

THE EFFECTS OF BIOLOGICAL RELEVANCE OF STIMULI ON
BLOCKING



BESTE İÇAĞASI

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THE EFFECTS OF BIOLOGICAL RELEVANCE OF STIMULI ON
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ABSTRACT

THE EFFECTS OF BIOLOGICAL RELEVANCE OF STIMULI ON BLOCKING

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This thesis project aims to investigate the blocking effect in human participants by manipulating the biological relevance of stimuli as being arbitrary or within the frame of discriminative fear conditioning paradigm. The blocking effect was examined via four studies which differentiated in terms of the biological relevance of stimuli that were employed in the experiments. In the first study, only arbitrary (geometric figures) stimuli were used, whereas in the second study, only ecological stimuli (snake pictures) were used. For the third and fourth study,

both arbitrary and ecological stimuli were used together. The difference between the studies was the phase that arbitrary or ecological stimuli were presented. Precisely, in the third study, CSs were arbitrary, while the novel stimuli were ecological, whereas in the fourth study, CSs were ecological, while the novel stimuli were arbitrary. A three-phase procedure involving acquisition, blocking, and test was adopted. In acquisition phase, participants were acquired conditioned fear responses through discriminative conditioning paradigm; in blocking phase, compound stimuli were presented; and in test phase, each stimulus was presented alone and skin conductance responses, *US* expectancy ratings, and arousal ratings obtained were recorded as dependent variables. Manipulation analyses were conducted by using dependent *t*-test for SCRs and Wilcoxon signed-rank test for *US* expectancy ratings and arousal ratings. Results indicated that biological relevance of the stimuli had a big influence on the occurrence of the blocking effect.

Keywords: blocking effect, biological relevance, discriminative fear conditioning

ÖZET

BİYOLOJİK ÖNEMLERİ BAKIMINDAN FARKLI UYARICI TÜRLERİNİN BLOKLAMA ÜZERİNDEKİ ETKİSİ

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Bu çalışmada, insanda, laboratuvar koşullarında ayırt edici klasik koşullama yordamı aracılığıyla korku tepkileri edinilmiş bir uyarıcıya, yeni bir uyarıcının eklenmesi sonucunda ortaya çıkan bloklama etkileri, keyfi ve ekolojik uyarıcılar olmak üzere iki farklı uyarıcı türü bakımından incelenmiştir. Bu amaç doğrultusunda, keyfi ve ekolojik olmak üzere iki adet uyarıcı seti hazırlanmıştır. Keyfi uyarıcı seti geometrik figürlerden oluşurken, ekolojik uyarıcı seti yılan resimleri içermektedir. Çalışma kapsamında, işlem yolları aynı dört farklı deney yürütülmüştür. Deneyler sadece kullanılan uyarıcılar bakımından birbirinde farklılaşmaktadır. İlk deneyde sadece keyfi uyarıcılar kullanılırken ikinci deneyde sadece ekolojik uyarıcılar kullanılmıştır. Üçüncü deneyde koşullu

uyarıcılar keyfi, koşullu uyarıcıların yanına eklenen yeni uyarıcılar ekolojik iken; dördüncü çalışmada koşullu uyarıcılar ekolojik, koşullu uyarıcıların yanına eklenen yeni uyarıcılar keyfi uyarıcılardır. Literatürde sıklıkla kullanılan ve (1) ayırt edici klasik koşullama yordamı aracılığıyla koşullu korku tepkilerinin edinildiği edinim aşaması, (2) koşullu korku tepkileri edinilmiş uyarıcılara yeni uyarıcıların eklendiği bloklama aşaması ve (3) bloklama etkilerinin incelendiği test aşamasından oluşan üç aşamalı bir işlem yolu takip edilmiştir. Test aşamasındaki uyarıcı sunumlarına ilişkin ortaya çıkan deri iletkenliği tepkisi, *US* beklentisi ve uyarılma düzeyi bağımlı ölçümler olarak kaydedilmiştir. Test aşamasındaki uyarıcılara verilen deri iletkenliği tepkileri için bağımlı grup *t*-testi kullanılırken, *US* beklentisi ve uyarılma düzeyinin analizlerinde Wilcoxon işaretli sıra testi kullanılmıştır. Çalışma kapsamında incelenen biri otonomik diğerleri bilişsel olan üç bağımlı değişken için elde edilen bulgular, farklı uyarıcı türlerinin bloklama etkilerinin ortaya çıkmasında önemli bir rol oynadığına ilişkin birtakım kanıtlar sağlamıştır.

Anahtar sözcükler: bloklama etkisi, uyarıcı türü, ayırt edici korku koşullaması

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CHAPTER 1: INTRODUCTION

Each organism –from earth worms to humans– has to learn. The idea is simple: neither can we survive nor can we reproduce without learning. The ability of an organism to survive and reproduce constitutes the most vital foundations of evolution. In other words, having that ability is the greatest evolutionary goal for organisms. However, is it possible to achieve that goal unless an organism *learns* how to survive and reproduce? As an illustration, people who failed to distinguish predator from prey and people who could not succeed in choosing a fertile mate could not become our ancestors. On the other hand, people who learnt to adapt to changes in their environment, kept those adaptations and transferred them successfully to the next generations most probably became our ancestors.

However, while people are trying to learn a particular fact or thing, they are continuously being exposed to a bombardment of stimuli at the same time. Some of those stimuli carry important information, whereas some others do not. Organisms selectively attend to their environment by processing some stimuli and eliminating others (Kopell, Wittner, Lunde, Warrick, and Edwards, 1970). Therefore, they cannot

acquire information conveyed by each stimulus. If so, there should be a mechanism that filters out the stimuli which does not provide the organism with important information. Human information-processing system already has such a mechanism; that is selective attention (Broadbent, 1958). Actually, it is an adaptive mechanism for organisms to have selective attention, because if all information around could be attended equally, then the speed of information processing would be decreased. However, it would be ineffective in case of a potential danger. Under such circumstances, organism should be capable of detecting the impending threat rapidly and find out the fittest response in order to survive. Learning how to respond properly to environmental stimuli, which predict potentially destructive events, is an adaptive mechanism vital to survival of any organism (Olsson, Nearing, and Phelps, 2007). However, it would not be implausible to state that it is crucial for organisms to determine which stimuli are related to their survival (e.g., whether it is threatening or not) and reproduction (e.g., whether it contributes to organism's reproductive success or not). The stimuli related to survival and reproduction of the organisms could be referred to as biologically-relevant stimuli (Sakaki, Niki, and Mather, 2012).

The aim of this thesis is to primarily investigate the effect of biological relevance of stimuli on blocking effect in humans within the frame of fear conditioning paradigm. The biological relevance of the stimuli was manipulated by classifying them as arbitrary or ecological. Arbitrary stimulus set involved biologically-irrelevant stimuli, whereas ecological stimulus set involved biologically-relevant stimuli. Before stating the main hypotheses of the study, the insufficiency of temporal contiguity in forming association between two stimuli, the importance of

informational relations in conditioning, the definition of blocking effect, studies conducted on animals and humans and function of biological relevance of stimuli in blocking will be mentioned.

1.1 Temporal Contiguity: The Only Component of Conditioning

Classical conditioning has become one of the most extensively studied learning paradigms under the head of associative learning. Associative learning, as the name implies, occurs when an organism learns the association between two stimuli or events. A standard classical conditioning procedure includes the process of pairing two stimuli. First, there should be a stimulus that reliably elicits a characteristic response: That stimulus is called *unconditioned stimulus (US)* and the response is called *unconditioned response (UR)*. The term *unconditioned* is used to point out that the connection between stimulus and response is unlearned; in other words, it is innate (Mazur, 2006). The third element of the classical conditioning paradigm is referred to as *conditioned stimulus (CS)* which is initially neutral and it elicits no response. As a result of repeated pairings between the *CS* and *US*, previously neutral stimulus begins to elicit a response. The response elicited by the *CS* is called *conditioned response (CR)*. The term *conditioned* is used to signify that the *CS* will elicit a response only after conditioning takes place (Mazur, 2006). In a typical conditioning procedure, a previously neutral stimulus (e.g., a tone) is paired with a biologically-relevant *US* (e.g., food) and consequently begins to elicit a new pattern of behavior (*CR*; e.g., a change in salivation) (Lovibond and Shanks, 2002).

In laboratory conditions, experiments are designed as being either appetitive conditioning or aversive conditioning. In a standard appetitive conditioning

procedure, previously neutral stimulus (e.g., a light) is paired with an appetitive stimulus (e.g., food) (e.g., Wasserman, Franklin, and Hearst, 1974), whereas previously neutral stimulus (e.g., a tone) is paired with an aversive stimulus (e.g., shock) in a standard aversive conditioning procedure (e.g., Rescorla, 1968). A great number of studies have been conducted under the title of both appetitive conditioning, for example, in sexual responses (e.g., Holloway and Domjan, 1993; Mahometa and Domjan, 2005) and aversive conditioning, for example, in eye-blink responses (Woodruff-Pak, Papka, and Ivry, 1996; Wolf, Bauser, and Daum, 2012), autonomic responses (Öhman and Soares, 1993; Balderston and Helmstetter, 2010), conditioned emotional responses (Annau and Kamin, 1961; Suiter and Lolordo, 1971), taste-aversion learning (Garcia, Ervin, and Koelling, 1966), and fear conditioning (Rescorla, 1966; Yoshida and Kondo, 2012).

As mentioned above, associative learning suggests that organisms have a general ability to connect things that occur together (Jozefowicz, 2014) and in order to establish such a connection, stimuli or events should be associated to each other. The association between two stimuli, namely the *CS* and *US*, is established by means of temporal and signal relations. Temporal relations focus on when in time the stimuli occur relative to each other, while signal relations emphasize the informational relations between those stimuli (Domjan, 2005).

Temporal relations are the most referred relations in classical conditioning definitions (e.g., Morgan and King, 1966; Atkinson, Atkinson, Smith, and Hilgard, 1987; Klatsky, 1980; Gardner, 1982; Rosenhan and Seligman, 1984, as cited in Rescorla, 1988). It is supposed that whether two different stimuli are associated is largely determined by how close they occur together in time and if the interval

between two stimuli increases, then they will less likely be associated (Boakes and Costa, 2014). The temporal proximity of the occurrence of two stimuli is defined as temporal contiguity (Shanks, Pearson, and Dickinson, 1989). If the temporal gap between two stimuli is short, then the association will most likely occur (Tourangeau, Murphy, and Baker, 2005). In other words, if the delay between a *CS* and *US* increases in a classical conditioning task, the *CR* will be retarded (Allan, Tangen, Wood, and Shah, 2003).

There are three types of conditioning affiliated with temporal relations (see Figure 1). *Simultaneous conditioning* occurs when a *CS* and a *US* are presented at the same time. For example, a tone (*CS*) and a shock (*US*) are presented at the same time during the experiment. In theory, it involves perfect temporal contiguity between the *CS* and *US*. *Delayed conditioning* occurs when the *CS* is presented slightly before the *US* during the experiment. More precisely, the *CS* is presented and remains on until the presentation of the *US*. For example, a tone (*CS*) is presented and then the shock (*US*) is presented in the middle of tone presentation. It is called delayed because the occurrence of the *US* is delayed after the presentation of the *CS*. The important point in delayed conditioning is that there is no gap between the *CS* and the *US*. The third type of conditioning that is actually obtained from delayed conditioning is *trace conditioning* in which a gap is introduced between the *CS* and *US*; in this way, a delayed conditioning procedure becomes changed into trace conditioning. The gap between the *CS* and *US* is defined as trace interval (Domjan, 2005).

It could be advantageous to consider simultaneous conditioning as the most effective temporal relation to form an association, because it allows bringing the *CS* as near as possible to the *US* (Domjan, 2005). However, in many cases, presenting

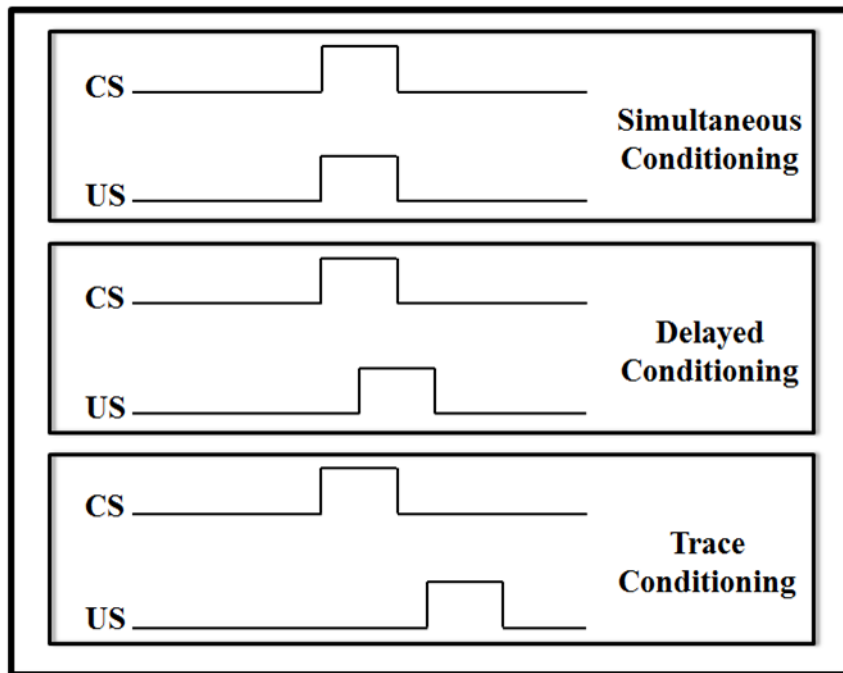


Figure 1. Procedures for simultaneous, delayed, and trace conditioning (adapted by Domjan, 2005).

the *CS* and *US* simultaneously does not provide strong evidence of conditioning. For example, in an earlier study of contiguity in human conditioning conducted by Wolfe (1932), an auditory stimulus (*CS*) and a shock to the finger (*US*) were paired and finger withdrawal to the auditory stimulus was measured as *CR*. By using this procedure, it was little simultaneous conditioning observed, whereas trace conditioning with trace intervals of up to 0.6 second was observed (as cited in Boakes and Costa, 2014). Compatibly, the study conducted by Smith, Coleman, and Gormezano (1969) revealed that groups trained with inter-stimulus interval (duration between the *CS* and *US*) of -50, 0, or 50 milliseconds did not demonstrate any evidence of conditioning, whereas groups trained with inter-stimulus interval of 100 milliseconds or longer demonstrated a remarkable increase in the frequency of responses over trials. These results could be interpreted as a failure of simultaneous conditioning to produce learning. It is possible that organisms are incapable of associating two stimuli that are presented at the same time; therefore a delay could be effective to form an association.

Traditional views have assumed that presenting two stimuli close in time is the most fundamental requirement in order to form an association. Once pairing the *CS* and *US*, a mental link is established between them. Presentation of one of those stimuli (*CS*) most likely activates a representation of that stimulus in memory and in turn activates a representation of the other stimulus (*US*). Consequently, a response that is proper for the second stimulus is elicited by the activation of the representation of that stimulus. Temporal coding hypothesis proposes that temporal contiguity is sufficient in order to form an association. However, components of

association are not limited to a mental link between representations of two paired stimuli (Miller and Barnet, 1993).

Although temporal contiguity is thought to be the main component of classical conditioning, it is neither necessary nor sufficient. Temporal contiguity is only a part of the procedure, because in some cases, conditioning might not occur even if the requirement of temporal contiguity is met. For instance, if order of the *CS* and *US* is reversed, which means the *US* will precede the *CS*, conditioning will not occur. Furthermore, Garcia *et al.* (1966) revealed that conditioning might take place even if the effect of temporal contiguity is minimal, because it was observed that rats could develop an aversion to a specific flavor even when the effect is experienced 12 hours later. These results imply that conditioning does not take place as a simple consequence of temporal pairing of *CS* and *US*. Instead, *CR* emerges if organism has acquired the ability to predict the occurrence of one stimulus -*US*- following the presence of another stimulus -*CS*- (van den Hout and Merckelbach, 1991).

Rescorla (1988) suggested that conditioning is determined by information provided by the *CS* about occurrence of the *US*. This suggestion might be reasonable because when the *CS* provides no information about the *US*, conditioning does not occur. For example, in a study, a rat is exposed to two apparent events: a tone served as a *CS* and a shock served as a *US*. In one of the conditions, those two events are uncorrelated in time which means that the tone provides no information about the forthcoming shock. In the other condition, however, the shock occurs only during the presentation of the tone. The difference between two conditions is based on the information that the tone provides. More precisely, the tone provides no information

about the shock in the first condition, whereas the tone is quite informative about the shock in the second condition. Although both conditions withhold the same contiguity of the tone with the shock, they separate from each other regarding the amount of information that the tone provides about the shock. Therefore, conditioning does not occur in the first condition, while it occurs in the second condition. It means that there should be another component that is responsible for the occurrence of conditioning.

From this point of view, it might be suggested that not only contiguity is responsible for conditioning, but also contingency. Signal relation between the *CS* and *US* has been defined as contingency (Rescorla, 1967). For example, in more complex circumstances, more than one *CS* could precede the *US* either as a form of simultaneous or a serial compound (Balkenius and Morén, 1999). In one of those circumstances, temporal contiguity would not be sufficient, because even if all the *CSs* share the same contiguity with the *US*, which *CS* provides information about the occurrence of the *US* will remain unclear. To illustrate, assume that additional *CSs* are included while the number of paired *CS/US* presentations is held constant. In such a case, the emergence of *CRs* is severely impaired because it will be harder to predict the occurrence of the *US* from the presence of the *CS* and therefore *CRs* become weaker (van den Hout and Merckelbach, 1991).

More clearly, if there is a contingency between two stimuli, it means that the occurrence of one stimulus could be predicted from the presence of other stimulus. In *CS/US* contingency, two probabilities are defined (see Figure 2). First one is the probability that the *US* will occur when the *CS* is presented [$p(US/CS)$]; the second one is the probability that the *US* will occur when the *CS* is not presented

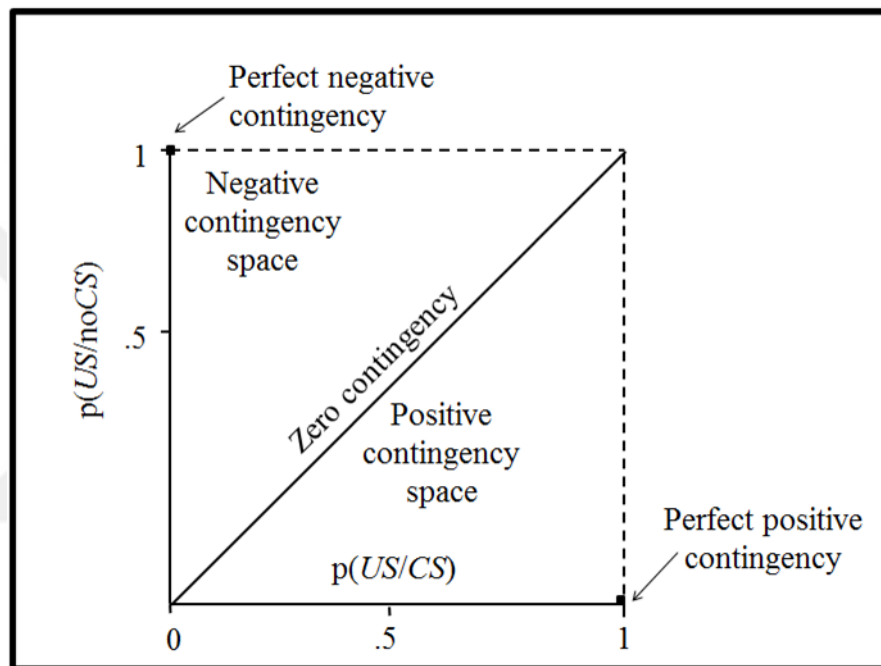


Figure 2. Contingency between a CS and a US (adapted by Domjan, 2005).

[p(*US*/no*CS*)] (Domjan, 2005). For example, green light is always a sign for motion in the traffic. In such a case, a perfect positive contingency takes place between the *CS* and *US*, because the *US* always occurs with the *CS*. On the other hand, a perfect negative contingency takes place when the *US* occurs in the absence of *CS*. For example, the red light signals the absence of motion in the traffic. Lastly, it is possible to mention zero contingency when the *US* occurs equally often with and without the *CS*. In that situation, *CS* has no useful information about the occurrence of the *US*. All these examples mentioned above suggest that conditioning involves prediction of the occurrence of a stimulus from the presentation of another stimulus. It is clear once again that it is not only contiguity that is crucial, but information that the *CS* provides related to the occurrence of the *US*. That is to say that conditioning emerges as a result of active processing of information (van den Hout, and Merckelbach, 1991).

From the evolutionary point of view, it is required that information processing should be functional. An organism will be capable of using predictive information provided by the presence of one stimulus to prepare for the occurrence of a forthcoming stimulus. Predictive information may also provide the organism with an understanding about the causal nature of the environment. The informational hypothesis proposes that conditioning develops only when one stimulus predicts the other stimulus (Miller and Barnet, 1993), indicating the importance of contingency. A similar emphasis on contingency was also made by Rescorla (1966) in a fear conditioning experiment with dogs. There were three groups in which the probability of occurrence of the *US* was the same in the presence and absence of the *CS* in random group; in excitatory conditioning group, the probability of occurrence of the

US in the presence of the *CS* was the same as in the random group but the *US* never occurred in the absence of *CS*. In the inhibitory group the probability of occurrence of the *US* in the absence of the *CS* was the same as in random group but the *US* never occurred in the presence of the *CS*. Subsequent presentations of those stimuli during a free-operant avoidance behavior experiment revealed that fear conditioning to the *CS* was observed in excitatory group, while inhibition of fear was observed in inhibitory group. However, there was no evidence of conditioning in random group although random group and excitatory group received the same number of *CS-US* pairings and differentiated only in that the *US* was uniquely paired with the *CS*. Also, the results of the study was replicated by Rescorla (1968), this time with rats, again indicating the importance of information provided by *CS*, because when the *CS* and *US* were presented in an unpaired manner, the occurrence of the *US* could not be predicted from the presence or absence of the *CS*.

