CONFIGURAL LEARNING IN RABBIT NICTITATING MEMBRANE CONDITIONING: ACQUISITION OF BICONDITIONAL DISCRIMINATION

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Abstract

The purpose of this study was to develop a new biconditional discrimination task using classical trace conditioning of the nictitating membrane response (NMR) in order to study configural learning in rabbits (N = 4). Multiunit activity (MUA) was also measured from hippocampus and prefrontal cortex, because in the light of previous research it can be assumed that these areas are involved in configural learning. Subjects were trained with sequential compounds (AA-, BB-, AB+, BA+) of two tones A (1000Hz) and B (2000Hz), and nine sessions were chosen for analysis. No signs of behavioral discrimination were shown, although results from hippocampus and previous research indicate that more training trials would have ensured the success. However rabbits did learn a conditioned NMR in response to all conditioned stimuli. These results are discussed in terms of added, inhibited and replaced elements views.

Keywords: Nictitating membrane conditioning, Rabbit, Configural learning, Biconditional discrimination, Prefrontal cortex, Hippocampus, Multiunit activity

1. Introduction

In the natural world, stimuli usually appear in complex compounds when they enter into association. In literature, this compound is often referred to as configuration. A variety of models have been proposed to clarify the processing of these compounds. Even though the consensus between theoretical approaches is still missing, three main lines of research can be distinguished from the studies made in last thirty years.

Early studies of Saavedra (1975) gave rise to Rescorla and Wagner's model of the configural learning in the seventies (Wagner & Brandon, 2001; Brandon, Vogel & Wagner, 2000). Model, here after referred as added elements model, proposes that there are elements which represent the separate stimuli, and configural elements which represent the compound of stimuli. The total associative strength of a compound stimulus is based on the summation of the associative strengths of its elements. By assuming this, the added elements model has been able to account for far wider range of empirical phenomena than have any of its predecessors. However, there remain certain discriminations for which

this model predicts the incorrect outcome, for example negative patterning, where single elements are reinforced but the compound of them is not (Pearce, 1994; 2002). It implies that a discrimination between two relatively different types of stimuli will be acquired more slowly than between two relatively similar types of stimuli. Empirical findings do not support this idea and it has been proved that discrimination is actually acquired more rabidly when the signals are relatively different (Pearce, 1994; 2002).

About fifteen years ago, Pearce (1987) proposed his theory of configural learning, referred here as inhibited elements model. To simplify, the basic assumption made is that the same number of elements represents each stimuli, and if these stimuli are coactive, the other stimulus inhibits half of the elements otherwise activated by the other. Compound stimuli are represented as configurations, and associations will develop between these configurations and unconditioned stimulus (US). Theory predicts that generalization will occur between similar configurations, which makes the discrimination between them slower. The similarity between two configurations is related to the number of common elements they share. Inhibited elements model has its difficulties in explaining empirical findings of phenomena called summation, where two stimuli are first separately paired with US, and then composed into a compound (Pearce & George, 2002). The responding during a compound has proven to be stronger than during either stimulus alone. It is possible for inhibited elements model to explain summation, but whether or not this explanation is justified remains to be determined (Pearce, 1994).

The third model of configural learning has its roots in the beginning of the 20th century, in Hull's theory of afferent neural interaction, although this so called replaced elements view was finally developed fairly recently by Wagner and Brandon (Wagner & Brandon, 2001; Brandon, Vogel & Wagner, 2000). The basic idea lies in a notion that every stimulus interacts with each other in the nervous system in a way that changes them into something different. Presence of one stimulus can make the representation of the second different than it would have been if it was presented alone. In effect, a compound stimulus involves both addition of unique configural elements (added elements model) and inhibition of elements otherwise activated by the constituent stimulus (inhibited elements model). In other words a stimulus can excite context-independent elements that will be activated every time the stimulus is presented, and context-dependent elements that will be unique to the trials with a particular compound. When two stimuli together each excite rather few context-dependent elements, then model makes similar predictions to the theory of added elements model, but when they excite a high proportion of such elements then the model is said to make predictions similar to those of inhibited elements model. The main problem with this new theory is, that it can be applied only to experimental designs in which first stimuli is paired with no more than two other stimuli, because of the inhibition effect. There is also some uncertainty about the conditions that

determine whether or not a perceptual interaction will occur between two stimuli or whether one stimulus will inhibit a large or a small proportion of the elements of another stimulus (Pearce, 2002). For example, replaced elements model can not explain results found by Pearce and George (2002), using autoshaping in complex negative patterning discriminations in pigeons, but results from Myers et al. (2001) using similar task in rabbits do support the predictions of this model. Despite the conflicting results, it can be seen as challenger for previous theories in configural learning.

Theories of associative learning can also be divided into elemental and configural ones, latter indicating that "the whole is different from the sum of its parts" and former "the whole equals the sum of its parts". Hull's view of neural interaction, Rescorla and Wagner's added elements model and Wagner and Brandon's replaced elements theory are examples of elemental ones, where the latter extends considerably the range of experimental findings that can be explained from an elemental perspective. In contrast configural approaches have recently been supported mainly by Pearce (Pearce, 2002).

