard to low spatial frequency pictures at different contrasts. Latency was found to be decreased to higher contrast, indicating that a more rapid processing to low spatial frequency stimuli of high amplitude was present. A statistical analysis of VEP as a function revealed that it was linearly related to contrast; hence evoking higher potentials in response to increasing contrast (Campbell & Kulikowski, 1972). Furthermore, a reaction time (RT) study showed that RT decreases to increasing contrast (Murray & Plainis, 2003). Therefore, processing of higher contrast is thought to take place more rapidly. Consequently, high contrast presented in low spatial frequency should generally elicit increased emotional responses. This can either be measured by an increased startle eyeblink response, higher ERP amplitude, faster RTs or more activation in the concerned brain areas. In the present study, the startle eyeblink response and reaction times will be measured to further investigate the influence of LSF high amplitude pictures. The terms contrast and amplitude will be used interchangeably throughout the paper.

Startle Eyeblink Response

There has been a variety of imaging studies investigating the nature and sites of fear reactions, especially of the amygdala. Additionally, physiological studies have often been used to examine the expression of fear and its consequences (Globisch, Hamm, Esteves, & Öhman, 1999; Öhman, 2009; van den Hout, de Jong, & Kindt, 2000). Physiological studies can have different forms, such as measuring the skin conductance response, heart rate, or startle reflex modulation. Higher skin conductance responses were observed in individuals with phobias while watching images representing the feared object (Öhman, 2009; van den Hout et al., 2000). Hamm, Cuthbert, Globisch, and Vaitl (1997) reported a higher eyeblink

magnitude in individuals with ophio- and arachnophobia watching pictures of snakes and spiders, respectively. As a result, startle reflex modulation has gained much evidence in the last years regarding emotional reactions. Some of the major advantages of this measure are its good temporal resolution (Åsli, Kulvedrøsten, Solbakken, & Flaten, 2009) and the independence of gender, age, language and voluntary actions (Wilbarger, McIntosh, & Winkielman, 2009). All in all, startle eyeblink response has been used as a reliable measure for automatically induced emotional reactions.

Commonly, startle responses are tested in affective or attention measures as the latency of an acoustic startle is relatively short with about 6 - 8 ms (Flaten, Nordmark, & Elden, 2005). In the brain, the nucleus reticularis pontis caudalis (nRPC), a small nucleus located in the pons, is assumed to function as the startle center. Supporting this theory, Lee, López, Melone, & Davis (1996) showed that electrical stimulation of the nRPC initiated a startle response. Moreover, the nRPC is connected to the muscles around the eye controlling the eyeblink reflex, such as the 'orbicularis oculi' muscle. In healthy individuals, a fear reaction is generally expressed with a heightened startle eyeblink response towards unpleasant stimuli. A loud noise for example is interpreted as a threatening situation, and a startle eyeblink response occurs. While startle eyeblink responses are enhanced by negative events, positive events associated with the startle probe (noise) usually reduce this reflex (Dichter, Benning, Holtzclaw, & Bodfish, 2010).

In the present study, experiment 1 focuses on the startle eyeblink response triggered by an acoustic probe of white noise. In contrast to prior studies however, the valence of the pictures is not the main focus here, but the feature of the picture presentation itself. Consequently, a higher startle eyeblink response is not expected towards negative pictures (angry and fearful faces), but especially to LSF pictures presented with higher amplitude in comparison to low amplitude.

Hypotheses

The human brain seems to be tuned to certain stimuli and cues to initiate a reaction. Öhman and Mineka (2003) put forward that our brain is recognizing threatening events faster via an unconscious route in the brain, to set off a rapid response. Evolutionarily, this has been important to avoid threatening situations and assure survival. Given formerly discussed literature, the hypotheses of the present study are based on findings that different features of a picture – in this case higher amplitude and LSF - lead to more neural stimulation and the activation of rapid emotional reactions via the magnocellular route (Campbell & Kulikowski, 1972; Maffei & Fiorentini, 1973; Reed et al., 1984; Vuilleumier et al., 2003). Faster and more intense reactions should be observable in an increased startle eyeblink response to higher amplitude pictures as well as faster reaction times. This effect should not be present for LSF pictures of low amplitude.

Regarding this, the first hypothesis sounds as follows:

Hypothesis 1: *LSF pictures of high amplitude are expected to provoke an increased startle eyeblink response in comparison to low amplitude LSF pictures*

To investigate whether features of the stimuli are influencing a faster route in the brain and hence a faster reaction, the startle eyeblink response will be measured at two different latencies. On half of the occasions, startle is measured shortly after picture presentation (250 ms), and for the other half after a longer time period (3,000 ms). If a faster, subcortical route is activated by high amplitude pictures regardless of emotional valence of the picture, this should present itself in a higher startle eyeblink response to high amplitude pictures in general. Consequently, reaction takes place before the emotional valence is consciously represented in the cortex. In this way, the features of a stimulus (high amplitude in LSF) are activating the fear response automatically. As a result, this effect should be absent when

measuring startle after longer latencies (3,000 ms), while here the conscious representation of the emotional valence should influence the fear reaction. Therefore, a main effect of emotional valence of the pictures is expected at longer latencies only. Here, the fear reaction is expected to be increased to negative images (afraid, angry) in comparison to happy or neutral faces. This leads us to the following hypotheses:

Hypothesis 2: *High amplitude LSF pictures are expected to increase startle eyeblink magnitude in comparison to low amplitude pictures regardless of emotional valence, when measured 250 ms after picture presentation*

Hypothesis 3: *A main effect of emotional valence is expected when startle is measured 3000 ms after picture presentation*