In line with those contingency related developments, earlier traditional approaches that tried to define classical conditioning with only temporal contiguity between the *CS* and *US*, started to be abandoned (van den Hout and Merckelbach, 1991). Modern theories emphasized the predictive or informational relationship between *CS* and *US* (Rescorla and Wagner, 1972; Mackintosh, 1975; Pearce and Hall, 1980 as cited in Eippert, Gamer, and Büchel, 2012). Actually, three substantial studies created a groundbreaking effect in the field of associative learning. All the studies reported that temporal contiguity between two stimuli was not sufficient in forming the association between stimuli. These studies could be united under three main titles: the contingency experiments of Rescorla (1966, 1968), the relative

validity experiments of Wagner, Logan, Haberlandt, and Price (1968), and the blocking effect described by Kamin (1968, 1969).

The results of these studies demonstrated that there is a competition between CSs to become associated with a US. If one CS (CS A) predicts the US better than another CS (CS X), then it will be more strongly associated with the US. Even if both precede the US with equal time; if CS A predicts the US better and CS X does not have predictive power, then the participant may not be capable of associating CS X with the US (Boakes and Costa, 2014). Such a procedure has already been employed in experiments to clarify the cue competition. One form of cue competition is the blocking effect and it might be considered as the best paradigm to explain the insufficiency of temporal relations and the importance of signal relations in forming associations.

1.2 The Blocking Effect: Why Does Conditioning Fail?

The blocking effect is defined as the decrease in conditioning to a stimulus as a result of presenting that stimulus with a CS that already predicts the US (Kamin, 1968, 1969). Briefly stated, when two stimuli (CS A and stimulus X) are presented together in compound and paired with the US, conditioning to one element of this compound (stimulus X) will be decreased if other element of this compound (CS A) was previously paired with the US. Therefore, although stimulus X is contiguous with the US, conditioning to stimulus X is blocked due to the fact that CS A already predicts the US reliably. There are number of reasons for this: (1) organisms could not attend both stimuli simultaneously (Mackintosh, 1975); (2) they could find the novel stimulus irrelevant, in other words, the second stimulus could not provide new

information to the organisms (Mackintosh, 1975); (3) the *CS* and novel stimulus could differentiate in terms of their biological relevance (Köksal, Domjan, and Weisman, 1994); or (4) one or more reasons might be together responsible for that decrease.

For a clearer comprehension of the term –blocking–, a real-life example might be illustrated before introducing the conditioned suppression procedure used by Kamin in order to demonstrate the blocking effect in laboratory conditions. For example, pollen allergy has usually manifested itself in chronic sneezes in spring when pollens begin to float in the air. A person who is allergic to pollen learns that sneezes begin by the time spring arrives. However, what happens if the person takes cold during the spring? Previous experiences of sneezes in spring will make that person attribute the cause of sneezes to spring rather than cold. In this example, the arrival of previously conditioned spring serves as *CS A* and it blocks the conditioning of cold which serves as stimulus *X*, despite pairing cold just as closely as the spring with the sneeze *US*. As the real-life example indicates, the blocking effect demonstrates that what the person learns about one stimulus is affected by the presence of other cues that were already paired with the same *US* (Domjan, 2005).

As mentioned above, Kamin used conditioned suppression procedure in order to explain the blocking paradigm. Conditioned suppression is an aversive classical conditioning procedure in which *CR* is measured by the suppression of positively reinforced instrumental behavior (Domjan, 2005). A typical conditioned suppression procedure involves: the instrumental conditioning trials in which rats, for example, learn to press a response lever in order to obtain food, the classical conditioning trials

in which a *CS* is paired with a *US*, and test trials in which the *CS* is presented alone. Briefly, an aversive stimulus, generally a brief shock, serves as a *US* in a typical conditioned suppression procedure. As a result of repeated pairings of a *CS* and the shock *US*, what is expected is the suppression of ongoing behavior during the presentation of the *CS*.

Since Kamin had introduced the phenomenon, a three-phase procedure involving two acquisition phases (i.e., *A+* and *AX+* trials) and a test phase has been employed in a standard blocking experiment. In Kamin's experiment, two groups of rats, a blocking group and a control group, were tested in a conditioned suppression procedure. In the first phase of the experiment (i.e., *A+* trials), blocking group receives a series of light (*CS A*) trials paired with shock; whereas the control group does not receive the light-shock pairings. In the second phase of the experiment (i.e., *AX+* trials), two groups of rats receive a light-tone (*CS AX*) compound paired with shock. In consequence of this procedure, the rats in blocking group showed a weaker *CR* to the tone (stimulus *X*) than the rats in control group (see Figure 3). This is all to say that little *CR* to the stimulus *X* was observed after *AX+* trials if it was preceded by *A+* trials, meaning that conditioning to stimulus *X* was blocked by the previously conditioned *CS A*. In this case, the contiguity between the tone and shock was not sufficient for the acquisition of a *CR* to the tone.

The blocking effect has revealed that repeated pairings of two stimuli is not sufficient for the formation of an association between those two stimuli. The discovery of the blocking effect has risen against the temporal hypothesis proposing that contiguity is sufficient in order to form an association between two stimuli,

Design of Kamin's (1968) Blocking Experiment				
Group	Phase 1	Phase 2	Test Phase	Result
Blocking	A ⁺	AX ⁺	X	no CR to X
Control	-	AX ⁺	X	CR to X

Figure 3. Design of Kamin's (1968) Blocking Experiment. A⁺ refers the trials in which light was paired with shock. AX⁺ refers the trials in which light and tone compound was paired with shock.

because it has been clearly shown that when a preconditioned *CS* was presented with a novel stimulus and they were paired with the *US*, little *CR* developed to the novel stimulus (Lotz, Vervliet, and Lachnit, 2009). Modern theories accentuating the informational relations have been trying to interpret the results of the blocking effect. For example, selective attention theory asserts that prior training with a stimulus would capture high level of attention to that stimulus; therefore there is a decrease in the probability of attending to any other stimulus presented simultaneously (Mackintosh and Turner, 1971). On the other hand, according to Kamin, no learning occurred related to the added element in the second phase of a standard blocking experiment, because the occurrence of the *US* was already totally predicted by the previously conditioned element. Actually, formation of an association between the *CS* and *US* is based on the surprisingness of the *US*: if the *US* is already predicted by another stimulus, then it is not necessary to form any further association with the new stimulus (Lotz, Vervliet, and Lachnit, 2009).

Actually, there are three main views that account for the blocking effect. Rescorla and Wagner (1972) suggested that a specific reinforcement can support a certain extent of conditioning and the associative strength of a stimulus increases through the conditioning trials. In a standard blocking procedure, the stimulus X may acquire little or no associative strength at the end of AX+ trials, because previously conditioned *CS* has already acquired associative strength close to maximum throughout A+ trials. More clearly, if a *US* is well predicted by a *CS*, then it becomes less effective at supporting any other learning. Therefore, the conditioning to stimulus X does not occur when it is presented as a part of a *CS* AX compound

which *CS A* fully predicts the *US*, meaning that it is less likely to form an association between stimulus *X* and the *US*, and hence observe a *CR* to stimulus *X* (Jones and Haselgrove, 2013). Pearce and Hall (1980), on the other hand, proposed that stimulus *X* displays a decrease in associability prior the training about the relationship between stimulus *X* and the *US*. This is due to the fact that even at the beginning of *AX+* trials, the *US* has already been well predicted by *CS A*; therefore the amount of conditioning to stimulus *X* is limited. The Pearce and Hall model, therefore, supposes that blocking takes place because the presence of *CS A* during *AX+* trials decreases the associative value of stimulus *X*. In brief, the presence of *CS A* successfully predicts the upcoming events, therefore stimulus *X* provides no further information about the *US* and because there is no surprise, stimulus *X* becomes redundant.

Mackintosh (1975) stated that if there is a preconditioned *CS A* that predict the *US*, when a compound stimulus (*CS AX*) is presented, predicting the same *US*; the failure of conditioning to stimulus *X* is not because the *CS A* already attracts the attention but is rather a result of the fact that novel stimulus signals no change in the *US*, predicting nothing more than *CS A* predicts. It could be considered that if this condition is not met, then blocking might not occur. In Kamin's experiment, because both *CS A* and *CS AX* predict a 1 mA shock, stimulus *X* gained little associative strength. However, if *CS AX* predicts a stronger shock than that was predicted by *CS A* in *A+* trials, or no shock at all, then significant excitatory or inhibitory conditioning occurs to stimulus *X*.

In the light of elaborative explanations accounting for the blocking effect, there has been an increase in the number of studies intended to investigate the basis of conditioned behavior. Those studies, in which animals such as pigeons, rats, and rabbits are used as subjects, have demonstrated the blocking effect successfully in both behavioral (e.g., Illich, Salinas, and Grau, 1994; Köksal, Domjan, and Weisman, 1994; Kim, Krupa, and Thompson, 1998) and neural level (e.g., Kim, Krupa, and Thompson, 1998; McNally, Pigg, and Weidemann, 2004). For example, Illich *et al.* (1994) revealed that rats presented with the CS AB compound after pre-training with CS A, failed to learn about CS B, whereas this was not observed in group without pre-training with CS A.

Although the blocking effect has been well demonstrated with nonhumans, it seems difficult to report the blocking effect in humans, because the studies conducted with humans have conflicting results. A number of studies have failed to demonstrate the blocking effect in humans (e.g., Lovibond, Siddle, and Bond, 1988), whereas a number of studies have been able to reveal results in which the blocking effect can be observed (e.g., Hammerl, 1993). According to Arcediano, Matute, and Miller (1997), the conflicting results observed in studies with human participants may have resulted from fundamental differences in information processing between humans and nonhumans or methodological differences between human and animal studies. First, although numerous learning theories have asserted the existence of a common interspecies mechanism; it might not reflect the reality since there are some fundamental differences between humans and nonhumans in terms of information processing. Second, the problem might have originated from the methodological

differences. Therefore, at least one of the reasons mentioned above would account for the conflicting results obtained from the studies conducted with human participants. However, when taking the interspecies common neurophysiological mechanism that makes learning possible into consideration, the origin of problem may have its roots in methodological differences.

Conditioned suppression procedure, for example, is frequently used as a behavioral measure in conditioning experiments with animals (e.g., Annau and Kamin, 1961) and it is known as a nonverbal procedure. On the other hand, verbal assessment of causal judgment is one of the most common ways of measuring the dependent variable in human participants (e.g., Tourangeau *et al.*, 2005). In such a procedure, participants are required to verbally express the probability of the occurrence of an effect from a preceding cause following the introduction of causes and effects. Dependent variables could lead to some problems when they are in form of verbal responses. For example, Matute, Arcediano, and Miller (1996) reported that there was a strong influence of the manner during the statement of test question on the results that were obtained. It could be the reason why the blocking effect cannot be observed in human participants. The blocking effect may be investigated only via nonverbal procedures like it has been studied with animals.

Nonverbal procedures have already been used to demonstrate the blocking effect in human participants, such as electrodermal conditioning (Lovibond *et al.*, 1988; Hinchy, Lovibond, and Ter-Horst, 1995) and eye-blink conditioning (Martin and Levey, 1991). However, Lovibond and his colleagues did not report any evidence of blocking by using electrodermal conditioning. Martin & Levey (1991)

reported conflicting results by providing evidence of blocking in within-subjects design but not in between-subjects design. Lack of evidence despite the usage of nonverbal procedures resulted from the three-phase-design of the blocking experiments. Hinchy *et al.* (1995) suggested that human participants may have difficulty in integrating the three phases. Although there is no interruption between three phases; participants might perceive them as independent experiments. The reason behind this might be that in the first phase stimuli are presented one by one, in the second phase they are presented in compound, and in the test phase they are one by one again. Therefore, Hinchy and his colleagues developed a single-phase-design in which CSs that are presented in the first phase are also presented in the second phase in order to maintain the associations acquired in the first phase. By this way, it is expected that participants will be able to transfer what they have already learned in the first phase into the second phase easily.

Previous failures stemming from a clearly separated phase structure and visual cues with semantic content (e.g., Lovibond *et al.*, 1988) might have diverted attention away from experimental contingencies. Therefore, a study was conducted by Hinchy *et al.* (1995) in which pre-training and compound training trials were intermixed and the transition to the test phase was masked for removal of phase boundaries. Along with a cognitive measure that was *US* expectancy, an autonomic measure (skin conductance response –SCR–) was also used in order to evaluate the relationship between autonomic and cognitive measures. These measures are usually seen as reflecting two distinct processes that are asserted to underlie human conditioning. The process indexed by autonomic measure is said to be primitive,

unconscious, and reflexive. On the other hand, the other process indexed by self-report expectancy measures is said to be higher-order and propositional (Dawson and Furedy, 1976; Razran, 1955 as cited in Hinchy *et al.*, 1995).

After the study conducted by Lovibond *et al.* (1988), it was assumed that the use of colored photographic slides of natural objects like mushrooms, lakes, and flowers made some of the participants establish a connection between slides based on their semantic content. Under such a case, participants could miss the main point of the experiment while they were trying to think about the content of slides and therefore the blocking effect would not be observed. Thus, Hinchy *et al.* (1995) employed simple colored squares as stimuli in order to prevent the establishment of possible connections about the content of CS. Participants were required to find out which individual colors caused shock, which avoided shock, and which were irrelevant to shock.

In the study, a blocking group was compared with an overshadowing group which served as a control group. Both of the groups received the same training except that the target cue was paired with the shock US in compound with a novel cue rather than a pre-trained cue in overshadowing group. More precisely, for blocking group, the target C cue was presented in compound with the A cue and the A cue was also reinforced individually and presented in compound with E. On the other hand, for overshadowing group, the target C cue was presented in compound with the D cue and the D cue was not reinforced again. In the test phase, both of the groups received one reinforced and two unreinforced trials. At the end of the test phase, the discrimination between A+ and C- would be interpreted as an indicator of

blocking in both autonomic and cognitive measures. It could be considered that masking transitions between phases and replacing meaningful photographic stimuli with simple color blocks are useful preparations for demonstrating the blocking effect in human participants.

In another study, Arcediano *et al.* (1997) investigated the blocking effect by using a conditioned suppression procedure in human participants. First, participants learned to press the space bar of a computer keyboard consistently; second, two phases of a standard blocking experiment were administered while participants were pressing the space bar; and finally, the target *CS* was presented in the test phase and suppression of space bar pressing behavior was measured. During the first phase, *CS A* predicted the occurrence of the *US*, while *CS B* predicted the absence of the *US* for blocking group, whereas *CS A*, *CS B*, and *US* were unpaired in control group. On the other hand, second phase of training and the test phase were exactly the same for both groups. During the second phase, a compound stimulus composed of *CS A* and stimulus X (*CS AX*) predicted the occurrence of the *US* and *CS BY* predicted the absence of the *US*. In the test phase, stimulus X was presented to all participants and suppression of space bar pressing behavior was measured. *CS B* and *CS Y* served as distractor stimuli and they never predicted the occurrence of the *US*. In order to prevent excessive generalization in simple computer-based tasks, the presentation of irrelevant stimuli together with the critical stimuli is observed frequently in human studies.

The experiment was conducted via a video game. In pre-training, the aim was to train each participant to press the space bar steadily. If participants press the space

bar before a Martian appears, an explosion occurs instead of the appearance of a new Martian. The aim of participants is to fill the screen with explosions and not with Martians. They are allowed to press the space bar once before appearance of each Martian. If the space bar was pressed more than one, instead of an explosion, a Martian appeared. Immediately after pre-training, two training phases and the test phase were administered. Those phases were combined with the operant task, in other words, the *CSs* and *USs* were presented in course of the Martian task. In conditioning trials, participants are told that the Martians have developed an anti-laser shield which reflects the shot back. Martians technique works only if participants shoot their laser-gun when the shield is connected. A white flashing on the screen indicates whether the shield is active or not. However, some other indicators help participants understand when the shield is about to be connected, but some false cues are displayed as well. Then participants are required to distinguish between the true and false indicators. The *US* was the white flashing on the screen; *CS A* and *CS B* were blue and yellow backgrounds; and stimulus X and Y were two distinct tones presented via headphones.

The results showed that participants in control group suppressed stimulus X more than the participants in blocking group during the test phase which was an indicator of the blocking effect. It was revealed that the blocking effect observed in animals is comparable to humans, because the blocking effect may manifest conditioned suppression procedure in both species. Moreover, the result provides a new line of evidence to the idea that there are learning and behavior processes shared by different species, meaning that methodological differences account for the

problems encountered during the study of human blocking better than fundamental differences in information processing between animals and humans.

Crookes and Moran (2003) aimed to examine the blocking effect in terms of age and gender differences by using a computerized task, namely the mouse in the house. In the task, there is a mouse icon being moved on the plan of a house in order to detect the location of invisible target squares which are cheeses. Participants are asked to form associations between two sets of color which serve as *CSs* and invisible target locations which serve as *USs* in order to find out the cheese. The game is composed of two phases: a conditioning phase and a blocking phase. As distinct from other studies, a blocking score is calculated for each participant based on the latencies in finding the cheese for each *CS*. The results showed that the blocking effect was observed in all age groups (6-8 years; 9-12 years; 13-17 years; 18-21 years; and 22+ years). On the other hand, females had higher blocking scores than males. This result contradicted findings from previous studies, however; the researchers noted that male participants were more experienced with using the mouse icon than female participants and they had more experience with computer games.

Prados (2011) also provided evidence that the blocking effect can be observed in humans. The aim of the study was to investigate cue competition effects such as overshadowing and blocking by using geometric cues. Participants are presented the image of the plan of a room on computer screen. The plan involves different locations marked by action buttons and participants receive the instruction that they need to obtain some food located in the room in order to feed the three blind mice of the story. Therefore, participants are required to single out the location of the food

among others. In the first phase, participants are asked to find where the food is located in one of the vertices of the blocking shape in blocking group, whereas in control group participants are required to find where the food is located by using the geometric properties of the target shape. In the second phase, participants are required to find where the food is located during the presence of the blocking and target shapes in both blocking and overshadowing groups. In test phase, all of the participants are required to identify where the food is located by using the target shape. For both experiments, the results showed that blocking group had lower performance in identification of where the food was located than the overshadowing and control groups, indicating that prior training of a shape that provides information about the goal location leads to blocking. In brief, consistent results obtained by Hinchy *et al.* (1995), Arcediano *et al.* (1997), Crookes and Moran (2003), and Prados (2011) indicated that the blocking effect can be observed in humans explicitly in behavioral level.

Eippert, Gamer, and Büchel (2012) conducted a study in an attempt to investigate the blocking effect via functional magnetic resonance imaging (fMRI) in order to examine human amygdala responses in aversive learning. As in other blocking studies mentioned above, the three-phase-procedure was followed; however a within-subject rather than a between-subject design was used. In the experiment, CSs are visual stimuli that are abstract colored shapes on a white background and US is painful electrical shocks. In the first phase of the experiment, participants receive only CS A and CS B presentations in order to demonstrate that CS A is paired with the US, whereas CS B is not. In the second phase of the experiment, stimulus X is

added to the presentation of *CS A* and they are presented together as a compound stimulus, as are *CS B* and *CS Y*. Both compounds which are *CS AX* and *CS BY* are paired with the *US*, while *CS A* and *CS B* are also presented alone in order to maintain the original associations which means that transitions between phases are masked.

As is known, the blocking effect is developed during the second phase, because the *US* is already predicted by *CS A* due to the fact that they are paired with each other in the first phase. When *CS AX* is presented as a compound, stimulus *X* becomes redundant because it does not provide additional information about the occurrence of the *US*. Therefore, *CS BY* is presented as a control condition. Although stimulus *X* is redundant, stimulus *Y* is not, because it actually predicts the *US*. In test phase of the experiment, stimulus *X* and *Y* are presented alone without pairing the *US* in order to test this assumption. Also, *CS A*, *CS B*, *CS AX*, and *CS BY* are presented again as paired with the *US*, except *CS B*, in an effort to maintain prior associations.

During the experiment, participants are required to perform a simple reaction time task that measures their vigilance level by pressing a button indicating which side *CS* appears on the screen (i.e., right or left). Moreover, participants are asked to rate the level of fear they are experiencing during presentation of each *CS* and also their awareness of contingency is assessed. Additionally, SCRs, heart-rate (HR) changes, eye-tracking data, fMRI data, and behavioral data are analyzed.

In neural level, amygdala responses indicated significant blocking effect within the context of aversive learning. Also, attentional factors played a role during the

development of the blocking effect and prefrontal areas were involved by flexibly altering their coupling to the amygdala. In behavioral level, significant fear ratings and *US* expectancy ratings were observed. In terms of autonomic responses, while acceleratory HR changes demonstrated the blocking effect, deceleratory HR and SCRs did not demonstrate such an effect. Furthermore, despite the fact that participants had significantly longer fixations on the predictive parts of the compound, they were still fixated to the parts which did not predict the *US*. This result is consistent with the results of another eye-tracker study which revealed that gaze duration was decreased for the blocked stimulus (Kruschke, Kappenman, and Hetrick, 2005). It would be concluded that participants detect the novel stimulus; however, because the novel stimulus does not provide any new information, it becomes irrelevant and conditioning to that stimulus does not occur.

As is seen, a great deal of evidence that reveal the blocking effect is available in literature. However, in some cases, the attenuation of blocking could also be observed. For example, if *CS AX* is paired with a stronger shock than that was predicted by the *CS A* during *A+* trial, a significant conditioning would occur to stimulus *X*. The stronger shock is found unpredicted or surprising and therefore served as an effective reinforcement. Also, as Kamin suggested, blocking might be attenuated also by adding a second shock after each compound presentation, thus the second shock becomes surprising again. As it can be understood, attenuation of blocking might take place only when the added stimulus signals a surprising *US* (Dickinson, Hall, Mackintosh, 1976). However, attenuation of blocking might also be observed by manipulating the biological relevance of stimuli.

1.3 How the Blocking Effect Functions When the Biological Relevance of Stimuli is Manipulated

The general process learning theory postulates that the principles of learning are valid across a wide range of stimuli and responses (Domjan, 2000). The main assumption of general process learning theory is that all events are equally relatable and they comply with common laws (Seligman, 1970). The principle of equipotentiality, which is considered as the foundation of general process learning theory, proposes that learning mechanisms are independent from the specific combinations of stimuli, responses, and reinforcements (Domjan and Galef, 1983). However, it had been revealed that rats have a tendency to associate gustatory or olfactory cues with internal outcomes, such as illness, even if there is a long delay between those cues and illness. On the other hand, they easily form an association between auditory, visual, or tactile cues and external outcomes, such as a shock (Garcia *et al.*, 1966). Therefore, it is possible to state that the principle of equipotentiality does not apply to all circumstances.

