University of Reading

Learned Safety Contingent on Cognitive Evaluation
Bridging the gap between extinction and cognitive reappraisal

Thesis submitted for the degree of Doctor of Philosophy

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September 2017

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ABSTRACT

Reduced ability to regulate emotions has been determined as a major factor in many psychological disorders. Traditionally, emotion regulation is investigated in paradigms using complex negative images, and cognitive reappraisal strategies to regulate emotion. However, it has been argued that the extinction of conditioned responses to simple stimuli shares many of the basic mechanisms involved in emotion regulation.

Studies have shown that safety signals can effectively reduce conditioned responses. This project aimed to expand on this idea by adding a cognitive evaluative element to reduce a conditioned response.

In this paradigm participants were conditioned to associate a simple stimulus with an aversive event. Subsequently, information was added to this stimulus that required cognitive evaluation to ascertain whether each trial was safe or maintained the risk of the aversive event.

On a neural level we found increased responses in a circuit associated with threat appraisal during dangerous trials. During safe trials on the other hand, we found activation in areas associated with some of the cognitive mechanisms involved in emotion regulation.

To investigate the potential of this paradigm for clinical research, we investigated the effect of trait anxiety on the physiological and neural correlates involved. We found that high anxious participants showed a pattern of responding that suggested increased sensitivity to threat as well as altered processing of safety information.

Taken together these findings show that conditioned responses can be reduced through cognitive evaluation and results suggest that some of the mechanisms and brain regions involved overlap with those recruited during emotion regulation tasks. Furthermore, high anxious participants showed a pattern of responding that is consistent with the idea that safety signals are not processed effectively in this group. We conclude that adaptations of this paradigm will be useful to further investigate the basic mechanisms involved in emotion regulation.

ACKNOWLEDGEMENTS

I would like to thank my supervisors Tom Johnstone and Tim Salomons as well as my external supervisor Matthew Whalley for their support throughout my PhD.

I would also like to say thanks to Shannon Hoare who helped me collect and analyse some of the data presented here.

In addition, a special thank you to Alex Macdonald for your support and kindness. I could not have done it without you.

Thank you also for any kind words, crossed fingers, paws or hooves throughout – you know who you are.

DECLARATION:

I confirm that this is predominantly my own work. In chapters 4 - 7, Shannon Hoare assisted with data collection and analysis of the pupil dilation data.

Birthe Macdonald

The research presented in chapters 2 - 9 is currently being prepared for publication.

1. Introduction

1.1 The Definition of Emotion

According to early researchers, emotions are defined as response tendencies that arise due to internal or external psychologically relevant events and manifest themselves as physiological changes (James, 1984, 1894). These changes discriminate between different emotions and can vary greatly between individuals (Scherer, Schorr, & Johnstone, 2001; Smith & Ellsworth, 1985). Emotions serve a variety of functions including evolutionary (Tooby & Cosmides, 1990), social communicative (Ekman & Paul, 1993) and decision making functions (Oatley & Johnson-laird, 1987). Fear for example can be seen as a protection mechanism in which the goal of the emotion as a response tendency is to protect the organism from harm (Panksepp, 1998). A response to a perceived threat includes a variety of behavioural and physiological mechanisms that prepare the organism to fight or flight (LeDoux, 1998), thus, ensuring its survival. The focus of this project is on two types of negative affective responses (learned fear and responses to negative images) and their regulation.

Barrett (2009) describes the view that although emotions exist as real, universal experiences, they must be constructed through a variety of simple mechanisms that facilitate the great variability of physiological, behavioural and cognitive processes that a single emotional category can encompass. This hints at their fluidity – the fact that they are continually constructed and the variability of the result supports the view that they are not fixed in their direction or intensity (Gross, 1998), and that they cannot currently be predicted by the sum of their components. This is consistent with appraisal theory (Frijda, 1986; Oatley & Johnson-laird, 1987; Scherer, 1993). Appraisal theory suggests that emotions are not the result of any specific event itself but of the individual's interpretation of the event and of any physiological responses to that event. This suggests that many complex processes are involved in emotion generation, i.e. processing of all available aspects of the situation, recall of similar and/or contrasting experiences, outcome prediction, processing of any physiological responses, to arrive at an appraisal. Consequently, this appraisal will be subject to updating itself as the individual's situation develops. Therefore, the question remains whether there is an aspect of readiness for regulation to any experience of emotion considering they have the potential to (and often do) constantly evolve while they are experienced.

This idea has recently also been expressed in neuroscientific ideas about emotion generation that there is a separation of the systems responsible for the generation of physiological, behavioural and cognitive responses to threat (LeDoux & Pine, 2016). It is beyond the scope of this thesis to make explicit distinctions between these systems, however, it does tie in with the idea that there is a need

for paradigms that simplify natural but complex processes to work towards being able to make such a distinction.

1.2 The Neurobiology of Negative Emotion

1.2.1 Emotion Generating Paradigms

This project's focus is on two types of negative emotion in particular: spontaneous negative emotion that occurs as a result of seeing complex negative stimuli such as emotional images, and learned aversive responses that are the result of fear conditioning.

During instructed emotion regulation studies, images depicting highly negative scenarios are used to evoke affective reactions in participants. These images are typically part of the International Affective Picture System (IAPS, Lang, Bradley, & Cuthbert, 2008)) and show scenarios like road accidents, injuries, interpersonal violence (e.g. Johnstone, van Reekum, Urry, Kalin, & Davidson, 2007; Ochsner & Gross, 2005). Other researchers have used film clips (Goldin, McRae, Ramel, & Gross, 2008), stress (Wager et al., 2009), or anger inductions (Mauss, Cook, & Gross, 2007). In these studies, participants are typically given training in how they should approach these images before testing. For the emotion eliciting condition they are usually asked to keep their attention on the image, consider it to be real and attend to whatever emotion it elicits. For the conditions that test emotion regulation, participants are usually given a specific strategy in order to achieve a reduction of their initial emotional reaction (see section 1.3.1.1).

In contrast, in fear conditioning studies, affective reactions are elicited by combining a previously neutral stimulus (CS+) such as a light or a tone with an aversive stimulus (US) such as an electric shock or a loud noise (Pavlov, 1927). This results in the CS+ starting to elicit an affective response in anticipation of the US.

The following section reviews the brain areas that have been shown to be involved in the generation of emotion.

1.2.1.1 The Amygdala

The amygdala is the area that is most commonly associated with responding to emotional stimuli, in particular those of negative valence. It was originally thought to be solely responsible for negative emotion, in particular, fear, since Kluver and Bucy (1936) found that removing the temporal lobes of primates lead to them displaying what Kluver and Bucy called "psychic blindness", approaching objects they would usually fear. Weiskrantz (1956) demonstrated that this was due to the removal of the amygdala. It is directly connected to an extensive network of brain regions involved in memory, sensation and cognition (Young, Scanneil, Burns, & Blakemore, 1994), which puts it in an ideal position to influence cognition and behaviour through emotional inputs (Anderson & Phelps, 2000;

Whalen, 1998). It has been shown to respond to emotional stimuli and those associated with them through conditioning (Davis, 1992; Maren, 2001; Maren, Phan, & Liberzon, 2013; Paton, Belova, Morrison, & Salzman, 2006), human faces showing fearful (Whalen, 1998) or surprised, negatively interpreted expressions (Kim, Somerville, Johnstone, Alexander, Andrew, & Whalen, 2003), but also neutral but novel stimuli (Blackford, Buckholtz, Avery, & Zald, 2010). This suggests a role as a significance detector that alerts the system to stimuli that have the potential for negative consequences (Whalen, 2007).

In studies of instructed emotion regulation it is reliably found to be more active when participants are attending to than when they are attempting to reappraise a negative stimulus (Buhle et al., 2014; Hartley & Phelps, 2010; Ochsner & Gross, 2005; Ochsner, Silvers, & Buhle, 2012).

In studies of conditioning it has been found to be necessary in the acquisition (but not verbal understanding) of conditioned responses (Bechara et al., 1995; Bechara, Damasio, Damasio, & Lee, 1999; Davis, 1992), and inhibited during their extinction (Amano, Unal, & Paré, 2010; Barad, Gean, & Lutz, 2006; Michael Davis, Walker, & Myers, 2003), thus, it is part of the circuit that generates both spontaneous and learned emotional responses and communicates these to the autonomic nervous system as well as to cortical regions.

1.2.1.2 The Hippocampus

The hippocampus is closely connected to the amygdala and is associated with contextual memory processes (Barrientos et al., 2002; Huff & Rudy, 2004; Matus-Amat, Higgins, Barrientos, & Rudy, 2004; Squire, 1992). It has been shown that it is essential for the context dependency of conditioning and extinction learning (Corcoran & Maren, 2001, 2004; Frohardt, Guarraci, & Bouton, 2000; Ji & Maren, 2005). Their reciprocal connections mean that the hippocampus can influence emotion encoding in the amygdala during instructed conditioning, i.e. when contextual information is vital for the acquisition. Consequently, amygdala activation can also influence memory encoding in the hippocampus (Phelps, 2004).

1.2.1.3 The Insula and the Salience network

The insula has also been implicated as an emotion processing area. Research has shown that its posterior part can be described as an area of interoceptive representation including internally and externally created sensations (Augustine, 1996; Craig, 2003). This is in line with research that shows direct anatomical ascending connections between the posterior insula and the spinal cord (Craig, 2002, 2003). Its anterior part, has been associated with interoceptive awareness during a heartbeat detection task (Critchley, Wiens, Rotshtein, Öhman, & Dolan, 2004), the integration of visceral information during decision making (Xue, Lu, Levin, & Bechara, 2010), and the resolution of

emotional interference in working memory (Levens & Phelps, 2010), supporting its association with processing visceral autonomic responses. An extreme example of a visceral stimulus is a pain stimulus. The insula has consistently been shown to play a role in the perception of pain and is part of a called "salience network" which also includes dorsal anterior cingulate (dACC), amygdala, thalamus and substantia nigra/ventral tegmental area (VTA) (Legrain, Iannetti, Plaghki, & Mouraux, 2011; Seeley et al., 2007). It has been suggested that this network is likely involved in detection and selection of relevant stimuli to guide behaviour (Craig, 2009; Craig, 2002), of which external or internal visceral experience is one. Thus, in emotion regulation tasks activation of the insula may represent the relevance and salience of the stimuli as well as the induced emotion through the processing of bodily representations.

In studies of instructed emotion regulation, the insula shows activation in the same contrast as the amygdala, i.e. when participants are attending to negative images compared to when they are regulating the response to them (Ochsner et al., 2012). It is possible that this activation is related to the processing of autonomic physiological responses such as increased heartrate or skin conductance while negative images are viewed, reflecting a salient event that requires attention.

In conditioning studies, the insula has been shown to respond to stimuli that predict a negative outcome (Büchel, Morris, Dolan, & Friston, 1998), although some say this is dependent on participants being consciously aware of the stimuli (Critchley, Mathias, & Dolan, 2002). Considering the insula's role in processing bodily states, it is also possible that this activation is dependent on the type of stimulus used with the processing or the expectation of painful stimuli being more likely to involve insula activation.

Consequently, in both conditioning and emotion regulation studies the insula seems to be involved in the processing of the affective stimuli's salience and resulting bodily states.

Together with the insula, the dorsal anterior cingulate has been shown to be involved in the generation of emotion. This area has been seen to be of particular importance during the generation of conditioned responses in conditioning studies. A meta-analysis showed that the dorsal ACC was an area that is generally involved in the generation of the CR in conditioning studies, whereas more rostral medial prefrontal cortex areas (mPFC) tend to be activated only when participants are explicitly made aware of the conditioning contingencies (Mechias, Etkin, & Kalisch, 2010). Like the insula, this area is also part of the "salience network"(Legrain et al., 2011) described above, suggesting a shared role in the detection of potentially threatening information. Dopaminergic neurons in the VTA are also involved in fear conditioning (Guarraci & Kapp, 1999), with subdivisions of dopaminergic neurons observed to increase firing to a CS+, while other neurons decreased firing

to this stimulus. In addition, a dopamine (D2) agonist injected after conditioning reduced fear potentiated startle in response to a conditioned light stimulus, supporting the idea that dopaminergic neurons in the VTA have a role in the expression of conditioned fear.

1.2.1.6 THE VENTROLATERAL PREFRONTAL CORTEX

Although the ventrolateral prefrontal cortex (vlPFC) is typically found to be involved in the reduction of negative emotion, it has recently been found that this area also has a role in the generation of emotion. Wager et al. (2008) found that the right vlPFC is involved in the regulation of emotion not only through connections with the amygdala, but also through those with the nucleus accumbens, an area of the brain that is typically biased towards positive emotion. Similarly, a recent meta-analysis found increased vlPFC activation when participants were focusing on the content of another's emotion (Lindquist, Wager, Kober, Bliss-Moreau, & Barrett, 2012), also suggesting an emotion-generative role for this brain area. Considering its relation also to emotion regulation it is possible that, rather than reducing emotion directly, this area is related to assessing emotional content and facilitating emotional aspects of an alternative appraisal during cognitive reappraisal tasks.

1.3 THE REDUCTION OF NEGATIVE EMOTION

There are two major different approaches to research into the regulation of emotion. The study of intentional or instructed emotion regulation through cognitive reappraisal is focused on top down processes and based on appraisal theory. It assumes that emotions can be changed through the application of explicit strategies to reappraise the stimuli or situations that gave rise to them. On the other hand, an approach that explores the bottom up processes involved in emotion and emotion regulation is the study of the conditioning and extinction of conditioned responses. In its most classical form, this approach studies the development of associative responses when a previously neutral stimulus (CS+) is combined with an aversive event (e.g. electric shock), and subsequently the reduction of this association when the CS+ is repeatedly presented on its own again (Pavlov, 1927). This is, therefore the study of more spontaneous emotion generation and regulation. Both approaches including their shared mechanisms and neurobiology will be reviewed below.

1.3.1 TOP DOWN EMOTION REGULATION

1.3.1.1 EXPERIMENTAL PARADIGMS

Cognitive reappraisal is typically studied using emotion eliciting stimuli (e.g. from the International Affective Picture system, IAPS) and giving participants a strategy to reinterpret those stimuli while physiological as well as behavioural responses are collected (see Buhle et al., 2014; Kohn et al., 2014

for recent reviews).

The images used in this type of study are usually divided into negative, positive, and neutral images and depict a variety of scenes. I will focus on negative and neutral images here as they are the ones relevant to studies in which participants are required to reduce negative affect. Negative images depict a variety of scenes including graphic violence, injuries, and accidents, evoking empathetic negative affect, as well as scenes that aim to induce feelings such as disgust, depicting vomit, human faeces etc. Neutral images often show landscape scenes, portraits, or plants.

In order to reduce negative affect to the negative images, participants are trained to use one of several strategies. A large number of studies investigates cognitive reappraisal which involves mentally changing the meaning of the image for example by imagining a positive (or less negative) outcome to the image or imagining the image to be part of a film (e.g. Modinos, Ormel, & Aleman, 2010; Ochsner et al., 2004). Other studies use suppression and instruct participants not to express the emotion they are feeling(e.g. Goldin, McRae, Ramel, & Gross, 2008; Jackson, Malmstadt, Larson, & Davidson, 2000), while still others use distraction, asking participants to think of something else that will have a calming effect (Delgado, Nearing, Ledoux, & Phelps, 2008, although this study did not use IAPS images).

The contrast of interest in these investigations is usually Negative Decrease > Negative Attend, with the Negative Attend > Neutral Attend used as a check that the negative images had the desired effect.

Even though results obtained using this type of task tend to be consistent, there are several issues that limit the claims that can be made about its results. The image stimuli used are very complex, their clarity and complexity can vary between images, and individual differences in participants' reactions to these images are expected. In addition, even though researchers tend to give detailed instructions and training in the strategies they ask participants to use to decrease their affective responses, they ultimately do not have control over what participants do, or whether they pay attention to the image at all. Even successful reappraisal itself involves several different mechanisms that contribute including the analysis of the current situation, the analysis of one's response to it, creating and selecting an alternative interpretation of the situation, its application, and the monitoring of the emotional response after the alternative interpretation has been applied to check the effectiveness of the reappraisal. Furthermore, the very nature of the negative images limits the use in certain patient populations (e.g. patients with posttraumatic stress disorder, PTSD) and increases the likelihood that some potential participants will decline their participation after seeing example images. Considering that the study of these patient populations and those with an anxious

disposition is vital to better understand the nature of psychiatric disorders that involve difficulty with regulating affect, a simpler and more controlled way of investigating it would be highly beneficial.

1.3.1.2 The Neurobiology of Top Down Emotion Regulation

Ochsner et al.,(2012) defined networks consisting of the amygdala and insula during emotion generation, and dorsolateral- and dorsomedial-, ventrolateral- and ventromedial prefrontal cortex (dlPFC, dmPFC, vlPFC, vmPFC, respectively) as well as dorsal anterior cingulate cortex (dACC), and inferior parietal corticex (IPC) to be involved in the regulation of emotion through cognitive reappraisal. They further suggested that temporal areas including the temporoparietal junction (TPJ), temporoparietal cortex (TP), Superior temporal gyrus and middle temporal gyrus (STG and MTG respectively) were involved in supplementary mechanisms during the process of emotion regulation. A meta-analysis by Kohn et al. (2014) analysed 23 studies on emotion regulation including several different regulation strategies (e.g. reappraisal, suppression, distraction). Their results support those reviewed by Ochsner et al (2012), finding a wide network consisting dlPFC, vlPFC, supplementaryr motor area (SMA), STG, ACC and angular gyrus (AG). A closer investigation of the function of each brain area revealed that each of the lateral areas is likely to contribute mechanisms that are not specifically related to emotion but instead serve more general functions. For example the dlPFC may be involved in the cognitive control. In contrast, the vlPFC is likely to be involved in emotion processing and generation as well as action inhibition, communicating with subcortical areas via ACC and anterior insula. The ACC cluster identified in this study is directly connected to subcortical areas and may integrate information from lateral prefrontal and motor cortex to implement emotion regulation. The SMA, AG and STG are likely to be involved in the application of the semantic strategies to regulate and the maintenance of their internal representations. These areas are also connected to subcortical emotion processing structures, suggesting a direct involvement in the regulation of their activation (see figure 1).

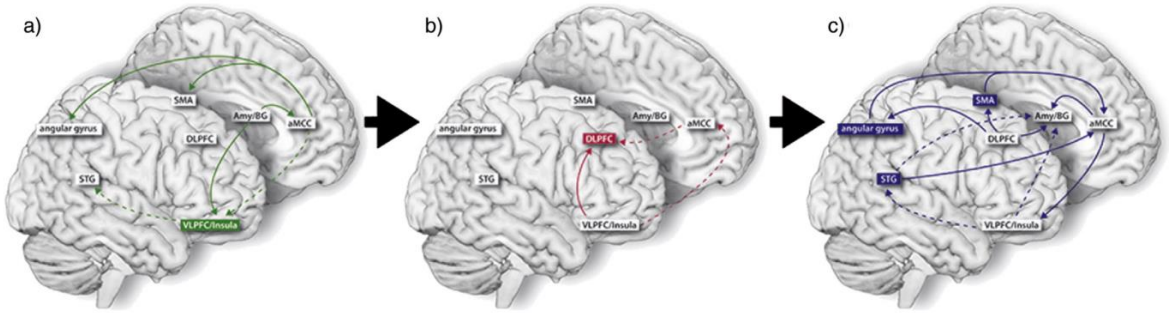


FIGURE 1. HEURISTIC MODEL OF NEURAL PROCESSING OF EMOTION REGULATION

THIS HEURISTIC MODEL OF NEURAL PROCESSING OF EMOTION REGULATION RELATES TO THE MODAL MODEL OF EMOTION (GROSS, 1998). AFFECTIVE AROUSAL IS RELAYED VIA AMYGDALA AND BASAL GANGLIA TO THE VLPFC AND THE ANTERIOR INSULA, AS WELL AS SMA, ANGULAR GYRUS AND STG (A). THE VLPFC INITIATES THE APPRAISAL AND SIGNALS THE NEED TO REGULATE THE EMOTION TO THE DLPFC (B). THE DLFPC PROCESSES THE REGULATION ITSELF AND GIVES A FEEDFORWARD SIGNAL (VIA THE AMCC OR DIRECTLY) TO ANGULAR GYRUS, SMA, STG, AMYGDALA AND BASAL GANGLIA, WHICH IN TURN PARTICIPATE IN THE GENERATION OF A (REGULATED) EMOTIONAL STATE (C).
FIGURE AND CAPTION REPRODUCED FROM KOHN ET AL. (2014). NOTE: AFFECTIVE AROUSAL IS DEFINED AS THE INITIAL RESPONSE TO AN EMOTION INDUCING STIMULUS.

The complexity of this network reflects the complexity of the process of emotion regulation: Kohn et al. (2014) suggest that the regulation of emotion is a multi-level process that involves different brain areas at different stages of the process. This includes attention to the stimuli, processing of the images shown and the initial emotional response, selecting an appropriate reappraisal strategy through memory and monitoring, applying this strategy and modulating the initial response, monitoring progress and maintaining goals in working memory. Consequently, the completion of each trial is very complex and requires the use of a large neural network and a simplification that would allow the investigation of the individual mechanisms that are involved and their neural correlates, would greatly further our understanding of this process.

1.3.2 Extinction of Conditioned Aversive Responses

1.3.2.1 Experimental Paradigms

Another process that serves to reduce an emotional response is the extinction of conditioned responses. During aversive conditioning, a conditioned stimulus (CS+) is repeatedly paired with an unconditioned stimulus (US) such as an electric shock, until the presentation of the CS+ alone evokes the same response as the US, the conditioned response (CR, Pavlov, 1927). During extinction, the US is taken away and responses that rose during conditioning trials are gradually reduced until they return to baseline levels. It has been suggested that this decrease in responding is due to the updating of the probability of the US occurring during every trial by comparison with previous trials (Miller & Matzel, 1988). However, the previously learnt response is not abolished during this process

(Bouton, 2004a). It has been shown that a CR acquired in a certain context and extinguished within a different context will return spontaneously if the subject is returned to the acquisition context after extinction (Bouton & King, 1983; Bouton & Bolles, 1979). Further, the CR also returns if the subject is placed into a new context after extinction, whether conditioning and extinction context were the same (Bouton & Brooks, 1993; Bouton & Bolles, 1979; Harris, Jones, Bailey, & Westbrook, 2000) or different (Bouton & Ricker, 1994; Tamai & Nakajima, 2000).

Not only spatial but also temporal context can induce spontaneous recovery of a CR (Bouton & Brooks, 1993; Pavlov, 1927). This means that if time is allowed to pass after extinction, without further exposure to the CS or US, the CR will return. Bouton (2004) discusses rapid reacquisition as further evidence that extinction is not an unlearning of the CS-US association. Following a period of extinction, a CR can be re-learned quicker than during the initial conditioning (Napier, Macrae, & Kehoe, 1992; Ricker & Bouton, 1996; Weidemann & Kehoe, 2003). Depending on the context, the CR can even be reinstated by only presenting the US, without the CS (Bouton & Bolles, 1979; Rescorla & Heth, 1975). Although critics have mentioned that the paradigms mentioned above have limitations that do not permit them to conclude definitively that extinction does not involve at least some unlearning of the CS-US relationship (Delamater, 2010), evidence from studies that used transfer of conditioning from pavlovian to instrumental (behavioural reinforcement) procedures shows that the relationship between a CS and a specific US is not destroyed by extinction (Delamater, 1996; Rescorla, 1996).

There are several strands of research that examine variations of the classic extinction paradigm by manipulating the contingencies under which the CS+ is removed. Reversal learning switches the CS+ - CS- contingencies after a period of conditioning so that the previously safe CS+ is now dangerous and vice versa. Consequently, the CR to the (previous) CS+ is reduced and a CR is established in response to the new CS+ (Rolls, Critchley, Mason, & Wakeman, 1996). Another example which investigates the effects of combining a CS+ and CS- on the CR is 'conditioned inhibition' (Rescorla, 1969) and describes the summation of a CS that reliably predicts a US and another CS which has never been associated with the same US. When presented together, they produce a reduction in CR. Further, there is a delay in subsequent establishment of the previous CS- as a CS+.

In Learned Safety (LS) paradigms, a previously neutral CS is paired with an aversive US in one group, and a period of safety from this US in the other. After a period of training, the CS alone is presented and (in mice) behaviour during the CS is compared to a pre-trial baseline (Pollak et al., 2008), resulting in reduced responses to the CS compared to pre-training in the LS group, and increased responses in the fear conditioning group.

Using SCR and fear-potentiated startle (a reflexive response to affective stimuli such as a CS+) as

measures of the CR, Grillon and Ameli (2001) found a reduction in SCR when a previously reinforced CS+ was preceded by a safety signal in low anxious participants. Consequently, CR's can be modulated not only by classical extinction but also through the use of safety signals, which means they are not fixed but flexible responses.

Taken together, these results suggest that the CS – US association is not destroyed completely through extinction or its variations, rather, it is updated with new information but can be reactivated rapidly. Thus, one could say this process is subject to an initial inhibition which stops the CR from occurring as the US stops (Barad, 2006). This suggests that the underlying mechanisms in extinction and instructed emotion regulation may be shared.

1.3.2.2 The Neurobiology of Extinction

Both lesion studies in animals and fMRI studies in humans have shown the amygdala to be vitally involved in both conditioning and extinction processes (Büchel et al., 1998; LaBar, LeDoux, Spencer, & Phelps, 1995; LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998; Phelps, Delgado, Nearing, & LeDoux, 2004). In fact, animal studies have shown that different subdivisions of the amygdala may serve different functions in this process and may be differentially involved in the inhibition of the CR: the lateral amygdala has been associated with attention to the CS whereas the medial central nucleus of the amygdala may be more involved in the generation of the response (Pare & Duvarci, 2012). Thus, During extinction, neurons in the lateral amygdala can regulate activity in the central nucleus to reduce amygdala output and behavioural responses (Ehrlich et al., 2009).However, in addition to the amygdala's own learning circuitry, evidence exists that vmPFC and hippocampus are also involved (Delamater, 2010; Quirk, 2011). The hippocampus is closely connected to the amygdala and its association with contextual memory processes (Barrientos et al., 2002; Huff & Rudy, 2004; Matus-Amat et al., 2004; Squire, 1992) suggests that it is involved in the continued representation of conditioning and extinction contingencies. This view is supported by the finding that the hippocampus is vital for contextually dependent conditioning and extinction (Corcoran & Maren, 2001, 2004; Frohardt et al., 2000; Ji & Maren, 2005).

The vmPFC is also directly connected to the amygdala and has been shown to be involved in the extinction of conditioned fear responses. Lesions in this area lead to increased responsivity to the CS+ both in the conditioning and extinction phase, and, thus, slowed down the extinction process (Milad, Vidal-Gonzalez, & Quirk, 2004; Morgan, Romanski, & LeDoux, 1993; Morgan & LeDoux, 1995). In contrast, stimulation of infralimbic neurons after conditioning reduced responses to the CS+ in rats (Milad et al., 2004; Quirk, Likhtik, Pelletier, & Paré, 2003). Findings from fMRI studies on extinction showed that while vmPFC did not show differential activation during CS+ and CS- trials in extinction, it did show increased activation in this contrast when participants were confronted with

the same stimuli in a delayed test of extinction (Phelps et al., 2004). This, however, does not rule out an active role for the vmPFC during extinction of the CR. Evidence that the vmPFC is involved in the processing of safe compared to dangerous stimuli comes from a meta-analysis of fear conditioning studies that looked at deactivated areas during fear conditioning and found parts of the vmPFC were deactivated during CS+ compared to CS- trials (Fullana et al., 2015). Taken together, the evidence from studies in animals and humans suggests an active role for the vmPFC in the inhibition of conditioned fear responses.

1.3.3 The Prefrontal Emotion Regulation Circuit – Detailed Review of Location and Function

1.3.1 Dorsolateral Prefrontal Cortex

During the regulation of emotion, a network of cortical brain areas is responsible for adapting the initial emotional response. The dorsolateral prefrontal cortex (dlPFC) has been implicated in working memory processes (Corbetta & Shulman, 2002) and the maintenance of goals and attention to the stimulus. MacDonald, Cohen, Stenger and Carter (2000) found the dlPFC responsible for the implementation of attentional control during a stroop task. A lesion study showed that injuries to the dlPFC resulted in increased levels of depression suggesting that the dlPFC is involved in the implementation of regulation in emotion regulation studies (Koenigs et al., 2008). Early lesion studies in primates showed that damage to this region resulted in performance deficits that were dependent on the delay between stimulus and action in working memory tasks. Lesions did not affect performance when delays between stimulus and required action were short but did affect it when delays were long. This suggests a role for the region in goal maintenance rather than the direct initiation of responses (Bauer & Fuster, 1976; Curtis & D'Esposito, 2003; Funahashi, Bruce, & Goldman-Rakic, 1993; Goldman-Rakic, 2011; Miller & Orbach, 1972). In addition, Miller and Cohen (2001) suggest that it is not only sensory information that is maintained by the dlPFC but also abstract task contingencies and rules. In addition, this area is activated when a fear conditioned CS+ is combined with a safe stimulus in learned safety paradigms (Pollak et al., 2010a) suggesting it may be involved in the inhibition of the learned fear response by maintaining the newly learned knowledge about the safety signal.

The inferior parietal cortex works in concert with the dlPFC to shift attention towards goal-relevant stimuli and manipulate working memory components accordingly, as summarised by several reviews (e.g. Colby & Goldberg, 1999; Shapiro, Hillstrom, & Husain, 2002).

1.3.2 Ventrolateral Prefrontal Cortex

The selection of goal appropriate information and responses has been associated with the ventrolateral prefrontal cortex (vlPFC, Badre & Wagner, 2007; Thompson-Schill, Bedny, & Goldberg, 2005). In particular, it has been argued, that this brain area is necessary for choosing a "weaker" alternative in favour of a more dominant response (Botvinick, Braver, Barch, Carter, & Cohen, 2001). Thus, in emotion regulation paradigms, the involvement of this area may reflect the selection of an alternative scenario to the one depicted in picture stimuli. In extinction paradigms in rats this area seems necessary only when extinction contingencies need to be remembered in an extinction test phase (Tian et al., 2011).The left vlPFC has also commonly been associated with external and internal speech production (Bookheimer, 2002; Hinke et al., 1993; Huang, Carr, & Cao, 2002) which is a component of many reappraisal tasks – participants are often required to create an alternative scenario in their head that can be applied to reduce the emotional impact of a stimulus, thus involving sematic processes. However, others argue that the seemingly language-specific vlPFC activations may be explained by more general regulatory activity (Thompson-Schill et al., 2005). Thus, their involvement in emotion regulation tasks can be explained by either theory. The right vlPFC is often found to be activated in studies with directly compare reappraisal through decreasing and increasing emotional reactions (Kim & Hamann, 2007; Ochsner et al., 2004; Urry, van Reekum, Johnstone, & Davidson, 2009). Thus, this area may be involved in the inhibition of the initial response in favour of the newly constructed one, which means it is not needed when participants are asked to increase their initial response (Silvers, Buhle, & Ochsner, 2014).

1.3.4 Dorsomedial Prefrontal Cortex and Dorsal Anterior Cingulate Cortex

In fear conditioning studies it has been shown that areas in rostral dmPFC are active in "instructed fear" studies but its activity is reduced under high working memory load when the threat stimulus cannot be actively processed, even though physiological responses to it remain stable (Mechias et al., 2010). Furthermore, rostral dmPFC activation is correlated with explicit threat evaluations but not physiological responses (Raczka et al., 2010), thus suggesting its involvement in the conscious evaluation of fear stimuli. This may be extended from fear- to all stimuli that are relevant to the self as rostral dmPFC areas have also been associated with representing one's own and other people's mental state (Amodio & Frith, 2006; Denny, Kober, Wager, & Ochsner, 2012; Olsson & Ochsner, 2008). Thus, in emotion regulation studies the involvement of this area may reflect a more general evaluation of the situation in the images used as negative stimuli, including mentalising and self-monitoring processes. In contrast, it has been suggested that the expression of physiological responses is related to activity in caudal areas of the dmPFC (i.e. dorsal ACC), though evidence is generally focused on sympathetic arousal rather than specific responses to CS+ and often also

involve areas in supplementary motor cortex and presupplementary motor cortex (SMA/preSMA, resp., Critchley et al., 2003; Gianaros, Van Der Veen, & Jennings, 2004; Nagai, Critchley, Featherstone, Trimble, & Dolan, 2004; Patterson, Ungerleider, & Bandettini, 2002). Conditioning studies have found that activity in dACC was also correlated with SCR to CS+ (Milad et al., 2007), further support that suggestion. In addition, Mechias et al. (2010) found that the dACC may be involved in general threat processing, as opposed to rostral dmPFC which requires conscious awareness of threat. Cognitively, dACC has been found to be involved in conflict monitoring when an external or internal event indicates that the focus of attention should be changed and a novel response is required (see Botvinick, Cohen, & Carter, 2004 for a review). Consequently, this area is likely involved in the updating of response tendencies during emotion regulation. The fact that it has not specifically been shown to be active in extinction studies may suggest a more cognitively based role of the dACC, however, this could also be due to labelling of this area since it is directly adjacent to more ventral parts of the ACC which are likely to be included in the vmPFC in extinction studies, associated less with conflict detection and more with conflict resolution on a neural level as outlined below.

1.3.5 Ventromedial Prefrontal Cortex

Finally, the ventromedial prefrontal cortex (vmPFC) has been described as a "system of systems" (Roy, Shohamy, & Wager, 2012) integrating information from several neural networks involved in affect, memory, attention and value to make sense of situations. Its connections to affect and regulation systems including the amygdala, periaquaeductal gray, raphe nuclei and spinal autonomic ganglia, as well as memory systems involved in the processing of both past experiences and anticipation of future events, and cortical systems involved in sensation, reward, mentalising, goal formation and maintenance put it in an ideal position to act as a "conductor" that integrates higher order cognitive processes with basic affective systems and turns information into action expressed as physiological, endocrine or motor responses, decisions, attentional shifts or behaviour (Amodio & Frith, 2006; Buckner & Carroll, 2007; Price & Drevets, 2010; Roy et al., 2012; Schoenbaum, Takahashi, Liu, & Mcdannald, 2011). In emotion regulation studies it has been shown to mediate the relationship between vlPFC and amygdala and more robust down regulation of the amygdala or insula has been associated with stronger correlations with vmPFC (Johnstone et al., 2007; Pitskel, Bolling, Kaiser, Crowley, & Pelphrey, 2011; Urry et al., 2006), a relationship that has also been shown to be moderated by participants' psychiatric status (Erk et al., 2010; Johnstone et al., 2007), genotype (Schardt et al., 2010) and reappraisal success (Wager, Davidson, Hughes, Lindquist, & Ochsner, 2008). However, a recent meta-analysis, the vmPFC did not find reliable activation in the vmPFC during emotion regulation (Buhle et al., 2014). The authors suggest that this null finding may

be due to possible involvement of the vmPFC in both initial emotion appraisal and reappraisal conditions. Alternatively, they also considered the possibility that rather than regulation of emotion specifically, vmPFC activation could be related to the maintenance of conceptual information when this is necessary to complete a reappraisal task. This is consistent with the idea of a "system of systems" which is involved in integrating information from different neural networks and applying them to form a response.

In addition to studies involving emotion regulation, the vmPFC has also been shown to track the value of a stimulus, showing stronger activation for stimuli with a higher positive value (Oya et al., 2005; Roy et al., 2012; Schoenbaum, Saddoris, & Stalnaker, 2007; Schoenbaum et al., 2011). In line with this, some researchers have found vmPFC activation to be diminished when participants are down regulating responses to positive stimuli (Kanske, Heissler, Schonfelder, Bongers, & Wessa, 2011; Kim & Hamann, 2007; Kober et al., 2010; Schardt et al., 2010; Winecoff, LaBar, Madden, Cabeza, & Huettel, 2011), further suggesting that the vmPFC reflects positive or more positive compared to initial evaluations of stimuli. Animal studies, however, have shown that stimulation of the infralimbic (IL) and dorsal peduncular cortices (ventral sections of the medial prefrontal cortex in rats) decreases heartrate and inhibits sympathetic tone (Owens & Verberne, 2001; Verberne, 1996), and inactivation inhibits parasympathetic function with no effect on sympathetic function (I.e. sympathetic tone, e.g. heart rate, will increase (Resstel, Fernandes, & Corrêa, 2004; Resstel & Corrêa, 2006). These results suggest that the vmPFC has a more direct role to play in the regulation of affective responses than just tracking value. Further research strengthening the idea that the vmPFC integrates contextual with affective information to arrive at an approriate response comes from animal studies of conditioning and extinction of fear. Damage to the IL increases the recovery of fear after extinction (Quirk, Russo, Barron, & Lebron, 2000), and prevents the reduction of stress responses that would normally occur when an aversive stimulus is under the organism's control (Amat et al., 2005; Baratta, Lucero, Amat, Watkins, & Maier, 2008). Human studies lend further support to the idea that the vmPFC combines information from different systems to alter a response if necessary. It is more activated during stimuli that used to be associated with an aversive CS+ but are now safe in reversal studies while the amygdala shows the opposite pattern (Schiller, Levy, Niv, LeDoux, & Phelps, 2008). It is decreased during the CS+ in studies on instructed fear (Mechias et al., 2010; Phelps et al., 2004) but more activated during instructed regulation of negative affect along with decreased amygdala activation and reduced skin conductance (Delgado et al., 2008). The vmPFC is involved in placebo analgesia suggesting a role in pain reduction through reappraisal (Diekhof, Geier, Falkai, & Gruber, 2011; Meissner et al., 2011; Wager, Atlas, Leotti, & Rilling, 2011) and activated during speech preparation in social stress tasks when negative emotion needs to be

regulated in order to fulfil the demands of the task (Wager et al., 2009). In addition, recent research in patients with bilateral lesions to the vmPFC has shown that this region is causally involved in the downregulation of amygdala responsivity in an instructed emotion regulation task (Motzkin, Philippi, Wolf, Baskaya, & Koenigs, 2015), lending support to the general consensus that it is an area that implements emotion regulation. Taken together, these findings suggest that the vmPFC is involved in the application of new information to facilitate changing the meaning of a given neutral or affective situation.

1.3.4 Extinction Processes as the Basis for Top Down Emotion Regulation

1.3.4.1 Overlapping Mechanisms

The basis for this thesis is the idea that the basic mechanisms and neurobiology of extinction and the conscious regulation of negative emotion are overlapping (Quirk & Beer, 2006). Thus, by investigating extended versions of extinction tasks, we may be able to find out more about the specific mechanisms involved in the regulation of negative emotions. This means it has to be clear where these processes overlap both functionally, and anatomically. I discuss this on the basis of figure 2 reproduced from (Ochsner et al., 2012). The figure below shows a detailed analysis of mechanisms that may be involved in the cognitive control of emotion including emotion generating, attention, working memory, language, value and conflict monitoring and inhibitory processes, represented in different cortical areas, and highlighting the complexity of cognitive reappraisal of emotion. Classical extinction, as discussed above, has been suggested to be based on simpler estimation of probability from one trial to the next. According to figure 2, it involves a) stimulus perception, b) tracking of the stimulus value, c) generation of the bodily states associated with it, and d) updating of the stimulus value in its current context (i.e. during each new trial compared to the last). This suggests that the processes involved in the extinction of a conditioned response are embedded within those involved in cognitive reappraisal of emotion and the latter is an extended, more complex version of the former.

