How hardwired are we against threats?
- An EDA study comparing modern and evolutionary fear-relevant stimuli.

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Abstract

The threat superiority effect refers to an ability to quickly and efficiently detect threatening cues in one’s environment. Hence, ensuing and appropriate behavioral defense responses entail greater chances of survival for an organism. Some researchers argue that natural selection has led us to automatically prioritize threats that would have been salient during the period of evolutionary adaptation; as for example snakes. However, others have also argued that activation of our defense response system is more flexible, thus able to also be triggered to dangers of more recent age: such as guns or airplane crashes. The present study has sought to impact this debate, by measuring the electrodermal activity (EDA) – more specifically the skin conductance responses (SCRs) – of subjects who were visually presented with both evolutionary (snakes and spiders) and modern (guns and knives) fear-relevant stimuli. The results demonstrated no significant difference between the two categories within subjects, suggesting that both evolutionary and modern threatening cues activate the defense response system in a similar manner. Although the results are preliminary, and would need further verification in higher powered studies, they can be seen to favor the view that our defense response system is flexibly adaptive in relation to the age of a given threat.

Keywords: Threats, Evolution, Fear, EDA, SCRs
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Introduction

Recurrent encounters with dangerous events out in the wild have contributed to the development of defense systems to effectively cope with environmental threats (Öhman, 1986; Öhman & Mineka, 2001). These defense systems are a result of natural selection. Hence, during evolution, better equipped species have acquired greater chances of survival in the wild so that their transport of genes have been able to carry on between generations (Wiedenmayer, 2009; Öhman, 1986; Öhman & Mineka, 2001). As a result of this, some animals exhibit a well-developed defense system already at birth: an ability for even young animals to trigger the appropriate defense behavior already when confronted with a predator for the very first time (Wiedenmayer, 2009). That is, the system is innate and ingrained from the time of evolutionary adaptation and does not require any specific experience or learning period for the animals (Wiedenmayer, 2009; Öhman & Mineka, 2001).

The human species as well have acquired efficient defense systems during our evolutionary adaptation to provide us with better chances of survival (Lobue & DeLoache, 2008; Öhman, Flykt, & Esteves, 2001). Activation of the defense system depends on environmental cues that indicate the presence of a threatening event or a dangerous predator. An ability that occur automatic – before we are consciously aware of the presented stimulus - and guide individuals’ perceptual attention faster toward these types of stimuli compared to neutral stimuli (Lobue & DeLoache, 2008; Öhman et al., 2001). Hence, individuals have a tendency to be faster and more efficient in locating and detecting fear-relevant stimulus (e.g. snakes and spiders) against a background of fear-irrelevant stimuli (e.g. flowers and mushrooms) compared to the other way around in a series of studies presenting visual stimuli to the participants (Lobue & DeLoache, 2008; Öhman et al., 2001).

The fact that evolutionary fear-relevant stimuli capture attention better and more efficiently than neutral stimuli is referred to as the threat-superiority effect (Brown, El-
Deredy, & Blanchette, 2010; Öhman et al., 2001) and have been demonstrated in various studies (Lobue, 2009; Lobue & DeLoache, 2008; Tomarken, Mineka, & Cook, 1989; Öhman & Dimberg, 1978; Öhman, Erixon, & Löfberg, 1975; Öhman et al., 2001). However, other studies have also investigated whether fear-relevant stimuli of more recent age – modern events, such as guns - would elicit the same behavioral defense responses at an automatic level (Blanchette, 2006; Brosch & Sharma, 2005; Brown et al., 2010; Flykt, Esteves, & Öhman, 2007; Hugdahl & Johnsen, 1989; Mühlberger, Wiedemann, Herrmann, & Pauli, 2006; Tomarken, Sutton, & Mineka, 1995).

This paper is going to investigate whether or not our automatic fear responses is triggered in similar manner independent on the age of the threat. That is, does more modern fear-relevant stimuli have the same effect on us as the evolutionary fear-relevant stimuli seems to have? At the time being, it seems to be inconclusive results as to whether this is the case, hence the question still remains widely debated from various perspectives. Also the neural processes relevant for the emotional state of fear, and its effects on us when encountering these objects and entities will be discussed as a way of understanding the underlying mechanism to the phenomena at an automatic level. An experimental study will then be conducted to investigate whether or not modern fear-relevant stimuli do have the same effects as the threatening stimuli with evolutionary significance by comparing how individuals react toward these type of stimuli as visually presenting the categories at a conscious level.
Evolution and Threats

The threat-superiority effect is commonly discussed and debated from various theories in the evolutionary framework of the emotional state of fear (Davey, 1992; Seligman, 1971; Tomarken et al., 1989; Öhman & Mineka, 2001). Some argue that due to the fact that better chances of survival always have been a crucial factor for reproduction, the development of more extensive fear of some animals or events in particular should be noticed in us humans.

The theories which suggest that this is the case, are referred to as having a strong evolutionary belief (Öhman, 1986). These sorts of theories mainly argue that dangerous events from the period of evolutionary adaptation have shaped the human gene pool through natural selection. Interestingly, some findings in particular have pointed out that snakes’ capture attention even more efficiently and faster than other evolutionary fear-relevant stimuli (Isbell, 2006). Furthermore, according to these theories of stronger evolutionary belief, the neural mechanisms of fear detection and the specific trigger of those functions are restrained by evolution and hard-wired into the organisms. That is, only threats relevant at the period of evolutionary adaptation can trigger an efficient and automatic detection of the threatening stimuli. Theories with a weaker evolutionary belief on the other hand, instead argue that the neural mechanisms are more flexible and not restrained by the age of the threat. These theories, claim that stimuli of both evolutionary as well as modern character would trigger an efficient and automatic detection in similar manner (Blanchette, 2006; Brown et al., 2010; Öhman, 1986).

A theory that has been influential for spawning other theories addressing fear and its relevance in the evolutionary framework of the emotional state of fear is the preparedness theory of phobias proposed by Seligman (1971). In his classic article, Seligman (1971) claimed that neither rats or people are blank slates from birth. Interestingly, studies conducted
on infants have demonstrated that they have a tendency to turn their heads faster toward images of snakes, rather than pictures presenting a flower (LoBue, 2009). This was also the case in relation to pictures presenting angry faces compared to happy faces. Studies such as these suggest that infants are able to detect the presence of threat in accordance with visual attention already at an early stage of development (LoBue, 2009).

Some of the most prominent sources of support for Seligman’s (1971) theory were obtained from the traditional fear conditioning paradigm but with the main focus on learning and unlearning processes of fear responses in relation to evolutionary threats: e.g. snakes and angry facial expressions (Öhman et al., 1975; Öhman & Dimberg, 1978). Because, according to Seligman (1971), the period of evolutionary adaptation would have contributed to the ability learn associations related to these types of threats more efficiently than others.

Generally, fear conditioning is a behavioral paradigm of fear acquisition where organisms learn to predict aversive outcomes and events (Davis, 1992; Öhman & Mineka, 2001). In the fear conditioning paradigm, a neutral stimulus is presented in contiguity with another more aversive event (unconditioned stimulus, US) - e.g. an electric shock - various times until the neutral stimulus becomes a conditioned stimulus (CS) by learning. When the CS and US have been presented together for a while, the organisms elicit fear responses toward the CS to help cope with the aversive event that usually follows (the US) – even without the occurrence of the US - a process known and referred to as fear acquisition (Davis, 1992; Öhman & Mineka, 2001). During the process of extinction, the association between the CS and US is unpaired by only presenting the CS alone various times, which after a while results in an absence of fear responses (Davis, 1992). However, Seligman’s (1971) theory took a step back from the traditional fear conditioning theories of fear acquisition, to put forth a focus on the acquisition of phobia in relation to evolutionary fear-relevant stimuli instead.
Based on these means, the preparedness theory of phobias proposed by Seligman (1971) argued that by the use of a threatening CS with evolutionary significance, the association between the CS and US would be faster and more easily acquired. Additionally, the linkage between the CS and US would also be more robust and resistant to extinction. Interestingly, this phenomenon can be seen clinically in individuals with phobia. Hence, the most common phobias in the world do have evolutionary roots (Isbell, 2006). Studies focusing on visual search tasks in relation to phobic participants have shown that these individuals are faster in detecting fear-relevant target associated with their specific fear as compared to normal individuals (Öhman et al., 2001). Indicating that phobic individuals frequently and automatically scan the surroundings for their specific phobias. These automatic and unconscious perceptual processes analyze the perceptual field without interfering with the ongoing cognitive processes, especially when scanning for fear-relevant stimuli (Öhman et al., 2001; Öhman & Mineka, 2001).

