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Effects of threatening pictures on encoding behavior of subsequent stimuli.

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Abstract

Research regarding episodic memory and emotion shows that emotion has a modulatory effect on memory. The aim of the present study is to investigate if exposure to emotionally loaded images could potentially change the way future neutral images are encoded, and if this exposure also modulates the memory experience of these images. In addition to this, data resulting from questionnaires regarding participants anxiety levels are also recorded and incorporated in follow -up analysis which examines the potential relationship between this exposure and individuals anxiety levels. To investigate the relationship between prior emotional experiences and memory encoding, the present study examines how a sequence of neutral and emotionally loaded images are encoded and how these images are subsequently recalled from memory. To capture the memory encoding process, eye-tracking is used to monitor participants' gaze behavior when they inspect and encode different images. Results of the present study offer novel insights into the relationship between emotion and memory and show that (a) memories of emotionally charged and neutral images were experienced differently; (b) that participants looked back to locations where a previous image was presented during encoding and that such gaze behavior had an effect on the subsequent memory experience; (c) a relationship between higher levels of anxiety and the tendency to look back at positions where emotionally charged stimuli were encoded.

Keywords: Eye movements, emotion, episodic memory, encoding, STAI, EmoPicS, Thank you!

I wish to express my sincere appreciation to my supervisors Roger Johansson and Sabine Schönfeld, not only for the opportunity to take part in this research project but also for all their invaluable advice and support guiding me in the right direction. I also wish to acknowledge the support and of my lovely friends and family, they kept me going on and this work would not have been possible without their input.

Vision is a complex interaction with our environment, it relies on learned information as well as being shaped by the biological constraints of our brain (Kapplas 2008). We use our eyes every day to navigate our environment, to scan and register those things that are relevant and informative to us and the more informative aspects usually attract more attention and also more fixations. There are however, many parts of our environment that can be informative to us, these are usually in flux and depend on our current goals and expectations (Buswell, 1935; Yarbus, 1967). If you are searching for a friend in a busy street, her red signature cap will be informative for your search while if you are driving a car down the same busy street the traffic signs and fast approaching cyclists will be more informative, still other more subtle aspects like something bright or a sharp contrast can inform our eyes where to look (Buswell, 1935). One aspect of our environment that in general seems to be informative to us is emotion and a connection between between gaze-behavior and emotion has started to emerge in the literature, (Öhman et.al., 2001; Nummenmaa et.al., 2006; Calvo & Lang, 2005; Simola et.al., 2015; Subramanian et.al, 2014) it shows that emotionally loaded stimuli typically attract more fixations compared to neutral stimuli.

A large body of research has established a tight relationship between memory formation and gaze-behavior (e.g., Olsen, Lee, Grady, Rosenbaum & Ryan, 2016; Liu, Shen, Ryan, 2017), where more fixations on an object usually leads to stronger encoding and in turn better memory. Eye movements can in this way be used as a means to study cognitive processes. Capturing what is attended to constitutes a premise for which information gets encoded into memory and which does not. Studying eye movements can thus lead to valuable insight about how we attend to and encode information. Where we look determines what we encode and remember, what we remember, and know influences where we look next (e.g Altmann, 2004, Kapplas, 2008; Yarbus, 1967). The aim of the present study is to investigate if emotionally loaded images could potentially change the way future events are encoded and subsequently remembered. The study will also explore if participants' anxiety levels could influence this encoding process.

Background

As an introduction, some of the basic definitions of the different components underlying the research question addressed in this paper will be explained. Starting with the memory systems and the connections to eye movements ending with a brief presentation of attentional bias relating to anxiety.

Episodic memory

The memory systems can be split up and categorized in many ways. The first level of classification is short-term or working memory, we use this type of memory when repeating a phone number to ourselves or the name of the classroom where the next lecture is held. It holds sensory, motor, and cognitive information for shorter periods of time. The second kind of distinction at this first level of classification is long-term memory, it is complex and can be divided up further into conscious/explicit and unconscious/implicit forms of memory, also described as declarative and procedural memories. Episodic memory is a type of declarative memory, which holds information about specific events, when and where those took place, related emotions to these as well as information about our own role in an event. If you are trying to remember the first time you learned to ride a bike, then you are using episodic memory. The other kind of declarative memory is semantic, which is memory for facts, language, and knowledge, like how many days there are in February (Squire, 2004). Episodic memory is different from other types of memory in several ways; it is often represented in the form of images and always has a perspective (field or observer). It makes remembering specific, which is an important part of memory that helps us understand how we fit in the world around us. Episodic memories are also subject to rapid forgetting and change, in general, episodic information is more summarized in character and generally more like a representation of an experience than a literal record (Conway, 2009). But before we can process any event (or fact) the information needs to be transformed into a form that can be stored in our brain.

Encoding and long-term memory. Encoding refers to the initial experience of perceiving information, it is the process of moving information from the temporary storage in working memory to the more permanent long-term memory. Even though long-term memory has no limit to how much information can be stored (Kolb-Whishaw, 2012), it is not possible to encode every stimulus we encounter. As such, one important aspect of encoding is that it is selective, i.e. we need to decide what information to attend to, where we fixate is a big decider for this and what we fixate on is what gets processed in working memory. Different mechanisms drive this selection, they can be described in terms of top-down and bottom-up processes. Bottom-up processes are driven by external features in the world that attract and pull our attention where top-down processes relate to our current goals, previous experiences, knowledge and expectations about the world, these direct our attention (Kolb- Whishaw 2012; Xue, 2018). Either or both of these processes are involved when selecting where to focus. Through this interaction the previous experiences we had are tightly connected to and influences how and what we are going to experience next.

For a memory to become a long-term memory it must first enter sensory memory, for instance iconic memory for visual information, from there it passes into working memory where the information needs to be held long enough for it to get stored in the different brain regions connected to the sensory organs involved. The last step of the memory process is consolidation, the process by which, encoded information becomes more durable over time (Baddeley, 2000). Since consolidation is considered a slow process, during which memories are not yet considered permanently set, this might allow an opportunity for an emotional reaction related to an event to influence its storage and increase the likelihood of it being remembered. The idea is that the way information is encoded affects how well it is remembered. The deeper the level of processing, the easier the information is to recall, emotion is one aspect that adds depth to encoding (for a review see McGaugh, 2000).

