

**Effect of awareness on learning in biconditional discrimination  
conditioning**

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## **TIETOISUUDEN VAIKUTUS OPPIMISEEN KAKSOISEHDOLLISTETUSSA EROTTELUEHDOLLISTAMISESSA**

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Tässä kokeessa vaikea ajassa tapahtuva erottelutehtävä, joka on melko helposti ratkaistavissa abstraktin tiedon avulla, esitettiin koehenkilöille. Koehenkilöt jaettiin kahteen ryhmään. Ensimmäinen ryhmä keskittyi vain esitettyihin ärsykkeisiin, kun toiselle ryhmälle annettiin myös samanaikainen lukutehtävä. Kahdesta äänestä, A:sta ja B:stä, muodostettiin neljä yhdistelmää (AA-, BB-, AB+, BA+, jossa + symbolisoi ” yhdistetty ehdolliseen ärsykkeeseen” ja – symbolisoi ”esitetty ilman ehdollista ärsykettä” ). Kokeen aikana mitattiin oikean silmän alta Orbicularis Oculi- lihaksen sähköistä aktiivisuutta (EMG) ärsykeyhdistelmän aikana. Yhdistelmistä tietoisuuden määrä mitattiin kysymällä molempien ryhmien osallistujilta ilmapuhalluksen ilmaantumiseen johtavaa sääntöä heti kokeen jälkeen. Muutokset lihaksen sähkönjohtokyvyssä analysoitiin oppimiseen liittyvien muutosten ja näiden muutosten mahdollisten tietoisuuden pohjautuvien osien löytämiseksi. EMG-aktiivisuudesta löydettiin erottelevia muutoksia juuri ennen ärsykettä. Molempien ryhmien jäsenet kehittivät testin aikana myös tietoisien havainnon ilmapuhallukseen johtavan ääniyhdistelmän säännöstä. Saadut tulokset tukevat väitteitä, joiden mukaan ihmiset 1) pystyvät käyttämään abstraktia informaatiota myös tiedostamatta, 2) ovat tietoisia kahden ärsykkeen samanlaisuudesta tai eroavuudesta, 3) pystyvät pitämään kohteiden ominaisuuksia muistissa jonkin aikaa. Tämän tyyppistä menetelmää ei ole kokeiltu aikaisemmin eikä siitä ole saatavana aikaisempaa tietoa.

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**Abstract**

In this study, difficult sequential discrimination task, which could be solved rather simply by using abstract information, were shown to the participants. Participants were divided in two groups. One group concentrated to incoming stimuli, while another group concentrated on a reading task. Two tones, A and B, formed four sequential combinations (AA-, BB-, AB+, BA+, where plus symbolizes “paired with the US” and minus symbolizes “presented alone”). During training, the electromyographic (EMG) activity of orbicularis oculi was measured below the outer canthus of the right eye. The rate of awareness was measured by asking the rule of coming of the airpuff from participants in both groups right after the end of the procedure. Changes in amplitude of EMG were analyzed in terms of learning-related changes and possible awareness-based behavioural correlates of these changes. Discriminative changes in EMG activity were found before US. Participants also developed a significant state of awareness of the rule in both groups during the test. Therefore results support the view, that humans 1) may use abstract learning strategies even masked, 2) are aware of the sameness or difference of two targets and 3) can keep the qualities of targets in memory for some time. This kind of procedure has not been tested before and no previous information is available.

*Keywords:* Awareness, Associative learning, Biconditional discrimination, Humans, Eyeblick conditioning, Sequential conditioning.