**Evolutionary Fear Relevant Stimuli versus Neutral Stimuli**

The ideas of Seligman (1971) inspired other researchers to also conduct laboratory experiments investigating the phenomena of biological preparedness in individuals (for review see: McNally, 2015). The most used method to test Seligman’s (1971) ideas of faster acquisition due to evolutionary significance of the CS was to expose groups of individuals to either fear-relevant CS (e.g. pictures of snakes, spiders or angry facial expressions) and fear-irrelevant CS (e.g. pictures of flowers, mushrooms or happy facial expressions) with a small electric shock as the following US ( McNally, 2015; Öhman et al., 1975; Öhman & Dimberg, 1978). Evolutionary fear-relevant stimuli used as CS did demonstrate to have a greater resistance to extinction, whereas fear-irrelevant or neutral stimuli extinguished almost immediately at the period of disassociation between the CS and US ( McNally, 2015; Öhman et al., 1975; Öhman & Dimberg, 1978).
Even though that the results were supportive to an increased resistance to extinction, what these types of studies didn’t do was to support the hypothesis that evolutionary fear-relevant stimuli would possess faster fear acquisition (Öhman et al., 1975; Öhman & Dimberg, 1978). It was further noted that fear-relevant stimuli generated increased skin conductance responses (SCRs) compared to the participants who were presented the neutral stimuli (Öhman & Dimberg, 1978). Furthermore, this was also the case when the subjects in the experiments had been verbally told that the stimuli could be followed by an aversive shock prior to the experimental session (Öhman et al., 1975).

The main ideas of the theory inspired researchers to develop newer research models and theories in relation to the evolutionary framework of the emotional state of fear, as researchers broaden their knowledge of the phenomena (for a recent review see: McNally, 2015).

Based on the fact, that the participants’ responses demonstrated a significant difference in larger SCRs toward fear-relevant CS when verbally instructed of an aversive shock following the stimuli, indicated that the results might have been due to selective sensitivity toward the threat (Tomarken et al., 1989). Tomarken et al. (1989) therefore argued that the previous experiments did not concern fear learning from the perspective of conditioning, but instead were selective sensitization to innate processes due to cues of danger. That is, a tendency for subjects to learn to associate some combinations of CS and Us over others more easily. Hence, according to them, the increased resistance of extinction with fear-relevant conditioned stimuli seen in earlier experiments were only a byproduct of covariation bias (i.e. an overestimation of random stimuli between fear-relevant stimuli and the aversive consequences) (Tomarken et al., 1989).

Tomarken et al. (1989) developed what is known as the illusory correlation paradigm to investigate individuals’ preparedness to overestimate the covariation between the
fear-relevant stimuli (CS) and the aversive shocks (US): as a way of examining fear-relevant selectivity. To be able to control for the possibility of a covariation bias, the illusory correlation paradigm shared some features with the fear conditioning paradigm previously used in relation to investigations of the significance of evolutionary stimuli over neutral stimuli (Tomarken et al., 1989). In addition to the traditional fear conditioning paradigm, the illusory correlation paradigm also asked the participants to estimate the association between fear-relevant stimuli (CS) and the aversive outcome (US) to see whether the results would indicate that selective associations were apparent. The participants were randomly presented slides of both fear-relevant and fear-irrelevant stimuli, which were followed by one of three outcome categories: an aversive shock, a tone or nothing. The participants were then told to determine the correlation between the outcome (US) and each type of slide (CS). Even though, no correlation was present, the participants tended to overestimate the magnitude of associations between the shock (US) and stimulus (CS) (Tomarken et al., 1989). Especially, in each of the experiments, subjects who were high in fear consistently overestimated the covariation when slides of their specific fear-relevant stimuli were followed by a shock. Or more specifically, participants high in fear of snakes rated the received shocks as more painful when it occurred after being presented pictures of snakes compared to neutral stimuli.

Findings as such, suggest that those individuals high in fear process information in a manner that serve to maintain or enhance their fear. These findings provided supportive evidence for the occurrence of selective association between fear-relevant stimuli (CS) and the aversive outcome (US), and especially for those subjects high in fear (Tomarken et al., 1989).

The results from Tomarken et al. (1989) studies suggested that even though the aversive properties may be important, other stimuli (not of evolutionary significance) may also induce covariation biases as long they are paired with an aversive shock. Tomarken et al.
(1989) especially pointed out that stimuli with a high semantic associability to be followed by a shock would induce these biases.

The main function of the selective processing of fear may be to reinforce an individual to mistakenly interpret relevant stimulus or events as more threatening than they may actual be. Because to mistakenly treating a dangerous event or stimulus as nonthreatening could turn out rather risky and costly in relation to survival (Tomarken et al., 1989). The overall results demonstrating the priority of evolutionary fear-relevant as compared to neutral stimuli is encouraging, also the fact that it might be due to processes of selective sensation. However, these types of studies have not controlled the aspect of whether or not these evolutionary fear-relevant stimuli also have priority over more cultural and modern fear-relevant events.

**Modern versus Evolutionary Fear Relevant Stimuli**

Evolutionary threatening stimuli require less conscious information processing than modern threatening stimuli, at least according to Öhman and Mineka’s (2001) theory of an *evolved fear module*. The evolved fear module points out a neural circuitry, evolved from the pressure of natural selection to constantly monitor dangers that we were recurrently encountered with during the period of evolutionary adaptation. Which mean that evolutionary fear-relevant stimuli such as snakes, spiders, heights and predators should be responded to more rapidly and efficiently than neutral and modern stimuli. The module involves an automatic search for dangerous environmental stimuli occurring outside of awareness, contributing to better chances of survival (Öhman & Mineka, 2001).

Öhman and Mineka (2001) mainly argue that natural selection has pressured animals and humans to evolve faster neural processes in relation to stimuli of evolutionary fear relevance. However, even though the fear module is an innate mechanism that grant priority to activation to evolutionary threats, the theory also suggests that threats of more recent age
can be feared by experimental learning. That is, by means of the conditioning paradigm, modern fears can be responded to just as efficient as a way of helping the organisms to increase their chances of survival.

Öhman and Mineka’s (2001) theoretical framework mainly points out a system, automatically activated and relatively immune to cognitive influences. A system that is especially sensitive to stimuli relevant from an evolutionary perspective that have evolved before the appearance of the cortex in mammals (which is why the system can work independently from cortical input). The fear module contains four main characteristics, all shaped by the time of evolutionary adaptation. These four characteristics are: selectivity of input, automaticity, encapsulation and a specialized neural circuitry (more discussed in the section ‘The Brain and Fear’) involved in the functions (Öhman & Mineka, 2001).

The selectivity of input derives from threatening events that were frequently encountered with during the period of evolutionary adaptation, making it rather selective with regard to the external cues of input (Mineka & Öhman, 2002; Öhman & Mineka, 2001). The neural mechanism responsible for the selectivity of input are able to limit the set of affective stimuli that need to be identified in order to elicit the correct and most appropriate defense responses (fight, flight or immobility) after only minimal processing. However, as for modern fear-relevant stimuli, these types of events require more extensive processing to activate the fear module. Which is why, according to the evolved fear module, these types of stimuli are slower responded to than stimuli from the evolutionary past (Mineka & Öhman, 2002; Öhman & Mineka, 2001).

The second characteristic of the evolved fear module is automaticity, a mechanism for fast and direct identification of fear-relevant stimuli. The automaticity of the module can grant priority to the neural processes that activate appropriate defense responses toward the stimuli, even in the absence of conscious awareness (Öhman & Mineka, 2001). The third
characteristic of the evolved fear module is known as encapsulation. Encapsulation refers to the fact that the evolved fear module is rather impervious to other higher cognitive processes. That is, once the functions of the fear module are activated, other cognitive processes have difficulties in interfering with the automatic processes. This explains why individuals high in fear respond fearfully to stimuli even though they might be considered harmless when reasoning about them (e.g. spiders) (Öhman & Mineka, 2001).

Other theories have also approached the question of whether or not modern fear-relevant activate our defense system in a similar manner as evolutionary fear-relevant stimuli have been demonstrated to do.

**Covariation bias studies.** Tomarken et al. (1995) investigated the hypothesis whether or not other types of stimuli also could induce the same responses as evolutionary fear-relevant stimuli. They did so by comparing the reported illusory correlations induced by snakes (evolutionary CS) with those induced by damaged electrical outlets (modern CS), when followed by an aversive shock (US). Compared to snakes, damaged electrical outlets might have a stronger association linkage to be followed by an aversive shock during contact, even if electrical outlets in general aren’t feared in the way that snakes are. However, Tomarken et al. (1995) found no indication of covariation bias for the modern fear-relevant stimuli (damaged electrical outlets), only for the evolutionary fear-relevant stimuli (snakes). Hence, the results implied that it might be that threatening stimuli from the period of evolutionary adaptation trigger subjects to overestimate those cues as being followed by an aversive outcome (US) more often than stimuli of more recent age.