Episodic memory and emotion. The brain structures associated with memory play crucial roles in regulating emotional behaviors as well. This interaction occurs at various stages of information processing, from the initial encoding and consolidation of memory traces to the long-term retrieval of these (for a review see LaBar & Cabeza, 2006). The brain structures associated with emotional behaviors is called the limbic system which is composed of two larger brain structures: Limbic cortex and the Hippocampal formation (Pierri- Lewis, 1995). Although all structures of the limbic system take part in regulating emotional behavior, the more interesting connections, however, when looking at episodic memory are between the hippocampus and amygdala (for a review see LaBar & Cabeza, 2006). Emotional learning has a strong influence on memory formation and its long-term consolidation; the amygdala, in particular, is a structure that directly modulates the relationship between memory and emotion, without which emotional effects on memory cannot occur. It can also affect explicit memory by modulating or enhancing the activity in other brain regions involved in memory in more indirect ways. The emotional information is processed via subcortical (unconscious) pathways that allow for faster transmission compared to the cortical (conscious) pathways in the cerebral hemispheres (for a review see McGaugh, 2000; McGaugh, Cahill, 1996; Tamietto & Gelder, 2010). Damages to any of these structures have been shown to affect both memory and emotion to different extents. Subject with normal amygdala function has been shown to remember the emotional content of a story in more detail than the neutral content, while subject with damage to the amygdala remembers the whole story in the same detail as the healthy group recalls the neutral content (Cahill, Babinsky, Markowitsch & McGaugh, 1995). The amygdala is an important structure that helps connect emotion to the rest of our experience, often without our awareness (Tamietto & Gelder, 2010).

The fact that emotion influences memory has been shown many times over in the literature, several findings show that participants' performance on long term memory tests were significantly better for emotional images and movie clips compared to images and clips with neutral content (Labar & Phelps, 1998; Subramanian, Shankar, Sebe, and Melcher 2014). Another interesting finding was that emotional content strengthens encoding for scene gist as well as weakening encoding of peripheral content (Rigss, Farb, McQuiggan, Anderson & Ryan, 2011; Everett & Koster, 2015). A more recent study by Tambini, Rimmele, Phelps and Davachi (2016) showed that neutral stimuli exposed 9–33 min after first being exposed to emotionally arousing stimuli were recollected to a greater extent during memory testing compared to those studied before emotional and after neutral stimulus exposure. The results indicate that an emotional experience can persist in time and bias how new, unrelated information is encoded. As mentioned above emotional aspects of an event add depth to encoding (for a review see McGaugh, 2000), it has a modulatory effect on memory in both spatial and temporal terms. The modulatory effect of emotion on memory is essential for us to be able to decipher which of the myriad of stimuli we encounter are important and which are not, which of them should be prioritized for encoding.

Just because something is emotionally loaded does however not mean it has been encoded, so how do we know if something has in fact been encoded or not? Several studies show that tracking eye movements can be used as a measure of this, as an indicator of the existence of a memory (Hannula et al., 2010). Recent research has shown that fixations made on a scene or picture is an adequate measure of what will subsequently be remembered; the number of fixations made on a picture or scene correlates with the level of encoding, with more fixations leading to deeper encoding (Olsen, 2016; Liu et.al., 2017). Eye movements can in this way be used as a means to capture what is attended to and thus forms the basis for which information gets encoded into memory. Studying eye movements can thus lead to valuable insight about how we encode information.

Eye movements and visual processing

Rays of light hit the photoreceptors of the retina where light is transformed into electrical impulses that travel through our eyes via the optic nerve, branching out in visual pathways that finally end up in the visual cortex in our brain for final processing (Yarbus, 1976; Kolb &

Wishaw, 2012). The process that turns rays of light into comprehensible objects, places, and faces depends on our eyes moving around, scanning our environment. We rapidly move our eyes across the scenes before us to explore and sample the world, these movements are called saccades. We engage in this behavior more than 170,000 times per day, making about 3 saccade movements each second (Kapplas 2008). Even though it feels like everything in front of us is in sharp focus, the fovea is the only part of our eyes that can detect details and color, where it is directed is the only area that we can actually see acutely, about 1.3 degrees of the visual field (Yarbus, 1967) corresponding to about a thumb, held up at arm's length. Doing this should give an indication of where the fovea is now directed and how much of what is in front of you actually is in focus. The function of the saccades is thus to move this small focus around, which is essential for good perception of what the world and the scene before us are like (Yarbus, 1967; Kolb & Whishaw, 2012).

So what determines where we look? Buswell (1935) noticed that viewers tend to cluster fixations to informative regions, but what makes a region informative? This is not constant and can depend on both external, physical properties of objects such as color or sharp contrasts but also on internal processes related to previous experiences and our expectations about the world. The external features that attract our attention can be described as bottom-up processes. By recording eye movements of participants watching scenes and pictures, several studies (e.g. Itti & Koch, 2000) have identified which features drive these processes (e.g., contrasts, light, edges, luminance). However, the bottom-up process is not the sole explanation to what drives attention. An example that shows that where we look is determined by more than bottom-up saliency is a finding by Altmann (2004), who demonstrated that participants often fixate on “blank spaces”, i.e. locations where goal-relevant objects had previously been encoded but is now blank and thus absent of any relevant information (Altmann, 2004). This shows that previous experiences have a strong influence on how eye movements are directed. This is what is called top-down processes, where attention is directed rather than attracted. In another classic example, Yarbus (1967) asked participants to determine the age of a group of people in a picture, participants would look more at their faces, in contrast to when they were asked to evaluate the material wealth of the group, fixations were more directed towards furniture and clothing (Yarbus, 1967). Task relevant instructions and goals also influence where we direct our eyes. The two processes described above control how attention is either covertly or overtly directed to the object of interest (Hendersson, 2007). It is important to note that these processes do not take place in isolation but unfold simultaneously and thus both of these determine which information will be encoded into memory when we experience a course of events.

Connecting emotion to eye movements.

The behavioral effects of emotion on attention has been shown in studies that use gaze as a measure of overt visual attention, people tend to fixate first and look longer at emotional pictures that are presented side by side with neutral pictures (Calvo & Lang, 2005; Nummenmaa, Hyönä, & Calvo, 2006). Emotional content draws eye fixations and strengthens memory for images with negative emotional content, this has been demonstrated in a study by Öhman, Flykt & Esteves (2001) where negatively loaded images attracted eye fixation faster compared to images with neutral content. More recent research (Subramanian, et.al, 2014) also found that different emotions can drive different kinds of gaze behavior. In a study with natural scene viewing, both the speed of participants' initial fixation but also the number of fixations and gaze duration were significantly enhanced for emotionally salient regions for unpleasant v.s pleasant images in a high arousal condition (Simola, Le Fevre, Tornaiainen & Baccino, 2015). In this case the emotionality of the stimuli causes a reaction where initial fixations are made faster, but it could also be a predisposition or sensitivity that causes a quicker reaction towards the stimuli.