During these years there have been intensive attempts to develop discrimination problems that could reveal the mechanism behind configural learning; among these are biconditional discrimination and negative patterning. On the other hand, there has been increasing debate about differences between theories and paradigms used in these studies (e.g. Pearce & George, 2002; Deisig, Lachnit, Giufra & Hellstern, 2001; Lachnit & Kimmel, 2000; Rescorla, 1999; Wilson & Pearce, 1992). Although Pearce (2002) has argued that it may be difficult to discriminate between the different theoretical assumptions on which elemental and configural theories are based by referring to experimental evidence, it does not mean that we should not study the mechanisms behind the configural learning. The 'either elementary or configural -debate' is important not only in the area of animal learning, but also in human associative learning areas. In the interest of diagnose and rehabilitation of learning related disorders it is important to know how these things happen. Furthermore, this issue is often relevant in discussion of psychobiological models of learning and memory (Lachnit, Reinhard & Kimmel, 2000). Therefore need for a new paradigm and information is always apparent.

Nictitating membrane (NM) conditioning, initially developed by Gormezano (1966), is a widely used and reliable task in the analysis of the associative learning. During NM-conditioning in rabbit, a tone (or light etc.) conditioned stimulus (CS) is presented, followed by an airpuff to the eye (or electric stimulation of related muscles etc.) as unconditioned stimulus (US). As consequence, the rabbit is forced to move its NM as an unconditioned response (UR). After repeated presentations of CS and US in pairs, a conditioned response (CR) develops to the CS alone. Conditioning in this task in the rabbit has been shown to relay on cerebellum (Steinmetz, 2000; Thompson & Krupa, 1994; Andesson & Steinmetz, 1994) but hippocampus becomes involved when the

temporal gap separates the CS and US (Wiess, Bouwmeester, Power & Disterhoft, 1999; Kim, Clark & Thompson, 1995; Moyer, Deyo & Disterhoft 1990; Solomon, Vander Schaaf, Thompson & Weisz, 1986), referred to as trace eyeblink conditioning. On the other hand, there is evidence that hippocampus might contribute also to learning configurations and relations, where multiple features of an experience are bound together into integrated memory (Davachi & Wagner, 2002; Whishaw & Tomie, 1991; cf. Moreira & Bueno, 2003). Lesioning of the cerebellum has been demonstrated to prevent learning and disrupt eyeblink conditioning-related hippocampal activity, although it is still unsure, which brain areas take part in this connection (Kronforst-Collins & Disterhoft, 1998). Prefrontal cortex (PFC) has been hypothesized to be a possible candidate for this connection. Extensive connections have been noted between the cerebellum and PFC through the thalamus and between hippocampus and PFC (Kronforst-Collins & Disterhoft, 1998; Doyère, Burette, Rédini-Del Negro & Laroche, 1993). PFC is, for example, involved in temporal processing (Chaillan, Marchetti, Delfosse, Roman & Soumireu-Mourat, 1997), behavioural planning (Tanji & Hoshi, 2001), spatial navigation (De Bruin, Swinkels & Brabander, 1997; Compton, Griffith, McDaniel, Foster &Davis, 1997), emotion (Deacon, Penny & Rawlins, 2003; Morgan, Romanski & LeDoux, 1993), decision making (Krawczyk, 2002) and short-term memory (Kronforst-Collins & Distehoft, 1998). It has also been found that prefrontal cortex supports configural learning rather than basic associative learning (Whishaw, Tomie & Kolb, 1992). There is a large amount of evidence to support the fact that hippocampus is necessary for trace eyeblink conditioning and that PFC functions as an information processor in many cognitive tasks. Therefore it would not be surprising if both of these areas found out to be important in the following configural learning design.

The present study was designed to develop a new biconditional discrimination paradigm using auditory stimuli in rabbit nictitating membrane response (NMR) conditioning. In biconditional discriminations all or the most of possible combinations of a set of elements is presented in a fashion of some compounds being reinforced and different compounds using the same elements being not. Number of presentations of the various compounds is balanced and therefore should not be possible to solve such discrimination elementally, because each element occurs as often in reinforced compounds as in nonreinforced ones. According to all three theories presented earlier, the biconditional discrimination should be possible, because of the configural elements that are unique to each compound. There is evidence that rats can learn an easy biconditional discrimination using stimuli from different modalities (Healey & Gaffan, 2001; Whishaw & Tomie, 1991). It has also been demonstrated that pigeons (Rescorla, Grau & Durlach, 1985), monkeys (Saunders & Weiskrantz, 1989) humans and even in honeybees (Lober & Lachnit, 2002) can master this type of task. Saavedra (1975), who can be seen as a pioneer of this paradigm, has revealed that rabbits can learn a biconditional discrimination using stimuli from two different

modalities (auditory and visual) in NMR conditioning, but apparently no one has studied this using only auditory information in sequential compounds in rabbit NMR conditioning.