The startle eyeblink response will give us insight into the intensity of fear reactions to certain features and stimuli. Additionally, it will be interesting to find out if reactions, expected to activate a faster route in the brain, will also be enhanced in real-time. To test whether a faster reaction to pictures of LSF in comparison to HSF can be observed, Holmes et al. (2005) conducted a reaction time study. The hypothesis that fearful LSF pictures elicit faster reaction times in comparison to happy faces, while no difference should be observable for HSF pictures, could be confirmed. Here it was proven that features like spatial frequency activate different routes in the brain, in this case faster processing of fear cues in LSF facial pictures. This difference was however absent for inverted pictures, pointing towards the involvement of facial recognition (Awasthi et al., 2011; Holmes et al., 2005). Taking this as a starting point and adding the assumption that higher amplitude activates neurons faster, we expect that LSF facial stimuli elicit faster reaction times when presented in high amplitude compared to low amplitude. Therefore, hypothesis 4 claims the following:

Hypothesis 4: *Reaction times are expected to be faster for high amplitude LSF facial pictures in comparison to low amplitude LSF facial pictures*

To test the given hypotheses, two experiments are conducted. In experiment 1 the startle eyeblink response is tested in response to LSF pictures high and low in amplitude, using electromyography (EMG). Here, the expected effect for amplitude should become clear. To test whether high amplitude pictures activate a faster route in the brain, leading to faster reactions, experiment 2 consists of a reaction time task. Here, faster reactions are expected after participants have seen LSF pictures of high amplitude in comparison to low amplitude LSF facial images.

Relevance

As previously discussed, our brain is specialized in recognizing threatening events and can react rapidly to those stimuli by initiating a fight-or-flight response. Without reaching consciousness, circuits in the brain make out the threatening element in a situation, and our body sometimes reacts before we even know what is threatening us. Evolutionarily, it is essential for survival to react rapidly in dangerous situations. However, it is still not completely understood, which feature of a threat is captured by the brain and interpreted as a potential hazard. To figure out, what feature of a stimulus our visual system is reacting to, the present study investigates emotional reactions to facial pictures presented with varying visual cues, like amplitude and spatial frequency.

If the hypotheses are proven right this could be a major step in the understanding of how fast and in which way the brain reacts to risk or danger. If reactions to certain features of a stimulus are enhanced and faster, this can give us more insight into what is influencing the different circuits in the brain. Confirming the hypotheses can give us a clearer picture of how

our brain works and reacts to fear, and how much the valence of a picture influences our reactions. If it can be shown that not only the valence of a stimulus, such as an angry face, is leading to an enhanced and faster fear reaction, but also – or even more – the feature of the stimulus itself, a better understanding of these processes in the brain can be obtained. Additionally, as anxiety disorders are one of the most common psychological problems in our society, it is of vast importance to understand the mechanisms behind it to provide better help. Therapies regarding phobias could possibly be adjusted, not only focusing on the valence of a picture, but much more in how it is presented and perceived.

Experiment 1

Materials and Methods

Participants

To recruit participants, flyers were hung up at different faculties of the University of Tromsø, Norway. Eighty-two students between 19 and 34 years participated in the study (M = 22.90, SD = 3.157 for age). Twenty-nine participants were male. All participants had normal or corrected-to-normal vision and normal hearing.

Table 1

Demographics of All Participants per Gender

Gender		N	Minimum	Maximum	*M*	*SD*
Male	Age	29	19	34	23.76	3.805
Female	Age	53	19	31	22.43	2.664
Total	Age	82	19	34	22.90	3.157

Note. *SD* = Standard Deviation.

EMG/Apparatus

The EMG activity was recorded with three sintered-pellet silver chloride AgCl miniature surface electrodes of 4 mm diameters, filled with Microlyte electrolyte gel (Coulbourn Instruments). Two electrodes were attached to the skin at the right orbicularis oculi muscle with an interdistance of about 1.5 cm, while the control electrode was attached to the forehead. The raw EMG signal was amplified by a factor of 50,000 and filtered (8 – 1,000 Hz), using a Coulbourn V75-04 bioamplifier. The signal was then integrated using a Coulbourn V76-24 contour-following integrator with a 10 ms time constant. A LabLinc V interface on a connected computer recorded the output. Noise stimuli lasted 50 ms and were presented at 95 dB over Sennheiser HD 250 headphones. Different latencies of noise presentation were given in semi-randomized order, such that acoustic probes were presented at either short (250 ms) or long (3,000 ms) lead intervals. To familiarize with the noise, five acoustic probes were presented before the first picture. A web camera was installed next to the screen, so that the whole session could be followed in real-time from the experimenter's room.

Stimuli

The stimuli used during the EMG session consisted of facial images (frontal view) showing four different emotional expressions (afraid, angry, happy, and neutral). Pictures were obtained from the Karolinska Directed Emotional Faces set (KDEF; Lundqvist, Flykt, & Öhman, 1998), originally consisting of 4900 standardized facial pictures of varying emotional valence. To preserve merely the facial features and avoid distraction, the images were converted to grayscale. By doing so, the contrast could be adjusted using the MATLAB function *imadjust* to increase contrast. Afterwards pictures were square-cut to center the nose in the middle of the picture. Pictures then were reduced to a size of 512x512 pixels.

Additionally a filter that excluded most of the hair, neck, and background was adjusted, using a two-dimensional Hanning-window (see figure 4). The LSF amplitude was estimated at around 0.5 - 2 cycles/degree of visual angle for all images of the picture set. The 10 lowest and 10 highest pictures of each category were then included in the study, resulting in 70 unique facial images. Pictures were selected likewise that no face was showing the same emotion more than once. The order of presentation was randomized.

Figure 4: The four columns (left to right) show examples of angry, afraid, happy, and neutral expressions in LSF. The faces in the top row have high amplitude, while the faces in the bottom row have low amplitude

Procedure

After signing the informed consent, participants sat down on a comfortable chair in the testing room in front of a computer screen. The experimenter instructed participants about the procedure, the duration, and nature of the experiment. It was especially emphasized that pictures should be followed at all times. Three electrodes were prepared with two-sided tape and gel. The skin below the orbicularis oculi muscle under the right eye and on the forehead

was cleansed before attaching the electrodes. Headphones were placed over the ears, the web camera placed in the right position, and instruction to look at the screen during picture presentation was given once more. Pictures appeared on the experimenter's as well as the participants' screen to detect possible disruptions in the presentation process. Moreover, the video from the web camera could be followed from the experimenter's room, so that errors in presentation and procedures could be detected and adjusted.