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**1.Introduction***1.1. Background*

An old but still unresolved problem for theories of conditioning concerns the manner in which associative strengths of elemental stimuli combine when the elements are presented in compounds. Although a wide range of empirical outcomes are in accordance with present theories, it is well known that simple additive combinations of associative strengths of components cannot handle nonlinear discrimination problems. One of the best known of these nonlinear

discrimination problems is biconditional discrimination (Saavedra, 1975).. In this procedure, animals are trained with an AB+, CD+, AD-, CB- (where plus symbolizes “paired with the US” and minus symbolizes “presented alone”) discrimination in which each element is reinforced on half of its presentations and non-reinforced on the other half. To solve it, animals have to learn to associate a reinforcer with specific configurations of stimuli (Saavedra, 1975).

One style of conditioning, although very close to biconditional discrimination, is differential conditioning. In differential conditioning, two CSs are presented. These CSs can be in the same modality (e.g. two tones of different frequencies) or in different modalities (e.g. a tone and a light) (Clark & Squire, 2000). When differential conditioning is used, one of the CSs always precedes and predicts the US. This is the CS+. The other CS is not predictive of the US and occurs alone. This is called the CS-. In differential conditioning, acquisition reflects a greater probability of responding to the CS+ than to the CS- (Clark & Squire, 2000).

In eyeblink conditioning, the responses are considered to be reflexive in nature. First, the unconditioned response (UR) is automatic reaction to the puff of air that is delivered to the eye. With repeated pairing of a CS and US a learned, conditioned response (CR) develops. This response is also thought to be automatic and, as such, does not require cognitive involvement (Clark & Squire, 2000). Nevertheless, though an eyeblink response can be involuntary, the eyeblink response can also be brought under voluntary control (Clark & Squire, 2000). If individuals become aware of the stimulus contingencies, they are also in a position to voluntarily blink their eyes to avoid the air puff. This circumstance has caused concern among experimentalists because it has been argued that the processes and characteristics of responses that are voluntary are very different from those that are involuntary or reflexive (Coleman & Webster, 1988).

### *1.2. The role of awareness*

A human participant can be consciously aware of something if and only if he or she can report it (Farber & Churchland, 1995). Disagreement on the role of awareness in conditioning has been prevalent. Some investigators have considered awareness of CS- US contingency essential to conditioning (e.g. Ross & Nelson,

1973) and others have considered it unessential and in a methodological matter even undesirable (e.g. Grant, 1973).

One of the most important parts of awareness is memory and how a person uses it. It is composed of several abilities that depend on different, namely declarative and nondeclarative, brain systems (Squire, 1992). Declarative (or explicit) memory provides the capacity for conscious recollection of facts and events. Nondeclarative (or implicit) memory is inaccessible to conscious recollection, but it is expressed through performance as skills, habits, and certain forms of classical conditioning. Simple delay classical conditioning of the eyeblink response is a well-studied example of nondeclarative memory. In delay classical conditioning, the conditioned stimulus is presented and remains on until the unconditional stimulus is presented. The two stimuli overlap and co-terminate. In trace classical conditioning, a silent (or “trace”) interval is placed before the presentations of the CS and US (Kim, Clark & Thompson, 1995).

An alternative approach by which to determine the role of awareness in human conditioning was to diminish such awareness by use of a concurrent (or masking) task that divided participants’ attention to the task (Ross, 1971). Attention has been referred to as selectivity in cognitive operations in which certain goals are in control of behaviour and certain stimulus information is thereby relevant (Duncan, 1995). Ross & Nelson concluded that discrimination learning may require an awareness of the CS –US relationship and that this awareness may be essential for some conditioning tasks and not others (Ross & Nelson, 1973).

These findings raise the possibility that awareness of the relationship between the CS and the US may be essential for some more complex conditioning paradigms and that division of attention may selectively disrupt awareness and performance of those forms of conditioning. Clark and Squire (1998) examined the role of awareness of CS – US stimulus contingencies in delay and trace discrimination learning. They concluded that conscious knowledge of stimulus contingencies is necessary for successful acquisition. Another discrimination study of Clark & Squire (1999) shows, that awareness of the CS – US contingency was essential for trace conditioning but irrelevant for delay conditioning. In another words, a person must be aware of the stimuli and keep it in memory during the silent interval.

