ON THE QUANTITATIVE EDGE OF FEATURE AND GESTALT – BASED ASSOCIATIVE LEARNING: LEARNING-RELATED ERP CHANGES IN THE HIPPOCAMPUS AND THE PREFRONTAL CORTEX DURING A COMPLEX AUDITORY DISCRIMINATION TASK IN RABBIT NM-PARADIGM

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PIIRTEISIIN- JA HAHMOON PERUSTUVAN ASSOSIATIIVISEN OPPIMISEN MITATTAVALLA RAJALLA: OPPIMISEEN LIITTYVÄT HERÄTEVASTEET HIPPOKAMPUKSESSA JA ETUAIVOLOHKOSSA MONIMUTKAISEN AUDITORISEN EROTTELUKOKEEN AIKANA KANIN VILKKULUOMIREFLEKSI -KOEASETELMASSA

Testinä olettamukselle että kehittyneemmät eläimetkin osaavat käyttää abstraktia informaatiota ainakin jossain määrin, vaikea peräkkäisten ärsykkeiden erottelutehtävä, joka on helposti ratkaistavissa käyttäen abstraktia informaatiota, suoritettiin kanin vilkkuluomirefleksi -koeasetelmalla yhdellä aistityypillä. Kaksi ääntä, A ja B, muodostivat neljä peräkkäistä ääniparia, joissa äänet alkoivat 500 ms välein, ja joista vain toisistaan eroavat äänet saivat vahvistusta (AB, BA). Opettamisen aikana aivojen sähköistä aktiivisuutta mitattiin kallonsisäisillä elektrodeilla kanien (N = 4) hippokampuksesta ja etuaivokuorelta, joiden kummankin uskotaan osallistuvan kyseisen tehtävän oppimiseen. Koesarjojen herätevasteet analysoitiin oppimiseen liittyvinä muutoksina sekä näiden muutosten käyttäytymisheijasteina. Erottelevia muutoksia löydettiin vain etuaivokuorelta 250 ms viiveellä kriittisestä, toisen äänen alkamisajanhetkestä. Näillä muutoksilla oli yhteys myös käyttäytymiseen, vaikka käyttäytymisaineistosta yksin ei löydetty oppimiseen liittyviä vasteita. Tulokset yhdessä aikaisempien tutkimusten kanssa tukevat näkemystä, jonka mukaan kanit 1) voivat mahdollisesti oppia näin vaikeita tehtäviä ja 2) mahdollisesti hyödyntävät abstraktia informaatiota. Kuitenkin lisätutkimuksia ehdotetuin systemaattisin muutoksin koeasetelmassa tarvitaan ennen lisäjohtopäätöksiä. Lisäksi tutkimuksessa esitellään uusi koeasetelma abstraktin assosiatiivisen oppimisen tutkimiseksi sekä esitetään ehdotus mahdolliseksi kognitiiviseksi mekanismiksi abstraktin tai suhteellisen oppimisen taustalla.

On the quantitative edge of feature and gestalt -based associative learning: learning-related ERP changes in the hippocampus and the prefrontal cortex during a complex auditory discrimination task in rabbit NM-paradigm

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Abstract

In this study, a difficult unimodal sequential discrimination task, which could be solved rather simply by using abstract information, was performed in rabbit nictitating membrane - paradigm to test the working hypothesis that animals are capable of using abstract information, at least to some extent. Two tones, A and B, formed four sequential, 500 ms stimulus onset asynchrony pairs with those being dissimilar receiving reinforcement (AB, BA). During the training, electroencephalography was measured via intracranial electrodes placed in the hippocampus and the prefrontal cortex, which both are believed to contribute to the learning of this task. Event-related potentials of the trials were analyzed in terms of learning-related changes and possible behavioural correlates of these changes. Discriminative changes were found only in the prefrontal cortex at the latency of 250 ms after the onset of the second tone. These changes were also behaviourally correlated, even though no discriminative behavioural responses were found in separate behavioural data analysis. Therefore these results, together with previous studies, support the view that rabbits 1) are able to learn a task of this level of difficulty and 2) may use abstract learning strategies. However, further studies with systematic modifications of the experimental procedure are needed before further conclusions. In addition, a new experimental paradigm to study abstract learning is presented and a possible cognitive mechanism for relational representations is proposed.

Keywords: abstract animal learning, associative learning, biconditional discrimination, Prefrontal cortex, Hippocampus, event-related potentials, learning-related changes.