Anxiety and eye movements.

Anxiety can be described as this kind of predisposition, the clinical condition is associated with a lower threshold for appraising threat (Mogg & Bradley, 1998). Another related but distinctly different aspect usually present in the anxiety literature is attentional bias. AB is described as the tendency to preferentially attend to threatening stimuli over other competing stimuli (Mogg & Bradley, 1998). Early cognitive views of anxiety propose that individuals with high levels of anxiety have an endurable automatic driven AB to threat compared to low-level individuals that tend to be threat avoidant (Bar-Haim, 2010). Later empirical work, however, shows that highly anxious individuals only sometimes exhibit AB to threats and sometimes not. ABs could, therefore, depend on multiple processes, including automatic salience-driven processes and goal-directed cognitive control, these can also be described in terms of bottom-up, stimulus-driven and top-down executive controls (Mogg & Bradely, 2016). These processes are, as mentioned in earlier sections, always at play.

One characteristic of AB is the expediting way in which threatening stimuli are detected. This feature of AB suggests that attention is drawn to the location of a threat stimuli more quickly and easily than to other stimuli. A second important characteristic of attentional biases is what is called difficulty disengaging (Fox, Russo, Bowles, Dutton, 2001; Salemink, van den

Hout & Kindt, 2007). According to this view, attention is maintained at the location of a threat stimulus and more effort is required to disengage. There are several different ways to assess AB in anxiety, Stroop tasks and the dot and probe tasks are two very common methods (MacLeod, Mathews, 1986; Williams, Mathews, & MacLeod, 1996). Eye-tracking is another method that is becoming increasingly more practiced within the field (Armstrong & Olatunji, 2012; Skinner, Hübscher, Moseley, Lee, Wand, Traeger, Gustin, McAuley, 2018).

Aim and hypothesis

Based on the literature reviewed above, some interesting questions can be asked. The consensus in the memory literature is that emotion has a modulatory effect on memory (for a review see McGaugh, 2000) and one could therefore expect that the presence of emotion will affect both encoding and memory experience.

The aim of the present study is to investigate if emotionally loaded images could change the way future events are encoded and subsequently remembered (Tambini et.al, 2016). To investigate this, participants will inspect a sequence of neutral and emotionally loaded images within the same context and recall these from memory. Upon recall they will answer questions relating to their memory experience of the images. To capture the memory encoding process and the influence of emotional images, eye-tracking is used to monitor participants' gaze behavior when they inspect and encode the images. Previous research has established a relationship between encoding and gaze-behavior (Olsen et al., 2016; Liu et.al., 2017), where more fixations lead to better encoding, as well as a connection between gaze-behavior and emotion (Öhman et.al., 2001; Nummenmaa et.al., 2006; Calvo & Lang, 2005; Simola et.al., 2015; Subramanian et.al, 2014), where emotionally loaded stimuli typically attract more fixations compared to neutral stimuli. Adding to these findings, previous research has shown that people frequently look back to locations, where a stimulus has previously been encoded, but is now absent (Altmann, 2004; Johansson & Johansson, 2014). Such "looking at nothing behavior" can thus be used to capture the reactivation of previous memories associated with a particular space, making it possible to track to what extent previous emotional information gets reactivated when novel non-emotional information gets encoded. The "looking back"(here after LB) behavior shares some resemblances with findings (Fox et.al., 2001; Salemink et.al., 2007) from the AB literature where individuals with higher levels of anxiety have a difficulty disengaging with emotionally loaded stimuli. Based on this, anxiety measures were also gathered from the participants to explore whether level of anxiety influences the encoding process.

In accordance with the research goals of the present study, the following hypotheses/expectations were formulated:

i) Participants' memory experience during recall will be different for the emotionally loaded images compared to the neutral images.

ii) Inspecting an emotionally loaded image before a neutral image will modulate the subsequent memory experience for the neutral image, but not the other way around.

iii) Inspecting an emotionally loaded image before a neutral image will influence encoding of the neutral image, but not the other way around. Specifically, it is predicted that participants will look back more towards the place where the previous picture was presented during encoding, when that picture was emotionally loaded, and that such gaze behavior will have a direct influence on the subsequent memory experience.

Additionally, it is expected that the looking back (LB) behavior exhibited during encoding will relate to the participants' level of anxiety. If anxiety takes any part in this interplay, how does that look, and can it help explain the relationship between emotion and memory experience?

Method

A total of 33 subjects, 14 women and 19 men participated in the present study, all students at Swedish universities with an average age of 23.06 years (SD 2.02). All participants stated that they either had no vision problems or had corrected for any visual errors. All participants gave informed consent and were remunerated for their involvement. Participants were recruited through social media. [1]

Apparatus and Material

The experiment was designed in Psychopy3 and was administered using the same program. The images used as stimuli were taken from the International Affective Picture System databases (IAPS; Lang, Bradley & Cuthbert, 2008) and Emotional Picture Set (EmoPicS; Wessa et al., 2010), which have previously been used in similar studies comparing emotional and neutral stimuli. Together, the databases consisted of 1554 images, from which 64 images, 32 neutral and 32 emotional were selected. The selection process for the images by Setenberg & Hidleman (2019) was based on values of valence and arousal parameters for each picture, where both values were based on a scale from 1-9 for each image, 32 image pairs were formed based on

[1] The original plan for this paper was to record additional data using another 30 participants but, due to the Covid19 outbreak, this was not possible. Instead, the analysis was based exclusively on a subset of the original data recorded by Stenberg & Hildeman 2019.

thematic similarities. In addition to the main experiment, participants performed an anti-saccade task (Hallett, 1978; Hallett & Adams, 1980), which also was designed in PsychoPy3.

Stimuli were presented to participants on a Tobii Pro Spectrum display, (EIZO FlexScan EV2451) with the resolution 1920 x 1080 pixels (52.8 x 29.7 cm). The participants were placed in front of the screen with their faces at a 63cm distance from the display and were instructed to position themselves so that their eyes mapped on to the center of a calibration circle visible on the screen. Chin and forehead supports were used, against which the participants placed their chin and forehead to stabilize and minimize head movements. Stimuli were presented on the screen through PsychoPy3, both during the main experiment and during an anti-sack task that followed. A focus of this study was long-term memory function and thus a distraction task was used to eliminate the possibility for the research participants to keep stimuli information active in the working memory. This was done using a Brown-Peterson task (Brown, 1958; Peterson, 1959), which has proven to be an effective way to clear working memory (Proctor & Fagnani, 1978).

