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Markku Penttonen

Classically Conditioned Lateralized Head Movements and Bilaterally Recorded Cingulate Cortex Responses in Cats

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ABSTRACT

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The aim of this study was to determine the effects of classical conditioning on lateralized head movements and bilateral cingulate cortex neural activity in the cat. For this purpose, a differential conditioning paradigm was developed, which allowed for experimental manipulations of the lateral position of the conditioned (CS) and unconditioned stimulus (US). One tone (CS+) was paired with a unilateral medial forebrain bundle (MFB) stimulation US activating the animal and eliciting approach movements, while another tone (CS-) was not. In different experiments, the CS+ was presented to both ears, to one ear only, or to each ear in random order. Differential conditioning was demonstrated as a head turn conditioned response (CR) of greater acceleration and shorter onset latency to the CS+ than CS-. Increases in cingulate cortex evoked responses and multiple unit activity were also greater to the CS+. Experimental manipulations of the US and CS lateralities resulted in specific CR directions of turn and cingulate cortex neural activity asymmetries suggesting brain-side specific responses in simple classical conditioning. The role of MFB activation for behavioral and neural plasticity is discussed. A new biological model for investigating conditioned lateralized behaviors and approach responses, the cat conditioned head turn response, is proposed.

Keywords: classical conditioning, auditory stimulus, medial forebrain bundle, head turn, cingulate cortex, cats

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Jyväskylä, February 1993
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LIST OF ORIGINAL WORK

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III Penttonen, M., Korhonen, T., Arikoski, J., Ruusuvirta, T., & Hugdahl, K. Activity-dependent neuromodulation of head turning and cingulate cortex neuronal activity in the cat. *Psychobiology,* submitted.

IV Penttonen, M., Korhonen, T., Arikoski, J., Ruusuvirta, T., & Hugdahl, K. (1993). Bilaterally recorded multiple-unit activity of the cingulate cortex during head turning conditioning with unilateral medial forebrain bundle stimulation. *Scandinavian Journal of Psychology*, in press.

CONTENTS

INTRODUCTION

The aim of these studies was to determine the effects of associative conditioning on lateralized head movements and bilateral cingulate cortex neural activity. For this purpose, a differential conditioning paradigm was developed, which allowed for experimental manipulations of lateral presentations of the conditioned stimulus (CS) and unconditioned stimulus (US). Specifically, a tone CS was presented symmetrically to both ears or asymmetrically to one ear. Unilateral medial forebrain bundle (MFB) stimulation, presumed to have asymmetrical effects on the two sides of the brain, was used as the US. Different combinations of the CS and US were used, and the effects were observed by recording the direction and extent of the head turn conditioned response (CR) and by measuring the bilateral cingulate cortex evoked potentials and multiple-unit responses.

The study design was similar to that used in our previous attempts to develop a cat model system for studying the neural basis of associative learning (Korhonen & Penttonen, 1989a, 1989b). Model systems of associative learning have been used widely in invertebrates and vertebrates (for reviews, see Byrne, 1987; Carew & Sahley, 1986; Farley & Alkon, 1985; Gabriel, Poremba, Ellison-Perrine, & Miller, 1990; Mpitsos, Collins, & McClellan, 1978; Thompson, Berger, & Madden, 1983; Thompson, Patterson, & Teyler, 1972; Schreurs & Alkon, 1991). In each model system, before neural analysis, a relatively permanent change in behavior after the temporal conjunction of two events has been demonstrated. For this purpose, the behavioral change has been required to be the result of paired presentations of CS and US in contrast to control procedures (Gormezano, Kehoe, & Marshall, 1983; Rescorla, 1988).

Different views have been expressed in defining associatively learned, and especially classically conditioned, behavior. Although some researchers have suggested that after paired CS-US presentations a new response to the CS should appear, there is no strict requirement that the CR should be similar to the unconditioned response (UR). According to this view, the CR should at least appear in the same response system as the UR (Gormezano et al., 1983). Other researchers have taken a more general view arguing that any changes in behavior, including changes in existing responses to the CS, can be defined as a CR (Rescorla, 1988). The more traditional approach of defining the CR as a response resembling the UR has been applied to one of the most successful vertebrate model systems, rabbit nictitating membrane conditioning (e.g., Thompson, 1986). The more recent definition of the CR as being any change in behavior has been applied to the analysis of one the most successful invertebrate model systems, the Aplysia siphon withdrawal response (e.g., Abrams & Kandel, 1988). In the present studies, the latter definition of CR has been adopted.

As a first step in developing our cat model system we started with a paradigm in which the head movements of the cats were classically conditioned (Korhonen & Penttonen, 1989a, 1989b). In this paradigm, a miniature loudspeaker was attached in front of the left ear and a freefield tone presented through the loudspeaker served as the CS during conditioning. The stimulation of the right or left MFB at the level of the lateral hypothalamus was used as the US. MFB stimulation was used as the US because of its efficiency as a reinforcer in self-stimulation studies and its ability to modify a wide range of behaviors (Olds & Fobes, 1981). In the standard paradigm, the left ear CS was followed after a short delay by the US during conditioning sessions and the CS and US were explicitly unpaired during control sessions.

The results showed that the cats rapidly acquired a head turn to the left as the CR and retained this response over daily sessions during paired conditioning. The control animals, receiving unpaired CS and US presentations, moved their heads during a few initial CS presentations only and later did not respond at all to the CS. A second CR also occurred in most cats after the initial head turn. This long-latency response could only reliably be determined in those animals which turned to the right, that is, to the side contralateral to that of the initial head turn CR. The results supported to some extent the idea that the long-latency response was similar to the head turn UR elicited by the MFB stimulation.

The present studies were designed to analyze more specifically the development of head turn CRs. In our previous studies, the tone CS and the MFB stimulation US had been lateralized stimuli. Also, the CRs had been lateralized head movements. Consequently, in the present studies the laterality of the CS, US and UR was systematically varied. In the present context, laterality means presenting the stimulus laterally either to the left or right side of the animal or to the left or right side of its brain. It also implies the recording of behavioral responses directed to the left or right or recording the neural response from either the left or right side of the brain. Similarly, symmetry and asymmetry refer to the balance and imbalance, respectively, in bilateral stimulus input, in the direction of response performance or neural recordings from the two sides of the brain.

Studies focusing on the lateralized effects of conditioning have generally been conducted within one of two approaches. The most extensively studied examples of the lateralization of conditioning are the learning, and especially retention, of visual discriminations in chickens (e.g., Rogers, 1986) and conditioned rotation in rats (e.g., Carlson & Glick, 1989).

One of the most successful animal models for the lateralization of sensory input is visual discrimination learning in the chicken. The visual system of the chicken is particularly suited to lateralized presentations of CSs, since visual information from one eye is almost entirely fed to the contralateral side of the brain (Cowan, Adamson, & Powell, 1961). By means of drug treatments and lesion methods, these studies have indicated that one side of the brain dominates over the other in discriminative food conditioning (Gaston, 1984; Gaston & Gaston, 1984; Howard, Rogers, & Boura, 1980; Rogers & Anson, 1979; Mensch & Andrew, 1986) and in passive avoidance conditioning (Barber & Rose, 1991; Bell & Gibbs, 1977; Patterson, Alvarado, Warner, Bennet, & Rosenzweig, 1986). Also, the greater activation of one side of the brain over the other has been shown by the 2-deoxyglucose method during passive avoidance conditioning (Rose & Csillag, 1985).

The conditioned circling response in rats has been used as a model of motor asymmetries with the consequent implicit assumption that conditioned asymmetric effects are mediated through asymmetric motor processes (for reviews, see Carlson & Glick, 1989; Glick, Jerussi, & Zimmerberg, 1977; Glick & Shapiro, 1985). These studies have revealed that after conditioning with food reward, rats have a persistent rotation bias which is related either to inherent individual rotation preferences (Glick, 1982; Glick & Hinds, 1984) or to a randomly selected direction of rewarded turning independent of the inherent directional preference (Szostak, Jakubovic, Phillips, & Fibiger, 1989; Yamamoto & Freed, 1984). At the population level, rats have not been found to circle preferentially to the right or to the left (Carlson & Glick, 1989).

Rotation preferences have been related to the asymmetrical distribution of dopamine between the different sides of the brain (Glick &

Shapiro,1985). To test this hypothesis, neurochemical activity has been measured in the brain ipsi- and contralateral to conditioned rotation, but the results have been inconsistent. Usually, increased dopamine activity has only been found in the side of the brain contralateral to the circling (Glick & Carlson, 1989; Yamamoto & Freed, 1982), although bilateral increases have been found in some studies (Schwarting & Huston, 1987; Szostak, Jakubovic, Phillips, & Fibiger, 1986; Szostak, Porter, Jakubovic, Phillips, & Fibiger, 1988).

In the above-mentioned studies, the neural basis of the acquisition of CRs has not been directly addressed. Thus, both the sensory and motor approach seem to offer only preliminary guide-lines for the present problem.

Compared to the chicken visual system, the cat auditory system is more complex for the presentation of lateralized stimuli. This is due to the fact that a tone presented to one ear activates auditory structures in both sides of the brain. The activation of contralateral structures, at least above the level of the superior olive is, however, greater compared to the activation of ipsilateral structures (Phillips & Brugge, 1985). Consequently, if a tone is presented simultaneously to both ears at equal intensity, there is no a priori reason to infer that one side of the brain is activated more than the other. The localization of a unilateral sound source requires further considerations. A tone presented in one auditory hemifield does not activate neurons in the contralateral sensory structures through decussating neural connections as is the case with the visual or somatosensory systems. Instead, auditory structures are activated in both sides of the brain, but with greater activation of the contralateral structures (Masterson & Imig, 1984).

For the purpose of the present study, the use of a tone CS offers a possible advantage. In cats, as in other species, the presentation of a lateralized tone stimulus induces orienting movements, such as head turning. It is assumed that the laterality of these movements, that is, the direction of turn, might be a simple index of the asymmetry of the perception of the tone. Furthermore, inherent individual perceptual or motor preferences in orientation may be identified by presenting the CS tones before conditioning and thus observing the direction of orienting movements before habituation.

Even though the MFB most obviously also has contralateral connections, in 2-deoxyclucose studies ipsilateral activation has been shown to be greater in structures to which the MFB is directly connected (Porrino, Huston-Lyons, Bain, Sokoloff, & Kornetsky, 1990). MFB stimulation at the level of the lateral hypothalamus is therefore presumed to be a unilateral US activating both the ipsilateral hypothalamus and ipsilateral MFB axons.

We have earlier observed that in addition to evoking forward directed approach movements MFB stimulation often has a tendency to elicit head turns contralateral to the side of the stimulation electrode (Korhonen & Penttonen, 1989a, 1989b). There is also extensive evidence that the stimulation of brain regions to which the MFB is either directly or indirectly connected more often elicits contralateral than ipsilateral head turns (for reviews, see Pycock, 1980; Yeomans & Tehovnik, 1988). It was thought, therefore, that the use of MFB stimulation would enable the direction of the head movement UR to be kept under experimental control.

Consisting of a large number of subpopulations of fibers, the MFB is one of the principal neural pathways interconnecting forebrain limbic structures and brainstem with the hypothalamus which critically controls many behaviors important for the survival of the organism (Swanson, 1987). Furthermore, as the MFB is reciprocally connected to a large number of structures at all levels of the brain (Nieuwenhuys, Geeraedts, & Veening, 1982), its inputs and outputs are numerous and complex. It is therefore ideal for integrating biologically important information and projecting this integrated information diffusely to many parts of the brain (Yeomans, 1988).

MFB stimulation is used as a replacement for natural reinforcers as it is thought to activate the same neural circuits which are activated during normal appetitive behavior. For example, while lesions in the lateral hypothalamus result in feeding and drinking deficits, its electrical stimulation evokes feeding and drinking (Swanson, 1987). Furthermore, the activity of lateral hypothalamic neurons is substantially altered by food-related stimuli. Therefore, the same neurons that are active during species-specific approach behaviors tend to be activated by MFB stimulation (Vaccarino, Schiff, & Glickman, 1989).

Since the discovery of self-stimulation in rats (Olds & Milner, 1954) electrical stimulation of the MFB has been extensively used as a reward for the bar-pressing response. In this contex, reward has been used to refer to the acquisation and maintenance of discrete responses. Those investigators of self-stimulation who by reward refer to both the motivational and reinforcing effects of the MFB stimulation have tended to use the terms reward and reinforcement interchangeably (Stellar & Stellar, 1986; Wise & Rompre, 1989). Where reward and reinforcement have been clearly separated reward has referred to motivation (White, 1989). Thus, the most basic feature of reward has been regarded as its capacity to elicit approach responses. Appetitive motivation, in turn, has then referred to the fact that brain stimulation elicits approach responses and thus increases incentive motivation (Carr, Fibiger, & Phillips, 1989). Reinforcement has been defined as the capacity of brain stimulation to increase the likelihood, that is the probability, of behaviors. However, as reinforcement also implies a change in association, its use in this specific meaning has been avoided in self-stimulation studies where the frequency of the target response is used as a dependent variable (Wise, 1989). This is because the probability of a response can be measured without reference to eliciting stimuli, and therefore associative changes between any two events need not be considered.