On the basis of research presented above and theoretical assumptions of associative learning, it can be assumed that rabbits could learn biconditional discrimination task using different sequential tone pairings as CS and airpuff to the eye as US. The hypothesis is that conditioned NM-responding increases as the training proceeds, and that animals learn to discriminate different stimulus types. The second purpose of this study is to analyze what happens in NM-responding and in multiunit activity (MUA) of prefrontal cortex and hippocampus during training trials as sessions go on. The hypothesis is that there can be found changes in the behavior and MUA, and that neural activity predicts and correlates with time-amplitude course of the NM-responses.

2. Methods

2.1. Animals

The subjects were four experimentally naïve, adult, New Zealand albino rabbits weighing 2,5-2,6 kg at the time of surgery. They were individually housed in metal cages on a 12:12 hour light-dark cycle with free access to food and water. All experimental procedures were performed during the light portion of the cycle. The experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC) regarding the care and use of animals for experimental procedures.

2.2. Surgery

The animals were anesthetised with intramuscular injections of ketamine-xylazine cocktail. (Ketaminol, 50 mg/ml, 5.6 ml; Rompun, 20 mg/ml, 2.2 ml; physiological saline 2.2.ml) The initial dosage was 3 ml and the anesthesia was maintained by additional injections of 1 ml every 20-30 minutes. After the deep general anesthesia had been achieved, the animals were placed in the stereotaxic instrument (Kopf Instruments) with bregma 1.5 mm above lambda. A longitudinal incision was made to reveal the skull onto which the headstage designed to hold the minitorque potentiometer was cemented with dental acrylic using four stainless steel anchoring screws. Five recording electrodes were implanted in the hippocampal and prefrontal area, two of them to the former area and three of them to the latter. Electroencephalography and MUA were monitored during the implantation procedure, and the electrode was lowered until typical activity of that area was observed. The electrode implantation procedure used here, is described elsewhere (Korhonen, 1991). Before finishing, a nylon loop was sutured into, but not through, the NM of the right eye. Analgesics (Temgesic, 0.3 mg/ml) were

provided right after surgery. The animals were given at least one week to recover after surgery before the actual experimental procedures.

2.3. Training procedure

Before the experiments, animals were adapted to the experimental situation by placing them at least once in a Plexiglas restraining box (Gormezano, 1966), located in a soundproof conditioning chamber. During the experiments, the NM-loop was linked by a rigid stainless hook to the swivel arm of the minitorque potentiometer for measuring NM-movement. The extension of the NM was transduced to voltage by the potentiometer (1 mm equal 1 V). Airpuff towards the cornea served as US, and sequential tone pairs as CSs (Figure 1).

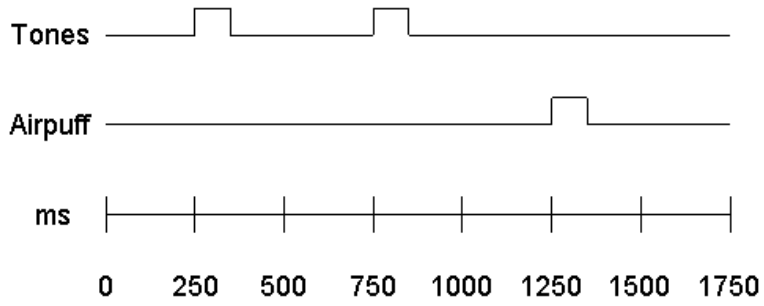

Figure 1. Schematic illustration of the biconditional discrimination training procedure.

The tones were directed to the rabbits left ear by generator placed outside the training box. The airpuff was delivered through a tube attached to the animal's headgear. Experiments were controlled by BRACE© computer program. All animals were observed constantly during the experiment in case of struggling or other problems. Four combinations of sequential CS-tones were used, consisting of two sounds 1000Hz and 2000 Hz, (78 dB) referred here to as CSA and CSB. Both tones and also US lasted for 100 ms. The interstimulus interval (ISI) and trace interval are shown in Figure 1. Intertrial interval (ITI) varied randomly ranging from 20 to 40 s (mean ITI 30 s).

Eight types of trial were used during the experiment, as presented in Figure 2. Training procedure started out with seven days discrimination training (phase I), where trial types from 1 to 4 were used. Types one and two served as CS- and types three and four as CS+. Each session consisted total of 88 trials, when the last eight of them were test trials (types 1-2 and 5-6). Subsequently (phase II) all four compounds were reinforced (trial types 3-4 and 7-8) and one session consisted total of 80 trials. This phase lasted from one to five days, depending on the animal's learning. Third phase (III) was equal to the first one, but it lasted only from two to five days.

Trial types	
Type 1	CSA + CSA
Type 2	CSB + CSB
Type 3	CSA + CSB + US
Type 4	CSB + CSA + US
Type 5	CSA + CSB
Type 6	CSB + CSA
Type 7	CSA + CSA + US
Type 8	CSB + CSB + US

Figure 2. Different types of trial used in the experiment.