Another study compared individuals with specific phobias (either spider- or aviophobic participants) to examine covariation bias and their physiological reactions triggered by presentation of either the specific evolutionary fear-relevant phobia (spiders) or the specific modern fear-relevant phobia (airplane crashes) (Mühlberger et al., 2006). Both the
flight-phobic and spider-phobic participants demonstrated a disorder-specific expectancy bias and enhanced SCRs elicited toward their specific phobias in the study. However, the participants with spider phobia exhibited an even greater bias of their specific fear-relevant CS when verbally told that the stimuli would be followed by an aversive US. This group also exhibited greater SCRs, event-related potentials (ERPs) and startle responses when compared to the group with flight phobia. That is, information of the situation had a stronger effect on evolutionary fear-relevant stimuli compared to modern fear-relevant stimuli (Mühlberger et al., 2006).

When considering these results, the data suggest that subjects have a pre-experimental expectancy that threatening cues of evolutionary relevance due are more associated with an aversive outcome, especially in those individuals high in fear of the specific stimuli (Mühlberger et al., 2006; Tomarken et al., 1995).

Visual search tasks. Blanchette (2006) conducted three experiments to examine how specific the evolutionary constraints are in relation to efficient detection of visual fear-relevant stimuli. In general, all of the experiments demonstrated that the participants targeted the fear-relevant stimuli quicker and more efficient than neutral stimuli, which gave supportive evidence for the occurrence of the threat-superiority effect. However, to investigate the effect of age in relation to attention and preattentive search, one needs to compare evolutionary stimuli with stimuli of more recent age. In these studies, the modern fear-relevant stimuli (e.g. knives, guns and syringes) demonstrated to be located and detected just as efficiently as the evolutionary fear-relevant stimuli (Blanchette, 2006).

One of the experiments conducted by Blanchette (2006), used both biological and non-biological threats (cartoons) as visual stimuli. The results from that experimental set-up were especially fascinating due to the fact that the results indicated that there was no difference in the threat-superiority effect even for biological versus non-biological stimuli.
Hence, it might be that as long as the stimulus is associated with threatening and dangerous cues - evolutionary or not, biological or not - activation of the threat-detection mechanism take place (Blanchette, 2006). As it has been argued by the threat-superiority effect, evolutionary fear-relevant stimuli are better attended to than neutral stimuli. Nevertheless, studies such as these seem to indicate that the threat factor are most important and not the evolutionary age of the threat (Brosch & Sharma, 2005).

The overall findings of the studies made by Blanchette (2006) demonstrated that the efficient detection system is not dependent or restricted to visual stimuli of evolutionary significance at the time the brain was shaped by evolution. Thus, it seems to extend to threatening and dangerous stimuli of the more modern time as well (Blanchette, 2006). These findings are inconsistent with a strong evolutionary belief and instead favor the weaker belief where more modern stimuli and events of fear relevance might as well activate the threat-superiority system (Blanchette, 2006; Brosch & Sharma, 2005).

If there is any difference in the neural mechanisms involved in the automatic search processes in relation to evolutionary versus modern dangers are not of knowledge today. It might be that the neural mechanisms of the automatic search processes for modern stimuli are more recently developed. However, Brown et al. (2010) conducted a study that compared emotional processing of modern and evolutionary fear-relevant stimuli by means of electroencephalographic (EEG) recordings of event-related brain potentials (ERPs) during a dot-probe task. In this experimental paradigm, a fear relevant cue facilitates attention to a following target and increases the amplitude of the P1 peak of the visual-evoked potential due to the target (Brown et al., 2010). The results indicated enhanced P1 peak when the following target appeared in the same location as the threatening cue, hence, attention was already allocated at that specific location. Moreover, this were the case for both modern and evolutionary fear-relevant stimuli. Even though the results did demonstrate an even stronger
effect for modern threats, the overall findings of the study seem to indicate similar neural processing of both modern and evolutionary fear-relevant stimuli (Brown et al., 2010). Nevertheless, further investigation is in need.

**Fear conditioning.** The expectancy theory, proposed by Davey (1992) supports the notion that cues related to evolutionary factors elicit greater SCRs than neutral stimuli. However, the theory also argue that modern fear-relevant objects or entities, paired with an aversive US, can elicit the same effects. Consistent with these arguments, a study conducted by Hugdahl and Johnsen (1989) demonstrated that modern fear-relevant CS (guns) as well as evolutionary fear-relevant CS (snakes) both contributed to just as strong conditioning responses in the participants. The subjects participating in the study were presented pictures of both snakes and guns, either directed toward or away from the participant to then be followed by a loud noise (US). The results in which indicated that a strong conditioning effect were especially apparent when the stimulus was directed toward the subjects (Hugdahl & Johnsen, 1989). Supporting the notion that stimuli of more recent age will induce just as strong responses when conditioned as evolutionary fear-relevant stimuli, especially when paired with an adequate aversive US that are closely linked with the CS.

However, other researchers (Flykt et al., 2007; Öhman & Mineka, 2001) have questioned whether the set-up of stimuli used in the study really represented selectivity of stimuli or whether the conditioning in fact only reflected an association between the CS and US. A need to conduct a study addressing the two categories effect at an automatic level is in place to examine whether both modern and evolutionary fear-relevant stimuli elicit similar defensive responses. A more stringent test should focus on investigating whether the effects of conditioning would survive and show persistence when processed outside of awareness. Flykt et al. (2007) conducted a study that investigated this possibility by the use of a backward masking paradigm: a set-up that prevent the subjects from consciously identifying
the CS during extinguishment. They were able to demonstrate that the subjects still elicited persistent SCRs, to both modern and evolutionary fear-relevant stimuli, when the stimuli were directed toward the participants. Flykt et al. (2007) proposed that it was the direction of the stimulus that appeared to be the most crucial factor in modulating the effects of masking on SCRs, and not the content of the stimulus presented to the participants. These findings suggest that fear-relevant stimuli – either evolutionary or of more recent age - can establish similar SCRs expectancies for fear-relevant stimuli during a fear conditioning paradigm: which are supportive to the expectancy theory proposed by Davey (1992).
The Brain and Fear

The neural system mediating our defense and fear responses to perceptual stimuli is the amygdala. The amygdala is a small structure consisting of grey matter located bilaterally deeply in the medial temporal lobe of the brain (LeDoux, 1994). As a part of the limbic system, the structure is considered a very important region for activation of defense behavior by means of controlling and maintaining the emotional processes such as fear, attention, motivation and autonomic functions (Yilmazer-Hanke, 2015). Hence, the amygdala is of crucial importance for the ability to detect and respond to environmental threats (LeDoux, 1994, 1998).

Amygdala activation occurs at an automatic level, and motivates the animal and human to cope with threatening events presented to them by means of attention and eliciting the appropriate physiological processes in relation to the threat (LeDoux, 1994, 2007; Öhman, 2005). These automatic responses are characterized by the release of stress hormones and changes in blood pressure and heart rate - responses that depend on activation of the autonomic and hormonal systems - but also freezing and inhibition of ongoing cognitive processes. What the most appropriate responses are, depend on the context in which the threat occurs at and the innate defense system of the organisms (Sah, Faber, Lopez de Armentia, & Power, 2003). For example, bilateral lesioning of the amygdala in rats have been demonstrated to change their reactions toward an otherwise threatening event (a cat). Making the rats approach the cat, thus, eliminating their appropriate defense system against the threatening stimuli (Blanchard & Blanchard, 1972). Moreover, lesions to the amygdala’s projecting targets: bed nucleus of the stria terminalis, lateral hypothalamus and the periaqueductal located in the midbrain have all been demonstrated to disrupt emotional behavioral and autonomic activity in rats during the fear conditioning paradigm (Laine, Spitler, Mosher, & Gothard, 2009).
Anatomically the amygdala consists of various distinct nuclei and connections to other regions, both cortical and subcortically mediated, of the brain (LeDoux, 1994; Yilmazer-Hanke, 2015). The underlying circuits and connections of the amygdala have been extensively studied during the years which have contributed to a rather detailed image of the neural system regulating fear and its behavioral defense processes (LeDoux, 1994).

Recent classification of the amygdala has distinguished that the structure consists of two major nuclear groups in terms of inputs and outputs of information (LeDoux, 1994; Yilmazer-Hanke, 2015). First, the **basolateral group of the amygdala**, with its coordinated expansion to the neocortical sensory association regions include subdivisions of nuclei as the **lateral** (LA), **basolateral** (BL, or also referred to as basal), and **basomedial** (or also referred to as accessory basal) (Yilmazer-Hanke, 2015). This nuclei group is often referred to as the basolateral complex when discussing all its sub-divisions as one entity. This group of nuclei contain cortical neurons connecting the region to various other more cortically mediated areas of the brain, making it receiving highly processed sensory information from temporal and parietal areas, but also from the neocortex (Sah et al., 2003).