For the present, no specific distinction is made between the terms reward and reinforcement. Instead, MFB stimulation is referred to as a US which activates the animal and elicits approach responses. Furthermore, behavioral training is defined as a procedure where an environmental stimulus, the CS, is paired with an MFB stimulation US. The reason for this is to avoid becoming involved in endless theoretical discussion on the differences between instrumental and classical conditioning (e.g., Macintosh, 1974, 1983), which might be expected given that the study of the behavioral effects of MFB has hitherto been based on instrumental conditioning procedures as against the classical conditioning procedure used in the present studies.

A specific advantage of using MFB stimulation as a US is the possibility both to investigate the effect of high-frequency stimulation on neuronal plasticity in a behaving animal and to correlate those neural changes directly to behavior. Long term potentiation, a long-lasting increase in synaptic efficacy resulting from high frequency stimulation of afferent fibers, usually observed in hippocampal slices, is generally thought to be one of the mechanisms of synaptic plasticity in the vertebrate cortex, and is, therefore, one of the most rapidly expanding research areas in neuroscience (see reviews in Baudry & Davis, 1991; Deadwyler & Landfield, 1988). The behavioral relevance of long term potentiation has not, however, been demonstrated directly. Instead, similar effects of specific treatments on long term potentiation and behavioral learning have been assumed to implicate similar mechanisms (e.g., Morris, Anderson, Lynch, & Baudry, 1986). In the present studies, the effects of behaviorally relevant high frequency MFB stimulation are determined on cingulate cortex neuronal activity during associative conditioning. MFB stimulation is considered to be behaviorally relevant as it activates the animal and induces orienting movements and exploration. In addition, the MFB provides a substantial afferent input to the cingulate cortex through diffuse cholinergic and catecolaminergic systems (e.g., Finch, Derian, & Babb, 1984; Nieuwenhuys et al., 1982; Vogt, 1985).

In our previous studies we recorded evoked potentials in the limbic system, including the cingulate cortex and the hippocampal formation, during conditioning, and found at 100-500 ms after CS onset a large negative deflection, which increased during paired conditioning (Korhonen & Penttonen, 1989a, 1989b). In the present studies, cortical evoked potentials were also recorded to determine whether conditioning would differentially effect the two sides of the brain. The cingulate cortex was selected as the target structure since it was thought to be easier to implant recording electrodes symmetrically in the left and right cingulate cortex than in the hippocampal formation. Both the cingulate cortex and the hippocampal formation are association cortices which receive multimodal sensory information from other association cortical areas. Furthermore, both limbic structures are innervated by the MFB (Swanson, Köhler, & Björklund, 1987; Vogt, 1985). Finally, the cingulate cortex and the hippocampal formation have frequently been implicated in learning and memory (e.g., Berger, Alger, & Thompson, 1976; Gabriel, Foster, & Orona, 1980; Segal, Disterhoft, & Olds, 1972)

The most extensive evidence concerning the involvement of the cingulate cortex in conditioning has been provided by Gabriel and his associates (e.g., Gabriel, Kubota, & Shenker, 1988). In an active avoidance paradigm, the rabbit has been trained to avoid a foot shock delivered to the grid floor of the running wheel by turning the wheel after the presentation of a tone CS. Based on multiple-unit activity recordings in the cingulate cortex and limbic thalamic nuclei with selective deafferentiation of recording targets, a limbic interaction model for conditioned response initiation has been proposed (Stolar, Sparenborg, Donchin, & Gabriel, 1989). This model includes a system for triggering, and another for inhibiting, the CR. The limbic thalamic nuclei and the cingulate cortex represent the core of the triggering system. The CS activates the limbic thalamic nuclei which, in turn, activate cingulate cortex output cells projecting to the motor system. Premotor areas, consisting of the neostriatum, superior colliculus, subthalamic area and pontine nuclei have been hypothetized to be the key structures ultimately activating the midbrain locomotor area controlling the spinal-cord locomotor pattern generators. In the present studies, the involvement of the cingulate cortex in response initiation is tested by neuronal recordings during head turn conditioning. Since an appetitive US instead of an aversive one is used the applicability of the model to a US of a different motivational sign is also tested.

The pairing of a tone CS with an MFB stimulation US was used here as the conditioning procedure. However, instead of separate paired conditioning and unpaired control groups, as in our previous studies, a single differential conditioning group was used here. All the animals were thus presented with two tone stimuli. One tone, the CS+, was always paired with the US, and the second tone, the CS-, was never paired. Each animal thus served as its own control for the conditioning effects (Rescorla, 1967). Since in two studies left and right MFB groups were formed according to the location of the stimulation electrodes, the number and size of the experimental groups could thus be kept at a reasonable level.

In addition to observing of the direction of the head turn URs and CRs by video monitors, the time-amplitude course of the turns could be measured with a movement acceleration transducer. The quantitative analysis of head movement CRs as changes in onset latencies and vigour was thus possible, allowing for a precise description of the CR topography.

AIMS AND HYPOTHESIS

The general aims of these studies were: (1) to investigate the effect of CS and US related neural activations on conditioned head movement acquisition and maintenance with lateralized stimulus presentation; (2) to determine if the head turn CR could be used as a biological model of lateralized behaviors; (3) to describe the neural changes in the cingulate cortex in relation to an MFB stimulation US; (4) to test the hypothesis that the cingulate cortex is a part of the CR initiation system in appetitive conditioning; and (5) to develop a model system for associative learning which incorporates the extensive knowledge on MFB obtained in self-stimulation studies into a classical conditioning paradigm for investigating the acquisition and maintenance of conditioned approach behaviors and associated neural mechanisms. For this purpose differential conditioning was performed to investigate the effects of the experimental manipulation of the laterality of the tone CS and the MFB stimulation US on lateralized head movements and bilaterally recorded cingulate cortex neural activity.

In Study I, the CS was presented symmetrically to both ears and the US was either left or right MFB stimulation (FIGURE 1). It was assumed that the symmetrically presented CS would not elicit any specific lateral orienting movements. It was also assumed that the US would elicit a head turn UR contralateral to the side of stimulation. The purpose of the study was to determine whether the URs were actually contralateral to the side of the US, and whether corresponding contralateral head turn CRs would appear. In addition, left and right US groups were formed to investigate whether URs, and consequently CRs, in opposite directions would appear in the left and right MFB stimulation group.

In Study II, an asymmetric CS was paired with a left or right MFB stimulation US. It was expected that the asymmetric CS would evoke ipsilateral orienting head turns before conditioning. Further, it was assumed that in each cat the MFB stimulation electrode would elicit contralateral head turn URs. The purpose of the study was to determine the relative contributions of the CS and UR to the conditioned responses by giving the CS+ tone to the ear opposite to the direction of the UR to the MFB stimulation.

In Study III, an asymmetric CS+ was presented alternatively to the left and right ear, and was associated with the right MFB US. Thus, the CS presentation techniques of Studies I and II were combined so that, although the CS was presented on each occasion asymmetrically, it was nevertheless symmetrical over presentations due to the equal probabilities of tone presentations to the left and right ears. The purpose of the study was to test whether conditioned responses similar to the UR would appear or whether, alternatively, the cats would develop head turns that were ipsilateral to the CS depending on the stimulated ear. All the animals were stimulated in the right MFB to investigate more closely the effect of a unilateral US on the formation of CRs to the ipsi- or contralaterally presented CSs.

In studies I, II, and III, cingulate cortex evoked potential responses were recorded bilaterally to determine whether differences exist between the brain sides ipsi- and contralateral to the US. Additionally, the neural activity of the left and right side of the brain was also compared. In Study IV, the behavioral training was similar to that of Study III but instead of evoked potential recordings, cingulate cortex multiple-unit recordings were performed.

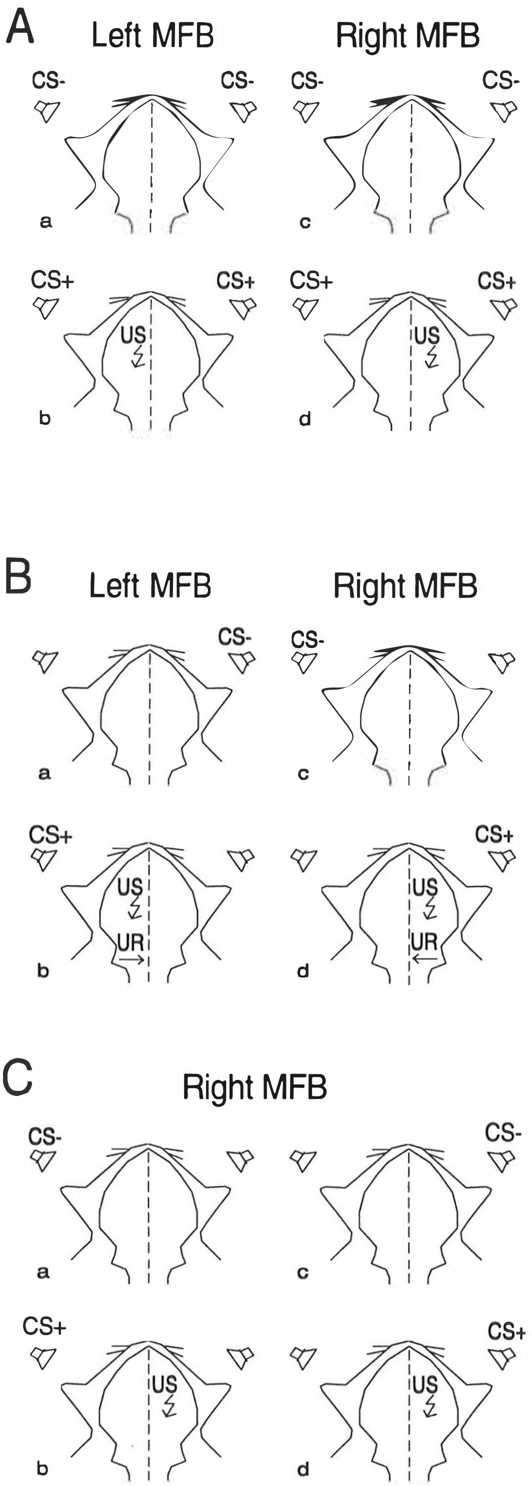

FIGURE 1 Experimental designs for differential head turn conditioning in the cat. Electrical stimulation of the medial forebrain bundle (MFB) was used as an unconditioned stimulus (US). Loudspeakers, placed on a holder on the cat´s head and positioned 2 cm in front of the left and right ears, delivered tone conditioned stimuli (CS). In each study, the CS+ was followed by the US while the CS- was presented alone. A. Discriminative conditioning with symmetrical tones of different frequencies as CSs (Study I). In half of the cats the US was left MFB stimulation (a and b) and in the other half the US was right MFB stimulation (c and d). B. Discriminative conditioning with asymmetrical tones as CSs (Stydy II). In each cat, a tone (CS+) contralateral to the direction of the head turn unconditioned response (UR) was paired with the US. Thus, in the left MFB stimulation group the left ear CS+ was paired with the US inducing right head turn UR (a), while the right ear CS- was presented alone (b). Correspondingly, in the right MFB stimulation group, the right ear CS+ was paired with the US inducing a left head turn UR (c), while the left ear CS- was presented alone (d). C. Discriminative conditioning with asymmetrical tones of different frequencies as CSs and right MFB stimulation as the US (Studies III and IV). CS+ as well as CS- was presented on some trials to the left and on other trials to the right ear in random order. Equal numbers of CS- tones to the left (a) and to the right ear (c) and CS+ tones to the left (b) and to the right ear (d) were delivered.

METHODS

For the implantation of the recording and stimulation electrodes, the cats were anaesthetized with sodium pentobarbital (40 mg/kg). Two or three recording electrodes were implanted symmetrically in both the left and right cingulate cortices. Stimulation electrodes were aimed at the MFB at the level of the lateral hypothalamus. In Studies I and II, two stimulation electrodes were implanted symmetrically in both sides, and in Studies III and IV, three electrodes were aimed at the right lateral hypothalamus. The recording electrodes were made of two Trimel-insulated nichrome wires, fitted in a dental needle and fixed with epoxy. The recording tips of the wires were cut transversely with scissors. The brain-stimulation electrodes were similar to the recording electrodes, except that the diameters of the wires and the outer diameter of the needle were greater.

The electrodes were implanted through holes, which were drilled to be just larger than the diameter of the needles (Korhonen, 1991). Two interconnected skull screws were fixed on both sides of the brain in front of the electrodes and they served as the indifferent reference electrode for the monopolar recordings. The brain stimulation electrodes were bipolar. All the electrodes were connected to two 15-pin D-type connectors, which were fixed with dental acrylic to the anchoring screws and the skull. A socket designed to hold a miniature loudspeaker in front of each ear during behavioral training was also fixed with dental acrylic in front of the connectors.