Afterwards, a group of individuals (n = 24) was randomly chosen to take part in a second part of the study. Here, participants were seated in front of a computer screen, and the pictures presented during the startle session were presented once more. To test the difference between objective and subjective feelings of fear, the Self Assessment Manikin (SAM; Bradley & Lang, 1994) in combination with a visual analogue scale (VAS) was conducted. Participants indicated on a continual line (VAS) how positive/negative they experienced each of the pictures seen before in the EMG session. Pictures were presented again, and participants could individually decide for how long they wanted to see each of the pictures. After that, the VAS appeared, and indication could take place via mouse click. Participants received compensation for their participation in form of two lottery scratch tickets, worth 50 Norwegian Crowns (~ 9 US $).

Data Analysis

First, the startle data was transformed using the proportion of difference values. This means that the raw startle data response of every participant was compared to the baseline response of the given participant. In that way, the influence of large variations in eyeblink response between the participants could be diminished, and comparison between the measurements was simplified. To analyze the difference between LSF facial stimuli of high contrast versus low contrast, a repeated-measures analysis of variance (ANOVA) was used.

This statistical method accounts for all within-subject variables plus their interactions. The advantage of a within-subject design is the reduction of variability and increased power to detect significant effect (Field, 2005). To measure the dependent variable *startle magnitude*, the different *valences* compared with the baseline object were used as a 4-level within-subject factor, while *amplitude* was used as a 2-level within-subject factor. A 2-level within-subject factor for *latency* was added.

For the subjective data the responses across each emotional valence were averaged. This was done for all valences divided into high and low amplitude. Consequently, eight different scores per participant were acquired. The variables were analyzed by a repeated-measures ANOVA, using *valence* as a 4-levels factor and *amplitude* as a 2-levels factor.

Results

An exploratory and frequency data analysis was conducted to determine if the startle score distribution was normally distributed. Although all valences reached significance on the Kolmogorov-Smirnov test of normality ($p < .05$), indicating that the assumption of normality was violated, the histograms looked roughly normal (Field, 2005). Removing outliers from the data did not contribute to any change of results, so that all data were included in further analyses. ANOVA is a quite robust method and due to the high number of participants ($n = 82$), the data should not be effected considerably by this possible violation of statistical assumptions (Field, 2005). Moreover, in this case, outliers occurred due to unpredictable strong blink responses, so that merely outliers in the positive direction could be observed. There were no outliers in the negative direction as the startle reflex is a forced response, which cannot result in ‘negative blinking’. In that way, we expected outliers only in the positive direction, hence stronger blink responses than the baseline average. Also for the

subjective data (n = 24) histograms appeared roughly normal distributed, and no data was removed from further analyses.

Startle eyeblink response

Given hypothesis 1, an increased startle eyeblink response to high amplitude pictures in comparison to low amplitude pictures was expected. The hypothesis was confirmed, and amplitude showed a main effect in the repeated-measures ANOVA, F (1, 81) = 4.41, p = .039 (see table 2). Furthermore, a strong main effect for latency was found, F (1, 81) = 30.33, p < .001, indicating that different latencies were leading to dissimilar reactions regarding the startle eyeblink response. Figure 5 clearly shows that longer latencies (3,000 ms) elicited a generally stronger startle eyeblink response than shorter latencies (250 ms). While there is no difference in response between the different emotions (p = .949), the interaction between valence and amplitude showed a trend towards significance (p = .077).

Table 2

Within-Subject Analysis for Valence, Amplitude, Latency and All Interaction Effects

Effect	Mean Square	*F*	Sig.
Valence	0.01	0.12	.949
valence · amplitude	0.17	2.31	.077
amplitude	0.45	4.41	.039*
valence · latency	0.05	0.49	.685
Latency	14.16	30.33	.000**
amplitude · latency	0.03	0.22	.640
valence·amplitude·latency	0.03	0.27	.849

Note. *significant at .05 level, **significant at .001 level.

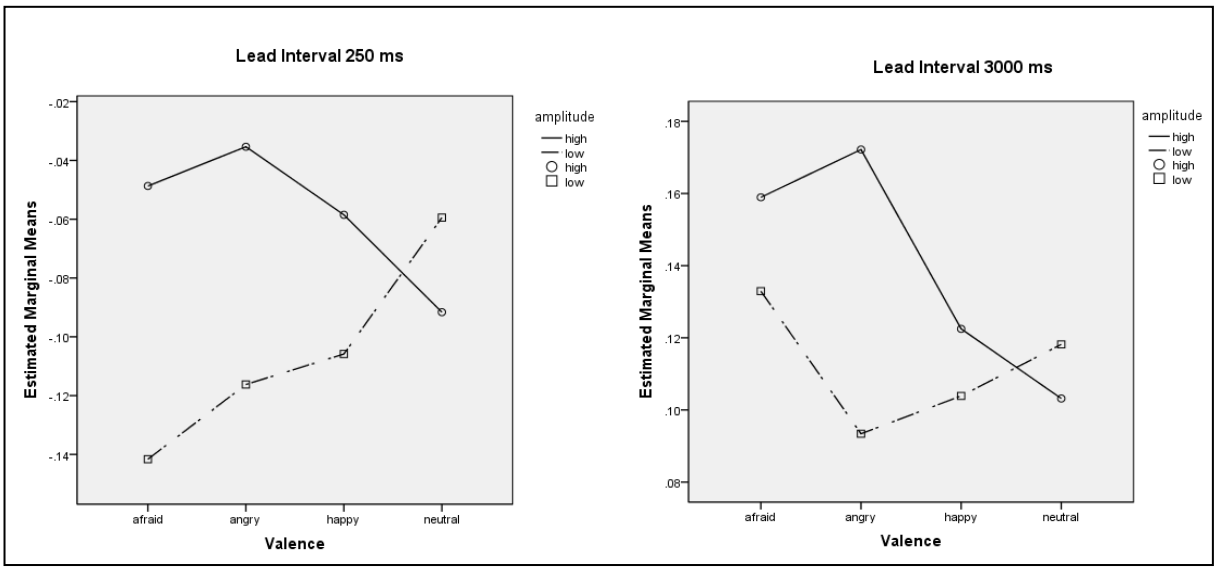

Figure 5: Startle eyeblink response magnitude for all four valences, for high (solid) and low (dashed) amplitude at both latencies (short and long lead intervals)