The second major nuclei group of the amygdala is the **centromedial group** with its downstream connections for the regulation of species-specific behaviors (e.g. fear and defense behavior, but also feeding and reproduction): based on a hierarchical organization. This is as well the area involved in the modulation of hormonal homeostasis (Yilmazer-Hanke, 2015). The centromedial nuclei group include sub-nuclei such as the **medial**- (ME) and **central** (CE) nucleus and contain neurons of striato-pallidal type connecting the region to subcortical structures of the brain. Additionally, the centromedial nuclei group is also receiving cortically sensory stimuli from olfactory cortex which is mainly processed by the ME (Sah et al., 2003).

The ME are the nuclei responsible for regulation of sexual behavior, food intake and defense responses by the use of its connections to hypothalamic regions. The CE instead
mainly modulate and control fear responses and the autonomic reactions through its connections with various brain stem and hypothalamic areas (Yilmazer-Hanke, 2015). The functions of the CE make it the major output nuclei of the amygdala as it receives inputs from all the other amygdaloid nuclei, hence, after processing the received information, the area contribute to eliciting the most appropriate behavioral outcome for the organism (Sah et al., 2003). It has been suggested that the function of the basolateral complex mainly is to evaluate the emotional content of the received sensory inputs whereas the function of the centromedial nuclei group is to produce attention and arousal toward the stimuli for improved detection of the threat (Sah et al., 2003).

The construction of the amygdala contributes to the important function of receiving input and sending output to various areas in the brain associated with the emotional processes related to the threatening and dangerous stimuli (LeDoux, 1998). Hence, the amygdala is the brain region that is able to receive sensory information from all input modalities: olfactory, somatosensory, gustatory, auditory and visual but also visceral information, at all levels (LeDoux, 1994, 1998).

Specifically, the LA located in the basolateral group is the subarea that receives the inputs from all the different sensory modalities. While the CE located in the centromedial group serve as the output nucleus of the amygdala, involved in the expression of innate emotional responses and those physiological responses especially related to the expression of fear (LeDoux, 1994, 2007). Neuronal stimulation – both electrical and chemical – of the connections from the CE to regions in the brain stem control both the autonomic and somatomotor responses (LeDoux, 1994; Sah et al., 2003). More specifically, as the brain receive signals of danger, it begins to send messages throughout the automatic nervous system to help regulate and maintain the activity of the bodily organs (both internal and external) to help cope with the demands of the situation (LeDoux, 1998). Damage to these connections
disrupts the behavioral and autonomic responses related to the emotional state of fear (LeDoux, 1994). The autonomic nervous system and its function associated with dangerous and threatening event will be more extensive described later on.

It is especially the linkage between the LA and the CE that allow the crucial communication between the basolateral and centromedial nuclei groups to trigger the defense system by means of activation of the hypothalamic-pituitary-adrenal (HPA) axis with its regulation of the bodily response of stress reactions (LeDoux, 1994). As the HPA activates, the pituitary gland located in the hypothalamus receives a signal to release the adrenocorticotropic hormone (ACTH). Additionally, the adrenal glands (located just above the kidneys) also begins to release a steroid hormone into the bloodstream to further help the animal or human to cope with the stressful or threatening event (LeDoux, 1994, 1998). The steroid hormone released into the blood flows to the brain where it binds to receptors in the amygdala, hippocampus, prefrontal cortex etc. Hence, the close linkage between LA and CE is of importance for the expression of appropriate defense behavior and survival of the animal by means of enhanced activation of heart, blood and sweats glands, which all occur automatically when confronted with fear (LeDoux, 1994, 1998).

In summary, the amygdala and its input and outputs connections, are part of a specialized neural system that allows fast activation of physiological processes related to all kinds of sensory stimuli associated with cues of threat or danger in our surrounding environment. These fear responses, shaped by evolution, occur at an automatic level even before conscious awareness and depend on the threat and its underlying system (Aggleton, 2000; LeDoux, 1998).

**Amygdala’s Role in Fear Activation at an Automatic Level**
The amygdala has the ability to process fear-related stimuli at an automatic level outside of our awareness of the behavioral, motivational and functional processes which are triggered by the threatening cues, especially in relation to our perceptual field (Aggleton, 2000; Öhman, 2005). Findings in relation to automatic fear activation before conscious awareness indicate that a detailed recognition of the affective stimulus is not necessary for activation of the appropriate defense responses (Zajonc, 1980).

Anatomical and physiological research of the human perception have found converging evidence of a visual system, subdivided into various visual pathways with rather different functions (Livingstone & Hubel, 1988). Despite that our perceptual field seems to be a unified and coherent scene, stroke patients can occasionally suffer rather selective visual losses depending on where in the brain the damage has occurred: e.g. loss of motion perception (caused by lesions to cortical area o V5 in the occipital lobe; Gazzaniga, Ivry, & Mangun, 1998) or loss of facial recognition, also known as prosopagnosia (caused by damage to the fusiform gyrus; Gazzaniga et al., 1998). These independent pathways analyze all the different aspects of the same retinal image. Hence, by knowing what particular aspect to investigate and the appropriate experimental paradigm to use, one can study the specific visual aspect that are of interest for the investigation (Livingstone & Hubel, 1988).

Early studies based on the fear conditioning paradigm discovered the possibility of a pathway which could transmit the necessary information directly to the amygdala from the thalamus and thereby bypassing the aid of the cortex. That is, a pathway that contributes to the possibility of eliciting fear responses against threatening events without conscious awareness of the stimuli presented (LeDoux, 1998). A pathway, able to bypass the cortex, would explain the fact that emotional responses can occur automatically without the involvement of higher cognitive processes (e.g. reasoning or thinking about them) (LeDoux, 1998).
Visual impaired individuals - namely blindsight patients - are known to be a key evidence of this possibility (Jolij & Lamme, 2005) Blindsight patients with lesions to their primary visual cortex are able to correctly “guess” the right attributes of the stimuli presented to them (Jolij & Lamme, 2005; Morris, DeGelder, Weiskrantz, & Dolan, 2001). Affective blindsight on the other hand refer to patients with damage to their striate cortex. These patients are as well unable to consciously perceive visual stimuli presented to them. However, when presented fear-relevant stimuli and emotional facial expressions, their amygdala indicate activation, as well as the superior colliculus of the midbrain and pulvinar nuclei: regions which both have valuable roles in visual attention (Morris et al., 2001). Studies conducted on affective blindsight patients do suggest that we are led by emotional information even in the absence of conscious awareness and that the outcome of the unconscious processes that occur probably affect our behavior toward the event (Jolij & Lamme, 2005).

In addition, there are now studies demonstrating that appropriate defense behavior and reactions can be interrupted in normal individuals -the same way as for affective blindsight patients - by the use of experimental manipulations. Jolij and Lamme (2005) were able to induce affective blindsight by the application of TMS to the striate cortex. Their results supported the suggestion of a subcortical pathway to the amygdala, a route with the function of processing “unseen” emotional and fear-relevant visual information in parallel to another cortical pathway with the main function of conscious identification. Hence, blocking the cortical routes by means of TMS demonstrated to block perception as such but not the affective discrimination of the stimuli presented (Jolij & Lamme, 2005).

In summary, studies based on the existence of affective blindsight indicate that there is an unconscious and subcortical visual pathway, able to processes affective and fearful information from our perceptual field. Thus, both humans and animals might have evolved a
direct pathway to the amygdala during the period of evolutionary adaptation, bypassing other cortices (Jolij & Lamme, 2005; Morris et al., 2001; LeDoux, 1998).

Findings such as these, fits well with the “two routes of fear” proposed by LeDoux (1998). LeDoux (1998) suggested that the amygdala has two rather different pathways of processing sensory information. These two pathways are referred to as the “the high road” and “the low road” of fear. These two have been suggested to work in parallel to transmit the relevant information to the amygdala (or more specifically, the LA) from the thalamus and cortex (LeDoux, 1998).

The ‘low road’ of fear is supposed to be sub-cortically mediated (shorter thalamo-amygdala) and able to process sensory information efficiently and automatically whereas the ‘high road’ fear is mediated cortically (longer thalamo-cortical-amygdala) and instead processes more detailed information (LeDoux, 1998). As the low road only involves one direct linkage between the thalamus and amygdala, the route cannot make any fine distinctions of the surrounding environment. The high road on the other hand, with its various links between amygdala and the cortex can instead make a rather polished image of the surrounding environment. However, the high road adds time in processing as compared to the low road, making it rather slow and dependent on attention toward the stimulus (LeDoux, 1998).

Nevertheless, even though the low road cannot make any full distinction of the specific stimulus which triggered amygdala activation, it can warn that something is located in the surrounding environment. Doing so by means of guiding the individual’s attention toward the dangerous event until the high road has made a full distinction and identified the stimuli (LeDoux, 1998). Hence, the ‘low road’ might have the reduced ability to analyze stimuli, however information travelling the shorter road are instead processed more rapidly -
without any conscious awareness of the specific threat - a crucial function for survival (LeDoux, 1998).