The measurements took place in a ventilated, electrically shielded box (60 x 48 x 58 cm). The box was illuminated by eight 5-W light bulbs located in the ceiling of the box. For monitoring the behavior of the cats, the door of the box was equipped with a window, and a videocamera was placed in front of the box. For later analysis, videotape recordings were made of all trials.

For the delivery of the symmetric and asymmetric tones, a special audio system was designed. Depending on the experiment, 1000 or 2500 Hz pure tones were used and delivered simultaneously or separately from the two loudspeakers attached symmetrically at a distance of about 2 cm in front of each ear. The balanced miniature loudspeakers were housed in a holder. For head movement recordings a custom-designed three dimensional acceleration transducer based on piezoelectric elements was also attached to the same holder. During behavioral training the holder was fitted to a socket which had already been fixed to the skull of each cat during surgery. The tones were delivered by two signal generators using a gate function to allow sine wave signals to begin at the same phase in each trial. The signals were fed to the loudspeakers through a battery powered audio amplifier. Before the training sessions, the intensities of the left and right ear tones were measured and balanced with a Brüel & Kjær type 2235 sound level meter.

Using a multichannel measurement system both the evoked potentials and multiple-unit activity were recorded during training. The pre-amplifier system consisted of an assembly of eight integrated, low-noise amplifiers coupled directly to the connector in the acrylic mass on the head of the cat. A flexible, shielded cable connected the animal to the signal conditioning system consisting of filters and amplifiers. The bandwidth of the pre-amplified signal (DC to 10 kHz) was divided by filters into evoked potentials (0.2 - 100 Hz or 0.2 - 50 Hz) and multiple-unit activity (500 - 5000 Hz) bandwidths and further amplified. Evoked potentials and multiple-unit activities were recorded as separate channels, together with the movement signal and timing pulses, onto a 14-channel instrumentation tape recorder for an off-line analysis of the recordings by a laboratory computer (PDP 11/23). In Studies III and IV the signals were fed directly from the signal conditioning system to an A/D converter in a IBM PC/AT compatible computer.

A microcomputer delivered the individual trials and controlled the operation of the video tape recorder and the signal generators. The computer also generated the brain stimulation pulse trains with an opto-isolated D/A converter. The trial identification data generated by the microcomputer were displayed on a video monitor and recorded on videotape.

After a recovery period of at least one week, the effect of the US was tested. The US consisted of electrical stimulation of the MFB at a pulse width of 0.5 ms (bipolar pulses), a train duration of 500 ms, and a pulse frequency of 100 Hz. The electrode inducing orienting and approach movements, which were usually accompanied by salivation, but without any aversive effects, was selected as the stimulation electrode. Where there was more than one such electrode in a cat, the electrode was chosen randomly. In Study II other criteria for the selection of the electrode were also imposed. Stimulation intensities ranged from 140 μA to 500 μA. After the selection of the brain-stimulation electrode the animals were given an opportunity to adapt to the experimental box.

In Study I, the US test session was presented before the conditioning sessions, and in other studies after conditioning. The US session consisted of 30 electrical brain-stimulation US presentations. A range of 40-80 s was allowed between trials (mean 60 s). This procedure was used to determine the behavioral characteristics of the UR.

A CS test session was given during the second day in Study I and during the first day in the other studies. The number of CSs and the duration of the intertrial intervals were the same as during conditioning. The only difference between the CS test and conditioning sessions was that no USs were presented during the CS test session. This phase was conducted in order to get the baseline levels for the tones used as CS+ and CS- in the conditioning phase, and to observe whether the cats had any inherent directional biases in orienting to the tones.

Differential conditioning took place either for 10 days (Studies I and II) or 4 days (Studies III and IV). One of the CS combinations was randomly selected as the CS- and the other as the CS+. The CSs varied between experiments. CS- trials consisted of the presentation of the 1,000 ms tone (1,500 ms on Studies III and IV). On CS+ trials the other tone overlapped during the last 500 ms with the stimulation US. Owing to the randomization of the trial sequences, in Studies I and II there were 60 trials during each daily session with approximately 30 CS- and 30 CS+ trials, and in Studies III and IV 80 trials with approximately 40 CS- and 40 CS+ trials. A range of 20-40 s (mean 30 s) was allowed between trials.

After the experiments, the animals were given a lethal dose of Nembutal. Using standard histological procedures the locations of the electrode tips were defined and compared against the coordinates of the stereotaxic atlas (Snider & Niemer, 1961).

SUMMARY OF CONDITIONING STUDIES

Study I: Symmetric CS

Our earlier findings showed that when a lateralized, left ear tone CS was paired with a MFB stimulation US, the cats rapidly turned their heads toward the CS. This occurred already during the first trials and the amplitude of these head turns did not decrease over the daily sessions (Korhonen & Penttonen, 1989a, 1989b). Moreover, during CS-only test trials, when the CS was presented without the US, some cats also performed a second, long-latency response which to some extent resembled the head movement UR to the brain stimulation. However, in those studies, the relation between the laterality of the CS and the laterality of the US, UR and CR was difficult to define. When the direction of the short-latency and long-latency responses differed, that is, when a left short-latency head turn was followed by a right long-latency head turn CR, it was possible to define and describe the relation between the lateralized CS and CR. However, this was found to be the case in only one out of six cats (Korhonen & Penttonen, 1989a) and in 6 out of 10 cats (Korhonen & Penttonen, 1989b). In the latter experiment the 6 cats were stimulated in the left MFB, and any brain side specific effects were therefore difficult to evaluate. This was further complicated by the fact that the long-latency responses appeared only infrequently before the US.

The present study was designed to determine more closely the properties of the CR in relation to the laterality of the US and UR. To minimize the lateral effects of the CS, the CS was presented symmetrically to both ears. Due to the non-directionality of the CS, it was expected that the short-latency and long-latency CRs observed in the earlier experiments would be replaced by a single CR. It was further expected that the UR to the MFB stimulation US would usually be a contralateral, or at least a lateralized, head turn. Consequently, it was predicted that the CR would also be a head turn in the same direction as the UR. Furthermore, left and right US groups were formed to study whether the effects of conditioning would be identical in both sides of the brain.

Finally, it was of interest to know if any differences in cingulate cortex evoked potentials existed between the left and right MFB US groups or between the left and right cingulate cortex, or whether there was any interaction between MFB stimulation and the cingulate cortex recording side. It was expected that differences between the sides of the brain contra- and ipsilateral to the brain stimulation US might appear because of the unilateral nature of the MFB stimulation.

Methods

The subjects were 16 adult cats. The CSs, delivered symmetrically through loudspeakers in front of each ear, were 1000 and 2500 Hz tones. After the standard US test session, the CS test session and 10 differential conditioning sessions, an additional laterality test session was given. During this session 60 CS+ tones were presented asymmetrically with approximately 30 tones to the left ear and 30 tones to the right ear in random order. The movement results were analyzed for six left and six right MFB-stimulated cats, and the evoked potential results for four left and for four right MFB-stimulated animals. The statistical analyses were based, as in Studies II - IV, on daily CS+ and CS- averages during conditioning sessions and CS test session, and on one US average during the US test session. After the baseline had been subtracted from all data points, a mean value for the period 128 and 328 ms from the tone onset was computed. The mean values of the movement and evoked potential signals were used as the dependent variables in the analysis of variance.

Results

During the US test session, the head movement URs were stereotyped, occurring during each trial with nearly similar characteristics and intensities. Five out of 12 animals turned their heads to the left, 2 to the right and 5 either upwards or forwards. The direction of a head turn was not, however, systematically dependent on the side of the MFB stimulation. During the CS test session, 3 out of 12 cats turned their heads to the left, and 1 to the right. Eight out of 12 cats showed a complete balance between the tones, displaying no consistent orienting head movements to the left or right. Instead, they turned their heads occasionally to the left, right or upwards. During the first trials, the orienting head movements were rather rapid and extended in all cats, but on subsequent trials they decreased, and habituated almost completely by the 10th trial. There were no differences in habituation rate between the cats stimulated in the left and those in the right MFB.

During the last conditioning session, the direction of the head movement CR to the CS+ tone was to the left in 11 out of the 12 cats, regardless of the original direction of the UR to the MFB stimulation or the direction of the orienting head movement during the CS test session. The five cats that initially turned their heads to the left as the UR acquired the same tendency as a CR during conditioning, while the two cats that had turned their head to the right and the four cats which had moved forward as the UR, showed left turning as a CR.

During the laterality test, when the CS+ tone was presented either to the left or right ear, 8 of the 12 cats still preferred turning to the left. In addition, 3 of these cats now also turned their heads to the left to the right ear CS+. The results also showed that the average extinction rate was slower when the tone was presented to the left ear than to the right ear.

Analysis of the head movement acceleration transducer recordings showed that during conditioning the cats increased their head movement responses both to the CS+ and CS-, but predominantly to the CS+. They also learned to differentiate the CSs during the second session of conditioning, and maintained this differentiation for the subsequent eight daily sessions. During the laterality test, the amplitude of the head movements was greater when the CS+ tone was presented to the left ear than to the right ear.

During conditioning, greater negative evoked potential deflections were found in the right than in the left cingulate cortex. Furthermore, greater negative deflections to the CS+ than to the CS- appeared only in the right cingulate recordings. No differences were observed between the sides of the brain during either the CS test session or the first conditioning session. Therefore, at the neural level, differential changes to the CS+ and CS- due to conditioning appeared only in the right cingulate cortex. During the laterality test, cingulate cortex negative evoked responses were greater when the CS+ tones were presented to the left ear, that is, to the ear ipsilateral to the predominant head turn CR.

Discussion and conclusions

Contrary to expectations the cats learned to turn their heads to the left as a CR regardless of the direction of the original UR or the side of the US. This conditioned left turning preference can not be attributed to an asymmetry in the hearing of the tone-CSs in the left or right ear, as most of the cats did not show any initial directional preference in orienting head movements to the CS during the CS test session.

It can still be argued, nonetheless that although the intensity of the bilateral tones were carefully equalized, and although the initial CS-test session indicated a symmetrical orientation in 8 of the 12 cats, there might have remained some hidden lateral imbalance in the perception of the tones. During subsequent conditioning sessions, this difference might have been magnified, appearing later as a preferred turn to the left. Therefore, the influence of asymmetric tones was approached more directly in Studies II and III.

When a tone is presented to one ear, a greater neural activation in the auditory structures, at least above the level of the superior olive complex, occurs in the contralateral side of the brain (Masterton & Imig, 1984). Correspondingly, during lateral turning, a greater activation occurs in the contralateral side of the brain (Yeomans & Tehovnik, 1988). Thus, the observations of head turn CRs to the left, greater negativity in the right than left cingulate cortex, slower extinction and greater amplitude of the head movement to the left ear CS+ may all be interpreted as indices of greater activation in the right side of the brain.

The results therefore suggest a population type of bias in conditioned responding when a symmetrical tone CS is associated with MFB stimulation. However, it seems reasonable to limit the tentative conclusion as to the greater excitability of the right side of the brain to a situation where a tone CS and brain stimulation US are paired, since no bias was found in orientation to the CSs alone. In addition, the nature of the US might have had some special, as yet unknown, effects. Furthermore, since studies on lateralization have indicated differences between species, and even subpopulations of a certain species, and since there have also been inconsistencies between laboratories (Glick, Carlson, Drew, & Shapiro, 1987), the present results need replication before their significance is more extensively discussed. However, in accordance with the present results, the greater involvement of the right side of the brain in self-stimulation of the lateral hypothalamus has been found in another paradigm (Bianki, Murik, & Filippova, 1989). The inactivation of the right cortex by the spreading depression method was found to decrease, and the inactivation of the left cortex to increase, the frequency of self-stimulation in rats.

Study II: Lateralized CS and UR

Study I showed that in most animals the head turn CRs were directed to the left in spite of the symmetric CS tone. This was carried one step further in Study II by asking whether CRs of different degrees would occur when a lateralized CS+ was presented either to the left or right ear.

To further specify the factors influencing the development of the direction of head turn CRs, the side of the CS+ and the direction of the UR were chosen to be opposite to each other. By using opposite lateralities, it should be possible to separate effects due to the CS versus effects due to the US. Accordingly, a restriction was imposed for the selection of the MFB electrode: the stimulation had to elicit a clearly observable lateralized head turn. Since it turned out that head turn URs contralateral to the stimulation side were more frequent, new animals were included until two groups of equal size were obtained. In one group, the cats were stimulated in the left MFB, hence turning their heads to the right. In the other group the cats were stimulated in the right MFB, hence turning their heads to the left. The formation of these two groups allowed for comparison of the effects of the left and right side USs.

Methods

The subjects were 15 cats. If more than one behaviorally effective electrode was found, the electrode associated with a contralateral head turn was selected. During conditioning, the CS+ tone was presented to the ear contralateral to the direction of the UR. The unilateral CS+ (1000 Hz) was accompanied by MFB stimulation. The CS- tone was presented to the opposite ear without MFB stimulation.