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1. Introduction

1.1. The animal associative learning research

With no doubt, psychology and related sciences are facing a huge challenge in trying to respond to the real-world need to understand brain mechanisms responsible for certain behaviours and to turn them to quantitative scientific theories (Shettleworth, 2001; for review, see: Brandon, Vogel & Wagner, 2001). Within the phylogenetical branch it is hypothesized that behavioural flexibility and the brain's anatomical and information processing complexity increases (Simpson, 1961). Although a lot is still unknown about the cognitive capabilities of simpler nervous systems, as the studies concerning e.g. marine mollusc *Aplysia* (Hawkins, Greene & Kandel, 1998) or honey bees (Komischke, Giurfa, Lachnit & Malun, 2002; Schubert, Lachnit, Francucci & Giurfa, 2002) indicate, psychologists studying higher animals such as mammals have made progress in conceptualizing different stages of cognitive processing, confirming experimentally behaviours reflecting these stages, and even in finding a causal connection to certain brain areas and molecular processes (Bao, Chen, Kim & Thompson, 2001).

An example of the localization of learning related plasticity in brain is the localization of the memory trace of conditioned nictitating membrane response (NMR) of a rabbit to the interpositus nucleus of the cerebellum by Thompson and his co-workers (McCormick, Clark, Lavond & Thompson, 1982; Krupa & Thompson, 1997; review: Kim & Thompson, 1997). Using systematic lesion studies and a well standardized paradigm originally developed by Gormezano (1966), they collected, with other NMR researchers, a vast amount of knowledge of the rabbit brain, its cognitive processes and the experimental paradigm used, to be taken advantage in future studies. As this paradigm became popular in laboratories, it is in the natural and practical interest of neuropsychologists to adjust the rabbit-NMR paradigm for studying also higher animal learning, as done in this experiment and in several others (e.g. Brandon, Vogel & Wagner 2000). While Thompson and his co-workers used delay conditioning, with co-terminating tone as a conditioned stimulus (CS) and airpuff as an unconditioned stimulus (US) to result a conditioned response (CR), others have used a trace paradigm in which there is a temporal gap between the CS and the US. Interestingly, this type of learning seems, contrary to delay learning, require an intact hippocampus (HPC; Beylin, Gandhi, Wood, Talk, Matzel & Shors, 2001; Mishina, 2003), but is also impaired by cortical lesions (Kronforst-Collins & Disterhoft, 1998). In the discrimination paradigm, the animal is required to respond to one stimulus or a compound of stimuli, but not to others. In turn, this type of learning requires an intact cortex, especially a prefrontal cortex (PFC; Deacon & Rawlins, 1996; Whishaw, Tomie & Kolb, 1992).

Formal theories conceptualizing how the stimulus becomes associated with the reinforcer have been proposed as from the end of the 19th century by several learning theorists starting from Thorndike and Hull, as presented by Pearce & Bouton (2001), and followed by Rescorla (Rescorla, Grau & Durlach, 1985), Pearce (1987) and Wagner (1981), resulting in increasingly sophisticated theories of

associative learning, which clarify the stimuli and their interactive effects on learning. Still many stimulus properties such as temporal separation (Kirkpatrick & Church, 1998), emotional value and structure (Pearce, 2002) of stimuli, have attracted attention only lately. Interestingly, as associative learning theories predict, animals learn, with proper repetition, rather difficult associative tasks, as negative patterning, consisting of different responding to separate stimuli as compared to their combinations (A-, B-, AB+), and, moreover, responding differently to any kind of combination of restricted amount of stimuli, causing considerable cognitive demand.

1.2. Gestalt, relational or abstract animal learning

The CS-US contingency can be construed so that there exists no physical correspondence with the reinforcement, but instead an abstract or a relational one. For example, the animal may be trained to respond only to a difference of 500 Hz of two sequential sounds, but not to any other difference presented. However, this type of learning has not yet been reported in classical conditioning studies. Instead proposed evidence for abstract learning is coming from studies using varying tasks and species (for review, see: Miller, Nieder, Freedman & Wallis; Flannery, 1997; Horwitz, Batista & Newsome, 2004) as in the case of numerosity understanding in rats, pigeons, raccoons, dolphins, parrots, monkeys and chimpanzees reviewed by Dehaene, Dehaene-Lambertz and Cohen (1998). This type of learning, independent of the physical identity of the stimulus, may require higher order representations to occur. These representations may include both uni- and cross-modal generalizations of sensed objects (Gestalts), being inevitably important also for animals, and might be one possible explanation for flexible animal behaviour (Shettleworth, 2001; Gärdenfors, 1996).

Historically, theories of higher cognition come from human studies and assume concept-representations, thus strongly indicating language or language-like processes and proposing that abstract learning should correlate strongly with the animal's language capabilities. Lately, however, it has been suggested that, with little modifications, associative learning theories can apply also to e.g. categorization and relational problems (Pearce & Bouton, 2001). This possibility is also supported by network models in which adding layers result in structural, or even abstract learning (Gorman & Sejnowski, 1988a). Somewhere between these lies the relational schema theory by Halford, Bain, Maybery and Andrews (1998) which argues that if the brain can discriminate within the dimension objects vary, representation of these objects can be sorted to form a relation and, then, manipulated with available operations. Also the postulation of animal numerical capabilities can be seen to share properties with the relational schema theory (Dehaene, 1993).