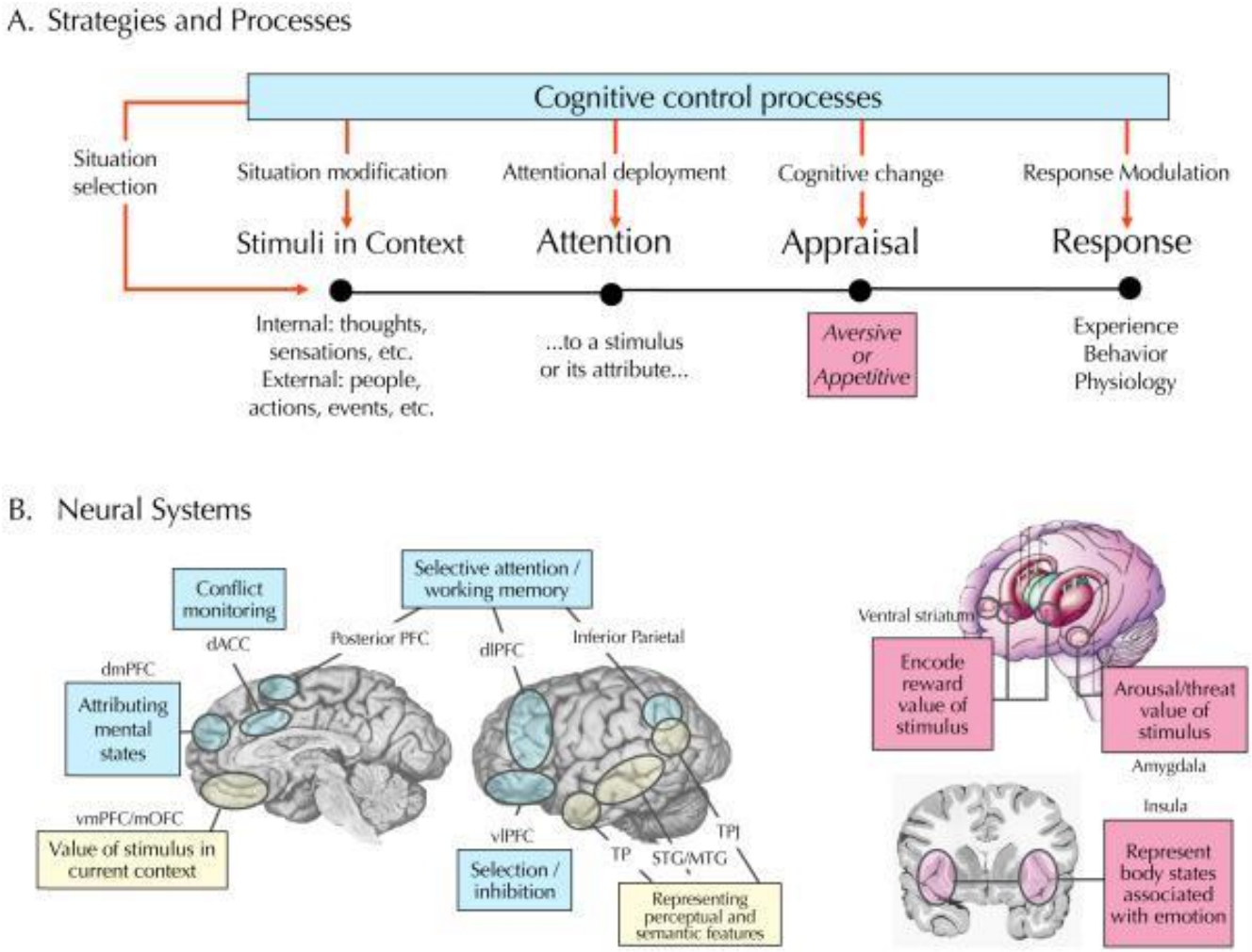


FIGURE 2. A MODEL OF THE COGNITIVE CONTROL OF EMOTION

A MODEL OF THE COGNITIVE CONTROL OF EMOTION (MCCE). A. **DIAGRAM OF THE PROCESSING STEPS INVOLVED IN GENERATING AN EMOTION AND THE WAYS IN WHICH COGNITIVE CONTROL PROCESSES (BLUE BOX) MIGHT BE USED TO REGULATE THEM. AS DESCRIBED IN THE TEXT, THE EFFECTS OF DIFFERENT EMOTION REGULATION STRATEGIES (THE RED ARROWS DESCENDING FROM THE COGNITIVE CONTROL PROCESSES BOX) CAN BE UNDERSTOOD IN TERMS OF THE STAGES OF OF THE EMOTION GENERATION SEQUENCE THAT THEY IMPACT. THE PINK BOX SEEN AT THE APPRAISAL STAGE IS MEANT TO INDICATE THAT NEURAL SYSTEMS INVOLVED IN GENERATING EMOTION SUPPORT THIS PROCESS.** B. **NEURAL SYSTEMS INVOLVE IN USING COGNITIVE STRATEGIES, SUCH AS REAPPRAISAL, TO REGULATE EMOTION (LEFT, BLUE BOXES), SYSTEMS INVOLVED IN GENERATING THOSE RESPONSES (RIGHT, PINK BOXES), AND SYSTEMS WITH AN UNDEFINED OR INTERMEDIARY ROLE IN REAPPRAISAL (LEFT, YELLOW BOXES). FIGURE AND CAPTION FROM** (OCHSNER ET AL., 2012)

1.3.4.2 Overlapping Neurobiology

There have been a number of studies that have investigated the neural correlates of paradigms which modulate extinction in some way. The results of these studies give some insight into the potentially shared mechanisms of extinction and emotion regulation on a neural level. Investigation of a learned safety (LS) paradigm in which participants learned to associate the CS with

a period of absence of the US, showed that caudate and dlPFC were active during learned safety whereas the amygdala responded to dangerous trials (Pollak et al., 2010a). However, this study was carried out in a between-group design with no within-group control condition. Thus, while these results are as hypothesized by the authors, they have to be treated with caution.

Conditioned responses can also be modified by instructed regulation. Delgado, Nearing, LeDoux, and Phelps (2008) instructed participants to expect an electric shock with one of two coloured squares. In a subsequent phase, they asked them to either "attend" to or "regulate" their initial reaction to seeing the cue by thinking about something calming in nature specific to the colour of the square. They found that SCR's were higher during CS+ "attend" than CS- "attend" trials, a difference that was abolished for CS+ "reappraise" and CS- "maintain" trials. On a neural level, they found increased amygdala activation during CS+ "attend" trials compared to CS+ "reappraise" trials, and vmPFC and dlPFC in the opposite contrast. They concluded, that participants were able to regulate their affective response when asked to do so, and that they recruited a set of brain areas that was more specific than that recruited in instructed emotion regulation studies, but extended compared to extinction. This supports the notion that the basic mechanisms as well as the neural networks involved in the extinction of affective responses and the instructed regulation of emotion through cognitive reappraisal overlap. Several questions arise when considering the design of this task. Instead of a standard conditioning procedure, Delgado et al. (2008) used explicit instructions to inform participants of the CS+/- contingencies. Although it has been shown that affective responses can reliably be evoked in this way, the underlying neural mechanisms are overlapping but not the same (see Mechias, Etkin, & Kalisch, 2009 for a meta-analysis). On a behavioural level, this is evident when CS+' are shown to participants at a duration below the threshold of conscious awareness. Participants who learned the CS-US contingencies through classical conditioning or observational learning show a response to these presentations, whereas participants who learned through instructions only do not (Olsson & Phelps, 2004). This implies that CS-US contingencies acquired through direct experience may be more robust than those acquired through instructions.

Another issue with the study by Delgado et al. (2008) is the continued complexity of the regulation instructions. Although the instructions to "think of something calming in nature" are more controlled than some that have been employed in instructed emotion regulation research, the resulting thought processes may still be too complex to pinpoint brain areas specifically involved in different underlying mechanisms. Thus, this project aimed to go one step further than Delgado et al. (2008). We used conditioned stimuli to elicit emotional reactions in participants. To simplify and control the regulation process, we then presented the conditioned stimuli within a context which

enabled participants to decide whether every given trial was a threat- or a safe trial. This way, we knew the information participants could use to (re)appraise each trial.

In summary, evidence suggests that the neural networks involved in the cognitive reappraisal of emotion and the extinction of conditioned responses overlap. The brain areas involved in stimulus perception depend on the type of stimulus used – in this case we assume both tasks use visual stimuli, thus, the visual cortex will be involved in their perception. However, one aspect of perception is also attention which has been shown to recruit inferior frontal and temporoparietal cortices to alert the brain to behaviourally relevant stimuli (Corbetta & Shulman, 2002). This system is, indeed, found to be active in both emotion regulation and extinction tasks (Armony, 2002; Silvers et al., 2014).

The original emotional value of the stimulus is encoded in areas such as the amygdala and insula in both tasks (Ochsner et al., 2012; Sehlmeyer et al., 2009) as discussed above. The knowledge and application of the new contingencies to regulate affective responses is filtered through a network comprised of dorsal and lateral PFC as well as ACC in emotion regulation (Silvers et al., 2014), whereas the top-down aspect of the reduction of the conditioned response may be reduced in extinction and expressed through reduced activation in amygdala, insula and ACC (Sehlmeyer et al., 2009), possibly via involvement of the vmPFC (Roy et al., 2012). This brief summary shows the discrepancy in the complexity of the two tasks, even though they serve the same goal. Thus, if it is possible to reduce the complexity of an emotion regulation task by selecting one process of regulation and embedding it in an extended extinction task, it may be possible to shed light on the individual neural circuits that are involved and draw new knowledge about possible pathways to regulation difficulties in psychiatric disorders.

1.4 Learned Safety Contingent on Cognitive Evaluation as a tool to bridge the gap between two approaches

1.4.1 The rationale of this project – why do we want to know?

Although there have been a number of studies that have started to bridge the gap between instructed emotion regulation and the extinction of conditioned responses, there is still a need for further investigation. This is particularly important in light of the variety of mental disorders that include symptoms of emotion dysregulation. Clinical therapies often include attempts to train people to reappraise situations (e.g. in cognitive behavioural therapies), however, a more specific approach would be beneficial. Even though the general problems with dysregulated emotion exists and presents similarly in several disorders, it is possible that the underlying problems vary on a physiological level.

1.4.2 Emotion Regulation and Anxiety

Anxiety disorders are one of the most common mental health diagnoses with the earliest onset (Kessler et al., 2005). They can be seen as disorders of emotion regulation since patients are unable to inhibit inappropriate emotions during triggering situations, and replace them with an adaptive response (Thayer & Lane, 2000). It has also been shown that patients with anxiety disorders tend to be hypervigilant to situations they see as threatening, which may act to exacerbate the regulation difficulty (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007). Although anxiety is a common human emotion that is associated with evolutionary advantages when it is an appropriate response to real threats, anxiety disorders such as generalised anxiety disorder (GAD) are characterised by constant unescapable worry (Tyrer & Baldwin, 2006). Thus, it is not the emotions themselves that make anxiety disorders maladaptive, it is their timing and intensity (Kring & Werner, 2004).

This does not mean individuals with anxiety disorders regulate their emotions less – on the contrary, it has been found that they are less acceptant of their emotions and tend to attempt to regulate them more (Campbell-Sills, Barlow, Brown, & Hofmann, 2006), but their attempts are ineffective or produce the opposite effect (Gross & John, 2003). In fact, it has been reported that the use of strategies that are associated with negative long-term consequences such as suppression and avoidance is more common in participants with anxiety than in healthy controls (Amstadter, 2008). Learning and using reappraisal techniques on the other hand, is associated with more positive outcome (Feldner, Zvolensky, Eifert, & Spira, 2003; Kamphuis & Telch, 2000). Thus, these techniques are part of the recommended treatment for anxiety disorders (National Institute for Health and Care Excellence, 2011).

Working memory processes are one essential component of regulating emotion that may be affected by high levels of anxiety. Researchers have shown that participants high in trait anxiety tend to perform worse in digit-span, reasoning and verbal working memory tasks (Darke, 1988; Eysenck, 1985; Eysenck, 1998; Colin MacLeod & Donnellan, 1993), suggesting that reduced working memory capacity may play a role in the reduced ability to regulate in individuals with anxiety. In addition, attentional biases for threat-relevant cues that occur in anxiety disorders (Barlow, 1991; Kring & Werner, 2004; MacLeod, Mathews, & Tata, 1986; Mathews & MacLeod, 1985, 1986; Mogg, Millar, & Bradley, 2000) may prevent individuals with anxiety disorders from re-focusing their attention on different, non-threatening aspects of a given situation and/or an alternative outcome. On a neural level this is supported by research from Davidson (1998), who stated that anxiety was associated with reduced right prefrontal activity. As described previously, it is thought that the prefrontal cortex is involved in inhibiting a response in favour of a newly constructed one (Silvers et al., 2014),

thus supporting the view that patients with anxiety are inflexible in their emotional reactivity and less able to change their initial appraisal than healthy individuals (Barlow, 1991; Kring & Werner, 2004).

Mochcovitch, da Rocha Freire, Garcia and Nardi (2014) reviewed functional imaging studies of emotion regulation in GAD patients and concluded that hypoactivation in ACC and PFC as well as reduced connectivity between amygdala and ACC contributed to deficient top-down control of emotion in this group. Even though the opposite has been found by one study (Blair et al., 2008), most found that prefrontal areas were less activated in GAD patients than controls in paradigms using IAPS images (Ball et al., 2013; Blair et al., 2012), or emotional faces and words (Etkin, Prater, Hoeft, Menon, & Schatzberg, 2010; Palm, Elliott, McKie, Deakin, & Anderson, 2011). This is in contrast to the previously described tendency by participants with anxiety to attempt to regulate their emotion more than their non-anxious counterparts. However, it is possible that these failed attempts to regulate are not expressed in higher activation of the regions that are involved. This observed hypoactivation of prefrontal areas could be explained by the possibility that increased threat hypervigilance stops participants actually engaging with the regulation attempt, thus decreasing its effectiveness. The fact that resting state studies show impaired connectivity between amygdala and regions in the PFC in anxious subjects (Kim et al., 2011) supports the idea that information cannot be processed efficiently and effectively, which may be a maintaining factor in the pathology of clinical anxiety.

1.4.3 This Project

This project aimed to start bridging the gap between the conscious, deliberate regulation of emotion explored in studies using images or other stimuli to evoke a reaction, and the more automatic process of extinction of conditioned responses to neutral stimuli in which previously learned affective responses are inhibited in a more gradual, automatic way.

There have been previous attempts to bridge this gap using uninstructed emotion regulation. Williams, Bargh, Nocera and Gray (2009) found that unconsciously priming a reappraisal goal helped participants reduce negative emotion during a stress induction (preparation and holding of a speech), particularly those who did not habitually use reappraisal to reduce emotion. Similarly, Mauss, Cook and Gross (2007) found that presenting participants with a reappraisal prime resulted in reduced reported anger as well as reduced physiological signs of stress during an anger provocation task. Finally, implementation intentions (self-regulatory strategies of how to respond to

certain stimuli) have also been shown to help participants reduce their emotional responses (Gallo, Keil, McCulloch, Rockstroh, & Gollwitzer, 2009). Studies assessing the neural correlates of spontaneous emotion regulation have found the dorsomedial prefrontal cortex to be involved in the resolution of conflict during an emotional stroop (Etkin et al., 2010) and the ACC associated with spontaneous regulation of emotion in older adults compared to younger adults (Dolcos, Katsumi, & Dixon, 2014). However, while these studies investigate a more natural process, the interpretability of these results is still limited due to the lack of control over the mechanisms involved.

Studies based on extinction have also been adapted to investigate how the presence of safe stimuli influences conditioned responses to a CS+ (Grillon & Ameli, 2001; Pollak et al., 2010; Rescorla, 1969), and the effects of instructed regulation on responses to a CS+ (Delgado et al., 2008). Both lines of research have found behavioural and physiological reductions in conditioned responses along with extended networks of brain activation compared to straight extinction studies, which is consistent with the idea that processing is more complex and deliberate in these paradigms. However, there are still several open questions. The conscious and active reappraisal of affective information is used as an effective tool in cognitive behavioural therapy for anxiety disorders (National Institute for Health and Care Excellence, 2011) and this strategy is similar to those investigated in studies on cognitive reappraisal. However, it is unlikely that healthy people employ these types of conscious, long winded strategies to protect themselves from excessive emotion in acute affective situations. While they may apply reappraisal strategies during times of low mood, in situations in which excessive emotion is not appropriate, it is more likely that their strategies include a diversion of attention to goal-relevant (i.e. safe) aspects of the situation as well as a more habitual reappraisal later in the emotion generative process (Troy & Mauss, 2011). In addition, the complexity of the instructed reappraisal process does not allow researchers to draw conclusions about the specific roles of each of the relevant brain regions, even when the emotion eliciting stimulus is tightly controlled. In contrast, paradigms that extend the extinction of conditioned responses by adding safety signals go some way towards investigating the instinctive application of new information during a previously affective situation but the deliberate component of using evidence to work out whether a situation is safe or not is missing from those studies. Thus, this project aimed to design a paradigm in which a conditioned stimulus is presented with additional information that participants have to manipulate to predict the outcome of each trial. This way, the cognitive manipulation that participants had to perform was known and exactly the same for each participant, thus, addressing some of the criticisms of the standard emotion regulation task and making the interpretation of eventual fMRI results more straightforward. In addition, developing a general framework for such a paradigm will enable researchers to investigate different types of processes and mechanisms

employed during emotion regulation, e.g. verbal and semantic processes, memory, working memory, spatial or social aspects of this process, allowing for the investigation of possible separate mechanisms that may contribute to the emotion regulation difficulties in different mental health disorders.

In contrast to the Delgado et al. (2008) study, the conditioned stimuli were acquired through experience rather than instruction. It has been suggested that this may lead to more robust and reliable conditioning (Fullana et al., 2015). In addition, anxiety disorders have been associated with negative early experiences, suggesting that such a conditioning procedure may better reflect similar processes on a neural level (Mineka & Zinbarg, 2006).

Due to constraints in the spatial and temporal resolution of fMRI data and ethical issues with testing humans, it is necessary to separate different processes by designing paradigms that enable researchers to separate complex processes into their basic components. That is what we have attempted here. As outlined above, this general design idea could be executed using any one of a number of processes. In this project as the first version of this task we decided to use a semantic based task. In this version, letters will serve as the CS'. In the second phase designed to enable participants to cognitively evaluate these stimuli, words belonging to two distinct categories (and including the letters) will be presented. Based on the category information, participants will be able to work out whether a trial is likely to be paired with the US or not.

1.4.4 Research Questions

This project aims to investigate 3 main research questions:

1) will participants be able to selectively extinguish their affective and physiological responses to a CS+ ONLY when this is presented with the appropriate additional information?

2) will part of the neurobiology involved in this task be shared with that involved in instructed emotion regulation and extinction?

3) will a group of participants high in trait anxiety show a pattern of responses on this task that differs from a group of participants low in anxiety?

This project aimed to answer these research question through three separate studies. The first study (chapter 2) aimed to test an initial design of our task using SCR as a dependent variable. This served to a) investigate the viability of the general idea of this project, i.e. that a simple cognitive manipulation can be utilised to modify a CR, and b) provide an opportunity to detect design flaws

and improve the task for subsequent studies (research question 1). The second study used an improved design to investigate the physiological and neural correlates of the CR reduction and compare them to those of an instructed emotion regulation task using BOLD fMRI, SCR, and pupil dilation (chapter 4 – 8; research question 1 and 2). Finally, the third study (chapter 9) investigated any differences in the physiological and neural correlates of this task between high and low anxious participants (research question 3).

2. Study 1: Physiological correlates of Learned Safety Contingent on Cognitive Evaluation

2.1 Study 1 - Abstract

In this study we trialled a novel paradigm that aims to bridge the gap between the research into the extinction of conditioned responses and the instructed regulation of emotion via a prescribed strategy. This was done through a process we called Learned Safety Contingent on Cognitive Evaluation (LSCCE). Participants were conditioned to associate a previously neutral stimulus (letter, CS+) with an aversive burst of white noise (US) and another letter with no aversive event (CS-). In a second phase, the same letters were presented within words belonging to two distinct categories. One group did not receive any more noise bursts during this phase which lead to the extinction of the conditioned responses (extinction group). The other group (LSCCE group) was told that the CS+ now only carried a risk of the noise occurring when it was presented in one (dangerous), but not the other word category (safe). The aim was to trigger an initial affective response which could then be regulated once the category had been worked out by the participant. Affective responses were assessed using skin conductance responses (SCR).

We found overall increased SCR's in response to CS+ (safe and dangerous) compared to CS- trials in the LSCCE group, and also increased SCR's in response to dangerous compared to safe CS+ trials. This suggests that the conditioned response (CR) created in the conditioning phase can be reduced through the cognitive evaluation of additional information and it is possible that extinction mechanisms are involved in this process.

Unfortunately, we did not find any effects in the extinction group, even in the early trials. It is possible that, due to the 100% reinforcement schedule in the conditioning phase, and the perceptually different trials in the extinction phase (compared to the conditioning phase), extinction was completed too quickly so that any differences between conditions could not be captured. Future experiments will use these results as a basis for an improved paradigm which will use a different reinforcement schedule in the conditioning phase and perceptually more similar trials, among other changes, to better capture changes in SCR as well as BOLD fMRI in future experiments.

2.2 Study 1 - Introduction

Classical conditioning occurs when a previously neutral stimulus (conditioned stimulus, CS+) is paired with an affective stimulus (unconditioned stimulus, US) until the CS+ elicits the same affective response as the US (conditioned response, CR) (Pavlov, 1927). This can be carried out using either positive stimuli such as food, leading to approaching or the strengthening of desired behaviours, or

negative stimuli such as a loud noise or an electric shock, leading to conditioned fear and avoidance behaviours such as freezing. The reduction of these conditioned responses is typically achieved through extinction, when the CS+ is repeatedly presented without the US until the CS+ ceases to evoke a CR. The extinction of conditioned aversive responses is an implicit and well-controlled way of testing how affective responses are reduced. In humans, this is often measured through physiological measures such as skin conductance responses (SCR, see Delamater, 2010 for a review).

This extinction effect does not seem to involve the abolition of the CS+ – CR relationship, however. Animals that undergo conditioning and then extinction of the CR one day will tend to show recovery of the CR the following day, albeit to different degrees (see Bouton (2002), for a review). This suggests that a trace of the CS – CR relationship is maintained through extinction, but down-regulated by another system responsible for the extinction effect. Thus, extinction is a form of fear-related learning that involves storing a modified representation of the affective meaning of the previously conditioned CS, and using this to regulate or override the CR.

There are several strands of research that examine variations of the classic extinction paradigm by manipulating the contingencies under which the CS+ is removed. One example which investigates the effects of combining a CS+ and CS- on the CR is 'conditioned inhibition' (Rescorla, 1969) and describes the summation of a CS that reliably predicts a US and another CS which has never been associated with the same US. When presented together, they produce a reduction in CR and a delay in fear conditioning to the previously established CS-. Using SCR and the startle reflex as measures of the CR, Grillon and Ameli (2001) found a reduction in SCR when a previously reinforced CS+ was preceded by a safety signal in low anxious participants. Kong, Monje, Hirsch, & Pollak (2014) reviewed studies investigating the neural pathways underlying what they call 'Learned Safety', conditioned inhibition specific to aversive US'. This typically involves training participants to associate one stimulus (CS+) with an aversive event and another with the absence, or a period of safety from this aversive event. In a test phase, both are combined and result in a reduced reaction to this combined stimulus. Thus, evidence exists that a previously established CR can be modified and inhibited without an extinction phase, through the addition of safety cues.

It is often assumed that such extinction processes are the basis of higher level cognitive reappraisal of emotion, which serves the same goal as the extinction of conditioned responses: to reduce an affective response. However, few studies have been published that explicitly attempt to bridge this gap. Delgado, Nearing, Ledoux, and Phelps (2008) investigated the instructed regulation of conditioned responses to coloured squares. They instructed participants that either a blue or a yellow square indicated a chance that an electric shock would occur. Before each trial participants

were instructed to either “attend” to their naturally occurring response to that trial, or to “reappraise” is by thinking of something calming in nature related to the colour. They found that SCR’s were higher during CS+ “attend” than CS- “attend” trials, a difference that was abolished for CS+ “reappraise” and CS- “attend” trials. On a neural level, they found increased amygdala activation during CS+ “attend” trials compared to CS+ “reappraise” trials, and vmPFC and dlPFC in the opposite contrast. They concluded, that participants were able to regulate their affective response when asked to do so, and that they did recruit a set of brain areas that was more specific than that recruited in instructed emotion regulation studies, but extended compared to extinction. This supports the notion that the basic mechanisms involved in the extinction of affective responses and the instructed regulation of emotion overlap. Several questions arise when considering the design of this task. Instead of a standard conditioning procedure, Delgado et al. (2008) used explicit instructions to inform participants of the CS+/- contingencies. Although it has been shown that affective responses can reliably be evoked in this way, it is unclear whether the two procedures really tap into the same mechanisms (this is discussed in more detail in the literature review, see chapter 1). In this study, we used a conditioning procedure.

In this study, we aimed to develop a version of conditioned fear extinction in which a simple cognitive manipulation would enable participants to predict whether a trial including a previously CS+ is “safe” or “dangerous”. Thus, in contrast to the studies mentioned above, it is not an additional CS associated with safety from an aversive event or an explicit instruction which determines whether the CS+ will be followed by the US, but additional information that participants need to cognitively evaluate in order to determine the threat value of the CS+. Because the meaning of the same stimulus that would ordinarily be seen as threatening is changed via a cognitive evaluation of additional information in this study, this strategy could be seen as a simplified version of those used in cognitive reappraisal studies, during which participants are often asked to reappraise aversive images in order to regulate their emotions (McRae, Ciesielski, & Gross, 2012).In these tasks, participants are asked to employ predefined strategies to change their appraisal of the images they see. Both the images and these strategies are complex and involve a number of cognitive processes to be completed successfully. This new paradigm aims to simplify the process and tap into the basic mechanisms that may be part of complex reappraisal.

We established a CR in participants through a standard Pavlovian conditioning procedure using letters (B and T) as conditioned stimuli. This was followed by a modified extinction phase during which these letters were presented within words belonging to two categories (birds and vegetables). This phase will be titled “learned safety contingent on cognitive evaluation (LSCCE) phase” from here

onwards. One of those categories was safe, i.e. no US would be delivered. Thus, the words needed to be cognitively evaluated to determine whether or not the US could be expected to occur subsequently to the CS+ or whether the CS+ could be reinterpreted as safe. The use of letters and words in this task is an attempt to tap into sematic processes during emotion regulation. Instructed emotion regulation tasks often use verbal strategies such as "think of a more positive outcome to the scenario depicted" (McRae et al., 2012), thus, this seemed to be an appropriate starting point for this investigation. However, we designed this task with an emphasis on its simplicity and potential for adaptation, to enable researchers to create different versions of this task to investigate different specific processes that may be involved in emotion regulation, and could be altered in patients with mental disorders who have difficulties regulating their emotion. In this particular method, the cognitive processes involved are limited to word categorisation, remembering safe and dangerous categories, and making a decision about each trial. Other versions of this task might include a different type of safe vs dangerous contingencies, e.g. a more memory based task, or a more abstract visuospatial task.

Emotional responses were assessed using skin conductance. Response patterns were then compared to a version of this task which was more similar to classical extinction to determine similarities and differences between the two versions of the task.

To check for possible differences between the two groups of participants that may affect their performance on this task, we also gave participants a number of questionnaires to assess their habitual emotion regulation strategies (Emotion Regulation Questionnaire, ERQ, Gross & John, 2003), their positive and negative affect (Positive And Negative Affect Scales – now, PANAS-NOW, Watson, Clark, & Tellegen, 1988) as well as tendency to worry (Penn State Worry Questionnaire, PSWQ, Meyer, Miller, Metzger, & Borkovec, 1990).

Skin conductance responses (SCR's) are a standard way of assessing autonomic affective responses in humans. In conditioning studies, SCR's tend to rise as the association with the CS+ is established, and fall when the association is taken away (Davey, 1992). We hypothesized that the new paradigm would enable participants to differentiate between safe and dangerous CS+ trials, resulting in a reduction of the CR. Thus, we predict that SCR's will be reduced during safe CS+ trials but maintained high during dangerous CS+ trials. In the group that received a version of the task that was more similar to a classical extinction, we expected to see higher SCR's in response to both categories of CS+ compared to CS- trials at the start. We expected this difference to be abolished at the end of the experiment once extinction had occurred.

2.3 Study 1 - Method

Participants

42 participants were tested in total, divided into 2 groups of 21 – one group that received the learned safety contingent on cognitive evaluation (LSCCE) part of the task (mean age = 25.4, 11 female), and one that received an extinction phase instead (mean age = 21.6, 16 female). Participants were recruited through the University of Reading Research Panel, flyers around the University of Reading, emails sent to different departments and word of mouth. Participants were randomly assigned to the two groups. All participants were psychologically healthy and had never had a diagnosis of a mental disorder. Participants were reimbursed for their time with a small monetary reward or course credit. This study was approved by the University of Reading Ethics committee (UREC 13/32). All participants provided informed consent and were fully debriefed upon completion of the study.

The Tasks

Conditioning

Both groups received the same conditioning procedure during which one of two letters (B and T) were paired with a burst of white noise, 100% of the time. The letter used as CS+ was counterbalanced between participants.
10 CS+ and 10 CS- trials were shown for 2000ms, with white noise onset 500ms into stimulus presentation with a 100% reinforcement schedule of the CS+.

After this first phase both groups were told that they would now see the same letters within words that belong to two categories (birds and vegetables). They were instructed to monitor the stimuli by pressing one button if the word belonged to one of the two categories and contained the CS+, and the other button if it belonged to one of the categories and contained the CS-. Two filler words which belonged to neither category were included to keep participants alert. This task was added to ensure participants paid attention to both the letter and the word categories. All instructions were included in the Eprime script.

Learned Safety Contingent on Cognitive Evaluation

To induce a reappraisal process, participants in the reappraisal group were told that they would not hear the white noise when the word they viewed belonged to one of the two possible categories. The reinforced letter as well as category were counterbalanced between participants.
During the Reappraisal phases, stimuli were presented in a random order and each stimulus was shown for 4000ms. Stimuli belonging to the “dangerous” category and containing the CS+ were

accompanied by white noise after 1500ms. 42 trials were shown, 10 each of dangerous CS+, safe CS+, dangerous CS-, and safe CS- trials (see table 2). Thus, the reappraisal group were shown 10 CS+ within a "safe" word during which no white noise was to be expected. 10 CS+ within a "dangerous" word were also shown, 5 of which were non-reinforced. These were used for analysis.
2 distractor items were also shown to keep participants engaged and ensure they were paying attention to both letter and word stimuli.

The inter trial interval (ITI) was jittered between 2000ms and 6000ms in preparation for a future fMRI experiment.

Extinction

Participants in the extinction group were not given any further instructions about the contingencies of the task. They were then presented with the same paradigm as the reappraisal group (i.e. they were also asked to press one button if the word belonged to one of the two categories and contained the CS+, and the other button if it belonged to one of the categories and contained the CS-, as well as withhold their button press for the filler words) but no further noise bursts were delivered.

Apparatus and Materials

Stimuli were presented using EPrime 2.0 (Psychology Software Tools, Pittsburgh, PA). White noise was delivered through a set of KYE Systems Genius SP-G06 Hi-Fi Speaker Systemat a level of 90dB.

The two letters used as CS' were "B" and "T". To provide the safe or dangerous context, 20 different words were chosen from 2 categories: vegetables and birds. The words were chosen on the basis of lists defined as typical members of these categories (Battig & Montague, 1969). Further words were chosen through online dictionaries. All words were validated by checking their frequency in the English language in a list based on the British National Corpus (Kilgarriff, 1997, for frequencies see table 1).

TABLE 1. MEANS AND STANDARD DEVIATIONS IN WORD FREQUENCY BASED ON THE BRITISH NATIONAL CORPUS (KILGARIFF, 1997). NO SIGNIFICANT DIFFERENCE WAS FOUND BETWEEN GROUPS OF WORDS WHEN THEY WERE DIVIDED BY LETTER (T(18) = 1.78, P = 0.092) OR BY CATEGORY (T(10) = -1.75, P = 0.1).

		Bird	Vegetable	Total
		Mean (SD)	Mean (SD)	Mean (SD)
T	Mean (SD)	211.2 (167.09)	374.72 (282.33)	292.7 (234.98)
B	Mean (SD)	79.2 (63.44)	208.6 (135.27)	143.9 (120.72)
Total	Mean (SD)	145.2 (137.97)	291.4 (226.22)	

Letters and words were presented on a grey background. Stimuli were prepared using PowerPoint. Letters and words were arranged in the centre of each slide, with letters in font size 66 and bold, and words in font size 44 (not bold, see figure 3).

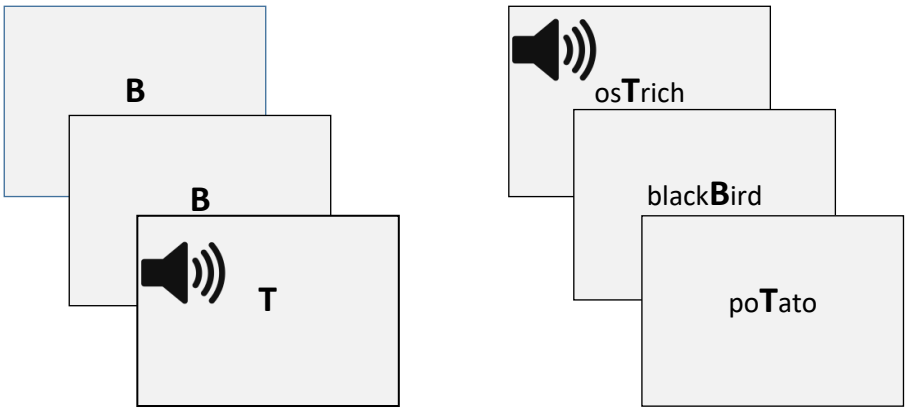


FIGURE 3. TRIALS IN THE LSCCE TASK. LETTERS ARE SHOWN IN BOLD IN THE MIDDLE OF THE SCREEN THROUGHOUT. IN THE LSCCE PHASE, LETTERS ARE EMPHASIZED WITHIN THE WORDS. IN THE EXTINCTION GROUPS, TRIALS WERE IDENTICAL BUT NO FURTHER SOUNDS WERE DELIVERED.

Skin conductance was recorded with Labchart 7 (ADInstruments, Dunedin, NZ) at a sample rate of 1000hz, using two stainless steel electrodes attached to the distal phalanges of participants' index and middle fingers of their non-dominant hand (Cacioppo, Tassinary, & Berntson, 2007). A low constant-voltage AC excitation of 22mVrms at 75 Hz was passed through the electrodes, which were connected to a ML116 GSR Amp, and converted to DC before being digitized and stored.

A questionnaire with a single question was used to assess the aversiveness of the white noise burst. Participants were required to mark on a 10-point Likert scale whether the sound was 1 (very aversive) to 10 (not aversive at all).

TABLE 2. DESIGN OF THE LSCCE TASK.

	Conditioning	**LSCCE Group**		**Extinction Group**	
		Safe	Dangerous	Birds	Vegetables
		(birds or vegetables)	*(birds or vegetables)*	*no further reinforcement*	
CS+	10 (all reinforced)	10	10 (5 non-reinforced)	10	10
CS-	10	10	10	10	10

Design

The design of this task was different for the two groups. In the reappraisal group we used a univariate design with 3 levels: CS+dangerous vs CS+safe vs CS-. In the extinction group we used a 2 factor design: CS+ vs CS-. Both groups underwent the same conditioning procedures but went on to complete either an extinction or a reappraisal procedure (see table 2.). Types of trials were compared within groups, contrasting (non-reinforced) CS+dangerous, CS+safe and CS- trials in the reappraisal group, and CS+ and CS- trials in the extinction group (all trials were non-reinforced in this group). Because we expected the responses to the CS+ to be reduced slowly in the extinction group, we included a time factor in a second step. Trials were presented in a random order throughout this task. Participants were randomly assigned to either the reappraisal or the extinction groups. CS+ and CS- as well as the safe and dangerous categories were counterbalanced between participants.

Procedure

After providing informed consent, participants were asked to rate the white noise and complete a number of questionnaires prior to commencing the task assessing their perception of the white noise burst and their participants' emotion regulation strategies, positive and negative affect, tendency to worry, and trait anxiety. The task and data collection procedures were explained and electrodes attached to participants' fingers. To avoid movement artefacts, participants were asked to remain as still as possible throughout. The task was then started and the experimenter left the room. After the task, the experimenter returned and removed the electrodes before debriefing the participant and answering any further questions.

SCR data analysis

Data was visually checked for motion artefacts and these were removed manually. SCR data was filtered using a band pass filter with a high cutoff of 1hz and a low cutoff of 0.01hz using a programme developed by Prof. Tom Johnstone, University of Reading (Johnstone, T. (2017, September 8). Psychophysiology Analysis Software. Retrieved from osf.io/4wsm3). Data was then analysed using a MATLAB (Mathworks Ltd.) script to identify the highest deflection from a 2 second pre-stimulus baseline within a 7 second window after each stimulus onset. Outliers were removed by transforming data into z-standardised scores across conditions and removing individual values that exceeded 3.29 or were smaller than -3.29, i.e. were larger or smaller than 99.99% of the values obtained in this experiment. Data from 3 participants in the extinction group was removed entirely (1 did not show responsivity throughout the task, 1 due to noise, and 1 due to a technical problem during data collection). Residuals were normally distributed, therefore data was not transformed but original data was analysed.

2.4 Study 1 - Results

Questionnaires

No group differences were found on any of the three questionnaires (ERQ, PANAS-now, PSWQ) or the stimulus aversiveness ratings (average aversiveness rating = 2.85). Only one participant rated the stimulus higher than 5 (See table 3 for Questionnaire scores).

TABLE 3. MEAN QUESTIONNAIRE SCORES.

Questionnaire	LSCCE group Mean (SD)	Extinction Group Mean (SD)	Cronbach's Alpha	T(37)	p
Stimulus Evaluation	2.67 (1.06)	2.83 (1.54)		-0.4	0.69
ERQ reappraisal	29.57 (5.02)	28.61 (6.36)	0.8	0.53	0.6
ERQ suppression	14 (4.53)	13.28 (4.99)	0.75	0.47	0.64
PANAS positive	29.62 (6.73)	25.5 (7.06)	0.88	1.86	0.07
PANAS negative	12.62 (3.65)	11.38 (1.82)	0.8	1.36	0.2
PSWQ	49.05 (17.33)	43.78 (15.84)	0.93	0.98	0.3

Behavioural Data

All participants were able to correctly perform the behavioural task throughout at above 80% accuracy (mean LSCCE group = 0.85, SD = 0.13; mean extinction group = 0.87, SD = 0.17). This difference was not significant between the two groups ($t(40) = 0.58$, $p = 0.57$).