Results

During the US test, seven out of eight cats stimulated on the left side turned their heads to the right, and six out of seven cats stimulated on the right side turned their head to the left as the UR. Five cats in the left MFB stimulation group and five cats in the right MFB stimulation group that showed contralateral turns were analysed further.

During the CS test session, before habituation of the head movements occurred, 6 out of 10 cats turned their heads in the direction of the tone. One animal invariably turned its head to the left, while in 3 animals the dominant direction could not be reliably defined due to rapid habituation. During conditioning, all the cats showed a fast and extended stereotypic head turn toward the CS+ tone and retained this response up to the end of the conditioning sessions. The cats responded in general also to the CS- by head movements. Thus, in only 3 out of the 10 cats, the head movements to the CS- were relatively minimal and slow compared to the responses to the CS+ tones. Analysis of the direction of the head turns to the CS- showed that three cats responded to the CS- by turning their head toward the tone and thus oriented toward the tone on both CS+ and CS- trials. In contrast, seven cats responded to the CS- tone by turning their head away from the tone and thus oriented toward the direction of the CS+ tone on CS- trials as well as on CS+ trials.

Even though the head movement acceleration amplitudes did not differ during the CS test session for the CS+ and CS- tones, they were greater to the CS+ than CS- during conditioning, and increased over sessions. Further, during conditioning the onset latency of the head movement was shorter to the CS+ than to the CS-, and was also shorter to both tones during the final compared to the first session.

Greater negative deflections were found in the cingulate cortex in response to the CS+ than to the CS-, but no differences between the left and right cingulate cortex or between the cingulate cortices ipsi- and contralateral to the MFB stimulation were observed.

Discussion and conclusions

As expected from Study I, the cats developed a head movement CR to the CS+, but the head turn was now invariably directed to the side from which the CS+ was presented. This suggests that a tone asymmetrically presented to the left or right ear laterally activates the nervous system as a CS during conditioning with an MFB stimulation US.

The amplitude of the head movement CR was equal whether the CS+ was presented to the left or right ear. These findings indicate that while the laterality of the CS+ contributed to the direction of the head turn CR, the lateralized presentation of the CS to the left or right ear did not have a differential effect on the amplitude of the CR. It can be similarly concluded that left and right MFB stimulation led to head movement CRs of equal amplitudes. Both of these latter conclusions are, however, confounded by the fact that the CS+ and US presentations were ipsilateral. Thus when the US was a left MFB stimulation the CS+ was presented to the left ear and correspondingly, when the US was a right MFB stimulation, the CS+ was presented to the right ear.

The results clearly demonstrated that the laterality of the CS was a more important determinant of the direction of the head turn CR than the laterality of the UR. This conclusion does not, however, exclude the possibility that responses resembling the UR had also developed. In the previous studies, UR-related CRs were also observed during CS-only trials, and they specifically occurred at the time of the omitted US (Korhonen & Penttonen, 1989a). In the present study, those long-latency CRs could not be observed since the CS+ was always presented together with the US.

Based on the assumption that effects of MFB stimulation are greater in the ipsilateral brain side, and that the effects of tone presentation are greater in the side of the brain contralateral to the ear stimulated, it can be argued that the CS+ and US might have predominantly activated conditioned foci on different sides of the brain. For example, the effects of the left MFB stimulation might have been greater in the left side of the brain, but the CS+ presented to the left ear might have activated the right side of the brain more. Since head turn CRs were ipsilateral to the CS+ ear, it can be argued that primary associative changes occurred in the side of the brain contralateral to the CS+ and US. Thus the ipsilateral US somehow modified the excitability of the contralateral structures involved in head turning.

The cingulate recording difference between the left and right side found in Study I was not replicated in Study II. On close examination this is less surprising, since there were large differences between the experiments. Whereas the CS was a symmetric tone in Study I, it was an asymmetric tone in Study II. Presumably, the asymmetric presentation of the CS evokes reflexive lateralized orienting to a greater degree than that evoked by symmetric presentation, with the result that the more subtle effects may be masked.

Study III: Lateralized CS presented ipsi- and contralateral to lateralized US

A left head turn CR to a symmetric CS was found in a majority of the cats in Study I. By contrast, in Study II, a head turn towards the asymmetric CS developed. In Study III, the acquisition of these apparently conflicting CRs was directly addressed by administering the CSs asymmetrically although presenting the CS+ and CS- to each ear equally over presentations. It was reasoned that even if there might be some small differences between the left and right ear tones, those differences would not be apparent when the tones were presented separately. Therefore, tones of different frequencies were used as CS+ and CS-. The CS+ and CS- tones were presented alternatively to the left and right ear in random order. Two contradictory predictions were formed. Based on the results of Study I, it was predicted that the cats would develop lateralized movements biased in one direction independently of the actual ear to which the CS+ tone was presented. According to the results of Study II, it was predicted that the cats would develop a head turn CR toward the particular ear to which the tone CS+ was presented.

In Studies I and II, no differences at all were found between cats stimulated in the left or right MFB. Therefore, in the present experiment the brain stimulation was always applied to the right MFB. It was expected that when all the cats were stimulated on the same side of the brain, a larger group would be made available for the analysis of the evoked potential responses recorded in the cingulate cortex ipsi- and contralateral to the brain stimulation US.

Methods

Adult cats were used in the study. During the CS test session, and during the subsequent four conditioning sessions, 1000 and 2500 Hz tones were presented either to the left or right ear at an equal intensity and in random order. One randomly selected tone (1500 ms, either 1000 or 2500 Hz) was used as the CS+ and the other tone as the CS-. The CS+ tone was presented randomly to either left or right ear, and it overlapped during the last 500 ms with the stimulation US. CS- trials consisted of the presentation of the other tone randomly either to the left or right ear. All the stimulation electrodes were implanted in the right MFB. Movements and evoked responses were analyzed in 12 animals.

Results

During the CS test session, all the cats initially moved their heads in response to the tones. Analysis of the direction of the orienting head turns before habituation showed that 11 out of 13 cats predominantly turned their heads in the direction of the tone. Regardless of the ear to which the tone was presented, two cats more often preferred to turn their heads ipsilateral (i.e. to the right) than contralateral to the side of the right MFB stimulation electrode. The habituation rate of the head movements to the tones presented ipsi- and contralateral to the stimulation electrode was equal.

During the final conditioning session, the responses to the CS+ tones were rapid head turns, without any other body movements. The head movements to the CS- tones were slower, they had a longer onset latency, and the displacement of the head was not as great as to the CS+ tones. Three cats turned in the actual direction of the CS+ and CS- tones. Ten cats, however, turned in one direction in response to the CS+ and CS- tones, regardless of the direction from which the tones were presented. Two of the cats that had turned ipsilateral to the side of the stimulation electrode during the CS test session continued with this response tendency during conditioning. Eight other cats showed unilateral CRs, although they had turned toward the tone during the CS test session. Two of these cats turned ipsilateral, and six contralateral, to the US in response to the CS presentations. Thus, those cats which during conditioning did not retain the bilateral orienting movements observed during the CS test session more frequently turned contralateral than ipsilateral to the US.

The head movement UR evoked by the US was not related to the head turn CR. A left head turn UR was observed in three cats; in one of these cats, the head turn CR was also to the left, but in one other cat, it was to the right. A head movement UR upwards or forwards was found in 10 cats, two of which also showed a slight bias to the right.

Both head movement onset latencies and accelerations were different in response to the CS+ and CS-. The head movement acceleration furthermore increased over sessions only in response to the CS+.

Whereas negative evoked potential deflections were equal in the cingulate cortex ipsi- and contralateral to the US electrode in the first conditioning session, during subsequent sessions the negativity was greater in the ipsilateral cingulate cortex. While in the contralateral cingulate cortex no differences in response to the CS+ and CS- were observed, in the ipsilateral cingulate cortex the negativity was larger in response to the CS+ than CS-, and this difference increased over sessions.

The relationship between cingulate cortex evoked potentials to MFB stimulation during the US test session, and to the CS+ during the final conditioning session, was analyzed by computing product moment correlations between the US and CS+ waveforms in the cingulate cortices ipsi- and contralateral to the MFB stimulation. This analysis was performed for nine animals with artifact-free US recordings. The evoked potential waveforms of the CS+ and US averages were similar to each other in both the ipsi- and contralateral recordings. This indicates that the form of the CS+ evoked potential responses closely corresponded to the form of the US evoked potential responses. In five cats, the ipsi- and contralateral cingulate cortex CS+ evoked potential waveforms were rather similar. In these cats, negative evoked potential deflections appeared in both sides of the brain in response to both the US and CS+, but were generally smaller in the contralateral than ipsilateral cingulate cortex. In four other cats, the CS+ waveforms between the two sides of the brain did not resemble each other. In these cats, a predominantly negative deflection was observed in the cingulate cortex ipsilateral to the US but a positive deflection in the cingulate cortex contralateral to the US. Taken together, in the ipsilateral cingulate cortex the US appeared to evoke negative potentials, and this negativity was recorded as a similar CS+ evoked potential response during the final conditioning session. Correspondingly, in the contralateral cingulate cortex, the US evoked a smaller negativity and even positivity, and the CS+ evoked potentials resembled these potentials during the final conditioning session.

Discussion and conclusions

In the present study the cats showed conditioned behavioral discrimination to the CS tones. This differentiation appeared as orienting head turns, that were more extended, of greater acceleration, and of shorter onset latency to the CS+ than to the CS-.

The present finding that a majority of the cats showed lateralized head movement CRs during the conditioning session corresponds to the findings of Study I, where symmetrical presentation of the CS+ resulted in lateralized responding. The present experiment specifically showed that lateralized movements appeared even though the CS+ was presented asymmetrically but with equal intensity and probability to each ear. Because the asymmetric CS+ was presented in a balanced sequence across the ears, the lateralization of the orienting head movements did not develop due to physical or perceived asymmetries of the CS+ as in Study II. Furthermore, some initial preference in orienting ipsi- or contralateral to the side of the US did not cause lateralization of the CRs, as no initial preference was found during the CS test session.

The appearance of lateralized orienting movements in response to the CS+ suggests that the pairing of the CS+ with unilateral MFB stimulation might have induced asymmetric changes in the brain structures involved in the neural control of auditory orientation. The imbalance in the neural activity between bilateral structures, usually with a greater activation of structures contralateral to the ear to which the tone is presented, is thought to be responsible for sound localization (Masterton & Imig, 1984). The pairing of the asymmetric CS+, presented to both ears on randomly alternating trials with MFB stimulation, may have lead to increased neural activity in the auditory structures located ipsilateral to the brain stimulation. This might have activated perceptual processes localizing the tone contralateral to the side of the US presentation, irrespective of the ear to which the tone was presented. This might explain why a head turn CR contralateral to the US was observed in the present study. Also, increased activation of ipsilateral non-auditory brain areas that control head turning may have occurred. The side of the brain contralateral to the direction of turn is believed to make a greater contribution to the turning response (Yeomans & Tehovnik, 1988). Thus, the neural structures involved in both sensory and motor control of orienting behavior may have exerted greater excitability ipsilateral to the US.

In Study I, a frequent CR was a head turn to the left with greater evoked potential responses in the right cingulate cortex. This was also observed in the present study, with a head turn to the left as the most frequent lateralized response. Furthermore, greater negative evoked potential deflections in the cingulate cortex ipsilateral to the MFB electrode means greater negative deflections in the right cingulate cortex. These convergencies between the two studies might indicate, that although the present results were interpreted to indicate a greater neuronal activation in the cingulate cortex ipsilateral to the MFB stimulation electrode, the possibility exists that also the left vs. right side dichotomy might have contributed to the results.

The analysis of the evoked potential waveforms supports the possibility of selective increases in brain activity ipsilateral to a brain stimulation US. The cross-correlations indicated that in some animals the CS+ waveforms differed between the cingulate cortices ipsi- and contralateral to the MFB stimulation. In contrast, the CS+ and US waveforms were quite similar to each other in both cingulate cortices in all cats. This would indicate that differences between the CS+ evoked potential waveforms between the two sides of the brain were related to differences between the brain sides in response to the brain stimulation US. Thus, due to the asymmetric effects of the US in the two cingulate cortices, the conditioned evoked potential changes were also asymmetric.

In conclusion, the present study basically showed that conditioning was greatest when the effects of a CS presentation and US stimulation were on the same side of the brain.

Study IV: Bilateral multiple-unit activity recording

In the previous studies, evoked potential recordings of the cingulate cortex were used to determine the effects of unilateral MFB stimulations on bilateral cortical neuronal activity. Although evoked potential recordings most probably reflect local neuronal changes, the possibility remains that the observed changes were due to volume conduction. That is, instead of local neuronal alterations, changes spreading from other brain regions might have been observed (Nunez, 1981). The asymmetries shown in Studies I and III can not, of course, be attributed to volume conduction from one side of the brain to the other since the asymmetries between the two sides of the brain should then have cancelled each other out. However, the observed asymmetries in the cingulate cortex may not have been primarily generated in the cingulate cortex, but may have been volume conducted from other structures located in the same side of the brain. For example, the hippocampal formation is known to be a strong evoked potential generator (Lopes da Silva, Witter, Boeijinga, & Lohman, 1990). To test the localization of the observed neuronal changes, cingulate cortex multiple unit activity changes were recorded. Multiple-unit activity was analysed in some of the cats that were used in Study III.

