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TAPANI KORHONEN

BEHAVIORAL AND NEURAL SHORT-LATENCY AND LONG-LATENCY CONDITIONED RESPONSES IN THE CAT

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ABSTRACT

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Differentation of associative short-latency, orienting ("alpha") and long-latency, delayed ("true") conditioned responses was attempted in cats in which both behavioral responses (specific head movements to the conditioned stimulus (CS) and to the unconditioned stimulus (UCS)) and neural evoked responses, mainly from the hippocampus, were simultaneously recorded. The time-amplitude characteristics and the latency of these responses were used as criteria. The experimental design comprised two groups which received paired conditioning (CC) and randomly unpaired stimulus control (CO) treatments in a balanced order. This design permitted the study of habituation, sensitization, associative learning, as well as the mutual interaction of these treatments (preexposure effects). The results showed that both nonassociative habituation and sensitization, and associative short-latency and long-latency learning could be demonstrated in these groups at behavioral and neural response levels, and that the order of treatments either facilitated (the CC-CO group) or retarded (the CO-CC group) subsequent paired learning. The results also indicated some specific interaction of the CS and UCS during paired learning. The UCS is proposed as having some modifying effect on the time-amplitude characteristics of the response to the CS during the interstimulus interval (ISI). The theoretical part of the thesis attempts to relate recent empirical findings in different areas of neurobiological research to traditional concepts of the theory of learning and conditioning. Sensitization is suggested as playing an important role in both nonassociative and associative learning and is also considered to represent a probable explanation for the nature of instrumental responses and learning. Deliberate elicitation of the short-latency behavioral response (a directed head head movement to the left) and the differentiable characteristics (latency and topography) of the unconditioned response in the present studies together with some preliminary observations of spontaneous head movements occurring during intertrial intervals (ITI) of the paired training sessions lend empirical support to these hypotheses.

Keywords: learning theory, conditioning theory, classical conditioning, conditioned response, alpha response, behavioral response, evoked neural response, brain stimulation, preexposure effect.

PREFACE

Contrary to my initial expectations, it took some years to set up the requisite research facilities and thus to commence the practical research into neural and behavioral responses. While my acquaintance with specific research methods proceeded gradually, my background studies of learning theory and neural mechanisms of learning proceeded faster and reached a point where I felt it necessary, after some preliminary studies, to increase the versatility of the experiments. Such demands and expectations in turn set higher standards for the instrumentation, and so on. Writing this thesis thus represents a point where I had to break this spiral for a while and report the results of the accumulated theoretical and experimental work.

This work has become possible due to a growing interest in physiological psychology and the favourable conditions for research at the beginning of the '70's in the Department of Psychology, University of Jyväskylä. Professor Carl Hagfors gave the initial training and impetus for the studies in this new field and has continued to lend advice in many difficult problems of instrumentation.

The development of the basic methods, instrumentation, and preliminary experimentation for the present thesis took place during a five year research period financed by the Finnish Academy. The main support has, however, come from the Department of Psychology itself as represented in the tolerant and unprejudiced attitudes of the successive heads of the department, professors Martti Takala, Isto Ruoppila, Lea Pulkkinen and, at present, Carl Hagfors.

During the preparation of this thesis, discussions with the previewers of the work, professor Eugene Sokolov, professor Trevor Archer and professor Carl Hagfors have offered guidance and inspiration. Especially important for my research efforts has been the collaboration with my colleague, Markku Penttonen, who has been of great help in the many problems of experimentation, extensive data manipulation, and editing. Consultations with Dr. Erkki Pahkinen helped in confirming decisions in the statistical design and analyses.

The University of Jyväskylä has also provided financial support for the publication of this thesis and accepted this work in their series "Jyväskylä Studies in Education, Psychology and Social Research".

Finally, concerning my dear wife and son, I hope that they have been burdened least of all by my research work, at best, perhaps, not even noticed it?

Jyväskylä, August 1987
Tapani Korhonen

CONTENTS

1 GENERAL INTRODUCTION

The present thesis consists of two theoretical papers ("Sensitization and associative learning" and "Neurobiological processes, neural responses, and associative learning"); three experimental reports (First, Second, and Third Study); and some general conclusions. All five papers have been presented as separate reports with their own references. The theoretical papers represent attempts to review traditional concepts of conditioning theory in relation to the latest findings in empirical research. Attention is particularly focussed on those aspects of neurobiological discoveries which have been made in the course of experimental brain research, which seems to be most relevant to the explanation and elucidation of the conditioning process.

In the first paper, "Sensitization and associative learning" the role of sensitization as a basic process for associative learning, together with its proposed significance as a probable source of spontaneous, instrumental responses is discussed and related to theoretical concepts of traditional conditioning theory. It is suggested that an understanding of the source of instrumental responses may be the key to a description of the operative associative mechanisms in neural networks common to both classical and instrumental conditioning.

The second paper "Neurobiological processes, neural responses, and associative learning" represents an overview of associative and nonassociative learning at the cellular and neural level from single ganglion cell preparations of invertebrate tissue to the latest vertebrate studies of conditioning. Recent findings concerning the neuroanatomical convergence of the conditioned stimulus (CS) and unconditioned stimulus (UCS) pathway systems at different levels of the nervous system, and a hypothesis postulating prewired neural connections as a prerequisite for associative learning are discussed. The difference between "alpha" and delayed, "true" conditioned responses in associative learning is also examined, and an attempt is made to find some explanation for their empirical differentation.

In the first experimental report (First Study), the postulated differences between short-latency "alpha" and long-latency "true" conditioned responses are empirically investigated. Both behavioral and evoked neural responses are recorded. Different ways of approaching the problem of the unconditioned orienting reflex to the CS, and the relation of this to the observed "conditioned" short-latency alpha response are discussed; and the experimental design is adjusted so as to yield at least a partial answer to these questions.

The second experimental study (Second Study) is in its major details a replication of the first study (First Study). Here, the number of training sessions was doubled in order to ascertain whether the evoked potential levels observed during paired training in the group which received first paired and then unpaired treatment (CC-CO group) were maintained over further sessions. The balanced order of treatments (CC-CO and CO-CC groups) also made it possible to examine the facilitating or retarding effects, on subsequent paired learning, of preexposure to unpaired CS and UCS presentations. The problem of the nature of the short-latency CR (is it a "true" CR?) was further studied in the Second Study using additional zero-delay ("backward") conditioning sessions.

In the Third Study, a specific form of interaction between the unconditioned response to the brain stimulation UCS and the time-amplitude course of the conditioned alpha response during the interstimulus interval (ISI) was studied in evoked neural and behavioral responses. Some observations in preliminary experiments in our laboratory (Korhonen & Penttonen, 1981a, 1981b) had suggested the presence of such interaction but these findings were considered only tentative until the experimental results of the First and Second Study were available. In the Third Study all relevant material concerning the effect of the UCR on the acquisition of paired conditioning during the ISI was derived from the preliminary experiments and from the First and Second Study of the present thesis. The specific features of the time-amplitude course of the ISI after paired training was compared to the changes observed during unpaired and backward treatments, and a neural "trace" hypothesis is discussed as an explanation.

Finally, the concluding chapter relates some topics suggested in the theoretical papers of this thesis to the empirical findings of the experimental studies (First, Second and Third Study). Additionally, some details of the instrumentation developed for these experiments are presented in three short technical reports (Appendices I, II, and III).

References

Korhonen, T., & Penttonen, M. (1981a). *Slow potential response in cats during classical conditioning.* (Report No. 242). Jyväskylä, University of Jyväskylä, Department of Psychology.

Korhonen, T., & Penttonen, M. (1981b). *Intracranial slow potential response in cats during classical conditioning: control and conditioning experiments compared.* (Report No. 244). Jyväskylä, University of Jyväskylä, Department of Psychology.

2 SENSITIZATION AND ASSOCIATIVE LEARNING

Studies of learning in invertebrates have emphasized the role of sensitization as a basic neural process for associative and non-associative learning. This suggestion is examined here in relation to the elements of associative learning (classical conditioning). The identification of sensitization also as a possible source for an evocation of instrumental responses reverses in fact the traditional view of the relationship between the classical and instrumental conditioning: instrumental responses do not indicate of the learning of any new association. Moreover, an instrumental response (R) can be paralleled with the orienting response (alpha) to a conditioned stimulus (CS) in classical conditioning. In this form, the operating associative mechanism in instrumental conditioning appears as identical with classical conditioning: neural representations of overt or covert stimuli are associatively coupled, not peripheral responses. The present evidence of the neuroanatomical convergence of related neural pathway systems (conditioned stimulus, CS and unconditioned stimulus, UCS) and the specific properties of these systems are used here for a formulation of a model of associative learning in which the properties and development of the alpha-response and "true" conditioned response (CR, representing properties of the unconditioned response system) are considered as separately identifiable but interacting components in the development of associative learning.

2.1 Introduction

Some fundamental elements of conditioning paradigms are analysed below in order to compare classical and instrumental conditioning under a common explanatory system. Such an attempt is not new (see e.g., Guthrie, 1935; Hull, 1943, 1952; Bindra, 1972, 1976), but recent progress in the neurobiological analysis of learning and neural learning in vertebrates allows some elaboration on earlier concepts and theories (Kandel & Schwartz, 1982; Alkon, 1984; Hawkins & Kandel, 1984; Thompson et al., 1984; Woody, 1984; Tsukahara, 1984).

Attempts have been made to analyze the nature of the conditioned response in the classical conditioning paradigm in many papers (e.g., Konorski, 1967; Bindra, 1972; Mackintosh, 1974 Gray, 1975; Dickinson & Mackintosh, 1978; Hearst, 1975, 1979) but the nature, origin and role of the instrumental response, R, as a component of associative learning has not gained so much attention. Traditionally, only the frequency of the occurrence of the R has been the main target of measurement.

However, if the concept of "spontaneity" of the R in an instrumental arrangement could be satisfactorly explained and the stimulus (and moreover, stimulus conditions) eliciting the R could, at least in principle, be identified, then a parallel between the roles of the CS (in classical conditioning) and the stimulus eliciting the response R (in instrumental conditioning) could be constructed. The R could then be regarded as a "CS", or more precisely, as a response elicited (or "emitted") by some, usually unknown CS, in a way similar to an alpha response (i.e., the orienting response to the CS) in the classical conditioning.

Thus far, this construct represents a formal foundation for the unification of the main elements of the classical and instrumental conditioning paradigms, but it leaves open the problem of the origin of the forces eliciting the R, that is, the problem of response evocation. One possible solution to this problem could be derived from recent invertebrate studies which have demonstrated the efficacy of a biologically significant stimulus (unconditioned stimulus, UCS) to sensitize the nervous system to yield either phylogenetically determined (= species-specific) and/or ontogenetically learned responses. This sensitization process has also been suggested as a basic cellular mechanism underlying classical conditioning and even higher forms of learning (Hawkins & Kandel, 1984; Kandel & Schwartz, 1982; Byrne, 1985).

A discovery of some fundamental elements of neural plasticity represents also minimal and necessary conditions for associative learning description. A basic necessary condition seems to be a pairing of a signalling stimulus (conditioned stimulus, CS) with a biologically significant stimulus, the UCS. The definite order of these events (the CS precedes the UCS) appears critical for true associative learning. The capacity of the UCS to sensitize rather nonspecifically the CNS is one essential feature. Another role of the UCS is to activate specific response pathways (unconditioned responses, UCRs) and so largely influence on the nature of the developing conditioned response (CR).

The role of the UCS as a possible determinant of the nature of the CR has been one of the main controversies in traditional conditioning theory. However, the uncritical use of the concept of "instrumentality" has led to some functionalistic explanations, according to which the CR is considered as preparatory (instrumental) with regard to the following UCS. Gormezano and Kehoe (1975, p. 172) have emphasized that such constructs represent post hoc explanations of the observed CR. The greatest weakness of the preparatory view is that it does not include a possibility of predicting a priori the nature of the developing CR. Hence, the preparatory view awaits an explanation of the origin and the determinants of such a CR.

As suggested above, the re-evaluation of the origin, nature and role of the instrumental response, R, creates new possibilities of relating it to the classical CS and CR. On the other hand, an analysis of the classical CR can be advanced on the basis of recent studies, which have offered empirical findings of the nature of the classical CR. Thus, in order to relate instrumental and classical conditioning to include as many common elements as possible, an exact description of the associative learning process is needed. An attempt to formulate such model is made in the present paper.

First, some relevant elements of classical and instrumental conditioning are described in brief in order to extract those features which appear essential for the model.

Second, a model of associative learning is suggested which utilizes the latest data of neural processes during learning, and especially those acquired in studies of the invertebrate nervous system.