Conditioning checks

No reliable comparison could be made between SCR's in response to CS+ and CS- during the conditioning phase, as the CS+ was 100% reinforced and thus, contaminated by the white noise.

LSCCE group

Only trials which did not include a burst of white noise were used in this analysis. A mixed effects GLM with 3 levels (CS+dang vs CS+safe vs CS-) was carried out on the SCR data from the reappraisal group which showed a significant effect of condition ($F(2,20) = 7.59$, $p = 0.002$, partial $\eta^2 = 0.27$, see figure 4, and table 4). Because the number of trials in each cell were unequal, sphericity could not be assumed in this case (Mauchly's $W = 0.703$, $p = 0.35$). Greenhouse-Geisser Correction of the main effect of condition still returned a significant result ($F(2,20) = 5.81$, $p = 0.012$, partial $\eta^2 = 0.23$). Post-hoc tests of this SCR data revealed that this effect was driven by the difference between both levels of CS+ and CS- (Responses to CS+dang was significantly greater than CS-: $F(1,20) = 9.92$, $p = 0.005$, Cohen's $d = 0.29$; Responses to CS+safe were significantly greater than CS-: $F(1,20) = 6.9$, $p = 0.02$, Cohen's $d = 0.19$). In addition, SCR's to dangerous CS+ trials were significantly larger than those to

safe CS+ trials (F(1,20) = 3.26, p = 0.043 (one-tailed), Cohen's d = -0.17).

Participants' questionnaire scores did not have an effect on these results.

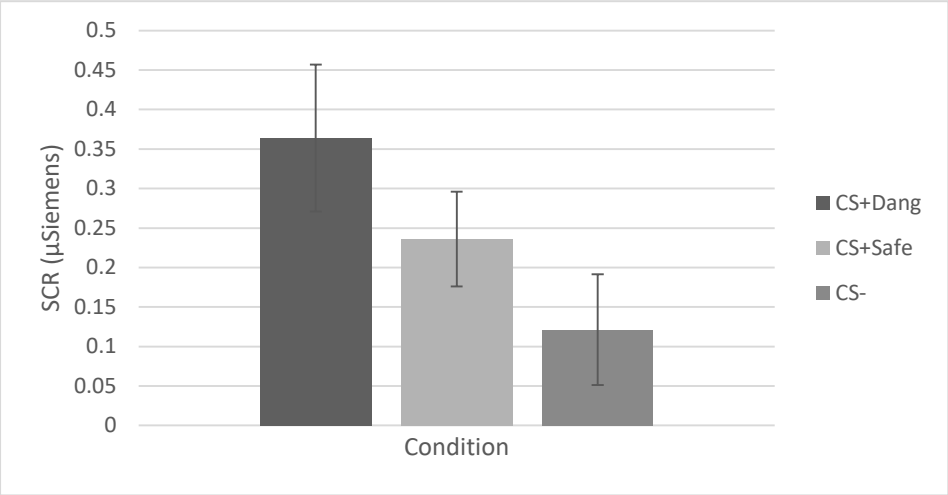


FIGURE 4. SCR IN THE LSCCE GROUP. ERROR BARS REPRESENT WITHIN SUBJECT 95% CONFIDENCE INTERVALS.

EXTINCTION GROUP

A mixed GLM with 2 levels (CS+ vs CS-) was carried out on the data from the extinction group. No significant difference was found between the two conditions (F(1,17) = 0.29, p = 0.6, partial η^2=0.013). The addition of a time factor revealed no significant effect of time (F(1,17) = 0.73, p = 0.41, partial η^2= 0.001) and no condition x time interaction (F(1,17) = 0.12, p = 0.73, partial η^2= 0.007).

To check whether there was a difference between CS+ and CS- trials in the first half of the extinction phase, we ran a separate mixed effects GLM on these trials and found no difference (F(1,17) = 0.22, p = 0.64, see table 4 for means).

Participants' questionnaire scores did not have an effect on these results.

TABLE 4. MEAN SCR IN µSIEMENS FOR EACH CONDITION. SCR ASSOCIATED WITH DANGEROUS CS+ TRIALS WAS SIGNIFICANTLY INCREASED COMPARED TO RESPONSES TO SAFE CS+ AND CS- SCORES. RESPONSES TO SAFE CS+ TRIALS WERE ALSO SIGNIFICANTLY GREATER THAN RESPONSES TO CS- TRIALS.

	Condition	Mean (CI)
Reappraisal Group		
	CS+safe	0.24 (0.16)
	CS+dang	0.36 (0.16)
	CS-	0.12 (0.12)
Extinction Group		
	CS+	0.21 (0.09)
	Early	*0.22 (0.12)*
	Late	*0.21 (0.12)*
	CS-	0.19 (0.09)
	Early	*0.19 (0.12)*
	Late	*0.21 (0.12)*

2.5 Study 1 - Discussion

The current study aimed to test a novel paradigm to investigate the specific mechanisms involved in emotion regulation. We used two letters (B and T) as CS+ and CS-, and a loud burst of white noise as US to evoke an affective response. These letters were then shown again within words belonging to two different categories (birds and vegetables). Two groups were tested: The reappraisal group was told that one of the categories represented safety from the white noise while the other remained dangerous. The extinction group was not told anything else and received no further bursts of white noise.

In the reappraisal group we expected to find increased SCRs in response to dangerous CS+ compared to safe CS+ and CS- trials.

In the extinction group we expected to find increased SCRs to CS+ compared to CS- trials in the early part of this phase, and no difference in the late part of this phase.

In the reappraisal group we showed an effective reduction of SCR in response to a previously conditioned letter when this letter was presented within a word belonging to a category participants knew was safe compared to a word which implied the danger of receiving a loud burst of white noise. This suggests that participants were able to reappraise this type of stimulus on the basis of cognitive evaluation of contextual information in order to determine its meaning, and that this modulated the CR, confirming our hypothesis. One possible explanation of this result is that mechanisms similar to those engaged in classical extinction may be at the basis of this CR reduction. This includes remembering the new contingencies, applying this knowledge on a trial by trial basis, and inhibiting the CR during safe trials. On a neural level this process is thought to be completed through processing both within the amygdala itself (Pare & Duvarci, 2012) as well as via inputs from the hippocampus (providing contextual information; Barrientos et al., 2002; Huff & Rudy, 2004; Matus-Amat et al., 2004; Squire, 1992) and vmPFC (integrating contextual and stimulus information and feeding this back to the amygdala; Fullana et al., 2015). However, this idea will have to be explored further using imaging techniques such as fMRI to investigate the neural circuitry involved in this process. Previous studies found SCR reductions when a learned safety cue was added to a previously reinforced CS+ (Kong et al., 2014, Rescorla, 1969), however, these cues did not have to be cognitively evaluated by the participants in order to determine safety or danger. Thus, this study extended those findings by adding an extra evaluative layer and making the paradigm more similar to those used in studies of instructed emotion regulation (e.g. Buhle et al., 2014).

These results complement the findings by Delgado et al. (2008) who used instructed fear conditioning to elicit aversive responses to one of two coloured squares, and then instructed participants to reappraise these by thinking of something calming in nature. They found that

participants were able to effectively reduce their affective responses through this strategy. On a neural level they found that in addition to amygdala and vmPFC, part of the dlPFC was also involved in the regulation of the CR. The task introduced here provides increased control over the reappraisal process compared to Delgado et al. (2008), thus, future studies will investigate whether similar PFC areas mediate the reduction of the CR in this paradigm.

Similarly, Hefner, Verona, and Curtin, (2016) told participants to expect an electric shock after a word printed in red but not if that word belonged to one specific category. They found decreased startle and faster reaction times during safe CS+ trials. Analysis of ERP components revealed, that threat information relating to word colour (i.e. CS+) was processed before the safety signal (i.e. word category), suggesting independent analysis of the components that make up this task. In our uninstructed conditioning task we found a similar effect – SCR's in response to both safe and dangerous CS+ trials were increased compared to CS- trials, and dangerous CS+ trials produced larger SCR's than safe CS+ trials. This also suggests that the information related to the letters (CS) had an influence on SCR independently from the information related to the word (safety cue).

In contrast, we found no significant difference between CS+ and CS- trials in the group that received the extinction conditions. We expected a difference in the early trials with the CS+ trials resulting in higher SCRs compared to CS- trials. The fact that we did not find this effect may be explained by a flaw in the design of this study, which would impact the extinction group in particular: The trials presented during the conditioning phase visually differed greatly from those presented in the extinction/reappraisal phase. In the conditioning phase we presented single letters, whereas in the extinction/reappraisal phase, words were presented for the entire duration of the trials. Further, while reappraisal group participants were informed of the danger of the aversive sound for one of the word categories when combined with the CS+, extinction participants were only asked to complete the accompanying task (to press one button if the word on the screen belonged to one of the two categories and contained the CS+, and the other button if the word belonged to one of the two categories and contained the CS-) with no further information and no further noise bursts. Because context is a vital factor in the maintenance of a conditioned effect (Bouton, 2004a), it is possible that the large change in context, coupled with no explicit instruction to pay attention to the combination of letter and word category, immediately abolished the conditioned response in the extinction group. In addition, we used a 100% reinforcement schedule during conditioning, thus, even if the conditioned effect persisted in the extinction group to start with, only few trials contained enough information for this to be abolished. This factor was taken into consideration in later versions of this task.

There are several potential limitations to this task and its results. As mentioned above, the trials in the conditioning and extinction phase were perceptually quite different, even though the conditioned letters were emphasised within the words. Thus, it is possible that the trials in the extinction phase were not similar enough to maintain a conditioned response when participants were not instructed of the continued threat associated with one of the letters. In future versions of the task this will be addressed by presenting the full words for only a short period of time, keeping only the letter on screen for the remainder of each trial.

In addition, while the 100% reinforcement schedule employed in the conditioning phase may have ensured that participants were aware of the contingencies during conditioning, it may have also played a role in further speeding up the extinction process. This would make the extinction process difficult to capture in SCR which is subject to great individual differences and trial by trial variability (Cacioppo et al., 2007) and, thus, differences between conditions can only be captured with a larger number of trials. Since a 50% reinforcement schedule is an effective way to train an association between a CS+ and a US (thus, producing a CR) and slowing down the extinction process across a larger number of trials (e.g. Phelps, Delgado, Nearing, & LeDoux, 2004), we will employ this in future task versions. Another issue was the small number of analyseable trials in this version of the task. Only 10 dangerous CS+ trials were presented to participants, therefore only 5 of those were non-reinforced trials and thus, uncontaminated by the white noise burst. As mentioned above, SCR is highly variable between individuals so the small number of trials will have reduced our power to find an effect. We will increase the number of trials to include in the analysis in future versions and make them equal across conditions.

Conclusion

This study used a novel task to assess the impact of simple cognitive evaluations on autonomic responses to letter stimuli that had previously been conditioned to an aversive white noise. The results suggest that participants were able to reappraise the CS+ and CR's were significantly reduced when the CS+ was presented with additional information they knew to predict safety from the white noise burst. As a next step, this paradigm will be improved by addressing the limitations mentioned above including changing the reinforcement schedule to 50% during conditioning, redesigning the trials in the extinction phase to be perceptually more similar to the conditioning trials, and increasing the number of trials over all. In addition, we will go one step further in investigating whether extinction mechanisms are applied during this task and collect BOLD fMRI in addition to SCR.

3. Study 2 Preparation

3.1 Pilot - Introduction

Although the results from study 1 point were a promising indication that our task works as intended, several issues with the design need to be improved on to be able to draw better conclusions. This chapter will go through some of the problems and explain the changes we made to the task to make it more interpretable. It will then introduce a pilot study to test a new task design.

Issue 1 – Conditioning checks not possible because CS+ reinforced 100% of the time
We employed this design in the first study to make sure that participants were absolutely aware of the contingencies for the second part of the study. However, a 50% reinforcement schedule has been shown to be as effective for learning (e.g. Phelps, Delgado, Nearing, & LeDoux, 2004) and because of the added uncertainty on each trial they may actually be seen as more aversive than a 100% reinforcement schedule. In addition, the 100% reinforcement schedule prevented us from assessing conditioning effects because all CS+ trials were confounded by white noise. Thus, in the next part of this project, we employed a 50% reinforcement schedule both in the conditioning and the reappraisal phase.

Issue 2 – Visual difference between trials in conditioning and reappraisal/extinction phase

Trials in the conditioning phase consisted of only the letter, whereas in the reappraisal phase the word was on the screen for the duration of the trial. Although the letter was emphasised in within the word trials, this extreme visual difference may have had an important influence on the way the CS letters in the LSCCE/extinction phase were perceived. Especially the fact that there was no difference between CS+ and CS- trials in the extinction group suggests that this trial structure needs to be adjusted. We therefore decided to alter the trial structure and make it more similar to those employed in emotion regulation paradigms. Instead of showing the word for the duration of the trial, we decided to show the letter on its own at the start and at the end of the trial, with the word appearing briefly after the first 500ms. The aim of this new design is to emphasize the letter as the primary signal of danger or safety, and the word category as additional information.

Issue 3 – Instructions

Before the LSCCE phase, participants were told the contingencies of the task including which word category was safe and which was dangerous. Although this ensured that participants were absolutely aware of the contingencies of the task, it did not facilitate any learning and may have contributed to habituation towards the end of the task. Thus, in the next instalment of the task we only told

participants that there was one safe and one dangerous category but not which one was which. The aim was to increase the uncertainty during the task and prolong the affective responses to the different trial types. In addition, this change enabled us to include baseline trials at the beginning of the LSCCE phase (before the safe – dangerous contingencies could be worked out by participants) to assess whether the conditioning effect was maintained when words were added to the trials.

Issue 4 – Between groups design

We initially chose a between groups design because we could not counterbalance the reappraisal and extinction phases without influencing the responses differentially. In the new task design we decided to add trials which only included the letter but no reinforcement to investigate how conditioned responses change in these circumstances.

Issue 5 – Sound as the US

Although the sound was rated as aversive by all participants, because the aim was to further examine this paradigm within an MRI environment, we were unsure about its effectiveness in interaction with the MR sounds. We therefore decided to move to electric shocks as US'.

3.2 Pilot - Method

Participants

5 participants (3 female, mean age 25.4) participated in this pilot study. They were recruited through word of mouth at the University of Reading.

Design

The conditioning phase was conducted as a 2 level (CS+ vs CS-) repeated measures design, whereas the LSCCE phase was constructed in a 2 x 3 (condition (CS+ vs CS-) x trial type (Safe vs Dangerous vs Letter Only)) repeated measures design.

APPARATUS

20 trials were presented to participants in the conditioning phase – 10 CS-, 5 reinforced CS+, and 5 non-reinforced CS+. Trials in the conditioning phase consisted of 4000ms of the letters. The US was a mild electric shock that was adjusted individually and delivered through two Ag-AgCl electrodes connected to an ADInstruments isolated stimulator built into a Powerlab 26T. A low constant-voltage AC excitation of 22mV$_{rms}$ at 75 Hz was passed through the electrodes, which were connected to a ML116 GSR Amp, and converted to DC before being digitized and stored.

In the reappraisal phase 20 CS-Safe, CS-Dang and CS+Safe stimuli, as well as 10 CS+Dangerous reinforced, CS+Dangerous non-reinforced, CS+Letter only, CS- Letter only were presented. Trials lasted for 5500ms. The first 500ms only the letter was presented, then the word appeared for 1000ms. Finally, the word disappeared again and only the letter stayed on the screen. Throughout

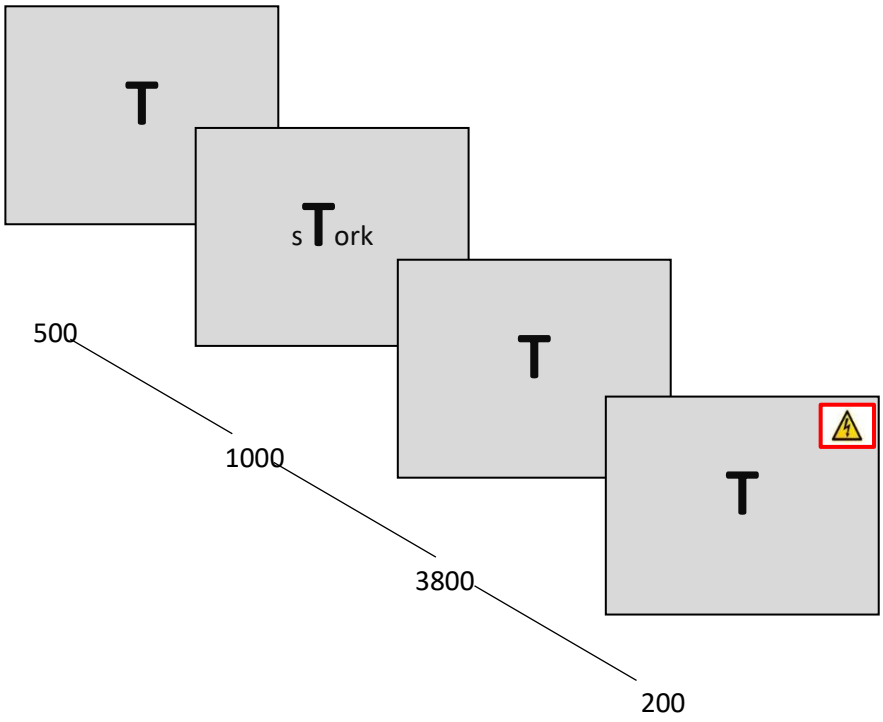


FIGURE 5. EXAMPLE OF AN LSCCE TRIAL.

the trial, the letter was emphasised in large and bold font (see figure 5 for an example trial). Intertrial intervals were jittered and ranged from 2000ms to 6000ms.

Stimuli were presented using EPrime 2.0 (Psychology Software Tools, Pittsburgh, PA).

Skin conductance was recorded at 1000hz using a different set of Ag-AgCl electrodes attached to the same ADInstruments Powerlab 26T via a PowerLab ML116 SCR amplifier input module.

PROCEDURE

After informed consent was obtained from each participant, the shock intensity was determined. An electric shock at a low intensity was delivered (0.5mV) and the intensity was increased in steps of 0.5mV. After each shock, the participant was asked to rate the sensation on a scale of 1 (“not painful

at all") to 10 ("extremely painful"). When they reached 8 on this scale, the intensity of the shock was reduced by 1 step and informed that this was the intensity the shock would remain at for the duration of the experiment.
Participants were then told to expect a shock to occur with one of the CS' during the conditioning phase, and also that one category was safe in the reappraisal phase. They were not given any further details.
After the task, participants were debriefed and thanked for their time.

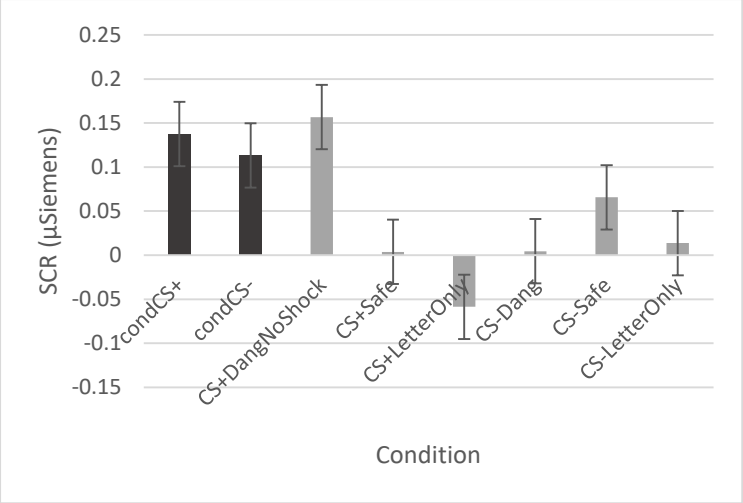
SCR DATA ANALYSIS

SCR data was analysed using a matlab script that determined the largest deflection for each trial from a pre-trial baseline within a 7 second time window from trial onset. The data was then examined visually and outliers were removed on a trial by trial basis. These peak values were then averaged across trials for each condition for each participant.

3.3 PILOT - RESULTS

Because of the limited amount of data in this pilot, no inferential statistics were carried out on these data and descriptive statistics are presented instead. We calculated standard errors across all trials, conditions and subjects to give an idea of the overall spread of the data.

The means in the conditioning phase were as expected: (Non-reinforced) CS+ trials (Mean SCR = 0.14μS) evoked higher SCR's than CS- trials (Mean SCR = 0.11 μS, see figure 6).
Of particular interest in the reappraisal phase was the contrast between dangerous and safe CS+ trials. The pattern was as expected: dangerous CS+ trials evoked higher SCR's than safe CS+ trials (CS+Dang Mean = 0.16 μS; CS+Safe Mean = 0.004 μS).
The negative mean of the CS+ Letter Only trial (-0.06 μS) is surprising, however, because of the small number of trials and participants, this is likely to represent an artefact. No further shocks were given during these trials so this may represent an extinction effect.



	Mean (µSiemens)
Conditioning	
CS+	0.14
CS-	0.11
Reappraisal	
CS+Dang	0.16
CS+Safe	0.004
CS+LetterOnly	-0.06
CS-Dang	0.004
CS-Safe	0.07
CS+LetterOnly	0.01

FIGURE 6. MEAN SCR IN THE PILOT STUDY.

THE RESULTS POINT IN THE EXPECTED DIRECTION. SCR TO CS+ TRIALS ARE LARGER THAN THOSE TO CS- TRIALS DURING CONDITIONING. DURING THE LSCCE PHASE, RESPONSES TO DANGEROUS CS+ TRIALS ARE LARGER THAN THOSE TO SAFE CS+ AND ALL CS- TRIALS. ERROR BARS REPRESENT STANDARD ERRORS ACROSS ALL RESPONSES.

3.4 Pilot - Discussion and next steps

In this pilot study we found the pattern of results that we expected based on the theory of the task. Participants seemed to be able to reduce their affective response to CS+ trials when they were presented with a context based "clue" that indicated no threat of shock. This suggests that CR's were successfully produced during the conditioning phase, and that participants were able to partially extinguish them during safe trials in the LSCCE phase. In addition, there doesn't seem to be a difference between safe and dangerous CS- trials. Taken together, this suggests that both letter and word category cues are processed by participants. This is a good basis for further investigation including the collection of BOLD fMRI data. However, in the course of the analysis we came across more issues that needed to be resolved to improve the task.

Issue 6 - Unequal numbers of comparable trials

Although the move to a 50% reinforcement schedule in the conditioning phase meant that we were able to check whether the conditioning worked, it meant we ended up with unequal numbers of trials in the conditions which decreased the power to detect differences in our contrasts. Thus, we increased the number of CS+ trials to solve this problem. We took the same approach to the reappraisal phase, increasing the number of dangerous CS+ trials. One concern with this approach then, is the balance between CS+ and CS- and CS+Safe and Dangerous trials. Participants may process these trials differently according to the amount of times they have been exposed to them. This can be partly remedied with the order of trials. In study 1 we had the same number of trials for each condition in the experiment and they were presented in a fully randomised order. Because the

likelihood of each trial occurring is equal, a fully randomised order is not problematic in that case. However, when the likelihood of one type of trial is more likely to occur, a pseudo-randomised design is more appropriate. There is evidence from similar types of tasks that a higher number of trials in one condition can be dealt with in that way (e.g. Delgado, Nearing, Ledoux, & Phelps, 2008).

Conclusion

The results of this pilot study were promising, suggesting that the aim to investigate emotion regulation on a basic level can be achieved with this task, and, thus, it is reasonable to take this a step further and investigate the neural circuits involved in this process.

4. Introduction to Chapters 5., 6., and 7.

4.1 Study 2 - Abstract

In this study we tested the neural correlates of a Learned Safety Contingent on Cognitive Evaluation (LSCCE) task designed to investigate the links between cognitive emotion regulation and extinction of conditioned fear in a simple and controlled way. Participants were conditioned to expect an electric shock during the presentation of one of two letters (CS+ and CS-). In a second phase, the same letters were presented within words belonging to two distinct semantic categories. Participants were told that one of these categories was safe. A contrast between safe and dangerous CS+ trials revealed a network of brain regions including left IFG, as well as bilateral temporal and parietal cortices, a network associated with word processing and working memory. Clusters in bilateral insula and anterior cingulate cortex (ACC), part of the network typically associated with pain, showed greater activation for dangerous versus safe CS+ trials. This suggests that the task elicited the expected anticipatory pain response during trials that remained dangerous, whereas a semantically based cognitive control mechanism regulated this response during safe trials. Results from this task were also compared with those from a modified version of an instructed emotion regulation task using negative IAPS images as affective stimuli. This task revealed the expected network of neural activation including amygdala and insula when negative images were attended to, and ventrolateral and dorsolateral prefrontal cortex as well as parietal regions when these images were reappraised. Even though a comparison of the two results showed no overlap between the tasks, a post-hoc analysis revealed that the left IFG cluster found in the LSCCE task was also active during the instructed emotion regulation task. These findings support the idea that this type of new task can be used to investigate the underlying mechanisms involved in the complex process of emotion regulation.

4.2 Study 2 - General Introduction

Studies of emotion regulation typically use highly affective images (e.g. from the International Affective Picture system, IAPS (Lang et al., 2008)) to evoke reactions. Participants are then instructed to either attend to the emotional content of these stimuli, or regulate their initial reaction using strategies they received training in beforehand. The ability of this paradigm to evoke consistent behavioural, physiological and neural responses suggests that it is a valid and reliable way to investigate emotion regulation. There are many benefits of using natural and complex images such as the IAPS images to elicit affective responses similar to those that may occur in real, extreme situations, as well as strategies often akin to those taught in cognitive behavioural therapy (CBT) to reduce these reactions. However, the complexity of both stimulus and (instructed) regulatory

response also makes it difficult to pinpoint the underlying basic mechanisms that contribute to this process. The initial appraisal of the images requires visual search, attention, and semantic processing as well as emotion generation and maintenance. Reappraisal of the initial affective response to the negative images then requires generation and application of an appropriate strategy, using working memory and attention, possibly also drawing on episodic memory, downregulation of the response, and monitoring of regulation success (Buhle et al., 2014; Kohn et al., 2014). Consequently, studies using variants of this paradigm consistently report an extensive cortical network to be active during regulation compared to attend conditions, including , ventro-and dorsolateral prefrontal cortices (vl- and dlPFC), associated with working memory and attentional processes, as well as emotion processing, posterior parietal regions, linked to internal imaging, language processing and memory, and vmPFC, dorsal medial PFC and ACC, areas which have been linked to the integration of cortical information and are assumed to play a direct role in the inhibition of the affective response (Kohn et al., 2014). Subcortical regions such as the amygdala and insula, associated with emotion processing and response generation, tend to be more active during attend compared to regulation conditions (Buhle et al., 2014). In addition, the dorsal ACC has been found to be part of a fear conditioning circuit, and is likely related to the generation of the physiological fear response (Etkin, Egner, & Kalisch, 2011; Mechias et al., 2010). More rostral areas of the dorsal ACC , in contrast, have been associated with the conscious appraisal of threat, for example in instructed conditioning studies (Kalisch & Gerlicher, 2014; Mechias et al., 2010). The wide range of psychiatric disorders including anxiety, depression, posttraumatic stress disorder (PTSD), and schizophrenia (Amstadter, 2008; Gross & Levenson, 1997; Gross & Muñoz, 1995; Joormann & Vanderlind, 2014; Khoury & Lecomte, 2012) that can be described as disorders of emotion regulation, further suggests a variation of mechanisms that are likely to be involved in emotion regulation and may be altered when problems arise. There is, thus, a need to investigate emotion regulation at a level which enables us to control both the adverse nature of the affective stimulus used, as well as manipulate the processes that give rise to stimulus appraisal and response regulation.

Like instructed emotion regulation, the extinction of conditioned responses (CR's) serves to reduce an affective response. Prior to extinction, the conditioning phase typically involves pairing a previously neutral stimulus (CS+) with an aversive, unconditioned stimulus (US), with an additional unpaired neutral stimulus used as a control (CS-). Eventually, the CS+ will elicit the CR, even when the US is not present (Pavlov, 1927; Rescorla, 1968; Watson & Rayner, 1920). During extinction, the US is removed, and over a number of trials the CS+ ceases to elicit the CR. Human and animal studies have shown that amygdala activity to the CS+ increases during the conditioning phase and decreases during extinction, suggesting that it is involved in the processing of the predictive meaning of the

CS+ (e.g. Kim & Jung, 2006). However, during extinction, the CS-US association is not unlearnt, instead, a representation of the new contingencies (i.e. US omission) is formed which inhibits the original CR. This is evident by the return of conditioned fear in rats on the day after initial fear conditioning and extinction as well as the role of context which can modulate conditioning and extinction (Bouton, 2002; Milad & Quirk, 2012). The ventromedial prefrontal cortex has been shown to play a vital role in the extinction process, however, there is currently no consensus on its specific role. Although some argue that it is directly involved in extinction (Gewirtz, Falls, & Davis, 1997; Morgan et al., 1993), it has since been found that it is involved in the recall or maintenance of an extinguished response, facilitating faster extinction during a recall test in a subsequent session (Milad & Quirk, 2002; Phelps et al., 2004).

In summary, it can be said that both the extinction of conditioned fear, and the instructed regulation of emotion serve the same goal, the reduction of negative affect. In addition, the mechanisms involved (i.e. the evaluation of the situation and the individual's response, the reduction of this response and monitoring of the result), as well as the brain areas involved in both processes (i.e. the amygdala, insula, vmPFC) also overlap (Hartley & Phelps, 2010).

Nonetheless, few studies have jointly examined these two paradigms of emotion regulation to ascertain the extent to which mechanisms and neural circuits involved in both processes overlap. Delgado, Nearing, Ledoux, & Phelps (2008) asked participants to either attend, or apply a strategy similar to classic instructed emotion regulation (i.e. to think of something calming in nature), to an aversively conditioned stimulus (conditioning was completed through instruction). As predicted, they found a network consisting of amygdala, vmPFC and dlPFC to be involved, with higher amygdala responses during "attend" trials, and, in turn, increased dlPFC and vmPFC during regulation trials. This vmPFC and amygdala activation overlapped with that found in a parallel experiment investigating the extinction of conditioned responses. In a connectivity analysis they also found vmPFC activation to be correlated with both amygdala and dlPFC activation, further supporting the notion that the dlPFC influences the activation of the amygdala via the vmPFC. Similarly, studies of conditioned inhibition and learned safety which involve adding a conditioned "safe" stimulus to a CS that predicts an aversive event, find a reduction of the CR in humans and animals, and a similar network of brain areas to be involved (Bouton, 2004; Kong et al., 2014; Rescorla, 1969).

Thus, evidence suggests that the neural mechanisms involved in emotion regulation and the extinction of conditioned responses overlap, but no study has attempted to compare the two in a single study. The following study is an attempt to do that by comparing extinction through cognitive evaluation, an extension of a classical extinction paradigm, with an instructed emotion regulation task.

5. Study 2.1 - The neural correlates of Learned Safety Contingent on Cognitive Evaluation

5.1 Study 2.1 - Introduction

Learned Safety Contingent on Cognitive Evaluation (LSCCE) combines elements of emotion regulation and extinction paradigms, replacing the instruction to regulate (Delgado et al., 2008) or the safety signal (Kong et al., 2014; Rescorla, 1969) with safety information that depends on the participant performing a simple cognitive, in this case semantic, manipulation. We combined one of two letters (B and T; CS+ and CS- respectively, counterbalanced across subjects) with an electric shock during the conditioning phase of this paradigm. During the LSCCE phase, we then presented the same letters within words belonging to two distinct categories. Participants had to learn which one of those categories was safe (i.e. not associated with an electric shock) to be able to assess the risk of receiving an electric shock during each trial. This assessment involves word categorisation, remembering the safe category, and deciding whether each trial is safe or dangerous. We predicted that safe trials would result in spontaneous down-regulation of the CR, whereas the CR would be maintained during dangerous CS+ trials. We collected BOLD fMRI, skin conductance (SCR) and pupil dilation throughout this task.

We hypothesized that this would result in increased activation in vlPFC and dlPFC (and vmPFC), as well as areas related to semantic processes and semantic decision making during safe compared to dangerous CS+ trials, representing the cognitive and affective evaluation of the additional information and the implementation of new stimulus-affect contingencies. On the other hand, we expected limbic brain regions associated with affective responses such as the amygdala and insula to be more active during dangerous than safe CS+ trials. Considering evidence from comparisons of instructed and uninstructed fear conditioning studies, we also expected some involvement of dorsal ACC (Kalisch & Gerlicher, 2014; Mechias et al., 2010). Based on the evidence presented by Kalisch and Gerlicher (2014), as well as Mechias et al. (2010), we expected dorsal ACC to show activation both during the conditioning phase, as well as during dangerous CS+ trials in the LSCCE phase. Furthermore, participants were required to cognitively evaluate the stimuli in the LSCCE phase, thus, we introduce a conscious appraisal component. Following the line of evidence above, it is possible that this also involved activation in rostral ACC. In addition, we assessed functional connectivity through a psychophysiological interaction (PPI) analysis and expected to find negative correlations between prefrontal and limbic regions during safe CS+ trials compared to during CS+ dangerous trials.

Psychophysiological responses such as skin conductance and pupil dilation have also been used to

investigate emotion regulation. There has been controversy about whether pupil dilation represents emotional arousal with some saying that it does (Bradley, Miccoli, Escrig, & Lang, 2008; Hess & Polt, 1960) and some stating it reflects cognitive effort (Koelewijn, Zekveld, Festen, & Kramer, 2012; Papesh & Goldinger, 2012). Urry, van Reekum, Johnstone and Davidson (2009) directly compared these two autonomic responses and found that while skin conductance reflected emotional arousal, pupil dilation reflected cognitive demand during effortful intentional emotion regulation. As the current paradigm is designed to induce the spontaneous reduction of conditioned responses through simple word categorization, it lends itself to further investigating the nature of SCR and pupil dilation in emotion regulation.

Based on Urry and colleagues (2009), we expected increased skin conductance responses during CS+ compared to CS- during conditioning, and dangerous CS+ compared to safe CS+ trials during LSCCE. We expected no significant difference between CS+ and CS- during conditioning in pupil dilation as the cognitive demand is the same for both stimuli. During LSCCE we expected pupil dilation to be increased during CS+ compared to CS- trials, due to increased cognitive demand while participants figure out whether CS+ was safe or dangerous (in contrast to CS- trials which were always safe).

In addition to the above we added physiological noise parameters into the fMRI analyses of these tasks. There has been a debate recently about whether some neural activation, especially in the amygdala, attributed to psychological processes may, in fact, be due to physiological noise from heart rate and respiration, which can also vary between different experimental conditions (Boubela et al., 2015). Thus, we tested whether adding these parameters made any difference to the activation contrasts in these tasks.

Finally, to be able to control for possible effects of current mood, habitual emotion regulation strategies, tendency to worry, trait anxiety, and intolerance of uncertainty we collected this data from each participant using questionnaires (Buhr & Dugas, 2002; Gross & John, 2003; Meyer et al., 1990; Spielberger, Gorsuch, & Lushene, 1970; Watson et al., 1988).

5.2 Study 2.1 - Method

Participants

20 (8 male) participants took part in this study. Participants were recruited via the University of Reading Research Panel and through university-wide emails. Participants were eligible to take part if they were between 18-55 years old, were right-handed, and had never been diagnosed with a mental disorder. All participants were screened for their suitability to be part of an fMRI study and signed a consent form before commencing the study. Mean age of the participants was 27 years

(range: 18 – 44). Participants received £10 and a picture of their brain to thank them for their participation.

The Task

Conditioning Phase

During the first part of this experiment one of two letters (B and T) was paired with an electric shock 50% of the time. The letter that served as the CS+ was counterbalanced between participants. Based on our previous research, this phase consisted of a total of 30 trials: 10 CS-, 10 reinforced CS+, and an additional 10 non-reinforced CS+. Trials lasted for a total of 4000ms during this phase of the task, and the electric shock co-terminated with the reinforced CS+ trials.

Learned Safety Contingent on Cognitive Evaluation (LSCCE)

During the LSCCE phase of this experiment, participants saw a total of 142 trials: 20 each of "safe" and "dangerous" CS-, and and 20 safe CS+, 20 reinforced dangerous CS+ and an additional 20 non-reinforced dangerous CS+. 2 trials contained words that did not belong to either of the two categories to ensure that participants were paying attention throughout. For the first 500ms of each trial, the letter was presented on its own. The word then appeared around it (with the letter in the correct place but emphasized) for 1000ms. Finally, the word disappeared and the letter remained on screen by itself for an additional 4000ms. During reinforced trials the electric shocks were delivered so they co-terminated with the trial. Also included in this phase were 20 trials that did not contain a word (10 CS+ and 10 CS-) to investigate how adding the words would influence physiological and neural activation in no-word trials. These trials were never reinforced.

Instructions

All instructions were given prior to task commencement. Participants were told that they would see two letters during the first phase, one of which would be associated with a risk of electric shock. They were then told that after a short break, they would see the same letters again, and this time a word would appear briefly. Most of these word would belong to two distinct categories: plants or animals, and one of these categories was safe. They were then asked to determine which category was safe during this phase, and to keep the contingencies in mind throughout. They were also asked to focus on whether they thought they might receive an electric shock or not during each trial. They were also told that 3 brief breaks were included in the task but that contingencies would not change. They were not given any additional information about the letter only trials.

Finally, they were told to press a button on the rare occasion that they saw a word that did not belong to one of the categories.

Design

This study was carried out in a within subjects 2 x 2 (Letter (CS+ vs CS-) x trial category (safe vs dangerous)) design. The main contrasts of interest compared CS+ and CS-_ during the conditioning phase, and dangerous CS+ (CS+dang) with safe CS+ (CS+safe) trials during the LSCCE phase. Only non-reinforced trials were included in this analysis.

Individual differences were assessed using a range of questionnaires. Further, the letter-only trials were included to assess how the LSCCE conditions affected responses during those trials.
Letters representing the CS+ and CS- as well as "safe and "dangerous" categories were counterbalanced, and stimuli were presented in a pseudo-random order, ensuring that no condition occurred more than twice in a row.

Apparatus and Materials

Conditioning stimuli (CS+ and CS-) were the letters "B" and "T" and the unconditioned stimulus (US) was a mild electric shock (10 pulses at 100hz).
During the LSCCE part of the experiment, participants were presented with the same two letters, briefly surrounded by words. Most of these words were either animals or plants; two words were fillers from neither category to ensure that participants paid attention.
10 different words were selected for each condition on the basis of lists defined as typical members of these categories (Battig & Montague, 1969). Further words were chosen through online dictionaries. All words were validated by checking their frequency in the English language in a list based on the British National Corpus (Kilgarriff, 1997, for frequencies see table 5).

TABLE 5. MEANS AND STANDARD DEVIATIONS IN WORD FREQUENCY BASED ON THE BRITISH NATIONAL CORPUS (KILGARIFF, 1997). NO SIGNIFICANT DIFFERENCE WAS FOUND BETWEEN GROUPS OF WORDS WHEN THEY WERE DIVIDED BY LETTER (T(38) = 1.51, P = 0.14) OR BY CATEGORY (T(38) = -1.41, P = 0.17). WORD CATEGORIES DEPICTING SAFE AND DANGEROUS TRIALS WERE COUNTERBALANCED ACROSS PARTICIPANTS WITHIN EACH EXPERIMENTAL GROUP.