Anxiety was measured using the State-Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983), designed to measure the participants' anxiety level in the moment. The STAI state anxiety scale has good internal consistency, ranging from .86 to .95 (Spielberger et al., 1983). In the current study the Cronbach alpha coefficient was .89.

Design

The whole experiment consisted of 32 blocks divided into two separated conditions, the conditions were set for free (16) and fixed (16) viewing, where the first condition meant that the subjects were free to look anywhere on the screen during all phases of the experiment (both encoding and retrieval). During the fixed condition, participants were instructed to look at a fixation cross during the recall phase. The conditions were not intermingled, this means that half of the participants started with the fixed condition and half with the free, upon completion of 16 blocks the conditions were switched. Participants were randomly assigned to their starting condition.[2]

Each block consisted of an encoding phase with two images, a distraction task, a recall phase, and finally three questions that should be answered for each image. Each image pair consisted of a neutral and a negative image. Saliency and arousal were controlled by paring

[2] In this study only data relating to the free viewing blocks of the experiment were used.This is because the gaze restriction during retrieval (fixed) was not part of the current research questions. Fixed and free conditions where thus not intermingled and could be analysed separately.

pictures based on similarities. Data from the eye trackers include fixations and duration of fixations. The questions were designed to collect data regarding the participants' experience of their memory.

The main experiment was designed to take about 35 minutes to complete and the entire procedure, including training task, anti-failure task, memory test, and self-assessment form, took about 60 minutes.

Procedure

Initially, the participants were informed about the purpose of the study. Upon entering the lab, they were instructed to sit down at a computer where they could read through and fill out a consent form which explained the terms of their involvement. Subsequently, participants entered their age and gender on the computer, after which a training session preparing for the real experiment was initiated. Instructions were visible on the screen, before, as well as during the training session which they manually clicked away from. After the training session, new instructions appeared on the screen indicating to participants to raise their hand whereupon an experimental leader came and calibrated the eye movement equipment to the participants' eyes, and after this the experiment began.

Encoding. The experiment consisted of and could be divided into two interdependent phases, the first phase being the encoding phase, the participants' task was to inspect pictures appearing to them at different places on the screen (see Figure 1). Each trial started with a fixation cross appearing in one of three boxes on the screen, the box at the lower part of the screen (hereafter referred to as box B). All three boxes were held constant throughout the experiment, the other two covering the upper half of the screen, one on the left side (box L) and one on the right side (box R). After fixating for one second on the fixation cross an arbitrary symbol (# or @) appeared in either box L or box R, the symbols functioned as retrieval cues for the next phase. After viewing the recall symbols for two seconds, a picture immediately appeared in the same box. Next, the second retrieval symbol appeared for two seconds in the box that was previously empty, followed by another picture. Each picture pair was followed by a distraction task in the form of the Brown-Peterson task. The participants were instructed to count backwards for 10 seconds starting from a randomized three-digit number appearing on the screen before them, which concluded the first phase of the experiment.

Retrieval. In the second phase the participants' task was to recall the pictures from the encoding phase. After the distraction task, a fixation cross appeared in box B for one second, whereby either # or @ was displayed for two seconds, which, in the encoding phase had been associated with an image. During the retrieval phase, participants were free to look wherever they wanted on the screen while trying to recall the image from memory.

After one retrieval, the following three questions regarding recall, discomfort, and avoidance appeared on the screen:

> *1. "Was your memory image vivid, clear, and detailed - almost as if you could see the image in front of you again?"*
> *2. "How much discomfort do you experience from this memory?"*
> *3. "How much would you like to avoid thinking about this image?".*

Participants were asked to answer on a scale of 1-6 , 1 indicating "not at all" and 6 indicating "very much". The same procedure was repeated for the second picture in the image pair which concluded one block. This concluded the main experiment.

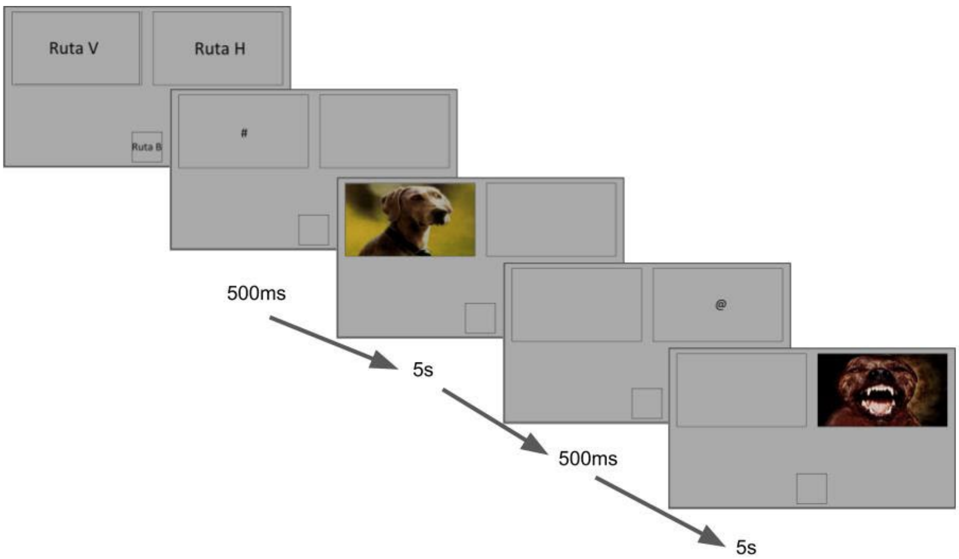

Figure 2. Paradigm for encoding one image pair. During the encoding phase, two images are presented to participants and their discovery was to remember which character was linked to which image and then can recall the correct image when the character presenters in the recall phase.