Following the challenges to the general process learning theory, one of the possible explanations that accounts for the failures of learning in specific circumstances suggests that there are “biological constraints on learning”. If there is a constraint on a phenomenon, it means that there is a limitation or boundary condition for its emergence (Domjan and Galef, 1983). The reason why organisms lack the ability to associate all stimuli, responses, and reinforcements might be their specialized adaptations (Domjan, 2000). The ability of organisms to associate stimuli and reinforcements could be constrained when particular stimulus-reinforcement

combinations may fail to produce changes in responding, whereas other combinations end up with remarkable changes in behavior and this is referred to as stimulus-reinforcement interaction (Shapiro, Jacobs, and LoLordo, 1980). As in rats, humans also could not attribute the reason of their nausea, for example, to a song that they have just listened. It might be derived from an adaptation that is necessary in order to survive, because it is functional to use gustatory cues rather than auditory cues to detect poisonous food that was associated with nausea in ancestral environment. It would be plausible to suggest that such an adaptation may be transmitted to modern-day humans.

An interaction between stimulus and reinforcement in pigeons was noted by Foree and LoLordo (1973). A group of pigeons are trained to press a pedal either to avoid shock or to get food when they are presented with a compound auditory-visual discriminative stimulus. Then, the elements of the compound stimulus that composed of red house light and a pure tone are presented one by one. In the test phase, it was revealed that the auditory stimulus controlled much more responding than the visual stimulus in shock avoidance condition did, whereas the visual stimulus controlled more responding than the auditory stimulus in appetitive condition did. Therefore, it might be concluded that the dominant stimulus in avoidance condition was the tone, while in appetitive condition, it was red house light. This result may be explained again within the context of evolution since pigeons have to search the environment visually in order to detect the location of food. Therefore, visual cues might serve as effective stimuli in appetitive conditioning.

Another line of evidence against the general process learning theory is provided by manipulating the nature of *CS*, precisely, the degree to which *CS* is a natural predictor of the *US* in the ecology of the organism (Domjan, Cusato, and Krause, 2004). Actually, it means that the more ecologically relevant *CS*, the more rapid conditioning takes place (Domjan, 2016). It could be due to the fact that ecologically relevant stimulus is meaningful to the organism either in terms of survival or in terms of reproduction. For example, Cusato and Domjan (1998) revealed the effect of a taxidermically designed female head as *CS*. A terry cloth object with female cue and a similar terry cloth object without female cue were compared in terms of *CR* they elicited. The terry cloth object with female cue was more likely to trigger conditioned copulatory responses which were grabs, mounts, and cloacal contact than the terry cloth object without female cues did. It is possible to say that the terry cloth object with female cue serves as an ecologically relevant stimulus, whereas the terry cloth object without female cues serves as an arbitrary stimulus meaning that it does not have any inherent relation to the *CS* (Domjan *et al.*, 2004).

In the context of the blocking effect, most of the studies have been conducted with food or shock reinforcement and arbitrary *CSs* were selected except for a few studies. As mentioned above, conditioning is observed easily if *CSs* and *USs* are relevant to each other than arbitrarily combined *CSs* and *USs*. Therefore, it may be assumed that relevance of stimulus might also affect the process of blocking. For example, conditioning of an added stimulus that is more relevant to the *US* may be less readily blocked by a *CS* that is already paired with the *US* (Köksal *et al.*, 1994).

The study conducted by Köksal *et al.* (1994) examined the blocking effect by manipulating the nature of the added CS in the sexual response system of male quail. The US is accessing to a live female quail, while the CS was the same audiovisual cue in all of the experiments. After pairing the audiovisual cue with the access to a live female quail, a novel stimulus (CS2) is added. In consecutive experiments, the added CS takes a more quail-like form and becomes more effective in supporting copulatory behavior. In the first experiment, CS2 is a rectangular wood block that does not look like a female quail and cannot support copulatory behavior. In the second experiment, CS2 is a terrycloth object that does not have quail parts but can support copulatory behavior. In the third experiment, CS2 is a terrycloth object that has a taxidermically designed head of a female quail. The terrycloth-only CS2 that is used in the second experiment had more rapid conditioning than the rectangular wood block that was used in the first experiment did; however the blocking effect was observed for both stimuli. However, a taxidermically designed head of a female quail that was used in the third experiment cannot be blocked by a previously paired arbitrary audiovisual cue. It shows that an arbitrary stimulus can become insufficient to block an ecological stimulus.

LoLordo, Jacobs, and Foree (1982) investigated the blocking effect in pigeons within the scope of fear conditioning paradigm. A red house light CS is presented in the first phase, a compound stimulus that is composed of the red house light and tone is presented in the second phase and both stimuli are presented individually in the test phase of the experiment. The results indicated that the pre-training of the visual CS does not block the formation of an association between tone and shock. It can be

concluded that the associations between an auditory CS and shock are resistant to the blocking effect. Actually, this result is not surprising, because Foree and LoLordo (1973) had already shown that auditory stimulus is effective in aversive conditioning of pigeons, whereas visual stimulus is not. It means that auditory stimulus is relevant for pigeons in aversive conditioning. Thus, as in sexual conditioning (Köksal *et al.*, 1994), an ecologically relevant CS is less susceptible to the blocking effect also in fear conditioning.

In this thesis project, the blocking effect was investigated by manipulating the biological relevance of stimuli as being arbitrary or ecological within the frame of fear conditioning paradigm. Arbitrary stimulus set was composed of geometric figures, while ecological stimulus set was composed of snake pictures as CSs. Although fear might be elicited by a lot of stimuli, extreme fears tend to gather around objects and situations which are fear relevant in a phylogenetic rather than an ontogenetic aspect (Marks, 1969; Seligman, 1971 as cited in Öhman and Mineka, 2001). Therefore, pictures of snakes were employed in the experiment as ecological stimuli, both because picture of a snake generates fear and it has a biological relevance to participants from phylogenetic point of view.

In the first study, all stimuli were arbitrary, whereas in the second study, all stimuli were ecological. In the third study, CSs were arbitrary, while new stimuli were ecological. On the other hand, in the fourth study, CSs were ecological, while new stimuli were arbitrary. By manipulating the biological relevance of stimuli, it was basically assumed that (1) when both the CS which had been already paired with the US and the new stimulus are neutral (i.e., arbitrary), then the new stimulus might

be blocked by the *CS*; (2) when both the *CS* which had been already paired with the *US* and the new stimulus are something meaningful to the organism (i.e., ecological), then the new stimulus might be blocked by the *CS*; (3) when the new stimulus is something meaningful to the organism (i.e., ecological) while the *CS* which had been already paired with the *US* is neutral (i.e., arbitrary), then the new stimulus might not be blocked by the *CS*; and (4) when the *CS* which had been already paired with the *US* is something meaningful to the organism (i.e., ecological) while the new stimulus is neutral (i.e., arbitrary), then the new stimulus might easily be blocked by the *CS*.

For the first two studies, it was aimed to test the standard blocking procedure, therefore it was expected to demonstrate the blocking effect. However, for the other studies, it was aimed to test the biological relevance of the stimuli on blocking. Specifically, for the third study, it was expected not to demonstrate the blocking effect due to the stronger biological relevance of new stimuli compared to *CS*s. For the fourth study, again it was expected to demonstrate the blocking effect due to the weaker biological relevance of new stimuli compared to *CS*.

CHAPTER 2: METHOD

This thesis project aims to investigate the blocking effect by manipulating the biological relevance of stimuli as being arbitrary or ecological and creating physiological fear responses in human participants in laboratory conditions within the frame of fear conditioning paradigm. The blocking effect was examined via four studies which differentiated in terms of the biological relevance of stimuli that were employed in the experiments. Accordingly, only arbitrary stimuli (geometric figures) were used in the first study, whereas only ecological stimuli were used in the second study. When it comes to the third and fourth study, both arbitrary and ecological stimuli were used simultaneously. However, the difference between these studies was the phase that arbitrary or ecological stimuli were presented. In order to put it more explicitly, in the third study, *CSs* were arbitrary, while the novel stimuli presented together with *CSs* were ecological, whereas in the fourth study, *CSs* were ecological, while the novel stimuli presented together with *CSs* were arbitrary. There was no difference between the studies with regards to research design, psychophysiological stimulation and assessment, data acquisition system, procedure,

or preparation of skin conductance data which was collected for analysis.

2.1 Participants

In all studies, a total of 120 undergraduate and graduate students, and academic staff (61 females and 59 males) of İzmir University of Economics participated in the experiment by using a convenience sampling technique. The age range of the participants was between 18 and 36 years ($M = 21.67$, $SD = 3.18$) (see Table 1 for detail).

Before the experimental process, a number of elimination criteria were determined in order to assign participants and to acquire eligible data. These elimination criteria were:

- having a cardiovascular disease and/or cardiac pacemaker
- having a neurologic and/or psychiatric diagnosis or treatment
- having a history of phobic disorder
- having a visual impairment
- having participated in any experiment with electrical stimulation within a year

Individuals who had one or more of those elimination criteria were not allowed to participate in the experiment. Also, if there was a case of power outage, machinery breakdown, or misplacement of electrodes during the experimental sessions, data acquired by those participants were removed from analyses.

Table 1. *Detailed information of participants based on gender and age.*

	Number of Participants	Age Range of Participants
Study 1	16 females and 14 males	18 and 27 years ($M = 21.43$, $SD = 2.22$)
Study 2	14 females and 16 males	18 and 35 years ($M = 22.83$, $SD = 4.04$)
Study 3	13 females and 17 males	18 and 36 years ($M = 21.60$, $SD = 3.72$)
Study 4	18 females and 12 males	18 and 26 years ($M = 20.80$, $SD = 2.04$)

2.2 Stimuli, Apparatus and Material

2.2.1 Stimuli

The arbitrary stimulus set was created by using Windings type font and symbols found in Microsoft Office 2010. Hence, the arbitrary stimulus set composed of six different geometric figures which were white and on a black background (see Figure 4)

The ecological stimulus set composed of six snake pictures (see Figure 5). Those pictures were obtained from the International Affective Picture System (IAPS). In the process of picture selection, a certain type of affective ratings –arousal– which were found in IAPS Manual were used and six snake pictures with arousal ratings of 5.5 and over on a 7-point scale formed ecological stimuli. A mild electrical stimulation was used as *USs*.

2.2.2 Participant Evaluation Form and Inform Consent Form

A participant evaluation form composed of a number of questions that intended to assess whether a candidate for participation had any of elimination criteria which were mentioned in *Participants* section (see Appendix A). By means of this form, participants were required to give information about previous and current psychological, neurological, and cardiovascular health and wellbeing, their medication status, visual acuity, and participation history in previous experiments. A few sample question were given in the below.

- Have you ever been participated in any other experiment? If so, please give brief information about that experiment and date of your participation.

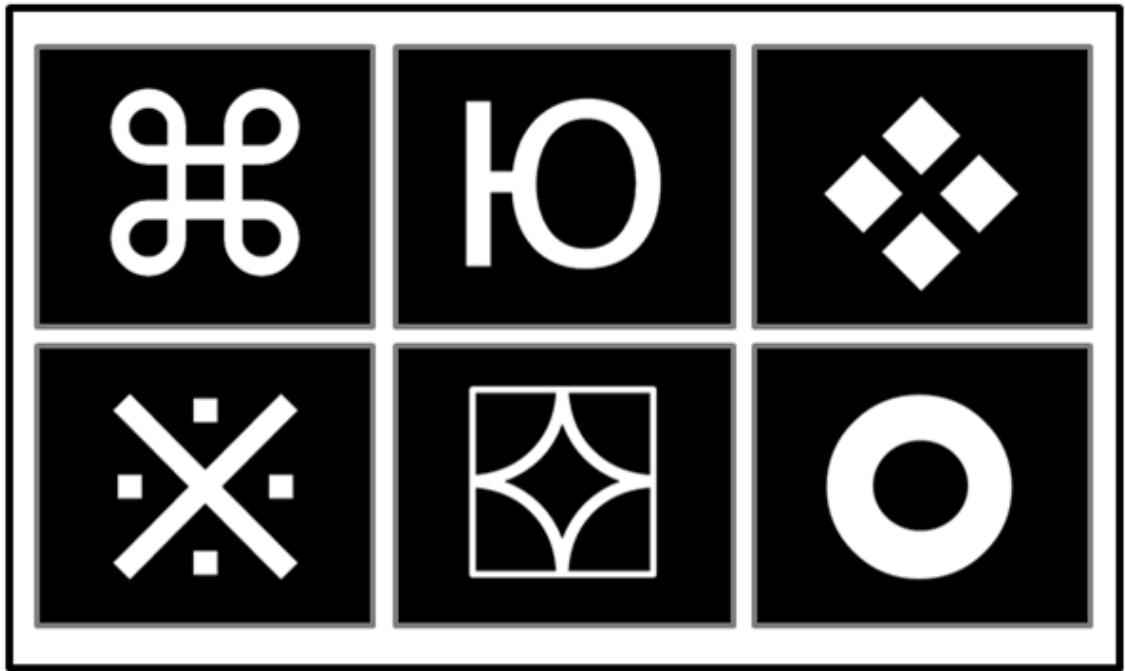


Figure 4. Arbitrary stimulus set.



Figure 5. Ecological stimulus set.

- Have you ever been diagnosed with any psychological disorder?
- Have you ever been on medication?
- Do you have any visual impairment such as myopia, hyperopia, or astigmatism?

An informed consent form was developed to be used in the process of informing participants about the aim of the study and the procedure of the experiment (see Appendix B). With this form, participants were explained that accepting or rejecting to participate was voluntary. Therefore, the participation was completely based on a voluntary basis and if any participant wanted, then that participant would quit the experiment.

2.2.3 Stimulus Presentation Program

Within the scope of this thesis project, four experiment programs were prepared by using SuperLab™ (Version: 4.5, Cedrus, Inc.), experiment builder software. Each of the experiments started with a five-minute relaxation period where the participants were asked to relax. The first two minutes of this relaxation period included a number of instructions in order to ensure that the participants adapted to the experimental setting and the measurements were taken accurately. Rest of the relaxation period included a presentation of a countdown which demonstrated how much time remained until the beginning of the experiment and the participants were required to relax as much as they could. After the end of the relaxation period, experimental trials began.

In the acquisition phase, within the scope of discriminative conditioning paradigm a *CS* (*CS A*) which was paired with the *US*; a *CS* (*CS B*) which was not

paired with the *US*; and a *CS* (*CS C*) which was also not paired with the *US* were presented. In the blocking phase, a new stimulus (*CS X*) was added to the *CS A* which had already been paired with the *US* in the acquisition phase of the experiment; a new stimulus (*CS Y*) which was paired with the *US* was added to the *CS B* which had not paired with the *US* previously; and a new stimulus (*CS Z*) which was not paired with the *US* was added to the *CS C* which had also not paired with the *US*. Thus, three compound stimuli which were (*[CS A + CS X]*, *[CS B + CS Y]*), and *[CS C+ CS Z]* were presented in the blocking phase of the experiment. Lastly, in the test phase, each stimulus (*CS A*, *CS B*, *CS C*, *CS X*, *CS Y* and *CS Z*) was presented alone –in other words they were presented separate from each other– and the physiological fear responses, *US* expectancy scores, and arousal levels of the participants were recorded. The inter-trial-intervals were 10 seconds; the presentations of the *CS*s took 4000 milliseconds; and the presentations of the *US*s began in the last 200 milliseconds of the presentation of the *CS*s. All these phases were identical except for the stimuli presented in all of the experiments.

2.2.4 Research Design

The design of the study was repeated measures design. *SCR*s, *US* expectancy ratings and arousal ratings obtained during the presentation of test stimuli in the test phase of the study were recorded as dependent measurements. Accordingly, *SCR* was measured with an electrodermal activity (EDA) data acquisition system (Model: GSR100C / MP150WSW-G BIOPAC Systems, Inc.); *US* expectancy was measured via a five-point Likert scale; and arousal was measured by using an arousal scale

which had been used for the purpose of evaluating the arousal level of the stimuli found in the database of International Affective Picture System (IAPS).

2.2.5 Psychophysiological Stimulation and Assessment

A bar electrode (Model: EL350; BIOPAC Systems, Inc.) was employed in order to develop the physiological fear responses in the participants. The bar electrode was placed to the right inner wrist through the medium of a plaster (see Figure 6a). Before placing the electrode to the right inner wrist, the area of the electrode was cleaned by means of a cotton with alcohol and then a small dollop of electrode cream (Model: EC2; Grass Technologies) was put to the electrode.

In order to convey the electrical stimulation, a linear isolated stimulator (Model: STMISOLA; BIOPAC Systems, Inc.) which was charged by a stabilized current was employed. This device meets the standards of “Safety: UL 3101-1, CSA C22.2 No. 1010-1, EN 61010-1 Other European Standards: EN 55011, EN 50082-1 European Directives: 73/23/EEC, 89/336/EEC”. Before the beginning of the experiment, the level of electrical stimulation was adjusted by the participants with the assistance of researcher. The initial level of electrical stimulation was about 20V. After 20V was tested, the participants were required to adjust the level of electrical stimulation to an “uncomfortable but not painful” level within three trials. The electrical stimulation during the test trials was given for 100 milliseconds, whereas the electrical stimulation during the experimental trials was given for 200 milliseconds. The highest stimulation level in the experiment, which was 60V, was determined on the basis of previous studies (Schiller et al., 2010).

Disposable snap electrodes (Model: EL507; BIOPAC Systems, Inc.) were applied in order to measure SCR derived from EDA. Two pieces of electrodes with isotonic gel were applied to the palm of the left hand (see Figure 6b).

2.2.6 Data Acquisition System

In course of the experiments, EDA was acquired by means of MP150WSW-G Data Acquisition System which was linked to the Bionomadix Wireless Pulse and EDA Amplifier BN-PPGED with a Universal Interface Module UIM100C (BIOPAC Systems, Inc.). For the purpose of connecting MP system to the computer operating stimulus presentation programs in order to isolate digital inputs and outputs to and from the MP system, an isolated digital interface (Model: STP100C; BIOPAC Systems, Inc.) module was used.

AcqKnowledgeTM (Model: 4.2; BIOPAC Systems, Inc.) software was employed in order to record the data and to make offline analysis of the data possible. This software was run by another computer (Intel[®] CoreTM i5- 2400CPU, 3.10 GHz, 4 GB of RAM) which was connected to a 21.5" monitor, with a screen resolution of 1920*1080 pixels, and refresh rate of 60 Hz in a control chamber, which was found next to the experimental chamber where the experimental task was conducted, for real-time monitoring of the measurements.

2.3 Procedure

The studies about the blocking effect which have been conducted with human participants have usually included a three-phase procedure (Arcediano *et al.*, 1997; Prados, 2011; Eippert *et al.*, 2012). Therefore, the experiments in this thesis project

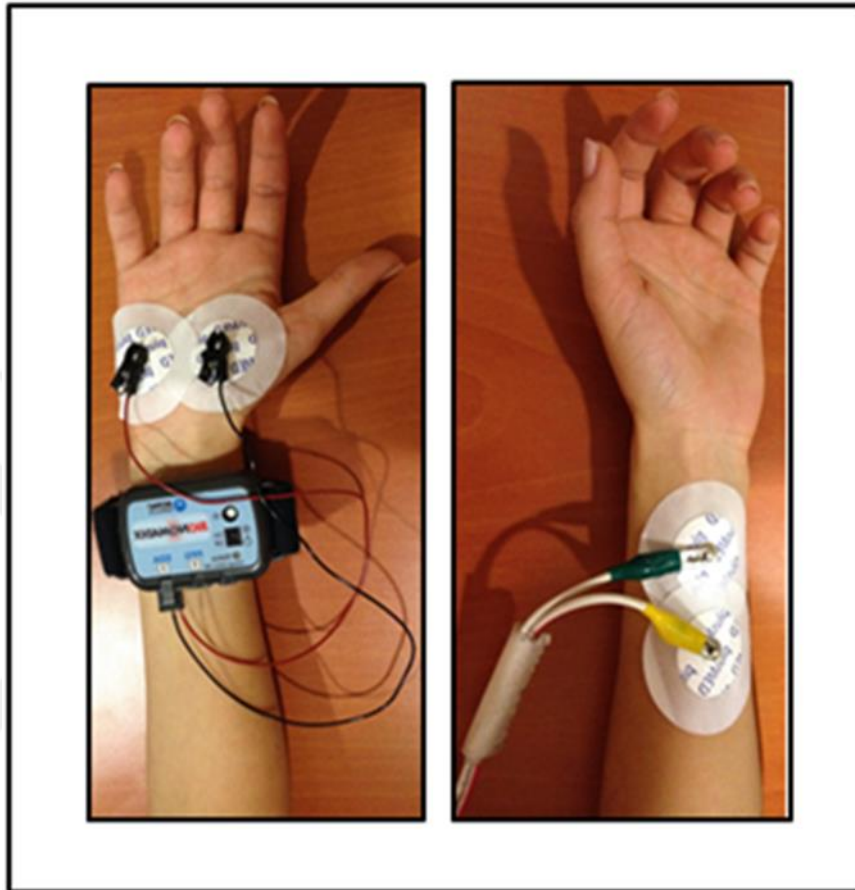


Figure 6. a) The bar electrode was placed to the right inner wrist for electrical stimulation, b) Disposable snap electrodes applied in order to measure SCR.

were also designed as a three-phase study including acquisition, blocking, and test phase. In acquisition phase, participants acquired conditioned fear responses through discriminative conditioning paradigm via arbitrary or ecological stimuli. In blocking phase, in order to create compound stimuli, a new stimulus was added to each of the CSs. In test phase, in order to test the blocking effect, each stimulus was presented alone. All experimental sessions were performed in adjacent (experimental and control) chambers with sound insulation (see Figure 7). The computer which SuperLab™ (Version: 4.5, Cedrus, Inc.) was installed was located in the experimental chamber, therefore the stimuli presentations were made in the experimental chamber. The participants took their position in the experimental room in order to start the experiment via a keyboard below the monitor. SCRs were obtained via the Acqknowledge 4.2™ software which was installed on the computer in the control chamber. Also, a video camera recorded all experimental sessions. In this way, the researcher could be informed whether the participants fulfilled their duty in accordance with the experimental terms and conditions.