		Animal	**Plant**	**Total**
		Mean (SD)	Mean (SD)	Mean (SD)
T	Mean (SD)	204 (175.09)	246.1 (256.11)	225.05 (214.61)
B	Mean (SD)	84.2 (61.18)	198.8 (144.1)	141.5 (122.75)
Total	Mean (SD)	144.1 (141.67)	222.45 (203.71)	

For the CS+ trials, one of those categories was safe, i.e., not associated with an electric shock. This resulted in 4 different types of trials: CS+safe, CS+dangerous, CS-"safe" and CS-"dangerous" (no electric shocks were delivered during CS- trials). To maintain the 50% reinforcement schedule, we also included an equal number of CS+dangerous, nonreinforced trials. Only these trials were used in the analysis. In addition, some trials did not contain a word (CS+LetterOnly and CS-LetterOnly trials).

20 trials of each condition were presented, resulting in a total of 142 experimental trials including the two filler trials. Trials in the LSCCE phase began with the presentation of the letter only for 500ms. Following this, the word was presented for 1000ms before the letter appeared on its own again for a further 4000ms (see figure 7). During the letter – only trials the letter was visible for the entire duration of the trial, 5500ms. Automated electric shock delivery was carried out using a macro in ADInstruments LabChart software which received a marker from the EPrime PC via a parallel port, and triggered shock delivery at the intensity specified prior to the task. Reinforced CS+ trials co-terminated with the electric shocks.

Stimuli were created using PowerPoint, Letters and words were arranged in the centre of each slide, with letters in black font size 66 and bold, and words in font size 44 (not bold, see figure 7) on a light grey background.

Stimuli were presented using EPrime 2.0 via a fibre-optic goggle system, screen resolution 1024 x 768(NordicNeuroLab AS, Bergen, Norway).

The electric shock US was delivered through 2 Ag-AgCl electrodes attached to participants' right middle and ring fingers and connected to an ADInstruments Isolated Stimulator built into Powerlab 26T. The intensity was determined by the participant themselves prior to the start of the task through a standardised procedure described below.

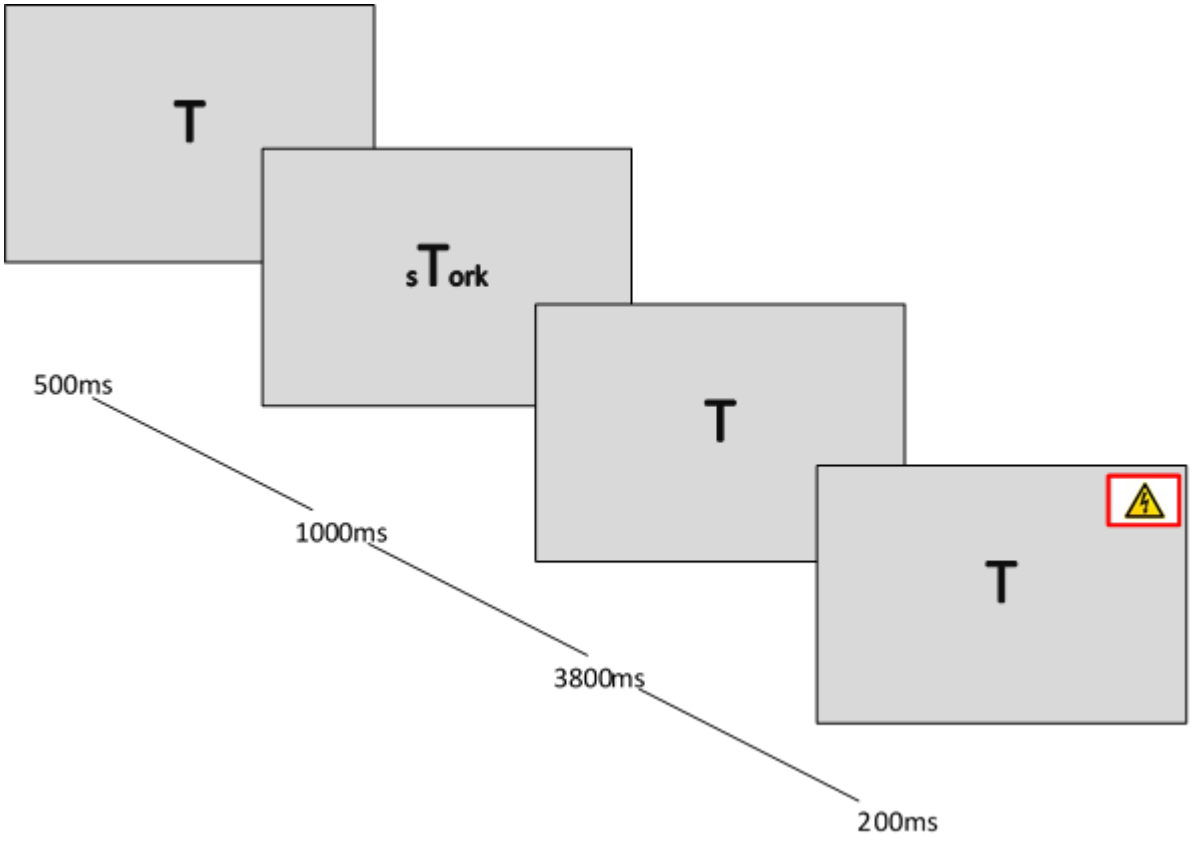


FIGURE 7. STRUCTURE OF AN LSCCE TRIAL. THE LETTER CS+ IS PRESENTED BY ITSELF BEFORE THE WORD APPEARS ON SCREEN. AFTER THE WORD DISAPPEARS THE LETTER REMAINS ON SCREEN FOR A FURTHER 4000MS. DURING REINFORCED CS+ TRIALS, THE TRIAL CO-TERMINATES WITH AN ELECTRIC SHOCK.

PHYSIOLOGICAL DATA COLLECTION

Skin conductance response (SCR) data was recorded at 1000hz with Labchart 7 using 2 Ag-AgCl electrodes on the distal phalanges of the middle and ring fingers of the participant's non-dominant hand (Cacioppo et al., 2007) and connected to an ADInstruments Powerlab 26T via a PowerLab ML116 SCR amplifier input module. A low constant-voltage AC excitation of $22mV_{rms}$ at 75 Hz was passed through the electrodes, which was converted to DC before being digitized and stored. Finger pulse was collected through an ADInstruments pulse transducer attached to participants' left index finger. Respiration data was collected using an ADInstruments Respiratory Belt Transducer placed around the participants' chest. Pupil data was acquired continuously at 60 Hz using an IView X system (v.1.3.3.31).

FMRI DATA COLLECTION

Structural and functional data were collected on a 3T Siemens Trio MRI scanner with 12 channel head matrix coil at The University of Reading Centre for Integrative Neuroscience and Neurodynamics (CINN). Functional scans consisted of a t2*-weighted gradient echo, echoplanar imaging (EPI) sequence (37 interleaved transverse slices, phase encoding P to A, 3 mm thickness,

128*128 matrix; 192 mm field of view; TR: 2000ms, TE: 30ms, Flip Angle: 90°; 212 whole-brain volumes). A high resolution whole-brain three dimensional structural image was also acquired using an MPRAGE sequence with 176 x 1 mm slices. (1*1*1 voxels size, TE: 2.9 ms, TR: 2020 ms, TI:1100 ms, FOV: 250 mm, Flip Angle: 90°).
Fieldmaps to be used to correct for magnetic field distortion were acquired using a gradient echo sequence (P to A, 3*3*3mm voxel size, TE1: 5.19ms, TE2: 7.65ms, TR: 400ms, FOV: 192mm, Flip Angle: 60°).

PROCEDURE

Informed consent was obtained from each participant before commencing the experiment. Participants then filled in the Positive And Negative Affect Scale (PANAS-NOW, Watson, Clark, & Tellegen, 1988) to assess current mood and, together with the experimenter, completed the initial screening form to ensure MRI safety.

Prior to starting the task, the stimulator electrodes were attached to participants' fingers, a shock at very low intensity (0.5mV) was delivered and the intensity was increased in steps of 0.5mV. After each shock, the participant was asked to rate the sensation on a scale of 1 ("not painful at all") to 10 ("extremely painful"). When they reached 8 on this scale, the experimenter reduced the intensity of the shock by 1 step and informed that this was the intensity the shock would remain at for the duration of the experiment (this procedure is based on that used by Delgado et al., 2008).

Subsequently, the sensors to collect skin conductance, heart rate, and respiration were attached. Before participants were set up in the scanner and the task was started, they completed the second screening form as well as a final metal check.

After completing the task, participants were asked about the contingencies of the task, i.e. which letter was the CS+ and which category was dangerous, and their answers were recorded. They then filled in a questionnaire to assess how they felt during each type of trial as well as how they felt throughout the task (7-point Likert scales, 1 = "not stressed", 7 = "extremely stressed", as well as 1 = "not bored" to 7 = "extremely bored", and 1 = "not sleepy" to 7 = "extremely sleepy"), as well as a range of standardised questionnaire to assess trait anxiety (State Trait Anxiety Inventory, STAI, Spielberger, Gorsuch, & Lushene, 1970), tendency to worry (Penn State Worry Questionnaire, PSWQ, Meyer, Miller, Metzger, & Borkovec, 1990), emotion regulation capacity (Emotion Regulation Questionnaire, ERQ, Gross & John, 2003a) and intolerance of uncertainty (Intolerance of Uncertainty Scale, IUS, Buhr & Dugas, 2002).

They were then verbally debriefed and given a debrief sheet to take home with them.

Questionnaire analysis

Questionnaires were scored according to instructions for each questionnaire. Scores were correlated with data in the following analyses to detect possible effects of individual differences in mood, state anxiety, intolerance of uncertainty, worry and emotion regulation strategies on skin conductance and pupil dilation on SCR and BOLD fMRI.

SCR data analysis

Data was visually checked for motion artefacts and these were removed manually. SCR data was filtered using a median filter with a width of 3 to remove artefacts resulting from the electric shock, and a bandpass filter with a low cutoff of 0.01Hz and a high cutoff of 1Hz (Johnstone, T. (2017, September 8). Psychophysiology Analysis Software. Retrieved from osf.io/4wsm3). SCR data was analysed using a MATLAB script that detected maximum deflection from a 2 second pre-trial baseline using a window of 7 seconds from trial onset. The mean value for each condition and participant was identified and exported into SPSS for analysis.

One participant's SCR data had to be excluded from the analysis due to excessive noise. Two participants' data was excluded because they did not show an SCR in response to the US (electric shock) during the task. This resulted in a final sample size of 17 for the SCR analysis of the LSCCE task. Residuals were normally distributed, therefore data was not transformed but original data was analysed.

Pupil Diameter data analysis

Pupil dilation data was cleaned and processed using the same software developed by Professor Tom Johnstone (Johnstone, T. (2017, September 8). Psychophysiology Analysis Software. Retrieved from osf.io/4wsm3). Blinks were identified and eliminated using local regression slopes and amplitude thresholds. Missing data points were then estimated using linear interpolation and a median filter of 5 samples was applied to smooth the signal. Mean responses were then calculated for a window equivalent to the length of the trial, by modelling each condition as a linear combination of sine and cosine functions (e.g., a Fourier Basis Set) of order 6. It is important to note that mean pupil dilation responses reported throughout this report are deviations from a pre-trial baseline. The true value is a proportional measure relative to the camera field of view. Due to the use of a light grey background for the fixation cross in the ITI's, pupil data is negative throughout this study.

fMRI data analysis

FMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). The following pre-statistics processing was

applied; motion correction using MCFLIRT(Jenkinson, Bannister, Brady, & Smith, 2002) ; non-brain removal using BET (Smith, 2002) ; spatial smoothing using a Gaussian kernel of FWHM 5mm; grand-mean intensity normalisation of the entire 4D dataset by a single multiplicative factor; highpass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma=50.0s), and B0 fieldmap unwarping (Jenkinson, 2003).

Single subject analysis

A fixed effects general linear model was used to analyse individual subject data. Regressors were created for each condition by convolving a stimulus boxcar function with the standard FSL gamma function. Temporal derivatives were included in this glm. Motion estimates were added as regressors to control for head displacement. Trials that included an electric shock were modelled separately (1 regressor for this condition) but not used for analysis.

Registration to a standard space was performed using a two stage procedure with FLIRT (Jenkinson & Smith, 2001; Jenkinson et al., 2002). The mean functional volume for each participant was registered to the individual's high resolution structural image using 6 degree of freedom (DOF) BBR white matter boundary mapping. In a second step the individual's high resolution structural image was normalised to the Montreal Neurological Institute (MNI) template brain using a 12 DOF affine transformation. These two transformations were combined and used for subsequent registration of that participant's contrast images to MNI space before higher-level group analysis.

Group analysis

Comparison of contrasts across participants was carried out using Mixed Effects (FMRIB's Local Analysis of Mixed Effects, FLAME 1) with automatic outlier de-weighting and Random Field-based cluster thresholding. Results were corrected for multiple comparisons with a familywise error of $p<0.05$.

Physiological Noise Modelling

All fMRI analyses were also carried out with the addition of regressors controlling for physiological noise (heart rate, respiration). We used PNM (Birn, Diamond, Smith, & Bandettini, 2006; Brooks et al., 2008) to model physiological noise. After PNM stage 1 (data import into FSL's PNM module, wave form modelling using Fourier series, and automatic peak detection), peak detection was manually checked and corrected before running stage 2 (generation of physiological noise EV's). Its output was included in Feat as voxelwise regressors.

Connectivity analysis

An analysis of functional connectivity was carried out using clusters that showed activation during the contrasts of interest as seeds. Timeseries were extracted for each cluster using Featquery. Interaction terms with CS+safe and CS+dangerous timing were entered as additional EV's in the original Feat single subject design. In this interaction term the condition EV was centred, and the seed timeseries EV was demeaned. A contrast between the two new interaction EV's was then created and subsequently analysed at group level.

5.3 Study 2.1 - Results

Questionnaires

All participants reported being aware of the exact contingencies of the task and correctly identified the CS+ as well as the dangerous category.

Participants responses about how stressed they felt during each type of trial were analysed in 2 x 2 (CS (CS+ vs CS-) x trial type (safe vs dangerous)) repeated measures ANOVA. We found a significant effect of CS ($F(1,19) = 107.5$, $p < 0.001$, partial $\eta^2=0.85$), trial type ($F(1,19) = 102.64$, $p < 0.001$, partial $\eta^2=0.84$), and a significant CS x trial type interaction ($F(1,19) = 16.1$, $p < 0.001$, partial $\eta^2=0.46$). Inspection of the means revealed that overall, responses following CS+ trials were higher than those following CS- trials. Thus, subjective reports suggest that conditioning was effective. Follow up t-tests revealed that participants felt significantly more stressed during dangerous (5.2) than safe (2.6) CS+ trials ($t(19) = 9.58$, $p < 0.001$, CI of difference = 0.26, Cohen's d = 2.2), and during dangerous (2.0) than safe (1.2) CS- trials ($t(19) = 3.1$, $p = 0.006$, CI of difference = 0.27, Cohen's d = 0.88). The difference between dangerous and safe CS+ trials was larger than that between dangerous and safe CS- trials (see table 6 for means).

All participants reported being able to read the letters and words, and using both letters and word categories to work out whether each trial was safe or dangerous. All participants were also able to accurately perform the behavioural task.

TABLE 6. QUESTIONNAIRE SCORE MEANS. SUBJECTIVE STRESS LEVELS AS WELL AS BOREDOM AND SLEEPINESS WERE ASSESSED ON A 7-POINT LIKERT SCALE GOING FROM 1 (NOT AT ALL STRESSED/BORED/SLEEPY) TO 7 (EXTREMELY STRESSED/BORED/SLEEPY).

	Mean (CI)	Cronbach's Alpha
Shock Intensity	4.78 (3.28)	
Subjective stress level during -		
- CS+ dang	*5.2 (0.26)*	
- CS+ safe	*2.6 (0.26)*	
- CS- dang	*2 (0.27)*	
- CS- safe	*1.2 (0.27)*	
Boredom	3.6 (1.9)	
Sleepiness	4 (2.05)	
STAI	39.55 (8.7)	0.88
PANAS positive	28.45 (6.6)	0.9
PANAS negative	13.4 (3.3)	0.78
ERQ Reappraisal	31 (9.9)	0.79
ERQ Suppression	14.9 (6.5)	0.81
PSWQ	46.7 (17.8)	0.96
IUS	59 (20.2)	0.95

Physiology Results

Conditioning Checks

Skin Conductance

To establish whether a conditioned autonomic fear response had been learnt by participants in the conditioning phase, a univariate mixed effects ANOVA was performed on the first and last 5 trials of each condition to compare SCR during CS+ (no shock) and CS- trials. This showed no significant effect of condition in early or late conditioning (Early: $F(1,16) = 0.39$, $p = 0.54$, partial $\eta^2 = 0.02$; (Mean difference CS+ - CS- = 0.052, CI = 0.2); Late: $F(1,16) = 0.5$, $p = 0.49$; (Mean difference CS+ - CS- = 0.007, CI = 0.1)).

To further check whether an aversive response occurred when participants briefly saw words of both categories with the CS embedded prior to the start of the extinction phase, a paired t-test was performed on the first 2 CS+ and CS- trials of the extinction phase, both including one word of each category prior to any US being delivered (participants were not aware which category was safe and which was dangerous at this stage). There was no significant difference between the two CS' ($t(16) = 1.14$, $p = 0.27$, Cohen's d = -0.71), however, the means show the expected direction (CS+ = 0.15, ; CS- = 0.08,Mean difference = 0.07, CI = 0.08).

Pupil Diameter

A two-tailed paired t-test compared pupil diameter data in CS+ (no shock) conditioning trials with that during CS- conditioning trials. Pupil dilation was not significantly greater ($t(14) = 2.82$, $p = 0.78$, Cohen's d = 0.27) during CS+ (no shock) conditioning trials (M = -0.002 mm, SD = 0.03) than during

CS- conditioning trials (M = -0.003 mm, SD = 0.028). Therefore, the presence of an autonomic conditioned fear response to the CS+ was not evident in the pupil diameter data (figure 3).

CS+DANGEROUS > CS+SAFE

SKIN CONDUCTANCE

To investigate the effects of LSCCE on SCR, a 2 x 2 repeated measures ANOVA was conducted with type of stimulus (CS+ and CS-) and type of trial (dangerous and safe) as within subject factors. This showed a marginally significant effect of trial type ($F(1,16) = 3.962$, $p = 0.06$, partial $\eta^2 = 0.2$) and a marginally significant stimulus type x trial type interaction ($F(1,16) = 3.957$, $p = 0.06$, partial $\eta^2 = 0.2$). A post hoc paired t-test was performed to compare SCR in CS+ dangerous trials with SCR in CS+ safe trials. This showed that SCR was significantly higher during dangerous (non-reinforced) CS+ trials (M = 0.17, SD = 0.15), than safe CS+ trials (M = 0.9, SD = 0.09, $t(18) = -3.3$, $p = 0.002$, one-tailed, Cohen's d = 0.89, see figure 8, table 7 for means).

Such a finding is consistent with participants being able to successfully decrease their emotional arousal to the CS+ when they identify, based on the word category, that there is no risk of receiving an electric shock.

To establish that the above effect was the consequence of successful regulation of the CS+ response when in the context of the safe word category, and not simply learning a new association between the word category and US, a further post hoc comparison compared SCR during CS- 'dangerous' trials, (M = 0.12 µS, SD= 0.09), and CS- 'safe' trials, (M = 0.1 µS, SD = 0.1). SCR was not significantly different between these two conditions, ($t(17) = -0.7$, $p = 0.49$, Cohen's d = 0.16). Such a finding indicates that the dangerous word category had an impact on emotional arousal only when paired with CS+ stimuli. Further, successful regulation during safe trials significantly reduced SCR to the CS+.

Because the trials in which the letters were presented by themselves during the reappraisal phase were visually different from the reappraisal trials, these were analysed with a separate t-test. This showed a non-significant difference in the expected direction (Means (SD's): CS+ = 0.1 (0.08); CS- = 0.05 (0.13); $t(17) = -1.1$, $p = 0.09$, one-tailed, Cohen's d = 0.37, see table 6).

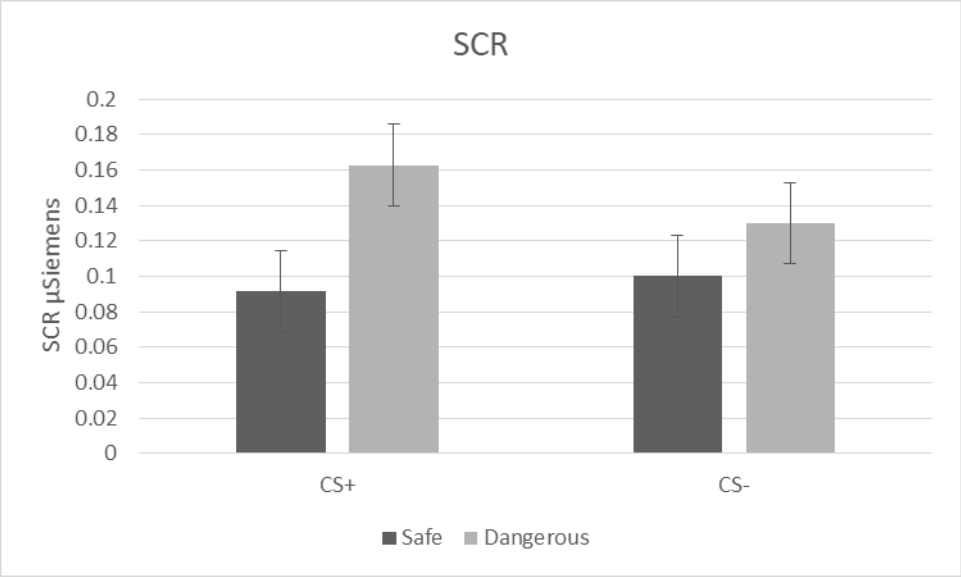


FIGURE 8. SCR ASSOCIATED WITH SAFE AND DANGEROUS CS+ AND CS- TRIALS. ERROR BARS REPRESENT WITHIN SUBJECT CONFIDENCE INTERVALS.

TABLE 7. MEAN SCR IN IN µSIEMENS, CIS AND SIGNIFICANCE TESTS. P < 0.01.** ***CONFIDENCE INTERVALS WERE CALCULATED PAIRWISE FOR THE MAIN CONTRASTS OF INTEREST.***

Conditioning	***CS- Mean***	***CS+ Mean***	***95% Confidence Interval (+/- Mean)***	***F (1,16)***	***p***
Early	0.033	0.09	0.1	0.39	0.54
Late	0.16	0.17	0.05	0.5	0.49
Reappraisal Baseline	***CS- Mean***	***CS+ Mean***		***t(16)***	***P***
	0.08	0.15	0.07	0.95	0.35
Reappraisal	***Safe Mean***	***Dangerous Mean***		***t (16)***	***p***
CS-	0.1	0.13	0.023	-0.7	0.47
CS+	0.09	0.16	0.032	-3.3	0.002**
Letter Only	***CS- Mean***	***CS+ Mean***		***t(16)***	***p***
	0.04	0.1	0.033	-1.8	0.09

PUPIL DIAMETER

A 2 x 2 repeated measures ANOVA indicated a significant main effect of stimulus, ($F(1,14) = 9.61$, $p = 0.008$, partial $\eta^2 = 0.41$) on pupil diameter. However, there was not a significant main effect of trial, ($F(1,14) = 0.04$, $p = 0.85$, partial $\eta^2 = 0.003$) nor a significant interaction between stimuli and type of trial, ($F(1,14) = 1.15$, $p = 0.3$, partial $\eta^2 = 0.08$). In line with predictions, pupil diameter was significantly greater in CS+ conditions (M = -0.019 mm) in contrast to CS- conditions (M = -0.024 mm, figure 9).

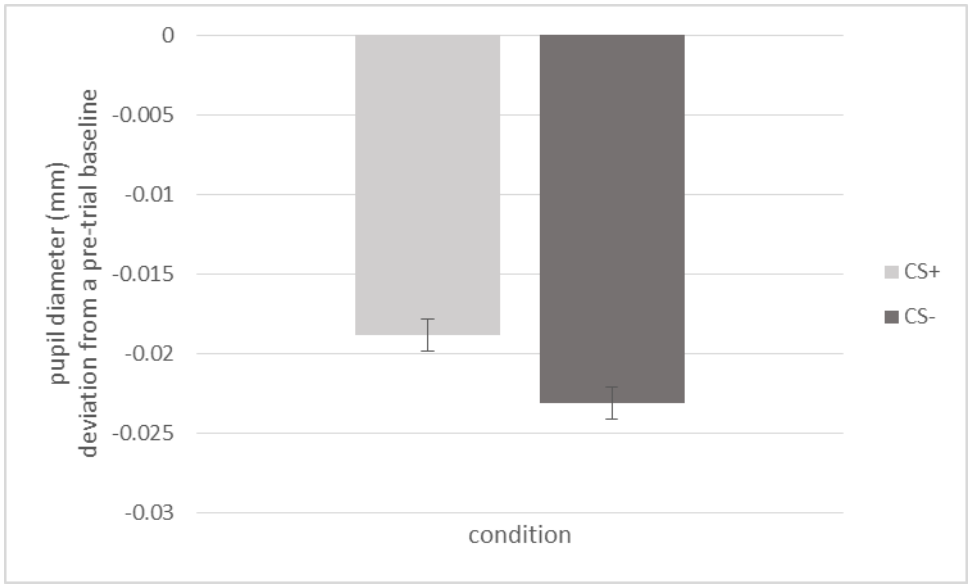


FIGURE 9. MAIN EFFECT OF CS ON PUPIL DIAMETER. ERROR BARS REPRESENT WITHIN SUBJECT CONFIDENCE INTERVALS.

fMRI Results

Conditioning checks

We did not find a main effect of condition during the conditioning phase that survived thresholding. At an uncorrected threshold of z=1.8 we saw a single cluster of activation in left anterior insula (see figure 10).

CS+dangerous > CS+safe

Dangerous CS+ trials compared to safe CS+ trials revealed clusters in left insula and right anterior cingulate (ACC, see table 8, figure 10). Contrary to our hypothesis, no significant amygdala activation was found.

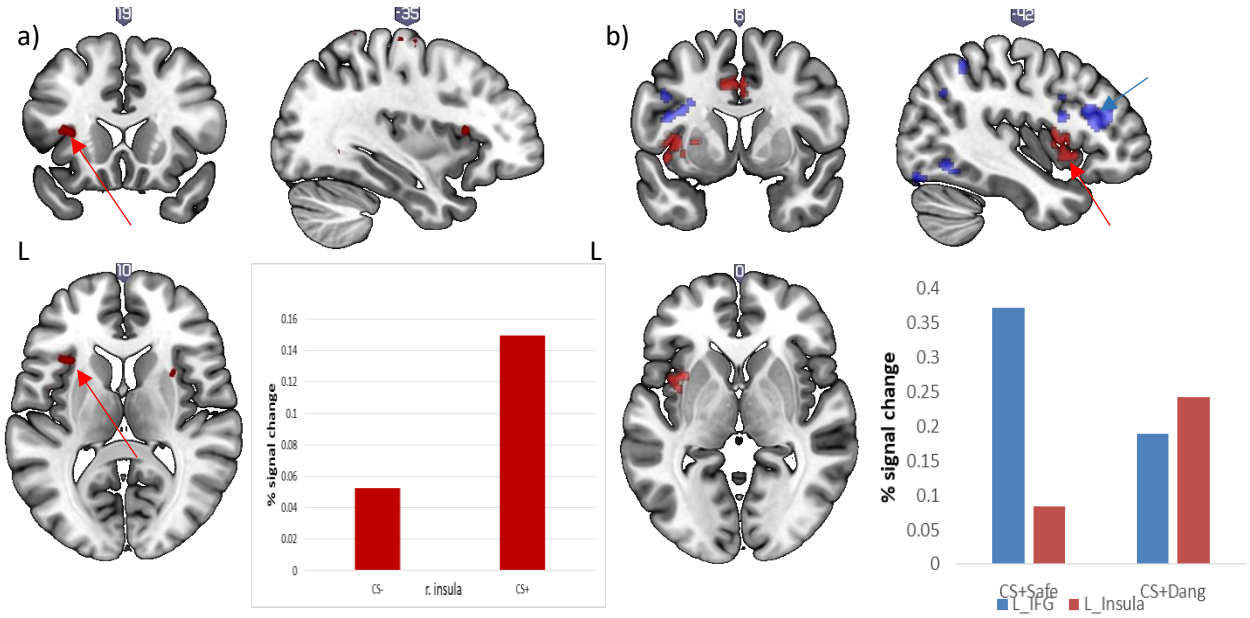


FIGURE 10. NEURAL ACTIVATION OBSERVED IN THE COMPARISON BETWEEN CS+ (NON-REIFORCED) AND CS- DURING THE CONDITIONING PHASE (A), Z=1.8, UNCORRECTED). B) SHOWS ACTIVATION DURING THE LSCCE PHASE: CS+SAFE > CS+DANGEROUS (BLUE) AS WELL AS CS+DANGEROUS > CS+SAFE (RED). AREAS THAT SHOWED INCREASED ACTIVATION DURING SAFE COMPARED TO DANGEROUS CS+ TRIALS INCLUDED LEFT INFEROIR FRONTAL GYRUS, BILATERAL INFERIOR TEMPORAL GYRUS AND SUPERIOR PARIETAL CORTEX. AREAS THAT SHOWED INCREASED ACTIVATION TO DANGEROUS COMPARED TO SAFE TRIALS INCLUDED THE LEFT INSULA AND DORSAL ANTERIOR CINGULATE. THE BAR GRAPH (C)) SHOWS THE PERCENT SIGNAL CHANGE IN THE LEFT IFG AND THE LEFT INSULA

CS+SAFE > CS+DANGEROUS

The opposite contrast revealed increased activation in a network including left inferior frontal gyrus (IFG), bilateral inferior temporal gyrus (ITG) and right superior parietal cortex during safe compared to dangerous CS+ trials (see table 8, figure 10).

Participants' Questionnaire scores did not have an effect on these results.

PPI RESULTS

We carried out a PPI analysis using the left insula, and left IFG as seed regions. We did not find differential connectivity for the comparison of safe and dangerous CS+ conditions.

TABLE 8. SIGNIFICANT CLUSTERS OF ACTIVATION IN THE MAIN CONTRASTS OF INTEREST, CS+DANG > CS+SAFE; AND CS+SAFE > CS+DANG. ALL CLUSTERS SURVIVED CLUSTER BASED THRESHOLDING AT 2.3. PEAK COORDINATES ARE PRESENTED IN MNI SPACE.

Contrast	Anatomical Region	Hemisphere	Cluster size (mm^3)	X	Y	Z
CS+ > CS- (z = 1.8, uncorrected)	Insula, Frontal Operculum	Left	344	24	75	34
CS+dang > CS+safe	Insula	Left	2376	-40	8	6
	Anterior Cingulate Cortex	Right	2152	4	10	42
CS+safe > CS+dang	Inferior Temporal Gyrus	Left	5608	-54	-54	-10
	Superior Parietal Lobule	Right	5456	30	-54	62
	Lateral Occipital Cortex	Left	4512	-26	-68	52
	Middle Frontal Gyrus and inferior frontal gyrus	Left	3352	-42	32	20
	Inferior Temporal Gyrus	Right	3144	52	-62	-18
	Precentral Gyrus	Right	3112	4	-26	66
	Cerebellum	Right	3032	6	-78	-26

5.4 Study 2.1 - Discussion

The aim of this study was to investigate the physiological and neural correlates of the LSCCE task. Participants were conditioned to expect a risk of electric shock during the presentation of one of two letters. In a subsequent phase, words belonging to two distinct categories briefly appeared. One word category signalled a safe trial, while the other category signalled continued risk of electric shock (i.e. a dangerous trial). Thus, participants had to cognitively evaluate the information given in each trial. As expected we found increased skin conductance in response to dangerous compared to safe CS+ trials as well as increased activation in a network of brain regions consistent with emotion processing including bilateral insula and right dorsal ACC. In contrast, in safe compared to dangerous trials we found decreased skin conductance and activation in a network of prefrontal brain areas including left MFG and IFG. In contrast to skin conductance, which reflected the affective component of the task, our findings were consistent with pupil diameter reflecting cognitive effort, with greater diameter during both safe and dangerous CS+ trials compared to CS- trials. We did not find any significant differences in functional connectivity with bilateral insula or left IFG, between safe and dangerous CS+ trials.

The fMRI analysis of the LSCCE task revealed increased activation in left insula and right dorsal ACC during dangerous (non reinforced) CS+ trials. These areas have previously been associated with the perception and anticipation of pain (Brooks, Nurmikko, Bimson, Singh, & Roberts, 2002; Porro et al., 2002), as well as with the processing of threat and the generation of physiological responses (Kalisch & Gerlicher, 2014; Mechias et al., 2010) which suggests that our paradigm worked in evoking a conditioned response. Further, Ochsner et al. (2004) found activation in bilateral insula while participants were attending to highly emotional images (compared to decreasing their emotional response). Taken together with the fact that SCR was increased during dangerous compared to safe CS+ trials, it can be concluded that the affective response evoked in anticipation of an electric shock was maintained during dangerous and reduced during safe CS+ trials.

In contrast to other conditioning studies (Delgado et al., 2008; Fullana et al., 2015) we did not find increased amygdala activation during CS+ presentations in the conditioning or LSCCE phase. One explanation for this finding is that the amygdala suffers from the same imaging restrictions as the vmPFC, being difficult to image. Alternatively, it is possible that the amygdala is involved in processing both safe and dangerous CS+ trials. It has been suggested that rather than processing negative emotion in particular, the amygdala serves as a "significance detector". Because the CS+ is still present in both safe and dangerous CS+ trials, the amygdala may still be involved in signalling a potential for a negative event, even when the trial is safe. In addition, evidence on amygdala

activation in conditioned fear is inconsistent, with a recent meta-analysis not finding evidence for increased amygdala activation during CS+ compared to CS- trials (Fullana et al., 2015). Thus, further studies would be beneficial to investigate the role of the amygdala in this paradigm. During the conditioning phase we found sub-threshold activation in the left insula. This brain area is commonly activated during CS+ presentations in conditioning studies (Büchel et al., 1998; Fullana et al., 2015). Thus, although this activation did not survive thresholding, this activation supports the idea that participants learned the association between the CS+ and the likelihood of electric shocks during the conditioning phase, and showed a CR. It is surprising, however, that we did not find stronger activation in this contrast. It is possible that our sample size was not sufficient to find a stronger effect. The next study will recruit a larger sample to increase our power to find an effect. Alternatively, it is possible that the number of trials was not sufficient, however, the number of trials is comparable to other studies that do find the effect (see e.g. Fullana et al., 2015), thus, this is unlikely. Taken together with the SCR results which did not show a significant difference between CS+ and CS- in acquisition, but did show a consistent difference in the expected direction during early and late acquisition, as well as the first (non-reinforced) LSCCE trials. It is, therefore, possible that we did not recruit enough participants to have the power to detect a significant effect.
We did find increased activation in a network including insula and ACC during dangerous compared to safe CS+ trials. This is consistent with findings relating to the experience and anticipation of painful stimuli (Legrain et al., 2011; Porro et al., 2002). In addition, the insula is also one of the areas commonly found during negative emotional processing in instructed emotion regulation paradigms (Buhle et al., 2014; Kohn et al., 2014), further supporting this conclusion. In addition, we found a cluster in dACC that showed increased activation during dangerous compared to safe CS+ trials. Activation in this area has been associated with general threat processing, being a common area of activation in both instructed and uninstructed conditioning studies (Kalisch & Gerlicher, 2014; Mechias et al., 2010). Part of this cluster of activation also overlapped with the rostral ACC area described by the authors as part of instructed threat processing. While participants did not receive explicit instructions about the safe – dangerous contingencies in this task, once they had worked these contingencies out (which should have happened within the first 5 trials that contained words), the task included a cognitive component which may be reflected in this rostral ACC activation.

Furthermore, the results showed increased activation in a network including a cluster spanning left MFG and IFG, bilateral temporal gyrus and right parietal cortex during safe compared to dangerous CS+ trials. During these trials, participants were first presented with the previously established CS+, which was then briefly presented within a word belonging to a “safe” category, before the CS+ alone was shown again. Thus, the word category provided information that participants could use to

evaluate the risk of shock during those trials by categorising the word, remembering the category contingencies, and using this information to decide whether each trial was safe or dangerous. Activation in middle and inferior frontal gyrus has been observed during word processing and semantic working memory tasks (see Nee et al., 2013 for a recent meta-analysis), as well as during emotion regulation studies when participants use a predefined strategy to reappraise an affective response (Buhle et al., 2014; Kohn et al., 2014). Here, this activation is present even though both conditions compared in this contrast involve a semantic decision – whether the trial is safe or dangerous. However, the letter - stimulus in question (CS+) always carried a 50% chance of receiving an electric shock during the conditioning phase. Thus, when this stimulus is presented in a word from the safe category, increased activation in these brain regions may reflect a greater amount of processing required to regulate or reverse the previously learnt contingencies. Similar processes may be at work in emotion regulation studies which commonly find activation in the same or adjacent areas when comparing conditions in which participants are asked to decrease an emotional response through reappraisal to when they are asked to attend (Buhle et al., 2014; Ochsner et al., 2004). This contrast also involves mobilising resources to generate and maintain an alternative stimulus meaning (or stimulus outcome) to one that was initially activated.
Activation in inferior temporal cortex has been associated with language processing (Ferstl, Neumann, Bogler, & von Cramon, 2008), which may reflect the analysis of the safe stimulus in this case. Right superior parietal lobule and precentral gyrus have been shown to be involved in motor inhibition (Thoenissen, Zilles, & Toni, 2002). A tentative explanation might be that this reflects the inhibition of a freezing response to the anticipation of an electric shock.

Inconsistent with our predictions, we did not find any significant activation in vmPFC in the CS+safe vs CS+dangerous contrast. Although this area has previously been found in studies of reappraisal (Diekhof et al., 2011), a later meta-analysis did not confirm this (Buhle et al., 2014). Similarly, the vmPFC has been found to be involved in extinction where it has been related to the processing of the new contingencies, facilitating faster and maintained extinction (Milad & Quirk, 2002; Phelps et al., 2004). However, the fact that vmPFC is not consistently found to be differentially active for CS+ and CS- during initial extinction suggests that it may be involved in the processing of both stimuli during this phase, i.e. monitoring that the CS- contingencies stay the same, while CS+ contingencies change. The same may be true for our study – Contingencies change for CS+safe (compared to the conditioning phase), but stay the same for CS+dangerous. Thus, if the vmPFC stores renewed contingencies, then it would be involved in both processes and a contrast between these conditions would not yield a significant result. However, the vmPFC is also an area that is difficult to image due to signal dropout in this part of the brain. In light of this it is also possible that we did not find

differential activation in safe compared to dangerous CS+ trials may due to a lack of power. Future studies with more participants may be able to provide further insight into the role of the vmPFC in this paradigm.