### *1.3. Aims of this study*

The present study was designed to develop a new biconditional discrimination paradigm using auditory stimuli in human eyeblink conditioning and to test this procedure in humans. One point of this procedure is, that stimuli is presented in sequential manner. In real life classical conditioning is too easy because, be it an animal or a human, participants can form connections simply by focusing on sameness or difference of stimuli and possibly achieved reward. Instead, real learning is combining several elements in a most appropriate manner. This style of learning is more effective, because learner can form several combinations from elements used and choose the most useful of them. Learner need not form new combinations everytime, but change available elements to achieve more useful combinations instead. Nowadays, there is no procedure to examine that kind of phenomenon. When Kukkola (2004) used this same sequential timing discrimination in rabbits as in present study, she found that rabbits could not learn a discrimination task similar to this. However, she found that some discrimination has happened at neural level and that rabbits would have learned it if training had continued (Kukkola, 2004). Apparently no one has studied this sequential tonal procedure in humans before.

On the basis of research articles about this subject and theoretical assumptions of associative learning, it can be assumed that humans could learn biconditional discrimination task using different sequential tone pairings as CS and air puff to the eye as US. The hypothesis is that conditioned responding increases as the training proceeds, and that humans learn to discriminate stimulus types. Also awareness about the stimuli increases during the training, because of the demandings of both trace conditioning and sequential timing procedure used.

## **2.Method**

### *2.1. Participants*

The volunteers of the experiment (N = 20; 14 female, 6 male) were normal, healthy university students ranging in age from 19 to 45 years (mean= 23,75; Md= 21). They were divided into two groups, ten persons for both groups. Non-masked group received the information freely without any interference. Masked group participants was interferenced by a reading task. The procedures of both groups were otherwise the same. The volunteers did not receive any credit for their participation in the experiment.

### *2.2. Design and Materials*

The design was a trace conditioning procedure with intertrial interval varying from 20 to 40 seconds in 10 second steps (Mean ITI= 30 s). The conditioning phase consisted of 80 paired tones. 40 of them was CS+ and 40 was CS-. After these were 6 CS+ test trials without US. Thus, the total number of the trials was 86.

The CS consisted of a combination of two tones, which were 1 kHz (Tone A) and 1.2 kHz (Tone B) of their frequencies. There were four combinations, namely A-A, A-B, B-A, and B-B. Combinations A-B and B-A were conditioned, whereas A-A and B-B were not. All combinations were presented randomly 20 times. The intensity of the tones was 78 dB (A-weighted) measured 50 cm distance from the surface of the speaker. The US consisted of an air puff to the outer corner of the right eye approximately 1 cm distance laterally from the eye. The intensity of the air puff was 0.5 bar (7.25 psi) delivered via a plastic tube (5 mm diameter), which was set at approximately 1 cm distance from the surface of the skin.

The duration of the CS was 100 ms and the US was 100 ms. In the trials the delay between the tones was 400 ms and the delay between the second tone and US was also 400 ms. The EMG measurement began 400 ms prior to the CS onset and continued for 1500ms.

The participants were randomly assigned to either of the two experimental groups. In both groups the same procedure was played. Group 1 concentrated to the tones while reading task masked Group 2.