In the last decades the nature of the brain's parallel functioning has become increasingly evident. Theories such as controlled and automatic processing (see review: Schneider & Chein, 2003), as well as multiple brain memory systems (White & McDonald, 2001), together with all their included modules, depict the brain as a system which have multiple distinct resources working in parallel and interaction. So when an animal is confronted with a task, the system capable of solving the task is

selected through an interactive, or a competitive, process. This leads to a situation where a task might always be solved using the simplest possible learning strategy. In the study of abstract learning, this sets high demands on the task design and there are examples where unwanted learning systems may contribute surprisingly well to the employed learning-task (Horwitz et al., 2004; Mélan, Castéras, Corbière & Gallo, 1995). This applies to studies of non-abstract learning as well (Gerlai, 2001). In the present task, two stimuli are presented in all of their four possible sequential pairs with the same likelihood, with only trials with a differing sound being reinforced, so that there is clearly no physical correspondence to be associated with the reinforcer.

1.3. Neural substrates for complex associative learning

Neural substrates, responsible for more complex learning than the delayed CS-US contingency, remain to be discussed. Surprisingly, it seems that several subcortical and cortical areas are working together even in simple trace learning (Kronforst-Collins, Disterhoft, 1998; Kotani, Kawahara & Kirino, 2003). It seems that several brain systems working interactively encode different properties of the memory (White & McDonald, 2001; Kim & Baxter, 2001) or that a shift between areas simply happens as a result of a general task demand depending on the difficulty (Beylin et al., 2001), the flexibility or the persistent need for a memory representation (see review: Eichenbaum, Schoenbaum, Young & Bunsey 1996). However, the general theory of the PFC function (Fuster, 1997), postulating that the PFC serves as a template for planning actions in time, based on sensory information from other brain regions, emotional information from amygdala and higher memory constructs formed by itself, seems to be in line with the detailed experimental evidence from studies of the recognition memory paradigm in rats (Eichenbaum et al., 1996; Ramus & Eichenbaum, 2000; Schoenbaum, Setlow & Ramus, 2003), configural discrimination learning in rats (Whishaw et al., 1992), timing discriminations with rats (Onoda, Takahashi & Sakata 2003), reversal learning with rats (McAlonan & Brown, 2003), and with abstract (Horwitz et al., 2004) and numerosity (Nieder, Freedman & Miller, 2002) learning in monkeys. Moreover, it is hypothesized that the rat PFC may be organized by the abstractness of the sensory processing with orbital areas encoding more featural and dorsolateral areas more abstract information (O´Reilly, Noelle, Braver & Cohen, 2002).

In contrast, the HPC has not received similar consensus for its function despite the fact that it has been long known to contribute to learning. Perhaps the most widely accepted functions reported concern memory consolidation (Alkire, Haier, Fallon & Cahill, 1998), spatial memory (Florian & Roullet, 2004) and context dependent effects (McDonald, Ko, Hong 2002). In addition, several task-related parameters, learning related activity and lesion-related impairments have been connected to the HPC during several task types including temporal coding in trace learning (Kronforst-Collins & Disterhoft; Kotani et al. (2003); McEchron, Tseng & Disterhoft, 2002), configural (Rudy & Sutherland, 1989) and relational learning (see review: Eichenbaum et al. 1996), but all of these positions have been challenged, or the HPC has not been confirmed to be necessary or sufficient for these behaviours.

When monitoring event-related potentials (ERPs) of the HPC and PFC in this study, it is expected that learning-related discriminative activity will emerge in both regions, especially in the PFC where the initial stimulus-response representation is believed to occur.

1.4. Aims of this study

In biconditional discrimination (BDT; Saavedra, 1975) the animal must learn to respond selectively to a certain combination of stimuli, but not to other combinations consisting of the same stimuli (e.g. AX+/BX-/AY-/BY+). In this study, BDT is formed by using only two different stimuli and presenting them in four possible orders. Moreover, the trials with differing sounds are reinforced and those being similar not, which hypothetically require abstract representations for solution of the discrimination.

The shift from compound association to abstract and more integrative representations is a major qualitative shift in cognitive processing and, thus, of great interest to the cognitive science. To start to investigate this topic under firm experimental science in our laboratory and to clarify if rabbits are capable of learning at all a task as difficult as this, as predicted similarly 1) by associative learning theories and 2) by several studies claiming that mammals are capable of abstract learning, this first exploratory study was conducted to look for learning-related changes from the NMR and from the ERPs of the HPC and PFC during training of the two-element and thus the most difficult version of BDT (Kukkola, 2004). Secondly, a rabbit-NM paradigm to study animals' capabilities to form abstract associative representations is evaluated.