The PPI analysis of functional connectivity of the left IFG, left and right insula clusters during the safe and dangerous CS+ trials did not yield any results. We expected a negative correlation between this area and those associated with emotion generation during safe CS+ trials. While it is possible that this correlation does not exist, it is also likely that the design of this task does not lend itself to revealing functional connectivity changes. PPI analyses work best for block designs or extended event related designs (O'Reilly, Woolrich, Behrens, Smith, & Johansen-Berg, 2012). Since the design of this task is event related but with relatively long trials, we decided to try this kind of connectivity analysis over one that would have involved more time in the scanner for participants, such as a DTI or resting state MRI, though the latter types of scan might afford more sensitivity to individual differences in connectivity that underpin physiological and fMRI responses in this task.

6. Study 2.2 - The neural correlates of a modified Instructed Emotion Regulation task

6.1 Study 2.2 - Introduction

We also compared the results to a modified version of an instructed emotion regulation task using IAPS images as affective stimuli. During this type of task, participants are asked to view affective and neutral images and either maintain their attention to them without attempting to change their response, or to reappraise the image using a predefined strategy.

Trials in event related versions of this task typically last for 8 (Ochsner, Bunge, Gross, & Gabrieli, 2002) to 12 seconds (Urry et al., 2006). Here, we shortened the trials to make the timeline as similar as possible to the ECE task. This was done both to test whether this task would still produce results that are comparable to those found with longer versions of this task, and to be able to more validly compare the results of the two tasks.
Studies using this type of paradigm typically find increased activation in the amygdala and insula while participants are attending to negative images, reflecting the processing of their emotional reaction, and increased activation in a network including medial and lateral prefrontal cortex as well as lateral temporal and parietal cortex (see Buhle et al., 2014 for a recent meta-analysis) when participants are down-regulating their response to these images, which has been associated with the retrieval and organisation of regulation information, and the inhibition of affective responses.

We expected to find the same pattern of medial and lateral prefrontal activation activation that is reliably found in the Negative Decrease > Negative Attend contrast in instructed emotion regulation tasks., During the Negative Attend > Negative Decrease contrast we expected to find increased amygdala activation. We also expected to find increased SCR during Negative Attend compared to Negative Decrease and Neutral Attend trials, representing emotional arousal, and increased pupil dilation during negative decrease compared to negative attend and neutral attend trials, representing cognitive effort (Urry et al., 2009).

6.2 Study 2.2 - Method

Participants

This task was part of the same session as the LSCCE task so the same 20 participants attended.

Design

We used a variant of the task originally developed by Jackson et al. (2000) to investigate instructed emotion regulation. Participants saw images depicting either negative or neutral scenes. During negative images they received instructions to either “decrease” or “attend” their initial reaction to

the image. To decrease their reaction to the image, they were asked to imagine a more positive outcome to the depicted scene, e.g. if an accident was shown in the image, to imagine that everyone involved fully recovered. When the image was neutral, they were always asked to "attend" to the image without changing the way they felt about it. The main contrast compared trials in which participants were asked to attend to negative images (Negative Attend) with those in which participants were asked to decrease their emotional reaction to the negative images (Negative Decrease).

Apparatus and Materials

40 negative and 20 neutral images from the International Affective Picture System (IAPS(Lang et al., 2008)) were presented in two blocks of 30 interleaved trials. Participants were asked to "decrease" their initial reaction to 20 negative trials, and "maintain" their initial reaction to 20 of the negative trials. They were always asked to "maintain" during neutral pictures. IAPS pictures are standardised and we selected highly negative and highly arousing pictures for this task (both ratings 1 – 9, 1= extremely negative/not at all arousing, 9= extremely positive/extremely arousing). The mean valence rating fir the negative images was 1.99, SD = 0.26, arousal mean = 5.95, SD = 0.74. The average valence rating for the neutral images was 5.1, SD = 0.37, arousal mean = 3.46, SD = 0.46. Trials lasted for a total of 6000 ms and instructions were presented through a set of Siemens (Siemens, Malvern, PA, USA) headphones for 1000 ms, 1000 ms into the presentation of the picture. The ITI was jittered between 2000 and 6000ms. Trials were presented through EPrime 2.0 in a pseudo-random order via a fibre-optic goggle system (NordicNeuroLab AS, Bergen, Norway) and the order of instructions was counterbalanced across subjects

Procedure

Prior to the scanning session participants received detailed instructions on how to complete this task. They were told that they would see negative and neutral images and hear instructions to either "decrease" or "attend" to their initial reaction to the image. In the event of an instruction to "decrease", they were asked to change their initial reaction to the image by imagining a more positive outcome to the image. In the event of an instruction to "attend" they were asked to attend to the image without changing their reaction to it. They were shown one explicit example of how to do this, and were then given a set of 5 practice trials. Following these, the experimenter checked that they had understood how to apply the strategy correctly by asking them what kind of things they had thought of when decreasing their reaction to the practice images and explaining again how participants should decrease their emotional reaction if this was not yet clear.

During the scanning session participants completed the LSCCE task first. Before the instructed emotion regulation task started, the experimenter reminded participants of the instructions and the

exact strategy they were asked to use. The task was only started once participants had confirmed that they had understood and were ready to complete this task.

A structural MRI scan was completed following this task, after which participants were removed from the scanner, debriefed and dismissed.

Analysis Procedures

SCR, pupil dilation and fMRI data was analysed following the same steps as described for the LSCCE task.

1 participants' SCR data was removed from the analysis due to noise and 1 participant did not wish to participate in the instructed emotion regulation task, resulting in a sample size of 19 for the fMRI, and 18 for the SCR analysis of this task. Technical difficulties meant that we could not obtain pupil dilation data from 5 participants so the final sample size for the analysis of pupil dilation data was 14.

6.3 Study 2.2 - Results

Questionnaires

Questionnaire data was analysed using a repeated measures ANOVA with 3 levels (trial type, Neutral Attend vs Negative Attend vs Negative Decrease). We found a significant effect of trial type ($F(2,18) = 36.86$, $p < 0.001$, partial $\eta^2=0.67$). Follow up t-tests revealed that participants felt significantly more stressed during Negative Attend than Negative Decrease trials ($t(18) = 4.44$, $p < 0.001$, Cohen's $d = 1.49$), and significantly more stressed during Negative Decrease than Neutral Attend trials ($t(18) = 4.31$, $p < 0.001$, Cohen's $d = 0.55$, see table 9 for means and CI's).

All participants reported being able to see the images and using the correct strategy to regulate their emotion when asked to do so.

TABLE 9. MEANS OF PARTICIPANTS' STRESS LEVEL DURING EACH TRIAL TYPE AND HOW BORED AND SLEEPY THEY FELT THROUGHOUT THE TASK. SUBJECTIVE STRESS LEVELS AS WELL AS BOREDOM AND SLEEPINESS WERE ASSESSED ON A 7-POINT LIKERT SCALE GOING FROM 1 (NOT AT ALL STRESSED/BORED/SLEEPY) TO 7 (EXTREMELY STRESSED/BORED/SLEEPY).

	Mean (CI)
Subjective stress levels during -	
Neutral Attend	*3.16 (0.28)*
Negative Decrease	*4.47 (0.4)*
Negative Attend	*6.32 (0.4)*
Bored	*2.58 (1.89)*
Sleepy	*3.42 (2.5)*

SCR RESULTS

To investigate whether an effect of emotional arousal exists between negative and neutral images, a two-tailed paired t test was conducted to compare SCR between negative attend and neutral attend conditions. Inconsistent with our predictions, there was no significant difference ($t(17) = -0.038$, $p = 0.97$, Cohen's $d = 0.095$) in SCR when participants were asked to attend to negative images, (M = 0.16 µS,), compared to when they were asked to attend to neutral images, (M = 0.17 µS, within participants CI = 0.27, see figure 11).

To investigate whether SCR is reduced as participants intentionally decrease negative emotion through reappraisal, a one-tailed paired t test was conducted to compare SCR during negative attend trials with SCR during negative decrease trials. In line with predictions, results indicated that SCR was greater ($t(17) = 1.5$, $p = 0.04$, one-tailed, Cohen's $d = 0.33$), when participants attended to their emotional response to negative images, (M = 0.16 µS), compared to when they aimed to decrease their emotional arousal, (M = 0.13 µS,within participants CI = 0.023), by reinterpreting the image with a better outcome. Such a finding is an indication of regulatory success as participants reduce their emotional arousal through the strategy of reappraisal (see figure 11).
Participants' questionnaire scores did not have an effect on these results.

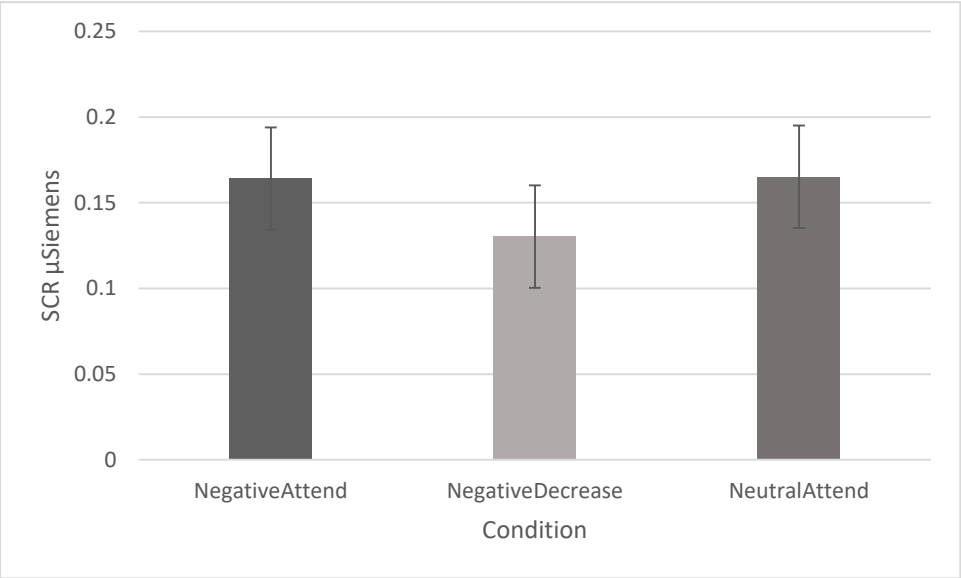


FIGURE 11. SCR IN µSIEMENS TO THE DIFFERENT CONDITIONS IN THE INSTRUCTED EMOTION REGULATION TASK. ERROR BARS REPRESENT WITHIN SUBJECT CONFIDENCE INTERVALS.

PUPIL DIAMETER RESULTS

To investigate the hypothesis that pupil diameter would be increased while participants were reappraising their emotional response to a negative image compared to attending to the image, a one-tailed paired t test was conducted. It showed reduced pupil diameter during Negative Attend (M = -0.048 mm, SD = 0.025) compared to Negative Decrease trials (M = -0.045 mm, SD = 0.024, t(13) = -2.03, p = 0.03, one-tailed, Cohen's d = 0.5). Such a finding demonstrates that pupil diameter is reflective of the increased cognitive resources that are required when participants consciously decrease their emotional response (see figure 12).

A further comparison indicates that pupil dilation was not affected by emotional arousal. Results revealed that pupil diameter was not significantly different (t(13) = 1.54, p = 0.15, Cohen's d = 0.76, Negative Attend M = -0.048 mm, Neutral Attend Mean = -0.052, within participants CI =- 0.002) when participants were asked to attend to negative images compared to when they were asked to attend to neutral images (M = -0.053 mm,within participants CI = 0.003).
Participants' questionnaire scores did not have an effect on these results.

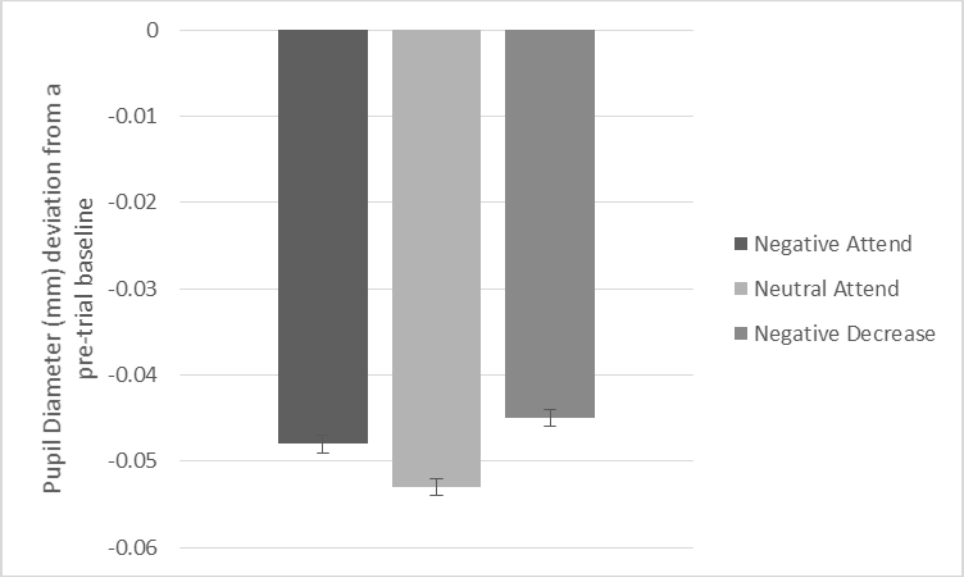


FIGURE 12. PUPIL DIAMETER IN THE INSTRUCTED EMOTION REGULATION TASK. ERROR BARS REPRESENT WITHIN SUBJECTS 95% CONFIDENCE INTERVALS.

FMRI RESULTS

NEGATIVE DECREASE > NEGATIVE ATTEND

For this contrast we found activation in a network including left IFG, left middle temporal gyrus (MTG) and bilateral dorsolateral prefrontal cortex (dlPFC, see table 10, figure 13).

For the opposite contrast (Negative Attend > Negative Decrease), activation was found in a network including bilateral insula and superior parietal cortex.

On the basis of previous research that used this task, an additional regions of interest (ROI) analysis was conducted using bilateral amygdala ROI masks and a small volume correction. This revealed a cluster in left amygdala (see table 10, figure 13).

Participants' questionnaire scores did not have an effect on these results.

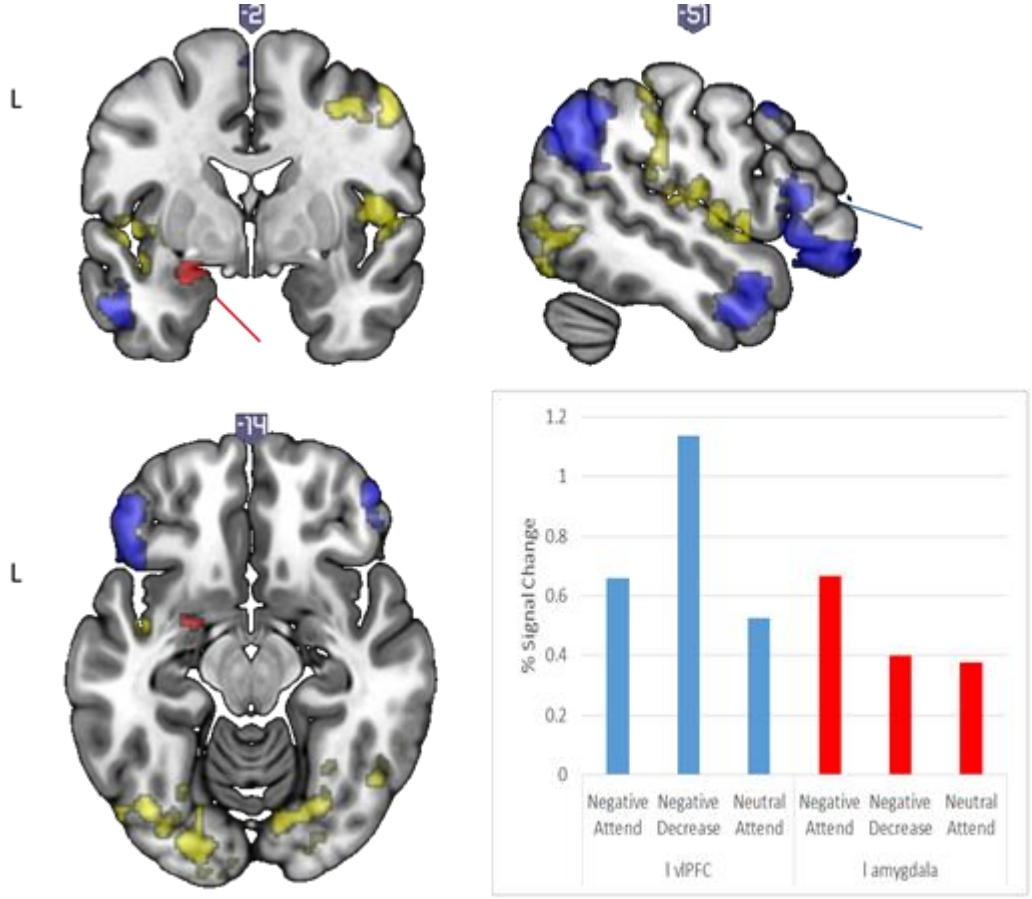


FIGURE 13. ACTIVATION IN THE INSTRUCTED EMOTION REGULATION TASK. NEGATIVE ATTEND > NEGATIVE DECREASE IS IN YELLOW, AMYGDALA ACTIVATION FROM AN ROI ANALYSIS ON THIS CONTRAST IS IN RED. BLUE SHOWS CLUSTERS THAT WERE ACTIVE IN THE NEGATIVE DECREASE > NEGATIVE ATTEND INCLUDING VLPFC, INFERIOR TEMPORAL GYRUS, AND LATERAL PARIETAL CORTEX.

TABLE 10. PEAK ACTIVATION IN THE INSTRUCTED EMOTION REGULATION TASK. ALL CLUSTERS SURVIVED CLUSTER BASED THRESHOLDING AT 2.3. PEAK COORDINATES ARE PRESENTED IN MNI SPACE.

Contrast	Anatomical Region	Hemisphere	MNI Coordinates (x,y,z)	Cluster size (mm^2)
NegativeDecrease > NegativeAttend	Middle frontal gyrus	left	-44, 8, 54	23592
	Lateral occipital cortex	left	-50, -58, 44	12760
	Lateral occipital cortex	right	58. -54, 38	7496
	Inferior frontal gyrus	left	-48, 40, -12	5912
	Superior frontal gyrus	right	20, 26, 62	4552
	Middle frontal gyrus	Right	2, 14, 50	2984
	Frontal pole	Right	46, 48, -12	2848
	Inferior temporal gyrus	left	-48, 0, -36	2576
NegativeAttend> NegativeDecrease	Lateral occipital cortex	left	-24, -90, 32	51256
	Parietal Operculum into central operculum and insular cortex	left	-60, -28, 18	17416
	Parietal Operculum into central operculum	right	60, -28, 22	8296
	Precentral gyrus	right	48, 8, 30	3336
ROI analysis Amygdala NegativeAttend> NegativeDecrease	Dorsal amygdala	left	-22,-2,-16	360

6.4 Study 2.2 - Discussion

In this part of the study we investigated neural and psychophysiological responses during an instructed emotion regulation task that was modified by shortening the trials so to make the design more similar to the LSCCE tasks we used. The reasoning behind this was twofold. Firstly, to be able to compare the neural activation across the two tasks, we needed to ensure the power to detect activation was as similar as possible. Because BOLD signal gets stronger over longer trials, we needed shorter trials in this task.

Secondly, due to the emotional nature of the images shown, decreasing the length of time they are on the screen, may save participants (especially those with mood or anxiety disorders) a considerable amount of distress and if the results are comparable, then a shorter presentation of the images may be a viable option in future clinical studies using this type of paradigm.

We expected to find increased SCR in response to Negative Attend compared to Negative Decrease trials, as well as in Negative Attend compared to Neutral Attend trials.

We also expected to replicate the neural patterns of activation found in previous research using this type of task, namely increased amygdala activation during Negative Attend compared to Neutral Attend and Negative Decrease trials, and a network of lateral PFC regions when comparing Negative Decrease to Negative Attend trials.

Even though our adaptation of the instructed emotion regulation task was shorter than previous versions, it revealed a neural pattern of results that is very similar to that described by Buhle et al. (2014). In particular, we found increased amygdala activation while participants were attending to the negative images compared to when they were decreasing their reaction to the negative images. The amygdala reliably shows activation in this contrast which has been linked to the processing of the significance and affective components of attending to highly negative images. In addition to the amygdala activation we found activation in bilateral parietal operculum which stretched into central operculum bilaterally, and into the insula on the left. These areas have also been linked to emotion processing, in particular with the processing of pain (Legrain et al., 2011), as well as the viewing of painful stimulation (Lloyd, Morrison, & Roberts, 2005). A number of images we used in this paradigm depicted painful scenarios, thus, it is possible that the activation is related to attending to these stimuli.

In the other contrast of interest we found increased activation in left vlPFC and dlPFC while participants were regulating the emotional responses to the negative images. These regions correspond to those commonly found in instructed emotion regulation studies using this contrast (Buhle et al., 2014; Kohn et al., 2014). Meta-analyses conclude that dlPFC is related to working

memory, reflecting the selection and application of regulation strategies, while vlPFC is likely involved in updating the updating of the emotional response.

As hypothesised we found increased SCR's while participants attended to emotional images compared to when they regulated their response to the images. In contrast, we found that pupil diameter was increased when participants decreased their emotional reaction to the images, compared to when they attended to them. This suggests that in contrast to SCR, pupil diameter reflects cognitive effort.

Interestingly, there was no difference in SCR between Negative Attend and Neutral Attend trials. And although SCR was marginally higher when participants were attending to the negative images compared to when they were decreasing their reaction to them, this effect was not as pronounced as we expected. It is possible that this is due to the decreased trial length. Processing the stimuli may have taken longer than expected and, thus, the SCR may have been slower to develop. In addition, participants had been lying down in the scanner for approximately 35 minutes at the start of the instructed emotion regulation task. Because SCR responsiveness is reduced when participants are lying down (McLaughlin, Goldman, Kleinman, & Korol, 1978), and drops further over time, it is possible that this skewed results.

Overall, these results show that the short version of this instructed emotion regulation task reveals psychophysiological and neural patterns of results that are largely consistent with those found in longer versions of this task. Thus, a comparison of this task with the LSCCE task is valid. In addition, this shorter version of the task may be beneficial when time in the scanner is limited, or when several tasks need to be completed in fMRI research.

7. Study 2.3 - Comparison between the LSCCE and instructed emotion regulation tasks

7.1 Study 2.3 - Introduction

The central objective of this study was to investigate whether the mechanisms involved in decreasing a conditioned aversive response overlap with those involved in the instructed regulation of responses to negative emotional images. Previous research compared results from a classical extinction paradigm with those from a paradigm in which participants used instructed regulation strategies to reduce a conditioned response (Delgado et al., 2008). The researchers found overlapping activation in the amygdala during early CS+ trials in the extinction task, and unregulated CS+ trials in the regulation task. In addition, vmPFC was involved in late extinction trials as well as regulated CS+ trials. This network was extended into dlPFC when participants regulated their response to a CS+. However, the Delgado study involved two completely different tasks with different stimuli. The aim of developing the LSCCE task is to investigate whether a single task can engage some of the mechanisms underlying more complex instructed regulation of affective images as well as extinction. Instructed emotion regulation tasks have been criticised for the complexity of both the emotion evoking stimuli used, as well as the regulation techniques. In contrast, the LSCCE task is highly controlled but still incorporates a regulatory element. Thus, if the LSCCE task incorporates some of the same mechanisms, then we predicted that the activation would overlap, but the LSCCE task should reveal more focal activation, restricted to areas related to semantic working memory processes.

7.2 Study 2.3 - Method

In this comparative analysis we aimed to find brain areas that were activated in both the LSCCE- and the instructed emotion regulation task. We used fslmaths to multiply the thresholded activation maps from the two contrasts of interest (CS+safe > CS+dangerous and Negative Decrease > Negative Attend). This way, voxels that were activated in one but not the other contrast would be multiplied by 0 and result in no activation in the resulting map, and voxels that were activated in both tasks would retain activation in the conjunction map.

Because the two prefrontal clusters resulting from the two contrasts of interest (left IFG in the extinction through cognitive evaluation task, and left vlPFC in the instructed emotion regulation task) were spatially very close together, we wanted to further investigate their activation during the two tasks. For each cluster, we extracted the mean % signal change from both the task we originally found them to be active in, and the other task (so for both the left IFG cluster from the ECE task, and

the left vlPFC cluster from the instructed emotion regulation task, % signal change from both tasks was extracted). These values were then compared using SPSS.

7.3 Study 2.3 - Results

Analysis of the group level activation maps from both tasks revealed no overlapping prefrontal areas. The mean within task condition differences (i.e. IFG CS+safe - CS+dang, IFG NegDec - NegSafe; vlPFC CS+safe - CS+dang, IFG NegDec - NegSafe) differences of the extracted % signal change values were then analysed in a 2 x 2 (cluster (left IFG vs left vlPFC) x contrast (CS+safe > CS+dangerous vs NegDec > NegAtt)) repeated measures ANOVA. We found significant main effects of cluster ($F(1,19)=24.88$, $p<0.001$, partial $\eta^2 = 0.57$) but not of contrast ($F(1,19)=2.35$, $p = 0.14$, partial $\eta^2 = 0.11$) and a significant cluster x condition interaction ($F(1,19)=26.6$, $p<0.001$, partial $\eta^2 = 0.58$). Post hoc t-tests revealed that the left IFG showed a significant difference difference between safe and dangerous CS+ ($t(19) = 5.22$, $p< 0.001$) but not between between Negative Decrease and Negative Attend trials ($t(19) = 0.45$, $p = 0.66$), and that the left vlPFC showed a significant difference between Negative Decrease and Negative Attend trials ($t(19) = 5.22$, $p < 0.001$) but not between safe and dangerous CS+ trials ($t(19) = 0.43$, $p = 0.67$). This confirms the voxelwise results i.e. each cluster showed differential activation within the task it resulted from but not during the other task. Visual inspection of the means reveals, however, that the left IFG is activated during both the Negative Decrease and Negative Attend conditions in the instructed emotion regulation task (see table 11 for means).

TABLE 11. MEAN % SIGNAL CHANGE FOR THE LEFT IFG AND LEFT VLPFC CLUSTERS IN THE 4 CONDITIONS OF INTEREST FOR THIS COMPARISON.

	Left IFG	**Left vlPFC**
	Mean % signal change	**Mean % signal change**
CS+Safe	0.37	0.03
CS+Dangerous	0.18	0.005
Negative Attend	0.37	0.59
Negative Decrease	0.38	1.02

7.4 Study 2.3 - Discussion

This comparison between left frontal activation resulting from the LSCCE and instructed emotion regulation task was carried out to investigate any shared mechanisms involved in the execution of both paradigms investigated in this study. As a first step we compared the activation maps resulting from the contrast of interest of each study (CS+safe > CS+dangerous in the ECE task, Negative Decrease > Negative Attend in the instructed emotion regulation task). The comparison of both tasks did not reveal any overlap in the neural network involved in both tasks even though the prefrontal

clusters are adjacent. As the two tasks differ greatly in the cognitive and attentional demands placed on participants, this is not necessarily surprising. While participants are required to process complex images during the instructed emotion regulation task as well as imagining a more positive outcome to decrease their initial reaction to them, the processes involved in the LSCCE task are less deliberative and the manipulation of the stimuli requires a very simple categorical decision. Thus, the network of brain areas involved is expectedly more defined and specific in the ECE task Further investigation of the activation in the left prefrontal clusters revealed that the IFG cluster found during safe CS+ trials (compared to dangerous) was, indeed, also active during both Negative Attend and Negative Decrease conditions in the instructed emotion regulation task. This suggests that it may be involved in an appraisal mechanism which may form part of the basis of appraisal in more complex emotion regulation tasks. The fact that this cluster is active both while participants are attending to negative images and while they are decreasing their emotional reaction to them illustrates the complexity of the emotion regulation task. Cognitive evaluative processes are involved both in the initial appraisal and in the regulation of emotion, thus, comparing the condition potentially masks brain areas that are part of this evaluative network. This further strengthens the suggestion that adaptations of the LSCCE task could be a useful tool to investigate the underlying mechanisms involved in emotion regulation with greater specificity.

8. Study 2 - General Discussion of chapters 5., 6., and 7.

The current study examined the effects of two different emotion regulation paradigms on psychophysiology and neural activation. The LSCCE task used conditioned stimuli (letters) to evoke an affective response, and added information that needed to be cognitively evaluated (words of two distinct categories) to inform participants whether each trial was safe or included a risk of electric shock. In contrast, in the instructed emotion regulation task participants either attended to, or used reappraisal to regulate their emotional reactions to affective images.

During the LSCCE task we found increased SCR in response to dangerous compared to safe CS+ trials. This difference was not present for CS- trials. In contrast, we found increased pupil dilation during CS+ compared to CS- trials. Similarly, during the instructed emotion regulation task we found increased SCR during Negative-Attend compared to Negative –Decrease trials; and increased pupil dilation to Negative-Decrease compared to Negative-Attend trials. Taken together these results support the notion that SCR and pupil dilation reflect separate aspects of emotion regulation with SCR reflecting emotional arousal and pupil dilation reflecting cognitive aspects (Urry et al., 2009), even when the experimental paradigm does not include an explicit instruction to regulate but instead uses a simple word categorization task to determine the risk of receiving an electric shock during each trial.

We did not find increased SCR in some comparisons where we did expect it. The lack of a significant difference CS+ and CS- in the conditioning phase of the LSCCE task is puzzling. It is possible that the relatively small number of trials along with the fact that participants had to figure out the contingencies during the conditioning phase prevented this effect from being significant. The fact that we found the expected effects in the LSCCE phase still suggests that conditioning worked. The fact that a trend in the expected direction also exists in the baseline trials and the letter only trials in the regulation/extinction phase is further evidence that the conditioning worked.

On a neural level, during the LSCCE task we found activation in bilateral insula and ACC during dangerous compared to safe CS+ trials, reflecting an anticipatory pain response (Legrain et al., 2011; Porro et al., 2002). During safe compared to dangerous CS+ trials we found activation in a cluster spanning left MFG and IFG, areas associated with semantic decision making and verbal working memory (Nee et al., 2013), consistent with the assumed mental processes involved in this task, and parietal areas associated with motor inhibition (Thoenissen et al., 2002).
The modified instructed emotion regulation task revealed activation in the amygdala during Negative Attend compared to Negative Decrease trials, which is commonly found in this contrast in

traditional emotion regulation tasks (Buhle et al., 2014; Kohn et al., 2014). The opposite, Negative Decrease > Negative Attend contrast showed activation in bilateral MFG, left IFG, bilateral lateral occipital cortex, frontal pole, superior and middle frontal gyrus, and inferior temporal gyrus. These areas are consistent with those that are typically found in this contrast in studies of emotion regulation and have been associated with the preparation and application of regulation strategies through attention, working memory, and inhibitory processes (Buhle et al., 2014; Kohn et al., 2014). Thus, we concluded that a short version of this task is also suitable to investigate emotion regulation processes.

We did not find overlapping activation between the two tasks. However, our results did suggest that left IFG was active during both Negative Decrease and Negative Attend conditions in the instructed emotion regulation task as well. It is possible that it contributes to evaluative processes involved in both conditions in this task. This highlights the need for highly controlled paradigms such as this LSCCE task to further investigate with a greater degree of specificity the underlying mechanisms and associated neural circuits.

Overall, this new paradigm is a controlled way of assessing the regulation of emotion through the evaluation of safety information. It removes many of the criticisms that have been addressed at studies of emotion regulation using IAPS images to evoke an affective response. It uses an individually adjusted electric shock to evoke as similar a response from all participants as possible, removing individual differences in responses that are present when images are used. Delgado et al. (2008) also used conditioned stimuli to evoke affective responses in participants. However, in contrast to the LSCCE task, Delgado and colleagues used instructed reappraisal to reduce emotional responses, which maintains some uncertainty about how exactly they are completing this task. In particular, they asked participants to think of something soothing in nature, a general strategy that does not manipulate the content of the CS+, but rather, a distraction strategy which does not involve modification of the stimulus itself, as in classic reappraisal studies (Ochsner et al., 2012). Other paradigms that have been employed to explore similar mechanisms include conditioned inhibition (Rescorla, 1969) and learned safety (Kong et al., 2014) in which participants are trained to associate one stimulus with an aversive US and another with the absence of that US. The two CS' are then combined and result in a reduction of the CR. In contrast, the safety cue in our paradigm needs to be cognitively evaluated by participants in order to figure out whether the CS+ (which is shown by itself again after the clue) is safe or associated with a risk of electric shock during each trial. Thus, the mechanisms employed during this paradigm are similar to, but more specific than those that have been indicated in cognitive reappraisal – a reinterpretation of a present affective stimulus. Because of its simplicity, this paradigm can be adjusted to investigate the different systems through which

reappraisal may be achieved, such as episodic memory, spatial manipulation, or prediction of future outcomes. That means it could be useful to investigate the impaired application of safety signals implicated in mental health disorders such as anxiety or posttraumatic stress disorder (Peri, Ben-Shakhar, Orr, & Shalev, 2000; Rauch, Shin, & Phelps, 2006).

9. Study 3 - The effects of trait anxiety on the physiological and neural correlates of Learned Safety Contingent on Cognitive Evaluation

9.1 Study 3 - Abstract

The aim of this study was to investigate differences in the physiological and neural correlates of the LSCCE task between participants high and low in trait anxiety. Clinical anxiety has been associated with increased reactivity in structures linked to emotion processing as well as on physiological measures. It has also been suggested that difficulties regulating emotions are involved in the maintenance of the disorder.

We recruited participants on the basis of their score on the trait version of the state-trait anxiety inventory and divided them into a high- and a low anxious group. Participants completed the Learned Safety Contingent on Cognitive Evaluation (LSCCE) task while skin conductance and BOLD fMRI data was collected. This task involves conditioning participants to expect an electric shock during the presentation of one of two stimuli (CS+/CS-, letters). In a subsequent phase, information is added to these stimuli that has to be cognitively evaluated to enable participants to determine threat level for each trial (words of two distinct categories, one signified safety, the other continued risk of electric shock). Finally, we added a continuation phase during which the same stimuli were presented but no further electric shocks were delivered.

We found that trials that included a threat of electric shock were associated with increased SCR's in the conditioning phase and with activation in bilateral insula as well as greater deactivation in the default mode network throughout. In contrast, safe CS+ trials were associated with activation in areas associated with semantic decision making.

We also collected diffusion tensor imaging data during this study to assess possible group differences in structural connectivity.

High anxious participants showed increased SCR's in the conditioning phase compared to low anxious participants, reflecting general hyperreactivity in this group. They also showed no difference between CS+ and CS- trials in the continuation phase whereas low anxious participants did.

On a neural level they showed no difference between safe and dangerous CS+ trials in a cluster in dorsomedial striatum and in vmPFC where low anxious participants did show a difference. These areas are associated with flexibility during switching and reversal tasks (dorsomedial striatum) as well as the integration of information to reduce a previously appropriate response (vmPFC) and this result may reflect possible reduced flexibility in high anxious participants' responses.

Finally, the high anxious group showed a tendency for increased activation in both bilateral insula as well as prefrontal areas compared to the low anxious group, further supporting the idea that anxiety

is related to hyperreactivity to threat, and that the emotion regulation difficulties they experience may not be due to decreased activation in the related neural circuits but that other mechanisms are responsible. It has been suggested that this inefficiency is due reduced connectivity between the neural areas involved in this process, however, our DTI analysis did not find support for this idea.

9.2 Study 3 - Introduction

Difficulties in regulating emotion are at the centre of a large number of psychological disorders including anxiety disorders (Gross & Muñoz, 1995). The two experimental paradigms that have most commonly been used to investigate deliberative and spontaneous emotion regulation processes respectively are instructed reappraisal tasks and the extinction of conditioned fear responses. Instructed emotion regulation paradigms typically use negative images to invoke emotional responses and a detailed instruction to either maintain, increase, or decrease participants' initial responses to these images for example by imagining a more positive outcome for negative images, or by distancing themselves from the situations depicted in these images (Ochsner et al., 2002; Urry et al., 2006). In contrast, in extinction research, a neutral conditioned stimulus (CS+) is first conditioned with an aversive unconditioned stimulus (US). After a number of pairings, the CS+ itself causes a conditioned response (CR, e.g. Büchel, Morris, Dolan, & Friston, 1998). Extinction follows this conditioning phase and involves removing the US, causing the CR to subside. However, this does not happen through unlearning of the CS-US relationship, rather, it happens through a new learning of the removal of this relationship, and inhibition of the CR (e.g. Bouton, 2004). Both instructed emotion regulation and the extinction of conditioned fear result in the reduction of an emotional response, instructed emotion regulation through conscious reappraisal, and extinction through automatic learning of new CS contingencies upon removal of the US.

On a neurobiological level there is consensus that areas including the insula and amygdala as well as dorsal and rostral ACC are involved in the generation of the affective responses (Buhle et al., 2014; Kalisch & Gerlicher, 2014; Maren, 2001; Mechias et al., 2010). Dorsal and rostral ACC have been linked to the intensity of pain and the subjective experience of pain, respectively (Roy et al., 2012), and some argue that in conditioning studies there is a distinction between non-conscious (dorsal ACC) and conscious threat appraisal (rostral ACC, Mechias, Etkin, & Kalisch, 2009). In addition, dorsal ACC is connected to motor areas and the spinal cord, as well as parietal cortex, suggesting a role for the processing and control of attention and action on the basis of sensory experience. In contrast, vmPFC is connected to medial and temporal cortex and has been linked to the construction of meaning from cognitive and sensory experiences (Roy et al., 2012). The rostral ACC is seen as a transition area that integrates the information from both networks. It co-activates with emotion-related areas such as the amygdala and nucleus accumbens, suggesting a role in shaping the

affective response together with the vmPFC (Roy et al., 2012). In summary, due to its direct connections to subcortical brain regions, the medial prefrontal cortex is in an ideal position to process information both bottom up and top down to influence affective responses and behaviour. This is further reflected in the typical patterns of responses during the reduction of affect in the extinction of conditioned responses and the instructed regulation of emotion.

In extinction, the inhibition of affective responses involves the amygdala itself as well as the ventromedial prefrontal cortex (vmPFC) and hippocampus (e.g. LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998; Sehlmeyer et al., 2009). In the instructed regulation of emotion, the network involved in the regulation is wider and more complex: it has been suggested that (among others) dorsolateral PFC (dlPFC) is involved in directing attention while ventrolateral PFC (vlPFC) is involved in the processing of the emotion as well as action inhibition, and the vmPFC integrates the information from lateral areas and may be involved in the implementation of emotion regulation (Buhle et al., 2014). Clinical studies using instructed emotion regulation paradigms have shown that patients with generalised anxiety disorder (GAD) and panic disorder (PD) showed reduced activation in the dmPFC and dlPFC compared to controls, both while attending to, and while regulating their emotional reaction to negative images. This suggests generally reduced PFC functioning in these disorders (Ball et al., 2013). Others showed this difference only in dorsal ACC, not in lateral PFC (Blair et al., 2012).