The same procedure was repeated for the second picture in the image pair which concluded one block. After completing all 32 blocks of the main experiment the anti-saccade task was administered; the participants were instructed to either follow a dot with their eyes on the screen as it moved from side to side or to disengage and look in the opposite direction. After the anti-saccade task participants were taken out of the lab to complete a memory questionnaire and the four self-assessment forms STAI, PTQ, HADS and DERS. [3]

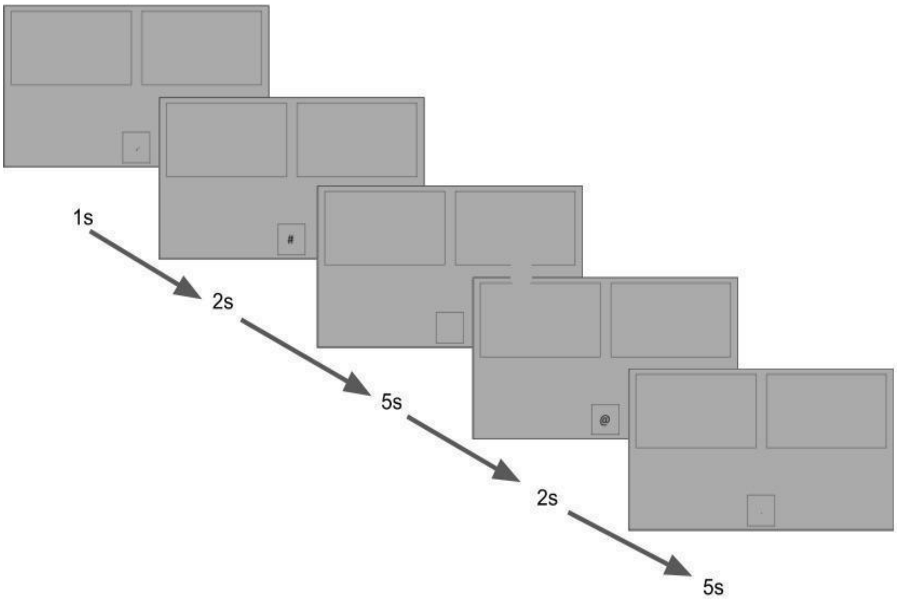

Figure 3. Paradigm for retrieval, fixated condition. The task was to remember the pictures presented during encoding.

Ethics

All participants received both verbal and written information about the study being voluntary, as well as their right to withdraw from the study at any time without giving any explanation. Participants were informed that no dead bodies and no sexual motives would occur, questionnaires regarding suicidal thoughts and traumatic memories were not selected. All participants gave written consent. Anonymity of the participants was ensured by assigning each person a number that was linked to their results (Stenberg & Hildeman, 2019). The present study complies with the informed consent that participants received in the original data collection, which states that the material can be used in research about memory and cognition. The consent form explains participants' right to withdraw at any time, that their identity can not be connected to their data, as well as how the data will be stored and that it will be used in analysis. They were

[3] Only the STAI questionnaire was used in this study, the other questionnaires were part of other research questions within the bigger research project.

informed that the data can in the future be presented and used in teaching and seminars. They were also informed about their right to contact the experiment leaders and to have theri data removed or to get information about the result presented in the paper. Everything the participants agreed upon at the time of data collection is compatible with the present study.

Data analysis

The eye tracking data was analyzed by measuring the number of fixations and fixation time in three predefined areas of interest (AOI). These AOIs corresponded to the places where a picture could be presented (box L, R). To be able to know anything about the relation between encoding behavior and rating scores. A rather clear bimodal distribution in the tendency to look back to the location where a picture was previously encoded was observed in the data: while some participants looked back at the previous location, others did not exhibit this behavior at all. The threshold for being categorized as exhibiting look back behavior was to, on average, have at least one fixation in the empty AOI across trials during encoding. This mean to have at least 1 fixation on average over the 16 trials in one block.

. Based on this bimodal data distribution, a binary between subject's variable was created, grouping the participants into either "LB" (n= 21) or "no LB" (n= 11). Based on participants' STAI scores a second binary between subject's variable was created, for high (n= 17) and low (n=13) levels of anxiety. Each participant's mean value STAI scores was calculated, and based on this calculation, a media distribution was made that divided the participants into either high or low group. The main type of analysis used in this study are repeated measures analysis of variance with follow-up paired samples t-tests in case of interaction effects. To correct for multiple comparisons, the analysis is made with Bonferroni correction. Assumption checks for the data in all analysis were made

Picture type and rating scores. It was expected that the participants' memory experience during recall would be different for the emotionally loaded images compared to the neutral images. Therefore, the first step in the analysis was to see whether rating scores (RS) for the neutral picture differed compared to the threatening pictures. This was done using paired samples t-tests. The RS corresponds to the three questions each participant answered at recall. This was done to see if the participants actually experienced any difference between the threatening and neutral pictures which was essential to establish in order to move forward with the other hypotheses.

Picture type, order and rating scores. To investigate memory experience, three separate 2x2 repeated ANOVAs were set up, using Picture type (neutral, threat) and Order (1st, 2nd) as factors. The ANOVAs were matched to each of the different RS (vividness, avoidance and disturbance) which were set as dependent variables. The analysis measured the difference in RS for the neutral pictures presented as first compared to presented as second, and where threatening pictures were used as a control (i.e. no order effects were expected for the threatening pictures). This part of the analysis was one of the main research questions and was designed to establish whether the memory experience of a neutral image would be modulated when presented after a threatening image, but not the other way around.

Picture type, order and encoding behavior. To investigate encoding behavior two separate 2x2 repeated measures ANOVA were set up using Picture type (neutral, threat) and Order (1st, 2nd) as factors with number of fixations made and fixation time spent on a picture as dependent variables. The analysis measured if the encoding behavior differed depending on the type of picture (neutral vs. threat) and the order (1st vs. 2nd) in which the images were exposed. This analysis was thus capable of directly investigating if the encoding behavior (as measured by fixation data) for a neutral picture was modulated by a previous threatening picture.

Looking back and order. To investigate LB behavior, two separate 2x2 repeated measures ANOVA were set up in the same way as above, using Picture type (neutral, threat) and Order (1st, 2nd) as factors and with number of fixations made in the empty AOI (were an image had previously been presented) as the dependent variable. The goal of this analysis was to examine if the previous image would cause participants to look back to where it had recently been presented when encoding the new picture and if such behavior interacted with Picture type.

In the next step, the participants were categorized as either "LB" or "no LB" based on how many fixations on average they made in the empty AOI (i.e. the area where the previous picture had been presented). The threshold for being categorized as having LB behavior was to on average, at least have one fixation in the empty AOI across trials.

Looking back, order and rating scores. To investigate memory experience and LB behavior, three separate 2x2 repeated measures ANOVAs matched to each of the RS (vividness, avoidance and disturbance) were set up, for both neutral and threatening pictures. The ANOVAs were set-up with Order (1st, 2nd) as a within subjects' factor and with LB behavior as a between subjects' factor (LB, no LB) on the dependent variable RS. The goal of this analysis was to investigate if the LB behavior was related to the subsequent memory experience, with the assumption that LB behavior indicates that aspects of the previous pictures were reactivated when encoding the new picture.

The analyses described above were conducted to test the three main hypotheses of this paper. In addition, an exploratory analysis was also conducted to examine the potential influence of anxiety on the LB behavior.