2.2 The nature of the CS

The conditioned stimulus in classical conditioning is usually defined as an initially "neutral" stimulus which later acquires signalling properties in respect to the following UCS. Although signalling is the most characteristic feature of the conditioned reflex, the signalling property of the CS should not be strictly bound to the nature of the CS. Moreover, the CS could be regarded as a triggering stimulus, the functionality of which is largely dependent on several internal and external factors with an effect at the moment of the delivery of the UCS. Anokhin (1974, p. 23) has referred to such an influence as an "afferent synthesis" which "as an initial and crucial stage of any conditional response, represents an organic union at the individual neuron level of the following types of excitation: dominant motivation; situational afferentation of excited states retrieved from memory; and finally (especially in a situation in which one is working with conditioned reflexes), the presence of triggering excitation".

The triggering nature of the CS can be clearly observed in experiments in which a direct stimulation of the cortex has been used as the CS. Doty (1961) and Grigoryan (1983) found that a foreleg flexion CR of a dog could be induced after conditioning by the stimulation of any chosen cortical point used as a CS, that is, independent of the nature of the response (alpha-response) elicited by the CS.

The triggering features of the CS are usually explicitly noted only in the context of classical conditioning where the CS is said to elicit (after learning) the conditioned response. Usually, the initial "neutrality" of the CS is emphasized and the observed response to the CS (alpha-response) has been considered a problem from the point of view of the experimental control procedures. Most often the alpha-response has been identified as an orienting response to a novel stimulation (CS), with an attempt being made in a typical conditioning experiment to habituate it off in order to prevent the expected experimental effect (CR) from becoming mixed with the changes of the CS defined as nonassociative. However, the CS may represent a triggering stimulus which has already acquired a capacity to elicit some CR. In such a case, the CS can be considered as a pre-existing, conditioned stimulus which not only is capable to elicit an unconditioned alpha response to the CS but is also able to "elicit" a conditioned response. Thus, the ability of the CS to act as a "truly" neutral stimulus should be regarded as theoretical in most classical conditioning arrangements; in fact, the experimenter should be prepared to wait for the occurrence of some "unexpected" responses to the CS.

The appearance of such an "alpha-response" could on some occasions be regarded analogous to the instrumental response (R) in instrumental conditioning. Although, from the point of view of the observer, the experimental subject in an instrumental conditioning situation seems simply to "emit" a definite response, it might in fact have been "elicited" by some external (or internal) stimulus. Konorski (1967, p. 358) used a denotation Sx "as an original factor provoking the movement M". Also Bindra (1972, p. 467) stated "that operant performance does not arise from "response reinforcement" but from the acquisition of a strong contingency between the incentive and the particular stimuli that evoke the operant response". On the basis of the evidence acquired in invertebrate studies (for a review, Hawkins & Kandel, 1984; Farley & Alkon, 1985), the function of the primary incentive stimulus (UCS) and the development of a conditioned incentive stimulus (CS) can correspond to the sensitization process. On the other hand, the response that has been elicited either passively or actively (e.g., a leg flexion of a dog induced either by the pulling of a string or by a mild shock to the leg) can also acquire signalling properties with regard to the following UCS (Miller and Konorski, 1928; Davydova, 1979).

Mackintosh (Mackintosh, 1974; Mackintosh & Dickinson, 1979) has formulated a model for the conditioning process according to which instrumental conditioning can be viewed as learning of a response (R) – reinforcer (UCS) association, analoguous to a stimulus – reinforcer association in classical conditioning. Most important in this kind of reasoning is that a response can be regarded to represent a central representation similar to that elicited by the CS in classical conditioning.

The role of the proprioceptive feedback from a response is obviously not an essential or even necessary condition for learning, although it may be an operating component of associative learning. This has been verified, for example, in curare experiments in which peripheral responses of the autonomic nervous system have been instrumentally conditioned (Miller, 1969). In addition, the studies using a CS applied directly on the cortex have shown that even peripheral afferent circuits are not needed for conditioning to occur (Loucks, 1936, 1938; Loucks & Gantt, 1938; Rutledge & Doty, 1955; Doty, Rutledge, & Larsen, 1956; Doty & Giurgea, 1961; Ellen & Powell, 1966; Hori, Toyohara, & Yoshii, 1970; VanDercar, Elster, & Schneiderman, 1970; Asdourian & Andrezik, 1971; Asdourian & Preston, 1971; Woody & Yarowsky, 1972; Khananashvili, Silakov, Zarkeshev, & Usova, 1977; Tsukahara, 1979, 1981a, 1981b, 1982; Grigoryan, 1983).

To summarize, in classical conditioning the stimulus (CS) properties have traditionally attracted the main interest; attempts have been made to extinguish (i.e., habituate off) the response (alpha) properties of the CS before the conditioning treatment. Razran (1971, p. 52) has suggested that this method cannot be recommended because, first, the alpha response is nevertheless dishabituated at the moment the UCR joins it, and, second, because some response to the CS is actually needed to effect the conditioning process.

In instrumental conditioning a quite opposite approach has been usual: the stimulus properties have been ignored, and the response (usually observable behavioral) has been of main interest. The acceptance of the assumption that there are no responses without stimuli (this question will be discussed in the next section) puts the instrumental response into a similar position as signalling events in the conditioning paradigm as the CSs: instrumental responses are elicited by some stimulus (internal or external) while, on the other hand, also a "neutral" conditioned stimulus elicits an alpha-type orienting response or a conditioned, pre-existing response, depending on its earlier acquired associations.

2.3 The nature of the instrumental response

2.3.1 Stimulus as a neural representation

In instrumental conditioning the usual procedure has been that the experimenter observes overt behavior and waits for the occurrence of some particular response, which thereafter is then selected as the instrumental criterion response. In many cases, this response seems to appear "spontaneously", that is, the experimenter rarely detects or has not even prepared to observe the stimulus conditions which might, in fact, have "elicited" the response.

Instead of paying too much attention to external stimuli or overt responses, it would be more important to emphasize the role of neural events in the brain accompanying such responses. It is evident that a stimulus is not perceived if it elicits no neural responses in the central nervous system (CNS), and, correspondingly, no overt response can be expected to occur without any preceding activity in the CNS.

However, as suggested above, it is also obvious that neither peripheral stimuli nor the behavioral execution of peripheral responses are necessary for conditioning. Rather convincing evidence for this conclusion has been accumulated from direct CNS stimulation experiments in which the overt appearance of peripheral skeletal responses has been blocked by the use of some paralysing agent, such as curare. Thus, a general definition of stimulus as proposed by, for example, Gray (1975, p. 2): ... "the physical energy change does not necessarily cause any alternation in subjects behavior (e.g., it may not have been noticed), and if it fails to do this it cannot be regarded as a stimulus", must be considered rather restricted, because only overt behavioral responses are then included.

Moreover, the changes in the CNS activity, elicited by some stimulus or stimulus condition, could also be defined as "responses". Emphasis on a central neural event as the main unit in an associative learning description could help to avoid many problems of definition which have been encountered when "stimulus" and "response" concepts have been utilized in a conditioning process description.

2.3.2 Are there real "spontaneous" responses?

The problem of spontaneity: responses without stimuli?

Razran (1971 p. 84) has stated that ... "stimuli do not exist for an organism until they are reacted to. Reactionless stimuli are even less tenable than stimulusless reactions". Further, Razran has suggested that even in the reinforcement design (instrumental conditioning) the antecedent reaction (R) is evoked by some existent stimulus (Razran, 1971).

Hearst (1975), referring to Konorski and Miller (1937) and Guthrie (1935), suggests that every behavior has its controlling stimuli, whether they can be identified or not. Furthermore, Hinde (1966) has suggested that there is no sharp dividing line between spontaneous and stimulus-elicited behavior. The execution of most responses inevitably produces some correlated change in external stimuli: pecking a key or pressing a lever is necessarily correlated with a close view of a key or a lever (Mackintosh & Dickinson, 1979).

Thus, every response (also instrumental, R) has an internal or external origin. The "spontaneity" concept in this context can be regarded as an indication of the apparently uncontrolled occurrence of the response rather than its origin.

Spontaneous responses during interstimulus interval (ISI) and intertrial interval (ITI)

The appearance of different, usually as conditioned defined, responses during the ISI of classical conditioning has occasioned different assumptions concerning their origin. Depending on the temporal position and nature of these responses, an attempt has been made to attach them either to the CS (i.e., directed to the CS), as in autoshaping, defined more broadly as "sign-tracking" by Hearst & Jenkins (1974), or to the UCS, defined as "goal-tracking" by Boakes (1977).

Simple time conditioning experiments with pigeons (Staddon & Simmelhag, 1971) have already revealed that upon the whole different responses may occur during stimulus intervals. In these experiments the grain in the food magazine was available every 12 seconds, independent of the behavior of the pigeon and without any external stimuli preceding the UCS delivery. The pigeons showed certain responses, such as jumping into the air, wing flapping and head and limb movements quite frequently throughout the early sessions of training. These responses were, however, later replaced by such activities as pecking at the wall or at the continuously unilluminated key. Staddon and Simmelhag (1971) called these latter activities "terminal" and the other, more variable activities "interim". Because interim responses occurred early in the training, then decreased and seldom occurred at the moment of the UCS delivery, their properties resembled the "preparatory" responses suggested by Konorski (1967). The temporal characteristics of the "terminal" response, that is, pecking behavior, can be paralleled with the properties of Konorski's consummatory activities, for they developed later and occurred in close conjunction with an expected UCS (Hearst, 1979, p. 46).

The development of "spontaneous" responses and the final form of the terminal responses during time conditioning have a close connection to the autoshaping phenomen. In autoshaping (the CS and UCS are presented as in classical conditioning using typically an ISI of 8 seconds), "instrumental", spontaneous responses seem to develop, although only a typical classical conditioning is arranged by the experimenter. As several observations seem to indicate, in autoshaping the UCS can usually be identified as the source of these "instrumental" responses: pigeons peck a lighted key (CS) in a similar way to the food-UCS showing species-specific approach responses typical to the UCS used. Because it is assumed that these "instrumental" responses quite often represent features elicited originally by the UCS, it could be asked to what degree these responses should be considered as classical, "real" CRs.

Wasserman (1979) has stated that, despite the formal similarity of autoshaping and classical conditioning (both involve the pairing of a neutral external stimulus with the presentation of a biologically significant stimulus, independently of the animal's responses to the stimulus), in autoshaping motor responses to a specific direction (to the CS) have been of specific interest. However, the directness of responses to the CS is not a sufficient basis for the distinction of the classical CR from autoshaping although it may be indicative concerning the origin of the autoshaping response. Pavlov (1934) had already observed a close correspondence between a classical CR (salivation) and motor responses. He found out that hungry animals went up to the lamp and licked it when a light stimulus was used as a CS. The CS in autoshaping seems to act as a signal of the UCS, even though the UCS itself does not elicit approach and contact movements in the same way as a heat lamp (Wasserman, 1979), positive brain stimulation (Peterson, Ackil, Frommer, & Hearst, 1972), or the delivery of water directly through a cannula into the beak acts as the UCS. These observations should indicate that a) the CS acts as if it were an UCS, and b) the UCS sensitizes typical, species-specific and pre-existing (learned) approach responses which appear as directed movements to the UCS and/or to the CS (a surrogate of the UCS).

The discussion whether CS-UCS or response-reinforcer association is more probable in autoshaping is not the main issue. More important is to discover whether the response occurring during ISI is instrumental (i.e., preparatory) with regard to the following UCS or whether it arises from the UCS, that is, to discover the determinant of the response R and the actual operative association which develops after the response R begins to occur in close conjunction (preceding) the UCS. Miller and Konorski (1928) had already discovered that, depending on the nature of the UCS (appetitive or aversive), the dog would start to lift its leg spontaneously or would inhibit the response, respectively.

One solution to the problem of the origin of these spontaneous, as species-specific, definable responses can be derived from the neurobiological data concerning the sensitization process in invertebrates (Hawkins & Kandel, 1984). The role of sensitization in response evocation and in conditioning, in general, will be discussed later in this paper.

The UCS as a source of species-specific responses

Anokhin (1974) suggested that a single UCS can be composed of several sequential impulses of different sensory receptors, so that in food UCS, for example, lingual receptors give tactile - thermal - chemical impulses at different latencies. Under proper conditions tactile impulses may become the signal (CS) for a forthcoming chemical stimulation of lingual receptors, and so forth. Food does not become a reinforcing factor because of its sensory qualities alone but in the background a series of sequentially developed phenomena exists which, as a result of phylogenesis, have become an "inherited", temporally "compressed" series. What was initially a slow sequence of events becomes in due course a rapidly and rather unconditionally occurring sequence of neural representations of these events. The UCS acts as a stimulus which signals through inborn (pre-existing) connections the real chemical terminal effect (food) on living cells in the organism, while the CS acts as a signal of the development of a new neural connection between the CS and the UCS. Thus, a single UCS such as food elicits a number of "unconditioned" activities which are directed to the terminal effect. "Both (of the usually used, that is, alimentary and defensive) 'unconditioned reflexes' are in fact highly developed unconditioned activities involving a vast number of apparatuses and mechanisms which develop successively until the full realization of the adaptive end effect" (Anokhin, 1974, p. 26).