The medial PFC region implicated in anxiety patients' reduced ability to voluntarily regulate emotion is also important for the extinction of affective responses (see Milad, Rauch, Pitman, & Quirk, 2006, for a review). Notably, part of the character of anxiety disorders is that the absence of external negative consequences to situations that are feared by patients with anxiety does not reduce their anxiety as it might do in healthy individuals. A meta-analysis by Duits et al. (2015) showed that although there was no difference in the response to clues predicting a negative event (CS+) between patients with anxiety and controls, the former group showed increased responses to clues NOT predicting this aversive event, (CS-), either due to a generalisation of the threat from the CS+ to the CS-, or to an inability to inhibit a fear response to the safe stimulus. In extinction, patients with anxiety continued to show fear responses to the CS+ even though the US did not occur in this phase. They also showed increased and sustained discrimination between CS+ and CS- in this phase compared to controls whose responses to the CS+ decreased and the difference between responses to CS+ and CS- subsided. Thus, Duits et al. (2015) suggest that patients with anxiety may need more occurrences of safe versus dangerous cues than controls before being able to distinguish between safe and dangerous cues, thus, the conditioned effect emerges later and persists for longer. Studies of fear generalisation have shown that hippocampus and vmPFC show activation to stimuli that are similar to the CS+ (Lissek et al., 2014), which may contribute to healthy participants' ability to

discriminate between CS+ and CS-, as well as their ability to extinguish responses to the CS+, both processes that involve inhibiting a response. The generalisation of fearful stimuli is common among patients with anxiety disorders (see Dymond, Dunsmoor, Vervliet, Roche, & Hermans, 2015, for a systematic review). A study investigating this phenomenon in patients with anxiety and healthy controls found that patients showed reduced recruitment of the vmPFC compared to controls when stimuli were similar but not the same as the CS+ (Greenberg, Carlson, Cha, Hajcak, & Mujica-Parodi, 2013). Thus, if vmPFC in particular is less responsive in patients with anxiety, it is possible that the pattern of responding in patients with anxiety disorders found in the meta-analysis by Duits et al. (2015), i.e. increased responding to CS- during conditioning and sustained responding to CS+ during extinction may be due to altered responding in the vmPFC. Support for this idea comes from a study of conditioning and extinction in patients with posttraumatic stress disorder (PTSD) who showed reduced discrimination between CS+ and CS- in vmPFC during extinction (Bremner et al., 2005).

These results, specifically the altered involvement of vmPFC in extinction as well as instructed emotion regulation, suggest that a common mechanism may underlie the difficulties of patients with anxiety disorders in both instructed emotion regulation and in conditioning and extinction. This may also explain why anxiety patients are often unable to regulate their emotion in non-threatening situations despite the knowledge that they are safe. Extensive exposure treatment as well as cognitive behavioural therapy can be useful measures to alleviate some of the symptoms of anxiety disorders, possibly by engaging and strengthening mechanisms involved in reappraisal and extinction.

In the current study, we aimed to combine elements of instructed emotion regulation and the extinction of conditioned fear into a conditioning task in which complete extinction is preceded by a phase in which the context of the CS+ has to be cognitively manipulated by participants to figure out whether the US may occur or not. The previous two studies have already established that healthy participants were able to successfully regulate a CR when additional information was presented that participants knew to predict safety. As a next step, we recruited a group of participants that scored highly on the trait version of the State Trait Anxiety Inventory (STAI, Spielberger, Gorsuch, & Lushene, 1970) to complete the same task and compared them to a group of participants that scored low on the STAI. We aimed to examine whether the two groups would show differences in their psychophysiological and neural responses during this task.

We previously found that the threat of shock in non-reinforced CS+ trials (both during conditioning and during dangerous trials in LSCCE) was reflected in the activation in the insula, and that a cluster in left IFG showed increased activation during safe CS+ trials, possibly indicating an inhibitory

response. In line with these results and previous research, we hypothesised that participants high in anxiety would show increased activation in the insula and decreased activation in prefrontal areas during these conditions compared to low anxiety participants. We also expected participants high in anxiety to show higher SCR's to dangerous trials than those low in anxiety.

Due to anxiety patients' tendency to generalise fear provoking stimuli (Dymond et al., 2015), we expected participants who scored highly on the STAI to be less able to differentiate between safe and dangerous CS+.

In addition to this, we were also interested in possible connectivity differences between the two groups. Structural connectivity is commonly assessed using diffusion tensor imaging (DTI), a technique based on water diffusion in the brain. Water diffuses more readily along, as opposed to across fibre tracts, due to myelin acting as a barrier to water diffusion. By measuring the fractional anisotropy (FA) of water diffusion – the degree to which it is diffusing more in one direction than another – DTI can provide information about the strength and structural integrity of the white matter tracts in the brain – greater FA along a white matter tract implies greater connectivity. (Alexander, Lee, Lazar, & Field, 2007). The majority of studies related to emotion regulation examining structural connectivity using DTI have focussed on a corticolimbic tract called the uncinate fasciculus (UF), which runs between the hippocampus and amygdala and the ventromedial prefrontal cortex. Some have found a positive correlation between trait anxiety and fractional anisotropy in this tract (Montag, Reuter, Weber, Markett, & Schoene-Bake, 2012) and link this to compensation for reduced hippocampal volume. However, they did not measure hippocampus size in this study, nor did any of the participants have clinical anxiety so this interpretation has to be considered with caution. Other studies found the opposite relationship between FA and trait anxiety, suggesting reduced connectivity between limbic and prefrontal brain areas (Baur, Hänggi, Langer, & Jäncke, 2013; Kim & Whalen, 2009). This is more in line with the idea that the communication between these areas is interrupted in anxiety disorders, giving rise to emotion regulation difficulties. In this study, we used DTI to assess differences in the FA of the UF between two groups of participants high and low in trait anxiety. We predicted lower FA in the high anxious group of participants, compared to the low anxious group. Although some studies report amygdala involvement in fear conditioning (Delgado et al., 2008; Phelps et al., 2004), others report variable activation of this area (Yin, Liu, Petro, Keil, & Ding, 2018) and a recent meta-analysis did not find any evidence for consistent amygdala activation during fear conditioning (Fullana et al., 2015). In addition, we did not find amygdala activation in study 2, so we do not expect a relationship between FA in the UF and BOLD. Thus, this DTI analysis is taking a more exploratory approach as outlined above.

9.3 Study 3 - Method

Participants

Participants for this study were selected on the basis of their score in the state version of the State Trait Anxiety Inventory (STAI, Spielberger, Gorsuch, & Lushene, 1970). This was administered electronically to 1st and 2nd year students at the University of Reading via the Reading University research panel. 20 low anxious (range 29-35, mean = 32.25(3.23)) and 20 high anxious (range 49-70, mean score =54.05(5.55)) participants were selected to take part in this study. The difference between these two groups was statistically significant ($t(38) = 15.19$, $p < 0.01$, Cohen's $d = 4.97$, overall Cronbach's $\alpha = 0.94$), high anxious participants scored significantly higher on this measure than low anxious participants. Due to sampling restrictions, all participants were female and most were psychology undergraduates who received course credit or £10 and a picture of their brain in return for their participation. All participants were screened for their suitability to be part of an fMRI study and gave written informed consent before commencing the study. Participants with a current diagnosis of a mental health disorder were excluded from the study. Mean age of the participants was 20.18 years (range: 18 – 45(low anxious group mean = 19.1 (0.91), high anxious mean = 21.25 (6.11), $t(38) = -1.56$, $p = 0.14$ (equal variances not assumed), Cohen's $d = -0.61$).

This study was reviewed and approved by the University of Reading Research Ethics Committee.

The Task

Conditioning Phase

During the first part of this experiment one of two letters (B and T) was paired with an electric shock 50% of the time. The letter that served as the CS+ was counterbalanced between participants. This phase consisted of a total of 30 trials: 10 CS-, 10 reinforced CS+, and an additional 10 non-reinforced CS+. Trials lasted for a total of 4000ms during this phase of the task, and the electric shock co-terminated with the reinforced CS+ trials.

Learned Safety Contingent on Cognitive Evaluation

During the LSCCE phase of this experiment, participants saw a total of 102 trials: 20 each of "safe" and "dangerous" CS-, and and 20 safe CS+, 20 reinforced dangerous CS+ and an additional 20 non-reinforced dangerous CS+. 2 trials contained words that did not belong to either of the two categories to ensure that participants were paying attention throughout. For the first 500ms of each trial, the letter was presented on its own. The word then appeared around it (with the letter in the correct place but emphasized) for 1000ms. Finally, the word disappeared and the letter remained on

screen by itself for an additional 4000ms. During reinforced trials the electric shocks were delivered so they co-terminated with the trial.

Continuation

We also wanted to explore how physiological and neural activation would change when the US was removed from all trials. Thus, we added an additional set of trials with the same structure as the LSCCE phase but no further electric shocks. During this continuation phase, a further 40 trials were shown: 10 each of safe and dangerous CS+ and CS-. However, no further electric shocks were delivered. The trial structure remained the same as in the LSCCE phase. This part of the task served to inform about whether the processing of the contingencies would be maintained when the dangerous CS+ ceased to be reinforced.

Instructions

All instructions were given prior to task commencement. Participants were told that they would see two letters during the first phase, one of which would be associated with a risk of electric shock. They were then told that after a short break, they would see the same letters again, and this time a word would appear briefly. Most of these word would belong to two distinct categories: plants or animals, and one of these categories was safe. They were then asked to determine which category was safe during this phase, and to keep the contingencies in mind throughout. They were also asked to focus on whether they thought they might receive an electric shock or not during each trial. They were also told that 3 brief breaks were included in the task but that contingencies would not change. They were not given any additional information about the extension/continuation phase.
Finally, they were told to press a button on the rare occasion that they saw a word that did not belong to one of the two categories.

Design

This study was carried out in a mixed 2 x 2 x 2 (group(high anxious vs low anxious) x Letter(CS+ vs CS) x Category (dang vs safe)) design. The main contrast of interest was CS+safe vs CS+dang (only trials that did not contain an electric shock were used for this comparison). Group differences in mood, state anxiety, intolerance of uncertainty, worry and emotion regulation strategies were assessed using a range of questionnaires.
Letters representing the CS+ and CS- as well as “safe and “dangerous” categories were counterbalanced, and stimuli were presented in a pseudo-random order, ensuring that no stimulus type occurred more than twice in a row.

Apparatus and Materials

Conditioning stimuli (CS+ and CS-) were the letters “B” and “T” and the unconditioned stimulus (US) was a mild electric shock (10 pulses at 100 Hz).

10 different words were selected for each condition on the basis of lists defined as typical members of these categories (Battig & Montague, 1969). Further words were chosen through online dictionaries. All words were validated by checking their frequency in the English language in a list based on the British National Corpus (Kilgarriff, 1997, for frequencies see table 5).

Stimuli were prepared using PowerPoint, Letters and words were arranged in the centre of each slide, with letters in black font size 66 and bold, and words in font size 44 (not bold, see figure7) on a light grey background (see figure 14).

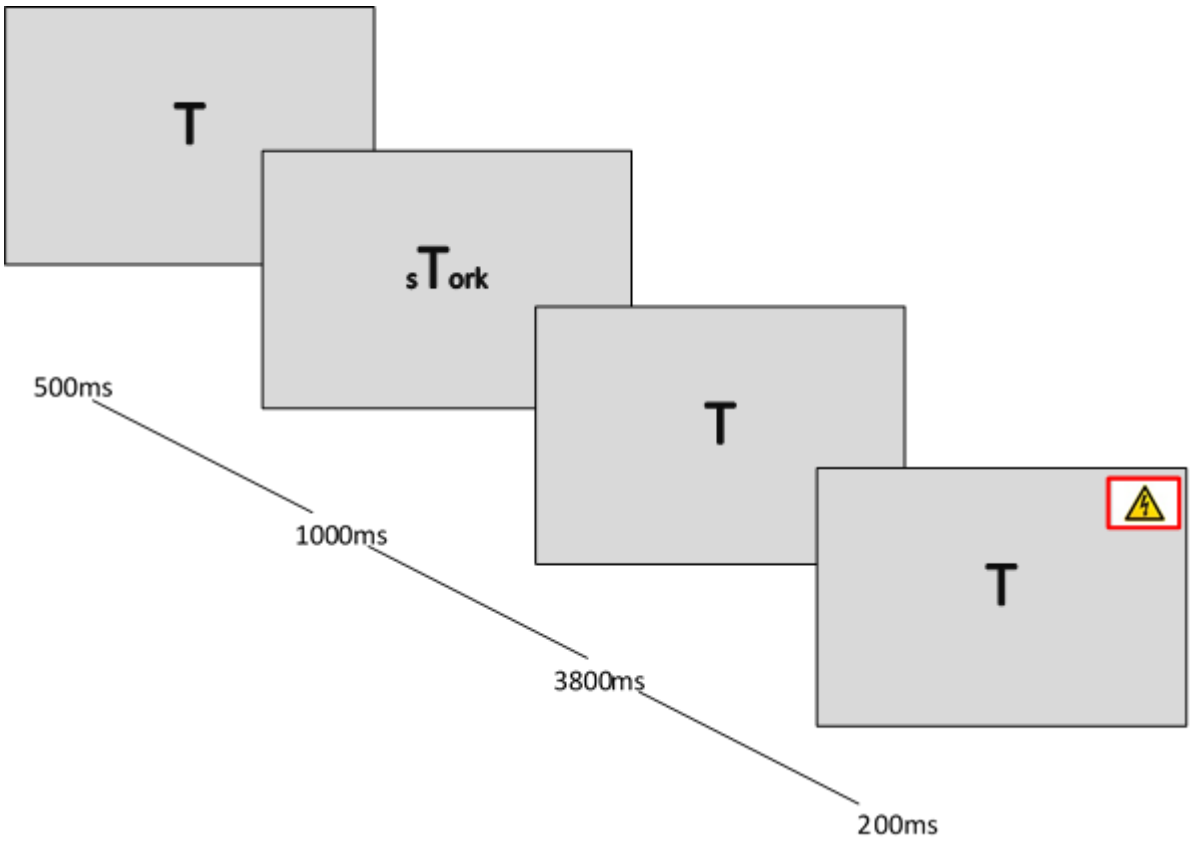


FIGURE 14. STRUCTURE OF AN LSCCE TRIAL. THE LETTER CS+ IS PRESENTED BY ITSELF BEFORE THE WORD APPEARS ON SCREEN. AFTER THE WORD DISAPPEARS THE LETTER REMAINS ON SCREEN FOR A FURTHER 4000MS. DURING REINFORCED CS+ TRIALS, THE TRIAL CO-TERMINATES WITH AN ELECTRIC SHOCK.

Stimuli were presented using EPrime 2.0 via a mirror and a BOLD screen (Cambridge Research Systems, Rochester, UK, screen resolution 1024 x 768). Automated electric stimulation was carried out using a macro in LabChart which received a marker from the EPrime script to trigger stimulation at the intensity specified prior to the task. Reinforced CS+ trials co-terminated with the electric shocks. Shocks were delivered through 2 Ag-AgCl electrodes attached to participants’ right index and

middle fingers and connected to an ADInstruments Isolated Stimulator built into Powerlab 26T. Automated electric shock delivery was carried out using a macro in ADInstruments LabChart software which received a marker from the EPrime PC via a parallel port, and triggered shock delivery at the intensity specified prior to the task. The intensity was determined by the participant themselves through a standardised procedure described below.

PHYSIOLOGICAL DATA COLLECTION

Skin conductance response (SCR) data was recorded at 1000hz with Labchart 7 using 2 Ag-AgCl electrodes on the distal phalanges of the middle and ring fingers of the participant's non-dominant hand (Cacioppo et al., 2007) and connected to an ADInstruments Powerlab 26T via a PowerLab ML116 SCR amplifier input module. A low constant-voltage AC excitation of 22mVrms at 75 Hz was passed through the electrodes, and converted to DC before being digitized and stored.

FMRI DATA COLLECTION

Structural and functional data were collected on a 3T Siemens Trio MRI scanner with 32 channel head matrix coil at The University of Reading Centre for Integrative Neuroscience and Neurodynamics (CINN). Functional scans consisted of a t2*-weighted gradient echo, echo planar imaging (EPI) sequence (37 interleaved transverse slices, phase encoding P to A, 3 mm thickness, 128*128 matrix; 192 mm field of view; TR: 2000ms, TE: 30ms, Flip Angle: 90°; 212 whole-brain volumes). A high resolution whole-brain three dimensional structural image was also acquired using an MPRAGE sequence with 176 x 1 mm slices. (voxel size: 1*1*1, TE: 2.9 ms, TR: 2020 ms, TI:1100 ms, FOV: 250 mm, Flip Angle: 9°).

Fieldmaps were acquired using a gradient echo sequence (P to A, 3*3*3mm voxel size, TE1: 5.19ms, TE2: 7.65ms, TR: 400ms, FOV: 192mm, Flip Angle: 60°).

DTI DATA COLLECTION

To assess structural connectivity, we collected DTI data after the task was completed (60 interleaved slices, encoding direction P>A, FOV 256mm, voxel size: 2x2x2mm, TR: 7800ms, TE: 87ms, 2 averages, 30 directions, diffusion mode: MDDW, b-value 1 = 0 s/mm^2, b-value 2 = 1000 s/mm^2).

PROCEDURE

Informed consent was obtained from each participant before commencing the experiment. Participants then completed a computerised version of the Positive And Negative Affect Scale (PANAS-NOW, Watson, Clark, & Tellegen, 1988) to assess current mood and, together with the experimenter, completed the initial screening form to ensure MRI safety.

Prior to starting the task, the stimulator electrodes were attached to participants' fingers, a shock at very low intensity (0.5mV) was delivered and the intensity was increased in steps of (0.5mV). After each shock, the participant was asked to rate the sensation on a scale of 1 ("not painful at all") to 10 ("extremely painful"). When they reached 8 on this scale, the experimenter reduced the intensity of the shock by 1 step and informed that this was the intensity the shock would remain at for the duration of the experiment.

Subsequently, the electrodes to collect skin conductance were attached to participants' left index and middle finger. Before participants were set up in the scanner and the task was started, they completed the second screening form as well as a final metal check.

After completing the scan, participants were asked complete computerised versions of a follow up questionnaire as well as a questionnaire to assess to assess how they felt during each type of trial as well as how they felt throughout the task (7-point Likert scales, 1 = "not stressed", 7 = "extremely stressed", as well as 1 = "not bored" to 7 = "extremely bored", and 1 = "not sleepy" to 7 = "extremely sleepy") and whether they were aware of the task contingencies. They also filled in a range of standardised questionnaires to assess trait anxiety (Stait Trait Anxiety Inventory, STAI, Spielberger, Gorsuch, & Lushene, 1970), tendency to worry (Penn State Worry Questionnaire, PSWQ, Meyer, Miller, Metzger, & Borkovec, 1990), emotion regulation capacity (Emotion Regulation Questionnaire, ERQ, Gross & John, 2003) and intolerance of uncertainty (Intolerance of Uncertainty Scale, IUS, Buhr & Dugas, 2002).

They were then verbally debriefed and given a debrief sheet to take home with them.

SCR DATA ANALYSIS

Data was visually checked for motion artefacts and these were removed manually. Prior to analysis, the SCR data was filtered using a median filter over 3 data points to remove artefacts resulting from the electric shock. Data was then filtered using a high pass filter with a cutoff at 0.2, and slow drift was corrected by creating a 100 second moving average which was then subtracted from the data (Johnstone, T. (2017, September 8). Psychophysiology Analysis Software. Retrieved from osf.io/4wsm3). SCR data was analysed using a MATLAB script that detected maximum deflection from a 2 second pre-trial baseline using a window of 7 seconds from trial onset. An average value for each condition and participant was identified and exported into SPSS for analysis. Residuals were normally distributed, therefore data was not transformed but original data was analysed.
Data from a number of participants had to be removed due to issues with the data quality. Participants who did not show a response to the electric shocks were also removed from the SCR

analysis. This resulted in 11 low anxious and 9 high anxious participants for this analysis. 2 participants did not complete the continuation phase of this task resulting in 9 high anxious and 9 low anxious participants in the analysis of this phase.

fMRI data analysis

FMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). The following pre-statistics processing was applied; motion correction using MCFLIRT(Jenkinson et al., 2002) ; non-brain removal using BET (Smith, 2002) ; spatial smoothing using a Gaussian kernel of FWHM 5mm; grand-mean intensity normalisation of the entire 4D dataset by a single multiplicative factor; highpass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma=50.0s), and B0 unwarping (Jenkinson, 2003).

Single subject analysis

A fixed effects general linear model was used to analyse individual subject data. Regressors were created for each condition by convolving a stimulus boxcar function with the standard FSL gamma function. Temporal derivatives were included in this glm. Motion estimates were added as regressors to control for head displacement. The main contrasts of interest for the LSCCE task compared CS+ and CS- during the conditioning phase, and dangerous CS+ (CS+dang) with safe CS+ (CS+safe) trials during the LSCCE phase. Only non-reinforced trials were included in this analysis. Trials that included an electric shock were modelled separately (1 regressor for this condition) but not used for analysis.

Registration to a standard space was performed using a two stage procedure with FLIRT (Jenkinson & Smith, 2001; Jenkinson et al., 2002). The mean functional volume for each participant was registered to the individual's high resolution structural image using 6 degree of freedom (DOF) BBR white matter boundary mapping. In a second step the individual's high resolution structural image was normalised to the Montreal Neurological Institute (MNI) template brain using a 12 DOF affine transformation. These two transformations were combined and used for subsequent registration of that participant's contrast images to MNI space before higher-level group analysis.

Group analysis

Comparison of contrasts across participants was carried out using Mixed Effects (FMRIB's Local Analysis of Mixed Effects, FLAME 1) with automatic outlier de-weighting and Random Field-based cluster thresholding. Results were corrected for multiple comparisons with a familywise error of $p<0.05$.

Based on the results of the previous study we hypothesized that the left IFG would be more active during safe compared to dangerous CS+ trials. We therefore completed a second group level analysis using the IFG cluster from the previous study as a region of interest (ROI).

DTI ANALYSIS

DTI data was preprocessed and analysed using FSL(Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012). Data was eddy current and motion corrected using EDDY (Andersson & Sotiropoulos, 2016), registered to structural space and to standard space using FLIRT (Jenkinson et al., 2002; Jenkinson & Smith, 2001) and FNIRT (Andersson, Jenkinson, Smith, & Andersson, 2007; Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012). Diffusion tensor models were fitted using DTIFIT. Tract based functional anisotropy (FA) was calculated to compare the two groups both on a wholebrain level and using the left and right uncinate fasciculus as ROIs using a mask derived from the JHU White Matter Tractography atlas in FSL.

9.4 STUDY 3 - RESULTS

QUESTIONNAIRES

All participants accurately reported which letter and category were safe. There were no significant differences between groups on the perception of the stimuli or how they felt throughout the experiment (see Table 12).

All participants were able to accurately perform the behavioural task.

A 2 x 2 x 2 (CS (CS= vs CS-) x trial type (safe vs dangerous) x group (low anxiety vs high anxiety)) mixed ANOVA revealed a significant effect of CS ($F(1,38) = 135.92$, $p < 0.01$, partial η^2=078) and trial type ($F(1,38) = 52.02$, $p < 0.01$, partial η^2=0.58), as well as a marginally significant CS x group interaction ($F(1,38) = 2.88$, $p = 0.1$, partial η^2=0.07). Follow up t-tests revealed that low anxiety participants reported feeling significantly more stressed during dangerous than safe CS+ trials ($t(19) = 3.44$, $p < 0.01$, Cohen's d = 0.77) as well as feeling more stressed during dangerous than safe CS- trials ($t(19) = 3.94$, $p< 0.01$, Cohen's d = 0.91).

Similarly, high anxiety participants reported feeling more stressed during dangerous than safe CS+ trials ($t(19) = 3.76$, $p < 0.01$, Cohen's d = 0.84), as well as feeling more stressed during dangerous than safe CS- trials ($t(19) = 2.35$, $p = 0.03$, Cohen's d = 0.76, this difference is not significant after application of a Bonferonni correction).

High anxious participants reported using suppression to regulate emotion marginally more than low anxious participants ($t(38) = 1.92$, $p = 0.06$), feeling more worry ($t(38) = 2.54$, $p = 0.02$), and being less tolerant of uncertainty ($t(38) = -3.76$, $p = 0.001$, see table 13 for all means).

TABLE 12. QUESTIONNAIRE SCORE MEANS. SUBJECTIVE STRESS LEVELS AS WELL AS BOREDOM AND SLEEPINESS WERE ASSESSED ON A 7-POINT LIKERT SCALE GOING FROM 1 (NOT AT ALL STRESSED/BORED/SLEEPY) TO 7 (EXTREMELY STRESSED/BORED/SLEEPY).

	Low Anxiety Mean (SD)	High Anxiety Mean (SD)	Cronbach's α	t(38)	p	Cohen's d
Shock Intensity	3.54 (1.6)	2.46 (1.3)		2.34	0.03	0.74
Perception of -						
- CS+ Dang	*3.4 (0.14)*	*2.95 (0.12)*		*1.67*	*0.1*	*0.52*
- CS+ Safe	*2.45 (0.14)*	*2.05 (0.12)*		*1.43*	*0.16*	*0.45*
- CS- Dang	*1.15 (0.08)*	*1.1 (0.13)*		*0.3*	*0.76*	*0.1*
- CS- Safe	*1.75 (0.08)*	*1.7 (1.13)*		*0.15*	*0.88*	*0.05*
Boredom	2.35 (1.04)	2.45 (0.89)		-0.33	0.74	0.1
Sleepiness	3.2 (1.7)	3.65 (0.93)		-1.04	0.31	0.33
PANAS positive	30.56 (6.35)	28.85 (6.23)	0.87	0.85	0.4	0.27
PANAS negative	14.25 (3.51)	15.15 (3.57)	0.79	-0.8	0.43	0.25
ERQ Reappraisal	28.9 (4.76)	26.8 (6.46)	0.82	1.17	0.25	0.37
ERQ Suppression	11.8 (4.26)	14.3 (3.97)	0.71	-1.92	0.06	0.61
PSWQ	54.15 (10.73)	53.9 (11.03)	0.94	-2.54	0.02	0.03
IUS	51.6 (13.24)	69.4 (16.53)	0.95	-3.76	0.001	0.8

SCR RESULTS

CONDITIONING

Due to an unfortunate flaw in the presentation program, the first CS- trial was always preceded by a reinforced CS+ trial with a short ITI, leading to a consistent shock-related SCR overlapping with the first CS- trial. Because of this, we removed the first CS- trial as well as the first unreinforced CS+ trial (so as not to bias comparisons) from the analysis of the SCR.

A 2 x 2 x 2 (CS (CS+ vs CS-) x group (low anxious vs high anxious) x time (early vs late)) mixed effects GLM revealed main effects of condition $F(1,18) = 10.03$, $p = 0.005$, partial $\eta^2 = 0.36$), time ($F(1,18) = 7.37$, $p = 0.014$, partial $\eta^2 = 0.29$), and group $F(1,18) = 4.39$, $p = 0.05$, partial $\eta^2 = 0.2$). CS+ trials were associated with higher SCR's than CS- trials, late trials resulted in higher SCR's than early trials, and the high anxious group showed increased responses compared to the low anxious group throughout (see table 14 for means).

Learned Safety Contingent on Cognitive Evaluation

Inspection of the means revealed that responses to safe CS+ were not lower than those to dangerous CS+ throughout the LSCCE phase (see table 14) inconsistent with our hypotheses.

Continuation

The continuation phase was analysed using a 2 x 2 x 2 x 2 (CS (CS+ vs CS-) x trial (safe vs dangerous) x time (early vs late) x group (high anxious vs low anxious)) mixed ANOVA.

This analysis revealed a main effect of CS ($F(1,16) = 5.44$, $p = 0.03$, partial $\eta^2 = 0.25$, CS+ was associated with higher responses than CS- trials) and a marginally significant main effect of time ($F(1,16) = 3.92$, $p = 0.07$, partial $\eta^2 = 0.2$, late trials were associated with higher responses than early trials). It also revealed a significant CS x group interaction ($F(1,16) = 4.98$, $p = 0.04$, partial $\eta^2 = 0.24$). Follow up t-tests showed that while low anxious participants showed significantly increased responses to CS+ compared to CS- trials in this phase ($t(8) = 2.4$, $p = 0.04$, Cohen's $d = 1.23$), high anxious participants did not ($t(8) = 0.16$, $p = 0.87$, Cohen's $d = 0.005$, see table 14, and figure 15 for means).

Participants' questionnaire scores did not have an effect on these results.

TABLE 13. SCR MEANS AND WITHIN SUBJECTS CONFIDENCE INTERVALS (CI) BY CONDITION AND GROUP. WE FOUND SIGNIFICANT EFFECTS OF CONDITION, TIME AND GROUP DURING THE CONDITIONING PHASE (ALL P'S < 0.05), AND A MAIN EFFECT OF CONDITION (P = 0.03), AS WELL AS A MARGINALLY SIGNIFICANT EFFECT OF TIME (P = 0.07) DURING THE CONTINUATION PHASE.

	Low Anxiety	**High Anxiety**
Conditioning	Mean (CI)	Mean (CI)
CS-		
Early	*-0.05(0.99)*	*-0.04(0.11)*
Late	*0.05(0.09)*	*0.09(0.1)*
CS+		
Early	*0.11(0.09)*	*0.19(0.1)*
Late	*0.05(0.97)*	*0.09(0.1)*
LSCCE		
CS-Safe		
Early	*0.04(0.05)*	*0.05(0.05)*
Late	*0.03(0.04)*	*0.02(0.05)*
CS-Dang		
Early	*0.06(0.05)*	*0.05(0.05)*
Late	*0.03(0.04)*	*0.03(0.05)*
CS+Safe		
Early	*0.05 (0.05)*	*0.04 (0.05)*
Late	*0.03 (0.05)*	*0.04 (0.05)*
CS+Dang		
Early	*0.02(0.04)*	*0.03(0.05)*
Late	*0.03(0.04)*	*0.02(0.05)*
Continuation		
CS-Safe		
Early	*0.03(0.05)*	*0.03(0.05)*
Late	*0.002(0.05)*	*0.008(0.05)*
CS-Dang		
Early	*0.01(0.05)*	*0.02(0.05)*
Late	*0.03(0.05)*	*0.04(0.05)*
CS+Safe		
Early	*0.03 (0.05)*	*0.02 (0.05)*
Late	*0.08 (0.05)*	*0.03 (0.05)*
CS+Dang		
Early	*0.06 (0.05)*	*0.01 (0.05)*
Late	*0.08 (0.05)*	*0.04 (0.05)*

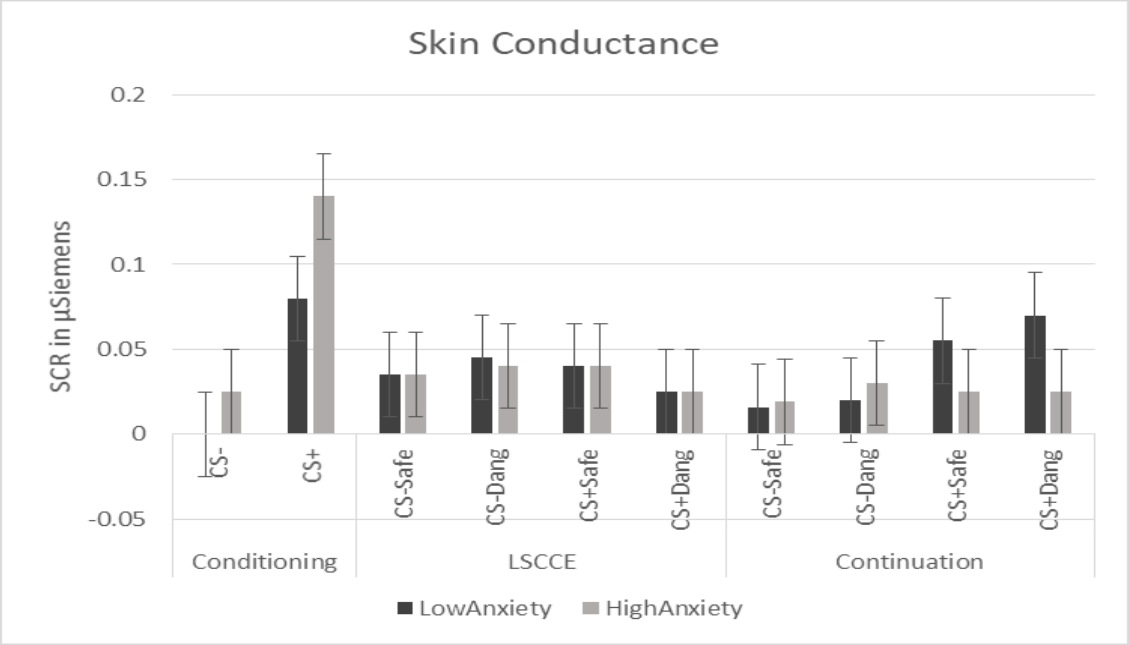


FIGURE 15 SKIN CONDUCTANCE RESPONSES STUDY 3 BY GROUP. VALUES FOR LOW ANXIOUS PARTICIPANTS ARE SHOWN IN DARK GREY, VALUES FOR HIGH ANXIOUS PARTICIPANTS ARE SHOWN IN LIGHT GREY. ERROR BARS REPRESENT WITHIN SUBJECTS CONFIDENCE INTERVALS.

fMRI RESULTS – WHOLE GROUP

CONDITIONING

CS- > CS+

This contrast revealed a large cluster including a large area in occipital cortex, cuneus, precuneus, lingual gyrus, hippocampus and amygdala for the whole group of participants (table 14). Further investigation revealed that the activation in amygdala and hippocampus as well as some of the midline activation was driven by deactivation during CS+ trials, rather than activation during CS-. In addition, a cluster in MFG survived thresholding. This cluster also stretched into vmPFC and ACC. This activation was driven by increased activation in CS-.

CS+ > CS-

No clusters survived thresholding in the opposite (CS+ vs CS-) contrast. However, sub-threshold activation in bilateral anterior insula was revealed at an uncorrected threshold of z = 2.3 (see figure 16).

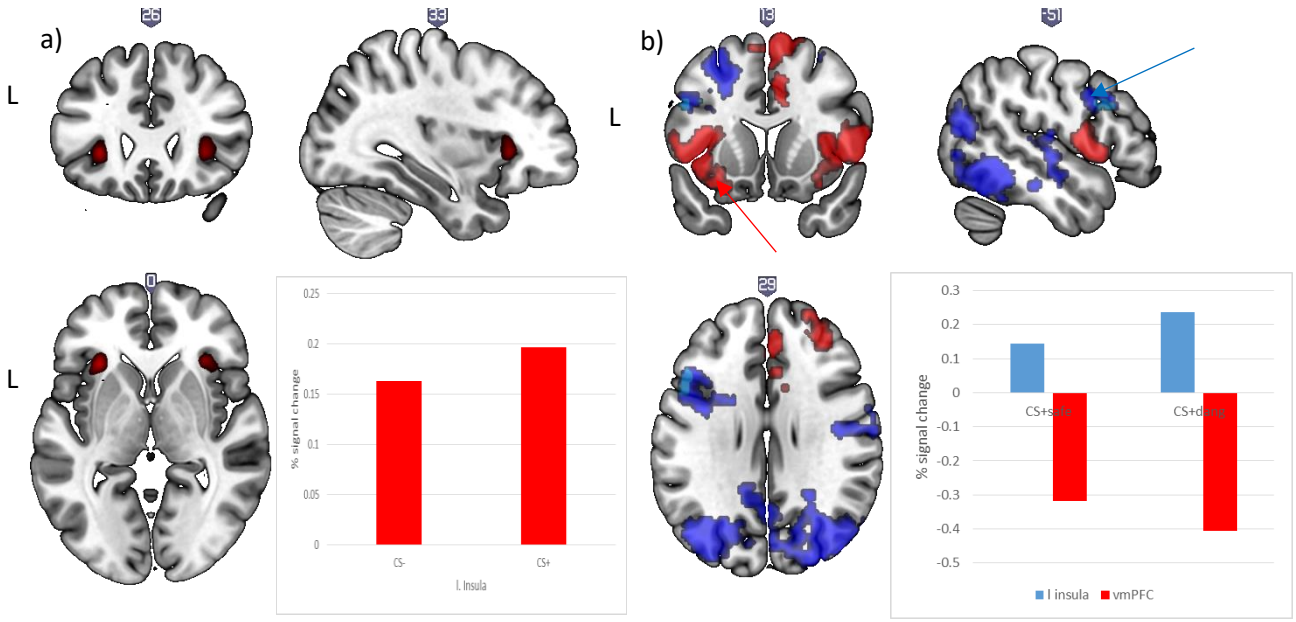


FIGURE 17. PATTERN OF ACTIVATION DURING EMOTION GENERATION AND LSCCE. A) SHOWS BILATERAL INSULA ACTIVATION DURING CS+ TRIALS IN THE CONDITIONING PHASE (Z = 2.3 UNCORRECTED). B) SHOWS THE PATTERN OF ACTIVATION DURING DANGEROUS COMPARED TO SAFE CS+ TRIALS IN THE LSCCE PHASE. AREAS MORE ACTIVE DURING SAFE CS+ TRIALS SHOWN IN BLUE INCLUDE THE LEFT MFG AND INFERIOR FRONTAL GYRUS, MIDDLE FRONTAL GYRUS AND INFERIOR TEMPORAL GYRUS. AREAS MORE ACTIVE DURING DANGEROUS CS+ TRIALS SHOWN IN RED INCLUDE BILATERAL INSULA AND FRONTAL OPERCULUM, AS WELL AS DORSAL ANTERIOR CINGULATE (Z = 2.3 CORRECTED). THE LEFT IFG CLUSTER REVEALED IN THE ROI ANALYSIS IS SHOWN IN LIGHT BLUE (B)).

CS+SAFE > CS+DANG

During the safe CS+ trials relative to dangerous CS+ trials, we found increased activation in bilateral inferior temporal gyrus and right dorsolateral prefrontal cortex (SFG/MFG/IFG). We also found vmPFC, posterior cingulate and precuneus, bilateral hippocampus and amygdala to be more activated during safe than dangerous CS+ trials (see figure 16 for activation maps, and table 14 for peak coordinates). Further inspection of this effect showed that the activation in the midline structures including vmPFC, posterior cingulate and precuneus, was driven by greater deactivation in dangerous than safe CS+ trials.

Follow up tests of the activation in amygdala and hippocampus revealed that both high and low participant groups showed significant differences between safe and dangerous CS+ trials in these clusters, high anxious participants showed a stronger effect (see table 15 a) for means and t-tests).

The ROI analysis on the IFG cluster revealed that part of this cluster was also more active during safe compared to dangerous CS+ trials, replicating the results of the previous study.

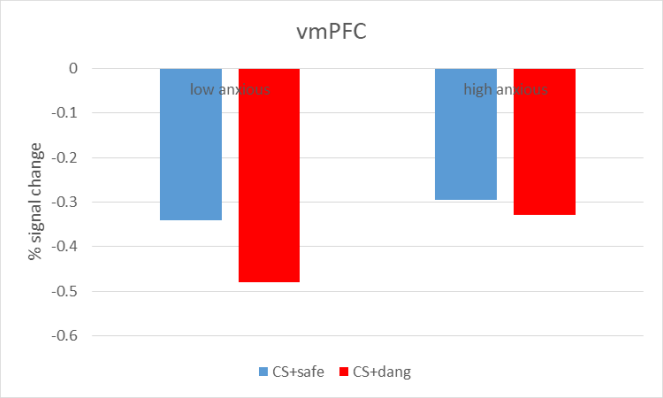


FIGURE 18. PATTERN OF RESULTS IN THE VMPFC. OVERALL, THIS CLUSTER SHOWS GREATER DEACTIVATION DURING DANGEROUS COMPARED TO SAFE CS+ TRIALS, DRIVING THE CS+SAFE > CS+DANG EFFECT.