2. Methods

2.1. Animals

The subjects were four ($n = 4$) experimentally naive 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. The recording electrodes were implanted in the hippocampal and prefrontal area. Electroencephalography (EEG) and multiple unit activity (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 (Fig. 1.) as CSs. The tones were directed to the rabbits left ear by a 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.
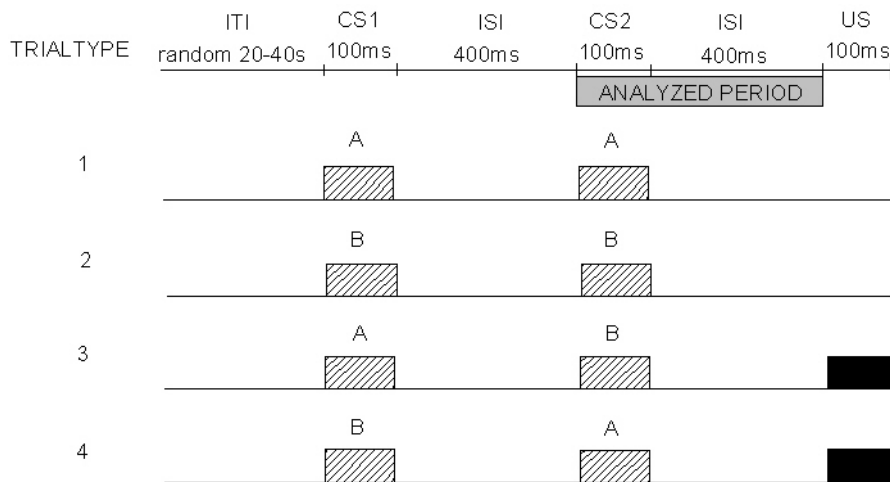

Fig. 1. Schematic illustration of the discrimination training procedure, A = tone 1000 Hz, B = tone 2000 Hz. The trial types were presented in pseudorandom order.

The training procedure is presented in Fig. 1. Four combinations of sequential CS-tones were used, consisting of two sounds of 1000 Hz and 2000 Hz (78 dB), referred to here as A and B. Both tones, and also the US lasted for 100 ms. The interstimulus intervals (ISI) were 400 ms (see Fig. 1) and the intertrial interval (ITI) varied randomly, ranging from 20 to 40 seconds (mean ITI 30 s). Eight types of trials were used during the experiment, four of which are presented in Fig. 1. The training procedure started out with a seven days' discrimination training (phase I), where trial types from 1 to 4 were used. Types 1 and 2 served as CS- and types 3 and 4 as CS+. Each session consisted of a total of 88 trials. Subsequently, for one to five days (phase II), all four types were reinforced and each session consisted of 80 trials in total. The sessions of this phase were excluded from the analysis. Third phase (III) was equal to the first one, but it lasted only for two to five days. As the experiment was terminated before the behavioural learning was achieved, a compensating performance-related *post hoc* analysis was performed (see chapter: data-analysis).

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. The 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. The signal for EEG recording was amplified by a factor of 20 000, band-pass filtered (0.1-100 Hz) and digitized at the rate of 500 samples/s. Afterwards, the signals were low-pass-filtered (30 Hz) with Mathlab-application to get rid of the high-frequency noise. Trials with NM-movement exceeding 0.5 mm during a period of 125 ms prior to the CS1 were excluded from the analysis. Eight sessions were selected for analysis, the first seven from phase I and one from phase III. Neural activity was analyzed only from the beginning of the second tone (CS2) to the beginning of the US. The ERPs were baseline corrected by subtracting each value in this period from the average of a period of 250 ms before the CS2. SPSS 11.5 for Windows was used for all numerical processing. With the aim of detecting accurately learning-related changes in the PFC and HPC data, a Principal Component Analysis (PCA) with VARIMAX rotation was conducted to the covariance matrices of the CS2-onset to US-onset period. For the PCA, this period was averaged to 50 time points, to be treated as variables, and 128 cases were constructed, consisting of 4 subjects x 4 trial types x 8 sessions. ANOVA for repeated measures was used in the analyses of the NMR and the component

factor loadings of ERP data, separately for each component. The significance level was 0.05.

The behaviourally correlated analysis of the PFC data was conducted as a *post hoc* -analysis. In this phase adaptive CRs were calculated as being a 0.5 mm NMR in the period of 100 ms before the US and then averaged to form four groups presented in table 1, depending whether there was an adaptive response and whether the trial was reinforced or not. As the group CR+CS- had smallest number of trials (53), the same numbers of trials was chosen by random from the group CR+CS+ and by taking consecutively the preceding or the following trial, compared to the CR+CS- -group, from the groups CR-CS+ and CR-CS-. Then the groups were brought together in the same picture and compared with each other by eye. In addition, a Chi-Square test was conducted to determine if the trials of the CR+CS+ -group were evenly distributed across sessions.

		CR	
		+	-
CS	+	71	1133
	-	53	1230

Table 1. Distribution of the trials of eight sessions when grouped by reinforcement (CS) and conditioned response (CR).