Methods

The behavioral training and movement acceleration recordings were similar to those of Study III. In short, during the CS test session and during the four conditioning sessions, 1000 and 2500 Hz tones were presented either to the left or right ear at equal intensity and in random order. CS- trials consisted of presentations of one randomly selected tone either to the left or right ear. The other tone served as the CS+, and it was also presented to either the left or right ear. During conditioning, the MFB stimulation US followed the CS+ and co-terminated with it. All the stimulation electrodes were implanted in the right MFB. Movements and multiple-unit responses were analyzed in five animals.

Analysis of multiple-unit signals was performed on recordings filtered to 300-5000 Hz and sampled at 10000 Hz using an IBM PC/AT compatible microcomputer. Multi-unit activity was further analyzed with a program simulating analog level detectors. After the parameters representing the rising and falling ends of the multiple-unit spikes had been defined interactively, the computer formed multi-unit histograms for each trial by counting the number of spikes in each consecutive 20-ms bin. Separate averages were computed for the CS+ and CS- tones presented to each ear of the CS test session and for the conditioning sessions. To compute the standard scores of the multi-unit histograms, the mean value and the standard deviation of the 500-ms pre-CS bins of each of the four session averages were computed for each channel. The mean value was then subtracted from each pre- and post-CS bin and the difference was divided by the standard deviation. These standard scores thus represented the neural activity at each 20-ms bin normalized with respect to the pre-CS baseline activity.

Results

The behavioral results replicated those found in Study III. Briefly, there were no differences between the ears in habituation rate during the CS test session. One cat moved its head as a CR in the direction of the tone presented to the left or right ear. Four out of the five cats showed a unilateral head turn CR, which was not related to the side of the tone CS or the direction of the UR. A head turn ipsilateral to the MFB stimulation was found in one cat and a contralateral head turn in three cats. Behavioral discrimination of the CSs was observed during conditioning as greater movement acceleration to the CS+ than CS-.

The multiple-unit activity data did not show any differential effects. Instead, the multiple-unit activity increased over sessions to both the CS+ and CS-, which suggests an effect due either to the paired presentation of the CS+ and US and/or to the presentation of the US only. Furthermore, the multiple unit activity was larger in the cingulate cortex ipsilateral than contralateral to the US. Since no interaction between conditioning and side of the cingulate cortex was observed, the results suggest that the greater increase in multiple-unit activity in the ipsilateral cingulate cortex was due to sensitizing effects.

Discussion and conclusions

The present experiment showed that multiple-unit activity increased in both cingulate cortices across conditioning sessions, but that neuronal activity was augmented in the cingulate cortex ipsilateral to the MFB stimulation. Even though these results did not demonstrate greater differential changes in the ipsilateral cingulate cortex, they imply that the negative deflections found in Study III were due to increased activation of the cingulate cortex neurons.

The present results demonstrated that neural changes are local, since the multiple-unit electrodes selectively recorded the activity of only a few neurons at the tip of the electrode. In general, whereas evoked potential responses are regarded as representing the activation of a large amount of neurons, multi-unit activity represents the activation of a small sample of neurons, and sometimes the activation of single neurons (Abeles, 1982). Accordingly, two different but related indices of cingulate cortex neuronal activity imply an asymmetrical activation of the cingulate cortex when a tone and unilateral MFB stimulation are paired. A closer study of the relation between the evoked potentials and multiple-unit activity was not attempted here since it would require simultaneous recordings of neural activity in different layers of the cingulate cortex.

The present results exclude the possibility that the asymmetric changes were due to overt movements. Even if increases in motor activity had been connected to increases in multiple unit activity in response to the CSs, the greater multi-unit responses in one cingulate cortex cannot be attributed to a general increase in motor activity.

GENERAL DISCUSSION AND CONCLUSIONS

Main findings

The primary aim of the present thesis was to determine the effects of associative conditioning on lateralized head movements and bilaterally recorded cingulate cortex neural activity. For this purpose, a differential conditioning paradigm was used, which allowed for experimental manipulation of the lateral position of the CS and US.

The present studies demonstrated that lateralized head movement CRs appeared during classical differential conditioning in all studies, with the direction of the CR related to the laterality of both the CS and US. In summary, at the behavioral level, the present studies demonstrated: (a) that the laterality of the CS made an important contribution to head turn CRs (Study II); (b) that although the primary neural activations related to the CS and US, presentations need not necessarily occur in the same side of the brain (Study II); the occurrence of these activations in the same side of the brain may have an important facilitatory role for the acquisition of a head turn CR, and may determine the direction of the CR (Study III); and (c) that there seems to be an inherent asymmetry in the cat brain which favors the acquisition of left-sided head turn CRs, although the present studies do not specify the structural or neurochemical basis for this asymmetry (Study I).

The present studies therefore showed that cats have a tendency to develop consistent and persistent lateralized head movements, or side preferences, to auditory stimuli paired with an MFB stimulation US. Although the laterality of the CS most clearly defines the direction of these CRs, the laterality of the US also has an effect that should be attended to in future studies. The appearance of a tendency to turn to the left with symmetric CS presentations only after conditioning was in line with other behavioral studies which have shown that the asymmetry in side preference is apparent only after extended training (Castellano, Diaz-Palarea, Barroso, & Rodriquez, 1989; Castellani, Diaz-Palarea, Rodriquez, & Barroso, 1987).

Both asymmetries and symmetries in cingulate cortex neuronal activity were observed. By using symmetric CSs, as in Study I, greater differential evoked potential changes were observed in the right compared to the left cingulate cortex. This implies an asymmetry at population level. In contrast, differences between the ipsi- and contralateral cingulate cortices to MFB stimulation were demonstrated in Study III, implying that the MFB US has a unilateral effect. These latter results may, of course, further indicate an asymmetry between the left and right cingulate cortex, since larger evoked potential changes observed in the cingulate cortex ipsilateral to the right MFB stimulation consequently mean larger changes in the right cingulate cortex.

With respect to the unilateral MFB stimulation US, it was further shown in Study III that the evoked potential waveforms recorded in response to the CS+ were similar to the waveforms recorded in response to the MFB stimulation. In particular, some evidence was found that the similarity of the CS+ and US responses within each cingulate cortex was greater than the similarity of the CS+ responses between the cingulate cortices. This further supports the idea that the conditioning procedure affected the two sides of the brain differently.

However, asymmetries in cingulate cortex neural activity were not found in Study II, where the CS+ and the US were presented on the same side. Two opposite tendencies can be thought to have caused these effects. Whereas the CS+ presented to the ear ipsilateral to the MFB may have more strongly activated the contralateral structures involved in the perception of the tone and performance of the orienting movements, the MFB may have more strongly activated the ipsilateral side of the brain. Due to the fact that such activations are important for the formation of a CR, and that they occurred in the opposite sides of the brain, it can be speculated that the experimental design of the study may have not been optimal for the appearance of conditioned neural asymmetries.

A close causal relation between the behavioral and neural measures was not demonstrated in the present studies, although some indirect evidence suggesting that both measures share common elements emerged. Primarily, the head turns were more frequent contralateral to the cingulate cortex in which the greater evoked potential response was observed (Study I). Together, these results imply that the conditioning affected neural activity asymmetrically from the low up to the highest brain levels. To further clarify the neural basis of the head turn CR, recordings from structures located at different levels of the brain would be of importance. In addition, the use of electrical stimulation as a CS could be attempted, particularly with respect to structures involved in head orientation to sounds.

The fact that the cingulate cortex is an important component of the limbic system, receiving its main input from cortical association areas and from the subiculum of the hippocampal system, and that it projects back to those areas (Swanson, 1983; Swanson et al., 1987), implies that the changes observed in the present studies might also have occurred in other intimately connected structures. Since the auditory input to the hippocampus is through the cingulate cortex, the input to the hippocampal formation might also have been affected. Correspondingly, the hippocampus may also have contributed to the cingulate cortex neural changes due to extensive outputs to this area through the subicular cortex. The asymmetries demonstrated in the present studies can thus be tentatively be regarded as an indication of changes occurring in the whole limbic system due to close interconnection between the structures. The fact that the cingulate cortex is innervated by the medial forebrain bundle, as are other structures of the limbic system, implies the possibility of simultaneous changes occurring in many limbic areas.

Because so many structures are involved in the activation of the MFB, it should be possible to find other US sites with more specific neural connections than the lateral hypothalamus. For example, the stimulation of the MFB can activate noradrenergic output from the locus coereleus, serotonergic output from the dorsal and median raphe nucleus or dopaminergic output from the ventral tegmental area and the substantia nigra. The MFB is further connected to the diagonal band of Broca and the medial septum, and is thus in a position to influence the cholinergic input to the cingulate cortex (Vogt, 1985). It remains to be clarified which of these neuronal systems primarily contributed to the neuronal activation of the cingulate cortex during conditioning in the present studies.

Lateralization of function

Despite numerous demonstrations of anatomical, neurochemical and functional differences between the two sides of the brain in animals and humans, it is still not known how these asymmetries are important for the behavior of an animal (Glick & Shapiro, 1985; Hellige, 1990). For the understanding of human lateral brain asymmetries, however, animal models are particularly valuable, as demonstrations of brain asymmetries in animals imply that they are neither unique to humans nor completely dependent on the development of language (Springer & Deutsch, 1989). In the cat, the right hemisphere has been found to be larger than the left (Kolb, Sutherland, Nonneman, & Whishaw, 1982) and a slight right paw preference has been observed (Tan, Yaprak, & Kutlu, 1990). The present studies suggest the dominance of the right side in spatial and emotional behavior in the cat. This interpretation is based on the fact that the turning response is an elemental spatial behavior, and that MFB stimulation functions as a strong emotional event.

The present results, of course, require further qualification. A larger sample size with experimental subjects balanced for sex, age and paw preference would be needed to more definitively prove these tentative results. Furthermore, to exclude the possibility that this asymmetry resulted from the specific rearing environment (Denenberg & Yutzey, 1985), cats from other laboratories would also need to be used as subjects.

The observation of a conditioned left turning bias in the cat is in accord with the observations of a rotational bias to the left in left-hemisphere dominant human adult females and males of mixed dominance (Bracha, Seitz, Otemaa, & Glick, 1987), in children (Glick, 1992), in hush-babies (Larson, Dodson, & Ward, 1989), and in mice (Ward, 1991). The preference for turning in one direction may be an important component of the sense of direction, and might thus also enable discriminations between left and right (Zimmerberg, Strumf, & Glick, 1978).

Since most cats showed conditioned head movements in one direction only, the present results imply that it is not only possible to induce individual experience-dependent spatial asymmetries with MFB stimulation, but that those asymmetries are also consistent and persistent. If the MFB activation is assumed to be of primary importance for developmental and adult neuronal network modifications (von der Malsburg & Singer, 1988), then the present conditioned lateralized orienting tendencies would imply that MFB might also be involved in the lateralization of functions during ontogeny. The asymmetry might not necessarily have to be apparent at birth, but due to the cooperative activation of neural networks together with the MFB modulation, small initial differences might be expected to self-amplify ultimately leading to asymmetric functions (Singer, 1990). Thus, even though inheritance might set important boundary conditions, for example by controlling the degree or strength of brain asymmetry, the expression of the actual asymmetry might be more related to experience (Collins, 1985).

The most popular explanation for human hemispheric specialisation is that, after adaptation of an upright stance, a right hand preference was assumed, followed by the lateralization of language to the left hemisphere. Other cognitive functions, such as the spatial, were, consequently, more or less forced to be specialized in the opposite, right hemisphere (Corballis, 1989). The validity of this explanation can, however, be questioned if spatial orientation in non-human animals without language, for example in cats, is lateralized in one side of the brain (Hamilton & Vermeire, 1991).

In developing human therapeutic interventions for brain dysfunction the observation of the unilateral effect of MFB stimulation has potential importance. Supposing that the patient has unilateral deficit in higher level sensory processing, a unilateral MFB effect could be selectively utilized for the partial restoration or replacement of the deficient function. The unilateral effect could, for example, be tried without electrode implantations by applying the principle of second-order conditioning (Pavlov, 1927). In in the first phase of second order conditioning, one CS is paired with a US, and in the subsequent conditioning phase this CS is paired with a new CS comprising another stimulus. It is assumed that some features of the US are transferred to the CS in the first phase of conditioning, and that this CS then functions as a US in the second phase. This results in the formation of an association between the second CS and the CS paired with the US in the first phase of conditioning. While it might not be possible to lateralize the effects of natural USs, by presenting the CS unilaterally in the first phase of conditioning, it might be possible to transfer the reinforcing features of the US unilaterally to the CS. For example, supposing that linguistic stimuli activate more the left side of the brain, due to the close reciprocal connections of the cortex and subcortical structures, those stimuli might acquire a greater capacity to activate the left MFB system as a US in the second conditioning phase. If the CS used in the second conditioning phase includes aspects of deficient perceptual processing, a reorganizition in their neural representation might occur during that phase.