Although, instead of two distinct neuroanatomical pathways described by LeDoux (1998), other more recent models propose another explanation for the neurobiology involved in visual processing of the emotional state of fear at an automatic level (Dehaene, Changeux, Naccache, Sackur, & Sergent, 2006; Lamme, 2006). These models are instead based on the coordinated activity between the brain regions during visual processing. They have especially pointed out a fast feedforward sweep where the incoming sensory information flows through the visual system (rapid responses outside of awareness) and a later stage of recurrent processing (visual awareness), in which the signals are sent back to the lower cortices via feedback connections (Lamme & Roelfsema, 2000). Models such as these suggests that feedforward activity in sensory cortices associated with environmental cues would be sufficient for a priming effect, but not for conscious perception. Hence, implying that affective processing in relation to visual information instead would have a cortical basis without any need of a subcortical pathway for affective processes (Dehaene et al., 2006).

The Measurement of Fear Activation at an Automatic Level

The defense system in animals and humans, as discussed so far, relies on quick and appropriate motor responses associated with the threatening stimuli. A mix of responses - affective, cognitive, behavioral and physiological - which all requires fast activation of metabolic support from the autonomic nervous system (Öhman, 1986) as briefly mentioned before (p. 21). The defensive responses when confronted with threatening events depend on the processes of the brain to quickly trigger the appropriate actions required of the event. When all these autonomic sub-systems are coordinated, they contribute to an efficient and goal-directed behavior - occurring even without any conscious awareness - to confront the environmental threat (Hagemann, Waldstein, & Thayer, 2003).
The autonomic nervous system resides in the spinal cord where it influences the activity of visceral organs, blood vessels, and sweat glands of the whole body (Canero & Hermitte, 2014) with the key function of maintaining the homeostasis of the body and regulating the autonomic reflexes occurring outside of voluntary control (McCorry, 2007). The autonomic nervous system branches out to two anatomically and functionally distinct sub-systems: the parasympathetic system and the sympathetic system. These two systems co-work to maintain homeostasis of the body: with opposing effects depending on the event that take place. That is, enhancing the activity of one of them while simultaneously inhibit the activity of the other (McCorry, 2007).

The effects on the bodily organs due to activation of either the sympathetic- or parasympathetic nervous system are rather different (Canero & Hermitte, 2014; McCorry, 2007). Activation of the sympathetic nervous system increases the heart rate by means of epinephrine and norepinephrine, whereas activation of the parasympathetic instead, by the use of acetylcholine, decreases heart rate (Canero & Hermitte, 2014). Thus, it is the parasympathetic division predominantly regulates the “rest and digest” functions. That is, bodily functions during times of rest and energy saving (McCorry, 2007). The sympathetic division, on the other hand, is instead dominant during emergency reactions. When activated by strong emotions - such as fear - physiological “fight-or-flight” responses are triggered (Canero & Hermitte, 2014; McCorry, 2007). These responses enable us to react with appropriate and useful actions required to cope with the threatening event, as for example fleeing or fighting against an enemy or danger, but also freezing as a way of becoming a less visible target for the predator (Canero & Hermitte, 2014). That is, the overall function of the sympathetic nervous system is to prepare our body and organs for stressful and physical activities (McCorry, 2007).
As already mentioned, the two branches of the autonomic nervous system innervate all the sweat glands distributed across our skin (Hagemann et al., 2003). Here, unmyelinated postganglionic sympathetic axons project to eccrine sweat glands on the skin, found with the highest density at the palmar and plantar regions (Critchley, 2002). As the sympathetic nervous system innervate the activity of the sweat glands, one can measure the changes in skin conductance - by means of these sweat glands - as an indirect measurement of neural activity, a measurement technique known as electrodermal activity (EDA).

EDA is an umbrella term referring to the autonomic changes in electrical properties of the skin in response to sweat secretion (Boucsein et al., 2012). Depending on the amount of sympathetic activation, the sweat will rise in the ducts as a result and the skin resistance will change (Dawson, Schell, & Filion, 2000). The higher degree of activation, the lower the resistance: which in turn yield observable changes in skin conductance (SC) by passing a low and constant voltage through a pair of electrodes placed on the surface of the skin. The measurement principle is of Ohm’s law (Dawson et al., 2000):

\[ SR = \frac{V}{I} \]

That is, skin resistance (SR) is equal to the voltage (V) applied between the two electrodes placed on the surface of the skin when divided by the current (I) as passed through the skin (Dawson et al., 2000). The measurement unit of EDA is in microsiemens (µS): the larger the value, the greater the levels of SC (Boucsein, 1992).

Recording SC data by passing an external current between the two electrodes is best known as the exosomantic method (Boucsein, 1992; Boucsein et al., 2012; Dawson et al., 2000). Methods of exosomantic recordings applies either a direct current (DC) or an alternating current (AC) to the participants’ skin (Boucsein, 1992; Boucsein et al., 2012). DC measurements uses a constant voltage or current to focus on the passive properties of the system whereas AC measurements mainly focus on the oscillatory signals. A third method is
the *endosomantic method* which does not involve any external current and only record the potential differences of the skin (Boucsein, 1992; Boucsein et al., 2012; Dawson et al., 2000). But the most widely applied EDA method in research today is by far the exosomantic DC recordings (Boucsein, 1992; Boucsein et al., 2012).

Basically, the EDA complex include both the measures of the skin conductance level (SCL, a constant and slowly moving tonic background level of the signal) and skin conductance responses (SCRs, a rapid phasic change in the signal) as a result from sympathetic neuronal activation (Boucsein, 1992; Boucsein et al., 2012; Critchley, 2002; Dawson et al., 2000). If phasic SCRs occur in absence of a presented stimuli is it referred to as non-specific SCRs (NS-SCRs) whereas SCRs that can be traced to a presented stimulus are referred to as event-related SCR (ER-SCR) (Boucsein, 1992; Critchley, 2002; Dawson et al., 2000). An SCR which begins between a time window of one to four seconds after presenting an unexpected and aversive stimulus is considered to be an ER-SCR (Boucsein, 1992; Critchley, 2002; Dawson et al., 2000; Venables & Christie, 1980).

EDA has become one of the most used recording methods of emotion-related sympathetic activity due to its sensitivity to changes in arousal associated with emotion, cognition and attention (Boucsein, 1992; Boucsein et al., 2012; Critchley, 2002). EDA responses are for instance elicited by threatening stimuli, but also by stimuli with motivational significance such as: wins and losses, love and hate, memory recall and otherwise cognitive loading work (Critchley, 2002). Even the fear conditioning paradigm to masked conditioned and unconditioned CS stimuli can elicit SCRs noticeable for the EDA method to register (Flykt et al., 2007).

The origin of our sweating due to sympathetic activation arise from various independent brain regions as for example the cortex, the basal ganglia, diencephalic structures (thalamus and hypothalamus), the limbic system and from structures of the brain stem.
The hypothalamic regions and brain stem has as previously mentioned a crucial role in the involvement of homeostatic control. In addition is the hypothalamic regions under the control of the limbic system which possesses a key role for the neurophysiological basis of emotional and motivational phenomenon (Boucsein, 1992). Nevertheless, amygdala activation is most likely to reflect the affective processes occurring in the brain when confronted with threatening events (Dawson et al., 2000).

When it comes to recordings of EDA, both the exosomantic and the endosomantic method is performed with two electrodes (Boucsein, 1992; Boucsein et al., 2012; Dawson et al., 2000). The preferred and most reliable recording sites on the skin for EDA are from the glabrous skin of the palms of the hand or the volar surfaces of the fingers (see Figure 1) but also the soles of the feet (Boucsein, 1992; Boucsein et al., 2012; Dawson et al., 2000). It is mainly because of that the electrodes easily can be attached at these areas, that these areas are rather stable for movement artifacts and possess a sufficient size to attach the electrodes (Venables & Christie, 1980). Distal phalange sites on the non-dominant hand is recommended due to the placements greater responsivity and sweat gland activity as compared to the other placements (Boucsein et al., 2012; Dawson et al., 2000).

Figure 1. Three electrode sites for recording EDA located at the hand
Figure 1. Three electrode sites for recording EDA located at the hand. Recording site 1 involves volar surfaces on distal phalanges, recording site 2 involves volar surfaces of medial phalanges, whereas recording site 3 involves thenar and hypothenar eminences of palms. Picture adapted from: Dawson et al. (2000). The electrodermal system. In J. T. Cacioppo, L. G. Tassinary, & G. G. Berntson (Eds.), Handbook of psychophysiology (3rd ed., pp. 159-181). New York: Cambridge University Press.

The Current Study

Taken together, current empirical evidence for the impact of evolutionary threats versus modern threats is elusive, even though it has been studied for more than a decade. In relation to the experimental study conducted in this master project, the main focus will be on the participants elicited SCRs as an indication of psychophysiological arousal evoked by the threatening stimuli presented to them. The experiment will try to answer the question whether or not modern fear-relevant stimuli have the same effects as the evolutionary threatening stimuli or if threats originating from the period of our evolutionary adaptation have an advantage over those of more recent age.