3. Results

3.1. Histology

The locations of the electrode tips are presented in Fig. 2. The tips of the HPC electrodes were located in the CA1 region in all rabbits and the tips of the PFC electrodes in the anterior cingulate cortex (Brodman's area 24). One electrode from each rabbit from both areas was chosen to data-analysis.
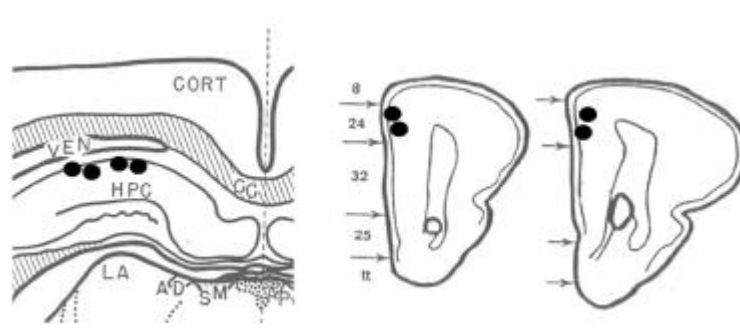

Fig. 2. Locations of the hippocampal and prefrontal cortex electrodes. The hippocampal electrode tips (on the left) were located in the CA1, the prefrontal electrode tips (on the right) were located in the anterior cingulated cortex (Brodmann´s area 24).

3.2. Behaviour

No significant differences were found in the peak analysis of the NMR data in the CS1-US period in relation to the TRIALTYPE ($F_{(1,3)}$ = 1.71, p = 0.28), the SESSION ($F_{(3,9)}$ = 1.67, p = 0.24) or their interaction ($F_{(3,9)}$ = 0.24, p = 0.86), even though a clear learned response was seen before CS2 (Fig 3, right picture).

3.3. Principal component analysis of the CS2-US period of the HPC and PFC

The PCA extracted six components from the HPC, none of which was learning-related, when the data was studied by repeated measures ANOVA. Instead, in the PFC, four components out of six turned out to be learning-related. The first component ($F_{(3,9)}$ = 21.59, p < 0.001), being TRIALTYPE*SESSION –related, increased towards the end of the period and was interpreted as autocorrelation (van Boxtel, 1998). Two of the components, namely component 3 ($F_{(1,3)}$ = 14.46, p < 0.05), co-occurring with the CS2, and component 4 ($F_{(1,3)}$ = 24.16, p < 0.05), peaking at the time of the CS2 offset, were TRIALTYPE -related and. Component 5, was TRIALTYPE*SESSION -related ($F_{(3,9)}$ = 4.02, p < 0.05) at the latency of 250–350 ms post the CS2 onset. Surprisingly, this interaction showed a somewhat fluctuating profile (Fig. 3. on the right). When looking session by session at the ERP-curves of individual rabbits, no consistent grand average-like difference between trial types could be detected by eye, suggesting that the grand average curve consists of a considerable amount of variation.
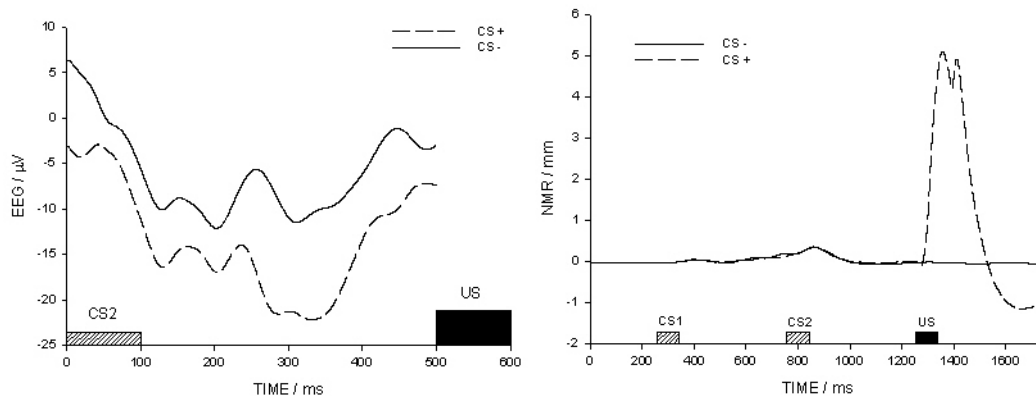

Fig. 3. The picture on the left shows the averaged PFC ERPs of CS+ and CS- trials of eight sessions. In picture on the right, the overall eyeblink movement is shown for CS- and CS+ -trials (average of 8 sessions). The response to the CS2 was not significant in the peak-analysis of variance.