Cingulate cortex neuronal responses

While the amygdala (Davis, 1992; Kapp, Whalen, Supple, & Pascoe, 1992; LeDoux, 1992) and hippocampus (Berger, Bassett, & Orr, 1992) have also been implicated in learning and memory, the cingulate cortex was preferred here as a limbic recording target due to its location in the midline of the brain, which simplified the bilateral implantation of the recording electrodes. Furthermore, as there are so few demonstrations of synaptic plasticity in the neocortex compared to the hippocampus, the five-layer cingulate cortex was selected due to its similarity with the neocortical association areas. For performing evoked response studies, the biggest disadvantages of the cingulate cortex over the hippocampus are directly related to these advantages. First, since the cingulate cortex resides in the medial wall of both hemispheres, the different cell layers of the cingulate cortex can not be reached with the same electrode, as they are located perpendicular to the tract of a vertically implanted electrode. This eliminates the possibility of performing a current source density analyzis for locating the current sinks and sources which are the physical causes of evoked potentials and EEG waves (Mitzdorf, 1986). The location of current sinks and sources is of primary importance when inferring whether the recorded evoked potentials are locally generated or volume conducted from other structures. Current source analyzis is also helpful when inferring how different synaptic inputs have contributed to the potentials. Second, the influence of different synaptic inputs on the evoked responses is more difficult to estimate in the five-layer cingulate cortex than in the three-layer hippocampus.

Due to the variation in the recording electrode locations in each study, it was not possible to determine whether consistent differences existed between the recordings from different layers. This would have provided some clues about the location of sources and sinks. The large evoked potential amplitudes and the increases in multiple unit activity, however, suggest that the negative field potentials may have represented excitatory synaptic influences on the apical dendrites of pyramidal cells. As it may be difficult to construct a multicontact electrode for recording the full depth of the cingulate cortex simultaneously, it might be helpful in future studies to record also from other limbic sites where it is easier to prove the local generation of evoked potentials.

Negative evoked potential deflections similar to our cingulate cortex responses have also been intracranially recorded in midline association cortical structures and in the hippocampus in the cat (Katayama, Tsukiyama, & Tsubokawa, 1985; O'Connor & Starr, 1985), and in the hippocampus and the amygdala of epileptic patients (Halgren et al., 1980; McCarthy, Wood, Williamson, & Spencer, 1989), in response to infrequent stimuli in the context of frequent stimuli. These cat and human studies have simultaneously recorded on the scalp a positive deflection similar to P300, one of the most extensively analyzed human event-related potentials, which appears as a positive deflection in the range of 240-700 ms on the scalp (see Hallgren, 1990 for a recent review). Although other studies have also recorded P300-like potentials on the scalp in cats (Buchwald & Squires, 1982; Csepe, Karmos, & Molnar, 1987; Wilder, Farley, & Starr, 1981) and in nonhuman primates (Arthur & Starr, 1984; Neville & Foote, 1984), in only a few studies has the neural origin of the P300 been investigated with direct neural manipulations. These studies have shown that lesioning of the medial septum in cats (Harrison, Buchwald, Kaga, Woolf, & Butcher, 1988), or locus coereleus in monkeys (Pineda, Foote, & Neville, 1989), leads to the disappearance of the P300-like potentials recorded on the scalp. Consistent with the observations of the importance of the cholinergic (Harrison et al., 1988) or noradrenergic (Pineda et al., 1989) components of MFB activation for the P300 formation, the present results showed that the activation of the MFB directly evoked P300-like negative potentials in the cingulate cortex, and that, after conditioning, similar negative responses appeared in response to the CS+. As all limbic structures in which P300 like potentials have been recorded, including the cingulate cortex, the hippocampus and the amygdala, are innervated by the MFB (Nieuwenhuys et al., 1982), these results strongly suggest that the MFB is involved in the generation of P300-like potentials in limbic structures through widely distributed modulating neural connections, and that these P300-like potentials may also represent synaptic plasticity.

Conditioned response initiation

The present studies showed selective increases in cingulate cortex evoked potential and multiple unit activity magnitudes in response to the CS+ compared to the CS-. The results are thus in line with previous observations of the involvement of the cingulate cortex in differential avoidance conditioning in rabbits (Gabriel et al., 1988). Based on evidence obtained from multiple-unit activity recordings with selective deafferentation of recording targets, Gabriel and his associates have proposed a limbic interaction model to explain CR initiation (Stolar et al., 1989). This model proposes that, through its reciprocal connections with the hippocampus and the limbic thalamic nuclei, the cingulate cortex forms a triggering system for avoidance CR initiation. Limbic thalamic nuclei are assumed to mainly have an excitatory, and the hippocampus an inhibitory role, in controlling the cingulate projections to the premotor systems. The cingulate cortex is thought to be involved in response initiation by its connections to the striatum, superior colliculus, substantia nigra and pontine nuclei ultimately activating the midbrain locomotor area. The system proposed by Gabriel is, furthermore, compatible with the conditioned response modification model proposed by Berger and his associates (Berger et al., 1992). This model, in turn, is based on the observations of detrimental effects of selective cingulate gyrus and hippocampal lesions on the acquisition of the classically conditioned nictitating membrane response (Berger, Weikart, Bassett, & Orr, 1986). The main focus of the model is on the connections from the hippocampus through the cingulate cortex to the same premotor system as suggested by Gabriel and also to the cerebellum. As the memory trace of the nictitating membrane CR, or at least a part of it, is thought to be located in the cerebellum (Thompson, 1986), the limbic system is thought to modulate behavioral responding also through the modification of CS information transmitted to the cerebellum through the ventral pons.

Although the present studies do not necessary directly support the models of Gabriel and Berger, and as both of them are still based on rare experimental evidence, the results are not only compatible with those models but also add the initiation and modulation of approach movements in general, and lateralized movements in particular to the model.

The previous models are extended here to appetitive CRs by using the neural evidence obtained in self-stimulation studies. The MFB self-stimulation directly activates a fast-conducting myelined descending fibre system (Shizgal, Bielajew, & Rompre, 1988). This primarily activated system synapses in the ventral tegmentum and activates, in turn, the ascending mesolimbic dopaminergic system (Phillips & Fibiger, 1989). The dopaminergic system projects to the basal forebrain, amygdala, prefrontal cortex, parts of the hippocampus and the cingulate cortex, and is currently the only neurochemically identified reward pathway (Stellar & Rice, 1989). One of the major targets of the mesolimbic dopamine system, the nucleus accumbens located in the basal forebrain, has been implicated to form an interface between the limbic and the motor system (Mogenson, 1987; Willner, Ahlenius, Muscat, & Scheel-Krueger, 1991). It is supposed that limbic structures influence motor systems through the nucleus accumbens (the ventral striatum), and that the mesolimbic dopamine system controls these limbic influences at the nucleus accumbens.

On the basis of the present observations regarding neuronal changes in the cingulate cortex together with the above-mentioned facts, a limbic-striatial CR initiation model is proposed here. During head turn conditioning with an MFB stimulation US the mesolimbic dopamine projections to the cingulate cortex are presumed to contribute to the neural changes in the cingulate cortex, and the cingulate cortex projection to the striatum are supposed to initiate the CR. Alternatively, the conditioning dependent changes in the nucleus accumbens may directly contribute to approach response initiation, so that the activation of the cingulate cortex importantly modulates CR initiation. The involvement of the cingulate cortex and nucleus accumbens system in the approach response initiation is supported by anatomical evidence showing that the nucleus accumbens has strong projections to the tegmental pedunculopontine nucleus, a part of mesencephalic locomotor region controlling the spinal-cord locomotor pattern generators (Mogenson, 1987). These nucleus accumbens projections at first synapse in the two major basal forebrain nuclei, substantia innominata and lateral preoptic area (Mogenson, Swanson, & Wu, 1983) and from there they course through the MFB to the midbrain locomotor region (Busher, Schungens, Wagner, & Huston, 1989).

In addition to its influences through the cingulate cortex, the hippocampal system may also be directly involved in the modulatation of conditioned response initiation. Innervations from the subiculum, the area through which the main output from the hippocampal formation courses to subcortical stuctures, and from the entorhinal cortex, the main cortical input to the hippocampal formation, end massively in the nucleus accumbens (Swanson et al., 1987). This suggest that also the hippocampal system, by integrating highly processed supramodal cortical input with the MFB subcortical influences, directly influences this ventral striatal nucleus involved in modulating locomotor activity associated with response intitiation (Mogenson et al., 1983).

The proposed limbic projections to the subcortical premotor systems for CR initiation also include as targets many structures which have been implied in the control of lateral eye, head and body movements, that is, the dorsal striatum, substantia nigra, superior colliculus, and pontine nuclei (Yeomans & Tehovnik, 1988). Furthermore, as the nigrostriatal projection from the substantia nigra to the striatum has been implicated in conditioned circling (Glick & Shapiro, 1985) and self-stimulation reward (Vaccarino et al., 1989), so the response initiation to a specific direction by turning is conveniently included in the present conditioned approach response initiation model.

For the study of the initiation of the CR, the present procedure is an important simplification over differential avoidance conditioning in rabbits, on which the limbic interaction model by Gabriel and associates is based. The avoidance conditioning procedure is an aversive one where the rabbit receives a foot shock delivered through the grid floor of the running wheel if the animal does not turn the wheel before the scheduled delivery of the foot shock after hearing the tone CS+. The first complexity in the avoidance procedure is related to the fact that the UR to the foot shock US has not been described in any one of the published papers, suggesting that that the UR as such has probably not been considered of any importance in the acquistion of conditioning. Supposing that the behavioral effects of the foot shock are struggle followed by immobility, as in rats during classical tone/foot shock conditioning, then the freezing CR observed in the rat might also be expected to be a component of the CR to the CS+ (Bolles & Collier, 1976). However, as a stepping response results from avoidance conditioning, it is possible that the stepping response is either related to the US-initiated struggle response or it is a component of the orienting response to the tone. The latter possibility is, however, difficult to evaluate as responses to CS presentation have never been described in the rabbit. Furthermore, if stepping response acquisition can not be related to preexisting responses to the CS, to the UR, or some other effects of the US, then it would appear that CR initiation cannot be explained by any known external stimuli and, therefore, that an objective description of the neural substrates of CR initiation is a difficult task.

One other inherent difficulty in the avoidance procedure is that the stepping response performance is supported by the omission of the US. As it has proved difficult to determine the neural substrates of learning and memory when a CS is consistently paired with a US, it may be much more difficult if the omission of the US forms a significant part of the reinforcement of the response.

The stepping CR occurs in the 5-s interval between the start of the tone CS and the omitted US. While response probabilities alone have been presented for the stepping CR, it appears that the relation between the most important factors of response initiation, latency and time amplitude course, and neuronal activity has not yet been defined for the stepping response. Furthermore, the direction of the stepping response has not been reported. As stepping responses obviously occur forward or backward they are symmetrical responses, which excludes the possibility of performing comparisons between the two sides of the brain in relation to the lateral direction of response.

In the present procedure, the CS and US-related behavioral effects are described, the CS+ is always presented with the US, responses are described in relation to CS onset, and the presentation of the CS+ in front of one ear results only in a stereotypical head turning toward that side.

The differential conditoning of head turning is thus a promising model for conditioned response initiation.

Relation to self-stimulation

The behavioral effects of MFB stimulation on the cats of the present studies are similar to those observed in rat self-stimulation studies. In the rat, along the full length and width of the MFB, stimulation activates the animal, producing active sniffing and exploration with few aversive side effects (Yeomans, 1988). Consequently, in the rat, vigorous self-stimulation is rapidly acquired from the electrode placements extending from the basal forebrain nuclei to the ventral tegmentum. Also the stimulation thresholds for bar-pressing are low and responding remains stable over long periods at those sites (Stellar & Rice, 1989). In cats, when stimulation electrodes associated with signs of withdrawal had been rejected, the behavioral observations indicated signs of positive affect during stimulation. More specifically, exploration, sniffing and salivation were observed when the stimulation was repeated several times. Although it was not possible to demonstrate conditioned preference to the experimental box (Carr et al., 1989), conditioned fear at least was excluded as no reluctance to step into the experimental box was observed in any cat during the conditioning sessions. Furthermore, if the stimulation had induced aversive effects, then it might have been expected that the frequency and amplitude of the head turn responses should have decreased during conditioning indicating withdrawal (Bolles, 1972b). On the contrary, when a unilateral CS+ was used in Study II, all the cats acquired head turn CRs toward the side of the CS+.