STAI, looking back, order and rating scores. To investigate if the LB behavior was related to higher levels of anxiety, participants were first divided into groups, either high or low depending on their scores on the STAI state questionnaire. Participants with higher scores correspond to higher levels of anxiety and vice versa for participants with lower scores. One repeated measure ANOVA was set up, with Order (1st, 2nd) as a within subjects' factor and with LB behavior (LB, no LB) and STAI (high, low) as between subjects' factors.

Results

Picture type and rating scores: Memory experience of neutral and threatening pictures

To see if participants rated the neutral and threatening pictures differently, the rating scores (RS) for each person was analyzed using paired samples t-test for each of the three categories of RS (vividness, avoidance, disturbance). The results from these showed that participants reported to recall the threatening pictures more vividly (M = 4.591, SD = .769) compared to the neutral pictures (M = 4.316 SD = 0.814), t (32) = 4.44, p <.001. The same was true for wanting to avoid thinking about the threatening pictures (M = 2.548, SD =.947) compared to neutral (M = 1.326, SD =*.345*) t(32) = 7.83, p < .001 as well as finding them more disturbing (M = 2.873, SD = .947) compared to neutral (M= 1.339, SD = .349) t (32) = 10 p < .001. After recalling both images in one pair, participants rated the threatening pictures higher on all three RS. These results confirm the first hypothesis, that the threatening images do indeed have the expected effect.

Picture type, order and rating scores: the influence of threat on memory experience of neutral pictures

The analysis investigating if a threat image can modulate the memory experience of the following neutral image consisted of three separate 2x2 repeated measures ANOVAs. The results from the analysis show a main effect for Picture type (neutral, threat) but not for Order (1st, 2nd). The threatening pictures were rated significantly higher on all three rating scores (see table 1). The Picture type effect is consistent with the result from previous analysis and there was no effect of order on either of the RS categories. The order in which the pictures were presented thus had no effect on how they were later rated at recall, i.e. a neutral picture preceded by a threatening picture did not get different rating scores compared to a neutral picture presented first (i.e. when not preceded by any other picture). Picture type was therefore independent of Order for memory experience and the second hypothesis was thus not supported.

Table 1. Analysis of variance (ANOVA) difference in rating scores depending on order and picture type.

		df	*F*	η_p^2	*p*
Vividness	Picture type	30	14.433	.317	<.001**
	Order	30	.481	.015	.493
	Interaction	30	.127	.004	.724
Avoidance	Picture type	30	61.449	.665	<.001**
	Order	30	1.608	.214	0.049
	Interaction	30	.019	.001	.890
Disturbance	Picture type	30	101.594	.766	<.001**
	Order	30	.196	.006	.660
	interaction	30	2.869	.084	1.00

*. The difference is significant at the <.001 level.

Picture type, order and encoding behavior: the influence of threat on encoding-behavior of neutral pictures

The analysis investigating if encoding behavior was modulated by which type of picture was presented and in which order the pictures were presented consists of two separate 2x2 ANOVAs. It was expected that a neutral picture presented after a threatening picture would have fewer fixations compared to a neutral picture presented first.

The analysis shows that Order had an effect on fixations F (1,31) =13.194422, p =.001, η_p^2 = 0.229: when a picture is presented first, it attracts more fixations compared to when it's presented second.

Order also influences fixation time (F (1,31) =15.602, p <.001, η_p^2 = 0.335): the pictures presented first, compared to the pictures presented as second, attract significantly more fixations and that their duration is significantly greater.

Picture type, on the other hand, did not have any effect on fixations (F (1,31) =.021, p =.883, η_p^2 =.001) or fixation time (F (1,31) =.134, p =.716, η_p^2 = .004) meaning that fixations made on neutral pictures presented first and threatening pictures presented first were not significantly different.

Looking back and order: can a previous image cause participants to look back?

To investigate if a previous picture could cause participants to look back, two repeated measure ANOVAs were conducted. The analysis showed that the LB behavior, as measured by number of fixations, was significant for Order F (1,31) =14.915, p =.001, η_p^2 = 0.325, but not for

Picture, F (1,31) =.642, p =.429, η_p^2 = .020. The same was true for fixation time, with a significant main effect of Order F (1,31) =15.633711, p <.001, η_p^2 =. 335, but not for Picture, F (1,31) =.618704, p = .438, η_p^2 = .020). This means that participants looked more in the other AOI when a picture had previously been associated with this particular space, but that the effect was independent of picture type.

Rating scores and look-back: does looking back influence memory experience? The analysis investigating the LB behavior related to the subsequent memory experience consists of three separate 2x2 repeated measures ANOVAs. Results showed that the LB group rated the neutral pictures higher when they were preceded by a threatening picture, on both avoidance (M = 1.681, SD = 498) F (1,30) = 7.830, p =.009, η_p^2 = .207 and disturbance (M = 1.670, SD = .630) F(1,30) = 6.691, p =.015, η_p^2 = .182. This effect was not present for the threatening pictures (p: s <.221), i.e. when those pictures had been preceded by a neutral picture. These results lend support to the third hypothesis, indicating that participants who, during encoding, looked back to the AOI where the threatening picture previously had been presented, rated their memory of the succeeding neutral picture as more disturbing and that they would, to a higher degree, like to avoid thinking about it. This effect was not present for the participants who did not exhibit the LB behavior to an AOI where a threatening picture had been presented (p:s >.412), and the effect was specific for the neutral pictures being preceded by threatening pictures (i.e. it was not a general order effect). See Figure 2.