Emotions are considered to be closely linked by association to the stimulus, responses and semantic concepts (Lang, Greenwald, Bradley, & Hamm, 1993). Due to the
close linkage, cues of input that match the stored concepts activate the appropriate actions and responses. These cues of input can be of various kinds, such as, actual events, specific memories or as simple as pictures (Lang et al., 1993). Hence, presenting pictorial stimuli have demonstrated to yield different action pattern dependent of the emotional meaning of what is located on the pictures (Dimberg, 1982). As when presenting individuals with pictures demonstrating angry or fearful faces versus happy facial expressions. The major observable difference between these two emotional categories were that the negative pictures increased corrugator activity (frown) while pleasant pictures demonstrated to induce zygomatic tension (smile) (Dimberg, 1982). Additionally, in studies presenting affective stimuli, either perceptual or imaginal, SC has been demonstrated to directly vary with the reported arousal (Greenwald, Cook, & Lang, 1989; Lang et al., 1993). That is, the pictorial information seems to match the actual properties of the object or event and activate cognitive representations associated with the appropriate emotional responses (Lang et al., 1993).

The typical set-up of experiments conducted to answer the question of whether evolutionary fear relevant stimuli and events are better responded to than more modern stimuli have involved fear conditioning (Hugdahl & Johnsen, 1989; Öhman & Mineka, 2001) and the backward masking paradigms (Flykt et al., 2007) but also the covariation bias paradigm developed by Tomarken et al. (1989) have been used (Mühlberger et al., 2006; Tomarken et al., 1995).

Studies such as the backward masking paradigm have approached the question from the perspective of affective processing in the absence of visual and conscious awareness: as the processes at the automatic level precede perceptual recognition and semantic identification. However, a problematic factor that arises with these models are that the detection threshold between the participants are not accounted for. That is, it does not control for the participants’ actual awareness of the stimuli presented to them (Lähteenmäki, Hyönä,
Koivisto, & Nummenmaa, 2015). Additionally, learning as a measurement of fear – as in the fear conditioning paradigm - is costly in terms of the neural processes that is required during the conditioning paradigm. Hence, a more direct measurement in relation to the emotional state of fear is in need (Öhman & Mineka, 2001). Moreover, a recent released paper from Lähteenmäki et al. (2015) obtained contradictable results against those experimental models which argues that affective processing can occur outside of individuals’ awareness. Because, the results of Lähteenmäki et al. (2015) demonstrated that recognition can’t occur in the complete absence of visual awareness: indicating that conscious recognition is of importance for affective processing

So as for being sure affective and emotional processes really is the independent variable, the set-up of the current study conducted in this master thesis will use pictorial stimuli presented to the participants at a conscious level (Dimberg, 1982; Greenwald et al., 1989; Lang et al., 1993; Lähteenmäki et al., 2015). Even at a conscious level, the fear and defense processes should be relatively immune to the influences of cognitive factors. Hence, once the neural mechanism has been activated – even before identification of the specific stimuli - the fear and defense responses will follow (Öhman & Mineka, 2001). The automaticity and encapsulation of the fear module, as brought up by Öhman and Mineka (2001), refer to the fact that fear of a threatening and dangerous stimuli can be activated directly without any detailed analysis of the specific stimulus (automaticity) and that once initiated, the fear response will be elicited resistant to conscious cognitive control (encapsulation).

One other factor this master thesis will control for is, as Hugdahl and Johnsen (1989) pointed out, the effects of directing the presented pictorial stimuli toward the participants. Hence, it might be that fear-relevant stimuli directed toward the participants are more threatening than stimuli presented to the side. Generally, angry faces have been demonstrated
to yield greater and more robust conditioning effects as compared to happy facial expressions (Öhman & Dimberg, 1978). The effect has been demonstrated to be even stronger when the faces were glaring directly at the subjects during presentation (Dimberg & Öhman, 1983). So the current study will make use of the considerations that Hugdahl and Johnsen (1989) brought up and present the pictorial stimuli directly toward the participants on the screen as a way of maybe obtaining even greater effective responses, since the pictures would be more of a direct threat (snake-bite or robbery).

When considering the specific measurement procedure for the automatic responses, the dependent variable in previous studies has mainly been SC. Especially due to the fact that the SCRs elicited does not only reflect fear as such. Rather the elicited SCRs in relation to these types of studies also reflects emotional processes related to the emotional state of fear: such as attention, arousal and interest. This will also be the case in relation to the current study (Dimberg, 1982; Greenwald et al., 1989; Lang et al., 1993; Öhman & Mineka, 2001).
Method

Participants

19 healthy young adults (12 males and 7 females with combined mean age of 25.32±4.68 SD) participated in the study after giving a written informed consent in agreement with the Declaration of Helsinki (http://www.wma.net/en/30publications/32doh/index.html). The participants were recruited from internet, e.g. social media and student groups linked to the University of Skövde. The majority of the participants were right-handed (17 of 19 participants), no one reported any history of mental or neurological disorders. All participants were Swedish native-speaking former or current university students who all had normal or corrected eyesight. All participants were asked if they had any phobic tendencies prior to experiment to be able to control for relevant confounding factors due to extreme fearfulness. Two of the participants reported phobic tendencies (ladders and centipedes), however none of the reported phobias that were relevant for the study and the participants were allowed to proceed with the experiment. However, two participants were excluded from further data analysis: one was discarded due to some unidentified form of significant electrical interference, clearly visible in the recorded signal (see Appendix A) and the second participant because falling asleep during the experimental session, leaving a total of 17 participants (11 males and 6 females with a total mean age of 24.47±2.9 SD) for further analysis.

Design

The experimental design was 2 x 2 factorial within subject, created and presented by the use of E-prime 2.0 software (Psychology Software Tools, Pittsburgh, PA). The presented stimuli were constructed using four different categories of colored pictures: two categories were modern threatening pictures (guns and knives) whereas the other two categories of
stimuli were evolutionary threatening pictures (snakes and spiders), each category consisting of 22 visually different pictures. All the pictures used in the study were directed toward the participants as a way of increasing the threat factor (Flykt et al., 2007; Hugdahl & Johnsen, 1989). Additionally, the two evolutionary categories were presenting spiders and snakes in their natural habitat, against the ground or floor whereas the two modern categories included humans or only human hands holding the knives and the guns. The pictures for the study were obtained from The Geneva Affective Picture Database (GAPED; Dan-Glauser & Scherer, 2011), Google search and taken with own camera (for distribution, see Table 1.). GAPED is a database consisting of 720 pictures with either positive, negative or neutral contents. In relation to the study conducted here, pictures from the negative categories of spiders and snakes were used (see extra notes in Table 1 for the selected visual stimuli). The pictures taken by own camera were in the categories of guns and knives. All sessions presented all the pictures (N=88) fully randomized and the size of the pictorial stimuli presented on the screen were 22 cm x 38 cm.
Table 1. Distribution of obtained pictorial stimulation.

<table>
<thead>
<tr>
<th>Type of Directed Stimuli</th>
<th>Frequency</th>
<th>Extra Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPED</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snakes</td>
<td>5</td>
<td>103; 118; 048; 035; 034</td>
</tr>
<tr>
<td>Spiders</td>
<td>9</td>
<td>012; 054; 062; 136; 137; 138; 144; 145; 157</td>
</tr>
<tr>
<td>Google Search</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spiders</td>
<td>13</td>
<td>See Appendix B for examples</td>
</tr>
<tr>
<td>Snakes</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Guns</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Knives</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Taken with own Camera</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guns</td>
<td>6</td>
<td>See Appendix B for examples</td>
</tr>
<tr>
<td>Knives</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Demonstrating where the pictures used in the study were obtained from. Extra notes indicate the specific picture taken from the GAPED but also remarks to appendix B for examples of the pictorial stimuli obtained from google search and taken by own camera.

The pictures were presented for 5 seconds after a 1 second fixation point, located at the center of the screen. The participants of the study were instructed to fixate on the fixation point as it appeared on the screen. Between these stimuli epochs a waiting screen appeared for 14s to act as a interstimulus intervals (ISIs) (Boucsein et al., 2012; Breska, Maoz, & Ben-Shakhar, 2011). In general, a typical ISI for SCR measures range around 10 – 60 seconds due to the SCR slow properties (Boucsein et al., 2012; Breska et al., 2011). The structure of the study resulted in a time epoch of 20s (the stimulus-onset asynchrony; see Figure 2) between each stimulus onset, making it possible for the signal to recover to baseline (Boucsein et al., 2012).
Figure 2. Schematic Details of the Experimental Design Used in the Study.

Figure 2. Demonstrating examples of the different threatening categories. Demonstrating the time epoch of 20s between the 88 stimulus onsets (14s waiting screen; 1s fixation point, 5s presentation the pictorial stimuli used in the study) as a way of act as a ISI during data recording of the SCRs.