The effect of an unconditioned stimulus, in addition to its own specific effects, could most properly be described as a nonspecific sensitizing influence on the UCS-pathway system. This sensitization elicits different inherited and learned species-specific responses.

The separation of one learned component of the UCR complex has been demonstrated in an experiment in which the attempt of Aplysia to bite or swallow food was hindered by wrapping the food in a plastic net. Because the net-enclosed food cannot be swallowed, it was rejected and the animal eventually began to stop responding to food, that is, the contact of food to lips of Aplysia became a negative CS (Susswein & Schwarz, 1983).

Culler (1938), for example, described the variety of unconditioned responses observed in dogs during the first shock: quick gasping, yelping, a hasty withdrawal of the foot, adduction of the tail, whining, barking, biting, snapping, twisting, jerking and occasional evacuation. In addition to these inborn response chains, there are several earlier learned responses which become intermingled with this sequence during the lifetime of each individual. The dependence of the unconditioned response on external stimulus conditions has been shown in several connections. Even the direct rewarding brain stimulation effect can cause variation in responding if the environmental cues are changed (Valenstein, Cox, & Kakolewski, 1968).

An understanding of the multiple nature of the UCR becomes essential when an attempt is made to relate the nature of the conditioned response to the complex of the responses representing the effector system which becomes elicited by a given UCS.

2.3.3 The role of the R as an element of an association

Defining classical and instrumental conditioning.

In line with the Pavlovian tradition, Konorski (1967) assumed that in classical conditioning connections between the CS and UCS are formed in the brain between central representations of these stimuli. This view still represents the main theoretical account of classical conditioning to this day (Mackintosh, 1974; Dickinson & Mackintosh, 1978).

In instrumental conditioning the experimenter arranges the presentation of a reinforcer contingent on the occurrence of a particular response. The degree of the level of control of the "emitted" response can vary from a "free operant" to a passively elicited movement (i.e., mechanically lifting the dog's leg as an instrumental response) in the presence of some discriminative stimulus (as used by Miller and Konorski in an early experiment in 1928). Accordingly, the response R can be treated as a CS and the operative associative mechanism is then composed of the response (R) and the reinforcing stimulus, that is, the UCS ("response-reinforcer" association; Mackintosh & Dickinson, 1979). How far a distinction between classical and instrumental associations can be maintained thus largely depends on how the response R is defined.

The development of response-reinforcer association.

The unintentional reinforcement of occasional responses (e.g., food approach) during conditioning has been termed in some contexts "parasitic" instrumental conditioning (Konorski, 1967), or "superstitious" behavior (Skinner, 1948). More precisely, Konorski (1973) describes spontaneous responses which are elicited by environmental stimuli and maintained through an association to the UCS. Konorski states that "it should be noted that in the first stage of CR training the animal performs the instrumental movement not only to the CS but also during the intertrial intervals. This means that the CR is first formed to the environmental compound stimulus". Such "parasitic" responses can be regarded as examples of responses which are "elicited" either as responses to "old", pre-existing conditioned stimuli or as responses representing the typical inherited response repertoire of each species.

Bindra (1976, p. 245) suggested that the primary force behind the appearance of certain responses is the motivational state created by the reinforcing stimulus (UCS). This motivational state makes some responses eliciting stimuli highly potent, leading to the formation of a learned contingency between the response-eliciting stimulus and incentive (motivational, unconditioned) stimulus through a stimulus-stimulus association. This suggestion has remarkable similarities to the "species-specific" response concept suggested by Bolles (1970, 1972a, 1972b). The species-specific responses thus represent responses which have a highly inherently determined priority to occur as the most probable "spontaneous" responses during the ISI.

The occurrence of parasitic responses (Konorski, 1973), the motivational view (Bindra, 1976) and the "species-specific" response concept (Bolles, 1970) come close to the suggestion of sensitization as the primary force eliciting pre-existing responses as a result of increased sensitivity of many CS-pathways in the nervous system. The significance of sensitization in associative learning and its neurobiological basis have recently been emphasized in invertebrate studies (e.g., Hawkins & Kandel, 1984).

As suggested above, the controlled "emission" of the response R can also be induced "unconditionally" using passive or active "elicitation" of this response. The introduction of the CS can be regarded as an equal procedure in classical conditioning: it represents a controlled emission of a response. This response has been defined as an orienting response or an "alpha"-response.

Close to these lines of reasoning has become also Razran (1971, p. 105) when suggesting a "new look" at the comparison of classical and instrumental conditioning. An important feature of his proposition was that also in instrumental conditioning the response R is considered as being evoked by some existent stimulus which corresponds to the CS in the classical design.

Responses as classical CSs

If the aim is to parallel the kind of response-reinforcer association as referred to above with the stimulus-reinforcer association, it should be possible to show that a response shares properties similar to those of a stimulus in its ability to act as the signalling part of paired stimuli. Mackintosh and Dickinson (1979) have argued that there is substantial evidence establishing the ability of the response to act as a cue in a similar way as the CS. For example, the rats can use differences between such patterns of behavior as washing, rearing or scratching as a cue to signal which of several alternative responses will be reinforced (Beninger, Kendall, & Vanderwolf, 1974).

Mackintosh and Dickinson (1979) conclude that if an animal's own responses can serve as discriminative stimuli signalling reinforcement, then it would be possible for them to act as CSs eliciting classical CRs. Miller and Konorski (1928) first reported this effect: during the course of instrumental conditioning of the leg flexion response for food reward, they observed that the performance of the response was accompanied by salivation. The leg flexion response thus acted as if it were a classical CS. In fact, a close temporal parallel existed between the initiation of active flexion responses (i.e., successful instrumental conditioning) and the first appearance of a classical CR correlated with the response. A similar function of response disappearance was observed during extinction. Davydova (1979) has later made similar observations.

Thus far, according to the view of Mackintosh and Dickinson (1979), it might be relatively safe to conclude that the response-reinforcer association can be parallelled to the stimulus-reinforcer association because both the CS and R can acquire the property of eliciting a CR similar to the response normally elicited by the actual reinforcer. In the classical conditioning paradigm this association is sufficient to explain the observed change in the organism's behavior. As Mackintosh and Dickinson (1979) state, however, the parallel association between the response and reinforcer does not directly produce the behavior for which an explanation is desired in instrumental conditioning (i.e., the increase or decrease in the rate of the instrumental response). "Once the dog has flexed his leg, he will perhaps salivate if the flexion response has been associated with food, but what we need to explain is why he flexes his leg" (Mackintosh & Dickinson, 1979, p. 162).

It is true that in instrumental conditioning a change in the frequency of the occurrence of a definite response R is observed, but such a change does not necessarily indicate anything concerning associative learning itself. In order to explain the observed change in behavior (increase or decrease in response frequency), the nature and intensity of the UCS are obviously the most important determinants of the response evocation. Instead, if the idenfication of some associative learning within a given instrumental conditioning arrangement is sought, the frequency measurement of the R does not indicate that phenomenon; the response R should not be considered as a CR but as a "CS" which may become able to elicit some true CR.

Autoshaping experiments could be regarded as examples of associative learning in which the response pattern initially elicited by the UCS also becomes, through the CS-UCS association, elicited by the CS. After the CS-UCS association has been developed, another operative associative mechanism also becomes possible: the response-reinforcer association. Providing that the response has enough "discriminative" properties (from the point of the CNS), it can act as a "CS". If this association develops, then the CS in a discriminative type of paradigm (Sd - R - Sr) may become redundant and could possibly be eliminated completely: the animal thereafter triggers its own trials. Such a process has been exemplified in the experiments of Konorski (1967): leg flexions (which begin to occur "spontaneously") begin to elicit salivation CRs.

2.3.4 Sensitization as a basic process in conditioning and its relationship to instrumental conditioning

Dykman (1976) has emphasized the neglected role of the sensitization process in explanations of conditioning. He has argued that there is no valid basis for considering sensitization and conditioning as separate processes. Dykman (1976) has proposed a theory of learning in which sensitization is the basic construct. The starting point in his theory is that stimuli (CS and UCS) have a mutual interference and/or augmentation so that the properties dominant in either stimulus will become augmented. Dykman (1976) has supposed that the properties of developing CR are determined by both the UCS and CS, depending on which of these stimuli has dominant properties. Thus, they reflect either alpha-response or UCR properties, or some compound of these effects. It follows then that the CR is rarely completely literal substitute for the UCR because, a) the CS usually has some dominant properties of its own and is thus influencing the form of the CR and, b) the sensitization effect of the CS on the UCS-pathway seldom reaches the intensity of the effect achieved by the direct excitatory effect of the UCS. Although these conclusions concerning the nature of the developing CR might be plausible, the concept of dominance as a determinant of the nature of the CR is not needed if the changes in the appearance of the alpha-response, and the classical CR are considered as differentiable events. If only behavioral measures are used, there remains a problem of defining the exact moment of the occurrence of the CR. Hence it follows that the alpha- and "true" conditioned response features are usually confounded.

The most interesting part of Dykman's theory is the concept of mutual interference and augmentation of the CS and the UCS. The sensitizing effect of the UCS with regard to the CS as a neurobiological process has been verified in invertebrate studies (see, Kandel & Schwartz, 1982; Hawkins & Kandel, 1984), which have provided a new neuroanatomical, physiological and neurochemical basis for a description of sensitization. Dykman's theory does not, however, pay specific attention to the sensitizing effect of the CS-pathway on the UCS-pathway, although he acknowledges the importance of the possibility of mutual augmentation. He has in fact started from the assumption that the balance between the dominant properties of the CS and UCS determines the power of the CS to act as a sensitizing stimulus with regard to the following UCS.

However, the possibility that the CS could sensitize after paired presentations with the UCS the UCS-pathway system would appear essential for true associative learning (classical conditioning). Young, Cegavske and Thompson (1976), for example, have found that the excitability of motoneurons in the abducens nucleus (the final common path for the nictitating membrane response in the rabbit) was enhanced by the sounding of a brief tone. This experiment also demonstrates a) that a CS can have sensitizing effects on the UCS and, b) that there are also pre-existing connections between sensory and motor pathways. Studies in invertebrates seem to indicate that learning is based on a modification of a "hard-wired" pre-existing nervous network. The specific constraints the prewiring concept sets for an explanation of conditioned alpha and "true" CRs are discussed later in this paper.

An important additional feature necessary for the development of a classically conditioned response is that of a temporal specificity or temporal order of the CS and UCS. Several recent experiments have suggested that no real "backward" conditioning can occur in a well-controlled experiment, thus indicating that a temporal order of the CS and UCS is one critical property for true associative learning. The mutual interference/augmentation principle suggested by Dykman (1976) includes a directionality hypothesis and actually joins (in one CR) the results of different phases of conditioning: the dominant stimulus (CS or UCS) determines the form of the developing CR. On the basis of invertebrate experiments, on the other hand, it could be suggested that the development of the alpha-response and the development of the true classical CR may represent different but connected phases of neural learning. However, Dykman's suggestion of mutual interference and/or augmentation can be useful in describing the way through which the CS elicits the CR providing that the CS has acquired during conditioning sensitizing properties with regard to the UCS. The assumption of complete bidirectionality can obviously be refuted on the basis of the determined order of electrophysiological and biochemical events necessary for associative learning (see Kandel & Schwartz, 1982). This constraint excludes, of course, the possibility of real "backward" conditioning and is supported by recent observations acquired in both invertebrate and vertebrate (e.g., eye-lid and nictitating membrane) experiments (Tsukahara, 1982; Voronin & Markevich, 1982; Woody, Kim & Berthier, 1983).

2.4 The nature of the conditioned response

Different responses appearing during the ISI of classical conditioning could be defined as either "real" CRs or, on the other hand "spontaneous", "parasitic" (Konorski, 1967), "preparatory" (Konorski, 1967), "sign-tracking" (Hearst and Jenkins, 1974) or "interim" (Staddon & Simmelhag, 1971) responses depending on their nature (compared to the UCR) or on their temporal location during the conditioning paradigm (short or long latency). The reason for such a dichotomy obviously stems from the observation that some of these responses resemble more closely the UCR and some less. Responses appearing temporally close to the UCS and directed to it have been called ”consummatory” (Konorski, 1967), ”terminal” (Staddon & Simmelhag, 1971), ”respondent” (Skinner, 1938), ”goal-tracking” (Boakes, 1977), or more traditionally, ”substitution” CRs, although no specific temporal constraint for the location of the substitution CR has usually been defined.

As these examples show, the classification of responses occurring during ISI has been based on the different properties of these responses. The apparent functional nature of the response, its directionality, or its topography (time-amplitude course) can be taken as determining in which category the observed response should be classified.

After a short recapitulation of the two traditional views of the nature of the CR, a list of different types of criteria for a basic classification is given below.