To test hypotheses 2 and 3, two repeated-measures ANOVA were conducted for short and long latencies, respectively. No main effect for amplitude was found during short latencies, however pointing towards a trend (F (1, 81) = 2.49, p = .118). In comparison, at longer latencies (3,000 ms), the main effect of amplitude did not reach significance either (F (1, 81) = 1.13, p = .291). Also, the three-way interaction between valence, amplitude, and latency did not reach significance (p = .849).

Subjective data

The subjective data test, measuring the difference experienced by the participants towards the pictures and their emotional valences, revealed a clear significant effect for emotional valence (F (3, 21) = 115.40, p < .001). While amplitude did not reach significance (F (1, 23) = 1.47, p = .237), an interaction effect was found for valence x amplitude

(F (3, 21) = 14.78, p < .001). As expected, negative images were experienced as less positive, while pictures with happy expressions scored highest on the positivity scale. Neutral images took a middle position. In general, pictures with higher amplitude elicited more extreme values than low amplitude pictures. In other words, negative images were experienced as even more negative when presented in high amplitude. Alongside, happy facial images reached a more positive score when presented in high amplitude compared with low amplitude (see figure 6).

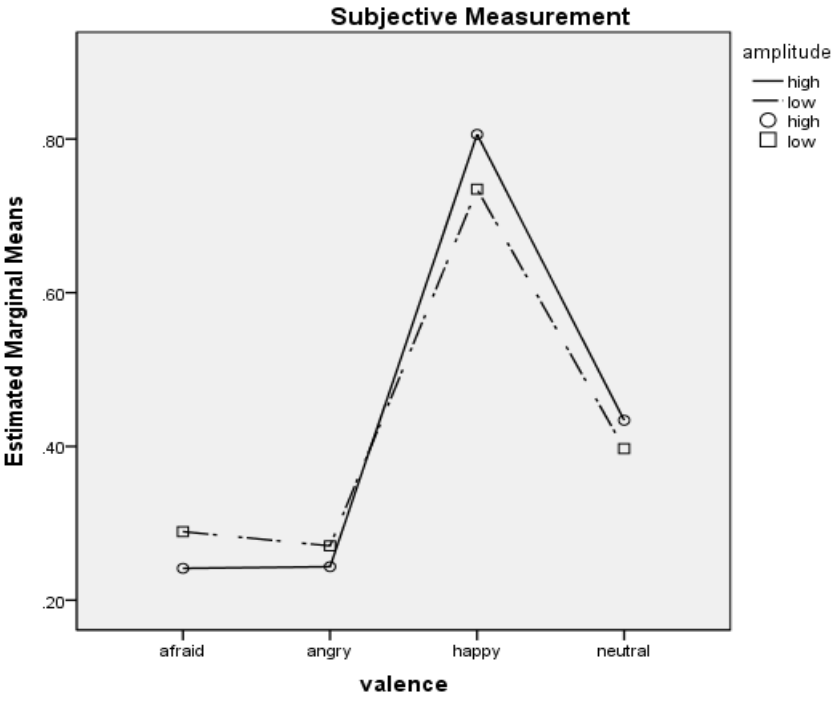

Figure 6: Averaged subjective evaluation of different valences
at high (solid) and low (dashed) amplitude

Discussion

In experiment 1, the startle eyeblink response to LSF pictures high and low in amplitude was measured. As expected, a generally higher eyeblink response was observed when a white noise was elicited while watching pictures with high amplitude in contrast to

low amplitude. Previous studies have shown that LSF pictures activate faster routes in the brain than HSF pictures (Bar et al., 2006; Holmes et al., 2005; Vlamings et al., 2009; Vuilleumier et al., 2003). Due to more neuron-firing to high amplitude stimuli in comparison to low amplitude, a similar effect was expected for LSF pictures high in amplitude but not for those of low amplitude. To test this reaction, the startle eyeblink response was chosen as a robust measurement for physiological and emotional reactions. The outcome indicates that there are routes in the brain reacting more intense to certain features of stimuli. In this case, higher contrast of a facial stimulus initiated a stronger reaction in the brain (a stronger eyeblink response), thus expressing an enhanced emotional reaction regardless of the valence of the picture.

Different emotions were presented in the pictures, ranging from faces showing afraid, angry, happy, to neutral expressions. No main effect of valence was found, indicating that the valence of a picture did not influence the startle eyeblink response. Although negative images combined with an aversive stimulus are known to elicit stronger responses (Dichter et al., 2010), the current finding is in accordance with the hypothesis that it is stimuli features, rather than valence, influencing processes in the brain leading to emotional reactions. Nevertheless, a significant effect of emotional valence was found in the subjective data. Participants had to indicate how positive or negative they experienced the given pictures. As expected, negative images (afraid and angry faces) were experienced as more negative than happy faces, while neutral faces were scoring somewhere in between. Hence, a difference in valence was observed subjectively while this difference could not be confirmed by enhanced startle eyeblink responses.