**Procedure**

After completion of the pre-experimental information the participants were seated in a quiet laboratory room at the Department of Cognitive Neuroscience, University of Skövde. The participants were placed approximately 1 meter in front of a LED computer screen (HP Z23i IPS Display with an illumination of 250 cd/m²) and attached with two small special conductive plastic electrodes (g.GSRsensor, Austria; http://www.gtec.at/) on the non-dominant hand, placed at the distal phalanges by the use of velcro straps (Recording site 1; see figure 1). The area of contact was not pretreated with any gel by recommendation of the manufacturer (g.tec, Austria; http://www.gtec.at/). The setup is exosomantic as the two electrodes measure the electrical conductivity of the skin by applying a constant direct current voltage (electrode voltage approximately 400mV) between them. Hence, the automatic emotional responses activate the sweat glands located on the subjects’ skin, which in turn results in increased skin conductivity between the two electrodes (g.tec, Austria; http://www.gtec.at/). Continuous SCR data was recorded with a sampling rate of 256 Hz, a lowpass filter of 30 Hz, and a notch filter of 50 Hz to reduce high frequency noise. The
amplifier used during the data recording was a g.USBamp (g.tec, Austria; http://www.gtec.at/).

Before each session, the participants were asked to remove any electronic devices and to avoid any unnecessary movement during the recording session. During the experimental session, the experimenter sat outside of the laboratory room and monitored the presentation of stimulus and the recording of the physiological data. The recording session took approximately 35 min in total, with a 5-minute pre-experimental baseline recording after instructions and attachment of electrodes as the participants were instructed to be in a relaxed state.

Due to cooperation with another master degree student to find and share participants, a concept was needed to counterbalance the order effects that the experiments might contribute to one another when presenting them after each other to the participants. It was decided that: if one experiment was presented first, then the other students’ experiment was run first to the next participant the following experimental session. In addition, a need for a break were in place in between the two experiments so that the participants could get some air, stretch their legs and in general regain some energy until the second experiment started. A 5-10 minutes’ break was added to account for boredom and tiredness that the time consuming experimental session could contribute to. During the break, the electrodes were unattached.
Results

Data analysis

For analyzing the obtained data, the program Ledalab Software (version 3.4.8; http://www.ledalab.de/) written in MATLAB (version R2014, http://www.matworks.com/) were used. Ledalab software possess the function to analyze a large amount of EDA data automatically by the use of what is called Batch-mode where one can preprogram the settings for analyzing the recorded data obtained from the participants of the study. A trough-to-peak analysis was performed on the collected data individually by the use of Ledalab’s automatic batch-mode. The classic trough-to-peak analysis is a standard peak detection method that are well used for analysis in research measuring SCRs (Boucsein, 1992; Boucsein et al., 2012). The method of trough-to-peak analysis defines the SCR amplitude as the SC differences in value at its peak and at the preceding trough (Boucsein, 1992). Amplitude refers to the mean value of those trials in which a measurable and observable response have occurred (Dawson et al., 2000). The settings were set to analyze all data with a threshold over 0.01 µS occurring in a response window of 1-4 seconds after stimulus onset (Boucsein, 1992; Boucsein et al., 2012), it was also down sampled from 256Hz to 16Hz and smoothed for further analysis as generally recommended (Boucsein, 1992). The results of the analyze resulted in an overview of the elicited SCRs of significance (N=352 triggers, over the threshold 0.01 µS, see Table 2), which were used for further statistical analysis (next section).
Table 2. Distribution of significant elicited SCRs.

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent (%)</th>
<th></th>
<th>Frequency</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Directed Gun</td>
<td>92</td>
<td>26.1</td>
<td>Modern Triggers</td>
<td>180</td>
<td>51.1</td>
</tr>
<tr>
<td>Directed Knife</td>
<td>88</td>
<td>25</td>
<td>Evolutionary Triggers</td>
<td>172</td>
<td>48.9</td>
</tr>
<tr>
<td>Directed Snare</td>
<td>81</td>
<td>23</td>
<td>Evolutionary Triggers</td>
<td>172</td>
<td>48.9</td>
</tr>
<tr>
<td>Directed Spider</td>
<td>91</td>
<td>25.9</td>
<td></td>
<td>172</td>
<td>48.9</td>
</tr>
<tr>
<td>Total</td>
<td>N=352</td>
<td>100</td>
<td>Total</td>
<td>N=352</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2. Distribution of significant SCRs elicited (over 0.01 µS) after 1-4s after onset of stimuli presentation extracted from participants’ data after the trough-to-peak analysis.

**Statistical analysis**

Statistical analysis was made with IBM SPSS Statistics (version 23.0) and level of significance was set at p=.05. A test for normality, the Shapiro-Wilk test, revealed that the obtained data were not normally distributed. Normally, studies measuring SCRs arithmetically log the data to make it normally distributed by means of square of root transformation or natural log (Dawson et al., 2000; Venables & Christie, 1980). However, the data was still very skewed after both types of log, which resulted in the use of non-parametric test when statistically analyzing the obtained data.

**Evolutionary versus Modern Threats.** A Mann-Whitney U-test (the non-parametric alternative to independent t-test) was done to determine if there were any difference in mean amplitude of the significant SCRs (µS) between the directed evolutionary fear-relevant stimuli (snakes and spiders) and the directed modern fear-relevant stimuli (guns and knives). Mean amplitude of the significant SCRs (µS) for directed evolutionary fear-relevant stimuli (median=.151, n=172) and directed modern fear-relevant stimuli (median=.128, n=180) were not statistically significantly different, U =14,881, z=-.628, p=.53. Although the graphic overview demonstrated that the direction of mean amplitude of
significant SCRs (µS) were favoring greater automatic responses in relation to evolutionary fearful stimuli (see Figure 3).

Figure 3. Graphic overview over the two categories of directed threatening stimuli.

![Figure 3](image)

Figure 3. Histogram demonstrating the mean amplitude of significant SCRs (µS) of the two categories of threatening stimuli presented directed toward the participants. The evolutionary category has a mean of .37±SD=.66, while the modern category has a mean of .28±SD=.45.

Comparison of the Four Categories of Stimuli. Also a Kruskal-Wallis test (the non-parametric alternative to ANOVA) was run to determine if there were a difference in elicited SCRs (µS) between all four of the different directed stimuli categories. The test showed no statistically significant difference in elicited SCRs (µS) between the different stimuli categories directed toward the participants, $\chi^2_{3} = 2.682$, $p=.443$. Although the graphic overview demonstrated that the direction of mean amplitude of significant SCRs (µS) were favoring greater automatic responses in relation to the presented threatening stimulus of evolutionary significance (see Figure 4).
Figure 4. Graphic overview over the four categories of directed threatening stimuli.

![Figure 4. Histogram demonstrating the mean amplitude of significant SCRs (µS) of the four categories of presented threatening stimuli. Pictures presenting a directed gun demonstrate a mean of .3 \pm SD=.52, directed knife demonstrate a mean of .26 \pm SD=.35, directed snake demonstrate a mean of .38 \pm SD=.76 and directed spider demonstrate a mean of .37 \pm SD=.56.](image-url)
Discussion

The study conducted in relation to this master thesis had in mind to investigate whether or not fear-relevant stimuli of more recent age (modern threats) would have the same effect as threatening stimuli from the period of evolutionary adaptation have on our defense system. The focus of the study was to measure the elicited SCRs of the subjects who participated in the study, as an indication of automatic physiological arousal evoked by the emotional state of fear. The results of the study did not demonstrate any significant differences in amplitude of the elicited SCRs between the two categories, nor when comparing the four types of stimuli (directed spider, directed snake, directed gun and directed knife) presented to the participants during the experimental session with each other. However, the present study suggested a slightly larger effect in mean amplitude for the fear-relevant stimuli present during the period of evolutionary adaptation when compared to the threats of more recent age. This effect was nevertheless minimal and non-significant. Thus, the results of the current study should be interpreted with caution.

The non-significant difference in mean amplitude between the two categories of stimuli could be due to the low statistical power which followed the use of non-parametric tests in the statistical analysis (McKillup, 2012). Also even though a within-subjects design improved the power by controlling the individual differences between the subjects, the sample size turned out quite small - with only 17 participants. A much larger sample size would provide a better estimate whether or not correctly retain the null hypothesis (no differences in mean). In other words, as increasing the power, the risks of a type II error decrease. Hence, based on these statistical factors, the study presented here might have failed to detect an effect that is present (McKillup, 2012).