2.4. Histology

After the experiments, animals were anesthetized with an intramuscular injection of ketamine-xylazine cocktail, the same cocktail that was used in the initial surgery. After that, they were given a lethal dose of pentobarbital and perfused via the ascending aorta with saline followed by 10 % formalin. The brains were removed, and then fixed in formalin solution for at least one week. Frozen coronal sections of 0,1 mm were taken from the sites of the electrodes. Slices were mounted on gelatinised slides and stained with cresyl violet. The locations of the electrodes were determined according to the stereotaxic atlas (Shek, Wen & Wisniewski, 1986).

2.5. Data Analysis

The data was gathered by using BRACE© computer program. The signal analysis was based on a 1750 ms sampling period. Nine sessions were selected for analysis, seven from phase I and two from phase II, total of nine trials. The NMR was measured as the maximum extension of the NM during the CS-period. Trials with NM-movement exceeding 0.5 mm during period of 125 ms prior to the first CS (CS1) were rejected from the analysis. MUA was band-pass filtered (500-6000 Hz) and digitised at the rate of 15 000 samples/s. Frequencies of spikes were calculated using a custom programmed DTVee for Windows program. After setting a spike frequency threshold (approximately 15 spikes / second), the frequencies exceeding this threshold were counted per 10 ms bin. The behavioural and neural activity was measured from the beginning of the CS1 to the beginning of the US as total and in four 250 ms periods. SPSS 11.5 for Windows was used for all numerical processing. ANOVA for repeated measures and Paired samples

t-test were used in the analyses. All data was analysed in consideration of different trial types.

3. Results

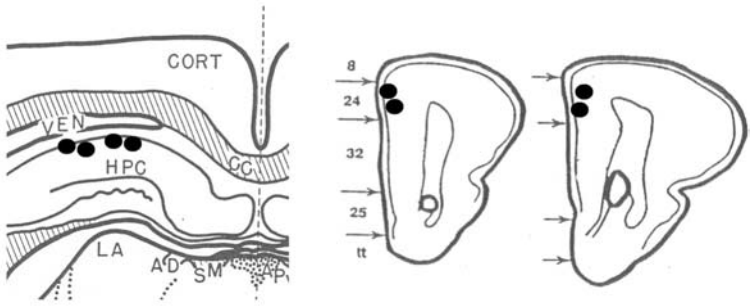

Figure 3. Locations of the electrode tips

Locations of electrodes are presented in Figure 3. Hippocampal electrodes were located in the CA1 region in all rabbits. Prefrontal electrodes located in the anterior cingulate cortex (Brodman's area 24) were also chosen for the analysis of MUA. One electrode per rabbit was used from each area in the analyses.

Left hand side of Figure 4 demonstrates the averaged NM-responses in the first and last sessions taken for analysis. During the training sessions there was an overall increase in NMR, measured from the CS-period [$F(1,3) = 1.98$, $p < 0.05$, one-tailed test].

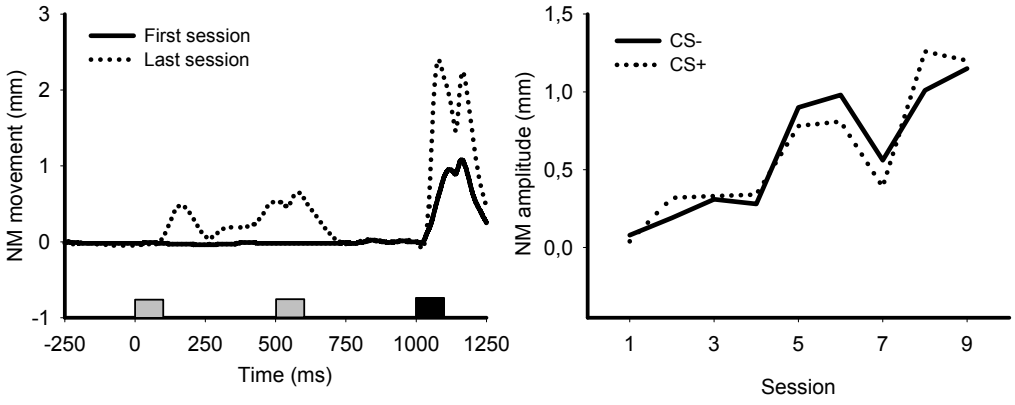

Figure 4. Averaged behavioral responses during the sessions 1 and 9, and behavioral responses to CS- and CS+.

During training, there were no significant differences in NMR between CS+ and CS- trial types (right hand side of Figure 4) or between trial types one through four either. CS-period was divided into four equal parts and no significant increases or differences between reinforced and nonreinforced trial types, in NMR were found in these, except in the first quarter, where responding to CS+ and CS- trials changed significantly during training [$F(1,3) = 2.61$, $p < 0.05$, two-tailed test]. These results indicate that biconditional discrimination training had an overall conditioning effect on behavior, but discrimination between CS+ and CS- did not happen.