CS+DANG > CS+SAFE

In the CS+Dang > CS+Safe contrast we found activation in left precentral gyrus and left insula, right insula, and supplementary motor cortex, as well as left parietal operculum (table 14, figure 16). In addition, we found sub-threshold (z = 2.3, uncorrected) activation in dorsal ACC (MNI coordinates: 8, 8, 36). This activation is consistent with that found in study 2 for the same contrast.

CS-DANG > CS-SAFE

We did not find differences between safe and dangerous CS- trials in these areas.

CONTINUATION PHASE

CS+SAFE > CS+DANG

The pattern of responses in this contrast was similar to the pattern of responses during the safe and dangerous CS+ trials in the Reappraisal phase. Participants showed increased vmPFC activation, driven by greater deactivation in dangerous CS= trials. We further found left MFG/IFG activation during safe CS+ trials. We carried out the same ROI analysis using the left IFG from study 2 and found part of this cluster also showed increased activation during safe compared to dangerous CS+ trials. This activation was partly but not fully overlapping with that shown in the wholebrain analysis.

CS+DANG > CS+SAFE

In this contrast we found increased activation in bilateral insula, as well as dorsal ACC and SFG. The insula activation overlapped with that found in the same contrast during the LSCCE phase (table 14, figure 18).

Participants' questionnaire scores did not have an effect on these results.

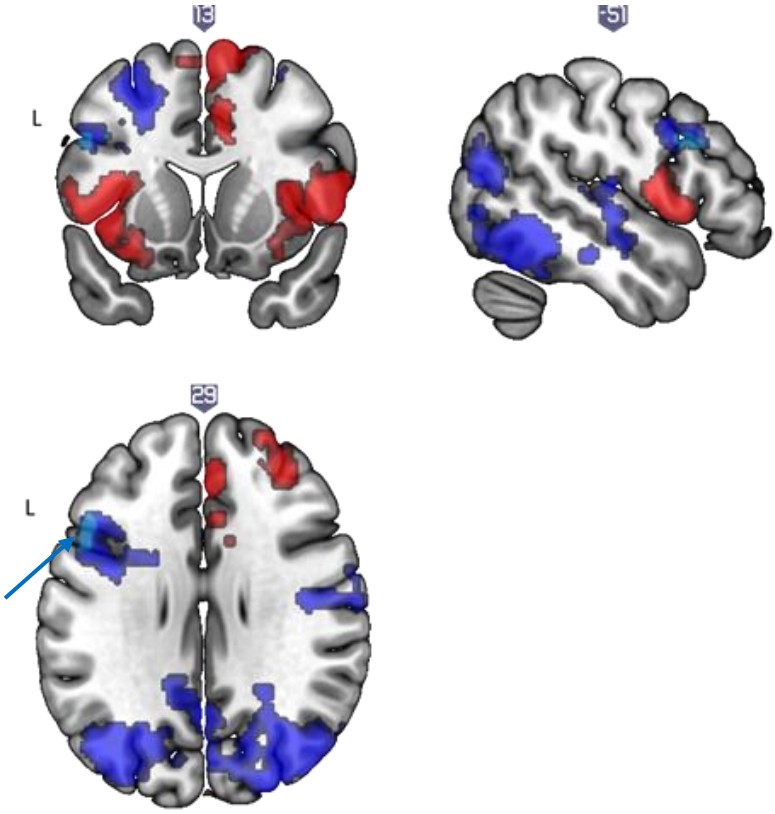


FIGURE 19 PATTERN OF ACTIVATION IN THE CONTINUATION PHASE. AREAS THAT SHOWED INCREASED ACTIVATION DURING DANGEROUS COMPARED TO SAFE CS+ TRIALS ARE SHOWN IN RED, INCLUDING BILATERAL INSULA AND ACC, AS WELL AS RIGHT VLPFC, AREAS THAT WERE MORE ACTIVATED IN SAFE THAN DANGEROUS CS+ TRIALS ARE SHOWN IN BLUE, INCLUDING BILATERAL IFG, AND MFG, ITG, AND SPC. THE IFG CLUSTER RESULTING FROM THE ROI ANALYSIS IS SHOWN IN LIGHT BLUE (ARROW).

TABLE 14. PEAK ACTIVATION IN THE MAIN CONTRASTS OF INTEREST (CS+ > CS- DURING CONDITIONING; CS+SAFE > CS+DANG, CS+DANG > CS+SAFE DURING LSCCE AND CONTINUATION. ALL CLUSTERS SURVIVED CLUSTER BASED THRESHOLDING AT 2.3. PEAK COORDINATES ARE PRESENTED IN MNI SPACE.

Contrast	Group	Anatomical area	Hemisphere	Cluster Size (mm^3)	X	Y	Z
Conditioning							
CS- > CS+							
	Whole Group	lateral occipital into precuneus, cuneus, fusiform, postcentral gyrus, lingual gyrus, hippocampus, dorsal amygdala	bilateral	248808	-46	-70	32
		vmPFC, ACC	bilateral	56600	8	56	4
		middle temporal gyrus into superior temporal gyrus, into precentral gyrus	right	4800	54	-8	-16
LSCCE							
CS+Safe > CS+DangNS							
	Whole Group	superior lateral occipital cortex into posterior cingulate into precuneus	bilateral	37920	44	-72	30
		superior frontal gyrus into middle frontal gyrus and inferior frontal gyrus	right	18576	28	24	56
		superior lateral occipital cortex	left	14200	-48	-72	28
		middle frontal gyrus into superior frontal gyrus	left	12760	-30	18	58
		cerebellum	right	10848	8	-86	-22
		inferior temporal gyrus into middle temporal gyrus into fusiform gyrus	left	9672	-52	-58	-12
		vmPC into ACC	right	8576	4	54	0
		middle temporal gyrus	right	4544	58	-52	-10
		postcentral gyrus	right	4424	8	-44	76
		parahippocampal gyrus, hippocampus, amygdala	right	3800	26	-32	-16
		cerebellum	left	3448	42	-68	-36
		amygdala into hippocampus	left	3296	-24	-2	-20
	ROI LSCCE	IFG	left	648	-44	26	20
	LowAnx > HighAnx	Putamen, caudate nucleus	right	2872	18	12	2

CS+DangNS > CS+Safe							
	Whole Group	parietal operculum	left	5912	-56	-28	20
		precentral gyrus	left	3896	-40	-14	56
		supplementary motor cortex	right	3616	6	-4	68
		precentral gyrus	right	3472	52	-2	52
		central opercular cortex, precentral gyrus into insula	left	3384	-52	-4	6
		insula into precentral gyrus	right	3152	44	4	0
Continuation							
CS+safe > CS+dang	*Whole Group*						
		lateral occipital cortex, into right premotor cortex, left hippocampus, amygdala	bilateral	152008	24	-84	44
		middle frontal gyrus into inferior frontal gyrus and superior frontal gyrus	left	6096	-26	12	46
		vmPFC	medial/bilateral	4456	-4	40	-12
		middle frontal gyrus into precentral gyrus	left	4344	-46	10	32
	ROI Extinction	IFG	left	736	-48	8	32
CS+dang > CS+safe							
		superior frontal gyrus into dACC	bilateral	16736	-10	2	70
		Insula	right	13784	32	22	-4
		Insula	left	11560	-28	20	8
		dorsolateral prefrontal cortex	right	3208	34	42	28

fMRI Results – Group Differences

Wholebrain group differences were found in the CS+Safe > CS+Dang contrast in the right dorsal putamen and ventral caudate. Further investigation of this effect revealed that low anxiety participants showed higher activation in this cluster during safe (mean = 0.105) than dangerous (mean = 0.04) trials, whereas high anxiety participants showed more activation during dangerous (mean = 0.24) compared to safe (mean = 0.17) trials (see figure 19).

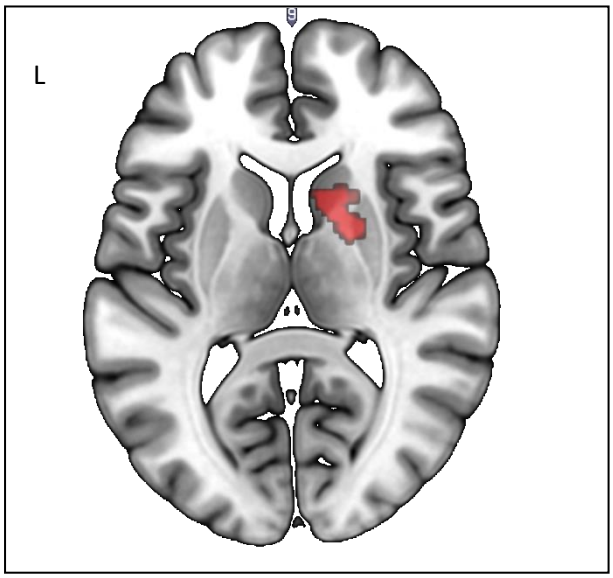


FIGURE 20. AXIAL VIEW OF THE CLUSTER IN RIGHT DORSOMEDIAL STRIATUM THAT SHOWED DIFFERENTIAL ACTIVATION IN PARTICIPANTS HIGH AND LOW IN ANXIETY.

To investigate whether there were any group differences that did not survive voxel-wise thresholding in vmPFC, bilateral insula and bilateral IFG, we extracted the % signal change from these clusters. We carried out paired t-tests, separately for the two groups and found that while low anxious participants showed a marginally significant difference between safe and dangerous CS+ in bilateral insula and bilateral IFG, high anxious participants showed significant differences in these clusters (safe > dangerous in bilateral IFG; dangerous > safe in bilateral insula). In contrast, low anxious participants showed greater deactivation during dangerous compared to safe CS+trials in vmPFC, whereas high anxious participants did not show a difference here.

TABLE 15. GROUP DIFFERENCES IN EXTRACTED ACTIVATION DATA (% SIGNAL CHANGE).* SIGNIFICANT AT P<0.05

	CS+safe Mean	CS+dang Mean	t(19)	p
a)				
Left Amygdala				
Low Anxiety	0.02	-0.05	2.17	0.04*
High Anxiety	0.11	-0.007	3.62	0.002*
Right Amygdala				
Low Anxiety	0.0003	-0.09	2.81	0.01*
High Anxiety	0.03	-0.06	2.24	0.04*
Left Hippocampus				
Low Anxiety	-0.05	-0.09	1.8	0.09
High Anxiety	0.07	-0.27	2.92	0.009*
Right Hippocampus				
Low Anxiety	-0.08	-0.16	2.29	0.03*
High Anxiety	-0.03	-0.12	3.37	0.003*
b)				
Right Insula				
Low Anxiety	0.07	0.14	-1.84	0.08
High Anxiety	0.17	0.34	-3.48	0.003*
Left Insula				
Low Anxiety	0.07	0.15	-1.87	0.08
High Anxiety	0.22	0.32	-3.09	0.006*
vmPFC				
Low Anxiety	-0.34	-0.5	3.18	0.005*
High Anxiety	-0.3	-0.33	0.69	0.5
Left IFG				
Low Anxiety	0.12	0.05	1.93	0.07
High Anxiety	0.26	0.17	2.29	0.03*
Right IFG				
Low Anxiety	0.13	0.02	1.95	0.07
High Anxiety	0.15	0.01	2.24	0.04*

MEDIATION ANALYSIS

To establish whether this data provided any evidence for an indirect influence of IFG on the insula clusters via the vmPFC, we planned to do a mediation analysis. We initially calculated difference scores between safe and dangerous CS+ trials for each cluster. We then ran correlations between the cluster scores and found no significant correlations. Therefore, we did not conduct the mediation analysis.

DTI RESULTS

No differences in structural connectivity as indexed by FA were found between groups on the wholebrain level or with an ROI analysis of the uncinate fasciculus.

9.5 Study 3 - Discussion

This study aimed to test whether there were any differences in physiological responding during the LSCCE task between participant groups with high and low scores on the trait version of the STAI (Spielberger et al., 1970). This version of the LSCCE task involves conditioning participants to expect an electric shock during the presentation of one of two letters (B and T). In a second phase, they see the letters again, this time embedded in words belonging to two distinct categories (plants or animals), one of which signalled a safe trial. Thus, during each trial, participants had to assess the word category, remember the safety contingencies, and apply this knowledge to decide whether the trial is safe or dangerous. We collected SCR and BOLD fMRI data while participants completed this task.

SCR results

Overall we found that participants showed higher SCR's to CS+ compared to CS- trials, thus, we can conclude that the conditioning worked in the way we expected.

In the LSCCE phase we expected participants to show increased SCR's in response to dangerous CS+ trials compared to safe CS+ trials. However, this is not what we found. Instead, the means showed the opposite direction in this case. One possible explanation of this is the quality of the data which was very problematic in this study and we had to complete several filtering steps to reduce scanner noise. If noise is, indeed, to blame for this result, then it is important to note that the result in the conditioning and continuation phases also need to be treated with caution.

In the continuation phase we found no difference in SCR between safe and dangerous CS+ trials. If there had been a CS+dang > CS+safe effect in the LSCCE phase, we would have expected this difference to still be apparent in the early trials of the extinction phase, however, this was not the case. We did find a distinction between CS+ and CS- trials, driven by the low anxious group suggesting that the overall conditioned effect was maintained in this group but not by the high anxious group. While the high anxious group showed increased responses compared to the low anxious group in conditioning, this was not the case in the continuation phase. The fact that high anxious participants did not show a difference between different trial types in the continuation phase is consistent with the suggestion that anxiety can be associated with fear generalisation. However, high anxious participants did not show increased responses compared to low anxious participants overall in this case. Therefore this result is not wholly in keeping with previous studies on anxiety that report generally increased responses during fear generalisation in high anxiety participants (Dymond et al., 2015).

In addition, participants showed increased SCR's in late trials. Because they were specifically told that contingencies would not change throughout the experiment, it is possible that this reflected an

increasing expectancy of an electric shock the more time passed since their last experience of an electric shock in the LSCCE phase. Looking at the means only, it could be suggested that this late > early difference is driven by responses to late CS+safe trials, so a tentative explanation could be that participants expected a reversal of the contingencies. However, we did not ask participants about their experience of this phase, so this is purely speculative. Future versions of this task may consider including self-report measures of participants' subjective experience at different points throughout the task to help interpret unexpected results such as this. Overall, these SCR results are not consistent with our hypotheses and, due to the quality of the data, need to be treated with caution. Future experiments using SCR as an indicator of affect in this task are needed to assess whether a LSCCE task does reliably induce the pattern of responses that we expected originally.

fMRI results

We expected a pattern of responses similar to those found in study 2 (see chapter 5), i.e. that the insula and dorsal ACC (dACC) would show increased activation during CS+ compared to CS- trials in conditioning, and to CS+dang compared to CS+safe trials in LSCCE, and that a network including lateral prefrontal areas would show the opposite effect, i.e. increased activation during safe compared to dangerous CS+ trials.

We did find increased insula activation during CS+ compared to CS-, and increased insula as well as dACC during CS+dang compared to CS+safe trials, supporting the idea that these areas are involved in processing the anticipation of an electric shock during these types of trials. This has previously been shown in conditioning studies (Büchel et al., 1998; Critchley, Mathias, Dolan, et al., 2002) as well as studies involving painful stimuli (Legrain et al., 2011; Seeley et al., 2007) and may be related to processing the affective component of the expected stimulation.

The fact that the activation in bilateral insula and dACC during CS+ trials is consistent with that found in other conditioning studies, together with the fact that SCR was increased during CS+ compared to CS- trials, suggests that participants successfully learned the CS+ - US relationship and acquired a CR.

In addition to the bilateral insula and dACC, we found parietal and somatosensory motor areas activated in this contrast, possibly involved in the anticipation of the somatosensory component of the electric shock and the preparation or inhibition of a motor response (Peyron, Laurent, & García-Larrea, 2000). In contrast to our results from study 2, we did not find rostral ACC activation in this contrast. This has previously been associated with conscious threat appraisal (Kalisch & Gerlicher, 2014; Mechias et al., 2010), and conscious appraisal of the stimulus contingencies is necessary to evaluate threat or safety in this paradigm, thus, the lack of activation in this area is unexpected. However, compared to the instructed conditioning studies that gave rise to the idea that conscious threat appraisal involves the rostral ACC, participants in this study were required to cognitively

evaluate the stimuli to determine danger or safety during each trial. Thus, if the activation in rostral ACC is related to the recall of instructions and the processing of threat in relation to those instructions, then it is not necessarily surprising that the cognitive evaluation of a stimulus according to experience may involve a different neural network.

In the opposite contrast (CS+safe > CS+dang) we found increased activation in right SFG, MFG, and IFG during safe than dangerous CS+ trials. These areas have consistently been reported in studies of instructed emotion regulation (Buhle et al., 2014; Kohn et al., 2014), suggesting that the mechanisms contributing to the LSCCE process are extended compared to those involved in extinction. As in study 2 we found activation during this contrast in inferior temporal gyrus, associated with language processing (Ferstl et al., 2008). This lends support to the idea that this area is involved in the processing and analysis of the safe stimulus.

A region of interest analysis also revealed that the left IFG cluster that was active in the CS+safe > CS+dang contrast in study 2 (see chapter 5), was also differentially active in this study. These dlPFC regions found in this contrast have previously been associated with working memory, and cognitive control (Ridderinkhof, van den Wildenberg, Segalowitz, & Carter, 2004) as well as instructed emotion regulation (Buhle et al., 2014; Kohn et al., 2014), all of which require the maintenance of the learned task contingencies and the inhibition of affective and behavioural components of the response. The left IFG cluster has been associated with sematic decision making in particular (Nee et al., 2013). This supports the idea that this area is involved in the processing of the word categories in this study, and that additional activation in this area during safe CS+ trials (compared to the dangerous category), may reflect the application of the knowledge about the meaning of the safe category to facilitate an inhibition of the CR. It is possible that the semantic information processed in IFG and temporal areas as well as the risk information signalled by the insula is combined in vmPFC. We found greater activation in vmPFC during safe compared to dangerous CS+ trials which is consistent with both conditioning (Fullana et al., 2015) and instructed emotion regulation studies (Buhle et al., 2014; Kohn et al., 2014). It has been suggested that vmPFC is involved in integrating cognitive information from lateral prefrontal areas and the application of updated knowledge about stimulus contingencies to change a previously appropriate response (Roy et al., 2012). Lateral brain areas may influence the activation of the subcortical emotion processing areas via the vmPFC (Johnstone et al., 2007; Pitskel et al., 2011; Urry et al., 2006). Although this suggestion is compatible with the network of brain activation we found in safe compared to dangerous CS+ trials, it could not be confirmed with a mediation analysis in this study.

One puzzling finding was the increased activation of amygdala and hippocampus during safe compared to dangerous CS+ trials. Further examination of the pattern of this activation revealed

that this finding was driven by increased amygdala activation during safe CS+ trials, particularly in the high anxiety group. The amygdala has been shown to be involved in the processing of ambiguous information (Kim et al., 2003). In addition, it has been shown that anxiety is related to intolerance of uncertainty (Dugas, 2001) and intolerance of uncertainty, in turn has been shown to affect safety processing during extinction learning (Morriss, Christakou, & van Reekum, 2015). In the LSCCE task, although participants learned that the CS+ is associated with the risk of an electric shock in the conditioning phase, the addition of new contingencies and the conflicting information of a dangerous CS+ but a safe category word may have induced a feeling of uncertainty, which might be greater in the high anxious participant group.

Overall, the pattern of activation we found in the safe compared to dangerous CS+ contrast is consistent with those reported in studies of instructed emotion regulation (Buhle et al., 2014; Ochsner et al., 2012), suggesting that the general mechanisms engaged in this LSCCE task are similar to those involved in instructed emotion regulation in that relevant cognitive information is processed in a network of lateral cortical brain regions and applied to reduce an affective response.

Interestingly, the CS+safe > CS+dang contrast revealed more extensive activation that included vmPFC, posterior cingulate, and precuneus. Further investigation showed that these regions were all deactivated (relative to baseline) in both CS+safe and CS+dang conditions, but significantly more so in the CS+dang condition. These regions make up part of the default mode network, a network of brain regions that is typically found to be activated while participants are at rest (Greicius, Supekar, Menon, & Dougherty, 2009). When task demands rise, this network tends to get deactivated (Singh & Fawcett, 2008). The pattern of activation we found in this network is consistent with that idea. The areas associated with the default mode network showed the greatest decrease during dangerous CS+ trials, i.e. when participants were aware that they might receive an electric shock. Thus, rather than a widespread network of brain areas involved in processing the safe stimulus, this widespread area of activation reflects the fact that the dangerous CS+ was the type of trial that has the greatest potential significance for participants. In addition, during CS+dang trials, the insula activation we found overlaps with a network of brain regions that has been labelled the salience network (Menon, 2015) that also includes the dorsal ACC. This further supports the idea that the deactivation in default mode network areas may be related to increased salience of and attention to the stimulus and its potential outcome during dangerous CS+ trials.

The only area that showed differential activation between the low and high anxiety groups on a wholebrain level was a cluster stretching between right dorsal putamen and ventral caudate. Grahn et al. (2008) argue that the caudate and putamen are part of the ventral striatum and the limbic

cortico-striatal loop which is involved in the interplay between cortical regions and limbic regions to affect goal-directed behaviour through learning of action-outcome contingencies. The cluster we found spans both a ventral portion of the caudate and a dorsal/medial portion of the putamen. While the ventral caudate has been related to affective processing, the dorsal putamen is associated with cognitive processes. The dorsomedial striatum as a whole, which both regions can be seen as part of, has been implicated in flexible behaviour and switching when task contingencies change (Grahn, Parkinson, & Owen, 2008). In this study, low anxiety participants showed higher activation in this area in response to safe compared to dangerous CS+. In contrast, high anxious participants showed the opposite pattern of responses and a reduced difference between safe and dangerous CS+. Taken together, this may reflect reduced flexibility in the responses in high anxious participants while low anxious participants are able to flexibly update the contingency information.

Although we did not find any further group differences on a wholebrain level, we did investigate the activation in bilateral insula and IFG as well as vmPFC separately for the two groups. In the bilateral insula, the high anxious group showed greater discrimination between safe and dangerous CS+ than the low anxious group. In light of research showing that anxiety is associated with hyper-activation in areas involved in emotion processing (Graham & Milad, 2011), this could be seen as greater sensitivity to potential threat in the high anxious group. Furthermore, high anxious participants also showed greater discrimination between safe and dangerous stimuli in the left IFG cluster which is thought to be involved in the evaluation of the word stimulus. It has been shown that anxiety is associated not with reduced attempts to regulate negative emotion, but ineffective emotion regulation (Campbell-Sills et al., 2006; Gross & John, 2003). Similarly, in patients with major depression, cognitive reappraisal of emotion is associated not with reduced activation in areas involved in this process but with ineffective processing in these areas (Johnstone et al., 2007). It is, therefore, possible that safety processing involved greater effort in high anxious participants than it did in low anxious participants, illustrated by greater discrimination between safe and dangerous trials both in bilateral insula and in left IFG.

In contrast, while the low anxious group showed significantly greater deactivation in vmPFC during dangerous compared to safe CS+ trials, high anxious participants did not show this difference. This cluster is part of the default mode network which typically shows greater deactivation when task demands rise. However, in high anxious participants, this brain area did not discriminate between safe and dangerous stimuli. Taken together, high anxious participants show reduced activation in the dorsomedial striatum, possibly reflecting reduced flexibility with changing task demands. They also show increased discrimination between safe and dangerous CS+ trials both in bilateral insula and left IFG, which is consistent with the idea that anxiety is linked to greater sensitivity to potentially

threatening situations, and that safety processing is ineffective. Finally, high anxious participants do not show differential activation to safe and dangerous CS+ in the vmPFC where low anxious participants do. The vmPFC has been linked to the integration of information from lateral brain areas to influence subcortical emotion processing areas during cognitive reappraisal (Johnstone et al., 2007; Urry et al., 2006). In addition, in patients with an anxiety disorder, the vmPFC has been found to show reduced discrimination between CS+ and CS- trials during extinction (Bremner et al., 2005). This suggests a key role for this brain area in the processing of safety signals.

In the continuation phase, we found that the pattern of activation in the CS+safe > CS+dang contrast largely overlapped with that found in the LSCCE phase of the study but the size of the network was reduced. In addition, the vmPFC activation is shifted ventrally, and part of the left IFG cluster that was further investigated in the ROI analysis now survived thresholding. In contrast, the anterior part of the right SFG-MFG-IFG cluster now does not survive thresholding.
In the CS+dang > CS+safe contrast we found a similar pattern of results to the LSCCE phase – the bilateral insula/operculum activation is situated more anterior and lateral compared to that found in the LSCCE phase, while the precentral cortex part of those clusters is maintained. In addition we found dorsal ACC activation during dangerous CS+ trials in the continuation phase which we did not find in the LSCCE phase. This suggests that the processing of the safe-dangerous contingencies is largely maintained throughout the continuation phase when participants are explicitly told that these would not change throughout. In future studies it would be interesting to investigate how this processing changes with different instructions, e.g. informing participants that no more electric shocks will be given during the final phase, or switching the contingencies with or without informing participants. This would provide further evidence about flexible responding as well as safety learning in healthy, sub-clinical, or clinical populations.

In this study we did not find structural connectivity differences, as indexed by FA, between groups on a wholebrain level, or in the UF. FA in the UF has previously been found to be correlated with trait anxiety (Kim & Whalen, 2009; Modi et al., 2013) so this finding is somewhat surprising. However, we did not identify the UF on the basis of individual participants' data and instead used an atlas ROI which may have resulted in unreliable localisation of the tract on an individual basis. If there is individual variation in the exact anatomy of the UF, then tract localisation should be carried out on the single subject level in future (Kim & Whalen, 2009; Modi et al., 2013).

In summary, during conditioning we found increased SCR as well as increased activation in the insula during CS+ compared to CS- trials. In addition, we found these differences in neural activation between safe and dangerous categories only in trials including a CS+ and not in trials including a CS-,

which implies that the CR to dangerous CS+ trials was a result of both letter and category information. This suggests that the conditioning procedure resulted in a reliable CR which was maintained during dangerous CS+ trials. In the LSCCE phase we found no support for increased SCR's during dangerous compared to safe trials. However, the pattern of brain activation shows increased insula and dorsal ACC during dangerous compared to safe CS+ trials, replicating the results from study 2. In addition, during safe compared to dangerous CS+ trials we found activation in a network of brain areas associated with language processing and semantic decision making and working memory, specifically, in right and left MFG and IFG as well as inferior temporal gyrus. We also found greater activation in vmPFC, an area associated with the integration of information from a wide variety of cortical areas which affects behavioural responses via subcortical structures. This pattern of activation is largely consistent with that found in emotion regulation studies, suggesting that the interplay between cortical and sub-cortical regions in the LCSSE task may be similar to that found in instructed emotion regulation. In addition, the vmPFC, posterior cingulate, and precuneus activation in safe compared to dangerous CS+ trials was driven by greater deactivation during dangerous CS+ trials. These areas are part of the default mode network which shows activation during rest and deactivation when task demands rise. Thus, the increased deactivation here is likely to reflect the increased potential importance of the dangerous CS+ trials (i.e. the awareness that an electric shock may be received during this condition).

On a wholebrain level, we found that high anxiety participants showed no discrimination between safe and dangerous CS+ trials in the dorsomedial striatum, where low anxiety participants showed increased responses to safe compared to dangerous CS+ trials. This area has been associated with response switching and the application of changing contingencies. Thus, it is possible that this reflects perseverative tendencies in high anxiety participants, although this cannot be confirmed by activation in other areas. High anxious participants also showed no differential responses to safe and dangerous CS+ trials in vmPFC suggesting altered safety processing in this group.

One puzzling result was significantly increased amygdala activation during safe compared to dangerous CS+ trials, driven by high anxiety participants. It is possible that this can be explained by the fact that safe CS+ trials included conflicting information (dangerous CS but safe word category) which may have induced an added level of uncertainty. High anxiety participants showed a greater difference in this area and did report increased intolerance of uncertainty in this study so this amygdala activation may be indicative of increased arousal due to uncertainty.

CONCLUSION

Taken together, the results from this study support the idea that specific, simple cognitive manipulation tasks can help inform research about specific mechanisms involved in emotion

regulation, and that sub-clinical populations show patterns of responses that differ from those of healthy participants. Future studies should consider designing versions of this task that aim to specifically involve mechanisms that are thought to be altered in particular patient groups. This will further increase our understanding of emotion regulation difficulties in different affective disorders.

10. General Discussion

In this project we developed a paradigm designed to investigate basic processes involved in emotion regulation. Being unable to effectively regulate emotion has been implicated in a large number of mental health disorders (Amstadter, 2008), and treatment for mental health disorders, for example anxiety disorders, utilises training in emotion regulation techniques ("Generalised anxiety disorder and panic disorder in adults: management (CG113)," 2011). However, little is known about the underlying mechanisms that contribute to instructed emotion regulation. Studies investigating instructed emotion regulation are based on appraisal theories that propose that emotions are determined not by the absolute meaning of a specific event but by the individual's interpretation of the event and of any physiological responses to that event (Frijda, 1986; Oatley & Johnson-laird, 1987; Scherer, 1993). Thus, many processes contribute to the generation of emotion, i.e. processing of all available aspects of the situation, recall of similar and/or contrasting experiences, outcome prediction, processing of any physiological responses, to arrive at an appraisal. According to appraisal theorists, the very processes that give rise to an emotion are also those that can change it at any point in its generation. The change of an emotion through cognitive mechanisms, e.g. the reinterpretation of a situation according to new information, is what is referred to as cognitive reappraisal. However, although several mechanisms have been suggested that may contribute to emotion generation and regulation through appraisal, a specific understanding of these mechanisms is lacking. On a neural level it has been suggested that a wide network of brain regions is involved in the generation and reappraisal of emotion. Studies have found that the processing of negative emotion in particular involves brain areas such as the amygdala and the insula while cognitive reappraisal involves a wide network of cortical brain regions including dorso- and ventrolateral prefrontal cortex which are thought to influence the experience and expression of emotion via the ventromedial prefrontal cortex (Buhle et al., 2014; Kohn et al., 2014). Although the neural network involved in cognitive reappraisal is reliably found across experimental studies, the complexity of both the underlying processes and the tasks typically employed to investigate their neural correlates make the specific role/s of each brain region difficult to ascertain.

It has been suggested that emotion regulation is an extension of the extinction of conditioned responses, and utilises some of the same mechanisms (Quirk & Beer, 2006). Paradigms that extend classical extinction, for example by adding safety cues to conditioned stimuli as in conditioned inhibition and learned safety paradigms (Kong et al., 2014; Pollak et al., 2010; Rescorla, 1969) show that an initially conditioned response (CR) can be modified through the addition of a safety cue, resulting in the reduction of the CR. This reduction suggests that the meaning of the conditioned

stimulus (CS+) is altered under these circumstances, and thus that similar mechanisms may be at work in extinction and more deliberate emotion regulation. In addition, when the emotion eliciting stimulus is as simple as a coloured square given an affective meaning by instructed conditioning, researchers have found that individuals are able to regulate their affective responses in the same way that they are able to in emotion regulation tasks that use images to elicit affective responses, by applying cognitive reappraisal strategies (Delgado et al., 2008). On a neural level, conditioning and extinction have been found to involve a network including the amygdala, insula and dorsal ACC during the acquisition of a CR. The vmPFC has been implicated in extinction learning, serving to integrate old and new information and update CS-US contingencies to inhibit the CR (Sehlmeyer et al., 2009). Learned safety paradigms as well as the regulation of conditioned responses extend this neural circuit into dorsolateral prefrontal cortex which is thought to be involved in higher level cognitive processes such as the evaluation of the situation (Delgado et al., 2008; Kong et al., 2014; Pollak et al., 2010b). This project attempted to extend these findings by using conditioned stimuli to elicit an affective response, and safety cues to a proportion of CS+ trials. However, instead of using an additional conditioned safe stimulus as the safety cue, we used information which needed to be cognitively evaluated itself to allow participants to determine whether each trial was safe or dangerous. Specifically, we used letters as CS', and words belonging to two distinct categories as additional information. For each trial, the word category reflected safety or danger.

To this end we conducted three separate studies. The first study investigated the physiological correlates of the Learned Safety Contingent on Cognitive Evaluation task and as an opportunity to improve its initial design. In the second study we investigated the neural correlates of the task and compared them to the neural correlates of an instructed emotion regulation task. Finally we conducted the same study recruiting groups of participants that scored high and low on the trait version of the state-trait anxiety inventory (STAI, Spielberger, Gorsuch, & Lushene, 1970) to explore the effect of trait anxiety on the physiological and neural correlates of the LSCCE task.

In the following, I will summarise each of the experimental chapters before I compare the overall project with extinction and emotion regulation studies, and finally move on to the broader implications and remaining questions arising from this project.

10.1 Review Study 1 and Pilot

The first study of this project aimed to test the initial version of our task using skin conductance as an index of affective responses.

Participants were informed that one of the two letters they would see was associated with an aversive white noise burst during the conditioning phase. Participants were divided into two groups for the next phase – one received the LSCCE condition, whereas the other group received an

extinction condition. Both groups saw the same letters as in the conditioning phase but embedded in words. The LSCCE group were informed which of the two word categories (birds or vegetables) was safe and that the other category, in combination with the CS+, still contained a risk of the white noise occurring. The extinction group were given no further instructions on danger or safety during the second phase. We hypothesised that participants in the LSCCE group would show decreased SCR's during safe compared to dangerous trials, and that participants in the extinction group would show increased SCR's during CS+ compared to CS- trials in the early trials of the extinction phase, and a reduced or no difference during later trials.

The decision to use a 100% reinforcement schedule was made to ensure that participants were consciously aware of the contingencies of the paradigm, however, this meant that we could not assess whether conditioning occurred during this phase. Nonetheless, the results from the LSCCE group suggested that it did: We found that participants showed stronger SCR's in response to dangerous than safe CS+, and responses to safe CS+ were stronger than to CS-. This result implies both that conditioning occurred initially, and that CR's can be reduced via the simple manipulation of additional information provided with CS+. In the extinction group we found no difference between SCR's to CS+ and CS- in the early or late trials. It is possible that this is due both to the fact that the stimuli in the extinction phase were perceptually different from those in the conditioning phase, as well as the lack of additional instructions during this phase, leading to rapidly reduced SCR's. Overall, the results of this first study with a new paradigm pointed in the direction we expected, thus, the next step was to adjust and pilot the paradigm before continuing with an fMRI study to examine the neural correlates of this task.

We made a number of changes to this paradigm before we ran a behavioural pilot for the fMRI study, again, using SCR as the dependent measure. We increased the number of trials overall to increase power. We also reduced the reinforcement schedule during the conditioning phase to 50% since reduced reinforcement schedules have been shown to be effective in classical conditioning (Büchel et al., 1998; Phelps et al., 2004) and this allowed us to assess whether a conditioning effect occurred initially. The design was changed to a within group design, with non-word trials interspersed throughout the LSCCE phase to investigate how the safe-dangerous contingencies in the CS+ would affect trials with no additional information.

We ran the pilot on 5 participants and found the expected pattern of results: In the conditioning phase, participants showed higher SCR's in response to (non-reinforced) CS+ than CS- trials. In the LSCCE phase, mean responses were higher for dangerous than safe CS+ trials, with a reduced difference between dangerous and safe CS- trials, both associated with lower SCR's than dangerous CS+ trials. Thus, we felt this improved design was appropriate for use in an fMRI study.

10.2 Review Study 2

The main aims of this study were twofold: 1) We wanted to extend our data to include fMRI and 2) compare the results to those of a more standard instructed emotion regulation task. The results from study 1 as well as the pilot study indicated that a reduction of the CR could take place through cognitive evaluation, however, investigating the neural circuits involved in the LSCCE task and a more typical instructed emotion regulation task may start to answer the question whether the same, or similar mechanisms are involved in both processes. To be able to compare the two paradigms we shortened the standard emotion regulation paradigm to make it more comparable to our LSCCE paradigm. During this study we collected SCR and pupil dilation as well as BOLD fMRI. This enabled us to assess which physiological measure was an additional useful indicator of affective responses for these tasks.

Although we could not show a conditioning effect at the end of the conditioning phase or in the baseline trials of the LSCCE phase, the mean SCR showed the expected direction in both phases, suggesting that conditioning did have the desired effect of enabling participants to distinguish between CS+ and CS- and producing a CR. In the LSCCE phase we found that SCR distinguished between safe and dangerous CS+ trials, showing increased responses during dangerous compared to safe CS+ and CS- trials. In contrast, we found no difference between safe and dangerous CS- trials. This suggests that SCR reflects affect – in the CS+ trials only, the word provided information about shock probability, and SCR was high when a 50% probability of electric shock was present. It was lower in all conditions where this was not the case.

Pupil diameter on the other hand, distinguished between CS+ and CS- trials but not between safe and dangerous CS+ trials. We concluded that pupil diameter was, therefore, a measure of cognitive effort rather than affect since participants needed to figure out the word category for both levels if the CS+, whereas in the CS- they did not have to take the word into consideration, consistent with Urry et al. (2009) who found a similar pattern when directly comparing SCR and pupil diameter during a study of instructed cognitive reappraisal.

Our fMRI results suggested that processing of the threat of shock involved the insula. This ties in with research suggesting that the insula is part of a salience network which has been linked to the processing of pain and pain expectancy, the processing of aversive CS+ in conditioning studies and the processing of negative images in studies of emotion regulation (Büchel et al., 1998; Buhle et al., 2014; Critchley, Mathias, Dolan, et al., 2002; Kohn et al., 2014; Legrain et al., 2011; Seeley et al., 2007) In addition, we found a cluster in right dorsal anterior cingulate, during dangerous CS+ trials. Activity in this area has been associated with threat processing (Mechias et al., 2010). We also found activation in the insula during CS+ (compared to CS-) trials during conditioning, however, this

activation was not as consistent. In study 2 in particular, this activation was below the z= 2.3 threshold. We concluded that this was most likely due to the sample size in this study. During safe CS+ trials we found activation in left IFG as well as dmPFC, temporal and parietal areas, suggesting that these areas were involved in processing safety and regulating the CR. Left IFG as well as inferior temporal gyrus have been linked to language processing and semantic decision making (Ferstl et al., 2008; Nee et al., 2013), suggesting that in this semantically based task, these areas were involved in processing the safe word category. This network of brain areas is similar but more defined than those found in instructed emotion regulation studies (Ochsner et al., 2012), but does not include the vmPFC and ACC areas previously associated with extinction learning and emotion regulation (Diekhof et al., 2011), consistent with another meta-analysis of emotion regulation that did not find consistent activation in the vmPFC (Buhle et al., 2014).

Our version of the instructed emotion regulation task did not produce the SCR effects that we expected – participants showed no differential responses between negative and neutral attended trials, and the difference between negative trials in which the initial emotional response was maintained, and those in which the response was decreased was only marginal.
The neural pattern of responses, however, was comparable with that described by Buhle et al. (2014) with the amygdala showing increased activation during negative compared to neutral, and negative attend compared to negative decrease trials; and vlPFC, dlPFC and posterior parietal regions being more active when participants decreased compared to attended negative images.