Before the start of the experimental session, individuals were taken to the participant waiting room and they were required to fill up a participant evaluation form (see Appendix A). Individuals who stated having any previous or current psychological, neurological, or cardiovascular diagnose, being on medication, history of participation in previous psychology experiments that were conducted by using similar arbitrary or ecological stimuli or electrical stimulation were not allowed to participate in the experiment. Individuals who became entitled to participate were given inform consent form (see Appendix B) and a participant code which was used

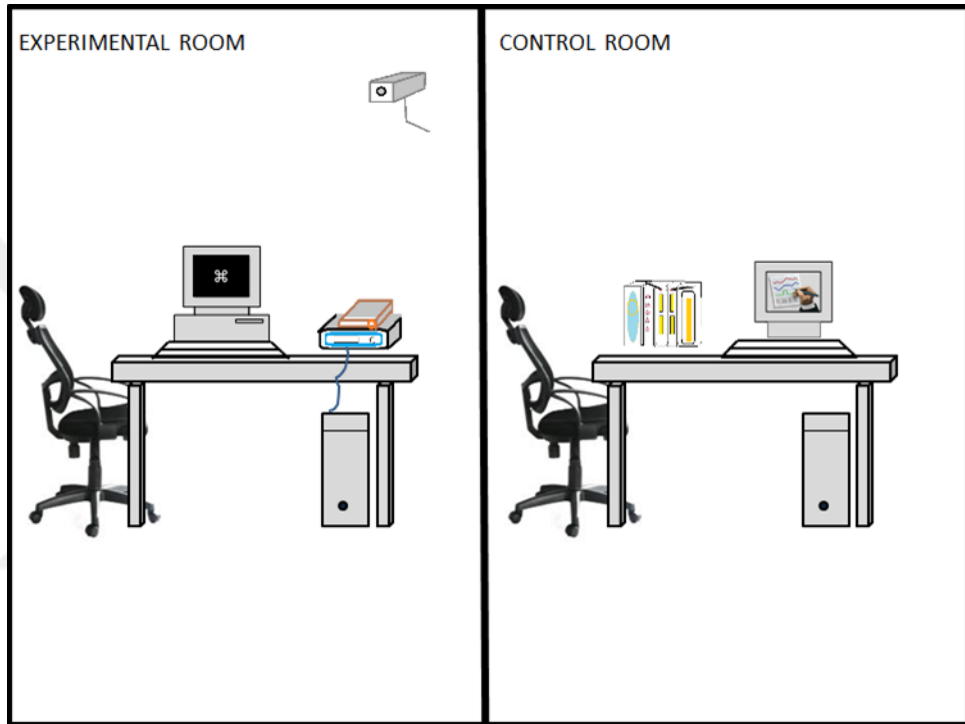


Figure 7. Experimental setup.

throughout the study instead of their names. The participants were assigned to the one of the experiments randomly.

After the form filling processes, the participants were taken to the experimental chamber and verbally kept informed about the aim of the study and the tasks that they would encounter during experiment. They were seated in front of the computer. The stimulator was adjusted to “ON” position and electrical stimulation electrode was attached to the right inner wrist of the participants before stimulus presentation program was started. As mentioned in the psychophysiological stimulation section, level of the electrical stimulation was set to a level which was “uncomfortable but not painful”. Once the participants determined the level of electrical stimulation, they were informed that the determined level of the electrical stimulation would be delivered to their right inner wrist during the experiment. For the purpose of measuring SCR, disposable EDA electrodes were placed to the palm of the left hand, to be more precise, to the thenar and hypothenar eminence. Before placing the electrodes, right inner wrist, and the palm of the left hand were cleaned with alcohol-soaked cotton and left for drying. EDA measurements are sensitive to the body movements; therefore those kinds of movements could easily cause motion artifacts. Hence, the participants were required to sit still during the experiment and use their right hand in case of pressing a key. Lastly, they were required to orient their attention to the computer screen and try to form associations between the stimuli on the screen and delivery of the electrical stimulation. All experiments started with a five-minute relaxation phase in order to adapt to the experimental environment and

also baseline of the EDA levels were measured simultaneously. Experimental sessions were recorded with a camera which was placed on the wall.

In the acquisition phase *CS A*, *CS B*, and *CS C*, and in the blocking phase [*CS A* + *CS X*], [*CS B* + *CS Y*], and [*CS C* + *CS Z*] were presented six times in random order. Also, as Hinchy *et al.* (1995) had suggested, presentations of *CS A*, *CS B*, and *CS C* were made two times in order to mask the transitions between phases. In the test phase, *CS A*, *CS B*, *CS C*, *CS X*, *CS Y* and *CS Z* were presented. Those stimuli appeared for 4000 milliseconds on the computer screen and SCRs, *US* expectancy ratings, and arousal ratings were recorded as dependent variables.

The only difference between the first and second experiment was the biological relevance of stimuli. That is to say that all stimuli were arbitrary in the first experiment (see Figure 8 for flow chart of the first experiment), whereas all stimuli were ecological in the second experiment (see Figure 9 for flow chart of the second experiment). Distinctively, for the third experiment, *CS A*, *CS B*, and *CS C* were arbitrary, while *CS X*, *CS Y*, *CS Z* were ecological (see Figure 10 for flow chart of the third experiment). In other words, the stimuli used in acquisition phase were arbitrary, whereas the added stimuli were ecological. On the other hand, for the fourth experiment, *CS A*, *CS B*, and *CS C* were ecological, while *CS X*, *CS Y*, *CS Z* were arbitrary (see Figure 11 for flow chart of the fourth experiment). It means that the stimuli used in acquisition phase were ecological, whereas the added stimuli were arbitrary.

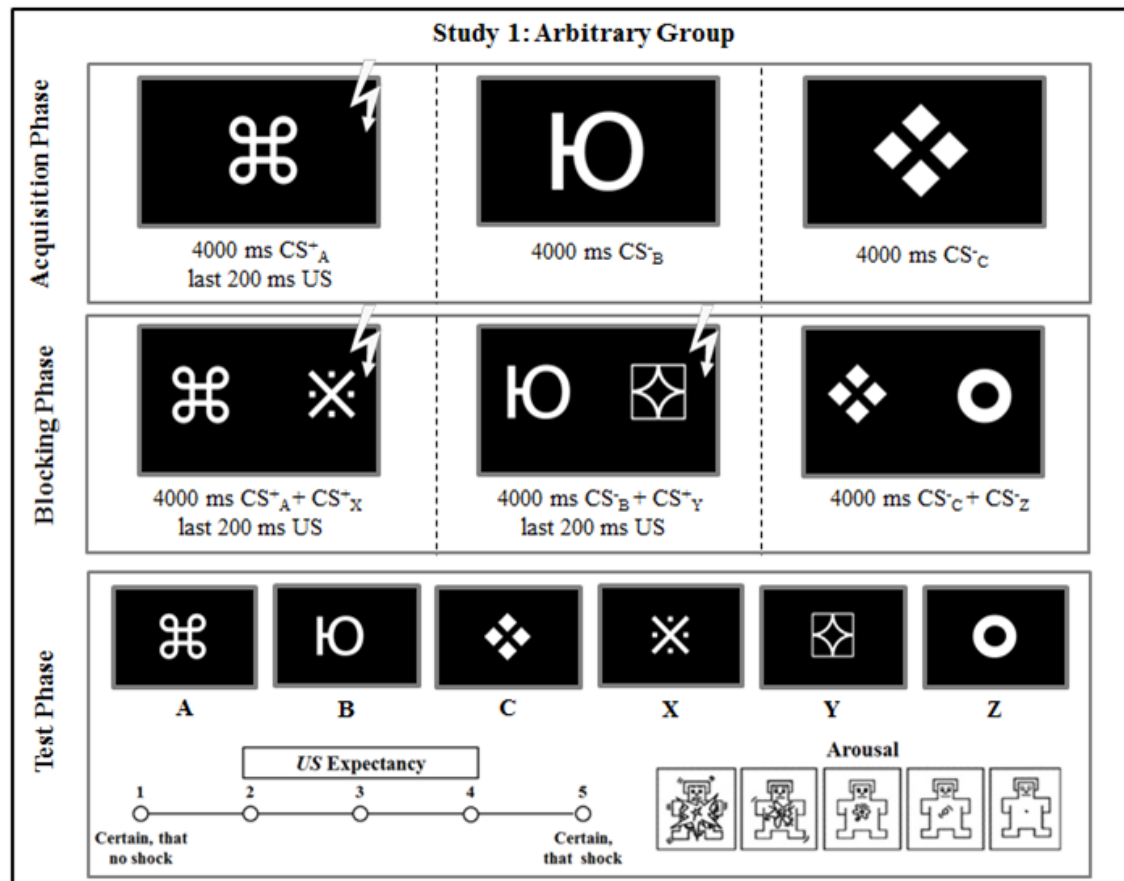


Figure 8. Stimulus presentation flow of the first experiment.

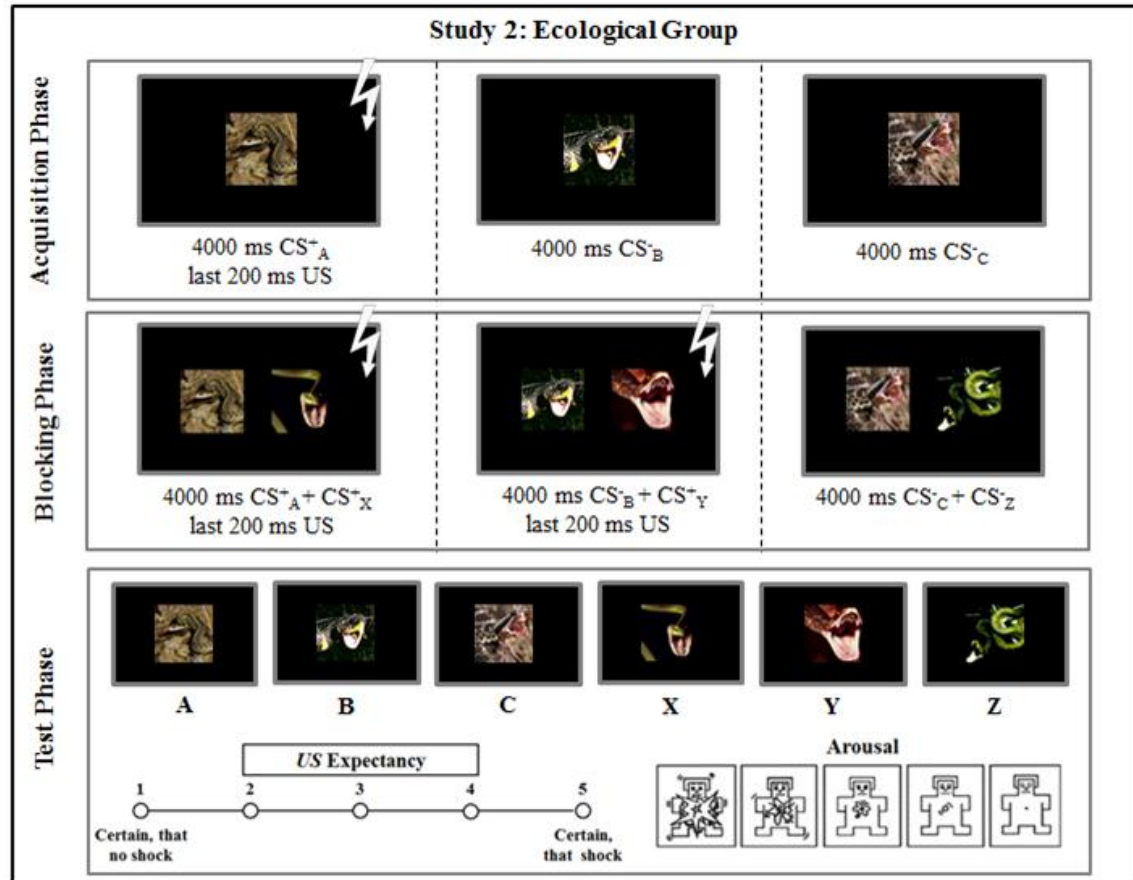


Figure 9. Stimulus presentation flow of the second experiment.

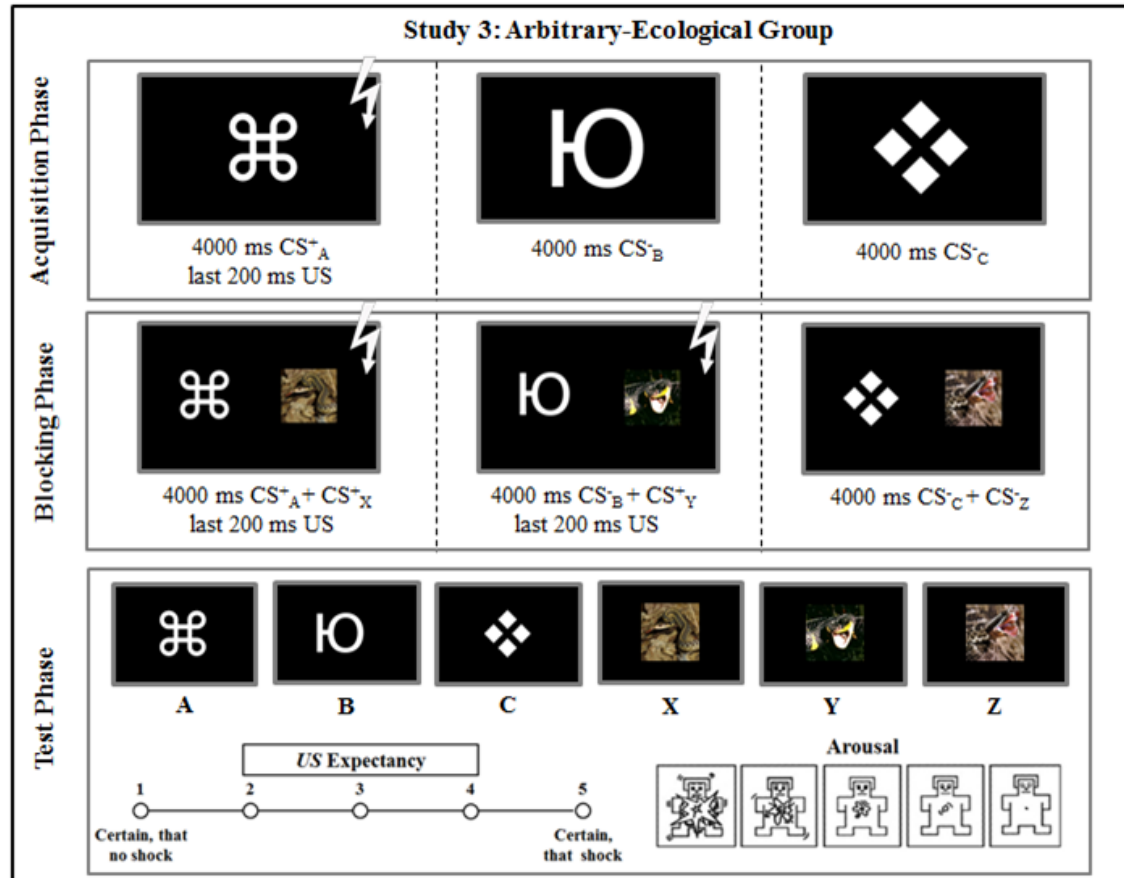


Figure 10. Stimulus presentation flow of the third experiment.

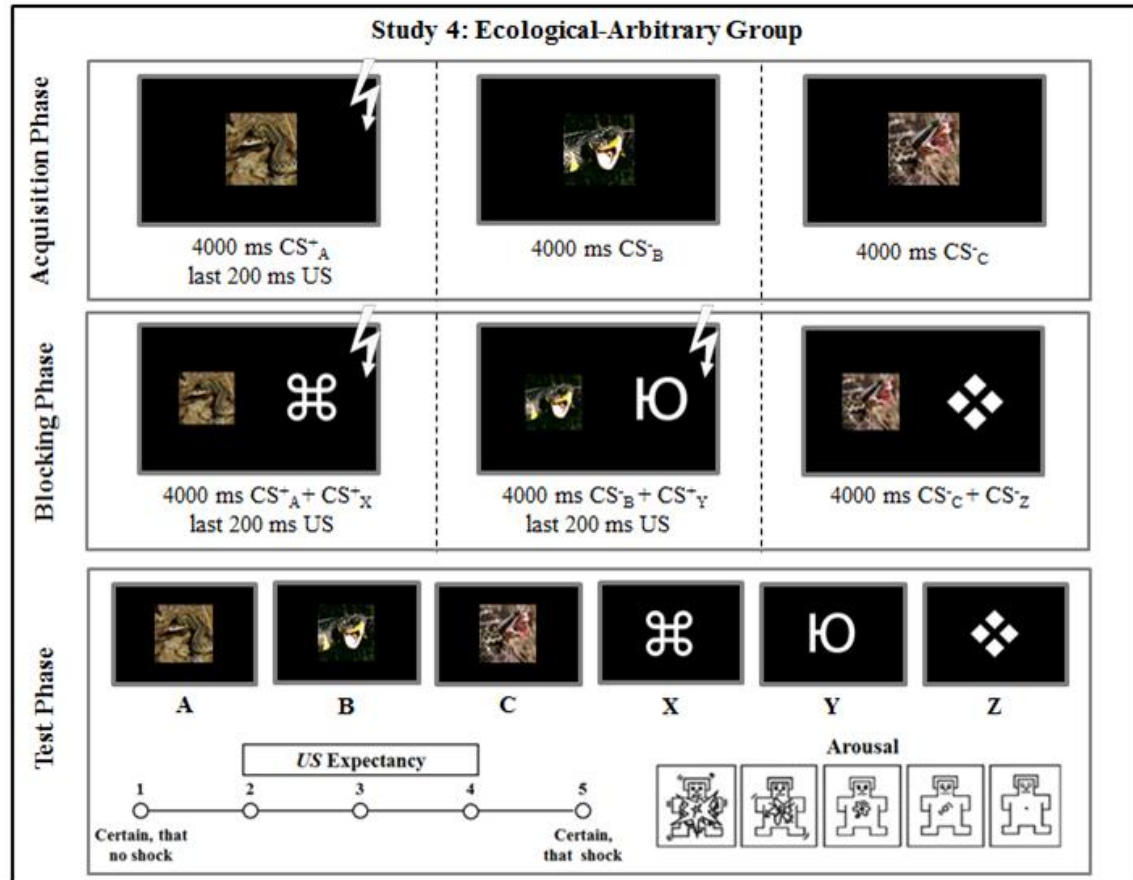


Figure 11. Stimulus presentation flow of the fourth experiment

2.4 Preparation of Skin Conductance Data for Analysis

During the experimental sessions, data acquisitions was done by means of MP systems and recorded via Acqknowledge™ 4.2 as explained before. A recorded data sample is shown in Figure 12. When this figure is examined, first channel indicates the EDA of the participant in course of the experimental session, and the other channels include the time periods of stimulus delivery made by SuperLab™, A, B, C, AX/XA, BY/YB, CZ/ZC, and X/Y/Z, respectively.

During the measurement of personal SCRs to the specific stimulus, level of response was designated from base to peak difference (amplitude, in microsiemens, μs) of the first response (waveform) that occurred within the 500ms and 5000ms time interval following the stimulus onset (see Figure 13). To be able to mention a waveform of a specific stimulus, base (beginning point) of the waveform should be inside this time interval and should have an amplitude value greater than $0.02\mu s$, which is equivalent to minimum SCR criterion.

2.4.1 Calculation of Acquisition Score

Prior to conducting a further analysis of the data, acquisition scores of the participants were calculated by using SCRs given to the CSs that had been presented in the first phase of the experiment. As a reminder, in the first phase, participants were required to comprehend the association between the CSs (CS A, CS B, and CS C) and the US (mild electrical stimulation). Thus, acquisition scores indicated whether participants had acquired the fear in course of the first phase or not. For each of the stimuli, amplitude of a response was calculated by subtracting peak microsiemens value from the base microsiemens value. Afterwards, square root

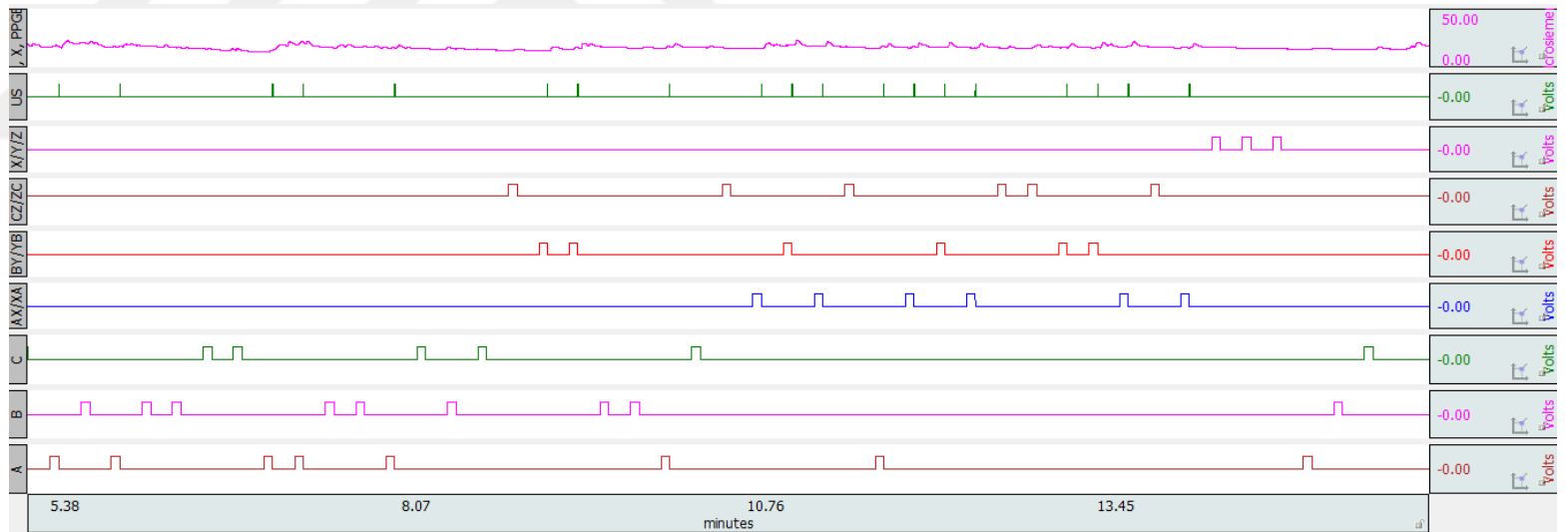


Figure 12. Sample data, recorded via Acqknowledge™ 4.2.