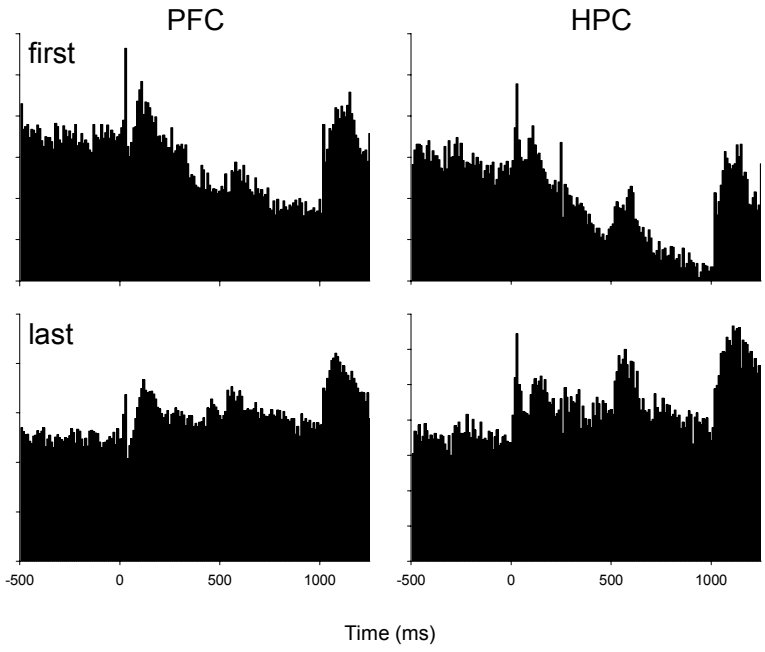

Figure 5. Averaged MUA during sessions 1 and 9 in PFC and hippocampus

Electrophysiological results from CA1 region of hippocampus (right hand side of Figure 5) show that during the training sessions there was an overall increase in MUA measured from CS-period [$F(1,3) = 6.68$, $p < 0.001$, two-tailed test]. When this time period was divided into four sections, there was a significant increase in all of these [in order of appearance [$F(1,3) = 2.83$, $p < 0.05$; $F(1,3) = 7.15$, $p < 0.001$; $F(1,3) = 6.71$, $p < 0.001$; $F(1,3) = 7.19$, $p < 0.001$, two-tailed tests] However, as training proceeded the only difference between trial types in MUA was found in the last quarter (1260-1500ms) as shown in the right hand side of Figure 6, where significant interaction between training sessions and CS+/CS- trial types was found [$F(1,3) = 2.15$, $p < 0.05$, one-tailed test]. Results from anterior cingulate cortex of PFC (see left hand side of Figures 5 and 6) showed

increase in MUA in two of the four sections, second [760-1000ms: $F(1,3) = 4.77$, $p < 0.01$] and last [1260-1500ms: $F(1,3) = 4.75$, $p < 0.01$], but no signs of discrimination were found. In both electrode sites the activation in the first quarter was greater than pre-CS baseline in all sessions, but in other three quarters it started out as smaller and changed in sessions 6-8 into greater than baseline. This phenomenon can also be seen in Figure 6.

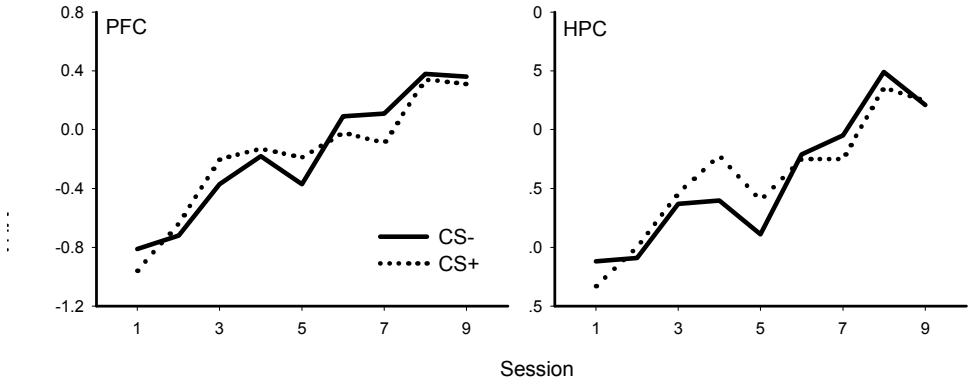

Figure 6. Averaged MUA of the last quarter of CS-period to CS- and CS+

4. Discussion

On behavioral level, rabbits did not learn the present biconditional discrimination task, but they did acquire conditioned NMR to tone stimuli in general. Electrophysiological results, especially from CA1 region of hippocampus, suggest that discrimination has happened at neural level and therefore, it can be assumed that it would have shown in behavior later on if training had continued.