2.4.1 Stimulus substitution view

The stimulus-substitution view, from the point of the neural pathway, includes an assumption that the CS and UCS have a common final pathway or that if they are separate, a site of convergence exists at some level of this pathway. Earlier theories assumed that this interaction occurred at the cortical level (Pavlov, 1928), but some recent findings seem to indicate that this convergence may be located at least in long latency CRs within the phylogenetically lower parts of the vertebrate nervous system. These studies seem to suggest that one possible site of convergence for a simple reflex (eye-lid response) is at the level of the brain stem and cerebellum and can be located in a relatively restricted area (McCormick & Thompson, 1984). The assumption of neuroanatomical convergence in the vertebrate nervous system is also supported by the recent findings of Tsukahara (1981, 1982) in the conditioning of a forelimb flexion response in the cat. A rather similar localization of conditioned reflex coupling was already suggested in earlier EEG studies (Fessard & Gastaut, 1958; Gastaut, 1958) and in the experiments of Anokhin (1961), who showed that very rapid changes occurred in respiratory components of the CR, thus revealing the participation of brain stem structures in the process of coupling.

Pavlov’s original account was that an initially indifferent stimulus, the CS, comes to elicit the response belonging to another stimulus, the UCS: one stimulus becomes a substitute for another. Konorski (1967, p. 303) stated that ”the classical conditioning is merely the formation of associations between neutral stimuli and biologically significant stimuli — those which give rise to overt unconditioned responses”. Furthermore,

... "in these conditions the neutral stimulus acquires the capacity of eliciting the same response as the UCS", and ... "thus by definition, the classical CR comprises only those effects which are elicited by the reinforcing agent". Konorski (1967, p. 269) assumed that the possible observed difference between the form of the CR and the UCR may hinge on how the UCS is presented.

Similarly, Anokhin (1974, p. 25) has defined the nature of the CR: "What is significant about the nature of the conditioned reflex is its relation to the unconditioned reflex, which is the invariable basis for the formation of the conditioned connection". A stricter interpretation of the nature of the CR is suggested by Anokhin (1974, p. 26) in his treatment of vegetative activity: "Unconditioned reflexes connected with any kind of vegetative activity comprise a special group. In this case there is no defense activity in its true sense. Rather, a type of vegetative activity originally evoked by an appropriate unconditioned stimulus is reproduced in the form of a conditioned reflex".

2.4.2 "Law of effect" view

As an alternative possibility to the substitution type CR development there is the view according to which the UCS functions as a response strengthener in classical conditioning. Hearst (1979) has differentiated two versions of this theme: response-reinforcer contiguity and response-reinforcer contingency views.

According to the response-reinforcer contiguity view, any response occurring during the CS or closely followed by the drive-reducing event, such as food delivery or pain termination, will be strengthened. Because the UCS evokes specific UCRs, the UCS increases the likelihood that especially responses identical with or resembling UCRs will occur and which may become the behaviors strengthened in this manner ("parasitic", "superstitious", instrumental responses can be counted as belonging to this category). This hypothesis does not, however, include any specific constraint for the nature of the CR to be learned; the role of the UCS is only that of inducing a motivating state, the reduction of which "strengthens" the preceding response (which is then defined as the CR). Furthermore, this view does not include any assumption concerning the possible modifying (i.e., preparatory) effect of the CR with regard to the following UCS. A useful feature of the contiguity view is that it treats the UCS as a motivating stimulus which can be assumed to act as a possible sensitizing source for the elicitation of "instrumental" (e.g., species-specific) responses during the ISI. It is not completely clear if the motivating and sensitizing property of the UCS can be made to correspond. The experiments of direct stimulation of the CNS, for example, seem to indicate that an assessment of the motivational properties of the UCS (e.g., cortical UCS inducing leg movement or eyeblink) is beoynd our present knowledge.

The sensitizing effect of the UCS in the contiguity view thus offers an explanation for why a definite kind of response (interpreted as the CR) may occur during learning, but it does not explain the operative associative mechanism in each case. There are in fact two possibilities for this purpose: a) no new responses are learned; the UCS simply elicits (through sensitization) either pre-existing CRs or inherited, species-specific responses, or b) some association develops between the response (R) and the reinforcer (UCS).

As shown in the preceding section, peripheral, overt components of the response R are not necessary for associative learning; the response-reinforcer association can be parallelled with the development of the classical CR as an association of central neural representations of these events.

The response-reinforcer contingency view of classical conditioning, includes the assumption that the CR definitely modifies the UCS. Mackintosh (1974) has termed this assumption a "response-shaping" hypothesis. In this hypothesis it is presumed that the CR occurs in close temporal contiguity with the UCS and precedes it. In contrast to the development of the stimulus-reinforcer association (substitution CR), the response-shaping hypothesis is incomplete in that there is no a priori way to predict which of the response candidates will become the CR.

Thus, if the CRs are reinforced according to the law of effect ("response-shaping"), it follows that there are relatively few constraints on their nature. The specification of which CR should emerge is at best intuitive and most often provided only after the fact (Mackintosh, 1974, p. 100). As Gray (1975 p. 43–44) puts it: "How can a response be instrumentally reinforced by preparation for a stimulus which is actually never followed". By contrast, the stimulus-substitution view allows explicit predictions about the relationship between the CR and the UCR.

2.4.3 Substitution vs. response-shaping views

As stated above, according to the stimulus substitution view, the CS acquires an ability to evoke some behavioral effects originally elicited by the UCS. This approach does not imply that the CR is actually strengthened by its consequences; the UCS does not act to "reinforce" prior responses, but to produce a set of responses (UCRs) that can be transferred to its substitute, the CS (Hearst, 1979).

Razran (1971, p. 184) has suggested that classical conditioning does not include reinforcement conditioning, since no "strengthening of the existing modified reactions may be disclosed in its typical paradigm. Statements that classical conditioning include reinforcement conditioning stem from theoretical analogies and not empirical evidence".

A kind of intermediate view of substitution and response-shaping views is represented in the "two-stage" CR hypothesis. Culler (1938) offered an interpretation that the CR appears as a copy of the UCS at the beginning of training but gradually develops into a different (preparatory) response.

A similar two-stage analysis was given by Maier and Schneierla (1942), who suggested that during the initial stage of conditioning stimulus contiguity was necessary for the development of an S-S association, which they termed "sensory integration". Conditioning may involve a second stage in which, through selective learning, the CR becomes increasingly specific the more effectively it leads to reward or attempts to escape punishment. They assumed that classical conditioning might also include a second stage, but they did not specify circumstances during which the "law of effect" could occur. These two-stage hypotheses are interpreted to represent classical-instrumental dimension while, in fact, responses of different origin might have been observed.

Both intermediate views can be more easily explained if the possibility of an additional response-reinforcer association is regarded as a further development of the original CS-UCS association. As a result of this development, the control of the initial CS (i.e., a CS-UCS association) is gradually transferred to the response R (i.e., the R-UCS association); the animal thereafter controls its own "trials".

2.4.4 Anticipatory CR concept

Anokhin (1974, p. 236) assumed that during the organization of a chain of stimuli connected with signalling principles, the impulses propagate over the brain from point to point much more rapidly than the actual external stimuli sequentially appear. The impulses anticipate the actual stimulus which will be acting on the central nervous system and arrive at those regions of the cortex which they are to excite. "This is how the concept of the adaptive role of impulses we named 'ancipatory impulses' came to be" (Anokhin, 1974, p. 236). The anticipatory reflection of reality parallels the sequential course of external events (Anokhin, 1974, p. 24). Anokhin (1974, p. 245) concluded that "it is evident that the formation of the action acceptor during the dispatch of the efferent impulses to the periphery can be accomplished only if the action acceptor contains an exact copy of the command to act sent by the efferent impulses to the periphery". "The action acceptor" (= "the acceptor of the results of action" = "anticipatory excitation" = "preparatory excitation" = equivalent of the goal, Anokhin, 1973, 1974) is defined as one kind of "copy" of the efferent unconditioned activity now elicited by a signalling stimulus, and the anticipatory nature of the CR becomes actualized as the compressed time scale between the signalling and unconditioned stimuli.

Kupferman (1981 p. 806) has defined the relationship of the conditioned and anticipatory CR concepts by the following: "When the conditional stimulus is repeatedly followed by the unconditional stimulus, the conditional stimulus comes to elicit responses (conditioned responses) that resemble the unconditioned responses. It is as if the conditional stimulus becomes an anticipatory signal for the occurrence of the unconditional stimulus, and the animal responds as if anticipating the occurrence of the unconditioned stimulus". These examples emphasize the point of view that the concept of anticipation is not necessarily synonymous with a functionally preparatory CR concept, because the development of the conditioned response is not dependent on its possible temporal occurrence before the UCS and on its possible modifying effects on the UCR.

2.4.5 Conditioned response and some criteria for an evaluation of its nature

In order to elaborate the evaluation of the nature of the CR, an attempt is made here to formulate some criteria. First, it may appear important to distinguish the topographical and latency features of the observed CR for a comparison of the similarity of the CR and UCR and their temporal relations. In addition, a separation of the time-amplitude course of short latency alpha and true conditioned response becomes at least in principle possible if the ISI is chosen sufficiently long.

Second, concomitant changes in neural activity can be used in comparing neural learning to the development of the overt behavioral response. This procedure is sensitive for an indication of "latent" neural learning. Another way to measure latent learning is the "test-stimulus" approach used by Walters, Carew, and Kandel (1979, 1981) in invertebrate studies. Moreover, the use of direct neural stimulation as the CS and/or UCS allows an assessment of the necessity of afferent and efferent circuits for learning.

Third, some neuroanatomical evidence of possible sites of neural convergence of CS- and UCS-pathway systems has been amassed relatively recently. Using combined neural multiple-unit recording, lesion and stimulation techniques, McCormick and Thompson (1984) have identified some rather limited regions in the brain stem and cerebellum as possible sites of the convergence of a simple reflex. Tsukahara (1981, 1982) has reported similar results in cats. In addition to these vertebrate results, more exact neuroanatomical and neurochemical data of convergence is available now, collected in several invertebrate studies (e.g., Hawkins &

Kandel, 1984). Invertebrate studies have thus far demonstrated only pairing specific short latency learning while in vertebrates the time interval learning and hence the long latency CR development has been verified. An assumption of a convergence of CS and UCS pathways in relation to a short-latency CR do necessarily disclose a possibility for interval learning or development of a CR which represents some typical features of the unconditioned response

Fourth, one group of criteria for the nature of the CR can be based on imaginative functionalistic explanations in which different, as adaptive, described functions, are adhered post hoc to the CR. Although the setting of an a priori criterion for the preparatory CR is difficult, even impossible, there are some ways of testing the observed CR, treating it as if it was preparatory with regard to the following UCS.

The significance of the topographical features of the conditioned response in the evaluation of the nature of the CR

In attempting to evaluate the appropriateness of either substitution or response-shaping views in an effort to explain the conditioning process, the topography of the CR may provide essential information of the basic neural associative process behind conditioning.

Mackintosh (1974, p. 64) states: "A response shaping interpretation of optimal ISI implies that conditioning will not occur at very short ISIs because the CR cannot occur before the onset of the UCS and cannot be reinforced (in the case of aversive UCS) by attenuating its effects."

In nictitating membrane (NM) experiments the minimum latency of the NM-response is 70–80 ms, while the average is over 100 ms. Smith, Coleman and Gormezano (1969) obtained conditioning using a 100 ms ISI, and Patterson (1970) observed conditioning at an ISI of 50 ms using an inferior colliculus stimulation as the CS. Highly significant conditioning occurred in these experiments, although most CRs were initiated after the onset of the UCS and according to the preparatory interpretation would therefore not have been reinforced at all (Mackintosh, 1974, p. 64).

Recent NM-experiments in which the multiple-unit neural activity of several brain structures has been recorded during classical conditioning have shown that neural changes can appear first in the unconditioned (UCS) period and later in the conditioned stimulus (CS) period (Berger, Laham & Thompson, 1980; Berger, Rinaldi, Weisz, & Thompson, 1983). While the peak latency of the conditioned NM response seems to follow the location of the UCS (i.e., CS-UCS interval), the onset latency decreases systematically away from the UCS toward the CS onset (Gormezano, Kehoe & Marshall, 1983). This means that the CR-UCS overlap decreases, contrary to expectations formulated according to the response-shaping hypothesis. Moreover, the temporal model of the conditioned neural response is very similar to the UCR in some brain sites (especially in the hippocampus). The experiments (Lavond, Clark, Holmes, & Thompson, 1981; McCormick, Clark, Lavond & Thompson, 1982; McCormick, Guyer & Thompson, 1982; Lincoln, McCormick & Thompson, 1982; Thompson et al., 1983; McCormick & Thompson, 1984) have shown that critical structures for the development of the conditioned NM-response are located in the pontine reticular formation and in the cerebellum. Lesions in these structures cause the disappearance of the conditioned NM-response but do not severe the unconditioned NM-response.

Cerebellar recordings in nictitating membrane conditioning studies have shown that the CR is strongly controlled by the CS-UCS interval in terms of onset latency and temporal morphology, and is always timed to be at maximum when the onset of the UCR occurs (Thompson et al., 1983, 1984b).