In addition to that, different latencies of measurement after picture presentation were taken into account. For this, the startle eyeblink response was measured at one out of two possible lead intervals after picture presentation (250 ms or 3,000 ms). Based on this, hypothesis 2 and 3 were pointing at the investigation of faster unconscious versus slower

conscious processes in the brain. However, the hypotheses were not confirmed. Nevertheless, a tendency for hypothesis 2 could be observed with amplitude showing a tendency towards significance for short latencies. This tendency was diminished at longer latencies indicating that longer conscious representation in the brain could have led to another fear response in regard to the pictures. In that case, valence of the facial stimuli should influence the startle eyeblink response much more than amplitude or spatial frequency of the given stimulus. Only after shorter latencies, amplitude and general features of the stimulus should influence an automatic fear reaction. At that time, the cognitive representation should not have reached the neocortex – and with that conscious awareness – yet. However, those hypotheses could not be confirmed by the current results, and further investigations are needed (see general discussion).

Experiment 2

Materials and Methods

Participants

Participants were recruited in the same way as in experiment 1. Thirty-four students between 19 and 34 years participated in the study (M = 23.62, SD = 3.385 for age). Fourteen participants were male. All participants had normal or corrected-to-normal vision.

Table 4

Demographics of All Participants per Gender (n = 34)

Gender		N	Minimum	Maximum	*M*	*SD*
Male	Age	14	20	34	24.71	4.196
Female	Age	20	19	29	22.85	2.519
Total	Age	34	19	34	23.62	3.385

Note. *SD* = Standard Deviation.

Stimuli and apparatus

The same picture stimuli as in experiment 1 were used. In contrast to only presenting one picture at a time in experiment 1, this time two pictures were shown at the same time to the participants. Pictures were paired in the following manner: The picture data set included four different emotions (afraid, angry, happy, and neutral) of two different amplitudes (high and low). Every high amplitude picture of the different valences was paired with each of the other four valences of low amplitude. With this balanced procedure, every kind of emotion with high amplitude was paired with every other emotion of low amplitude. Consequently, 16 pairs were presented (see table 5). For each pair, 10 random pictures of the data set were chosen and presented twice (to the right and to the left hand side of the screen). Hence, a total of 320 pairs (= 16 pairs x 10 pictures x 2 location) were included in the experimental session. Additionally, eight pictures were randomly chosen (two of each emotional valence) to include 32 test trials. Thus, a total of 352 pairs were presented throughout the whole experiment. The stimuli were presented and recorded using MATLAB R2012b with Psychophysics Toolbox.

Table 5

All 16 Possible Combinations of Picture Pairs

Afraid - high	Afraid - low
Afraid - high	Angry - low
Afraid - high	Happy - low
Afraid - high	Neutral - low

Happy - high	Afraid – low
Happy - high	Angry – low
Happy - high	Happy - low
Happy - high	Neutral - low

Angry - high	Afraid - low
Angry - high	Angry - low
Angry - high	Happy - low
Angry - high	Neutral - low

Neutral - high	Afraid - low
Neutral - high	Angry - low
Neutral - high	Happy - low
Neutral - high	Neutral – low

Note. Low = low amplitude, high = high amplitude.

Procedure

After signing the informed consent, participants were seated in front of a computer screen with a viewing distance of around 60 cm. The experimental session was divided into five blocks, and a little break was given after every 75 pictures. Before the first block started, four test trials were carried out to give participants the opportunity to practice and understand the task. Without participants' knowledge, the first 28 pictures of the first testing block were test trials as well, not taken into account for analysis afterwards. In that way, participants could get familiar with the pictures and the task. Including the test trials, there were a total of 352 picture pairs. Before each picture pair, a black screen appeared for a short time, randomly varying between 0.5 and 1 second. Afterwards, a fixation cross was visible in the middle of the screen for 750 ms. The screen then turned black for 100 ms, and subsequent picture presentation lasted for 30 ms. One of the pictures was then directly substituted by the probe

target, a rectangular bar, visible for 180 ms and presented either vertically or horizontally. Half of the times, the target replaced a picture on the left hand side, the other times on the right hand side. This was balanced in the manner that targets replaced pictures of each valence and amplitude equally. Afterwards, participants got 4 seconds to press one of two buttons (H for vertical, space for horizontal oriented bar) using two fingers of their dominant hand. Only one hand was used to avoid left and right confusion, possibly induced by the location of target presentation. Participants were instructed to press as quickly and as accurately as possible. After pressing one of the two buttons a white circle appeared in the middle of the screen, turning green after a correct response, and red when no or a wrong response had been given. After each block and in the end, feedback was presented on the screen, summing up how many targets were identified correctly (%) and showing the average reaction time. Stimuli were presented in random order. Participants received compensation for their participation in form of three lottery scratch tickets, worth 75 Norwegian Crowns (~ 13 US $).

Data Analysis

The first 32 trials, including the four test trials, were excluded from analysis. An exploratory analysis revealed two outliers with a total error rate of more than 30 percent. The two participants were therefore removed from further analyses, and the remaining data set (n = 32) did not reveal any further outliers. The remaining data with 320 trials was analyzed using repeated-measures ANOVA. Mean reaction times were established by averaging reaction times to pictures of all valences high in amplitude over all possible pairs of low amplitude. Hence, an average mean was established for each emotional valence of high amplitude compared with all possible picture pairs. The dependent variable *reaction time* could then be measured using *valence* as a 4-level and *amplitude* as a 2-level within-subject factor.

Results

Exploratory analysis revealed that the data (n = 32) was distributed normally, reaching no significance on the Kolmogorov-Smirnov test of normality (p > .05 for all variables). Additionally, the histograms looked normally distributed. The repeated-measures ANOVA with reaction time as the dependent variable revealed a main effect for amplitude (F (1, 31) = 5.60, p = .024). Higher amplitude led to longer reaction times compared with low amplitude (see figure 7). There was no interaction effect (p = .741) or main effect of valence (p = .538) (see table 6; for mean error rates see table A1 in appendix).