4.1. Behavioral results

Modern theories of conditioning have to deal with an old question which first came up in the psychology of perception: is the whole more than or even different from the sum of its parts? According to added elements view, in this paradigm there were four configural elements, aa, bb, ba, and ab, coming from the isolable cues A and B[1]. In biconditional discrimination, the specific configural cues acquire excitatory strength in the compounds that are reinforced (ab and ba) and inhibitory strength in those that are not reinforced (aa and bb). The associative

[1] For the sake of clarification and generalization, CSA is referred in this discussion as A and CSB as B.

strength of these configural cues can be expected to be large only if the isolable cues A and B are not allowed to pick up strength (Pearce, 2002; Wagner & Brandon, 2001; Brandon et al, 2000; Saavedra, 1975). It could be assumed that in the biconditional discrimination described here, the associative strengths of compounds were not very different, because all of them are composed only of two elements, and therefore the isolable cues A and B probably also gained at least some strength. CS+ pairs equalled the CS- pairs in elements that comprise them. Only thing unique to reinforced compounds were the configural cues ab and ba. Because of the rather few unique elements, the added elements model proposes that this biconditional discrimination task is very difficult to learn. All this does not however allow one to reject the possibility that other configural cues, common in both groups of compounds, such as "twoness", "difference" or "similarity" could be exerting stimulus control over responding, because these cues were also reinforced throughout training.

Pearce's inhibited elements view (Pearce, 2002; Wagner & Brandon, 2001; Brandon et al., 2000; Pearce, 1994; 1987) assumes that similarity between any pair of compounds, which dictates the degree of generalization between them, is equal to the ratio of the number of common cues to total cues in one configuration times the ratio of the number of common cues to total cues in the other configuration. By this rule there is not any similarity between CS- pairs, AA and BB in this study (0/2 x 0/2 = 0) and CS+ pairs, AB and BA were basically the same (2/2 x 2/2 = 1), therefore dictating a total generalization between them. Following the same rule, it can be counted that similarity between any CS- and CS+ pairing is 0.5 (eg. Pairs AA and AB, 2/2 x 1/2 = 0.5). According to inhibited elements model, the salience of all configurations is equal, and that the total number of theoretical elements in any compound is constant. Therefore the subset of theoretical elements representing any stimulus A that is active when A is in compound with one stimulus is statistically independent of the subset that is active when A is in compound with a different stimulus. Figure 8 shows what this means in the paradigm developed here.

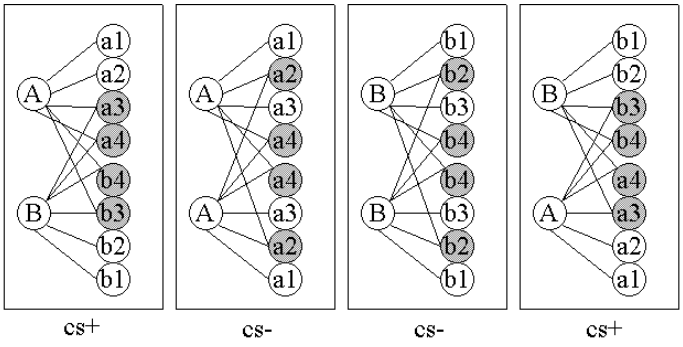

Figure 7. Illustration of inhibited elements view in present biconditional discrimination task. The elements inhibited in compound are darkened. (Modified from Wagner & Brandon, 2001; Brandon, Vogel & Wagner, 2000)

Note that CS+ and CS- trials share half of their active theoretical elements, in agreement with our computation of 50% generalization between them. However, there seems to be equal number of unique elements (a3 and b3 in CS- trials, and a2 and b2 in CS+ trials) in both reinforced and nonreinforced trial types. According to inhibited elements model any manipulation that enhances the similarity of the signals for reward and nonreward will make the discrimination between them more difficult to learn. As consequence, there will be substantial generalization of excitation from CS+ trials to CS- trials, and many more training trials will be needed before the inhibition association with CS- has developed to such extent that no response occurs on the nonreinforced trials (Pearce, 2002; Wagner & Brandon, 2001; Brandon et al., 2000; Pearce, 1994; 1987). Pearce has yet argued that the role of similarity in discrimination studies is not fully understood and needs further investigation (Pearce, 2002).

Replaced elements view by Wagner and Brandon (Pearce, 2002; Wagner & Brandon, 2001; Brandon et al., 2000) would explain present results using the ideas from both previous theories. Presentation of compound activates an element that is not activated by isolable stimulus, and at the same time inhibits the activation of an element that is activated by this stimulus alone, and vice versa. Each compounded stimulus causes the replacement of different elements, and that pair wise replacement process produces the necessary discriminable representations for a compound. This means that there are context-specific components in the representation of constituent stimuli as well as in the representation of compound stimuli. Each isolable stimulus is supposed again to have four elements. When they are presented in a compound, one of the original elements is replaced by a element, which reflects the compound. In the present study compounding A with B leads to the replacement of a4 by ab, where as compounding of A with A leads to the replacement of a3 by aa. According to this theory also, rabbits should learn the discrimination because there are elements that are unique to reinforced (a3, ab, b3, ba) and nonreinforced (aa, a4, bb, b4) compounds. Elements of one stimulus are more likely to be inhibited by another if they belong to the same, rather than to different modalities (Myers, Vogel, Shin & Wagner 2001; Kehoe & Gormezano, 1980). As consequence, this leads to the same interpretation of the present results as earlier two theories; more training trials are needed.