Neural CR developing before an overt behavioral response

In nictitating membrane conditioning studies the pyramidal neurons of the hippocampus of the rabbit develop a clear model of the behavioral response long before the behavioral CR itself appears, and this learned change invariably precedes (appears in earlier trials than the behavioral response) and accurately predicts subsequent behavioral learning performance (Thompson et al., 1983).

Additional evidence of this type of "latent" learning in the neural system has been acquired in some invertebrate experiments based on a "test-stimulus" approach. This method includes the use of another UCS-type test-stimulus (e.g., tail shock) to trigger different behaviors after conditioning (Walters, Carew, & Kandel, 1979, 1981). A test stimulus can reveal that some learning has occurred, even though no peripheral responses have yet appeared. Second, the test stimulus exposes a group of response pathways that represent inherited or learned complex effects adjoining to the UCS-type used. Third, the test-stimulus indicates that such a latent learning is a result of the sensitizing effect of the CS on the UCS-pathways. A verification of latent neural learning has important implications from the point of view of the interpretation of the nature of the CR. The preparatory hypothesis, concerning the nature of the CR, becomes rather difficult to defend: how can the animal learn a CR which has not at all appeared as an overt response and which can thus have no preparatory functions? It could, of course, be argued that the assumed instrumental effect of the CR is mediated centrally, but it could then be asked, how a mediation phenomen can be conceptualized among brain processes: what "mediates" what?

Direct stimulation of the CNS.

The similarity between the CR and UCR has been reported in studies in which a direct stimulation of the cortex has been used as an UCS. Giurgea (1953, 1955) and Doty and Giurgea (1961) showed that it was possible to use a direct stimulation of the motor cortex as the UCS which, after pairing with the CS, elicited a behavioral CR very similar to the UCR (head turning, limb movement etc.). Doty (1961) observed that during extinction these CRs were occasionally so identical with the UCR that the experimenter jumped to check equipment, thinking the postcruciate gyrus (where the UCS was applied in this experiment) had been stimulated through error. Brodgen and Gantt (1937) reported earlier similar observations using a cerebellar stimulation as the UCS.

Later, Mis, Gormezano and Harvey (1979) showed that direct abducens nucleus stimulation (eliciting an unconditioned nictitating membrane response) could be used as the UCS. A paired representation of the tone-CS and brain stimulation UCS yielded a conditioned response (nictitating membrane closure), while the unpaired control group failed to show associative learning.

Further, a recent experiment of Gonzalez-Lima and Scheich (1984) demonstrated that in rats a) direct midbrain reticular formation stimulation could act as the UCS (eliciting bradycardia in the heart rate and an increase in 2-deoxyglucose (2-DG) uptake as the UCR), and b) the developing conditioned response was similarly bradycardia and an increase in 2-DG uptake. CS-alone, UCS-alone, backward conditioning or unpaired controls showed no similar changes.

Testing the instrumentality of the CR

As stated above, the "preparatory" CRs according to the response-shaping hypothesis include an assumption that the CR necessarily precedes (or coincides with) the UCS in time.

One common argument has been that the CR succeeds in reducing the aversiveness of the UCS (eye closure from an air-puff, salivation from dryness of food). A number of researchers (e.g., Culler, 1938; Prokasy, 1965, 1984; Perkins, 1968) have argued that all classically conditioned responses may be reinforced in this way. "But the fact that some responses may be reinforced by their consequences in classical conditioning experiments does not imply that all CRs are strengthened by response-contingent reinforcement" (Mackintosh, 1974, p. 113). Furthermore, Mackintosh (1974, p. 114) states that "the fact that some CRs are adaptive is hardly surprising: most biological systems share this property". According to Mackintosh (1974, p. 114), a clear prediction on the basis of the response-shaping hypothesis would be that the probability of the CR will be affected by a contingency between the CR and UCS.

For example, if a flexion response of a leg takes place because of some reduction in the aversive effect of the following shock (response-shaping view), then the non-delivery of the shock-UCS (as it occurs in the avoidance situation) would be a still bigger instrumental reinforcement, and in the next trial, the flexion should be stronger or at least not weaker. Coleman (1975) examined the effects of CR-contingent decrements of the amperage of a shock UCS, using four different groups which all received the 5 mA shock if a CR did not occur on a trial. Three of these groups received a graded UCS intensity (0, 1.7, and 3.3 mA), while the fourth group received the 5 mA shock whether or not a CR occurred and served as a traditional classical conditioning group. Results showed that the completely adaptive group (if a CR, then no shock) also showed a CR acquisition almost identical to the other groups. In addition, the results of the second experiment (Coleman, 1975) in which the UCS intensity was varied according to the CR performance (increased, maintained at the same level, or decreased for different groups, respectively) showed, just as the first experiment, that the CR performance increased according to the UCS intensity, not according to the different adaptive contingency. The direction of these changes was clearly opposite to that expected from the preparatory hypothesis. More exactly, these experiments indicate that a reduction of the physical intensity of the aversive UCS is not reinforcing at all.

The possible instrumentally adaptive (preparatory) nature of the CR with regard to the following UCS can also be tested using an omission schedule in classical conditioning. On the omission schedule, the CS may initially signal reinforcement quite consistently, but as soon as CRs start to occur the probability of the UCS declines and the animal is essentially on a partial reinforcement schedule. If, for example, Pavlov's dogs salivated because they learned that salivation improves the taste of dry food (an instrumental, response-reinforcer relationship), the implication is that they could equally well have learned not to salivate if this had been the only way of obtaining food (Dickinson & Mackintosh, 1978, p. 588). However, as experimental evidence shows, dogs continue salivation even when salivation costs them food (Sheffield, 1965), pigeons will continue pecking although pecking causes the omission of food (Lucas, 1975; Woodard, Ballinger, & Bitterman, 1974), and rats may continue to make contact with a lever (Stiers & Silberberg, 1974). Most important, however, is the fact that animals may show a reliable acquisition of the CR when trained on a omission schedule from the outset of the experiment (in pigeons: Schwartz & Williams, 1972; Williams & Williams, 1969, jaw movements by rabbits: Gormezano & Hiller, 1972; licking in rats: Patten

& Rudy, 1967). The results of an experiment of Soltysik and Jaworska (1962) showed that an omission of the UCS weakened the flexion response (increased its latency) as it could be predicted on the basis of the stimulus substitution assumption (Gray, 1975).

One way of studying the possible instrumental role of a CR with regard to the following UCS has been through the blocking of the appearance of the CR by the paralyzing agent, curare. Black (1975) described a series of experiments in which curarized dogs were presented with the classical conditioning sequence of stimuli: a tone-CS followed by a shock-UCS. The animals acquired a conditioned heart rate response (acceleration) to the tone, although the EMG recordings showed a complete paralysis of skeletal muscles. Gray (1975, p. 35) suggests that it is very difficult to see how a paralyzed animal could alter the sensory effects of shock, and how the conditioned change in the heart rate itself could have such an effect.

Similarly, nictitating membrane conditioning experiments offer examples of classical CRs which obviously cannot act as "preparatory" with regard to the following UCS. In these experiments an air-puff (an aversive UCS) is delivered to temporal region of the cornea. Instead, the nictitating membrane extends from the inner canthus of the eye and rarely covers the midline of the pupil, thus leaving the temporal portion of the receptor surface of the cornea exposed (Gormezano, 1965).

2.5 Summary and conclusions

In order to make conclusions of the nature of the CR, the actual operating mechanism for associative learning in the nervous system should be identified. Neuroanatomical evidence acquired recently, mainly in invertebrate but also in vertebrate studies, suggests the possibility of an actual convergence of relevant pathways (CS and UCS) at some level of the nervous system.

Second, the UCS obviously elicits specific unconditioned responses and simultaneously widely sensitizes other relevant (to the UCS used) parts of the nervous system. Such non-associative sensitization might be regarded as a significant source for different, traditionally instrumental or preparatory classified, responses and, in addition, as a form of non-associative learning: the learning of conditioned alpha responses.

On the other hand, as suggested above, quite conclusive evidence exists which favours an associative learning paradigm in which the temporal proximity and definite order of two stimuli (the actual central representations of these stimuli) is assumed as the only operative associative mechanism. The close correspondence of the temporal occurrence of the UCS and CR indicates the presence of a neural timing mechanism which controls the temporal features of the conditioned response topography. The data which have shown that neural learning develops in earlier trials, well before the behavioral CR, and that the topography of the developing CR closely predicts the features of the behavioral CR, indicate that the nature of the CR is closely related to the properties of the UCS effector system. The evidence of latent neural learning, direct manipulation of suggested instrumental properties of the CR, and omission experiments seem to show rather strongly that the instrumental, preparatory type CR hypothesis is hard to defend, at least on empirical grounds: the CR does not simply obey the Law of Effect.

2.5.1 Classical-instrumental relationships

A traditional way of separating classical and instrumental conditioning as different learning processes has been to claim that the CR in classical conditioning bears at least a broad similarity to the UCR, while in instrumental conditioning the response R (thus paralleled with the CR) need have no fixed relationship to the UCR. For example, bar pressing for a food reward does not resemble responses to food. Although bar-pressing is obviously not a component of the UCR complex to food, it might be closely related to food approach behavior which represents one component of the UCS effect.

In addition, the use of such an UCS as food, for example, yields some problems in attempts to define temporal characteristics of unconditioned and conditioned responses. Other types of UCSs, such as the direct stimulation of brain structures or aversive stimuli, in general represent a much more immediate effect on the CNS. Furthermore, the use of such stimuli makes possible the use of very short interstimulus interval paradigms, which minimize the possibility of the intervention of other responses during the ISI. The identification of these other responses, that is, "instrumental" or those interpreted as "preparatory", appearing during the ISI is important for a comparison of operative associative mechanisms in the classical and instrumental conditioning paradigms.

As referred to above, the UCS seems to have a dual role: First, it elicits a definite UCR which, at least in more developed animals, is in fact usually a complex of many responses. Responses belonging to this effector system are either inherited responses which have developed through evolution (such as food ingestion, swallowing, even salivation and different neurosecretory responses), or, the during ontogeny of each individual, acquired, learned responses which precede and act as conditioned signals of the following UCS (a response-reinforcer association, as Mackintosh, 1974, 1979 has suggested).

Second, the UCS acts as a sensitizer of relevant existing stimulus pathways in the CNS. This means that in typical classical conditioning the

UCS, through its sensitizing power, increases sensitivity to stimuli in the temporal proximity of the UCS, including the possible CS-pathway which, instead of habituation to repetitive stimuli, may increase its sensitivity (i.e., show orienting or alpha-responses) on trials following the UCS experience. As a result of this sensitizing effect the animal is likely to produce responses ("instrumental") which are specific to that UCS.

Hence, if an aversive UCS is used, in all probability different "species-specific defense responses", as Bolles (1972) calls them, can be observed. Correspondingly, responses appearing in the proximity of the appetitive UCSs would be called "species-specific approach responses". Common to both these responses is that they are possible candidates for an "instrumental" or "preparatory" response appearing in the interstimulus interval of classical conditioning. As should be evident, however, none of these responses is necessarily the acquired, new conditioned response, resulting from an association of two events.

More generally, associative learning can be described as a process. Habituation therefore represents the basic phenomen for neural plasticity which can be manifested on a single neuron level, and is not necessarily dependent on the activity of other cells or pathways.

Sensitization represents a more advanced property in the modifiability of the nervous network. It is a process that in an instant of time can restore the sensitivity of habituated neurons and increase the activation of existing (learned or inherited) pathways. As referred to above, the sensitization process also represents a possible mechanism for the evocation of "instrumental" responses. The concept of instrumental conditioning thus needs some revision: instrumental conditioning as a separate associative learning mechanism does not in fact exist; the appearance of instrumental responses or an increase in their frequency is not as such an indication of associative learning. The principle of the possible operative associative learning mechanism (response-reinforcer type association) is already fully described by the operative associative mechanism included in classical conditioning.

Associative learning (classical conditioning) thus includes elements of more elementary learning (habituation and sensitization) but, in addition, it represents a more complex process for learning: one stimulus (in addition to its own response properties, that is, alpha responding) acquires the ability to increase the activity of other CNS-pathways (including the UCS-pathway). As a result of this process, the plasticity of the organism increases considerably. In practice, the conditioning process also includes the occurrence of other sensitized, learned instrumental responses, the form of which is largely dependent on the type of the UCS used in each situation and on earlier learning experiences (associations). These responses (or more exactly, the neural representations) can, in turn, also become as signals for the occurrence of the UCS.

2.5.2 What is the picture that emerges on the basis of the evidence presented?

1. Neurobiological evidence acquired mainly in invertebrate studies indicates that habituation and sensitization represent a possible fundamental neural mechanism necessary for both non-associative and associative learning.

2. Neurobiological evidence suggests that associative, short latency alpha-learning can also be seen as a basic preliminary neural process for delayed, "true" associative learning.

3. "True" associative learning can thus be defined as learning in which the activation of one pathway system (CS) also enables the activation of another pathway system (UCS), hence also eliciting, in addition to its own response pathway responses, features that initially belonged to the UCR.