Table 6

Within-Subject Analysis for Valence, Amplitude, and Interaction Effect

Effect	Mean Square	*F*	Sig.
valence	98.823	0.72	.538
amplitude	465.316	5.60	.024*
valence · amplitude	46.163	0.41	.741

Note. *significant at .05 level.

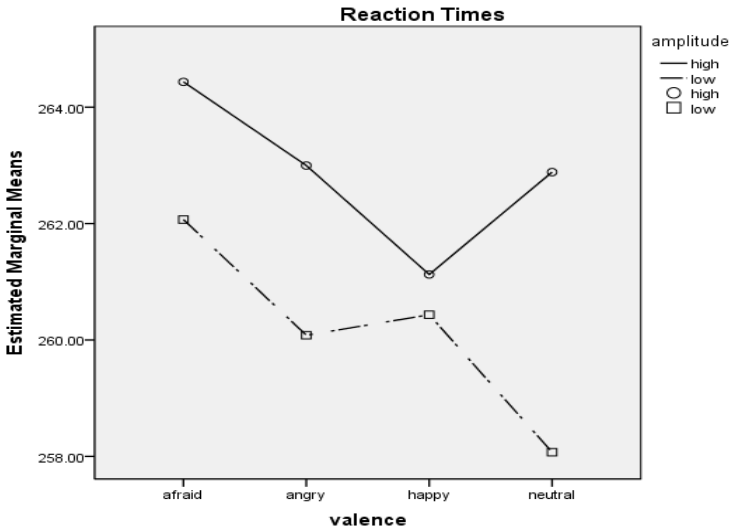

Figure 7: Reaction time means (ms) for all valences, presented with high (solid) or low (dashed) amplitude

Discussion

Hypothesis 4 stated that faster reaction times were expected to high amplitude LSF pictures while slower reactions should occur to low amplitude pictures. To test this, a reaction time task was conducted, in which participants had to press a button as fast as possible after presentation of an orientation bar. The orientation bar was presented either on the left- or right-hand side after short presentation of a picture pair, one facial image with high and the other one with low amplitude, showing dissimilar emotions. When the probe target replaced a high amplitude picture, a faster reaction was expected due to activation of the faster subcortical route in the brain. Nevertheless, this hypothesis was not confirmed by the given study. Quite the contrary, the complete opposite was found: Higher amplitude led to significantly slower reaction times. This happened regardless of emotions expressed in the pictures as neither a main effect for valence nor an interaction effect between valence and amplitude was present.

Although the expected effect was not confirmed by experiment 2, an interesting effect has been found. The fact that higher amplitude in LSF facial stimuli is leading to slower reaction times than low amplitude pictures will without doubt need further discussion and investigation. A possible explanation could be that lower amplitude pictures lead to a reduced masking of the probe target, and hence an easier and faster processing of those pictures. Holmes et al. (2005) found that HSF stimuli contain less contrast information than low spatial frequency pictures, which consequently leads to faster undistorted reactions. With this, the authors explained enhanced response latencies discovered for HSF pictures in general. In other words, higher contrast leads to more neuron-firing, but it also leads to a stronger cognitive representation of the stimulus. This will further lead to slower reaction times as observed in the current study. This theory is additionally supported by the findings of Baek (2012), who observed that attention improved perceptual sensitivity when stimuli high in contrast were presented. According to that, enhanced attention in visual perception could

additionally lead to a longer and more intense representation, which consequently leads to longer reaction times. On the other hand, lower contrast might not be processed as thoroughly. Hence, attention or visual processing may be diminished, leading to a less disrupted reaction and thus enhanced reaction times in comparison to high amplitude stimuli.

Furthermore, previous studies have shown that low luminance contrast is activating the magnocellular pathway (Nieuwenhuis, Jepma, Fors, & Olivers, 2008). Consequently, lower amplitude pictures, as presented in experiment 2, could have activated the faster route leading to faster reaction times. Furthermore, as demonstrated by Fox et al. (2001), an enhanced dwelling time for threatening events might have led to slower reactions times for high amplitude pictures (see general discussion for further elaboration).

General Discussion

In the present study, we conducted two experiments measuring responses to low spatial frequency pictures with high and low amplitude. Experiment 1 investigated the startle eyeblink response, induced by a loud noise, using EMG. The expected outcome of greater startle magnitude to pictures with high amplitude in comparison to low amplitude (hypothesis 1) was confirmed. Furthermore, measurement of the startle eyeblink response after longer latencies (3,000 ms) in comparison to short latencies (250 ms) revealed a significantly stronger eyeblink response. However, hypothesis 2 and 3, stating that a main effect of amplitude should only be found for measurement after shorter latencies while longer latencies should only show a main effect for valence, could not be proven by the present study. In experiment 2, reaction times were measured in regard to high and low amplitude pictures of low spatial frequency. A main effect for amplitude was found, indicating shorter reaction times for LSF pictures of low contrast in comparison to high contrast. Hypothesis 4, stating

the opposite, could thus not be confirmed. Nevertheless, a significant and interesting effect was found.

Based on previous findings, putting forward that enhanced emotional reactions have been observed in regard to LSF facial pictures, the present study used LSF as the primary stimulus feature. Moreover, former studies have shown that effects have been especially strong for facial stimuli in comparison to other pictures (Awasthi et al., 2011). Therefore, merely facial pictures were included in the present study, using pictures from the KDEF data set (Lundqvist et al., 1998). Nevertheless, in former studies little attention has been paid to other cues visible in picture presentation, such as orientation and amplitude. Therefore, amplitude was chosen to be included in the study - combined with LSF stimuli. Few studies have investigated the effect of different amplitudes on fear reaction before. Hence, it was interesting to see how much amplitude influences emotional reactions when presented through LSF facial images. The startle eyeblink response was chosen to measure emotional reactions, due to its advantages in temporal resolution and its good validity (Wilbarger et al., 2009; Åsli et al., 2009). Using this method, it was possible to measure the different responses and the intensity of an emotional reaction regarding high and low amplitude in LSF facial images. Additionally, the component of different latencies of measurement could be added to investigate whether reactions intensify over time, or might act on different routes in the brain.