Additionally, due to the small sample size – 11 males and 6 females – a control for differences in the emotional neural processing due to the sex were not considered in the study.
As suggested by the research of Whittle, Yücel, Yap, and Allen (2011) there are some differences in relation to the sex of the subjects during these kinds of studies. Hence, research has shown that the perceptual emotional processing of females is more associated with greater levels of subcortical and temporal activation than males. Males on the other hand, have greater levels of frontal and parietal activation during the same processes. Differences as these may contribute to faster and more accurate processing of emotional detection and recognition for females (Whittle et al., 2011). Furthermore, differences in activation, dependent on the presented category of emotional stimuli, could as well be apparent. Thus, the neural mechanism of the female sex appears to be more involved during reactivity to negative emotional stimuli (such as fear). Whereas in males, the neural mechanism instead reacts more to stimuli associated with positive stimuli or negative stimuli with cues of interpersonal hierarchy (threatening scenes or faces) (Whittle et al., 2011). Future research of automatic emotional processing might need to consider including the gender of the subjects as a dependent variable in their studies. It would especially be of use if the number of subjects participating is higher, otherwise, with a low amount of participants there might not be enough collected data to get any statistical power to the results as in the present study.

Some other factors need to be considered before any firm conclusions are being made. Even though the amplitude of SCRs is a well-used measure of amygdala activity, attention and emotional arousal during the emotional state of fear. The measurement technique, when used to measure physiological response passive image task, might not be a specific indication of the emotional processes that occur (Laine et al., 2009). SCRs can be spontaneously triggered by other task related factors involved during recording of the data: e.g. daydreaming or movements. Hence, neural activity in the amygdala and its modulation of sympathetic arousal can occur regardless of any stimuli presentation. The fact is that the recorded data might not only reflect the functions of fear, arousal and attention but also
various other functions, triggered in the absence of external stimuli (Laine et al., 2009). Nevertheless, fear as such, are a rather strong emotion and some of the elicited and recorded SCRs when presenting subjects to threatening stimuli can go as high as 8 µS (in relation to the threshold of 0.01 µS used in the present study) (Boucsein, 1992; Boucsein et al., 2012; Dawson et al., 2000). In addition, the recorded responses due to spontaneous neural activity are limited by the use of down-sampling and smoothening of the data (Boucsein, 1992; Boucsein et al., 2012; Dawson et al., 2000). Hence, properly taking care of the data control the interference from spontaneously triggered SCRs.

The behavioral responses of our emotional states due to amygdala activation are not stimulus-specific, it is much more complex (Öhman & Mineka, 2001). To really study the emotional state of fear as such, one might need to consider additional methods of measurements and not only rely upon the results based on the elicited SCRs. As for example, one might include measurements of other closely related behavioral responses of fear such as heart rate acceleration, the activity of the facial corrugator muscle - all of which are important behavioral components of the defense system (Öhman & Mineka, 2001). The current study lacked additional measurements and only relied upon the results on the SCRs elicited by the different categories. Nevertheless, the method of EDA is still a useful measurement to investigate the differences of psychophysiological changes associated to the presented stimuli. Due to the sensitivity of the technique, EDA is still one of the most widely used measurements for emotion-related sympathetic activity and arousal in psychology for the changes that occur (Boucsein, 1992; Boucsein et al., 2012; Critchley, 2002).

Another factor that might have influenced the results of the study is that many of the subjects participating in the study mentioned that they played a fair amount of videogames in their spare time. The problem that arise, is that the choice of pictorial stimuli used in the modern category might not have been as affective as a threat due to this. Mainly because
habituation for those subjects who regularly play first-person shooting games. Some researcher has also questioned the set-up of using guns directed toward the participants (Öhman & Mineka, 2001). Mainly because a pointed gun may be of more extreme character than presenting attacking snakes (Flykt et al., 2007). A pointed gun might as well be deadly from a distance whereas a snake in attack position most often can be coped with by withdrawal from the danger zone (Öhman & Mineka, 2001). More research is in need to make any firm conclusions whether or not this may be the case.

In general, the choice of pictorial stimuli might be a factor that could have influenced the results in many ways. Many of the pictures used - namely all which weren’t obtained from GAPED (Dan-Glauser & Scherer, 2011) - haven’t been rated according to arousal and valance. This lack of control contributes to the fact that the differences in emotional arousal between the categories and type of directed stimuli are not of knowledge.

Although both spiders and snakes are used in the category of evolutionary fear-relevant stimuli in the present study, only snakes have been concluded to be counted as a recurrent threat for humans during the period of evolutionary adaptation (Isbell, 2006). Just recently were snakes also demonstrated to guide attention faster toward these types of threatening stimuli in relation to visual search task, as compared to spiders. That is, snakes may consist of stronger cues of danger than spiders which might have been influencing the evolutionary category used in the current study (Öhman, Soares, Juth, Lindström, & Esteves, 2012). As it is now, it might be that the pictures used in the present study were not sufficiently emotionally salient or that other differences were apparent. Specifically, there might be an alternative explanation for the results rather than age of threat which cannot be controlled for. However, this is just speculations and seems to not have been a problem for aforementioned studies addressing these types of stimuli. It could however be reasonably that the average
emotional salience of the different images ought conceivable to have an impact on relevant physiological ANS responses.

The current study could have included an epoch in which the participant could rate the emotional arousal of the picture after presentation or a pilot study with rating. Hence, if the pictures used would have been rated according to emotional salience, one would be able to better investigate the correlation between the factor of threat and the obtained results of the study.

Another consideration for future research of the emotional state of fear is to take into account how an individual perceives a stimulus. Other factors than just the degree of danger could be linked to the etiology of fear (Armfield, 2006). The first property of a stimuli that one need to take into account is the disgust-evoking properties that some animals might cause. Findings have found that fear of spiders in individuals co-vary to both fear-evoking animals (e.g. snakes) and disgust-evoking animals (e.g. insects). Suggesting that the fear of some animals instead derive from disease-avoidance processes and create misleading results when the focus in mind is to investigate the variable of fear (Armfield, 2006). Another property of a stimuli that one might need to take into account in future studies is unpredictability, that is, the uncertainty of a stimuli: as for example its identity and movements. As well as the uncontrollability properties of a stimulus, specifically, the feeling of lack of control that can affect individuals by means of anxiety (Armfield, 2006). A combination of these properties - disgust, unpredictability and lack of control - could contribute to individuals’ perception of being vulnerable and in turn elicit greater activity of the individuals’ defense system (Armfield, 2006). The current studies used stimuli directed toward the participants to increase vulnerability and the threatening factor, however, future studies might consider using stimuli presented as movies or in a virtual reality to better cope to individuals perceived vulnerability and adequately investigate the complexity of the emotional state of fear.
However, even though several factors may have influenced the results of the current study, another answer to the lack of significant difference between the categories might be apparent. That is, *phenotypic plasticity*, in which the defensive behaviors and responses continuously change throughout development and the lifetime of the animal or human (Wiedenmayer, 2009). Phenotypic plasticity contributes to the fact that mammals adaptively can respond to dangerous environmental threats by means of experiences. This plasticity shape their defense system by influencing the perceptual and motor components of the behavioral defense responses. As some mammals already are able to exhibit useful behavioral responses (fight, fleeing or freezing) the first time encountered with dangerous events, other can also learn by social experience. Learning by social experience - fear learning from family members and environmental conditions - in turn modifying the behavioral defense responses in later life as they learn about the properties of the situation. These molecular and cellular processes of neural development and how they are associated with the defense system are still poorly understood (Wiedenmayer, 2009). Hence, the threat-superiority effect associated with modern fear-relevant objects and entities, could be a function of the phenotypic plasticity.

**Conclusion**

The fact that the results of the present study suggested a slightly larger effect in mean amplitude for evolutionary fear-relevant threats must be interpreted with caution. It might be due to several influencing factors between the used stimuli. Nevertheless, the absence of any significant difference between the two categories are consistent with the studies demonstrating a flexibility of the threat-superiority effect. That our brains are not restricted to dangerous and threatening events with evolutionary significance, but extends to events which did not threaten our survival during the period of evolutionary adaptation. However, if replication is of interest, one should increase the statistical power by the various means noted above.
One thing is certain, the emotional state of fear is a multi-component adaptive system, developed during the period of evolutionary adaptation. Although the results of the current study do not settle the matter, it might be of major importance for our survival that we react just the same to dangerous objects and entities of more modern relevance and not only those threats relevant during evolution.
References


Appendix A

Above. Inserted picture from Ledalab Software demonstrate the collected SCRs data from participant 11 during recording: zoomed in to a time interval of 54.92 seconds. Clearly displaying an external factor influencing the signal. Red lines indicating presentation of pictorial stimuli.

Above. Inserted picture from Ledalab Software demonstrate the collected SCRs data from participant 11 during recording: zoomed in to a time interval of 20.42 seconds. Clearly displaying an external factor influencing the signal. Red lines indicating presentation of pictorial stimuli.
Appendix B

Below. Examples of the pictorial stimuli used as modern threats (directed gun and directed knife) that were taken with own camera.

Below. Examples of the pictorial stimuli used as modern threats (directed gun and directed knife) that were taken from Google search.
Below. Examples of the pictorial stimuli used as evolutionary threats (directed spider and directed snake) that were taken from Google search.