3.4. Performance-related ERP analysis

To analyze further the nature of these changes, a NMR performance comparison of the ERP activity by trial type was conducted as suggested by Alkire et al. (1998; see chapter: "Data-analysis" in "Methods"). In this analysis, distinctive negative spike in the group CR+CS+ was seen in comparison to other groups in time range of the maximum variation of the discrimination -related component 5, suggesting that this spike of negativity is correlates with discriminative behaviour. Moreover, the Chi-Square test indicated that the CR+CS+ -trials were not evenly distributed across sessions ($?^2(7) = 31.42$, $p < 0.01$), but instead had a tendency to occur in later sessions.
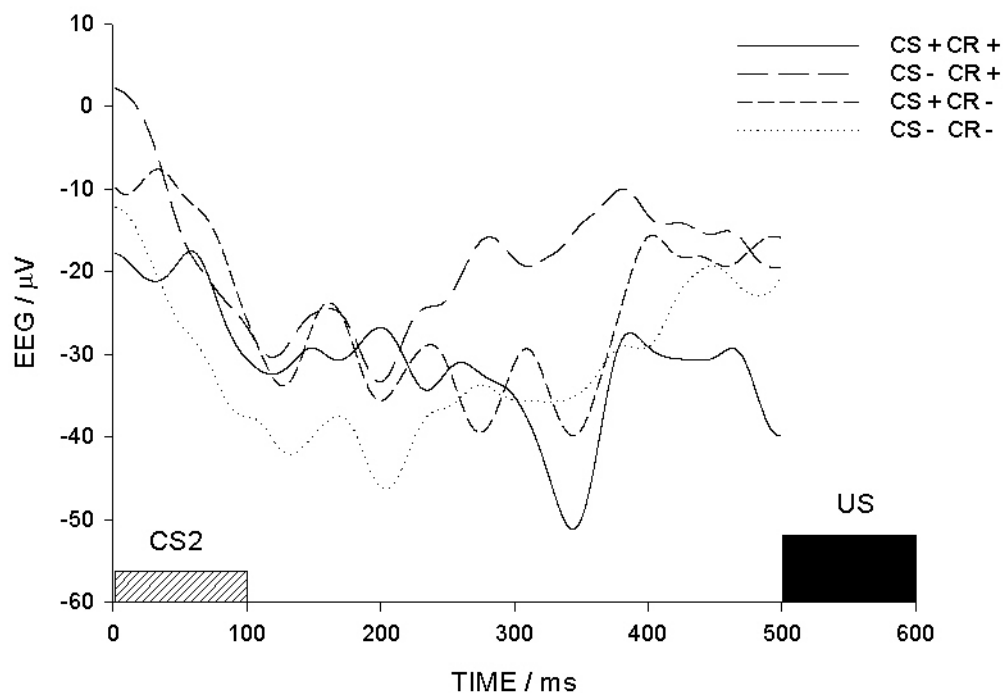

Fig. 4. Performance-related ERPs of PFC. The average of 53 trials classified to groups on the basis of whether the trial was reinforced or not (CS+, CS-) and whether there was an adaptive response or not (CR+, CR-). Notice the distinctive negative spike at the latency of 350 ms in the CS+CR+ group.

4. Discussion

Even though the experiment was terminated before the behavioural learning was achieved, significant differences on the neural level were detected. Surprisingly, on the behavioural level, no statistical significance was found in the peak analysis of the CS1 to US period, despite of the clear sign of learned CS1-CS2 contingency in the grand average curve. Contrary to Kukkola´s MUA study, no learning-related changes were found in the HPC but instead in the PFC. This PFC activity showed increased

negativity at the latency of 250 - 350 ms after the CS2 onset, showing a connection to discriminative behaviour, when studied in the *post hoc* picture analysis (see Methods). Together, these results support the hypothesis that a rabbit can, at least at the neural level, learn this task, possibly using abstract learning strategies.

4.1. Comparison of the results with Kukkola (2004) study

Firstly, as mentioned, this study used one session less than Kukkola (2004) causing differences in the results. This is evident in the NMR data, in which only nine sessions showed significant responding to the CS1-CS2 contingency. The PFC difference in brain activity between the trial types found in this study developed during the first five sessions, turning to similar values after that. This unstable development of the discrimination may explain why no effect was found in the analysis of nine session data. On the other hand, Kukkola (2004) found a beginning discrimination in the HPC.

Secondly, in this study a PCA was conducted to the HPC and PFC data, instead of the ANOVA for averages of four blocks of the CS1-US period used in the Kukkola study. Clearly, the PCA method is a more sensitive tool for detecting covariations in data and should not give false-positive results (van Boxtel, 1998). Therefore, it is surprising that the PCA extracted no learning-related component from the HPC, as found in the Kukkola study. The HPC effect seen in her study was present only in the last sessions, suggesting the possible influence of one more session on the statistical analysis.

Third, differences in the ERP and MUA sampling methods may have had an effect on the results. Given that these two studies used same electrodes and that changes in the MUA may be due to a more local neuronal activity than the ERP, it is possible that changes in the HPC occurred in the very place of electrode, resulting in smaller relative changes in the HPC ERP data, rather than in the MUA, due to the influence of a more distant activity too (Buchwald, Weber & Holstein). This implies then that changes found in the PFC were distant from the electrode location because they were not seen in the MUA.