A comparison between the LSCCE and instructed emotion regulation task showed that the left PFC clusters associated with safe CS+ trials and trials in which participants decreased their initial response to negative images did not overlap, but were directly adjacent. In addition, the cluster found in the LSCCE task was also active in both the negative attend and negative decrease conditions in the instructed emotion regulation task. This suggests that general evaluative processes that are necessary for the appraisal and reappraisal of complex images (that the IFG cluster may be involved in) are masked during this task because they are involved in both Negative Attend and Negative Decrease trials. Thus, it supports the idea that more tightly controlled paradigms such as our LSCCE paradigm can be a useful tool to more specifically investigate the underlying processes of complex emotion regulation.

Overall, we concluded that the network of brain areas we found in the CS+safe vs CS+dangerous contrast in the LSCCE task was consistent with but more focussed than that described in meta-analyses of instructed emotion regulation studies (Buhle et al., 2014; Kohn et al., 2014). Similarly, the altered version of the instructed emotion regulation task also replicated previous research using

longer versions of this paradigm (Kim et al., 2003; Ochsner et al., 2002; Urry et al., 2006). Finally, the fact that we could show activation in the left IFG cluster from the LSCCE task in the instructed emotion regulation task suggests that the mechanisms involved in the LSCCE task may indeed be also involved in the instructed emotion regulation task.

10.3 Review Study 3

The objective of study 3 was to test how trait anxiety affects physiological and neural responses during the LSCCE task. We recruited participants based on their scores on the trait version of the STAI (Spielberger et al., 1970), selecting those with high and low scores on this measure.

The LSCCE task was modified only slightly for this study. Instead of including non-word trials interspersed within the LSCCE phase we added a continuation phase at the end, during which the letters were still presented with words, but no further electrical shocks were delivered. The idea behind doing this was to investigate whether the pattern of responses, both physiological and neural would be maintained when the US was removed.

During the conditioning phase we found that CS+ trials were associated with higher SCR's than CS- trials as expected. In the LSCCE phase, we expected to find higher SCR's in response to dangerous compared to safe CS+ and CS- trials. We did not find this effect, in fact, overall, the mean SCR associated with dangerous CS+ were not higher than those associated with the other conditions. During the LSCCE continuation phase we expected to find a sustained difference between dangerous and safe CS+ and CS- trials at least during the early portion of this phase. We found, that overall CS+ were associated with higher SCR's than CS-, and late trials were associated with higher responses than early trials. Further, this effect was driven by the responses of the low anxious group, as the high anxious group did not show different responses during CS+ compared to CS- trials. It is possible that this due to an expectation that an electric shock would still be delivered during this phase, however, we did not explicitly asses this, thus, in combination with the general quality of the data, this result should be considered with caution.

On a neural level we largely replicated the results from study 2, finding that the insula was involved in processing the anticipation of the electric shock, and bilateral IFG and ITG were involved in the reduction of the CR in safe CS+ trials. In addition, we found greater deactivation in midline areas that are part of the default mode network during CS+ compared to CS- trials in the conditioning phase, and during dangerous compared to safe CS+ trials during the LSCCE phase.

One puzzling finding during this study was deactivation in amygdala and hippocampus during dangerous CS+ trials. We speculated that this may be due to the conflict introduced by presenting a previously dangerous CS+ together with information that signifies a safe trial, however, this needs further investigation.

The only group difference we found on a wholebrain level was a cluster in right dorsomedial striatum related to switching and reversal tasks (Grahn et al., 2008). Low anxiety participants showed increased activation here during safe compared to dangerous trials, whereas high anxiety participants showed a reduced difference and the opposite pattern. This may reflect reduced ability to respond flexibly to changing task conditions in high anxiety participants. This also ties in with results of a closer investigation of the patterns of activation in bilateral IFG, vmPFC, and bilateral insula. High anxious participants showed increased discrimination between safe and dangerous CS+ in bilateral insula and left IFG compared to low anxious participants, possibly reflecting general hyperreactivity to potentially threatening situations as well as increased effort during the processing of safety signals. In addition, while low anxious participants showed differential responses to safe and dangerous CS+ in vmPFC, high anxious participants did not show this difference, suggesting that the vmPFC may be crucial to the processing of safety information.

An analysis of fractional anisotropy of the UF did not reveal a difference between the two groups, thus, we could not find support for the idea that trait anxiety is related to connectivity in this area.

10.4 General discussion of the results of this project

SCR results

Although in studies 2 and 3 we did not consistently find the SCR results that we expected (i.e. increased SCR for CS+ compared to CS-, and to dangerous CS+ compared to safe CS+ trials), the overall pattern of results is consistent with the idea that SCR reflects the affective component of the LSCCR task. During the conditioning phase, even when the differences between responses to CS+ and CS- were not significant, CS+ trials resulted in higher SCR's than CS- trials.

We also found that SCR was consistently increased to CS+ compared to CS- trials either during baseline trials at the beginning of the LSCCE phase, or throughout the LSCCE phase. This indicates that the addition of words to the letter CS' did not affect the CR.

During the LSCCE phase of the task, we found that SCR distinguished between safe and dangerous CS+ trials in studies 1 and 2. This suggests that, as expected, that additional information about the CS can be cognitively evaluated and the CR reduced during safe trials. We did not find this effect in the LSCCE phase in study 3. Although this was surprising, we did experience issues with data quality in this study which we concluded were most likely responsible for this result. Considering the results of the previous studies we are confident that the underlying processes are consistently as expected even though we could not show them in this instance.

Thus, in summary, SCR reflected affective responses throughout. Results from the conditioning phase suggest that CR's were reliably evoked through the conditioning process.

In addition, despite inconsistent results, the overall results suggest that this physiological CR can be reduced via the cognitive evaluation of additional information in the LSCCE phase.

fMRI results

During studies 2 and 3 we consistently found activation in the insula associated with CS+ compared to CS-, as well as dangerous compared to safe CS+ trials. This is consistent with the idea that the insula is involved in the processing of salient stimuli, i.e. those that have potentially important consequences for the individual, such as the anticipation of an uncomfortable electric shock (Büchel et al., 1998; Critchley, Mathias, Dolan, et al., 2002; Legrain et al., 2011; Seeley et al., 2007). In addition to studies investigating responses associated with visceral stimuli, this area has also been reported in studies using images to evoke affective responses (Buhle et al., 2014; Kohn et al., 2014). This underlines its general role in salience processing as opposed to the processing of pain in particular, although in this project this salience happens to be related to the anticipation of a painful stimulus.

In addition, we found activation in dorsal ACC during CS+ compared to CS- and dangerous compared to safe CS+ trials (though this activation did not survive whole-brain thresholding in study 3). This area has been linked to the implicit processing of threat (Mechias et al., 2010), and has been shown to be correlated with SCR in conditioning studies (Milad et al., 2007). This area also has direct connections to the autonomic nervous system, suggesting that it might not only be correlated with, but also directly involved in the control of physiological responses.

During CS+ trials in conditioning as well as during dangerous CS+ trials in the LSCCE phase in study 3 we found increased deactivation in cortical midline areas belonging to the default mode network. This network tends to be activated when participants are at rest and is thought to be involved in autobiographical memory and other types of self-referential thought. Activation in this network is reduced when task demands rise (Greicius et al., 2009; Singh & Fawcett, 2008). We concluded that the deactivation in areas associated with the default mode network during dangerous CS+ trials reflects the increased task demands associated with threat processing and the expectation of an electric shock. The vmPFC cluster we found in safe compared to dangerous CS+ trials in study 3 is part of that network and showed the same pattern of activation (i.e. greater deactivation during safe than dangerous CS+ trials). The vmPFC is associated with the integration of information received from lateral cortical regions and the communication of this information to subcortical regions (Roy et al., 2012). It has also been shown to be involved in the cognitive reappraisal of emotion (Kohn et al., 2014), although a recent meta-analysis did not find this (Buhle et al., 2014) and the extinction of conditioned responses (Sehlmeyer et al., 2009). Although the role of the vmPFC in extinction is disputed, with some researchers finding that it is involved in extinction directly, while others suggest

that it is linked to retention of extinction (LaBar et al., 1998), its involvement in updating changing task contingencies is evident in reversal learning studies where it shows activation when a previously dangerous stimulus no longer predicts danger (Schiller et al., 2008). However, the activation shown in most studies is expressed only in terms of contrasts, thus, it is unclear whether this is driven by activation or deactivation against baseline. It is, therefore, difficult to conclude with certainty, how our result relates to previous studies. Evidently, however, the contrast result matches those of previous studies that find vmPFC activation when safety cues are added to previously conditioned stimuli (Pollak et al., 2010b) as well as when participants were asked to regulate their emotion to previously conditioned stimuli (Delgado et al., 2008). We did not find differential activation for safe and dangerous CS+ in vmPFC in study 2, which may be due to the smaller sample size in this study (20 compared to 40 participants overall).

In both study 2 and study 3 we found left ITG, related to language processing (Ferstl et al., 2008), and IFG, associated with semantic decision making (Nee et al., 2013). We concluded that the ITG was most likely involved in the analysis of the word content whereas the IFG may have had an evaluative role, i.e. applying previously learned knowledge about the meaning of the category in terms of safe or dangerous contingencies and communicating this knowledge to other brain areas involved in response generation and/or reduction (especially in light of the fact that this area also seemed to be involved in evaluative processes in the instructed emotion regulation task). Although these processes would be involved in both safe and dangerous trials, it is possible that the conflict resulting from the combination of a dangerous CS but a safe word resulted in the need for further cognitive processing (and thus increased activation) to change the response, whereas in the case of a dangerous CS and a dangerous word, the previously learned CR remains the appropriate response.

We did not find amygdala activation during CS+ during conditioning, or dangerous CS+ trials during LSCCE in either study. In fact, in study 3 we found increased amygdala activation in safe (compared to dangerous) CS+ trials. Both aspects were unexpected. The amygdala has been shown to be activated during fear conditioning (Sehlmeyer et al., 2009), including in studies using electric shock as a US (e.g. LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998), thus, we expected to find amygdala activation during trials associated with the risk electric shock. However, other studies exist that also do not find amygdala activation in a wholebrain analysis during conditioning and instead find amygdala activation during CS – compared to CS+ trials during extinction (Phelps et al., 2004), similar to our results. They also reported insula activation during CS+ during conditioning. This suggests that while the amygdala may be involved in conditioning and extinction, its activation may not only reflect the predictive value of the CS+. Instead it is possible that its role is more complex and also incorporates information about the safe CS- stimulus to facilitate adaptive responding to

changing environments (Lichtenberg et al., 2017).

In study 3 we investigated any differences in this circuit between participants high compared to those with low scores on the STAI. On a wholebrain level we found that low anxious participants showed a significant difference between safe and dangerous CS+ trials in an area in the dorsomedial caudate (safe > dangerous), while high anxious participants did not show this difference. The dorsomedial caudate is associated with flexible responding when task demands change (Grahn et al., 2008). In addition, further investigation of the bilateral insula and IFG, as well as the vmPFC activation revealed that high anxious participants showed an increased difference between responses to safe and dangerous CS+ trials in bilateral insula (CS+dang > CS+safe) and left IFG (CS+safe> CS+dang). We concluded that this pattern of responding may be related to greater reactivity to threat in the high anxious group reflected in the insula. The increased activation difference between safe and dangerous CS+ in left IFG may, then reflect attempts to reduce that response. This supports the idea that reduced ability to regulate emotion may not be due to patients not attempting to regulate, but rather that they are not effective at doing so (Gross & John, 2003). In contrast, high anxious participants did not show differential responding in the vmPFC where low anxious participants did (CS+safe > CS+dang). This brain area has been shown to be involved in the integration of information from lateral brain areas and facilitate a reduction in emotion in studies of cognitive reappraisal (e.g. Johnstone, van Reekum, Urry, Kalin, & Davidson, 2007; Urry, van Reekum, Johnstone, & Davidson, 2009), and participants with an anxiety disorder have shown reduced differentiation between CS+ and CS- during the extinction of conditioned responses (Bremner et al., 2005). This suggests that the vmPFC may have a key role in the processing of safety information.

All in all, the neural network we found involved in this task, and, in particular, the safe vs dangerous CS+ contingencies, overlaps with that found in fear extinction and extends it into areas specific to language processing and category evaluation. Similarly, the network of brain regions we reported also overlaps with studies of cognitive reappraisal of emotion. This often includes insula activation in response to negative images, and vmPFC during cognitive reappraisal. While the lateral PFC clusters we found in the LSCCE and the emotion regulation paradigm in study 2 did not overlap, the cluster associated with safe CS+ trials also showed activation during Negative Attend and Negative Decrease trials in the emotion regulation task. This supports the idea that brain areas involved in simple cognitive evaluative mechanisms may not be captured in complex emotion regulation tasks in which the task conditions are not as tightly controlled, and strengthens the idea that simple tasks such as the LSCCE tasks can be beneficial to capture these underlying mechanisms.

10.5 THE LSCCE NETWORK

To summarise the results found in this project, the following section will sketch a model describing the interactions of the brain regions we have found to be involved in the successful reduction of the CR in the LSCCE task.

In this version of the task, based on semantic decision making, threat of electric shock involved in the insula and dorsal anterior cingulate, areas that are part of the salience network and involved in the processing of stimuli that have potentially important consequences for the individual, including pain and its anticipation (Legrain et al., 2011). During safe trials this activation was reduced and instead we found a network of IFG, ITG, and vmPFC that showed increased activation. In the vmPFC in particular (as well as in other parts of the default mode network), this increase in activation represented reduced deactivation compared to dangerous CS+ trials.

We suggest that the decrease of the CR is the result of interactions between these regions. It is possible that the ITG, which has been linked to language processing (Ferstl et al., 2008) is involved in the processing of the word content and its categorisation. This information is then passed on to the IFG, which has been linked to semantic decision making (Nee et al., 2013). The stimulus information may be evaluated in this area, i.e. the safe/dangerous contingencies are recalled and a decision is made about the trial in question. If the trial is safe, this information is then passed to the vmPFC. This area has been shown to be involved in both extinction (Sehlmeyer et al., 2009) and emotion regulation studies (Buhle et al., 2014; Kohn et al., 2014), and is generally thought to integrate information from different brain areas to alter a previously appropriate response (Roy et al., 2012). This function ties in with the idea that in the LSCCE task it may receive input from IFG when the word category is safe and integrates this knowledge with the danger signal from the insula and dACC that resulted from the presentation of the CS+, being involved in down-regulating insula activation as well as physiological responses. It is possible that this occurs via processes in the dorsomedial striatum, involved in response switching (Grahn et al., 2008) or back via the dACC which is directly connected to the autonomic nervous system (Roy et al., 2012).

A diagram illustrating a tentative suggestion of the circuit involved is presented in figure 20.

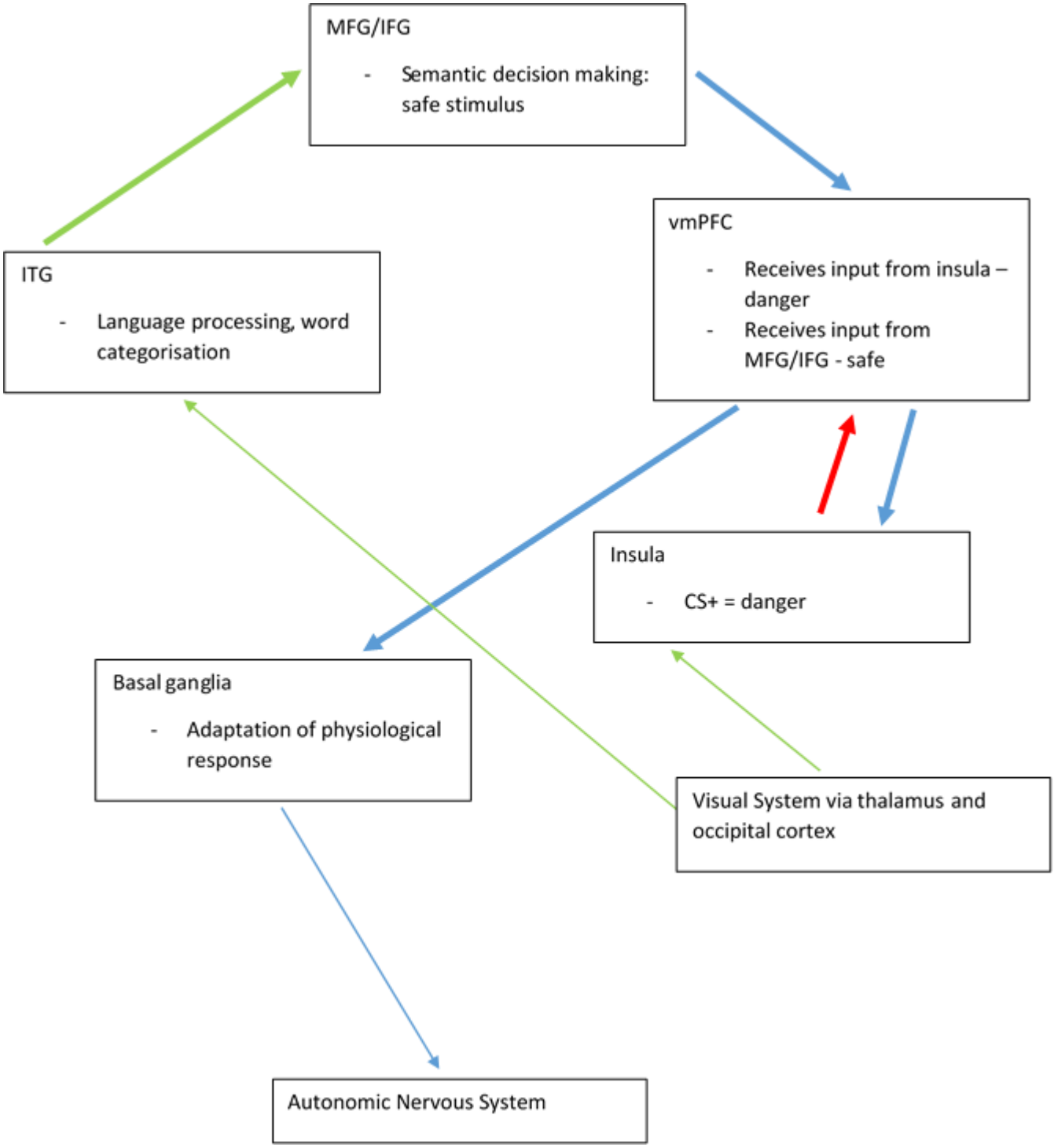


FIGURE 21. INTERACTION BETWEEN BRAIN AREAS DURING A SAFE CS+ TRIAL IN THE LSCCE TASK. INTERACTIONS SIGNALLING THREAT OF ELECTRIC SHOCK ARE REPRESENTED BY RED ARROW, INTERACTIONS SIGNALLING SAFETY ARE REPRESENTED BY BLUE ARROWS. GREEN ARROWS REPRESENT SIGNALS THAT DO NOT CONTAIN AFFECTIVE INFORMATION.

10.6 Implications for extinction research

This work can be seen as an extension of the extinction literature. There are several ways in which the extinction literature has been extended beyond the simple removal of the US. It has been shown that the modulation of the context in which conditioning and extinction occur can have an effect on the CR (Bouton, 2004). For example, moving a rat to a different cage after conditioning can speed up extinction, and returning it into the cage in which conditioning initially took place leads to a spontaneous return of the CR even after extinction has been completed. Furthermore, adding a stimulus that has been trained to be associated with a period of absence of the US to a CS+ trial also reduces the CR, both in rodent (Rescorla, 1969), and human research (Kong et al., 2014). This conditioned inhibition, or learned safety was the basis of the current project. Adding a safety cue to a CS+ results in the reduction of the CR when the CS-US association was intact previously, i.e. when no extinction has yet occurred. This suggests that the meaning of the CS+ changes through the addition of the safety cue, similar to instructed emotion regulation, in which participants are required to cognitively reappraise their initial reaction to a complex affective image. However, in learned safety paradigms this change does not require conscious cognitive evaluation of the two stimuli together, instead the reduction is the result of a separate conditioning process in which the safety signal is associated with safety from the aversive event. Thus, the aim of this project was to introduce a cognitive component into this process by adding a task that required a simple cognitive manipulation to enable participants to work out whether each trial was likely to be associated with the US or not. We found that, indeed, a partial extinction occurred in this way: During trials that participants knew were safe, i.e. never associated with the US, the CR was extinguished, while it was maintained during trials which still carried a 50% chance of the US occurring. Thus, adding this extra cognitive level to the process still facilitates extinction in the appropriate condition.

On a neural level classical extinction is typically associated with activation in vmPFC which is thought to influence the emotion eliciting areas (typically the amygdala or the insula, depending on the conditioning paradigm) and inhibit the CR (Sehlmeyer et al., 2009). Safe trials in learned safety paradigms have also been found to be associated with vmPFC activation as well as dlPFC (Pollak et al., 2010b) which is typically found in paradigms requiring an attentional or working memory component (Curtis & D'Esposito, 2003). It is likely that this reflects the increased need to monitor the contingencies during learned safety tasks.

In this paradigm we found a circuit that included this general network but was more specific to the processing of semantic information, reflecting the added level of conscious evaluation of the stimuli required during this particular task. This is consistent with the fact that knowledge of the contingencies can only be acquired through conscious cognitive evaluation of the stimulus

information, i.e. the processing of the word categories, and the reduction of the CR can only happen through the application of this knowledge.

Our results also support those found by Hefner, Verona, and Curtin (2016) who investigated safety signals using a similar paradigm to our LSCCE paradigm. Instead of using letters as CS', they used word colour, but like in this project, word category was used as a safety signal. Measuring ERP's they found that participants processed threat and safety information (i.e. word colour and category) in parallel, enabling them to use the safety information to regulate their responses to the threat when appropriate. Our results support this idea. We found that word category only had an influence on CS+, not CS- trials, suggesting that both letter and category information were processed throughout.

Finally, our results are also partly consistent with those found by Delgado et al (2008) who employed instructed fear conditioning using coloured squares as CS' and electric shocks as US', and asked participants to think of "something calming in nature" to reduce their affective responses to the CS+. They found a reduction in SCR's when participants reduced their responses, compared to when they maintained them. They also found amygdala activation when participants expected an electric shock, and vmPFC and dlPFC activation while they regulated their responses. While the vm- and dlPFC activation overlaps with the activation we found during safe CS+ trials in chapters 5 – 9, we did not find amygdala activation during dangerous CS+ trials, the trial type comparable to "attend" trials in Delgado's study. Instead, we found that a network associated with processing and anticipating visceral painful stimuli including the insula during dangerous CS+ trials. Delgado et al (2008) also reported activation in clusters in bilateral insula that overlap with those found in this project, which may be related to the processing of the threat of electric shock. Similarly, they found some of the same areas as we did when participants attempted to reduce their affect during CS+ trials, including left dlPFC (MFG) and vmPFC, even though the strategy they asked participants to use was more complex than that applied in the LSCCE task and more akin to distraction rather than reinterpretation of the CS+. Thus, possible conclusions about the specific roles these PFC areas may have in Delgado et al.'s study are limited. Considering the overlapping results in this LSCCE study, it is possible that vmPFC and dlPFC have a general role in emotion regulation which may be independent of task demands. This is consistent with the idea that both dlPFC and vmPFC have a direct role in regulating the responses of emotion generating areas (Pollak et al., 2010b; Urry et al., 2006). However, in the LSCCE task, the mechanisms through which the CR is reduced in safe trials are known (i.e. in this version this is done through word categorisation), and the left dlPFC cluster we found to be consistently activated during safe CS+ trials has been linked to semantic decision making. In addition, the inferior temporal gyrus cluster that showed the same pattern of activation is typically found during language processing (Ferstl et al., 2008; Nee et al., 2013). While this pattern of

activation does not rule out a general role in reducing negative affect (after all, that is still part of this task) this allows us to draw more specific conclusions about the processes that these brain areas are contributing to. Results from future versions of this task that may utilise cognitive evaluations based on different mechanisms (e.g. memory or attentional processes) will allow for further conclusions about which brain areas may be directly involved in emotion regulation, and which are specific to each version of this task.

10.7 Implications for Emotion Regulation Research

Instructed emotion regulation is typically investigated using negative images to create an affective response, and asking participants to regulate this response by applying specific strategies to reappraise the scenarios depicted in the images. This requires a variety of cognitive processes, including attention, working memory, visual processes and more, which is reflected in the wide network of brain regions (Buhle et al., 2014; Kohn et al., 2014) typically recruited during this task. Although emotion regulation processes have been shown to be affected in a range of mental health disorders (Gross & Muñoz, 1995), due to the complexity of the task, it is unclear exactly which mechanisms are altered in which specific disorders. In addition, both appraisal (i.e. Negative Attend trials) and reappraisal (i.e. Negative Decrease trials) involve cognitive evaluative processes. Therefore, a contrast of the two conditions will mask brain activation in areas that are involved in evaluative processes in both conditions. The LSCCE paradigm, on the other hand, removes some of the evaluative process from the emotion eliciting stimulus by using simple conditioned stimuli rather than complex images. Although participants are likely to form a conscious knowledge of CS+ and CS-, because the stimuli are so simple, the amount of cognitive processes required are limited to evaluating the visual properties of the stimuli. Furthermore, the cognitive evaluative process in the LSCCE phase is tightly controlled. Rather than having to find and apply a strategy to reinterpret a complex scene, participants only have to remember the knowledge of which category is safe and which is dangerous. The idea that fewer resources are needed in the LSCCE task compared to cognitive reappraisal tasks is supported by the fact that, compared to the networks typically involved in instructed emotion regulation tasks, the LSCCE task was associated with a more defined network that was directly related to the specific cognitive processes required (i.e. a network associated with language and semantic decision making, e.g. IFG, ITG, as well as possible integration of processes through vmPFC, although this needs further investigation). It is possible to use any simple stimulus as the CS, and any cognitive manipulation for participants to work out the safe/dangerous contingencies. Thus, a range of versions of this LSCCE task could be designed to probe how different cognitive processes contribute to more complex emotion regulation.

10.8 Problems with dependent measures during this project

To complement our fMRI results from studies 2 and 3 we used physiology data as additional dependent variables. However, we encountered several problems with the data we collected. These will be discussed in the following section.

Pupil Diameter

One of the objectives of study 2 was to further investigate whether pupil diameter does or does not reflect emotional arousal as both theories have been posited. Some study results suggest that it does carry an affective component (Bradley, Miccoli, Escrig, & Lang, 2008; Hess & Polt, 1960), others have found that it reflects cognitive effort (Koelewijn et al., 2012; Papesh & Goldinger, 2012). A direct comparison of pupil dilation and skin conductance in an emotion regulation paradigm concluded that it is more likely that pupil dilation is driven by cognitive effort (Urry et al., 2006). The combined results from both tasks in study 2 support the latter conclusion, that pupil dilation reflects an aspect of cognitive effort rather than emotional arousal. This is illustrated by increased pupil diameter while participants were decreasing their emotions compared to while they were maintaining them in the emotion regulation task. In the LSCCE task, pupil dilation did not distinguish between CS+ and CS- in the conditioning phase, or safe and dangerous CS+ trials in the LSCCE phase. Pupil diameter was increased during CS+ compared to CS- trials in this phase. Because both types of CS+ trials contained a cognitive component but CS- trials did not, i.e. word categories had to be taken into consideration during CS+ trials but not during CS- trials, this is further support to the idea that pupil diameter is linked to cognitive effort, not to affect.

Skin Conductance

As indicated above, skin conductance has been seen as a reliable measure of affect in psychological research. It is commonly used when participants are in a situation in which a threatening stimulus is presented that cannot be avoided because it has been argued that it is related to an inhibitory system (Cacioppo et al., 2007). This makes it a good choice for this paradigm. However, it is also very susceptible to interference from noise and due to differing physical attributes such as thickness of skin there is a large amount of difference in individuals' SCR. Below we will discuss our SCR findings throughout this project as well as some of the issues with collection and analysis.

SCR clearly showed the effects we expected in study 1, distinguishing between safe and dangerous CS+ trials. In this study, participants were sitting upright in front of a computer screen, so it was relatively straightforward to ensure they moved as little as possible during the experiment. However, the data collected alongside fMRI was more difficult. Participants were lying down in the scanner, which may have reduced their overall physiological responsiveness (McLaughlin et al.,

1978). The effects we expected were small, and, thus, more susceptible to noise introduced by MRI scanning.

We took several approaches to analyse this data. An initial crude analysis showed the effects we expected (increased SCR to CS+ compared to CS-, and to dangerous compared to safe CS+ trials), however, further filtering was needed before this data could be included. We explored several filtering options before we settled on a median filter across 3 datapoints to exclude noise from the electric stimulator, and a bandpass filter with a lower limit of 0.01Hz and an upper limit of 1Hz to exclude both low frequency drift and high frequency MRI noise for study 2. Because the signal to noise ratio was still low we used a model-based analysis fitting tent functions to the shape of the responses in the hope that this would capture the overall shape of the responses over the noise. However, the nature of the design with relatively short ITI's meant that the model was prone to including data from previous/subsequent trials which lead to large artefacts and we dismissed this method. This difficulty in the design was the result of a trade-off between obtaining good skin conductance and MRI data; in study 1 a number of participants informally reported that they felt bored during the task, thus, increasing ITI's to accommodate a complete return of the skin conductance to baseline would likely have increased boredom and may have lead to reduced quality and reliability of our MRI data.

We finally decided that a second-by second analysis of each trial would give us the best chance of reducing the noise in the data. We used a matlab script that calculated an average second by second deflection from baseline for each trial and used the maximum deflection within the post-trial window as the measure of SCR response. We used a time window that reduced the overlap between trials to 500ms, a timespan that is not sufficient for an SCR in the next trial to influence the data of the current trial, as a new SCR would not likely occur before 500ms from stimulus onset (Cacioppo et al., 2007). Although it is possible that the SCR would not be completed in this time window, because we baseline corrected on a trial by trial basis, and trials were in pseudorandom order, the mean peak value for each condition was not biased by the previous responses. Using this analysis method we found the expected pattern of results in study 2, i.e. increased SCR's to CS+ compared to CS- trials in the conditioning phase, and increased SCR's to dangerous compared to safe CS+ trials during the LSCCE phase.

In study 3, due to technical issues with the MRI scanner, the signal to noise ratio in the SCR data was further reduced. This meant that the previous filtering strategy did not remove enough noise to enable us to analyse the data. Thus, we used different filters, i.e. using a low pass filter with a cutoff at 0.2Hz, and slow drift correction by creating a 100 second moving average which was then

subtracted from the data. However, data from a large number of participants could still not be filtered sufficiently. This was reflected in the pattern of results we found in this study. Although we could show a conditioning effect, data in the LSCCE phase did not show the expected pattern. It is impossible to say with certainty that this was due to the quality of the data, however, we cannot rule it out. Therefore, in study 3, the SCR data did not allow us to come to any reliable conclusions.
It is difficult to say what measures could be taken to ensure good quality SCR data collection in the MRI scanner. Using equipment that is intended (as opposed to just safe) for use within an MRI scanner, and may provide additional shielding to reduce interference from scanner noise could be one way to improve data quality. However, participants will still be lying down in the scanner, reducing their overall responsivity. Thus, it may be useful to complement SCR data collected during MRI scanning with data collected outside the scanner when the expected effects are small.

10.9 Extinction, Learned Safety, or Cognitive Evaluation?

Throughout this project, one of the main discussion points in the team was the decision about a name for this task. Initially, the name involved extinction, since the task is based on the reduction of conditioned responses (e.g. “cognitive extinction”, “cognitively mediated extinction”). However, at later stages, this did not seem appropriate because we felt that extinction described an overall reduction of a CR, whereas in this task, this reduction should only occur during trials that have been evaluated to be safe, not on those where a risk of shock persists. For that reason we considered including a term such as “partial”, or “contingent” in the name (e.g. “partial extinction through cognitive evaluation”, or “extinction contingent on cognitive evaluation”). None of these suggestions seemed to fully describe the paradigm. We therefore decided to change our approach from extinction to focussing on safety rather than extinction per se, and arrived at the current name for the paradigm “Learned Safety Contingent on Cognitive Evaluation” (LSCCE). This name acknowledges of the learned safety literature based on extinction (Kong et al., 2014) and illustrates the added cognitive evaluative element of this study.

10.10 Future Directions

The main outcome of this study is the idea that it is possible to investigate the simple mechanisms involved in the reduction of an affective response through LSCCE tasks. Therefore, the main objective in future studies using this type of paradigm should be the design of additional versions of the LSCCE tasks to investigate a range of cognitive processes. This will be beneficial not only to emotion regulation research in healthy participants, but it also has the potential to contribute to the understanding of psychological disorders that include reduced ability to regulate inappropriate emotions.

One of the mechanisms that can have an influence on the appraisal and reappraisal of emotion is memory (e.g. Marsella & Gratch, 2003) and it has also been suggested that the emotional dysregulation experienced by patients with anxiety disorders may be due to a reduced ability to remove themselves from worry and focus on previous positive experiences (Mennin, Heimberg, Turk, & Fresco, 2005). In addition, memory processing is altered in individuals with mild cognitive impairment, which is thought to be a transition phase between normal functioning and the onset of Alzheimer's disease (Albert & Blacker, 2006). Disorders like Alzheimer's have also been linked to changes in mood and increased prevalence of affective disorders (Magai & Cohen, 1998). It is possible that the causes and factors maintaining these affective disorders differ to those linked to affective disorders in individuals without Alzheimer's. Thus, research into memory mechanisms as possible contributing factors to emotion regulation and dysregulation may be beneficial for the research into affective disorders in older people with Alzheimer's. To add a memory component to the LSCCE task, participants could be shown lists of words prior to testing. Letters could, once again, serve as conditioned stimuli. These could then be presented within words that participants saw before, and within words that they did not see before to determine safety/danger during these trials. This should involve both semantic processing areas like the left MFG/IFG found in this study, as well as those involved in word retrieval such as left frontal pole (Braver et al., 2001), or anterior vlPFC and perirhinal cortex (Simons & Spiers, 2003). These regions have been linked to semantic recollection, thus, overall, they should be activated when words are recognised compared to new words. This activation related to recollection should then interact with the safe/dangerous contingencies. It is possible that activation in these areas would be increased when familiar words signal a safe trial, whereas they may be reduced when they signal a dangerous trial. Based on the results from this project this would be due to the need to communicate this information when it is necessary for safety processing but for its relative irrelevance when the recollection does not have an impact on the meaning of the CS+.

Another possible extension of research into LSCCE related to memory would then be to make the remembered words relevant to participants' autobiographical memories. We previously conducted a script-driven imagery study to investigate the neural correlates of autobiographical memory in adolescents at risk for depression (Macdonald et al., 2016). This revealed altered patterns of activation in parts of the default mode network associated with autobiographical memory, including posterior cingulate and precuneus as well as reduced functional connectivity between those areas and prefrontal cortex suggesting inefficient prefrontal regulation of autobiographical memory. The current study revealed potential involvement of the default mode network in the processing of safe and dangerous trials in the LSCCE task. Thus, the role of this network may be investigated further by

introducing an autobiographical component to this task using, for example, words relevant to scripts of participants' personal experience. One issue that needs to be taken into account when designing alternative versions of the LSCCE task that tap into different mechanisms is the difficulty of the cognitive evaluation process. It has been found that a high cognitive load can suppress affective responses (Clarke & Johnstone, 2013; Van Dillen, Heslenfeld D.J., & Koole, 2009), thus, the demands on the participant must be kept simple so the emotion eliciting stimulus is still processed effectively.

10.11 Conclusion

Overall, this project showed that research on the extinction of conditioned fear can be extended using a simple cognitive evaluation to enable participants to determine whether a given trial is safe or dangerous. This resulted in reduced SCR in response to safe compared to dangerous CS+ trials as well as a neural network including the vmPFC, left ITG and bilateral IFG involved in the cognitive evaluation of safe CS+ trials and the reduction of the CR. The anticipation of threat during dangerous CS+ trials involved the insula and dACC. In addition, dangerous CS+ trials resulted in greater deactivation of brain areas associated with the default mode network, which may represent increased demands during the processing of threat.

Finally, we found an increased difference between safe and dangerous CS+ trials in bilateral insula and left IFG, in high compared to low anxious participants, as well as a reduced difference in dorsomedial striatum and vmPFC between safe and dangerous trials in high anxious participants. This suggests higher reactivity to threat in this group as well as reduced ability to process safety information. The results of this project suggest that basic mechanisms involved in the cognitive reappraisal of negative emotion can be investigated using the LSCCE task. Future studies should amend this task to investigate different processes involved in reappraisal.

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APPENDIX

WORD LIST

Study 1		
	B	T
Birds	Bluebird	Ostrich
	Blackbird	Parrot
	Buzzard	Pheasant
	Mockingbird	Stork
	Robin	Turkey
Vegetables	Broccoli	Carrot
	Cabbage	Lettuce
	Cucumber	Potato
	Basil	Tomato
	Bean	Turnip
Filler	Wardrobe	Plate
Study 2 and 3		
Animals	Bluebird	Caterpillar
	Blackbird	Cheetah
	Buzzard	Ostrich
	Caribou	Panther
	Cobra	Parrot
	Gerbil	Pheasant
	Lamb	Stork
	Mockingbird	Stork
	Robin	Tiger
	Zebra	Turkey
Plants	Banana	Carnation
	Basil	Carrot
	Bean	Lettuce
	Birch	Mint
	Broccoli	Palmtree
	Cabbage	Potato
	Cucumber	Tomato
	Mulberry	Tulip
	Raspberry	Turnip
	Rosebush	Watercress
Filler	Wardrobe	Plate

FOLLOW UP QUESTIONNAIRE STUDY 2 AND 3 (LSCCE TASK)

Follow-up questions

Did you have a strategy to complete this task (tick all that apply)?

- I monitored the word categories
- I monitored the letter
- Other:

How did you feel when the letter was "dangerous" but the word category was "safe"?

_____

1	2	3	4	5	6	7
Not stressed					Extremely stressed	

How did you feel when the letter was "dangerous" and the word category was "dangerous"?

_____

1	2	3	4	5	6	7
Not stressed					Extremely stressed	

How did you feel when the letter was "safe" but the word category was "dangerous"?

_____

1	2	3	4	5	6	7
Not stressed					Extremely stressed	

How did you feel when the letter was "safe" and the word category was "safe"?

_____

1	2	3	4	5	6	7
Not stressed					Extremely stressed	

FOLLOW UP QUESTIONNAIRE STUDY 2, INSTRUCTED EMOTION REGULATION TASK

Follow-up questions

Did you have a strategy to complete the second task?

_____

How did you feel when the picture was negative and the instruction was "attend"?

_____

1 2 3 4 5 6 7

Extremely negative Neutral

How did you feel when the picture was negative and the instruction was "decrease"?

_____

1 2 3 4 5 6 7

Extremely negative Neutral

How did you feel when the picture was neutral and the instruction was "attend"?

_____

1 2 3 4 5 6 7

Extremely negative Neutral

How bored did you feel during this task?

_____

1 2 3 4 5 6 7

Not bored Extremely bored

How sleepy did you feel during this task?

_____

1 2 3 4 5 6 7

Not sleepy Extremely sleepy