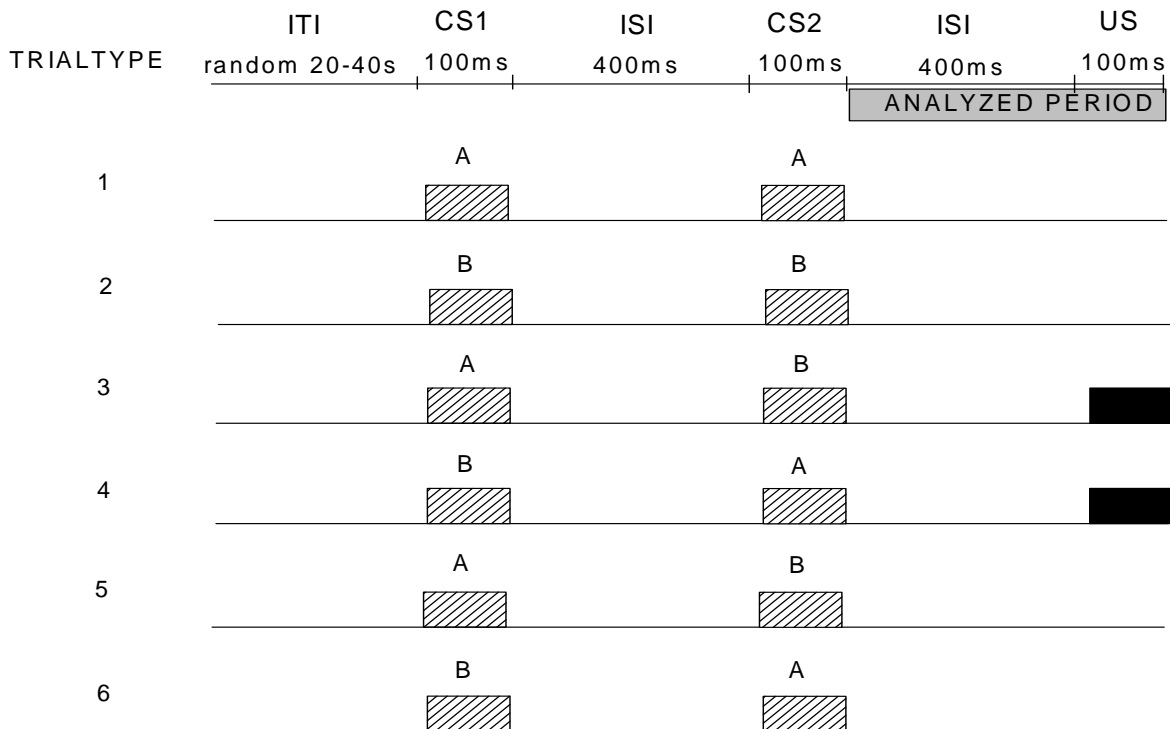


Fig.1. Schematic illustration of the discrimination training procedure, A = Tone 1000 Hz, B = Tone 1200 Hz. Trial types were presented in pseudorandom order. (Adopted and modified from Hautala, J.V. (2004) with kind permission).

### 2.3. *Signal processing*

The two electrodes used to measure the electromyographic (EMG) activity of orbicularis oculi below the outer canthus of the right eye were disposable Ag/AgCl electrodes filled with electrolyte pasta. A ground electrode was located at the midline of the participant's forehead.

The experiment was controlled and timed by the E-Prime 1.1 software on a computer which was equipped with Intel Celeron 500MHz processor. The procedure used was self-made by the E-Prime 1.1 program. The raw signal was amplified by a gain of 20000 and band-pass filtered from 60 to 1000 Hz. Data was collected on a computer which used Intel Celeron 900 MHz and DT Measure Foundry version 4.0 program. The signal obtained was then baseline corrected and rectified before digital filtering with 30 Hz cut-off on low-pass filter. The signal was then averaged over subject and the group for calculating the maximum amplitudes for analyses.

#### 2.4. Procedure

After arrival to the laboratory, the participants were verbally given a brief description of the experiment before placing the electrodes on and guiding them into the experiment chamber.

The experiment chamber was a room where the participants sat under a speaker approximately 50 cm above of them. Group 1 were told to focus on the tones and try to figure out when the air puff is coming. Group 2 did not receive that kind of information. The participants were also informed that they could abort the session whenever they felt like it. The air puff nozzle were placed and the electrodes were wired to the amplifier before the participants were randomly assigned either of the two groups, non-masked or masked. The test was started by the experimenter and it was aborted after the test trials of the experiment. After the testing procedure, the participants were asked, if they did learn the rule when the air puff is coming and when its not. All the answers are collected to measure the awareness of the reactions.