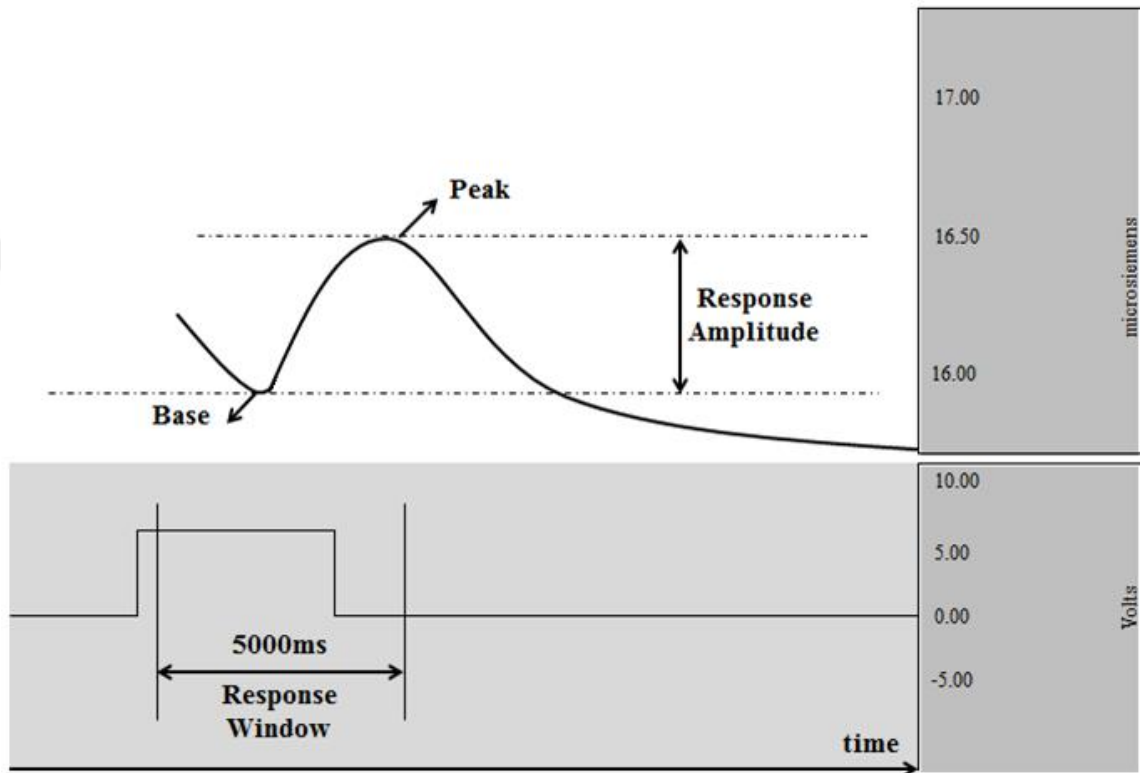


Figure 13. Measurement of SCR obtained from a single stimulus.

transformation was applied with the objective to normalize distribution for all 18 calculated values (6 CSs A, 6 CSs B, and 6 CSs C) because it is observed that amplitude variable may tend to have a negatively skewed distribution (Boucsein, 2012). Each transformed value of the CSs was divided by transformed values of 20 US that were averaged. In other words, each unique response given to the CSs was scaled with each participant's own UR. Difference scores were calculated between scaled CS that had been paired with the US and CSs that had not been paired with the US (i.e., CS A – CS B and CS A – CS C). Lastly, by averaging these difference scores, acquisition score was obtained (see Figure 14 for an example). The criterion suggested by Schiller et al. (2010) was used in order to decide whether acquisition occurred or not. From this point of view, participants who had acquisition scores “more than 0.10” were accepted as ones who developed the fear and showed CR to CS A, whereas participants whose acquisition scores was “less than 0.10” was excluded from further analysis.

2.5 Statistical Analysis

In order to conduct further analyses, a preliminary examination including distribution of the data was performed. As a consequence, extreme values were replaced with the mean score of that variable via mean imputation procedure. Extreme values were detected by using z -scores as suggested by Field (2009). For this, values of SCRs were converted to z -score and then z -scores with absolute value greater than 2.58 were selected as extreme values.

After preliminary examination, procedural controls were performed in order to investigate the acquisition of fear responses. A 3 (stimuli: CS A, CS B, CS C) x 6

O12 f_x $=(O10+O11)/2$

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	
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Figure 14. Calculation of acquisition score.

(trial numbers: 1, 2, 3, 4, 5, 6) repeated measures ANOVA was conducted to examine the difference in SCRs between CSs paired with the *US* and CSs not paired with the *US* through the trials of acquisition and blocking phase of the experiment. For any significant effect, planned contrasts (simple or repeated contrast) were used as follow-ups for repeated measures ANOVA. Lastly, manipulation analyses were conducted by using dependent *t*-test for SCRs and Wilcoxon signed-rank test for *US* expectancy ratings and arousal ratings.



CHAPTER 3: RESULTS

In this chapter, results of the analyses will be reported for all studies in an order as specified follow: (1) procedural control, (2) manipulation analysis.

Procedural controls were conducted to examine the pattern of conditioned fear responses obtained from SCRs as a consequence of acquisition trainings which were involved in the first and second phase of the experiment. For the first phase of the experiment, it was expected that SCRs obtained from *CS A* should significantly differ from *CS B* and *CS C*, because *CS A* was a reliable predictor of the *US*. In a similar manner, for the second phase of the experiment, it was expected that SCRs obtained from *CS AX* and *CS BY* should be significantly different from *CS CZ*, because in this phase both *CS AX* and *CS BY* were paired with the *US*. A 3 (stimuli: *A/AX*, *B/BY*, *C/CZ*) x 6 (trial numbers: 1, 2, 3, 4, 5, 6) repeated measures ANOVA was conducted in order to observe acquisition of fear responses for different *CSs* through the trials in the first phase and second phase, respectively. If *Mauchly's* test revealed that the assumption of sphericity was violated, based on the Epsilon (ϵ) value, degrees of freedom were corrected either by using Greenhouse-Geisser (if ϵ

< .75) or Huynh-Feldt estimates of sphericity (if $\epsilon > .75$) (as suggested by Field, 2009). For main effects and interaction effect, three follow-ups were performed. For the main effect of stimuli, simple contrast analyses were conducted as follow-up to display the expected difference among the stimuli. For the first phase, simple contrast analyses were performed based on the comparisons to the CS A, whereas for the second phase, simple contrast analyses were performed based on the comparisons to the CS CZ. For the main effect of trials, trend analyses were performed as follow-up to display the change in SCRs through the trials, which would indicate whether discriminative fear conditioning procedure worked in an expected manner. For the interaction effect between stimulus and trials, repeated measures ANOVA was conducted as follow-up to compare the stimuli for each of the trials. In order to control the familywise error, level of significance was corrected by using Bonferroni correction in which significance level was divided by the number of comparisons, because there were six trials, the significance level was determined as 0.008.

Manipulation analyses were conducted to reveal the effect of biological relevance of the stimuli on blocking by examining the SCRs, *US* expectancy ratings, and arousal ratings obtained in the test phase of the experiment. In order not to make familywise error, only pairwise comparisons that were helpful in demonstrating the blocking effect were made. Therefore, the pairwise comparisons involved the comparisons of CS A and CS X, CS B and CS Y, and lastly CS C and CS Z. For SCRs, dependent *t*-tests were performed for each pair of comparisons and one-tailed significance values were reported. For *US* expectancy ratings and arousal ratings, because these ratings were not normally distributed, Wilcoxon signed-rank tests were

employed as a nonparametric counterpart of dependent *t*-test. In an attempt to control the familywise error, again, level of significance was corrected by using Bonferroni correction in which significance level was divided by the number of comparisons made. Three pairwise comparisons were performed, thus the significance level was determined as 0.017 (see Table 2 for a general overview of the results).

3.1 Study 1: Arbitrary Group

3.1.1 Procedural Control

For the first phase of the experiment, results of the analysis revealed a significant main effect of stimuli (*CS A*, *CS B*, and *CS C*) on mean SCRs, $F_{(2, 58)} = 57.33, p < .05, \eta^2 = .66$. Simple contrast analyses showed that there were significant differences between *CS A* and *CS B* ($F_{(1, 29)} = 63.39, p < .05, \eta^2 = .69$) and between *CS A* and *CS C* ($F_{(1, 29)} = 97.58, p < .05, \eta^2 = .77$). Also, the effect of trials on mean SCRs was significant, $F_{(5, 145)} = 14.77, p < .05, \eta^2 = .34$. Trend analysis indicated a significant linear change in mean SCRs, $F_{(1, 29)} = 54.74, p < .05, \eta^2 = .65$. Lastly, a significant interaction between stimuli and trials was reported, $F_{(10, 290)} = 3.45, p < .05, \eta^2 = .11$.

Results of the analysis indicated that the mean SCRs did not vary among stimuli (*CS A*, *CS B*, and *CS C*) for the first trial, $F_{(1.64, 47.67)} = .99, p > .008$ and also for the second trial, ($F_{(2, 58)} = 4.96, p > .008$). When it comes to the third trial, results of the analysis showed that the mean SCRs varied among stimuli (*CS A*, *CS B*, and *CS C*), $F_{(2, 58)} = 11.73, p < .008, \eta^2 = .29$. As a result of simple contrast analyses, it was found that both the differences between *CS A* and *CS B* ($F_{(1, 29)} = 24.29, p < .05, \eta^2 = .46$) and between *CS A* and *CS C* ($F_{(1, 29)} = 12.83, p < .05, \eta^2 = .31$) were

Table 2. Results of SCRs, *US* expectancy ratings, and arousal ratings indicating significance level (the sign “x” indicates non-significant results; the sign “√” indicates significant results).

	SCR			<i>US</i> Expectancy			Arousal		
	CS A CS X	CS B CS Y	CS C CS Z	CS A CS X	CS B CS Y	CS C CS Z	CS A CS X	CS B CS Y	CS C CS Z
Study 1: Arbitrary Group	x	√	x	√	√	x	√	√	x
Study 2: Ecological Group	√	√	x	√	x	x	√	x	x
Study 3: Arbitrary - Ecological Group	x	√	√	√	√	√	√	x	√
Study 4: Ecological - Arbitrary Group	x	x	x	√	√	x	√	x	x

significant. SCRs during the presentation of *CS A* ($M = .94, SD = .29$) were observed to be higher than *CS B* ($M = .58, SD = .31$) and *CS C* ($M = .64, SD = .31$). For the fourth trial, results of the analyses displayed that the mean SCRs differed among stimuli (*CS A*, *CS B*, and *CS C*), $F_{(2, 58)} = 17.51, p < .008, \eta^2 = .38$. Simple contrast analyses indicated that both the differences between *CS A* and *CS B* ($F_{(1, 29)} = 39.79, p < .05, \eta^2 = .58$) and between *CS A* and *CS C* ($F_{(1, 29)} = 26.02, p < .05, \eta^2 = .47$) were significant. Higher SCRs during the presentation of *CS A* ($M = .95, SD = .25$) than *CS B* ($M = .54, SD = .37$) and *CS C* ($M = .55, SD = .34$) were expressed. For the fifth trial, results of the analysis revealed that the mean SCRs varied among stimuli (*CS A*, *CS B*, and *CS C*), $F_{(2, 58)} = 26.26, p < .008, \eta^2 = .48$. Simple contrast analyses indicated that both the difference between *CS A* and *CS B* ($F_{(1, 29)} = 38.00, p < .05, \eta^2 = .58$) and between *CS A* and *CS C* ($F_{(1, 29)} = 32.90, p < .05, \eta^2 = .53$) were significant. Higher SCRs during the presentation of *CS A* ($M = .97, SD = .26$) than *CS B* ($M = .47, SD = .36$) and *CS C* ($M = .45, SD = .41$) were reported. For the sixth trial, results of the analysis demonstrated that the mean SCRs differed among stimuli (*CS A*, *CS B*, and *CS C*), $F_{(2, 58)} = 28.61, p < .008, \eta^2 = .50$. Simple contrast analyses indicated that both the difference between *CS A* and *CS B* ($F_{(1, 29)} = 31.65, p < .05, \eta^2 = .52$) and between *CS A* and *CS C* ($F_{(1, 29)} = 43.12, p < .05, \eta^2 = .60$) were significant. Higher SCRs during the presentation of *CS A* ($M = .81, SD = .35$) than *CS B* ($M = .35, SD = .35$) and *CS C* ($M = .25, SD = .31$) were reported (see Figure 15).

For the second phase of the experiment, results of the analysis showed that the mean SCRs varied among stimuli (*CS AX*, *CS BY*, and *CS CZ*), $F_{(1.68, 48.78)} = 32.77,$

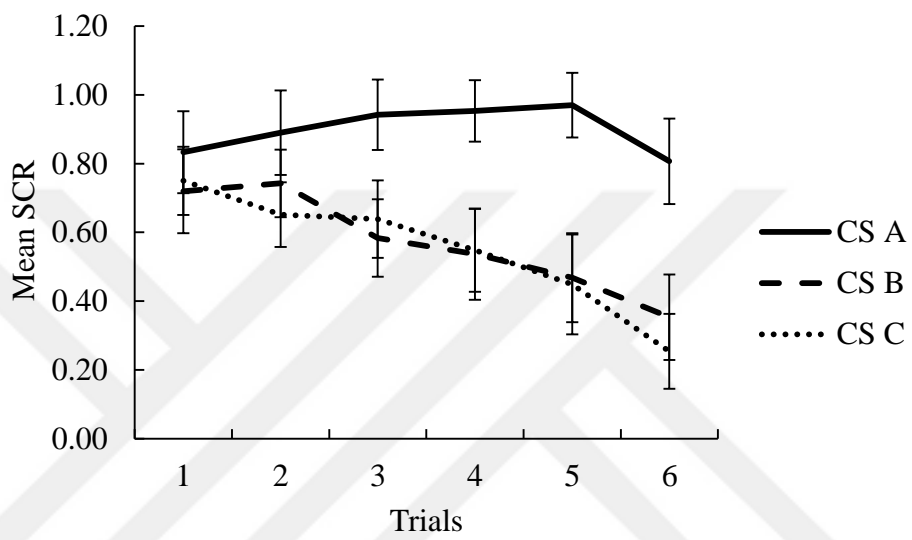


Figure 15. Mean SCRs for the first phase of the experiment in Study 1 (Error bars indicate 95% CI).

$p < .05$, $\eta^2 = .53$. It was observed that there was a significant difference between CS AX and CS CZ ($F_{(1, 29)} = 42.71$, $p < .05$, $\eta^2 = .60$) and also between CS BY and CS CZ ($F_{(1, 29)} = 33.36$, $p < .01$, $\eta^2 = .54$). A main effect of trials on SCRs was obtained, $F_{(4.59, 133.18)} = 17.07$, $p < .05$, $\eta^2 = .37$. Trend analysis indicated a significant linear change in mean SCRs, $F_{(1, 29)} = 58.13$, $p < .05$, $\eta^2 = .67$. On the other hand, the significant interaction between stimuli and trials was not found, $F_{(10, 290)} = .90$, $p > .05$ (see Figure 16).

3.1.2 Manipulation Analysis

3.1.2.1 Skin Conductance Response

For the test phase of the experiment, no significant difference between CS A and CS X ($t(29) = -2.15$, $p > .017$), a significant difference between CS B and CS Y ($t(29) = 4.84$, $p < .017$, $r = .45$), and no significant difference between CS C and CS Z ($t(29) = -.33$, $p > .017$) were found. The participants displayed higher SCRs during the presentations of CS Y ($M = .78$, $SD = .36$) than CS B ($M = .38$, $SD = .42$) (see Figure 17).

3.1.2.2 US Expectancy Ratings

For the test phase of the experiment, it was obtained that there were significant differences between CS A and CS X ($z = -4.04$, $p < .02$, $r = -.52$) and also between CS B and CS Y ($z = -4.28$, $p < .02$, $r = -.55$). However, the difference between CS C and CS Z ($z = -1.51$, $p > .02$) was not found significant. The participants stated higher US expectancy ratings for CS A ($Mdn = 5.00$) than CS X ($Mdn = 2.00$) and for CS Y ($Mdn = 4.00$) than CS B ($Mdn = 1.00$), respectively. (see Figure 18).

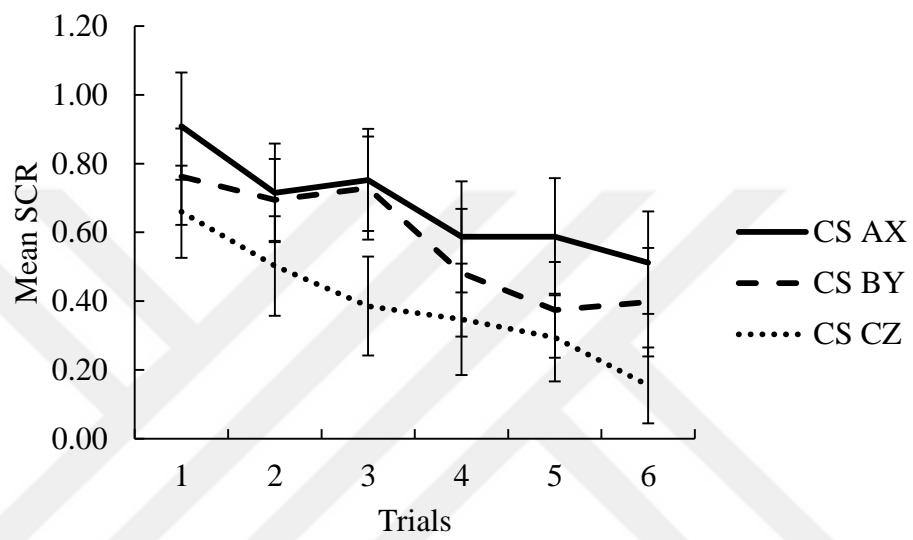


Figure 16. Mean SCRs for the second phase of the experiment in Study 1 (Error bars indicate 95% CI).

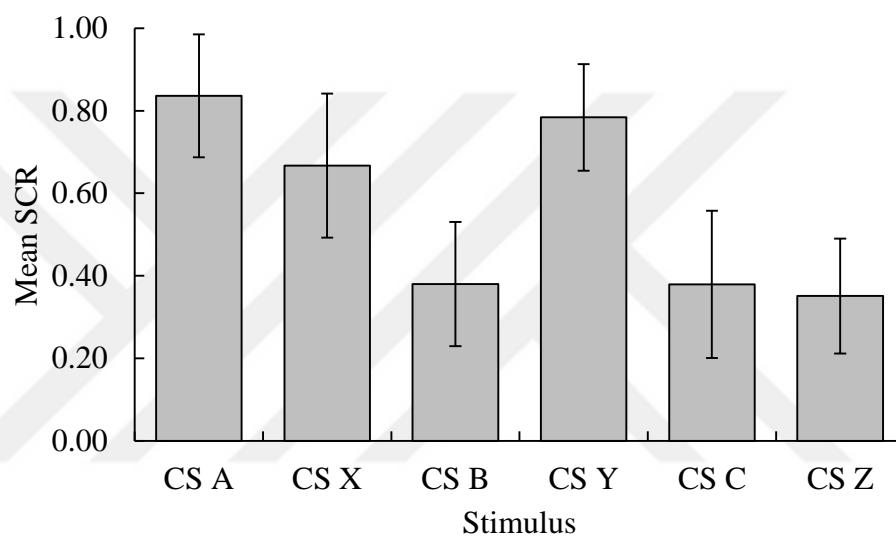


Figure 17. Mean SCRs obtained in the test phase of the experiment as responses to CS A, CS X, CS B, CS Y, CS C, and CS Z in Study 1 (Error bars indicate 95% CI).

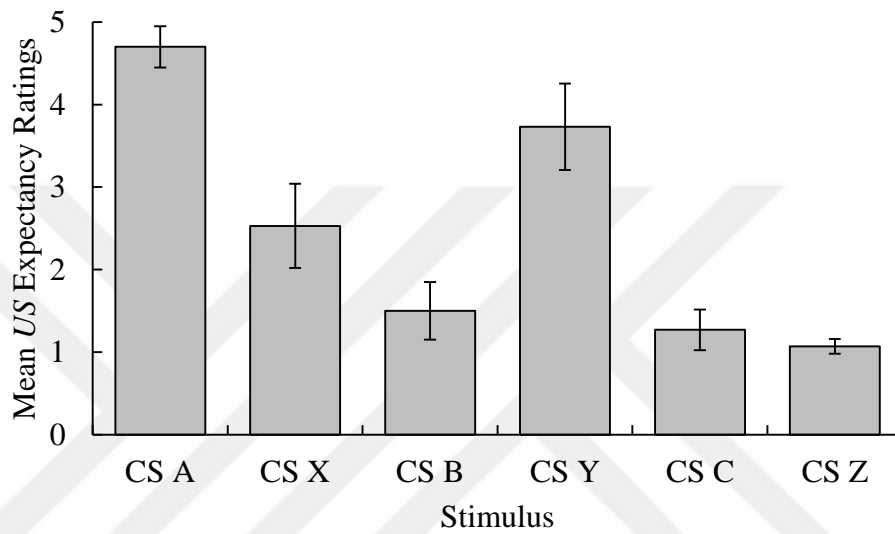


Figure 18. Mean *US* expectancy ratings obtained in the test phase of the experiment as responses to *CS A*, *CS X*, *CS B*, *CS Y*, *CS C*, and *CS Z* in Study 1 (Error bars indicate 95% CI).

3.1.2.3 Arousal Ratings

For the test phase of the experiment, it was reported that there were significant differences between CS A and CS X ($z = -4.02, p < .02, r = -.52$) and also between CS B and CS Y ($z = -3.06, p < .02, r = -.39$). On the other hand, the difference between CS C and CS Z ($z = -1.80, p > .02$) was not significant. The participants expressed higher arousal ratings for CS A ($Mdn = 4.00$) than CS X ($Mdn = 2.00$) and for CS Y ($Mdn = 2.00$) than CS B ($Mdn = 1.00$), respectively (see Figure 19).

3.2 Study 2: Ecological Group

3.2.1 Procedural Control

For the first phase of the experiment, results of the analysis showed a significant main effect of stimuli (CS A, CS B, and CS C) on mean SCRs, $F_{(2, 58)} = 70.58, p < .05, \eta^2 = .71$. Simple contrast analyses revealed significant differences between CS A and CS B ($F_{(1, 29)} = 90.96, p < .05, \eta^2 = .76$) and between CS A and CS C ($F_{(1, 29)} = 170.35, p < .05, \eta^2 = .86$). Also, a main effect of trials on SCRs was obtained, $F_{(5, 145)} = 5.15, p < .05, \eta^2 = .15$. Trend analysis indicated a significant linear change in mean SCRs, $F_{(1, 29)} = 9.45, p < .05, \eta^2 = .25$. Furthermore, a significant interaction between stimuli and trials was reported, $F_{(10, 290)} = 6.89, p < .05, \eta^2 = .19$.