Together these patterns of results permit the interpretation that learning was still ongoing in the non-discriminative CS1-CS2 contingency, revealed as the statistical significance of the NMR only in the data of nine sessions, accompanied by the early neural discrimination in the PFC seen only in the first five sessions, and followed then by discriminative activity in the HPC, which in turn, according to some studies (McEchron, Tseng & Disterhoft, 2003), predicts a forthcoming behavioural discrimination. These results support the view that the PFC contributes to the discriminative compound-CS-CR association.

4.2.1. Possible cognitive mechanisms responsible for the learning-related changes found

Firstly, it is possible that the four sequential tone pairs produced four unique memories to be reinforced directly with the reinforcer. This might happen in early

auditory pathways or in the primary auditory cortex through the process of sensory integration, and if it was possible to conserve the pitch information, this would arguably lead to solution of this task. This alternative would then lead us to look for quite sophisticated early information processing. However human ERP studies provide evidence against this possibility as this temporal integration window is shown to be less than 170 ms (Yabe, 2002; Loveless, Levänen, Jousmäki, Sams & Hari, 1996; Sussman, Winkler, Kreuzer, Saher, Näätänen & Ritter, 2002).

Several associative learning theories such as Rescorla-Wagner´s unique cue extension, referred to as the added elements model by Brandon et al (2000), Pearce´s (2002) configural learning theory (inhibited elements by Brandon et al.) and the replaced elements model by Brandon et al. (2000), all predict learning to occur in this task, as explained in more detail by Kukkola (2004). Although including context properties, these theories do not take account of the sequential nature of stimuli, (Pearce & Bouton, 2001) which casts doubt on this interpretation. Sequence-stimuli have been demonstrated to have a special, although much similar role to that of the context, for conditioning (Brandon et al., 2001) and only very recently, modifications to add temporal properties to associative theories has been made (Brandon et al., 2001).

The sequence-effect led to theorizing about the occasion setters to function as a cue for responding to subsequent stimuli (Bonardi & Ward-Robinson, 2001). Applied to the task at hand, preceding tone A would prepare the rabbit to respond to tone B (and *vice verca*) and would therefore explain the learning of this sequential task. Occasion setting provides the most suitable existing candidate for an associative mechanism behind the learning of a task of this kind.

4.2.2. Proposed model for relational learning

One development in neural information processing is the shift from feature-based to gestalt-based, more abstract representations. Fig. 5a shows schematically associative discrimination and generalization processes in the auditory frequency domain. Two tones, A and B, are more easily discriminated as a function of the frequency differences of the tones and, inversely, generalized, due to the dimensional organization of primary sensory cortices (Weinberger, 1998). Abstract learning in the same auditory frequency domain requires computation of the difference between the frequencies of the tones and thus, the forming of a working relation instead of bare identity (Fig. 5b and c). In this way the differences AD, CD, AC, CB and BD get the values 2500, 1500, 1000, 1000 and 500, respectively. Again, if they are capable of forming representations of these differences, the same associative discrimination and generalization processes may contribute between these representations (Fig. 5c).

Therefore, discriminating between AC and BD would turn out to be more difficult than distinguishing AC from AD. This rather mathematical view of relation may provide quantitative framework to study complex animal learning and possibly, the shift from combinations of identity to relational, stimulus' physical identity independent associative learning. However, it has to be noted that animals may be biased towards processing only relations relevant to their purposes, not in the
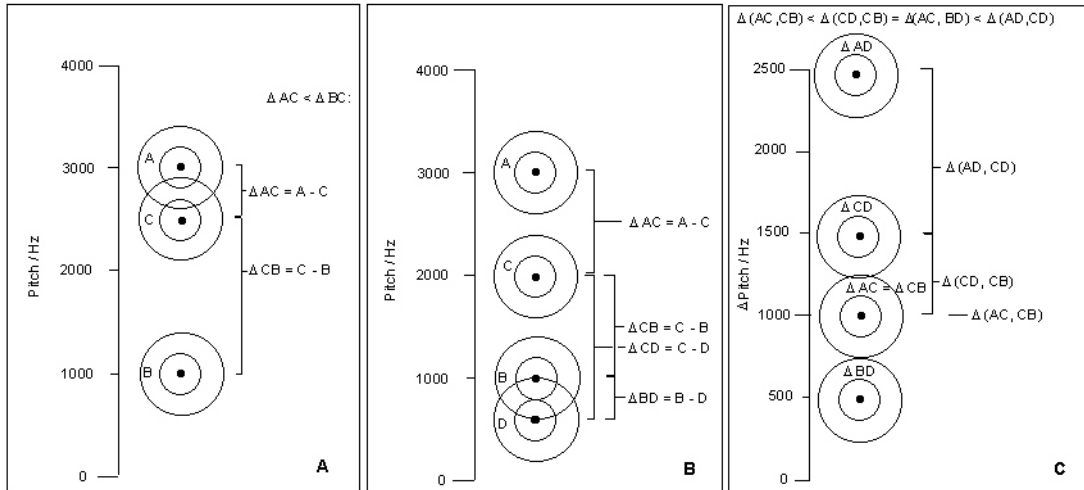
Fig. 5. Schematic illustration for pitch-representations in receptive fields of the auditory cortex a) sounds A and C are more easily generalized than B and C. If being discriminated, they can be connected to form configural representations. The differences between sounds illustrated in picture (b) are arranged according to their quantity in picture (c). Again, differences between BD and AC are generalized more easily than difference between CD and AD.

straightforward mathematical way depicted in the example. From this viewpoint it is possible that the brain is initially equipped to detect any difference as compared to the sameness.