#### 2.5. Data Analysis

The statistical analyses were performed by the SPSS 11.5 for Windows program. The data was divided into nine blocks, of which the first eight consisted of of the trials during the conditioning phase, ten trials in each block. Thus, the ninth block, that is the test trials, consisted of the last 6 trials.

In all the trials the CR was defined as the maximum value of the mean amplitudes of eyeblink between 1000 ms and 1250 ms from onset of the first tone.

The effects of the conditioning on CR amplitude was analyzed by using the analysis of variance (ANOVA) for repeated measures and the paired samples t-test for planned comparisons. The statistical design for the initial trials and the test trials was a 2 ( group: non-masked, masked) times 2 ( blocks: first, eighth). The design for the conditioning phase was a 2 ( group: non-masked, masked) times 8 ( blocks: blocks of ten trials).

Degrees of freedom were corrected by the Greenhouse-Geisser correction if needed. The significance level was .05 in all analyses.

### 3.Results

The EMG-response amplitude to the tone increased from the first to the last block in both groups. ANOVA for repeated measures showed a significant increase in amplitude of the CR between the blocks,  $F(2,19) = 16.48$ ;  $p < .001$  and between different stimulus types,  $F(2,19) = 8.43$ ;  $p < .01$ , suggesting more detailed comparison revealed that there was also statistically significant interaction between the blocks and stimulus  $F(2,19) = 8.08$ ;  $p < .05$ . Group was also a discriminative factor,  $F(2,19) = 4.91$ ;  $p < .05$ . Non-masked group learned responding faster and conditioned more powerful than the masked group, but both groups showed significant learning during the test. These results indicate that the conditioning had an effect, and that this effect was different between the groups. Comparing first and last block of aware participants of non-masked group to first and last block of non-aware participants of masked group, results showed that there is statistically significant difference between blocks,  $F(2,12) = 13.66$ ;  $p < .01$  and between stimulus types,  $F(2,12) = 5.19$ ;  $p < .05$ . These results indicate that a state of awareness is important in this procedure. Although aware participants of non-masked group learned better than non-aware participants of masked group, state of learning in masked group participants is equally significant.

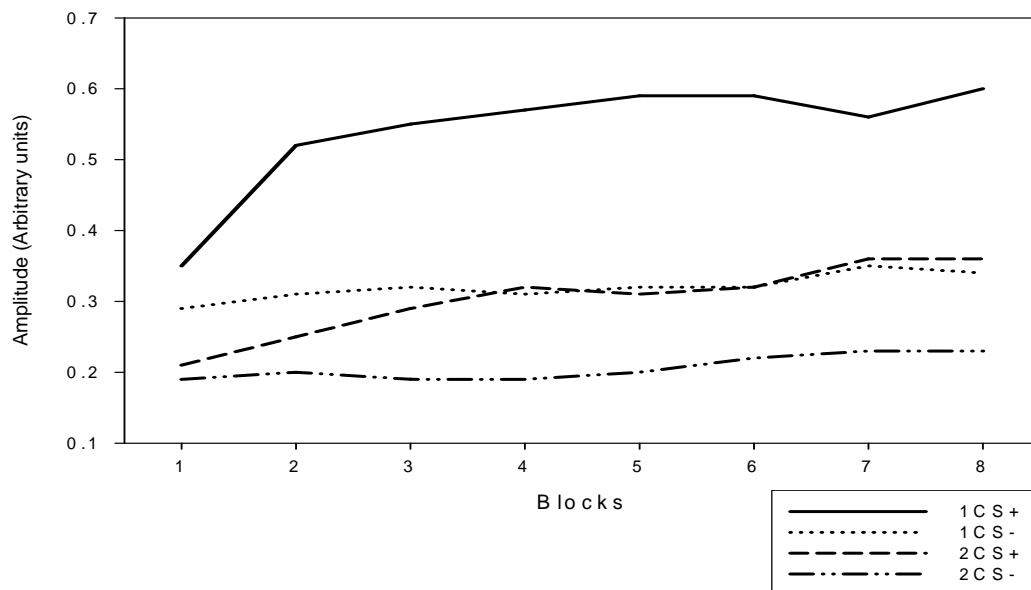


Fig.2 The growth of learning in amplitudes measured from the first to the last block.  
1 = non-masked group, 2= masked group.