For the first trial, results of the analysis indicated that the mean SCRs did not vary among stimuli (CS A, CS B, and CS C), $F_{(2, 58)} = 3.19, p > .008$. Following the first trial, results of the analysis showed that the mean SCRs varied among stimuli (CS A, CS B, and CS C) in the second trial, $F_{(2, 58)} = 6.73, p < .05, r = .19$. Simple

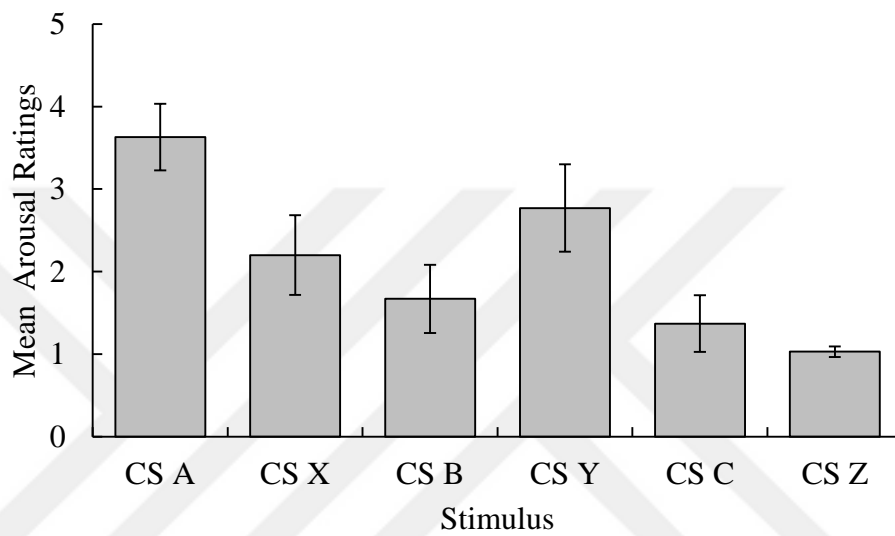


Figure 19. Mean arousal ratings obtained in the test phase of the experiment as responses to CS A, CS X, CS B, CS Y, CS C, and CS Z in Study 1 (Error bars indicate 95% CI).

contrast analyses indicated two significant differences in which between *CS A* and *CS B* ($F_{(1, 29)} = 6.02, p < .05, \eta^2 = .17$) and between *CS A* and *CS C* ($F_{(1, 29)} = 13.31, p < .05, \eta^2 = .32$). It was seen that participants had higher SCRs during the presentation of *CS A* ($M = .78, SD = .30$) than *CS B* ($M = .63, SD = .36$) and *CS C* ($M = .58, SD = .29$). For the third trial, results of the analysis showed that the mean SCRs varied among stimuli (*CS X*, *CS B*, and *CS C*), $F_{(2, 58)} = 10.99, p < .008, \eta^2 = .28$. Simple contrast analyses indicated that both differences between *CS A* and *CS B* ($F_{(1, 29)} = 22.75, p < .05, \eta^2 = .44$) and between *CS A* and *CS C* ($F_{(1, 29)} = 15.26, p < .05, \eta^2 = .35$) were significant. It means that higher SCRs were observed during the presentation of *CS A* ($M = .87, SD = .23$) than *CS B* ($M = .56, SD = .35$) and *CS C* ($M = .59, SD = .39$). For the fourth trial, results of the analysis displayed that the mean SCRs differed among stimuli (*CS A*, *CS B*, and *CS C*), $F_{(2, 58)} = 24.69, p < .008, \eta^2 = .46$. Simple contrast analyses indicated that both the difference between *CS A* and *CS B* ($F_{(1, 29)} = 39.19, p < .05, \eta^2 = .58$) and between *CS A* and *CS C* ($F_{(1, 29)} = 31.67, p < .05, \eta^2 = .52$) were significant. Higher SCRs during the presentation of *CS A* ($M = .93, SD = .27$) than *CS B* ($M = .49, SD = .37$) and *CS C* ($M = .62, SD = .37$) were reported. For the fifth trial, results of the analysis showed that the mean SCRs differed among stimuli (*CS A*, *CS B*, and *CS C*), $F_{(2, 58)} = 29.12, p < .008, \eta^2 = .50$. Simple contrast analyses obtained significant differences between *CS A* and *CS B* ($F_{(1, 29)} = 33.71, p < .05, \eta^2 = .54$) and between *CS A* and *CS C* ($F_{(1, 29)} = 54.23, p < .05, \eta^2 = .65$). Higher SCRs during the presentation of *CS A* ($M = .91, SD = .32$) than *CS B* ($M = .50, SD = .32$) and *CS C* ($M = .40, SD = .33$) were obtained. For the sixth trial, results of the analysis indicated that the mean SCRs differed among stimuli (*CS A*, *CS B*, and *CS C*), $F_{(2, 58)} = 22.57, p < .008, \eta^2 = .44$. In consequence of

simple contrast analyses, it was obtained that both the difference between *CS A* and *CS B* ($F_{(1, 29)} = 40.43, p < .05, \eta^2 = .58$) and between *CS A* and *CS C* ($F_{(1, 29)} = 36.85, p < .05, \eta^2 = .56$) were significant. Higher SCRs during the presentation of *CS A* ($M = .90, SD = .34$) than *CS B* ($M = .47, SD = .37$) and *CS C* ($M = .42, SD = .41$) were observed (see Figure 20).

For the second phase of the experiment, results of the analysis revealed that the mean SCRs varied among stimuli (*CS AX*, *CS BY*, and *CS CZ*), $F_{(2, 58)} = 20.35, p < .05, \eta^2 = .41$. There were significant differences between *CS AX* and *CS CZ* ($F_{(1, 29)} = 33.88, p < .05, \eta^2 = .54$) and also between *CS BY* and *CS CZ* ($F_{(1, 29)} = 17.29, p < .05, \eta^2 = .37$). A main effect of trials on SCRs was observed, $F_{(3.57, 103.55)} = 10.06, p < .05, \eta^2 = .26$. Trend analysis indicated a significant linear change in mean SCRs, $F_{(1, 29)} = 26.74, p < .05, \eta^2 = .48$. However, the interaction effect between stimuli and trials was not significant, $F_{(6.32, 183.17)} = .186, p > .05$ (see Figure 21).

3.2.2 Manipulation Analysis

3.2.2.1 Skin Conductance Responses

For the test phase of the experiment, there were significant differences between *CS A* and *CS X* ($t(29) = -5.01, p < .017, r = .46$) and between *CS B* and *CS Y* ($t(29) = 2.56, p < .017, r = .18$). However, no significant difference between *CS C* and *CS Z* ($t(29) = -.22, p > .017$) were obtained. The participants expressed higher SCRs during the presentations of *CS A* ($M = .97, SD = .35$) than *CS X* ($M = .58, SD = .37$) and *CS Y* ($M = .72, SD = .52$) than *CS B* ($M = .53, SD = .39$) (see Figure 22).

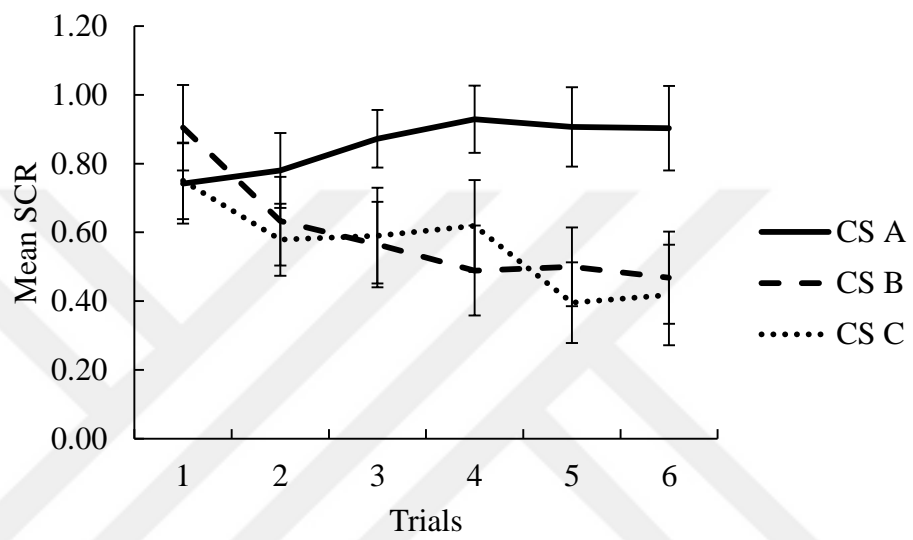


Figure 20. Mean SCRs for the first phase of the experiment in Study 2 (Error bars indicate 95% CI).

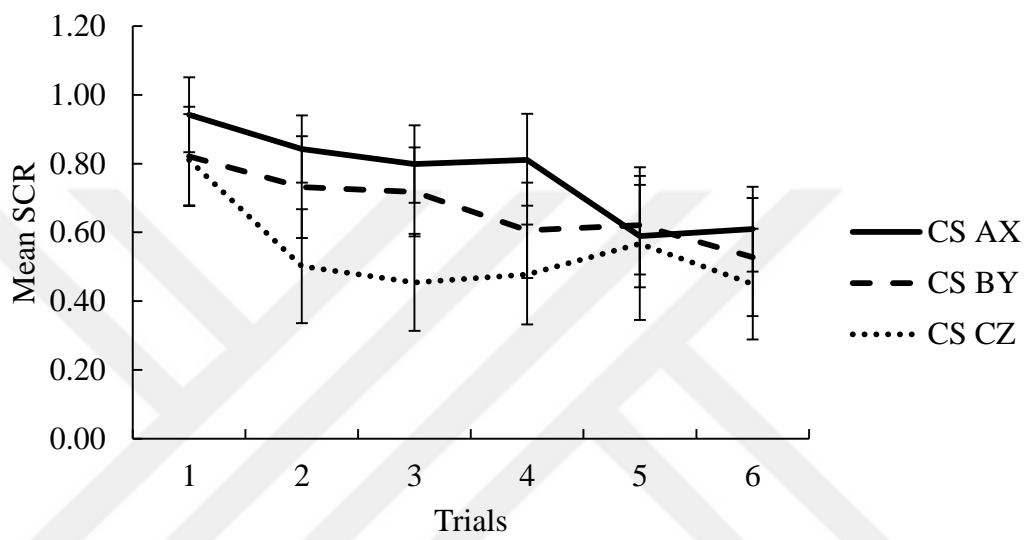


Figure 21. Mean SCRs for the second phase of the experiment in Study 2 (Error bars indicate 95% CI).

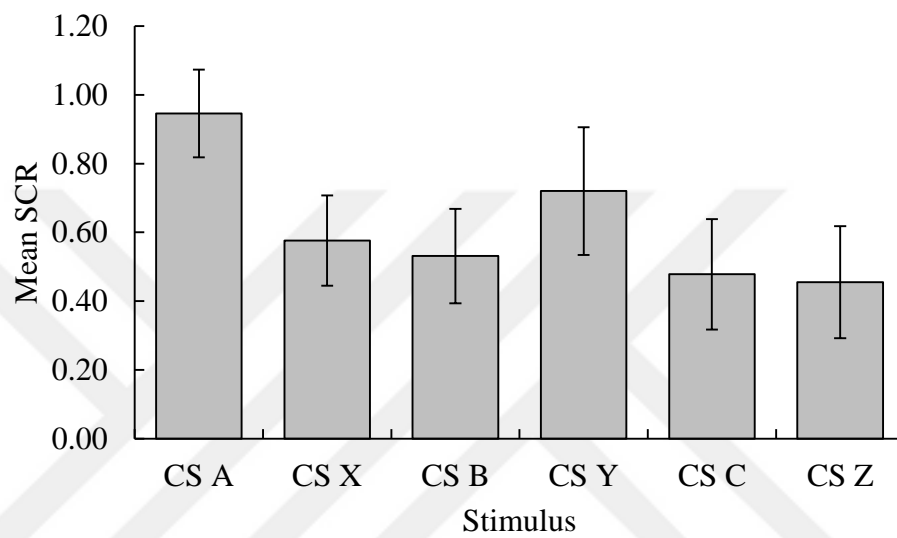


Figure 22. Mean SCRs obtained in the test phase of the experiment as responses to CS A, CS X, CS B, CS Y, CS C, and CS Z in Study 2 (Error bars indicate 95% CI).

3.2.2.2 US Expectancy Ratings

For the test phase of the experiment, results of the analysis implied that there was a significant difference between CS A and CS X ($z = -3.85, p < .02, r = -.50$). On the other hand, the differences between CS B and CS Y ($z = -1.88, p > .02$) and between CS C and CS Z ($z = -.03, p > .02$) were not found significant. Higher US expectancy ratings for CS A ($Mdn = 5.00$) than CS X ($Mdn = 2.00$) were expressed (see Figure 23).

3.2.2.3 Arousal Ratings

For the test phase of the experiment, it was revealed that there was a significant difference between CS A and CS X ($z = -2.52, p < .02, r = -.33$). On the other hand, the differences between CS B and CS Y ($z = -2.12, p > .02$) and between CS C and CS Z ($z = -1.30, p > .02$) were not significant. The participants stated higher arousal ratings during the presentations of CS A ($Mdn = 3.00$) than CS X ($Mdn = 2.00$) (see Figure 24).

3.3 Study 3: Arbitrary-Ecological Group

3.3.1 Procedural Control

For the first phase of the experiment, a significant main effect of stimuli (CS A, CS B, and CS C) on mean SCRs was obtained, $F_{(2, 58)} = 92.29, p < .05, \eta^2 = .76$. Simple contrast analyses demonstrated that there were significant differences between CS A and CS B ($F_{(1, 29)} = 164.76, p < .05, \eta^2 = .85$) and between CS A and CS C ($F_{(1, 29)} = 122.69, p < .05, \eta^2 = .81$). It was shown that there was a main effect of trials on SCRs, $F_{(3.48, 101.00)} = 4.98, p < .05, \eta^2 = .15$. Trend analysis indicated a significant linear change in mean SCRs, $F_{(1, 29)} = 7.49, p < .05, \eta^2 = .21$. A significant

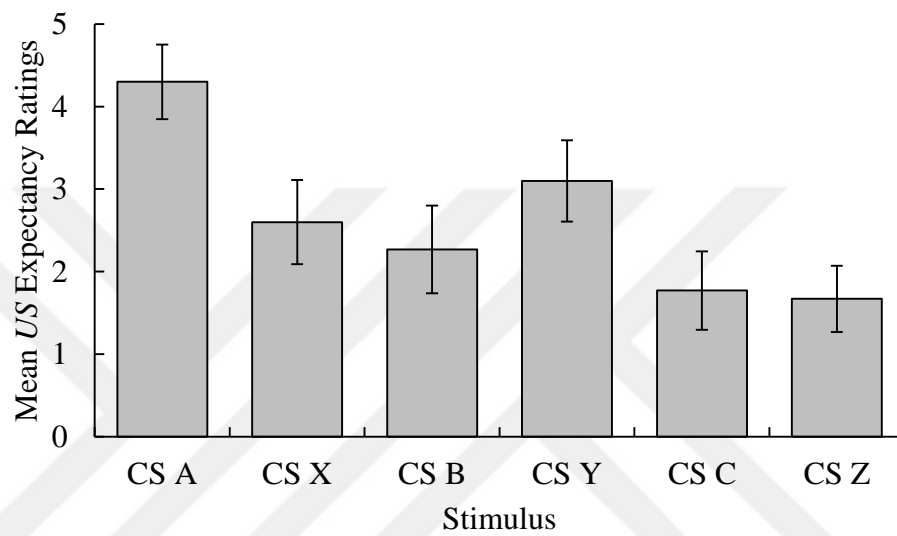


Figure 23. Mean *US* expectancy ratings obtained in the test phase of the experiment as responses to *CS A*, *CS X*, *CS B*, *CS Y*, *CS C*, and *CS Z* in Study 2 (Error bars indicate 95% CI)

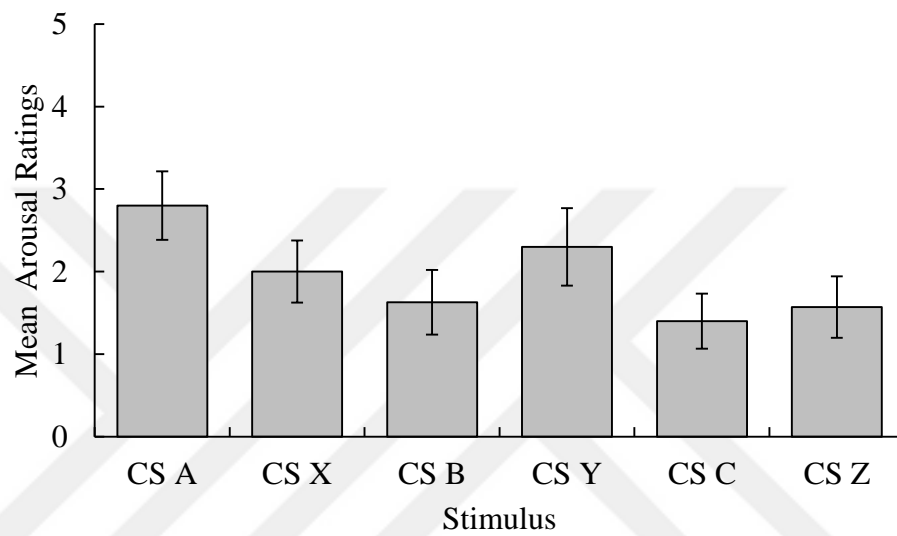


Figure 24. Mean arousal ratings obtained in the test phase of the experiment as responses to CS A, CS X, CS B, CS Y, CS C, and CS Z in Study 2 (Error bars indicate 95% CI).

interaction between stimuli and trials was reported, $F_{(6,22, 180,31)} = 7.64, p < .05, \eta^2 = .21$.

For the first and second trial, results of the analysis indicated that the mean SCRs did not vary among stimuli (CS A, CS B, and CS C), $F_{(2, 58)} = 3.53, p > .008$, and $F_{(2, 58)} = 4.71, p > .008$, respectively. For the third trial, results of the analysis revealed that the mean SCRs varied among stimuli (CS A, CS B, and CS C), $F_{(2, 58)} = 19.62, p < .008, \eta^2 = .40$. Simple contrast analyses showed both the difference between CS A and CS B ($F_{(1, 29)} = 36.44, p < .05, \eta^2 = .56$) and between CS A and CS C ($F_{(1, 29)} = 16.45, p < .05, \eta^2 = .36$) were significant. In other words, participants expressed higher SCRs during the presentation of CS A ($M = .96, SD = .49$) than CS B ($M = .38, SD = .40$) and CS C ($M = .50, SD = .37$). For the fourth trial, it was seen that the mean SCRs differed among stimuli (CS A, CS B, and CS C), $F_{(2, 58)} = 41.59, p < .008, \eta^2 = .59$. Simple contrast analyses implied that the differences between CS A and CS B ($F_{(1, 29)} = 64.93, p < .05, \eta^2 = .69$) and between CS A and CS C ($F_{(1, 29)} = 57.89, p < .05, \eta^2 = .67$) were significant. Higher SCRs during the presentation of CS A ($M = .86, SD = .32$) than CS B ($M = .24, SD = .28$) and CS C ($M = .38, SD = .35$) were reported. For the fifth trial, results of the analysis pointed out that the mean SCRs differed among stimuli (CS A, CS B, and CS C), $F_{(2, 58)} = 35.95, p < .008, \eta^2 = .55$. Simple contrast analyses indicated differences between CS A and CS B ($F_{(1, 29)} = 49.94, p < .05, \eta^2 = .63$) and between CS A and CS C ($F_{(1, 29)} = 59.73, p < .05, \eta^2 = .67$). Higher SCRs during the presentation of CS A ($M = .93, SD = .31$) than CS B ($M = .42, SD = .39$) and CS C ($M = .43, SD = .40$) were observed. For the sixth trial, it was found that the mean SCRs differed among stimuli (CS A, CS B, and CS C), $F_{(2,$

$_{58}) = 29.45, p < .008, \eta^2 = .50$. Simple contrast analyses indicated that *CS A* differed from *CS B* ($F_{(1, 29)} = 47.73, p < .05, \eta^2 = .62$) and *CS C* ($F_{(1, 29)} = 60.16, p < .05, \eta^2 = .63$). Higher SCRs during the presentation of *CS A* ($M = .89, SD = .34$) than *CS B* ($M = .43, SD = .45$) and *CS C* ($M = .38, SD = .34$) were displayed (see Figure 25).

For the second phase of the experiment, results of the analysis implied that the stimuli differed in terms of mean SCRs (*CS AX*, *CS BY*, and *CS CZ*), $F_{(2, 58)} = 7.22, p < .05, \eta^2 = .20$. There were significant differences between *CS AX* and *CS CZ* ($F_{(1, 29)} = 16.02, p < .05, \eta^2 = .36$) and also between *CS BY* and *CS CZ* ($F_{(1, 29)} = 5.94, p < .01, \eta^2 = .17$). A main effect of trials on SCRs was observed, $F_{(3.54, 102.55)} = 28.05, p < .05, \eta^2 = .49$. Trend analysis indicated a significant linear change in mean SCRs, $F_{(1, 29)} = 66.09, p < .05, \eta^2 = .70$. However, no significant interaction between stimuli and trials was reported, $F_{(10, 290)} = .63, p > .05$ (see Figure 26).

3.3.2 Manipulation Analysis

3.3.2.1 Skin Conductance Responses

For the test phase of the experiment, there was not a significant difference between *CS A* and *CS X* ($t(29) = 1.27, p > .017$), whereas significant differences between *CS B* and *CS Y* ($t(29) = 3.81, p < .017, r = .33$) and between *CS C* and *CS Z* ($t(29) = 2.30, p > .017, r = .15$) were obtained. The participants displayed higher SCRs during the presentations of *CS Y* ($M = .74, SD = .45$) than *CS B* ($M = .40, SD = .41$), and during the presentations of *CS Z* ($M = .47, SD = .45$) than *CS C* ($M = .28, SD = .33$) (see Figure 27).