Although the startle eyeblink response study showed clear significant results, indicating stronger blink responses to high amplitude pictures in comparison to low amplitude pictures of LSF, these results did not offer valuable clues to how fast this reaction occurred. Therefore, a second experiment was conducted, measuring the reaction time in regard to high and low amplitude LSF facial images. In the first experiment, the activation of a faster route to high amplitude pictures should have become obvious by comparing the measurement at two different latencies. However, those hypotheses (2 and 3) could not be confirmed. On the

other hand, in experiment 2, a significant difference could be established showing that reaction times were faster in response to low amplitude LSF facial images.

Hypotheses Revisited

First of all, the finding that different amplitudes are influencing the startle eyeblink response is of vast importance for the current research. As predicted and expressed in hypothesis 1, a generally stronger startle response was elicited by high amplitude LSF pictures, while low amplitude pictures led to diminished startle magnitude. This field of study has not been investigated a lot in the past. There has been a variety of studies regarding the influence of spatial frequency on emotional processes in the brain (Bar et al., 2006; Holmes, Green, & Vuilleumier, 2005; Vlamings, Goffaux, & Kemner, 2009; Vuilleumier et al., 2003). However, amplitude or other features of picture presentation have been investigated much less in the past decades. Therefore, it is of special interest to find this clearly significant effect of amplitude, indicating stronger emotional reactions to LSF pictures presented in higher contrast. Consequently, a possible connection can be seen between stimuli cues and emotional reactions in the brain. Furthermore, as EMG measurements are generally objective methods, this research is of high validity. Overall, there is a large possibility that high amplitude cues are in fact activating a different route than low amplitude pictures, shown in the different reactions measured in experiment 1.

Although experiment 2 could not confirm the expected outcome (hypothesis 4), a truly interesting effect has been found. Reaction times were significantly enhanced to low amplitude LSF pictures at all levels of valence. Prior expectations of faster reactions to high amplitude pictures were thus refuted. As previously mentioned, there are different possible factors that might have led to this unexpected outcome. Nieuwenhuis et al. (2008) state that low amplitude activates the faster, magnocellular pathway. As a result, activation of the faster

route could therefore have initiated a faster response. Additionally, this effect may also be seen in coherence of what was found in experiment 1. There was a significant effect of latency with stronger startle eyeblink response elicited when measured after a longer lead interval. Higher amplitude pictures, generally leading to more neuron-firing, could as well have been represented more strongly in the brain, leading to slower reaction times. In other words, high amplitude has a stronger cognitive representation, which leads to a stronger startle magnitude as well as reduced masking and slower reaction times.

Furthermore, Fox et al. (2001) found that attentional dwell time is enhanced to stimuli when presented with threatening cues beforehand. In this case it was negative images such as angry faces, which led to slower reaction times in detecting a target afterwards. No emotional valence effect was found in the present study; however, experiment 1 demonstrates that high amplitude pictures lead to stronger startle magnitude in comparison to low amplitude LSF pictures. As the startle eyeblink response measures emotional and fear reactions, with stronger blink magnitude indicating more fear, it can be stated from experiment 1 that high amplitude LSF pictures elicit stronger fear reactions than low amplitude pictures. Consequently, pictures presented with high amplitude in experiment 2 can be seen as threatening events leading to longer attentional dwell times, which as a result might have led to slower RTs when presented before the probe target. Accordingly, high amplitude LSF pictures draw attention, and it takes longer for participants to disengage those stimuli, and slower RTs could be observed. Moreover, Fox et al. (2001) suggest this reaction might occur due to evolutionarily developed behavioral freezing, as seen in animals when facing a potential hazard. This cognitive freezing to fear-inducing stimuli consequently leads to slower reaction times.

Another interesting outcome includes the finding of significantly stronger eyeblink responses measured after longer latencies (3,000 ms) in comparison to short latencies (250 ms). This effect is not unexpected, demonstrating a prepulse inhibition (PPI). Prepulse inhibition occurs when an acoustic, visual, or other sensory event is presented shortly before a

stronger startle eliciting stimulus, like a loud noise. This prepulse, presented about 30 – 500 ms before the startle stimulus, inhibits the upcoming startle response, initiating a reduced startle reflex (Braff, Geyer, & Swerdlow, 2001). The PPI is a universal event, happening in all mammals and across modalities. Therefore, a picture presented before a loud noise can lead to a diminished startle eyeblink response. Thus, the finding that the startle eyeblink response was significantly stronger at longer latencies in experiment 1 can be explained by PPI, inhibiting the startle eyeblink response at shorter latencies. When measured after only 250 ms the observed diminished response is therefore most probably due to prepulse inhibition of the presented picture while this effect is absent at longer latencies (3,000 ms). In accordance, Bradley, Cuthbert, and Lang found in their 1993-study that the startle eyeblink response is reduced to pictures of varying emotional valence when elicited before 1,000 ms, while the reflex increased afterwards. Additionally, a change in response to the different emotional valences could be observed. The significantly reduced startle magnitude at shorter latencies could be explained by prepulse inhibition similar to the findings in experiment 1 of the present study.

Furthermore, hypothesis 2 and 3 could not be confirmed by experiment 1. A main effect was expected for valence at longer latencies. After longer sensory processing, consciousness is reached, and automatic reactions should have less effect on emotional reactions. However, a main effect of valence was only found for subjective measurements. At short lead intervals a main effect of amplitude was expected, which could not be confirmed either. Subsequently, possible limitations are discussed that might have led to the given results.