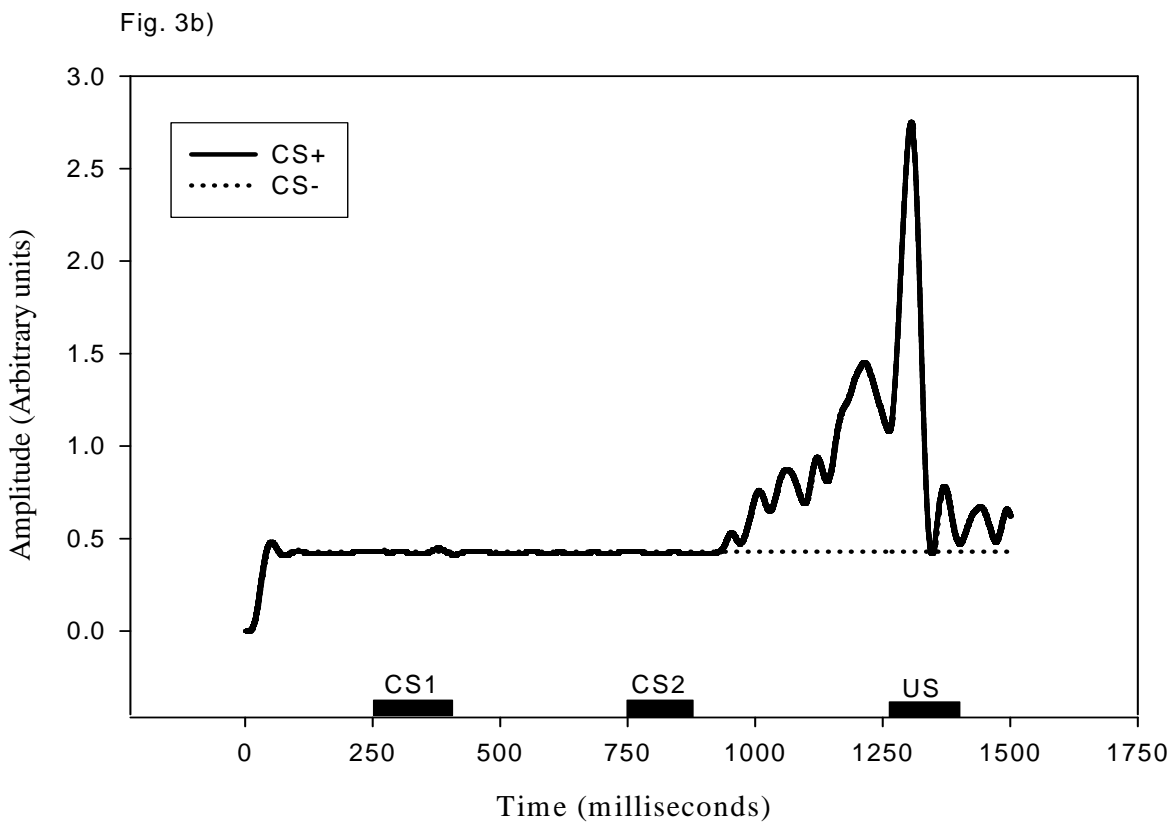