Applying the computational view of relation presented in Figures 5b and 5c to Halford et al.'s relational schema theory (1998) would lead to a relational solution of this task. According to them, prerequisite for relational learning is the capability to sort the representations of sensed objects within some dimension included in all these representation, within pitch, in this case. Then, the animal can use available procedures to manipulate these objects. Now, according to studies of primary cortices, all sensory stimuli are represented in the primary sensory cortices within different physical dimensions in receptive fields (Weinberger, 1998) and therefore, it can be argued, that also basic associative learning fulfills this Halford et al.'s prerequisite for relational arrangement. The primary cortex differentiates the afferent sensory input enabling the identification of stimuli from each other, thus making associative learning possible. This is not yet sufficient for relational learning, but systematic interaction employing the dimensional arrangement is needed between these representations. Thus, the core in integrating computational view of relation presented here into the relational schema theory is the specification of this interactive process i.e. Halford at al.'s mental procedures to a single procedure of subtraction, at least in the simple animal learning paradigm. With this modification, no language-like representations or procedures are needed for the study of abstract learning, and a quantitative experimental paradigm can be constructed. This could also explain why the sensory cortices are dimensionally arranged, because for bare identity it is sufficient that every stimulus have unique, but not arranged, representations in the

sensory cortex. This view is supported by Weinberger (1998), who argues that representations in receptive fields of the primary cortex, are in flexible use.

4.3. Found mismatch negativity-like response in the PFC and relational processing

Present changes in the PFC fall in the latency of the automatic change detector mechanism, found in several cortical and subcortical areas, namely the mismatch negativity (MMN; Näätänen, Paavilainen & Reinikainen, 1989; Ruusuvirta, Korhonen, Penttonen, Arikoski & Kivirikko, 1995; Astikainen, Ruusuvirta & Korhonen, 2001; Javit, Steinschneider, Schroeder, Vaughan & Arezze, 1994). Usually MMN studies are conducted in an oddball-paradigm where a repetitive standard stimulus has induced lasting memory representation and expectancy of this standard stimulus, which is sometimes contradicted by presenting a deviant stimulus. Importantly, the brain compares the preceding stimulus to the subsequent one and if they do not match, produces the MMN. Even though the MMN is usually thought to occur only after the presentation of several repetitive standard stimuli, small MMN effects have been found with only two sequential stimuli (Squires, Wickens, Squires & Donchin, 1976; Giese-Davis, Miller & Knight, 1991 in: Näätänen, 1992). This leads to the further interpretation that sameness and difference are exceptional examples of relational processing and very intrinsic to neural nets. The results of this study suggest this possibility as an MMN-like difference was detected in the PFC as a response to differing sequential stimuli. Also, the PFC MMN has been connected to unintentional attention shifting (Doeller, Opitz, Mecklinger, Krick, Reith & Schröger, 2003), but contradictory suggestions have also been made (Paavilainen, Mikkonen, Kilpeläinen, Lehtinen, Saarela & Tapola, 2003). Because of the behavioural correlation of the MMN-like response found in this study, our results suggest PFC MMN contribution to the latter memory comparison process also. Whether the content of the comparison process found is simply between consecutive stimuli or between the representation of compound-stimuli and the integrative representation of sequential sensed stimuli, remains to be determined in future studies. One solution to this problem is to present the used stimuli without the US; in this case, the MMN should be elicited by differing sounds.

4.4. Future directions

For future research, this exploratory study raises many questions, but also suggests ways how they can be answered: 1) to make sure if rabbits are capable of learning this type of biconditional discriminations, a study with more trials and shorter ISIs is needed. 2) To make sure if rabbits are able to make abstract discriminations, a study with random pitches of stimuli, while keeping the same percent of "same" and "different", is needed. If rabbits are shown to be successful in one of the tasks or both of them, 3) to determine how increasing or decreasing the abstractness or the ISI of the task affects learning, studies with varying relations of pitches and ISIs should be done. 4) Moreover, if rabbits have been able to learn an abstract task, it is relevant to study if it is possible to condition rabbits, not only to sameness but to any

difference of pitches. Also, more subjects, training sessions and comprehensive control procedures, including paired tones without contingency to the US, are needed. Neural activity from receptive fields of the auditory cortex and from the PFC should be monitored in these studies to find out if the neural site changes depending on the ISI or the abstractness of the task.

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