Limitations

Not all the data showed clear-cut results, and while the first hypothesis could be confirmed, hypothesis 2 and 3 were refuted. Hypothesis 4 gave the complete opposite, however significant, result. Clearly, no study is without limitations and possible restraints could have led to problems in data analysis, and interpretation. One limitation of experiment 1 includes the different latencies chosen. It was hypothesized that integration in the neocortex should not have taken place after 250 ms; while at longer latencies (3,000 ms) consciousness should be involved in sensory processing and cognitive representation. Those hypotheses (2 and 3) could not be confirmed. As Park et al. (2012) point out, encoding of HSF pictures in the visual cortex is already completed after 170 – 200 ms. This means that the slower, parvocellular pathway might be faster than expected, and integration of low amplitude stimuli could likewise be integrated much faster as well. Consequently, the expected difference between the two different latencies used in experiment 1 could not be found. Possibly after 250 ms already, the information has been integrated. Follow-up studies are needed to investigate whether shorter latencies might show a difference and confirm the given hypotheses.

Another factor influencing the outcomes of the present study is the fact that this field of study is a rather new one. Magno- and parvocellular systems have been detected, especially in the visual system. However, it is not completely clear how all the connections are set up. Today the two pathways are seen as fairly independent. Still, interconnections most probably exist between the two routes, and a strict segregation is unlikely (Barrett & Bar, 2009). This could also have affected the results of the given study. If information, usually sent through one channel, influences the other channel as well, this can lead to unexpected results. In other words, other results might have been found in experiment 1 regarding amplitude and valence at different latencies if a clear segregation of the two pathways had already been proven a

fact. Interconnections may have led to the unexpected results regarding hypothesis 2 and 3 as well.

Furthermore, the results of experiment 2 can most probably be explained by an attentional dwell effect, as suggested by Fox et al. (2001). However, the effect found in their study proposed that only individuals scoring high on an anxiety scale should show slower RTs to threatening stimuli. Participants of the current study were not tested for possible anxiety disorders, which might have influenced the results. Moreover, the fact that both high amplitude and low amplitude pictures were paired and thus presented next to each other at the same time might have influenced the perception of the stimuli. Possibly both location were attended, making it difficult to distinguish, which of the pictures influenced the subsequent reaction. However, as stimuli presentation was very short, and the probe target was only replacing one of the two pictures, attention should have been mainly led towards that image.

Concluding Remarks

In general, as this field of study is still a new one, clear predictions are difficult to make beforehand. Holmes et al. (2005) showed in a study similar to experiment 2 that faster reactions are elicited by fearful LSF pictures in comparison to neutral LSF pictures, while this effect was not present for HSF pictures. This indicates that there seems to be a faster route activated by frightening events, in this case fearful expressions, which reacts to special features like LSF. Especially the amygdala, as suggested by Vuilleumier et al. (2003), tend to play a major role in processing coarse information obtained via LSF pictures leading to an enhanced emotional reaction. The findings of experiment 1 support the theory that it is much more picture presentation than valence influencing our emotional responses. Previous studies have shown that negative events are associated with a higher startle eyeblink response when paired with an aversive event such as a loud noise (Dichter et al., 2010). Additionally, the

subjective study confirmed that different valences were perceived and rated of varying positivity. Therefore, recognition of the correct emotional valence was evident, and a difference in startle eyeblink response could be expected. However, valence did not show a significant effect in the EMG study. This indicates that - when presented with certain cues such as spatial frequency and amplitude - our brain is reacting more to these cues than what is presented emotionally on the pictures.

Regarding this, some interesting findings have been established by the current study. If our brain is primed to react to stimulus features much more than its emotional valence, this can be of substantial importance for current brain research and cognitive neuroscience. Until now, most of startle eyeblink response studies were concentrating on the effect of negative or positive expressions in the pictures. The fact that those might be influenced by its feature characteristics such as spatial frequency, orientation or amplitude, only got more in focus in recent studies. While spatial frequency and especially the difference between HSF and LSF have been investigated in more detail, the influence of amplitude on emotional and sensory processes is harder to find in the recent literature. Therefore, the significant effect found in experiment 1, is a solid first step. More investigation is needed to clarify effects of amplitude, in addition to orientation, to make implications for the future. Further confirmation of the hypotheses could then possibly lead to improved help for patients suffering from anxiety disorders, visual or sensory difficulties. As contrast sensitivity is a great issue regarding different neurological problems, such as MS (Baek, 2012), it might be an immense benefit to figure out the principles behind visual perception and how features of a stimulus are affecting our perception. Additionally, patients suffering from autism spectrum disorder might profit from a better understanding of facial perception, as distorted facial perception is a common problem, and many of their social problems occur in response to faces (Golarai, Grill-Spector, & Reiss, 2006).

Overall, the findings made by the present study are interesting in many ways. Probably the most important finding was made in experiment 1, finding a significantly stronger eyeblink response to high amplitude pictures. Additionally, a stronger response found at longer lead intervals could be explained by the prepulse inhibition effect present at shorter latencies. Furthermore, the unexpected significant result of experiment 2 brings up further questions regarding reaction times to different presentations of amplitude. Yet, further investigations are needed here to give better insight in this new field of research and make beneficial implications for the future. Especially, follow-up studies will be needed to clarify the effect of amplitude on reaction time.

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Appendix

Table A1

Mean Reaction Times (RT) in ms, and Mean Percentages (%) of Errors and Their Standard Deviations for Experiment 2 (n = 32)

	M RT	*SD* RT	*M* % error	*SD* error
AF-H	264.43	54.042	6.88	4.623
AF-L	262.07	55.902	8.36	5.944
AN-H	263.00	53.828	6.88	5.236
AN-L	260.09	57.384	5.00	4.445
HA-H	261.13	58.649	7.11	5.317
HA-L	260.43	55.976	5.31	5.149
NE-H	262.89	57.060	6.41	6.316
NE-L	258.07	54.486	6.72	4.728

Note. AF = afraid, AN = angry, HA = happy, NE = neutral, H = high amplitude, L = low amplitude.