4. The nature of the developing true CR is, of course, then largely determined by the properties of the relevant UCS-pathway system.

5 Responses occasionally appearing during the ISI (and also during the ITI), may be a) elicited by the sensitizing property of the UCS and thus represent either some components of the UCS system or pre-existing (learned earlier) CRs, or b) responses belonging to the CS system (alpha-response).

6. The occurrence of "instrumental" responses elicited by an unconditioned stimulus or representing pre-existing CRs does not indicate of the learning of any new association. Similarly, the development of a conditioned alpha-response do not probably represent true associative learning in a sense that it may only be an indication of the intensification of the alpha response, although it may also be a necessary step in the underlying process. However, it is possible that alpha learning and "true" associative learning are based on pre-wired connectivity in the CNS. Because some degree of convergence of the pathway systems (CS- and UCS-pathways) is needed for both phenomena the observed difference in the end-result may only be an indication of the efficacy of the connection to transfer the activation of one pathway system to another.

7. The constraints of a definite sequence (contingency) of events (CS before UCS) and a minimum optimal ISI and some degree of convergence of the CS- and UCS-pathways in the CNS describe the general limit conditions for associative learning.

8. In addition to these basic requirements, some kind of neural mechanism for time interval learning (ISI) is needed in order to yield delayed CRs. The interval learning ability is probably connected to endogenous oscillatory or pacemaker properties of single neurons (von

Baumgarten, 1970; Hoyle, 1980, 1982; Sokolov & Grechenko, 1981; Sinz, Grechenko, & Sokolov, 1982a).

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3 NEUROBIOLOGICAL PROCESSES, NEURAL RESPONSES AND ASSOCIATIVE LEARNING

Recent development in the neurobiology of basic processes of the adaptive behavior in invertebrates and the studies of the neural activity during conditioning in vertebrates have increased possibilities for a definition of sufficient and necessary conditions for the basic learning process. Such findings are reviewed here while attempting to extract some common principles for associative learning at different levels of phylogeny. Sensitization as a basic neural process for associative and non-associative learning is suggested and available evidence of the neuroanatomical convergence and properties of relevant neural pathway systems (conditioned stimulus, CS, and unconditioned stimulus, UCS) are tentatively related to the concepts of conditioning theories.

3.1 Associative learning in invertebrates

3.1.1 Learning in a single isolated ganglion neuron

Experiments on a single isolated ganglion neuron of the edible snail (Helix pomatia) have shown that plastic changes could be possible even in a neuronal somata without dendrites or synapses and with a broken-off axon (Sinz, Grechenko, & Sokolov, 1981, 1982, 1983; Sokolov, 1971, 1981; Sokolov & Willows, 1981; Grechenko, 1981; Grechenko, Sinz, & Sokolov, 1982).

In this preparation, in addition to habituation and sensitization, also an analogue of classical conditioning has been demonstrated. The conditioned stimulus (CS) was either a weak intracellular depolarizing pulse with an intensity of 0.5 nA and of a duration of 100 ms or a micro-iontophoretically applied asetylcholine stimulation to a surface site of the neuron body. The unconditioned stimulus (UCS) was a stronger intracellular stimulus of 2.2 nA and of 100 ms duration evoking an action potential of an amplitude of 83 mV as the unconditioned response (UCR). The interstimulus interval was 120 ms and the intertrial interval 10 seconds. Habituation and sensitization effects were demonstrated using a repeated application of the depolarizing current pulses.

A paired presentation of the CS (stimulation) and UCS produced an increase in the amplitude of the conditioned spike response (from 32 mV to 49 mV). The CR extincted to the initial level (32 mV) within 8 minutes. Subsequent reconditioning returned potential more rapidly to the 49 mV level and lasted 10-15 minutes after extinction. The authors have interpreted the results as a demonstration of associative conditioning, because repeated CS-alone applications, non-contingent (pseudo-conditioning), or a reversed presentation of the CS and UCS (backward conditioning) revealed no changes in the action potential amplitude (CR).

Similar results were obtained using paired chemical (acetylcholine, AHc) CS and electrical UCS stimuli (as above). The increased chemosensitivity to the CS appeared as an increase of the amplitude and also as an increase of the number of action potentials to the CS.

Because a single neuronal soma seems to have a capacity to undergo plastic changes formerly assigned only to the neuronal network, Sinz, Grechenko and Sokolov (1982a) conclude that the synaptic model could no longer be considered the exclusive one for memory traces. Hence, the authors introduce a "memory neuron" concept in order to emphasize the capacities of a single cell to perform associative functions and to retain this change.

The changing parameter in these "endoneural" conditioning experiments was the amplitude of the neural response to the CS. To be exact, according to the definition of classical conditioning ("true" associative learning) a pairing of a neutral CS to the biologically significant UCS, provides the CS with the property to elicit responses originally elicited by the UCR. Thus, the increase in the amplitude of the response to the CS (alpha-response) as such cannot be regarded as an example of true classical conditioning. Moreover, it should be considered as an example of associative alpha conditioning, because no new response was acquired for the CS. Nonassociative alpha learning (pure sensitization) was excluded on the basis of control procedures.

Interestingly enough, Grechenko and Sinz (1985) later reported an experiment in which a conditioned response similar to the unconditioned response was formed after paired representations of the serotonin (5-OT) stimulation of a chemosensitive point of the somatic membrane (CS) and depolarizing electrical stimulation of the membrane (evoking an action potential, UCR). The original (alpha) response to serotonin stimulation on the CS-alone trials was a hyperpolarization which, however, was reversed after the paired training showing an increased depolarization development. During the test trials (CS alone) of the last session (4th), the depolarizing response to the CS was increased to a spike generation level of action potential. Again, backward conditioning, unpaired representations, or repeated CS presentations indicated no learning specific changes: no depolarizing component to the serotonin-CS appeared.

Summary: These experiments seem to show that habituation, sensitization and simple associative learning are possible in a single ganglion neuron. The membrane of the ganglion neuron has, however, a complicated mosaic of chemosensitivity, owing to different transmitters (Sinz, Grechenko, & Sokolov, 1982). How the conditioned and unconditioned stimuli affect the membrane of the neuron depends on the membrane site to which transmitter is applied. Alkon (1984) has suggested that the site of heterosynaptic interaction (interaction between two different, CS and UCS, synaptic inputs) in associative learning could be localized on the common postsynaptic membrane of the dendritic branches or somata of a shared neuronal cell. Thus the postsynaptic membrane could provide a site for a convergence mediating interaction of associated stimuli. The changes in an isolated ganglion cell of Helix might then be interpreted to represent specific changes in chemosensitivity of converging CS and UCS effects on the neuronal membrane.

3.1.2 Learning in higher invertebrates: experiments with marine molluscan

Habituation

Habituation is probably the most fundamental behavioral change found in all animals. "It refers to a decrease in behavioral response when an initially novel stimulus is repeatedly presented" (Kandel, 1979).

Castellucci and Kandel (1974) have suggested that the biochemical mechanism for the habituation of gill withdrawal reflex in Aplysia is a progressive decrease in the amount of transmitter quanta released by the sensory neuron terminal onto their target cells.

Short-term and long-term habituation effects can be differentiated. Short-term habituation involves a transient decrease in synaptic efficacy while long-term habituation produces a more prolonged and profound change, leading to a functional disruption of most of the previously effective connections (Castellucci, Carew, and Kandel, 1977).

Sensitization

Sensitization is a form of behavioral arousal. Unlike habituation, sensitization affects a variety of related reflexes. Sensitization is a mirror image process of habituation: a strong or noxious stimulus enhances an animal's pre-existing reflex response (Kandel, 1979).

In this role, the sensitization process might also represent a probable source for the "instrumental" response evocation during the conditioning process. The unconditioned stimulus (UCS), as usually defined in conditioning experiments, is in most cases a complex of inherited ("species-specific") defensive or approach conditioned responses which may all become evoked by the sensitizing effects of the UCS. Thus, a strongly sensitizing stimulus, such as the UCS, can activate a group of pre-existing response pathways (to elicit the "instrumental" response, R) the action of which may then become associated with the following UCS effect. This is in fact the sequence of events occurring in conditioning arrangements, traditionally called "instrumental". However, in instrumental conditioning the association is thought to occur between a discriminative stimulus and the response R. The UCS thus acts only as an additional "catalyst" or "reinforcer" which "glues" the S-R association.

Sensitization is a more complex phenomenon than habituation and resembles classical conditioning in that activity in one pathway facilitates reflex activity in another. Unlike in classical conditioning, however, the reflex facilitation does not require a specific temporally contingent association of the CS and UCS. Dishabituation — the enhancement of a habituated response with a strong stimulation — is only a special case of sensitization (Kandel, 1979). Hawkins and Kandel (1984) have recently emphasized more explicitly the possibility that the cellular mechanisms underlying classical conditioning could be an extension of the mechanism underlying sensitization. They have also proposed that higher order features of classical conditioning might be based similarly on conditions of habituation, sensitization and classical conditioning.

Associative learning

Kandel and Schwartz (1982), for example, define two elementary forms of learning: nonassociative learning (habituation and sensitization), and associative learning (classical and instrumental conditioning). For associative learning two stimuli must be temporally associated: a weak or ineffective conditioned stimulus (CS) acquires behavioral significance only after it has been paired with a strong unconditioned stimulus (UCS). After conditioning the animal behaves as if the CS activates the UCR effector system in the same way as the UCS originally did. In contrast, nonassociative learning does not require temporal pairing of stimuli and does not teach the animal to expect any specific relationship between stimuli.

The identification of associative learning and elements of it in the classical conditioning paradigm appears to be no overwhelming problem. However, the identification of corresponding associative elements in instrumental conditioning has always been a disputed question. Thus, the confirmation of instrumental conditioning as associative learning presupposes first the discovery of necessary elements of this kind paradigm. Only after that it is possible to discuss the supposed associative nature of the instrumental conditioning. Invertebrate experiments, especially studies with molluscs, seem to bring some new insights to this problem. Above all, these findings appear important because the neurobiological basis of some critical events for associative learning has been clarified.

The associative learning ability has been, for example, demonstrated in a marine molluscan, Pleurobranchaea, which has shown a clear conditioned withdrawal response to food CS after pairing it with a head shock (Mpitsos & Collins, 1975; Mpitsos, Collins, & McClellan, 1978). It is interesting that in this experiment the CS was a stimulus that unconditionally elicited approach behavior, that is, a persistent and "unconditioned" alpha-response already existed. After learning both approach (alpha-response) and defensive (CR) response tendencies appeared occasionally during the same trial.

Carew, Walters and Kandel (1981b) paired a light tactile stimulus (CS) to the siphon of Aplysia, which elicits a feeble withdrawal of the siphon and gill, with a strong electrical stimulus to the tail (UCS), that produces a powerful withdrawal. After 15 pairing trials, the conditioned animals showed a longer withdrawal in response to the weak CS, both immediately after training and as long as 4 days later, than did the control animals (sensitized, CS-alone, UCS-alone and random CS-UCS paired). When the animals were tested 24 hours later, the paired group's responses were still significantly higher than all the other animals. However, the UCS-alone control animals also showed significant sensitization of siphon withdrawal compared to the other groups. Thus, the siphon withdrawal reflex can express both classical conditioning and sensitization. The demonstration of true associative learning in this experiment remained partly open because both the alpha-response and unconditioned response were very similar.

Differential conditioning (siphon-CS vs. mantle-CS) in Aplysia (Carew, Hawkins, & Kandel, 1983) and other higher order forms of associative learning, previously thought to be restricted to vertebrate learning, have also been verified in invertebrates. Sahley, Rudy and Gelperin (1981), for example, demonstrated blocking, second-order conditioning and preconditioning in the land snail (Limax).

Kandel and Schwartz (1982) have suggested that classical conditioning can be composed of some components that account for sensitization, and that in addition there is a mechanism for temporal specificity for associative learning. According to this hypothesis, cyclic AMP-mediated enhancement of transmitter release could serve as the basic mechanism for strengthening synaptic connections in associative as well as in nonassociative learning in Aplysia. Optimal enhancement might require that the CS and UCS be temporally paired.

Hawkins, Abrams, Carew and Kandel (1983) have found that after a series of pairing trials in which action potentials in a sensory neuron immediately precede activity in the UCS-pathway, the sensory neuron releases more transmitter than when action potentials in the sensory neurons are not paired with the UCS. Thus, at least some of the mechanisms for the temporal specificity of classical conditioning might appear within the sensory neuron itself.

The association of two stimuli depends on the timing of prior electrophysiological activity in the modulated (sensory) cells. Increased spike activity in the sensory neuron immediately before the neuromodulatory effect from the UCS obviously amplifies the amplitude and duration of the modulatory effects. This is a probable electrochemical mechanism of convergence, but the problem still remains how the facilitation of the relevant sensory CS-pathway acquires an ability to activate (sensitize?) also the UCS-pathway.