While the lever-pressing training before conditioning sessions might have additionally indicated the appetitive motivational effects of the stimulation and more closely related the present observations to MFB self-stimulation in the rat, bar-pressing training was not used for stimulation electrode selection, as it might have had unknown effects on the subsequent conditioning. Instead, the stimulation intensities were matched to result in approach responses of approximately equal magnitudes in each cat to a single stimulation train or multiple trains during initial US testing. US presentation might therefore be argued to be better controlled, for example, in nictitating membrane conditioning preparation, where air is directed at the eye of each animal at the same pressure. However, as the behavioral effect of the stimulation was considered to be more important than the stimulation intensity of the US, the intensities were adjusted to result in equal behavioral effects.

In studying the effects of MFB stimulation on learning, the present experimental procedure is advantageous over the self-stimulation procedure as it allows precise control over the timing and sequencing of stimulus events (cf., Gormezano, 1984). Most importantly, as presentation or omission of the US is independent of the CR occurrence, all behavioral and neural recordings are synchronised to CS and US presentations only. Furthermore, the tone CS is physically constant as it is delivered through miniature loudspeakers attached in front of the ears. This excludes, however, manipulation of tone location, as the tone source moves with the turning of the head. Other physical features of the CS, such as intensity, frequency and duration can, however, easily be related to CR acquisition. Accordingly, while in the present studies the specificity of the CRs to the intensity and duration of the CS was not tested, the results showed the CRs to be specific to the frequency and side of the CS presentation. Finally, the contextual cues are standardised in the present procedure since the cat remains in a single location throughout the measurements, there being no need to approach and press a lever.

In the present procedure, the stimulus antecedents to the CR can be defined at the start of training, which simplifies the analysis of CR acquisition and performance (cf., Gormezano, 1984). As the probability of the target response is the only dependent variable in self-stimulation studies, no external stimuli are either needed nor used for response definition and measurement (Skinner, 1938). The emphasis on response probability, however, excludes other response measures which presuppose a stimulus to which they are related (Wise, 1989). Latency and time-amplitude measures, common in classical conditioning studies, are not therefore defined in bar-press self-stimulation studies. In the present procedure, the definition of the target response can be restricted by selecting the CR from preexisting orienting responses to the CS during the CS test session. The acquisition of the CR, that is learning, can then be followed as changes in response amplitude and latency in relation to the CS. Moreover, as response acquisition is relatively slow it can be followed over trials before the CR performance attains an asymptotic level at the second or third session.

Rather than focusing on CR acquisition, that is learning, MFB self-stimulation research has been more centred on the performance of already acquired responses, and subjects with a stable level of responding have even been preferred in order to keep the variance of the response probability as small as possible (Wise, 1989). In self-stimulation studies the frequency of lever-pressing responses therefore more probably reflects motivational factors than those related to response acquisition. In addition to the fact that MFB stimulation and approach are connected, motivation has been central to self-stimulation studies, since a priming brain stimulation is quite often needed to initiate well-learned responding at the start of a session (Wise & Rompre, 1989). Finally, as self-stimulation studies have been more concerned about motivation, they have been less concerned about where the neural changes responsible for response initiation and execution actually occur.

Moreover, the performance of a learned response is easier to determine in this paradigm than in self-stimulation. After an asymptotic level of CR acquisition has been reached, the head turn is stereotypical, rapid and constant in latency, which simplifies the analysis of the performance of the response. As the cats start the head turning response from the same sitting and forward looking posture, and as they simply turn their heads, without any forward movements, the conditioned response can easily be differentiated from other responses.

From the point of view of motivation the present results are in accord with the incentive explanations of appetitive motivation (Bozarth, 1991). The results can thus be interpreted to come close to the view of Bindra (1976) that pairing of the CS and the appetitive US increases the incentive value of the CS, thus leading to the performance of approach responses as the increased incentive value of the CS "pulls" the animal. The results are also in accord to the ideas of Bolles (1972a) that if a biologically important appetitive event occurs in the presence of environmental stimuli, those stimuli will increase the likelihood of approach responses in the future.

CR acquisition most obviously results from synaptic efficacy changes in neuronal networks. Since no such circuit has yet been identified for the bar-pressing response, these neuronal networks have thus been supposed to be too complex for experimental analysis with current techniques (Stein & Belluzzi, 1988). However, as the head turn CR is a relatively simple response, the neural networks involved in CR acquisition should be easier to analyze. The present classical conditioning procedure simplifies this analysis because it allows greater control over the stimuli and their relations, a more precise quantification of the temporal properties of the response, and a slow development of the CR. The neural regions and circuits involved in the habituation of reflexive head turning to auditory stimulation have already been described in the cat (Jenkins & Masterton, 1982; Jenkins & Merzenich, 1984; Thompson & Masterton, 1978). The regions and circuits involved in eye, head and body turning have also been described from the forebrain to the brain stem in the rat and to a lesser degree in the cat (Pycock, 1980; Yeomans & Tehovnik, 1988). Furthermore, the final common path of the cat head turn has been described (Iwamoto, Sasaki, & Suzuki, 1990; Suzuki, Siegel, & Wu, 1989).

Behavioral and neuronal plasticity

Here the head turn CR formation has been interpreted to involve, at least partly, changes in the sensory processing of the CS. During the last decade, conditioning dependent changes in the sensory system have been studied extensively in invertebrates. Conditioning-related increases in the siphon withdrawal response in Aplysia and decreases in the phototactic response in Hermissenda have been demonstrated to be causally related to changes in the synaptic transmission efficacy of tactile (Hawkins, Abrams, Carew, & Kandel, 1983) and visual neurons (Farley, Richards, Ling, Liman, & Alkon, 1983), respectively. Although the involvement of sensory systems in memory trace formation has been largely disregarded in vertebrate model systems (e.g., nictitating membrane response, Thompson, 1986), their importance for information storage also in higher species has been argued by some researchers (Weinberger, 1990). For example, the magnocellular medial geniculate nucleus (the thalamic auditory nucleus) has been demonstrated to be important for classical conditioning of the pupillary dilation (Ryugo & Weinberger, 1978) and pressor response (LeDoux, Sakaguchi, Iwata, & Reis, 1986).

The present observations of MFB stimulation-related synaptic plasticity in adult cats may also be connected to the self-organization of sensory processing in young animals (Singer, 1990). Use of the sensory system together with the gating function of the central core, largely corresponding to the MFB, has been suggested to be essential for developmental as well as adult plasticity (Singer, 1990). In the present studies, the activation of the auditory system, together with the subsequent MFB stimulation, may have led to activity-dependent changes in those brain regions and circuits connected to auditory orientation, ultimately leading to these stereotypic head turn CRs. Also, the activation of the cingulate cortex by afferent input just before MFB stimulation may have led to these negative evoked potential responses, reflecting the reorganization of the neuronal network due to synaptic efficacy alterations. Direct recordings from the auditory system should conform the former possibility, and recordings from the cingulate cortex, or a related structure, with a multicontact electrode should more definitively prove the latter.

The observed CRs may represent perceptual learning (Hall & Honey, 1989). Thus conditioning may have modified the extent and degree to which the environmental stimuli were perceived. More generally, during differential conditioning the attention may also have become modified enabling the dimensions along which the CS+ and CS- differred to become dominant. Thus, when the frequency or laterality of the CS+ was perceived, a greater head turn CR appeared than when the features of the CS- were perceived.

The present results also focus attention on the distinction proposed by Konorski (1967), and recently more specifically analyzed by Wagner and Brandon (1989) and Wagner and Donegan (1989), that USs have both sensory and emotional/motivational components, and that for some CRs the sensory properties of the US are more apparent than for some others. An example of the primary contribution of the more specific sensory properties of the US on the CR is nictitating membrane conditioning in the rabbit, where the nictirating membrane extension over the cornea occurs as a UR and CR. An example of the influence of more general emotional properties of the US on the CR is the conditioned emotional response in the rat (Estes & Skinner, 1941). The consistent observation of the present studies was that the specific behavioral components of the US were not related to the CR formation. On the contrary, instead of the sensory properties of the US, those of the CS were of great importance for CR acquisition (Study II). Also a specific asymmetry in the neuronal networks responsible for spatial orientation appeared to contribute more to the response formation than the specific proporties of the US (Study I). Finally, the asymmetric distribution of the US-related nonspecific effects in the brain apparrently also contributed to the CR formation (Study III). Thus, in these studies specific properties of the CS and nonspecific properties of the US primarily contributed to CR acquistion.

The US-related specific properties were not, however, completely without effect. In Study II, the initial head turn CR in the direction of the asymmetric CS+ was followed by another CR, resembling the UR, frequently in one cat and infrequently in two cats. If the analysis of the neural substrates of these rarely observed long-latency CRs is considered important, their frequency might be increased by increasing the interval between the CS and US onset thus allowing for a more specific temporal separation of their neural effects. Furthermore, using the standard paired conditioning procedure, thus pairing the CS with the US on all trials, would probably lead to a faster conditioning of the long-latency responses. In the present studies, the CS- presentation might have had only a negligible effect on the observed CRs, but a larger suppressing effect on the long-latency CRs. In addition, the long-latency responses would most reliably be observed, as in our previous studies (Korhonen & Penttonen, 1989a, 1989b), during CS-only test trial presentations interspersed with the paired trial presentations. Finally, the essential neural circuits for coding the memory traces of the CRs observed in the present studies may be more directly accessible with MFB stimulation than those essential for the formation of long-latency responses. More specifically, while the MFB has direct widespread modulating influences all over the brain, the MFB stimulation may not directly activate the neural circuits coding the specific properties of the US. Accordingly, since our inital observations, classically conditioned head turn CRs, resembling our long-latency responses (Korhonen & Penttonen, 1989a, 1989b), have been recently obtained with a cerebellar stimulation US (Swain, Shinkman, Nordholm, & Thompson, 1992). Since the cerebellum and closely related structures are thought to be essential for the conditioning of nictitating membrane responses, long-latency head turns might be easier to condition in future studies by using stimulation electrodes implanted in the brain stem.

The present results support to some extent the idea that preexisting responses to the CS were facilated when the CS+ and the US were paired (Hawkins & Kandel, 1984). In the present studies, during the CS test session, a wide variety of head movements were observed and, as a rule, only one response, a head turn in a specific direction, was facilitated at the expense of other head movements during conditioning. It is reasonable to suppose that memory traces of this CR were formed in the sensorymotor pathways mediating the head turn CR, and that these memories were represented as an alteration in the efficiency of the same neural pathways. In addition to sensory and motor systems, synaptic efficacy changes also occurred in higher integrating systems, such as the cingulate cortex, an important component of the limbic system.

The synaptic efficacy changes, appearing as directed head turns at the behavioral and as cingulate cortex network connection changes at the neuronal level, are most economically explained by the principle of activity-dependent neuromodulation (Hawkins et al., 1983; Walters & Byrne, 1983). Thus those synaptic connections active before the activation of a modulating system were strengthened. The MFB forms an ideal modulating system as it has widespread access to input pathways located throughout the brain. The selectivity of synaptic plasticity can be attributed to the specific temporal relation between the CS+ and the MFB activation, which resulted in increases in synaptic strength at those sites that were active just before or at the time of the modulating effects of the MFB stimulation.

A long-lasting change in synaptic efficacy due to the high frequency stimulation of afferent inputs, or long term potentiation, is currently the most likely neural mechanism for synaptic plasticity in the mammalian cortex (Baudry & Davis, 1991). As long term potentiation has been demonstrated in brain slices using artificial electric stimulation, it has been difficult to obtain direct proof of its involvement in learning and memory. The present studies, however, demonstrate that a high-frequency stimulation of cingulate cortex afferent input results in long-lasting neuronal changes, presumably reflecting alterations in the synaptic connections in the cingulate cortex neuronal populations, with simultaneous changes in behavioral responding. As MFB stimulation, by inducing behavioral activation and approach movements, was a behaviorally relevant method for electrically stimulating cingulate cortex afferent input, and as the temporal pairing of this afferent input with sensory stimulation induced specific neural changes in the cingulate cortex with simultaneous changes in behavior, these cingulate cortex synaptic alterations have an obvious behavioral relevance. It is important, if the behavioral relevance of long term potentiation is to be determined, that it is more closely related to the present observations in future studies.

Cat conditioned head turn response

Lateralized stimulus presentations and response measurements in the present studies were primarily used to further develop our basic cat head turning model for the study of the neural basis of associative conditioning. The results of the present studies will therefore be discussed more closely in the context of associative conditioning and a refined model system will be formulated.

The head turn CRs observed in the present studies are not very easy to classify according to traditional definitions of conditioning (Guthrie, 1935; Hull, 1943; Pavlov, 1927; Skinner, 1938; Tolman, 1932). For example, a head turn CR cannot be regarded as an instrumental CR since, although the first head turn CRs occurred already after the first few CS+ presentations, implying an association between the head turn response and the US, the response acquisition occurred even when no specific procedural requirements were imposed on the occurrence of the head turn response (cf., Mackintosh, 1974).