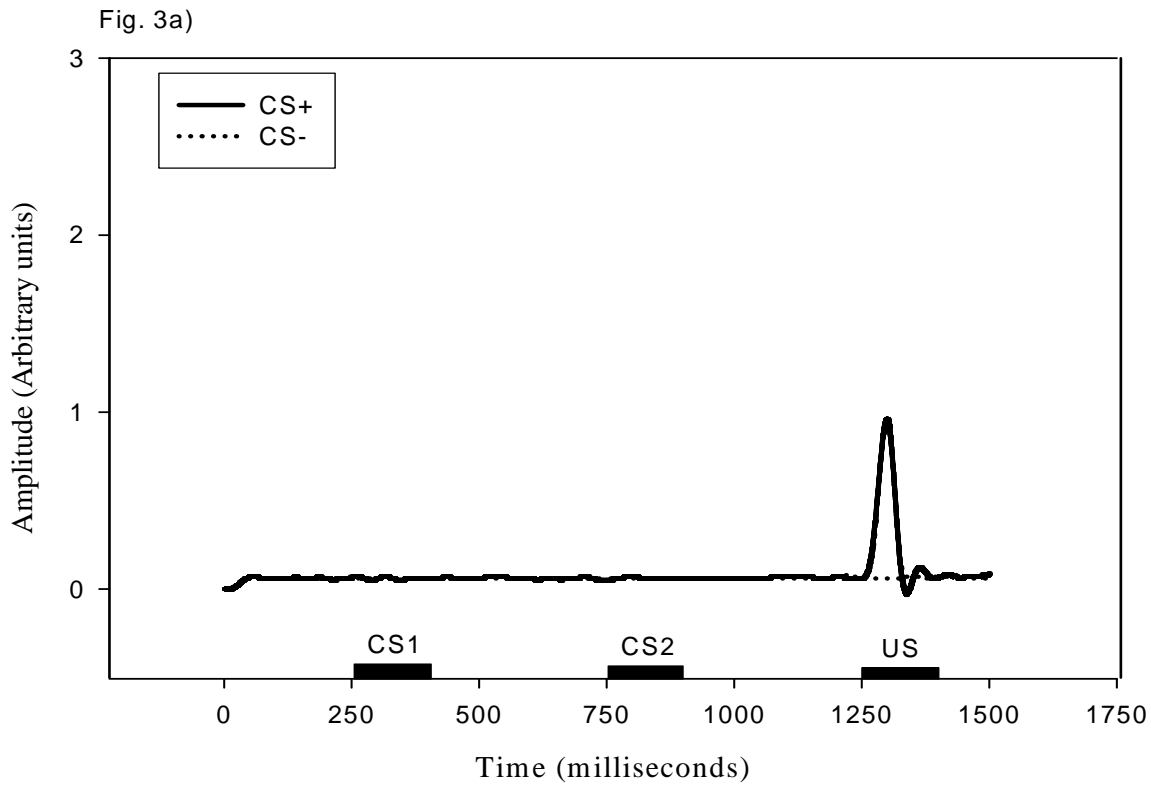