The assumption of a convergence of the CS- and UCS-pathways at a presynaptic button level as proposed by Kandel and his associates (Hawkins & Kandel, 1984; Kandel & Schwartz, 1982) in their model, or the localization of the convergence at the post-synaptic membrane level does not (as Alkon, 1984, has suggested), as such, determine the interpretation of the basic conditions for associative learning. If the CS- and UCS-pathways have such a spatially close connection on a common neuronal membrane making possible their interaction in a way suggested in the presynaptic model, then the fundamental property of a neuronal mechanism capable for sensitization and "true" associative learning might be described.

It is obvious that the Aplysia experiments have demonstrated a sensitization effect and as a closely related mechanism, the learning of the response of the CS-pathway (conditioned alpha-response). Similarly, the studies with another mollusc, Hermissenda, have demonstrated a conditioned suppression of a phototactic behavior to light CS after pairing with rotation UCS (Farley & Alkon, 1985; Alkon, 1984). Although these studies, as Aplysia experiments, seem mainly to indicate alpha learning, a later experiment with Hermissenda can be interpreted to represent "true" associative learning. In this study, the light-CS acquired a capacity to elicit the same motor behavior ("clinging") initially elicited by the rotation-UCS (Lederhendler, Gart, & Alkon, 1983).

Thus, as an additional and essential feature of associative learning (classical conditioning) should be considered the learning of a conditioned response which has properties of a response complex initially elicited by the second stimulus, the UCS. As many classical conditioning studies in higher animals have shown, the CS can be considered as a stimulus which after pairing with the UCS acquires the ability to elicit a "substitute" or "surrogate" (Hearst & Jenkins, 1974; Gamzu, 1971), or moreover a response complex or a "state" similar to the original UCR effector system.

Previous Aplysia experiments have demonstrated CS-pathway learning using the method of an additional test stimulus (e.g., tail shock) to trigger different behaviors after conditioning (Walters, Carew, & Kandel, 1979, 1981). This test-stimulus approach can reveal that learning has indeed occurred, even though no peripheral responses have yet appeared. Second, the test stimulus exposes a group of response pathways that represent inherited or learned complex effects adjoining the UCS-type used, and third, the test stimulus reveals that such a "latent" learning is a result of the sensitizing effect of the CS to the UCS-pathways. These latent learning discoveries may have important implications for the interpretation of the nature of the CR.

In some Aplysia studies there has been an overt conditioned response to the CS after training. Walters, Carew and Kandel (1981) used shrimp extract as the CS and a head shock as the UCS. During training the studied responses (head withdrawal, siphon withdrawal, inking and escape locomotion) appeared as UCRs to the head shock, but after training only head withdrawal occurred as the CR to the CS. Using a similar CS and UCS, Carew, Walters and Kandel (1981a) made similar observations: after training appeared defensive head withdrawal appeared as as the CR, a response similar to the unconditioned response to the UCS (head shock). Other related defensive behaviors (inking, siphon withdrawal and escape locomotion) were facilitated (when the test-stimulus was applied) as before (Walters, Carew, & Kandel, 1981). It seems then that the UCS (head shock) elicits, in addition to head withdrawal, other related defensive specific UCRs. Head withdrawal to the shock is apparently the first response to occur in this hierarchy.

Conditioned head withdrawal can also be acquired in Pleurobranchaea as a response to the food-CS after pairing it with a head shock (Mpitsos & Collins, 1975). The same experiment also showed that selective (to the oral veil) versus whole-animal stimulation respectively produced conditioned responses to food similar to the unconditioned responses to a particular type of stimulation. As an explanation for this general facilitatory effect these authors suggest that the CS elicits a conditioned fearlike central state that primes a variety of behaviors.

The general facilitatory effect exerted by the UCS is related to (inherited or learned) pathways (e.g., different approach responses towards food source, touching, tasting, biting of food, etc.). Aversive UCSs facilitate (sensitize) "species-specific" defensive responses (as termed by Bolles, 1972), or as in the case of positive UCSs, they could be called "species-specific" approach responses, respectively. The fear-construct thus has similar features to the concept of incentive motivation scheduled by Bindra (1972). Both concepts represent efforts to solve the problem of response evocation. Invertebrate studies seem to support a view according to which the sensitizing effect of the UCS provides a significant source for these, often as instrumental described, responses.

In Aplysia experiments, the recordings of the activity of identified motor neurons of different behaviors sensitized by the UCS have shown that the facilitation of these behaviors cannot be accounted for by the subthreshold effects of the CS on the motor neurons (Carew, Walters, & Kandel, 1981a). The membrane potential of the motor neuron did not differ in paired animals compared to unpaired controls. This should indicate that passive properties of the motor neurons are unchanged by conditioning. Instead, the CS enhances the synaptic input effect (i.e., facilitates presynaptically) of the stimuli that trigger defensive responses. Thus, the CS can exert a common action on all recorded defensive response systems.

The simple form of facilitatory effects of a biologically significant stimulus on the CS-alpha response pathway alone (sensitization) does not explain the temporally specific effect of the CS–UCS pairing. The "fear"-construct suggested by Walters, Carew and Kandel (1981) reflects the conditioned activation of defensive response systems. A schematic model of conditioned fear has been suggested by Walters, Carew and Kandel (1981). In this model a defensive central state ("fear") is initially elicited by the noxious UCS. Thus, an initially neutral sensory CS, when paired with the UCS, is proposed as becoming capable of eliciting the same defensive state (or at least some components of it) as the UCS. The exact mechanism by which this state is expressed is not yet known but some interneuron connections might be responsible for this effect.

3.1.3 Studies in other invertebrates

Other invertebrate studies, using insects, have reported the learning of instrumental responses in headless preparations. Horridge (1962) showed that the headless cockroach could learn to position its leg so as to minimize a shock administered whenever the leg dropped below a preset level. Willner (1978) confirmed the earlier results of Pritchatt (1968, 1970), which showed that headless cockroaches were able to learn instrumental leg lowering or leg raising responses to avoid shock. However, the retention of learned responses was short (10–15 min.).

Hoyle (1982) suggests that the leg extension is more difficult than flexion, which is a natural defense position. He has studied the motor neuron of the anterior adductor of the coxa of the locust and has found that the tonic firing rate of this motor neuron, which appears to have a stable intrinsic pacemaker property, can be modulated by a excitatory or inhibitory synaptic input. This indicates that learning either occurs within the motor neuron itself or is transferred to it. The final event is, however, altered pacemaker activity of the motor neuron (Hoyle, 1982). At least, one interneuron has been found which effectively modulates the motor neuron activity (Burrows & Siegler, 1978; see, Hoyle, 1982). Hoyle (1980) has reported that it is possible to change this basic pacemaker frequency by instrumental conditioning and with a negative stimulus (shock or loud noises) as the reinforcement. Warmth has also been used as a reward so that the insect has learned thermoregulation (Hoyle, 1979, 1980).

Horridge (1962) has doubted whether such preparations have demonstrated learning. Willner (1978) answers that obviously the headless cockroach can "learn" one of two alternative adaptive responses (leg lowering and leg raising), but because of an apparent lack of memory, it might be safer to leave the question of learning open.

Hoyle (1982) has tentatively discussed the possible mechanism of instrumental learning. Horridge (1962) already reported that cockroaches can exhibit several different spontaneous leg responses to a shock. The problem, then, is first to discover the origin of these spontaneous responses. Hoyle (1982) concludes that all instrumental responses must be initiated within the nervous system. "In natural operant conditioned learning the animal makes its own trials" (Hoyle, 1982, p. 208).

After the origin of spontaneous responses has been solved (at least hypothetically), the possible operative associative mechanism included in these "instrumental" conditioning experiments arises as the next question. Hoyle (1982) has suggested a "homeostatic" model which principally relies on the association of proprioceptive feedback ("efference copy" in "efference memory", p. 199) with the reinforcement. However, the proprioceptive feedback (as a signalling event) in the learning of a new association is not necessary, as several vertebrate experiments in which this feedback is either pharmacologically or surgically prevented have shown. One likehood is then that some external (e.g., a sensitizing stimulus like UCS) or internal effect is the source of spontaneous responding, the neural representation of which can also act as a signalling event ("stimulus") to be associated with the following, unconditionally elicited neural activity. The leg lowering might then acquire an ability to become associated with the UCS and thus act as a conditioned stimulus eliciting, as the original UCS, species-specific defence responses (leg raising in this case).

The true associative learning effect has been demonstrated in honeybees by Bitterman, Menzel, Fietz and Schäfer (1983). The CS in these experiments was an odour and the UCS was a compound stimulus consisting of a sucrose solution delivered to the antenna (eliciting antenna movements and the extension of the proboscis) and of the sugar solution "reward" delivered to the proboscis of the bee. The acquisition of the conditioned response (antenna movements and the extension of the proboscis) was very rapid, and its associative nature was confirmed by the use of differential conditioning and an explicitly unpaired control procedure. Neural recordings during sensitization and conditioning have shown that a multimodal convergence at the level of higher sensory neurons is a necessary condition for learning (Erber, 1981, 1983).

Remarkable in the experiments of Bitterman, Menzel, Fietz and Schäfer (1983) is the use of an omission contingency to eliminate the possibility of adventitious instrumental response-reinforcer contiguity ("response-shaping" hypothesis of classical conditioning) which had no adverse effects on acquisition.

In summary, the results of the experiments in insects seem to agree with other invertebrate studies: sensitization and some associative learning can be demonstrated and the "instrumental" responding can be considered to be a result of the sensitizing effect of the UCS.

3.2 Vertebrate conditioning studies

3.2.1 Spinal conditioning

In vertebrates, the spinal preparation represents a simplified model of the neuronal mechanism of learning. Using an electrical stimulation of a dissected superficial sensory peroneal nerve of the hindlimb of spinal cat as the CS and a strong shock to the ankle skin of the same leg as the UCS, it has been possible to demonstrate classical conditioning in this preparation (Patterson, Cegavske, & Thompson, 1973; Patterson, 1976). Explicitly unpaired or backward presentations of these stimuli did not induce learning. Forward pairing was a necessary condition for associative learning as it also appeared in invertebrate studies. Furthermore, spinal conditioning studies have shown some similarities with learning in the intact vertebrates. Patterson (1976) found that the optimal interstimulus interval (ISI) for spinal conditioning was 250 ms. This is very similar to what has been observed in intact animals (Smith, Coleman, & Gormezano, 1969).

Beggs, Steinmetz and Romano (1983) also showed that associative learning in spinal animals exhibits the acquisition and retention of a conditioned response in a way similar to conditioning in intact animals. As an exception, spinal animals do not show the latency shifts (delayed CR) to be seen in intact preparations. As the CR, spinal animals show an increase in the amplitude in the response to the CS (alpha-response).

Thus, no new connections are actually formed that were not present prior to training. However, as single ganglion cell and some invertebrate studies indicated, this effect is not a result of nonassociative sensitization only because forward paired animals showed learning but not unpaired controls or the backward conditioning group. Patterson (1980) suggests that "the spinal cord may lack the elegant machinery for response selection and adaptation of the cortex or upper brain stem, but the results of our work and Durcovic's (Durcovic, 1975; Misulis & Durcovic, 1982) studies suggest that the machinery for the basic pairing or associative effects of classical conditioning is present". The effect of optimal ISI needed for the best learning results demonstrates this similarity. Thus, spinal conditioning experiments arouse the same question as single ganglion cell studies above: Is the increase in the alpha-response an indication of some kind of associative learning? More generally, is the development of the conditioned alpha-response a necessary phase for the development of time-specific "true" associative learning?

In addition to classical conditioning, instrumental conditioning has been described in spinal animals. Earlier studies have shown that spinal vertebrates can be conditioned to acquire a leg withdrawal response (Buerger & Fennessy, 1971; Farel & Buerger, 1972). The acquisition of an instrumental leg lowering response in spinal rat has been demonstrated by Sherman, Hoehler and Buerger (1982). As Hoyle (1982) described several differerent responses to shock in the cockroach, so Sherman, Hoehler and Buerger (1982) state that both leg flexion and extension are probably components of the UCR to the shock in spinal rat. Thus, the instrumental performance (leg lowering or leg raising) does not necessarily represent the learning of a new association; instead, it may indicate (learned or inherited) pre-existing responses which may act as a signalling event (CS) in true associative learning.

3.2.2 Empirical findings in neural activity during conditioning in vertebrates

Nictitating membrane (NM) and eye-lid conditioning experiments in rabbits and cats and concomitant multiple-unit recordings of neural activity of different brain sites have provided important data, especially concerning some time-amplitude parameters of classical conditioning. In addition, those classical and instrumental conditioning experiments, which have used direct recording of single unit, multiple-unit or slow potential responses have also brought some new insights to the evaluation of the necessary minimum and limit conditions for associative learning.