Furthermore, as the CRs in many cases differed from the orienting responses measured during the CS test session, the head turn CRs did not solely represent pairing specific increases of the original UR to the CS. Additionally, as the latency of the head turn CRs decreased and the amplitude increased differentially over sessions, the CR was more than just a pairing specific intensification of the unconditioned response to the CS. Finally, as the form of the CR was specific to the CS and US combination used in the separate studies, the unconditioned properties of the CS did not exclusively explain the CR formation. Taken together, the head turning CR can not be regarded solely as a conditioned alpha response, that is, an intensification of the original response to the CS (Hawkins &

Kandel, 1984), or a Type A conditioned response, that is, a CR with relatively fixed onset and peak latency (Donegan & Thompson, 1991). The head turn CR is therefore qualitatively different from such widely studied alpha or Type A conditioned responses as the Aplysia siphon withdrawal response (Hawkins & Kandel, 1984), cat short-latency eyeblink response (Woody, 1984), cat pupillary response (Weinberger & Diamond, 1988), rat fear potentiated startle response (Davis, 1992) and rabbit heart rate response (Kapp, Markgraf, Wilson, Pascoe, & Supple, 1991).

The head turn CR can neither be regarded as a traditional classical CR resembling the UR, such as the nictitating membrane response, since the form and direction of the head turn CR was not in any consistent manner related to the UR of the US in any study. Furthermore, the peak amplitude of the CR was not located at the onset of the US as in nictitating membrane conditioning (Gormezano, 1984). The head turn CR has, however, some features which have usually been considered to be specific to nictitating membrane conditioning, thus making it an ideal model for associative conditioning (Gormezano et al., 1983). Primarily, although the CRs were not timed to the onset of the US, they were precisely timed to the onset of the CS. Moreover, as the amplitude of the conditioned head turn CR increased and the response latency decreased over conditioning sessions, extensive possibilities for a correlational analysis between behavioral and neural measures exist (Pascoe, Supple, & Kapp, 1991).

The head turn CR most closely appears to resemble responses observed in autoshaping studies (Brown & Jenkins, 1968). In a typical autoshaping experiment, an animal is classically conditioned with a localizable CS and with a food US. After a few paired trials, the animal approaches and contacts the CS, for example a lighted key in pigeon studies. In the present context, it is of interest to note that approach movements to and contact with a continuously lighted key has also been conditioned with lateral hypothalamic stimulation in rats (Peterson, Ackil, Frommer, & Hearst, 1972). One of the important features of autoshaping is that it provides a strict experimental stimulus control similar to classical conditioning for the study of approach or more specifically goal-directed (Wasserman, 1981) and signal-directed (Tomie, Brooks, & Zito, 1989), behaviors. Buzsaki (1982) has suggested a similarity between autoshaped responses and conditioned orienting responses toward a tone CS paired with the delivery of food to a dispenser in the cat paradigm (Grastyan & Vereczkei, 1974). In the Grastyan & Vereczkei study, the cats showed conditioned orienting head movements toward a loudspeaker located on a wall opposite to the food dispenser. Since similar conditioned orienting movements appeared in the present study when the food was replaced by MFB stimulation, the conditioned head turns seem to offer a reduced model for studying conditioned orienting movements and, more broadly, approach behaviors. Importantly, conditioned orienting responses appear not to be species and stimulus specific as they have also been observed in rats using both visual and auditory CSs. After paired conditioning with a food US, the frequency of orienting responses to a light CS (rearing) and to a tone CS (head startle) increased (Holland, 1977, 1990).

In spite of the vast number of behavioral studies of autoshaping, no model system for the study of the neural basis of associatively conditioned approach responses has been developed. In fact, all current vertebrate model systems, including the rabbit nictitating membrane response, cat short-latency eyeblink, cat pupillary response, rabbit heart rate response, rabbit avoidance behavior, and pigeon heart rate response, have used an aversive US (e.g., Thompson et al., 1983). The lack of a model system for approach behaviors may be related to the great variation in autoshaped responses and the complexity of each response (Schreurs, 1989). The cat head turn CR, however, seems to offer many attractive features for the formation of a successful approach response model system. Most importantly, it provides a simplified and controlled experimental situation for studying associative learning. For example, the cats remain in a constant position during intertrial intervals even without any specific restraints. In addition, the CRs are directed, discrete and well-timed responses occurring in the same form over trials. Moreover, the presentation of the CS and US is a well-controlled event. Finally, the most essential features of associative learning, including pairing specificity, stimulus specificity, long-term retention, and reversibility (Farley & Alkon, 1985), have already been demonstrated in this model system. As the head turn CR is consistent over trials with little variation between animals, the identification of the sensory, motor and integrative pathways involved in the associative conditioning of approach behaviors should be possible.

One argument against the validity of the proposed model system would be that no new, adaptive response, as in nictitating membrane conditioning, is formed during associative conditioning (Farley & Alkon, 1985). The head turn CR is, however, a highly adaptive response reflecting gradual changes in stimulus significance, allowing the localization of a particular stimulus in space and preparing the animal to select the most appropriate response to that stimulus in a constantly changing environment (cf., Buzsaki, 1982). The results obtained with this model may therefore also have implications for the understanding of motivational, perceptual and attentional processes.

For future studies of the neural basis of the cat head turn CR, a unilateral MFB stimulation US could be applied together with a tone CS to the ear contralateral to the US, possibly exclusively using right-side MFB stimulation, while recording neural activity from the ipsilateral side of the brain or lesioning ipsilateral structures. This model would combine the implications of the present studies to form a cat conditioned orienting head turn model system, a preparation showing a clear instance of associative learning in which neuronal analysis of conditioned approach responses is possible. Such a model may also explain the lateral differences found between the sides of the brain by showing how an association is formed and represented.

YHTEENVETO

Tutkimus on osa laajempaa yritystä selvittää ehdollistuneen lähestymiskäyttäytymisen hermostollista perustaa. Tämän tutkimuksen erityisenä tavoitteena oli määrittää klassisen ehdollistamisen vaikutukset kissan sivuttaisiin pään liikkeisiin ja molemminpuoleisesti mitattuun aivojen pihtipoimun sähköiseen toimintaan. Tähän tarkoitukseen kehitettiin tutkimusasetelma, joka mahdollisti ehdollisen ja ehdottoman ärsykkeen puolisuuden kokeellisen säätelyn.

Neljän tutkimuksen sarjassa yhteen ehdolliseen ääniärsykkeeseen liitettiin toispuoleinen keskimmäisen etuaivosidoksen sähköinen ärsyttäminen ehdottomana ärsykkeenä, kun taas toinen ehdollinen ärsyke esitettiin yksin. Sähköisen ärsytyksen ehdottomia vaikutuksia olivat eläimen viriytyminen ja lähestymiskäyttäytyminen. Tutkimuksissa mitattiin pään liikkeen suunta ja kiihtyvyys sekä molempien pihtipoimujen herätevasteet ja moni-yksikkövasteet.

Ensimmäisessä tutkimuksessa ehdollinen ääniärsyke annettiin samanaikaisesti molempiin korviin pienien kaiuttimien kautta. Yhden taajuiseen ääneen liitettiin vasemman tai oikean keskimmäisen etuaivosidoksen sähköinen ärsyttäminen; toisen taajuinen ääni esitettiin yksinään. Ehdollistamista edeltäneen tottumiskerran aikana kissat eivät osoittaneet mitään erityistä mieltymystä ehdollisiin ärsykkeisiin suuntautumisessa. Ehdollistamisen aikana useimmilla kissoilla ilmaantui vasempaan suuntautunut ehdollinen päänkääntövaste, johon liittyi oikeassa pihtipoimussa vasempaa pihtipoimua suurempi, negatiivisena jännitteenä mitattu herätevaste.

Toisessa tutkimuksessa ehdollisena ärsykkeenä käytettiin toispuoleista ääntä. Ehdottoman ärsykkeen tuottamiseen valittiin kultakin kissalta se ärsytysneula, johon johdettu sähkövirta sai aikaan ärsytysneulan aivopuoliskosta (esim. oikea) poispäin suuntautuneen pään kääntämisen (esim. vasempaan). Ehdolliseen ja ehdottomaan ärsykkeeseen liittyvien päänkääntöjen erottamiseksi toisistaan ehdollinen ääniärsyke esitettiin sähköärsytyksen tuottamalle pään kääntämiselle vastakkaiseen korvaan, kun siihen liittyi keskimmäisen etuaivosidoksen ärsyttäminen ja samanpuoleiseen korvaan, kun siihen ei liittynyt sähköärsytystä. Tällöin eläimet omaksuivat ehdolliseen ärsykkeeseen suuntautuneen pään kääntämisen ehdollisena vasteena. Pään käännön suunnan määräsi siten ehdollisen ärsykkeen toispuolisuus, ei sähköisen ärsykkeen aiheuttaman ehdottoman vasteen toispuolisuus. Eri aivopuoliskojen herätevasteet eivät eronneet toisistaan.

Kolmannessa tutkimuksessa toispuoleinen ehdollinen ärsyke esitettiin satunnaisessa järjestyksessä oikeaan ja vasempaan korvaan, ja siihen liitettiin oikean keskimmäisen etuaivosidoksen ärsyttäminen. Vertailuun käytetty, toista taajuutta oleva ehdollinen ärsyke esitettiin myös satunnaisesti vasempaan ja oikeaan korvaan ilman ehdotonta ärsykettä. Selvä vähemmistö kissoista ehdollistui kääntämään päätään siihen suuntaan, mistä ehdollinen ärsyke kulloinkin tuli. Suurin osa eläimistä kuitenkin tuotti yksisuuntaisen ehdollisen päänkäännön; ne kääntyivät etupäässä samaan suuntaan riippumatta siitä, kumpaan korvaan ehdollinen ärsyke annettiin. Enemmistö yhteen suuntaan kääntyvistä eläimistä käänsi päänsä ehdollisena vasteena sähköärsytyspuolelle vastakkaiseen suuntaan. Tulos viittaa ehdollistumisen tapahtuneen tehokkaammin kun ärsytysneulalle vastakkaiseen korvaan annettua ääntä on seurannut sähköärsytys. Tämä vaikutus liittynee vastakkaiseen korvaan annetun äänen ominaisuuteen tuottaa suurempia muutoksia ärsytysneulan puoleisissa hermorakenteissa hermoratojen risteämisen vuoksi, ja siten ehdollistumiselle olennaisten ehdolliseen ja ehdottomaan ärsykkeeseen liittyvien hermotoimintojen esiintymiseen samassa aivopuoliskossa.

Pihtipoimusta mitattujen herätevasteiden negatiiviset jännitemuutokset olivat suurempia ärsytetyn keskimmäisen etuaivosidoksen puoleisessa aivokuoressa. Lisäksi ehdollisen ja ehdottoman ärsykkeen tuottamien herätevasteiden aaltomuodot olivat keskenään samanlaisia molemmissa aivopuoliskoissa. Sitä vastoin ehdolliseen ärsykkeeseen mitattujen herätevasteiden aaltomuotojen samanlaisuus aivopuoliskojen välillä ei ollut yhtä suuri. Tulokset osoittivat, että ehdoton ärsyke tuotti erilaiset vaikutukset eri aivopuoliskoissa, ja että ehdollistamisen jälkeen tämä toispuoleisuus oli havaittavissa myös ehdollisen ärsykkeen tuottamissa vasteissa.

Neljännessä tutkimuksessa mitattiin moni-yksikkövasteet samassa asetelmassa kuin kolmannessa tutkimuksessa. Toimintapotentiaalien laukeamistaajuus oli ehdolliseen ärsykkeeseen suurempi sähköärsytyksen puoleisessa kuin vastakkaisessa pihtipoimussa. Vaikutus oli kuitenkin samanlainen molempiin ehdollisiin ärsykkeisiin, joten moniyksikkövasteiden toispuoleiset muutokset johtuivat herkistymisestä.

Kokonaisuutena tutkimuksien tulokset osoittavat kehitetyn ehdollistamismenettelyn tuottaneen käyttäytymiseen ja/ tai aivopuoliskojen sähköiseen toimintaan ehdollistuneita toispuoleisuuksia. Ensimmäinen tutkimus viittaa siihen, että kissan aivopuoliskojen välillä voi olla luontaisia eroja ehdollistuvuudessa ääniärsykkeisiin, kun keskimmäisen etuaivosidoksen ärsytys toimii ehdottomana ärsykkeenä. Kuitenkin toiset tutkimukset osoittavat, että kokeellisesti säädeltävissä olevat ehdollisen ja ehdottoman ärsykkeen puolisuudet sekä niiden yhdysvaikutukset merkittävimmin muovaavat ehdollistuneita käyttäytymis- ja hermostovasteita.

Tulosten pohjalta muodostettiin ehdollistuneiden lähestymisvasteiden hermostollisen perustan tutkimukseen soveltuva malli, ehdollistunut kissan päänkääntövaste, joka sisältää määrittelyt vakioisen toispuoleisesti suuntautuneen pään käännön ehdollistamiseen.

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