The problem seems to be that CS+ and CS- trials are too much alike which makes the discrimination hard to learn. Interesting enough, none of these theories pay attention to order of isolable stimuli. In the present study trial types 3 (AB) and 4 (BA) differed only on this order, and the way they differ from types 1 and 2 is that both of these CS- compounds are composed only of one isolable stimulus (AA and BB). The rule behind the task could be outlined as following: Every time the second stimulus is different from the first, trial is reinforced. Therefore rabbit should pay attention to the CS1 and then remember it for a short while in order to compare it to the CS2. This could also be seen as an example of relational

learning; a rabbit needs to use information of sameness and difference in order to solve this problem. There is evidence that at least two species, the African Grey parrot and chimpanzee, can use this kind of information to solve discriminations. However, in both cases animals received many years of training before they showed this ability, and it is not clear which aspects of their training were responsible for the success (Pearce, 1994).

Before turning into discussion of electrophysiological results, it can be concluded that the task used in this experiment was extremely difficult to learn and rabbits need probably many more training trials before they can master the discrimination. For example, Saavedra (1975) used 20 daily sessions of approximately 4,5 hour's duration in her biconditional discrimination task. Due to the summation principle of early elemental theories, responses to the different compounds should not differ because they consist of elements that all have received the same amount of reinforcement and comparable reinforcement histories. Present behavioral results are therefore in line with these theories. Differential responding to CS+ and CS- right after CS1 is probably a false positive result, because in both cases either one of the tones could be first and so there is not really any physical differences between these trial types (see Methods in this article for details).

4.2. Electrophysiological results

Use of the rabbit classical nictitating membrane/eyelid conditioning preparation has yielded more data concerning brain structures and systems involved in associative learning than any other paradigm or procedure (Steinmetz, 2000). Therefore it was chosen also for this experiment. Electrophysiological activity was measured from both the CA1 region of hippocampus and anterior cingulate cortex in PFC. It has been suggested that short CS duration (such as 100 ms in this experiment) coupled to a relatively long trace interval, could maximize the need for the rabbit to formulate an appropriate short-term memory trace in hippocampus, necessary for the correct association between the CS and the US (Moyer, Deyo & Disterhoft, 1990). Results from variety of studies indicate that ISI between 200 and 400 ms is optimal for learning (Port, Mikhail & Patterson, 1986). In the present experiment, the trace intervals between the three stimuli were 400 ms, but the interval from the CS1 to the US was as long as 900 ms.

Hippocampus becomes involved in the trace conditioning paradigm hundreds of trials before the animal acquires the behavioural CR (Solomon, Vander Schaaf, Thompson & Weisz, 1986). Its pyramidal neurons change their firing rates as a result of paired training. These firing patterns seem to mimic the amplitude and time course of the conditioned behavioral response as the animal learns the task. This activity increases very early in training and continues to grow thereafter. In the beginning of training, increased neuronal activity is present throughout the CS-US interval. But as training progresses, the activity shifts to later in the interval and appears to model the behavioural response. This has shown to be

conditioning specific phenomena, because these changes are not observed during unpaired pseudoconditioning (Moyer, Deyo & Disterhoft, 1990; Solomon, Vander Schaaf, Thompson & Weisz, 1986; Patterson, Berger & Thompson, 1979). Earlier studies have shown that responses to the CS always precede behavioral responses in latency (Berger, Basset & Orr, 1991). MUA from hippocampus in the present study seems to model the overall behavioral conditioned response to the tones, but there are also signs of discrimination in this activity which could not be seen in behavior.

During classical discrimination training, characteristic learning-related activity has been seen in the CA1 region of hippocampus on trials when CR was executed. As discrimination learning occurs and number of CRs to the CS- diminishes, learning related activity in the hippocampus on CS- trials also diminishes. There could be an increase in inhibitory hippocampal neuronal activity on CS- trials, thus causing an overall decrease in MUA, or hippocampus may simply become unresponsive to the CS- because it is not paired with the US. It has been suggested that this inactivation actually promotes acquisition of discrimination (Miller & Steinmetz, 1997). In the present study there was learning related hippocampal activity 250 ms before the onset of US, where signs of discrimination occurred, although they were not very clear. There was a rough tendency in CS- trials to be smaller in the MUA than CS+ trials in the last sessions of training. It is suggested that these changes in activity reflect early stages of learning the biconditional discrimination.