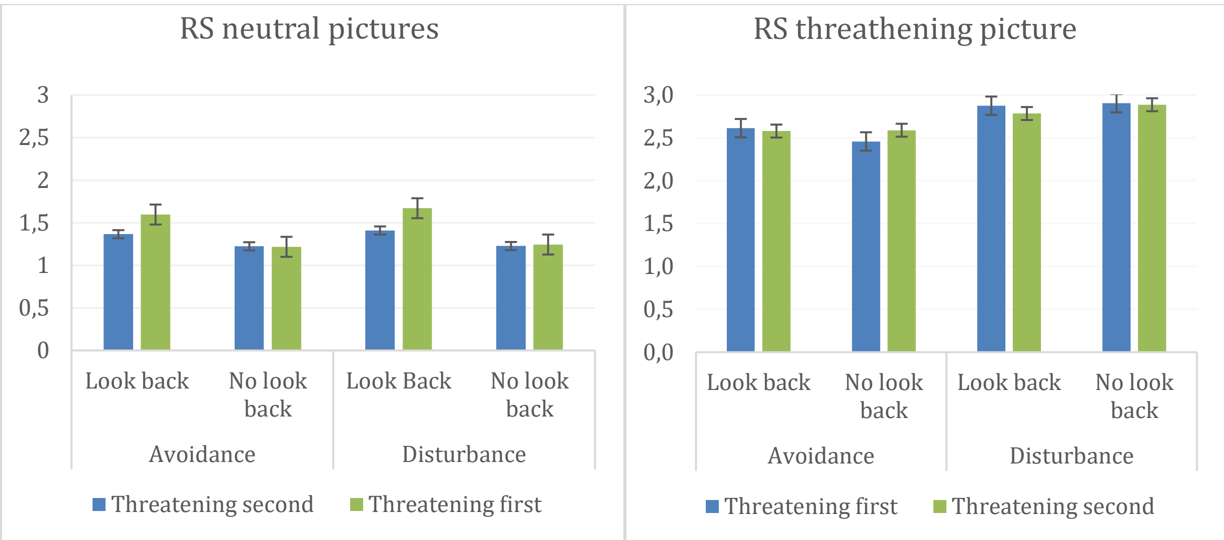

Figure 2. The difference in rating scores between the "LB group" and "no LB group" for neutral and threatening pictures.

STAI, looking back, order and rating scores: an exploratory analysis

To explore if the LB behavior was related to higher levels of anxiety, a repeated measures ANOVA with two between subject factors was used (LB behavior and STAI). Results showed an interaction tendency between the STAI group and Order on the dependent variable avoidance F (1,26) = 3.965, p= .057, η_p^2 = .132. The participants with higher STAI scores (more anxious) rated the neutral images higher on the avoidance parameter e.i they want to avoid thinking about the neutral picture to a higher degree when the neutral picture was preceded by a threatening picture but not the other way around. The participants that exhibited the LB behavior (more fixations in an empty AOI) also rated the neutral pictures higher on avoidance F (1,26) = 9.742, p= .004, η_p^2 = .237, this is consistent with previous analysis.

Discussion

The aim of the present study was to investigate how emotionally loaded events may change the way future events are encoded and subsequently remembered. Results show that the threatening pictures did indeed have an effect on participants' subsequent memory experience. They were rated as being more vivid and more disturbing to think about, and that the participants would have liked to avoid thinking about them to a larger degree. This confirms the first hypothesis, which was an essential step to establish that the two pictures types affected subsequent memory experiences differently. To investigate the main goal of the present study three analyses were then conducted.

First, it was expected that encoding a threatening image before a neutral image would modulate the subsequent memory experience of the following neutral image. However, the results offered no support for such an order effect.

Second, it was expected that encoding an emotionally loaded image before a neutral image would influence the encoding behavior of the subsequent neutral image, but not the other way around. The order in which pictures were presented had an influence on fixation and fixation time, leading to fever fixations and less time on the following picture. Contrary to the hypothesis, this influence was independent from the type of picture shown first; results offered no support for a picture type having an effect on encoding behavior.

Third, it was expected that participants would look back more towards the place where the previous picture was presented when that picture was emotionally loaded. While it was observed that participants did look back to the location where the previous picture was presented during the encoding phase, the type of picture presented had no influence on this behavior.

Finally, it was predicted that a tendency to look back towards the position where threatening pictures were previously shown would have a direct influence on the memory experience related to the neutral picture shown subsequently. The data confirmed this hypothesis: participants who looked back to the position of the threatening picture while being shown a neutral picture rated the latter with higher avoidance compared to those who did not exhibit this behavior.

On top of this the results of the follow-up analysis show that participants with higher STAI state scores rated the neutral pictures when shown after the threatening picture with higher avoidance, in a similar way as the participants that exhibited the LB behavior. However, no three-way interaction was reported.

Do treat pictures affect the encoding of neutral stimuli?

Previous research has established a link between fixations made on a picture and level of encoding and that emotionally loaded images attract more fixations thus leading to deeper encoding (Calvo & Lang, 2005; Simola et al., 2015; Öhman et al., 2001), it was therefore expected to see some difference in the encoding behavior for the neutral and threatening pictures. There was, however, no such difference in the fixation analyses in the present study.

The participants still recalled the threat images as more vivid compared to the neutral images, despite being subjected to roughly the same number of fixations. Previous research has shown that emotionally charged words and images are recalled better than neutrals (LaBar & Phelps, 1998; Cahill et al., 1995) it could therefore be assumed that the same would happen in this study. Even though participants felt that they could recall the picture more vividly when it was emotionally charged, compared to a neutral, on an actual memory test this might not have been the case. Emotions have also been shown in this study to have a reinforcing role for memory recall, it was, however, not possible to detect the influence of emotion on memory experience by only looking at gaze behavior. The number of fixations and the duration of those made on a picture were significantly greater for the pictures presented first, compared to the pictures presented second. It was expected that the presence of threat before a neutral image would modulate the memory experience and the encoding behavior of that neutral image (Tambini et.al, 2016), but it seems to be the presence of a stimuli in general and not whether the stimuli is emotionally loaded or not that influences gaze behavior. This is likely to be explained by more general abilities to control gaze, where the previously encoded picture to some degree is still active and influences gaze behavior of the second picture.

Another explanation for not finding a difference in fixations between the first threat compared to the first neutral picture could be because their values of arousal and valence were not extreme enough. Since the study has ethical guidelines to relate to, the images had to be adapted to something that was ethically justifiable to ensure that the participants did not risk being adversely affected to a degree that exceeded the scientific gain of the study in question. The consequence of this may be that the images were not sufficiently emotionally charged and when looking at rating scores for threatening pictures, there seems to be less agreement between participants compared to the ratings of neutral pictures where there is much more agreement and less variation. The results might be different if images with higher valence and arousal were used.

Do emotionally loaded images cause participants to look back to the place where this image was just presented?

Participants did indeed looked more in the other/empty AOI when a picture had previously been associated with this particular space, but contrary to expectations the LB behavior was not related to picture type, i.e. participants did not look back to a higher extent when the previously presented picture was a threatening one. This means that the emotional component of an image did not influence participants to look back more. The emotional component of the previous picture did, however, have an influence on the neutral picture the participants were "looking away" from. The participants that looked back to an AOI associated with a previous threatening picture, experienced the neutral pictures they were looking away from as more disturbing and wanted to avoid thinking about them to a greater extent. This effect was not present for the participants that did not show any LB behavior. Previous research has established that this "LB" or "looking at nothing" behavior is related to the recollection of information associated with a particular space (Johansson & Johansson, 2014; Richardson & Spivey, 2000; Stenberg & Hildeman, 2019) that is now empty. This behavior would thus mean that the previous picture was still active in memory or was reactivated from memory.