Fig. 3 EMG-responses in masked procedure participant before (Fig. 3a) and after (Fig. 3b) conditioning. CS1= First conditioned stimulus, CS2= second conditioned stimulus, US=unconditioned stimulus.

## 4. Discussion

This study clearly showed, that humans could learn biconditional conditioning procedure even if they did not attend to the stimuli. Both groups of this study learned the task. In this research the amplitude of the eyeblink response to the tone was increased from the first block to the last block of trials in both groups, although in non-masked group the growth of amplitude response was less significant. The awareness of the procedure and of the rule also increased rapidly during the test in non-masked group. Responses for stimuli are stronger in non-masked group than in masked group due to the awareness of stimuli they showed during the test. The masked group were actually not conscious of their learning, but they could reproduce the rule when asked. This result suggests that learning do not necessarily have to be conscious. In this procedure it confirms the hypothesis, that humans can learn present biconditional discrimination task.

### 4.1. *The role of awareness*

Awareness of stimuli is the main issue in this study. In Clark & Squire's (1998) study only those individuals who developed knowledge about the CS-US associations successfully acquired the task. In current study, participants in non-masked control group showed conscious action during the test, as was expected. What is more interesting, also the entire masked test group learned the rule of the coming of the air puff against Clarke & Squire's results. Another issue, when asked if the participants in masked group do think they learned the rule, is that they consciously did not know they had learned it. For example, a few subjects in a masked group assumed there were a relationship, but did not know what it was. This finding shows that successful learning does not require awareness. Almost all participants in non-masked group found it consciously and few even developed it further on during the last test block by waiting air puffs when it was not coming. It suggests that, upon entering the experimental situation, subjects consciously expected, and even looked for, a relationship between stimuli. Manns, Clark & Squire (2000) claimed that awareness was unrelated to differential delay conditioning. Further, using a trial-by-trial measurement of

awareness, they found that awareness and differential trace conditioning emerged concurrently (Manns, Clark & Squire, 2000). Furthermore, the degree of awareness after the first ten conditioning trials predicted the overall success of trace conditioning across the entire conditioning session (Manns et al., 2001). In the current research, in both groups amplitude response for CS+ does not strengthen Manns et al.'s (2001) claim but CS- responses does. If this phenomenon is due to the difficulty of biconditional discrimination, it should act similarly with both CS+ and CS- condition because of similar time intervals between CS1 and CS2. The influence of air puff may act a role by steering the awareness to the air puff in CS+ conditioning while in CS- conditioning participants learned that they could sigh with relief.

One development in information processing is shift from feature-based to gestalt-based, more abstract representations. Two tones, in this study A and B, are more easily discriminated as a function of frequency differences of the tones. Abstract learning requires computation of difference between frequencies of the tones and thus, to form working relation instead of bare identity (Weinberger, 1998). Again, if being able to form representations of these differences, the same associative discrimination and generalization processes may contribute between these representations. Therefore, discriminating between A and A, would turn out to be more difficult than A from B. This rather mathematical view of relation may provide quantitative framework to study complex learning and possibly, shift from combinations of identity to relational, stimulus' physical identity independent associative learning. However, it has to be noted that humans may be biased to process only relations relevant to their purposes, not in a straightforward mathematical way. From this viewpoint it is possible that human brain is initially equipped to detect any difference compared to sameness.

#### *4.2. Future use of the procedure*

As outlined in the introduction, there is need to enhance our knowledge about basic mechanisms that lie behind learning functions in order to understand human behavior. Although rather few studies have been conducted in order to study the role of awareness in biconditional configural learning, it is most likely to provide a model of a process where more complicated information is used in learning. In the interest of diagnose and rehabilitation of learning related

disorders it is important to know how these things happen (Lachnit, Reinhard & Kimmel, 2000). This research leaves open several questions. Does the difference of the stimuli have to be fixed or is it possible to discriminate continuously changed stimuli? Another question is that what is the limit when aging or amnesic people are capable of learning the rule? Major weakness of this study concerns the fact, that measurement of awareness is possibly not valid enough. Method of detecting awareness used in this study does not fully separate participants who actually developed awareness unconsciously during the test from those who developed it consciously after the examiner asked it. Conscious focusing of attention to stimuli during task in masked group is another issue that may lessen the validity of this study. Participants could have stopped reading for some time and concentrated their attention to stimuli instead.

In summary, the responses to the biconditional combinations were augmented from the first block to the last block of trials indicating that conditioning had an effect. The augmentation was stronger in the non-masked group than in the masked group. However, both groups learned difficult biconditional discrimination learning task. This is suggested to happen because of the awareness of stimuli and a flexible use of discrimination rules. There is also evidence that learning does not necessarily have to be conscious. Earlier studies on trace conditioning in humans is done on single-cue procedure, so there is no possibility for direct comparison. This study qualifies the hypothesis, that humans are able to discriminate different, more difficult stimulus types both consciously and sub-consciously.

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