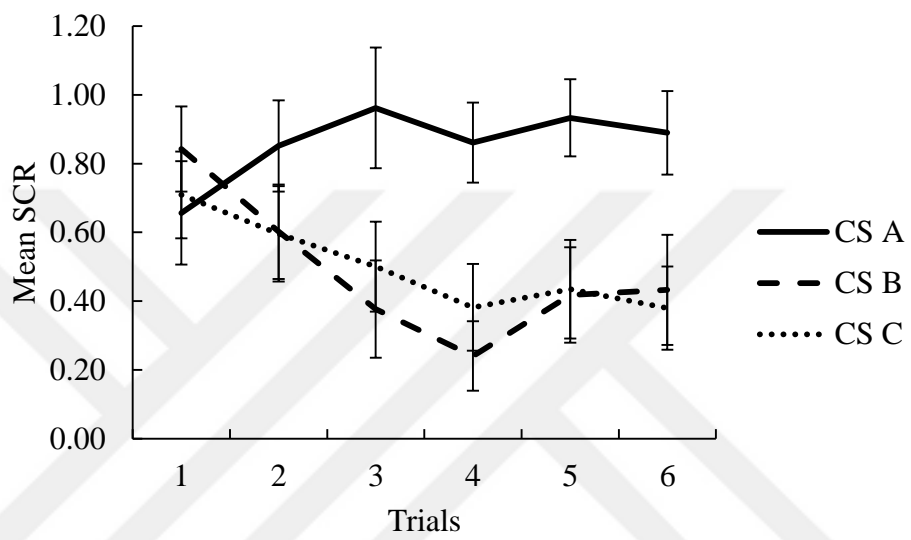


Figure 25. Mean SCRs for the first phase of the experiment in Study 3 (Error bars indicate 95% CI).

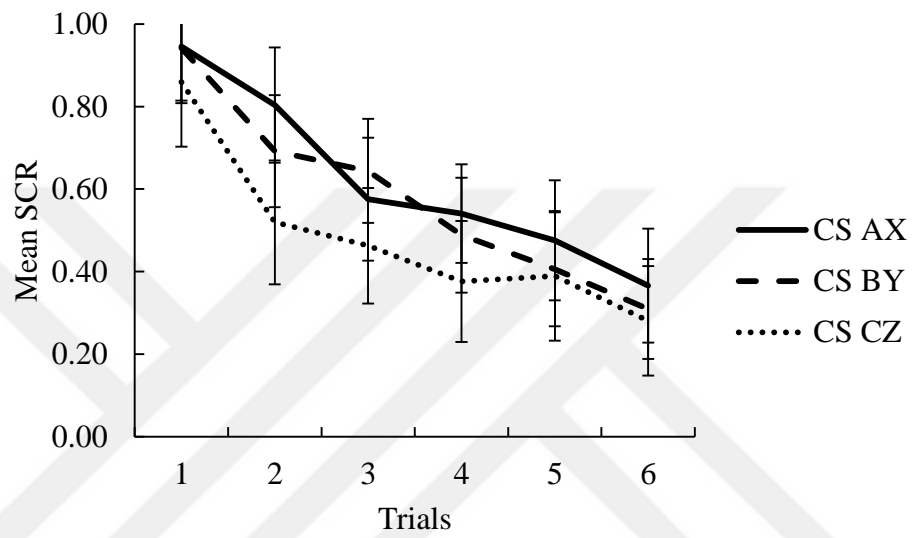


Figure 26. Mean SCRs for the second phase of the experiment in Study 3 (Error bars indicate 95% CI).

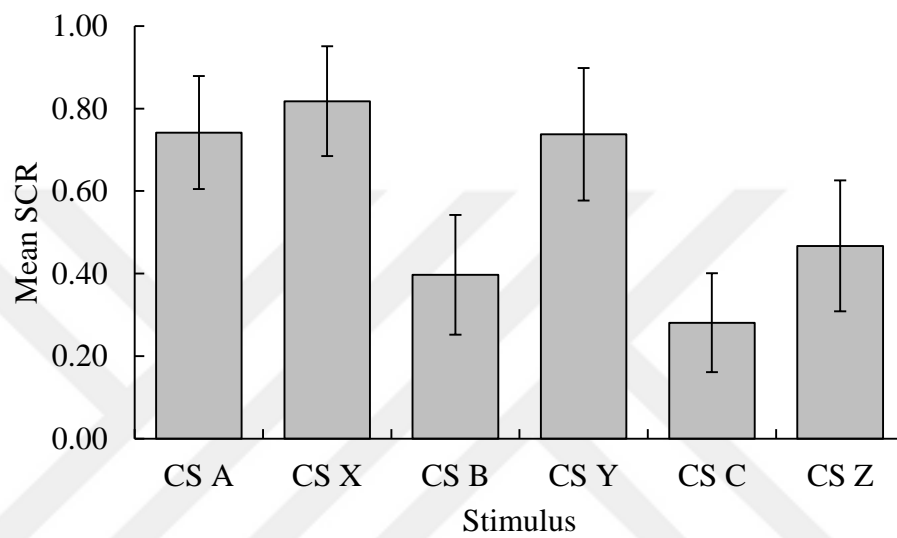


Figure 27. Mean SCRs obtained in the test phase of the experiment as responses to CS A, CS X, CS B, CS Y, CS C, and CS Z in Study 3 (Error bars indicate 95% CI).

3.3.2.2 US Expectancy Ratings

For the test phase of the experiment, significant differences between CS A and CS X ($z = -3.77, p < .02, r = -.49$), between CS B and CS Y ($z = -2.40, p < .02, r = -.31$), and between CS C and CS Z ($z = -3.17, p < .02, r = -.41$) were obtained. The participants had higher US expectancy ratings for CS A ($Mdn = 5.00$) than CS X ($Mdn = 3.50$), for CS Y ($Mdn = 4.00$) than CS B ($Mdn = 2.00$), and for CS Z ($Mdn = 2.00$) than CS C ($Mdn = 1.00$) (see Figure 28).

3.3.2.3 Arousal Ratings

For the test phase of the experiment, it was revealed a significant difference between CS A and CS X ($z = -2.53, p < .02, r = -.33$), no significant difference between CS B and CS Y ($z = -2.10, p < .02$), and a significant difference between CS C and CS Z ($z = -2.86, p < .02, r = -.33$). The participants had higher arousal ratings during the presentations of CS A ($Mdn = 3.00$) than CS X ($Mdn = 2.50$) and CS Z ($Mdn = 1.50$) than CS C ($Mdn = 1.00$) (see Figure 29).

3.4 Study 4: Ecological-Arbitrary

3.4.1 Procedural Control

For the first phase of the experiment, a significant main effect of stimuli (CS A, CS B, and CS C) on mean SCRs was obtained, $F_{(2, 58)} = 31.17, p < .05, \eta^2 = .52$. Simple contrast analyses showed that CS A differed from CS B ($F_{(1, 29)} = 52.11, p < .05, \eta^2 = .64$) and CS C ($F_{(1, 29)} = 34.97, p < .05, \eta^2 = .55$). Also, a main effect of trials on SCRs was found, $F_{(5, 145)} = 5.58, p < .05, \eta^2 = .16$. Trend analysis indicated a significant linear change in mean SCRs, $F_{(1, 29)} = 13.94, p < .05, \eta^2 = .33$.

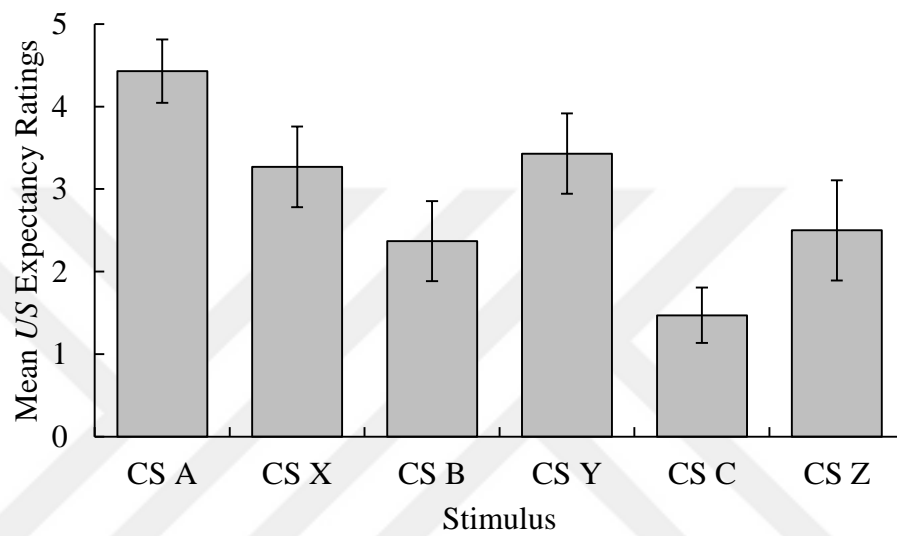


Figure 28. Mean *US* expectancy ratings obtained in the test phase of the experiment as responses to *CS A*, *CS X*, *CS B*, *CS Y*, *CS C*, and *CS Z* in Study 3 (Error bars indicate 95% CI)

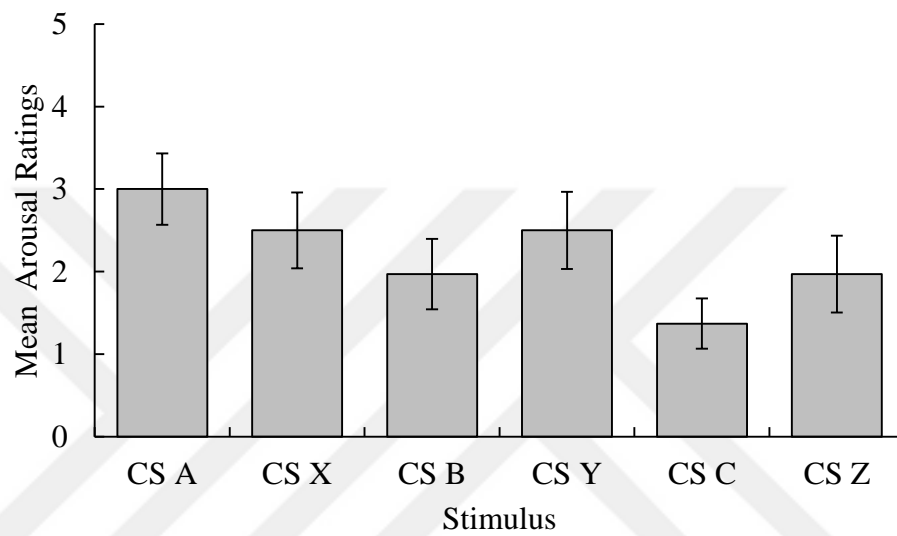


Figure 29. Mean arousal ratings obtained in the test phase of the experiment as responses to CS A, CS X, CS B, CS Y, CS C, and CS Z in Study 3 (Error bars indicate 95% CI).

Furthermore, a significant interaction between stimuli and trials was reported, $F_{(5.80, 168.12)} = 5.42, p < .05, \eta^2 = .16$.

Results of the analysis indicated that the mean SCRs did not differ among stimuli (*CS A*, *CS B*, and *CS C*) for the first trial, $F_{(2, 58)} = 1.76, p > .008$, and for the second trial, $F_{(2, 58)} = 4.97, p > .008$. When it comes to the third trial, results of the analysis revealed that the mean SCRs varied among stimuli (*CS A*, *CS B*, and *CS C*), $F_{(2, 58)} = 10.11, p < .008, \eta^2 = .26$. Simple contrast analyses implied that both the difference between *CS A* and *CS B* ($F_{(1, 29)} = 18.48, p < .05, \eta^2 = .39$) and between *CS A* and *CS C* ($F_{(1, 29)} = 16.70, p < .05, \eta^2 = .37$) were significant. Participants stated higher SCRs during the presentation of *CS A* ($M = .92, SD = .22$) than *CS B* ($M = .62, SD = .34$) and *CS C* ($M = .67, SD = .31$). For the fourth trial, results of the analysis displayed that the mean SCRs differed among stimuli (*CS A*, *CS B*, and *CS C*), $F_{(2, 58)} = 12.81, p < .008, \eta^2 = .31$. Simple contrast analyses showed significant differences between *CS A* and *CS B* ($F_{(1, 29)} = 21.81, p < .05, \eta^2 = .43$) and between *CS A* and *CS C* ($F_{(1, 29)} = 15.46, p < .05, \eta^2 = .35$). Higher SCRs during the presentation of *CS A* ($M = .92, SD = .21$) than *CS B* ($M = .50, SD = .40$) and *CS C* ($M = .65, SD = .40$) were reported. For the fifth trial, results of the analysis revealed that the mean SCRs differed among stimuli (*CS A*, *CS B*, and *CS C*), $F_{(2, 58)} = 13.61, p < .008, \eta^2 = .32$. Simple contrast analyses indicated that there were significant differences between *CS A* and *CS B* ($F_{(1, 29)} = 20.12, p < .05, \eta^2 = .41$) and between *CS A* and *CS C* ($F_{(1, 29)} = 18.18, p < .05, \eta^2 = .39$). Higher SCRs during the presentation of *CS A* ($M = .90, SD = .30$) than *CS B* ($M = .48, SD = .35$) and *CS C* ($M = .54, SD = .38$) were obtained. For the sixth trial, it was shown that the mean SCRs differed among stimuli (*CS A*,

CS B, and CS C), $F_{(2, 58)} = 32.85, p < .008, \eta^2 = .53$. Simple contrast analyses indicated that the differences between CS A and CS B ($F_{(1, 29)} = 71.25, p < .05, \eta^2 = .71$) and between CS A and CS C ($F_{(1, 29)} = 22.66, p < .05, \eta^2 = .44$) were significant. Higher SCRs during the presentation of CS A ($M = .91, SD = .24$) than CS B ($M = .33, SD = .30$) and CS C ($M = .54, SD = .35$) were observed (see Figure 30).

For the second phase of the experiment, results of the analysis demonstrated that the mean SCRs varied among stimuli (CS AX, CS BY, and CS CZ), $F_{(2, 58)} = 11.43, p < .05, \eta^2 = .28$. Significant differences were revealed between CS AX and CS CZ ($F_{(1, 29)} = 42.71, p < .05, \eta^2 = .60$) and also between CS BY and CS CZ ($F_{(1, 29)} = 33.36, p < .01, \eta^2 = .54$). A main effect of trials on SCRs was obtained, $F_{(5, 145)} = 17.46, p < .05, \eta^2 = .38$. Trend analysis indicated a significant linear change in mean SCRs, $F_{(1, 29)} = 45.14, p < .05, \eta^2 = .61$. However, the interaction between stimuli and trials was not found significant, $F_{(6.69, 193.86)} = 1.15, p > .05$ (see Figure 31).

3.4.2 Manipulation Analysis

3.4.2.1 Skin Conductance Responses

For the test phase of the experiment, no significant differences between CS A and CS X ($t(29) = -1.43, p > .017$), CS B and CS Y ($t(29) = 1.36, p > .017$), and CS C and CS Z ($t(29) = 1.67, p > .017$) were found (see Figure 32).

3.4.2.2 US Expectancy Ratings

For the test phase of the experiment, results indicated significant differences between CS A and CS X ($z = -3.81, p < .02, r = -.49$) and between CS B and CS Y (z

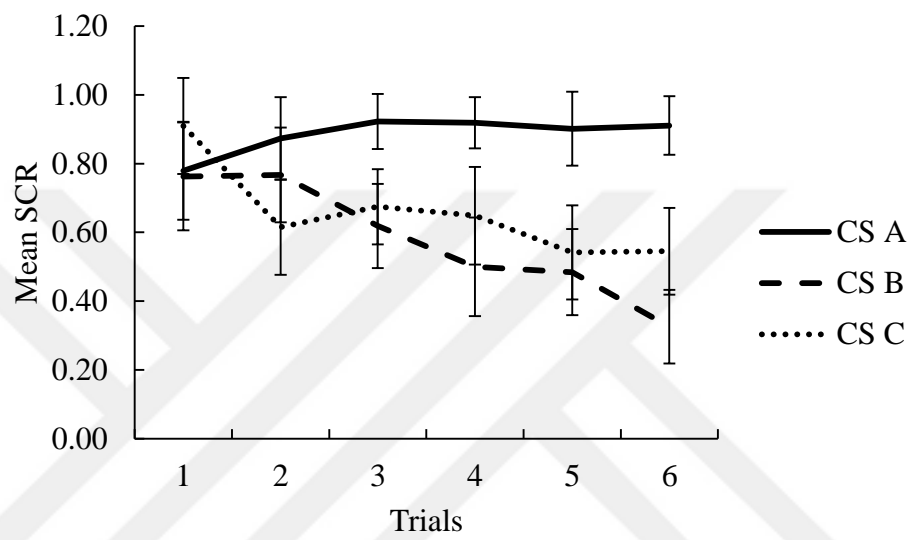


Figure 30. Mean SCRs for the first phase of the experiment in Study 4 (Error bars indicate 95% CI).

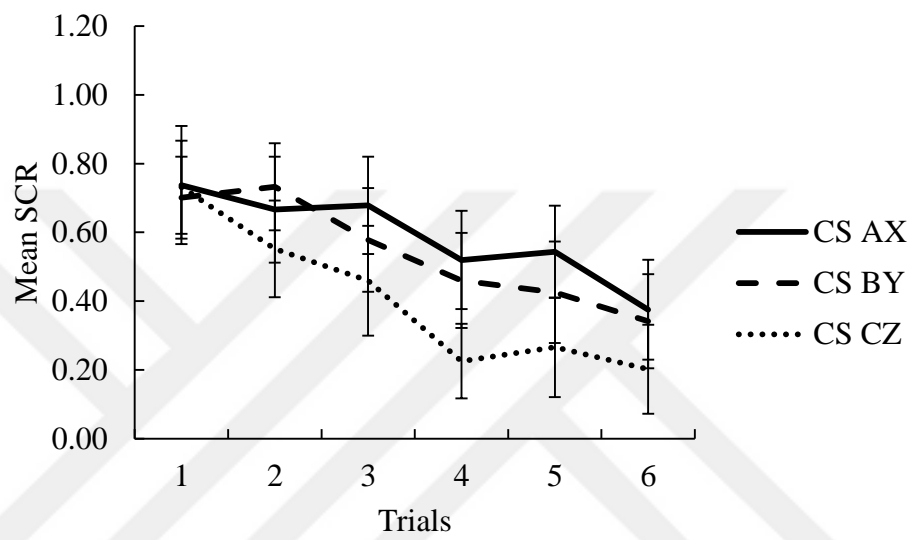


Figure 31. Mean SCRs for the second phase of the experiment in Study 4 (Error bars indicate 95% CI).

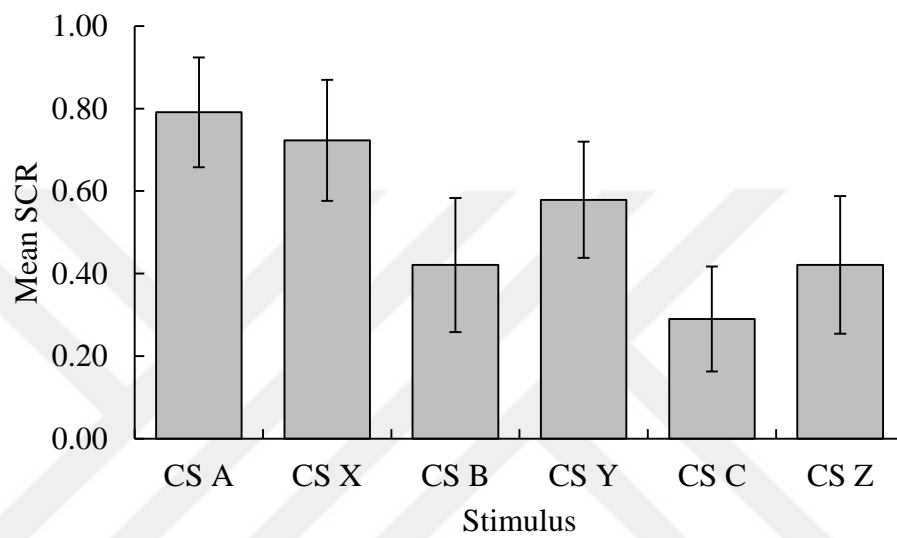


Figure 32. Mean SCRs obtained in the test phase of the experiment as responses to CS A, CS X, CS B, CS Y, CS C, and CS Z in Study 4 (Error bars indicate 95% CI).

= -2.59, $p < .02$, $r = -.33$). On the other hand, there was not a significant difference between CS C and CS Z ($z = -1.23$, $p > .02$). Higher *US* expectancy ratings during the presentations of CS A ($Mdn = 5.00$) than CS X ($Mdn = 4.00$) and CS Y ($Mdn = 4.00$) than CS B ($Mdn = 2.00$) were expressed (see Figure 33).

3.4.2.3 Arousal Ratings

For the test phase of the experiment, results of the analysis showed that there was a significant difference between CS A and CS X ($z = -4.10$, $p < .02$, $r = -.53$). On the other hand, the differences between CS B and CS Y ($z = -.11$, $p > .02$) and between CS C and CS Z ($z = -.96$, $p > .02$) were not significant. The participants had higher arousal ratings during the presentations of CS A ($Mdn = 3.00$) than CS X ($Mdn = 2.00$) (see Figure 34).