There are a large number of interconnections between the hippocampus and the PFC (Kronforst-Collins & Disterhoft, 1998). For example, anterior cingulate cortex of PFC and hippocampus are both important for successful acquisition of trace conditioned reflex (Weible, Weiss & Disterhoft, 2003). Investigators concerned also with the neuropsychology of aging have assessed the contributions of the frontal lobes and hippocampus for learning and memory. Research results strongly suggest that deterioration within these structures plays a prominent role in aged-related declines in learning, memory and cognition (Broersen, 2000; Winocur & Moscovitch, 1990). Interactions between hippocampal system and prefrontal cortex are also important in learning language-like rules, as human neuroimagining studies indicate (Opitz & Friederici, 2003). A similar type of activation in the two areas during a variety of tasks has been observed in many previous studies (e.g. Kronforst-Collins & Disterhoft, 1998), which was also seen in this experiment. Characteristic to all but the first time period of MUA in CA1 region and anterior cingulate cortex in the present study was the early inhibitory activation, which declined as training preceded and changed into excitatory later on. All discrimination tasks involve excitatory responding to one stimulus and inhibitory responding to another and experimental data as well as clinical observations suggest that learned behaviours involving changes in inhibitory responding are greatly affected by prefrontal damage (Chachich & Powell, 1998). Results from this study also indicate, that early in the learning process, some

inhibitory activation is needed to possibly identify the stimuli appropriately. In the first time period, 250 ms after the onset of the CS1, there was an increasing excitatory activation in both areas throughout all nine sessions, which is probably a sign of orienting alpha response. MUA showed two larger bursts during the CS-period, which were located before the CS2 and before the US. These are suggested to reflect different aspects of conditioning, behavioural responses in hippocampus and attention in PFC, as discussed later on.

Prefrontal cortex has four major functions. First of all, it retrieves sensory information to meet behavioral demands and secondly it exerts executive control of memory retrieval from sites of long-term storage. Thirdly, it actively maintains either sensory or memory information, and last but not least integrates or manipulates the retrieved or stored information for subsequent use. (Tanji & Hoshi, 2001) Although the nonprimate mammalian prefrontal cortex lacks the well-defined granular layer (IV) that typifies the one on primates, it has been suggested that homologies may be drawn between the PFC of rabbit based on the projection fields of the medial dorsal nucleus of the thalamus. Caudal area of medial PFC, including the anterior cingulated region, in nonprimate mammals is homologous to the primate dorsolateral PFC (Kronforst-Collins & Disterhoft, 1998; Joel, Weiner & Feldon, 1997). Human dorsolateral PFC is critical in making decisions that call for the consideration of multiple sources of information. It is needed for example in working memory, reasoning, integration of information, mediating relational information and categorization of novel stimuli (Rympa & D'Esposito, 2003; Krawczyk, 2002; Kronforst-Collins & Disterhoft, 1998). Much of the same as needed in the present experiment, although in a very simple manner. Damage in PFC area (e.g. in schizophrenia, Parkinson's disease, Huntington's disease) disrupts performance on tasks that require temporal ordering of information, response sequencing and the acquisition of rule-based behavior. (Chaillan, Marchetti, Delfosse, Roman & Soumireu-Mourat, 1997)

The medial PFC of the rabbit is commonly described as including the infralimbic, prelimbic and anterior cingulated cortices, and is located rostral to the genu of the corpus callosum. In rabbits the anterior cingulate cortex is involved in identifying behaviorally salient stimuli and mediating sustained attention. It is also involved in more complex orientation and selective attention tasks through interactions with other cortical areas (Weible, Weiss & Disterhoft, 2003). Present results from this area show an increase in MUA before the CS2 and before US, in a same way as was seen in hippocampus, although there are not any signs of discrimination. This activity so precedes two behaviorally meaningful stimuli. As noted earlier, only the CS2 in relation to the CS1 tells if the trial is going to be reinforced or not. Therefore this activity of PFC could reflect the attention that is paid to these stimuli during learning. It would be interesting to see what happens to this activation when the task is learned in the behavioral level. In sum these results do support the hypothesis that prefrontal cortex is somehow involved in

configural learning, possibly in focused attention during training. On the other hand, these results indicate that anterior cingulate cortex is not responsible for the discrimination needed in the present experiment.

4.3. Conclusion

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. Configural learning in rabbit nictitating membrane conditioning would seem to provide an animal model of a process where more complicated information is used in learning, because so much about its neural substrates is already known. However, rather few studies have been conducted in order to study neural mechanisms behind configural learning and experimental data, for example, from biconditional discrimination is still insufficient. These questions could yet be resolved with currently available methods, such as reversible inactivation or recording of neural activity. Because of the fact that present study has been very experimental in nature, there remain many unsolved questions for future research. More training trials are evidently needed, more subjects also. For purposes of electrophysiological results, one must have an unpaired control group. One question that arises form present study is that what if the conditioned stimuli were not as similar as they were here. Does it have an effect on learning. It would also be interesting to study the neural mechanisms behind the most common configural learning paradigm, the negative patterning, because to my knowledge there is not much done yet in that area. The results presented here are only suggestive, because of the small number of subjects, which inevitably affects the statistical analyses.

Taken together present results show that even though the signs of learning the biconditional discrimination in NMR were absent, it can be hypothesized that it would have happened if training had continued. Behavioural results support the hypothesis made by all three theories presented here; many training trials are needed before one can master this kind of difficult discrimination. However, the results from HPC support our original hypothesis that some discrimination had occurred already in these rather few training sessions. These electrophysiological results are therefore more in line with inhibited and replaced elements model than with added elements view.

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