Emotionally loaded image: a spill-over effect?

As we have seen, for participants who tend to look back toward the position of a previous threatening picture while being shown a neutral image, the emotional content associated with that space seems to get reactivated and influence their judgement of the neutral image shown subsequently.

Previous research has found that emotion adds depth to encoding and that exposure to emotionally loaded stimuli before neutral stimuli results in greater recollection compared to the opposite sequence (Tambini et.al., 2016). While no confirmation of this finding was present in our data, our results can be interpreted as to show that the emotionality of a previous experience can influence the emotional valence of a subsequent one in encoding.

This interpretation would be in line with long standing previous research on consolidation (for a review see McGaugh, 2000), according to which during the consolidation process memories are not yet considered permanently set, making it possible for prior emotional content to influence future unrelated neutral content, thus modulating that memory experience.

In summary, the act of shifting the gaze to the position of a previously displayed threatening image seems to make its emotional valence spill over to the neutral image displayed afterwards. In order to obtain a better understanding of the mechanisms underlying this finding, an exploratory analysis was conducted to investigate the relationship between the LB behavior and anxiety.

A marker for anxiety? It was expected to see some overlap between the high STAI group (more anxious) and the LB behavior when a threatening picture was shown before a neutral picture, in terms of higher tendency to look back in the "high" STAI group and higher avoidance ratings for the neutral picture in both groups.

While the "high" STAI group exhibited a higher tendency to look back at the position of previously exposed threatening pictures, this correlation was not statistically significant ($p=.057$). The lingering for the anxiety group could perhaps be explained in terms of difficulty disengaging (Fox et.al., 2001; Salemink et.al., 2007), an important characteristic of attentional biases.

On the other hand, both the high STAI and the "LB group" marked the second, neutral picture as more threatening. The results can be interpreted as that the participants in the high STAI group also wanted to avoid a neutral image more, which would be in line with the Attentional bias (AB) of anxiety, a tendency to preferentially attend to threatening stimuli over other competing stimuli (Mogg & Bradley, 1998). However, the results did not show a three-way interaction between Order, "LB group" and STAI group. Thus, the effects of anxiousness and LB behavior were independent of each other. Since the result was not significant ($p=.057$) and since the sample consists of relatively few participants ($N = 13$), the results should definitely be interpreted with great caution.

Limitations

This study exists within a larger research project and the data used was already recorded by a group within this project (Stenberg & Haldeman, 2019). The original plan for the study was to use the already recorded data as well as recording additional data from another 30 participants but due to the covid-19 situation this was not possible (see footnote 2). An issue arising from the limited sample of 30 participants is exemplified when splitting the sample into groups, low (N=17) and high (N= 13) groups for anxiety and into "LB" (N= 21) or "no LB" (N= 11) groups for viewing behavior. This is most likely causing a problem and the recommendations are therefore to continue with the original plan and record more data to see if the effect related to these subgroups have any real bearing or not. For what concerns the choice of data analysis the repeated measures ANOVA fit the design of the experiment, but due to the many tests administered this opened up for a type 1 error. Participants' ability to focus may have been affected during the course of the experiment, the main experiment was approximately 35 minutes long and maintaining focus on the screen throughout the experiment is essential, this may have led to practice and fatigue effects. This was on the other hand hindered as much as possible by randomizing the order in which the images were presented for each participant and counterbalancing the positions. As mentioned above there was much more variation in the threatening pictures RS compared to the neutral pictures and could definitely have influenced the internal validity of the study. This could be an indication that pictures need to be more graphic in nature, but the effect could also arise from the small sample size.

The generalizability of these results is subject to certain limitations. For instance, all participants were students at a Swedish university and of similar age, several of which also studied psychology, making them a very specific population. Cultural differences exist in many aspects of human behavior (Henrich, Heine, Norenzayan, 2019), although it might be more evident for social and behavioral science. When it comes to saccades and eye movement research has shown that this can also vary among cultures and countries (Chua, Boland & Nisbett, 2005; Knox, Wolohan & Helmy, 2017), differences in culture could therefore result in different viewing behavior. In any case the generalizability of this study should be interpreted with some considerations.

Conclusion

In this study eye movements were used as a way to study encoding of neutral and emotional images. The result of this study finds that a negative previous experience to some extent affects how we encode future events. There were no differences in the fixations made on neutral compared to threat images during memory encoding, however the findings of this study do confirm that participants' memory experience of neutral and emotionally loaded images is in fact different from one another. Whilst the results did not confirm that a negative previous experience directly affects the viewing behavior of neutral pictures, we did see an interesting effect for the participants looking back towards where the negative picture was just exposed. The participants that exhibited this behavior wanted to avoid thinking about the neutral image they were looking away from to a greater extent and found it more disturbing in general. In spite of its limitations, the study adds to our understanding of modulatory effects of emotion on memory encoding and offer novel insights into the relationship between emotion and memory and show that (a) memories of emotionally charged and neutral images were experienced differently; (b) that participants looked back to locations where a previous image was presented during encoding and that such gaze behavior had an effect on the subsequent memory experience; (c) a relationship between higher levels of anxiety and the tendency to look back at positions where emotionally charged stimuli were encoded. Notwithstanding the relatively limited sample in relation to the looking back and anxiety groups, this work offers some inspiration to future research investigating the relationship between memory encoding and emotion and if anxiety potentially influences this process. These results show that the encoding behavior of future neutral pictures is affected by the previous threatening picture although, not in all the ways this study predicted.

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Appendix 1

LUNDS UNIVERSITET

Tillåtelse att använda data från projektet ”Minnesbilder, kognition och välmående”

Jag godkänner härmed att mitt testmaterial får användas i forskning om kognition, minne och välmående. Detta innefattar såväl olika slags statistiska och kvalitativa analyser, som publicering av artiklar, böcker eller på vetenskapliga konferenser och seminarier.

Jag ger slutligen också mitt tillstånd till att datamaterialet används i undervisning om kognition och minne.

Jag har blivit informerad om

- att det är frivilligt att delta och att jag kan dra mig ur när som helst
- syftet med inspelningarna
- att mina data behandlas anonymt i all analys och redovisning
- i vilken form mina data sparas
- att jag har rätt att kontakta Svante Hildeman eller Philip Stenberg och få mina inspelningar borttagna ur undersökningen
- att jag kan kontakta Svante Hildeman eller Philip Stenberg för att ta del av undersökningens resultat

Ort och datum	Underskrift
____________________________	____________________________
	Namnförtydligande
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