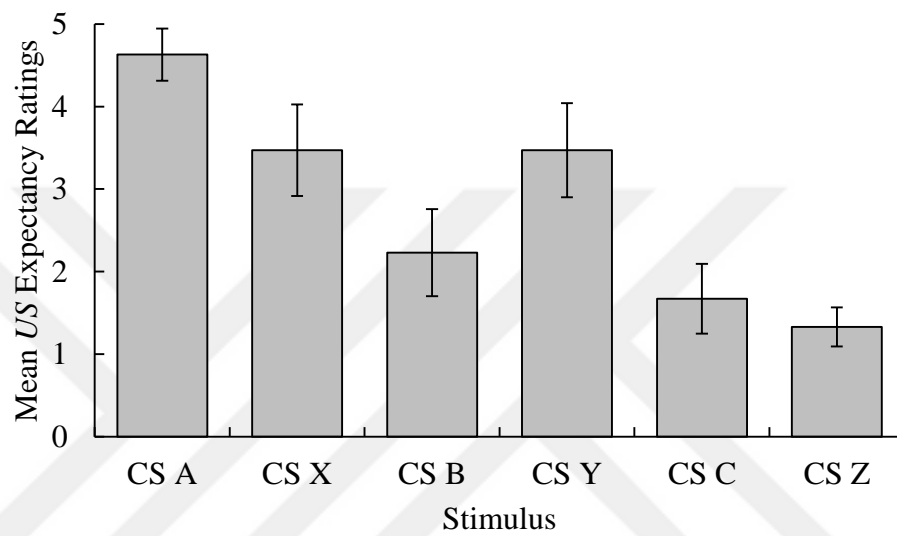


Figure 33. Mean *US* expectancy ratings obtained in the test phase of the experiment as responses to *CS A*, *CS X*, *CS B*, *CS Y*, *CS C*, and *CS Z* in Study 4 (Error bars indicate 95% CI)

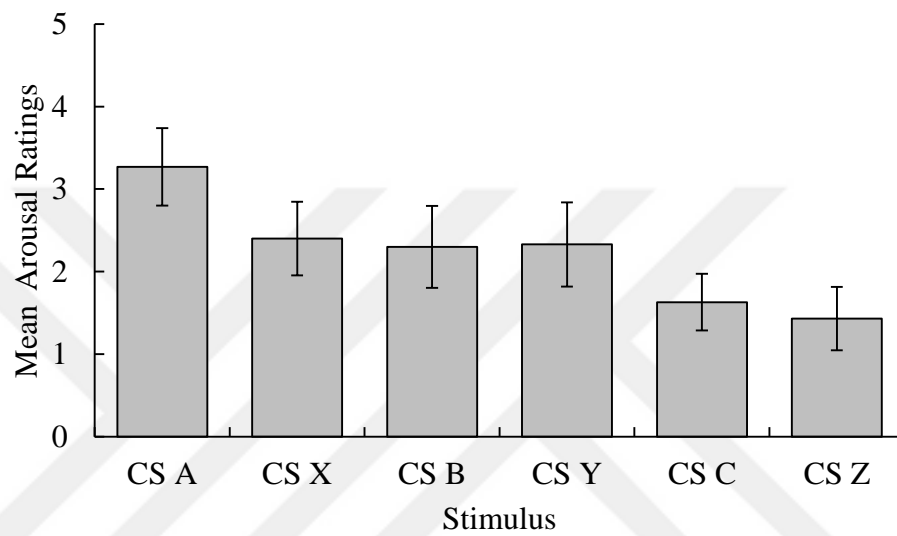


Figure 34. Mean arousal ratings obtained in the test phase of the experiment as responses to CS A, CS X, CS B, CS Y, CS C, and CS Z in Study 4 (Error bars indicate 95% CI)

CHAPTER 4: DISCUSSION

Evidence has indicated that conditioning to a stimulus might decrease following the presentation of that stimulus together with another that is already paired with the *US*, meaning that blocking occurs. Previous studies have revealed that the blocking effect could be demonstrated in humans both in appetitive or aversive conditioning. In this thesis project, main purpose was to demonstrate the blocking effect in humans by manipulating the biological relevance of stimuli within the scope of discriminative fear conditioning paradigm.

For this purpose, only arbitrary stimuli were used in the first study, whereas only ecological stimuli were used in the second study. In the third study, *CSs* were arbitrary, while added stimuli were ecological. On the other hand, in the fourth study, *CSs* were ecological, while added stimuli were arbitrary. In the first two studies, the effect of stimuli with same biological relevance on blocking was examined in a standard blocking procedure. It is important to note that since all stimuli shared the same biological relevance in those studies, blocking effect was expected to be

observed. However, for the other studies, the aim was to examine the effect of stimuli with different biological relevance on blocking. To be more precise, for the third study, since the added stimuli had stronger biological relevance compared to CSs, the blocking effect was not expected to be observed. For the fourth study, however, since the CSs had stronger biological relevance compared to added stimuli, again blocking effect was expected to be observed.

In acquisition and blocking phases, the only measure recorded was SCR, therefore procedural control was applied based on SCR. At the beginning of acquisition trials, it was expected that stimuli should not differ, because such a difference could lead to a bias in results obtained in the test phase. As a result of procedural control, it was revealed that mean SCRs were similar for all stimuli at the beginning of acquisition trials. Therefore, it would not be implausible to state that throughout the trials, a possible difference among stimuli might be interpreted as learning to those stimuli. Following the second trial of acquisition phase, it was revealed that the stimuli started to differentiate. In other words, conditioned fear responses elicited by CS A was higher than responses elicited by CS B and CS C observed in SCRs, indicating that discriminative fear conditioning paradigm works. For the blocking trials, again, it was demonstrated that the compound stimuli differed from each other in SCRs. CS CZ was different from CS AX and CS BY which were paired with the US. It was reliably concluded that discriminative learning took place in all studies.

The results provided by the first experiment revealed that despite higher SCRs during the presentation of CS A than the presentation of stimulus X, no significant

difference was observed. On the other hand, consistent with expectancies; CS B and stimulus Y significantly differed, whereas CS C and stimulus Z did not. Based on the comparison between CS A and stimulus X regarding SCRs, it should not be concluded that blocking did not occur, because the ratings of CS A were significantly higher than the ratings of stimulus X in *US* expectancy and arousal, indicating that blocking occurred. Although results obtained from different dependent measures seem to be conflicting, actually that is not the case. It has been argued that there are implicit and explicit processes underlying reasoning. Implicit processes are composed of a number of autonomous subsystems and shared with nonhumans, whereas explicit processes allow abstract reasoning and they are peculiar to humans (Evans, 2003). Dual process theory suggests that there is dissociation between implicit and explicit processes. According to Schultz and Helmstetter (2010), experimental contingencies end up both with a propositional learning and a conditioning process. Propositional learning prompts awareness of the contingencies (i.e., explicit process), while conditioning process prompts development of an autonomic response (i.e., implicit process). Due to the dissociation between the processes, they become independent from each other. Therefore, dual process theory accounts for the lack of evidence in SCRs while *US* expectancy ratings and arousal ratings provide support for blocking for the first study of this thesis project.

The second experiment provided evidence for human blocking in terms of both autonomic (SCRs) and cognitive (*US* expectancy and arousal) measures. It is critical to demonstrate blocking in this study due to the semantic content of the stimuli used. Previously, in the study conducted by Lovibond *et al.* (1988), the reason underlying

the failure of revealing any blocking was attributed to the semantic content of the stimuli composed of mushrooms, lakes, and flowers. It was considered that participants have a tendency to form association among slides based on their semantic contents. Therefore, the association that should be formed based on *CS-US* contingency might be missed while participants concentrate on the content of slides and thus no blocking might be reported. For this reason, due to experimental convenience, it has become a common trend to use arbitrary stimuli in blocking studies conducted with human participants instead of stimuli with semantic content. Therefore, there have been no studies in which ecological stimuli are used since the study of Lovibond *et al.* (1988). However, this study provides a new line of evidence that the blocking effect might also be demonstrated in both autonomic and cognitive measures by using ecological stimuli. It would be reasonable to indicate that if a working procedure is adopted, the blocking effect can be demonstrated in humans by using ecological stimuli with semantic content.

The first two studies provided a basis for the other studies in which arbitrary and ecological stimuli were used together. Considering the results of the first two studies, it might be concluded that both discriminative fear conditioning paradigm and stimuli used did not present any challenge in demonstrating of the blocking effect. Therefore, after the validation of the procedure and stimuli, other planned studies in which biological relevance of the stimuli was manipulated were conducted. For the third study, due to the stronger biological relevance of added stimuli compared to *CSs*, it was expected that blocking effect would not be easily observed. On the other hand, for the fourth study, due to the stronger biological relevance of

CSs compared to added stimuli, it was expected that blocking would be easily observed.

For the third study, as it was expected, mean SCRs did not differ between CS A and stimulus X. Also, not surprisingly, stimulus X had higher SCRs due to its biological relevance. Nonetheless, stimulus Y significantly elicited more SCRs than CS B, as was the case with stimulus Z and CS C. It is reasonable for stimulus Y to elicit more SCRs than CS, since predicting the absence of the US and being arbitrary makes CS B a weak stimulus compared to stimulus Y, which becomes a signal for the US when it is added to CS B in blocking trials. In case of CS C and stimulus Z, the reason behind the difference is that although both CS C and CS CZ did not predict the US, the arbitrary nature of CS C could not resist ecological stimulus Z. Regarding cognitive measures, results were parallel with the results obtained from SCRs to control and distractor stimuli (i.e., CS C and stimulus Z; CS B and stimulus Y, respectively), in other words, no significant difference between CS B and stimulus Y and between CS C and stimulus Z was observed. However, the difference between CS A and stimulus X was significant. After all, although cognitive measures could not provide expected results, SCRs demonstrated that blocking did not take place when an ecological stimulus was added to arbitrary stimulus.

Such results have important implications for the mechanism of unblocking. According to Dickinson *et al.* (1976), if intensity of the shock is increased in compound trials, a significant conditioning would occur to added stimulus. Also, as Kamin suggested, blocking might be attenuated also by adding a second shock after each compound trial. In other words, it is assumed that unblocking might occur only

when the added stimulus signals a surprising *US*. However, it was revealed that unblocking might also be demonstrated by manipulating the biological relevance of stimuli.

Köksal *et al.* (1994) suggested a different explanation for this finding based on the decrease in generalization from the blocking trials to the test trials. If the added stimulus is more salient than the *CS* presented in the first phase of the experiment, it is most likely to result in more *CR*. Within the scope of this study, because a snake was more salient than a geometric figure, presentation of the snake alone could easily trigger the representation of snake-geometric figure compound, and therefore participants may also develop *CR* to the snake. Köksal *et al.* (1994) also proposed that due to the similarity between the added stimulus and *US*, blocking of added stimulus might be prevented. In that study, the *CS* was an audiovisual cue, the added stimulus was a terrycloth object with a taxidermically designed female head, and the *US* was accessing to a live female quail. In terms of their physical features, the added stimulus was very similar to the *US* when compared to the *CS*. However, the similarity does not have to be in physical features only. Also, the added stimulus and *US* might be similar in terms of evolutionary basis. For example, the added stimulus and *US* were relevant to reproductive system in study with quails because both supported copulatory behavior. In this study, on the other hand, the added stimulus (picture of a snake) and *US* (mild electrical stimulation) were relevant to survival, because both involved a signal of threat. Thus, a meaningless arbitrary cue could not block the ecological cue that provided information in an evolutionary base.

For the fourth study, as mentioned above, due to the biological relevance of CSs, it was expected that the arbitrary added stimuli could easily be blocked by ecological CSs. Contrary to expectations; mean SCRs were not different between CS A and stimulus X. Although higher SCRs were obtained during the presentation of CS A than stimulus X, actually mean SCRs obtained from those stimuli were similar to each other. As mentioned above, the view accounting for unblocking asserts that it might occur only when the added stimulus signals a surprising US. However, there is no information about the surprisingness of stimuli added to CS in the blocking phase. Added stimulus with different biological relevance presented in the blocking phase might be surprising and therefore participants may have also attended to the added arbitrary stimulus in this study. Thus, conditioned fear responses to the added arbitrary stimulus might be obtained in the test phase regarding SCRs. When it comes to US expectancy ratings and arousal ratings, the blocking effect was revealed in an expected manner. Again, there is dissociation between autonomic and cognitive measures, supporting dual process theory.

Considering all studies, the results provided by this thesis projects have several implications. First, although a number of studies succeeded in demonstrating blocking effect in humans, there were also a considerable amount of studies that did not provide any evidence for human blocking. Probable reasons underlying why the blocking effect cannot be manifested in humans while it is clearly observed in nonhumans could unite under two titles: information processing errors and methodological errors. However, after it was suggested that blocking in humans might be considered as an analogue of blocking in nonhumans (Arcediano *et al.*,

1997), the view claiming that the difference between information processing between humans and nonhumans prevents the observation of blocking effect in humans has started to lose its effect. Therefore, methodological errors during the study of human blocking would be the reason behind the fail in human blocking. Since there is such a methodological difficulty in revealing the blocking effect in humans, in order to examine the effect of biological relevance of stimuli on blocking, first it was aimed to develop a procedure that could already work in a standard blocking experiment. Therefore, discriminative fear conditioning paradigm was employed based on previous studies (e.g., Eippert *et al.*, 2012) and it was again clearly revealed that blocking effect might be demonstrated by using this paradigm.

Second, the stimulus set of the experiments involved target, control, and distractor stimuli. Target stimuli were composed of *CS A* and stimulus *X*. It was expected to observe a difference in conditioned fear responses elicited by those stimuli in the test phase, indicating blocking occurred. Control stimuli were composed of *CS C* and stimulus *Z*. Since *CS C* predicted the absence of the *US* in the first phase and *CS CZ* also predicted the absence of the *US* in the second phase, those stimuli were used with the purpose of providing a control condition. Distractor stimuli were composed of *CS B* and stimuli *Y*. *CS B* predicted the absence of the *US* in the first phase, however *CS BY* predicted the presence of the *US* in the second phase. Therefore, it was expected to observe a difference between *CS B* and stimulus *Y* contrary to the difference between *CS A* and stimulus *X*. Precisely, it was assumed that stimulus *Y* elicited more fear responses than *CS B* did. By doing this, it was aimed to create a distraction to prevent generalization occurred in human research on

blocking due to the simplicity of the tasks employed. It is important to provide control in within-subjects studies in which there is not a control group. The design of the experiments in this thesis project included control condition and experimental condition together. Results indicated that using both control stimuli and distractor stimuli together with target stimuli might be an effective way to demonstrate blocking.

Third, as Hinchy *et al.* (1995) suggested, single stimulus presentations were made in order to mask the transitions between phases with intent to maintain the previously formed associations. It was crucial for the participants to integrate the phases and perceive the experiment as a whole. All stimuli were presented individually without *US* in the test phase in order to compare the target, control, and distractor stimuli in terms of SCRs, *US* expectancy ratings, and arousal ratings. Although the studies of human blocking have employed SCRs and *US* expectancy ratings as dependent measures (e.g., Hinchy *et al.*, 1995; Eippert *et al.*, 2012), for the first time, measurement of arousal level in addition to SCRs and *US* expectancy ratings was used in this thesis project as a dependent measure to demonstrate the blocking effect.

All in all, the results of this thesis project provide evidence for the effect of biological relevance of stimuli on blocking, however lack of evidence of blocking in SCRs in the fourth study is still disputable. Although there are several studies conducted in order to examine the effect of biological relevance of stimuli on blocking, only biological relevance of added stimulus is manipulated (e.g., Köksal *et al.*, 1994). If both *CS* and added stimulus are manipulated in terms of biological

relevance as in this thesis project, then it will be provided deeper knowledge about the effect of nature of stimuli on blocking. Research of human blocking has been a new issue compared to animal blocking and it has developed a new understanding by revealing explicit processes involved. In this thesis project, a new line of evidence that supports the dual process theory was presented by revealing the dissociation between autonomic and cognitive measures. According to Schultz and Helmstetter (2010), the reason of conflicting findings in humans between autonomic and cognitive measures is because different brain structures have roles in simultaneous implicit and explicit learning. Actually, this suggestion emphasizes the importance of using different measures in a study to be able to show the expected effect. Öhman and Mineka (2001) mentioned a three fear response systems that are cognitive, behavioral, and physiological to provide evidence of selective associations in fear learning. Therefore, the use of cognitive measures besides autonomic measure increases the opportunity to demonstrate the blocking effect in this thesis project. In the context of arousal ratings, to obtain expected results supports that use of arousal ratings contributes the demonstration of blocking. This thesis project takes the first step for further investigations by using effective stimuli and adopting a working procedure as well as employing different response systems in order to deepen the knowledge about how the nature of all stimuli affects the blocking.

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Appendix A

“Participant Evaluation Form” applied before the experiment.

İZMİR EKONOMİ ÜNİVERSİTESİ PSİKOLOJİ LABORATUVARI

KATILIMCI BİLGİ FORMU

CİNSİYET: YAŞ:

BÖLÜM / SINIF: /

Lütfen aşağıdaki soruları, durumunuzu en iyi yansıtan seçeneği işaretleyerek ve boşlukları doldurarak yanıtlayınız.

1. Yakın zamanda (son bir sene dahil) başka bir psikoloji deneyine katıldınız mı?
 Evet, hafta / ay / yıl önce içerikli bir çalışmaya katıldım.
 Hayır
2. Herhangi bir psikolojik rahatsızlık tanısı aldınız mı?
 Evet hafta / ay / yıl önce tanısı koyuldu
 Hayır
3. Rahatsızlığınızla ilgili kullandığımız ilaçlar var mı?
 Evet, isimli ilaç(lar)ı kullandım / kullanmaktayım
 Hayır

“Participant Evaluation Form” applied before the experiment (cont.).

4. Herhangi bir obje veya duruma karşı fobiniz var mı? (örn: belirli bir hayvan, yükseklik, kalabalık, dişçi vs.)

Evet, fobisi
..... hafta / ay / yıl önce tanısı koyuldu

Hayır

5. Herhangi bir nörolojik rahatsızlık tanısı aldınız mı?

Evet hafta / ay / yıl önce tanısı
koyuldu

Hayır

6. Rahatsızlığınızla ilgili kullandığınız ilaçlar var mı?

Evet, isimli ilaç(lar)ı kullandım /
kullanmaktayım

Hayır

7. Herhangi bir kalp rahatsızlığı tanısı aldınız mı?

Evet hafta / ay / yıl önce tanısı
koyuldu

Hayır

8. Rahatsızlığınızla ilgili kullandığınız ilaçlar var mı?

Evet, isimli ilaç(lar)ı kullandım /
kullanmaktayım

Hayır

9. Herhangi bir ameliyat / operasyon geçirdiniz mi?

Evet Ameliyat / operasyon:
Ameliyat / operasyon tarihi:

Hayır

10. Vücudunuzun herhangi bir yerinde protez / implant var mı?

Evet Protez / implant: bölgesinde

“Participant Evaluation Form” applied before the experiment (cont.).

Protez / implantın yapı

maddesi:

Hayır

11. Düzenli olarak kullanmakta olduğunuz ilaçlar var mı?

Evet, isimli

ilaç(lar)ı amacıyla kullanıyorum

Hayır

12. Herhangi bir görme bozukluğunuz var mı?

Evet

Miyop Derece: sol göz / sağ göz

Hipermetrop Derece: sol göz / sağ göz

Astigmat Derece: sol göz / sağ göz

Diğer

Hayır

13. Aşağıdaki seçeneklerden hangisi sizin için uygundur:

Gözlük kullanıyorum

Lens kullanıyorum

Gözlük ya da lens kullanmıyorum

“Participant Evaluation Form” applied before the experiment (cont.).

Aşağıdaki belirtileri bugün de dahil olmak üzere son bir hafta içinde ne ölçüde yaşadığınızı göz önünde bulundurarak yanıt veriniz.

	Hiç	Hafif	Orta	Ağır
Bedeninizin herhangi bir yerinde uyuşma / karıncalanma				
Sıcak / ateş basmaları				
Bacaklarda halsizlik, titreme				
Gevşeyememe				
Çok kötü şeyler olacak korkusu				
Baş dönmesi / sersemlik hissi				
Kalp çarpıntısı				
Dengeyi kaybetme korkusu				
Dehşete kapılma				
Sinirlilik				
Boğuluyormuş gibi olma duygusu				
Ellerde titreme				
Titreklik				
Kontrolü kaybetme korkusu				
Nefes almada güçlük				
Ölüm korkusu				
Korkuya kapılma				
Midede hazımsızlık / rahatsızlık hissi				
Baygınlık				
Yüz kızarması				
Terleme (sıcağa bağlı olmayan)				

Appendix B

“Participant Information Form” given before the experiment.

İZMİR EKONOMİ ÜNİVERSİTESİ PSİKOLOJİ LABORATUVARI

KATILIMCI BİLGİLENDİRME FORMU

Bu çalışmanın amacı, laboratuvar koşullarında keyfi ve/veya ekolojik uyarıcılar kullanılarak geliştirilen fizyolojik korku tepkileri aracılığı ile bloklama etkisinin incelenmesidir.

Çalışma sürecinde bilgisayar ekranından -belirli aralıklarla- birtakım uyarıcılar sunulacaktır. Bu uyarıcılardan bazıları, sağ el bileğinize bağlanacak olan elektrotlar aracılığıyla verilen hafif bir elektriksel uyarım ile sonuçlanacaktır. Elektrotlardan verilecek olan elektriksel uyarımın şiddetini araştırmanın başında -sizi rahatsız edecek, fakat canınızı yakmayacak bir düzeyde olacak biçimde- sizin belirlemeniz istenecektir. Bilgisayar ekranından sunulan uyarıcılara verdiğiniz fizyolojik tepkiler, sol elinizin avuç içine yerleştirilecek olan elektrotlar aracılığıyla ölçülecektir.

Çalışma kapsamında katılımcılardan elde edilen veriler isim kullanılmaksızın analizlere dahil edilecektir; yani çalışma sürecinde size bir katılımcı numarası verilecek ve isminiz araştırma raporunda yer almayacaktır.

“Participant Information Form” given before the experiment (cont.).

Katılımınız araştırma hipotezinin test edilmesi ve yukarıda açıklanan amaçlar doğrultusunda literatüre sağlayacağı katkılar bakımından oldukça önemlidir. Ayrıca katılımınızın psikoloji alanın gelişmesi açısından da bir takım faydaları bulunmaktadır.

Çalışmaya katılmanız tamamen kendi isteğimize bağlıdır. Katılımı reddetme ya da çalışma sürecinde herhangi bir zaman diliminde devam etmeme hakkına sahipsiniz. Eğer görüşme esnasında katılımınıza ilişkin herhangi bir sorunuz olursa, araştırmacıyla iletişime geçebilirsiniz

İZMİR EKONOMİ ÜNİVERSİTESİ

KATILIMCI İZİN FORMU

Çalışmanın amacını ve içeriğini katılımcı numarasına sahip katılımcıya açıklamış bulunmaktayım. Çalışma kapsamında yapılacak işlemler hakkında katılımcının herhangi bir sorusu olup olmadığını sordum ve katılımcı tarafından yöneltilen bütün soruları yanıtladım.

Tarih:

..... / /

Araştırmacının İmzası:

.....

Araştırmacının Telefon Numarası:

.....

Çalışmanın amacı ve içeriği hakkında açıklamaların yer aldığı “Katılımcı Bilgilendirme Formu”nu okudum. Araştırmacı çalışma kapsamındaki haklarımı ve sorumluluklarımı açıkladı ve kendisine yönelttiğim bütün soruları açık bir şekilde yanıtladı. Sonuç olarak, uygulama esnasında şahsımdan toplanan verilerin bilimsel amaçlarla kullanılmasına izin verdiğimi ve çalışmaya gönüllü olarak katıldığımı beyan ederim.

Tarih:

..... / /

Katılımcının İmzası:

.....