Olds and his colleagues used an appetitive (food reward) conditioning paradigm and recorded multiple-unit activity in rats. Using waveform discriminators they could select isolated "single-unit" responses from multiple-unit population activity. Olds and his associates (Olds, Mink, & Best, 1969; Olds, 1973; Disterhoft & Olds, 1973; Kornblith & Olds, 1973; Linseman & Olds, 1973; Segal, 1973; Olds, 1975; Disterhoft & Stuart, 1976, 1977; Brauth & Olds, 1977; Disterhoft & Buchwald, 1980) carried out several classical conditioning experiments designed to map the relevant brain structures for learning. The search for "learning centers" consisted of the analysis of two time series of neural activity during the interstimulus interval (1000 ms, later 280 ms). The first analyses were based on the short latency ("millisecond time series"), in which it was attempted to construct the sequence of the learning process in different brain sites by identifying the "shortest latency learned response" to the CS. The second way of study included trial sequence changes, in which the development of the CR over trials was followed and the neural activity during the whole ISI examined. As in nictitating membrane studies (see below) an interesting finding in the short latency studies was that some regions of reticular formation indicated very short latency changes after learning (see, Disterhoft & Buchwald, 1980). The trial sequence curves of many brain structures (especially in the hippocampus) showed a quite similar topography in the neural activity to that of the nictitating membrane conditioning studies of Thompson and his associates (see next chapter).

Olds and his coworkers attempted to relate the trial sequence observations to the corresponding behavioral response development so that they divided the CR development into different phases. During the first phase the rat began to orientate (after habituation in test trials) to the tone-CS and the neurons in the reward system became activated. During the second phase the rat began to move slowly toward the pellet dispenser at the CS presentation: arousal and motor neural regions were activated. During the third phase, well directed movements appeared toward the food dispenser and simultaneously hippocampal (CA3) and extrapyramidal neurons increased their activity. During the fourth and fifth phases refined anticipatory movements appeared and increased activity was shown in the hippocampus body, motor cortex and sensory systems. Relating these observations to the proposed conception of sensitization and associative learning, the first phase might be identified as a nonspecific effect of the UCS. During the second phase, species-specific approach responses appear as a result of sensitizing effect of the UCS on related UCR pathway systems. The third, fourth and fifth phase obviously represent then learning of response-reinforcer type association.

The use of food pellets as the UCSs in these experiments created a rather complicated sequence of different responses. The rat had to be trained first to eat pellets from a noisy dispenser. The gross movements of the animal were defined as the unconditioned response (UCR). This

UCR definition, however, is quite different from the traditional UCR identification, that is, the responses to the UCS (= food) are actually food ingestion, tasting, salivation, swallowing, etc., not the events (as approach movements, etc.) preceding the UCS. The approach to food, as probably also the responses which prepare for the reception of food (tasting, ingestion, even salivation) should apparently be considered as pre-existing CRs ("instrumental responses") which are elicited as the result of the sensitization effect of the UCS. A similar sensitizing effect of the food UCS to a preceding food approach (antenna movements) has been observed, for example, in honeybee experiments of Erber (1981, 1983, see above).

Another problem arises from the effect of approach movements with regard to the recorded neural activity. It might be argued that the observed conditioned response changes were affected by behavioral feedback. However, gross movements are obviously no problem in the short latency studies. One solution to this difficulty could be an exact trial by trial analysis of the appearance of the conditioned neural responses related to the simultaneous behavioral response development. This strategy has been used in the nictitating membrane experiments and the results show that neural responses develop earlier in trials during which no behavioral CR has yet appeared.

3.2.3 The nictitating membrane and eye-lid conditioning studies in rabbits and cats

Recent studies of the conditioned nictitating membrane (NM) responses constitute one of the most comprehensive sets of the conditioning experiments using multiple-unit recordings. These studies are based on the model of the classical NM-conditioning paradigm originally developed by Gormezano and his associates (Gormezano, Schneiderman, Deaux, & Fuentes, 1962; Gormezano, 1966, 1972; Gormezano & Moore, 1969). The properties and the parametric features of the NM-response are well characterized and this is the main reason why Thompson and his associates (see Thompson et al., 1976, 1980) have adopted this paradigm as their basic model system.

Thompson and his colleaques have reported a series of studies concerning the conditioning of neural multiple-unit responses during a short (250 ms) interstimulus interval (ISI). The aim of these experiments has been to find neural substrates for learning and, more specifically (Thompson, Berger, & Madden, 1983; Thompson et al., 1976, 1984a, 1984b), to find the neural substrate of the change in behavior, as opposed to the substrate of behavior, per se. By adopting a simple learning paradigm (classical conditioning of the NM response) they have attempted, first, to identify the critical structures in the brain for learning and, second, to analyse the function of these structures in order to relate their activity to behavioral conditioned responses.

The paradigm includes a tone-CS lasting 350 ms and an overlapping airpuff-UCS to the corneal surface, beginning 250 ms after the CS onset. The experimental animals have been rabbits and cats (Patterson, Berger, & Thompson, 1979). The animal is held motionless but not drugged or paralyzed during the 2-hr training session. Unpaired control groups receive a random sequence of tone CSs and air-puff UCSs so that the density of stimulation is the same as in paired conditioning animals. The behavioral response is the extension of NM measured by a micropotentiometer. The neural multiple-unit data are collected 250 ms prior to the tone CS (the pre-CS period), 250 ms of ISI (the CS period), and 250 ms from the beginning of the UCS (the UCS period).

As a brief summary, the main results of these studies are:

a) The neural conditioned response appears in the proximity of the UCS and shows a slightly decreasing onset latency and an increasing amplitude development as training proceeds. At the beginning of training the CR may occur during the UCS period.

b) The conditioned neural response can arise in a few trials and always precedes (by about 40 ms) the behavioral response in time (e.g., Berger, Laham, and Thompson, 1980).

c) The occurrence of the CR follows the length of the ISI, so that it appears at the end of the ISI or during the UCS period.

d) The conditioned neural response develops in some brain structures (i.e., in the hippocampus, brain stem and cerebellum) before any sign of the behavioral CR exists.

e) The length of the ISI is critical, so that no learning occurs if the ISI is shorter than 50 ms.

f) No "backward" conditioning takes place.

g) The hippocampus forms a temporal model of the behavioral response and precedes it in time (i.e., hippocampal changes appear in earlier trials).

h) The hippocampal multiple-unit changes predict the subsequent behavioral learning: if no hippocampal responses appear, the animal will not learn; if hippocampal changes develop slowly, the animal learns slowly. In short, the growth of the hippocampal unit response is an invariable and strongly predictive concomitant of subsequent behavioral learning (Thompson, et al., 1982).

Contrary to the traditional belief that the CS period is the only proper trial segment for the analysis of changes in learned behavior, the NM experiments seem to indicate that the UCS period is also indicative of the acquisition referring thus, except to learning of a true classical CR, also learning of a time-interval. Gormezano (1972) had already shown earlier that during learning the first conditioned behavioral NM response is developed in the UCS period. In addition to the confirmation of these observations in neural (hippocampal) recordings during NM conditioning, Berger and Thompson (1982) showed that extinction may also begin within the UCS period rather than during the CS period (again, first in neural activity, then in behavioral response).

Hoehler and Thompson (1980) have suggested that the hippocampus plays a critical role in the temporal aspect of learned behavior. Weisz, Solomon and Thompson (1980) showed, using a bilateral ablation of the hippocampus, that the animals were unable to learn a trace conditioned response (i.e,. where a period of no stimulation intervened between the CS offset and UCS onset). When these animals were then shifted to a standart short delay paradigm, they learned in the normal number of trials. Thompson et al. (1982) conclude that "neural trace" of the time interval may actually develop in the hippocampus. Early in training, long before the behavioral CR develops, hippocampal activity builds up and persists for the entire trace period. As behavioral learning develops, this unit response appears as a predictive model of the learned behavioral response in animals that learn. They emphasize that the "endpoint" of the learning-induced neuronal response in the hippocampus is essentially identical in both short delay and trace conditioning paradigms.

The latest studies of Thompson and his associates have more beneficially succeeded in the localization of brain structures which seem to be critical for the learning of short delay eye-lid conditioned responses. The activity evoked by both the conditioned and unconditioned stimuli and also the model of the learned behavioral response (as in the hippocampus) appeared in the cerebellum. Large removals of ipsilateral cerebellar cortex or small lesions of a superior cerebellar peduncle completely removed the well-trained CR (NM or eyelid) but had no effect at all on the UCR (McCormick, Lavond, Clark, Kettner, Rising, & Thompson, 1981; McCormick, Clark, Lavond, & Thompson, 1982; McCormick, Lavond, & Thompson, 1982; Thompson et al., 1982; Thompson, Berger & Madden, 1983; McCormick & Thompson, 1984). None of the lesioned animals showed any signs of relearning the original conditioned responses. If the cerebellum is the locus of the primary engram for simple learned responses, as Thompson, Berger & Madden (1983) suggest, then the relationship of higher brain structures (especially the hippocampus) to the cerebellum becomes interesting. There is a pathway from the hippocampus to cerebellum via the subiculum to the cingulate gyrys to the pontine nuclei of the brain stem (Berger, Milner, Swanson, Lynch & Thompson, 1980). Clark, McCormick, Lavond, Baxter, Gray and Thompson (1982) observed that cerebellar dendate nucleus lesions removed both the behavioral conditioned response and the learning-induced hippocampal response in the CS period. Thompson, Berger and Madden (1983) suggest that the learning-induced plasticity that develops in the hippocampus requires some type of input or influence from the cerebellum to express itself.

The role of the cerebellum in the development and maintenance of the conditioned response is also confirmed through observations in which anatomically close brain stem structures seem to covariate with the cerebellar functions. Smith (1970) had already reported that large unilateral lesions in the red nucleus markedly impaired a classically conditioned flexion response of the forelimb of the cat. Later, 'Tsukahara (1979, 1981a, 1981b, 1982), using a direct cerebrorubral stimulation as the CS and a shock to contralateral forepaw as the UCS, showed that the red nucleus appeared as the site of learned plasticity. These experiments indicated that the primary site of the conditioned change could be located as the corticorubral presynaptic modulation of the elbow reflex pathway. The topography of the developing CR was very similar to the time-amplitude course of the conditioned NM-response.

The eyelid and the NM conditioning represent largely the measurement of the same basic phenomenon. They both also show the same topography and features of the CR development. As in the NM experiments, the CR also develops initially in the eyelid conditioning experiments at the time of the onset of the UCS (periorbital shock) and gradually moves forward in time as training continues (McCormick, Lavond, & Thompson, 1982).

Woody and his associates have also measured the eyelid responses of the cat, but the UCR was now elicited by a glabella tap. The earlier experiments (Woody, 1970; Woody, Vassilevsky, & Engel, 1970; Woody, Vassilevsky, Owens, Baumgarten, von, 1974; Woody & Black-Cleworth, 1973; Brons & Woody, 1980), using only CS (click) and UCS (glabella tap) pairing showed that, contrary to NM-observations, a very short latency (about 20 ms) CR developed. The latency of this CR did not shift during training and the learned response developed very slowly. The development of the CR was not a result of general activation (sensitization), because unpaired or backward presentations of the CS and UCS did not produce long-term increases in the excitability of CR-related neurons. However, the unpaired presentations of the UCS produced transient increases in the excitability, referring to some degree of the sensitization effect of the UCS. The conditioned nature of this response was also verified by its selective function: if the CS was paired with a glabella tap, the eyelid response appeared as the CR; if it was paired with a stimulation of the nose, the nose twitch developed as the CR. Intracellular recordings of the motor cortex neurons showed decreases in membrane thresholds both in the UCS alone and paired groups but only CS-UCS pairing produced long-lasting decreases (Brons & Woody, 1980).

In more recent studies (Woody, Kim, & Berthier, 1983; Kim, Woody,

& Berthier, 1983) Woody and his associates added a hypothalamic brain stimulation reward after the glabella tap UCS and observed that now the acquisition of the CR developed quickly (within 20 trials) and a long-latency CR developed (>50 ms, compared to 20 ms in earlier studies) similar to NM conditioned responses. A discriminative paradigm was used in these experiments and it appeared that the hypothalamic stimulation also elicited eye-blinks as the UCR (as well as other related movements) and, in addition, increased eye-blink responses both to CS+ and CS- (= sensitization effect of the ESB), which with further training became smaller and less frequent.

Voronin (1971) has observed short latency CRs similar to those in the single neuron conditioning experiments. These responses to the CS appeared mostly to be results of sensitization but some cortical neurons showed "time-locked" cellular conditioned responses similar to the responses elicited previously by the cortical UCSs. In addition, these CRs occurred at a time corresponding to the occurrence of the UCS (Voronin, 1971). The CS and the UCS were direct cortical stimulations. The CS stimulation mainly elicited only a small inhibitory postsynaptic potential, and the UCS (UCS-electrode from 3 to 12 mm away from the CS-electrode) elicited a prominent spike discharge and also evoked a contralateral foreleg movement. In one case, an inhibitory conditioned response consisting of the disappearance of some spike to the CS occurred. As in the experiments of Woody, Voronin observed that motor cortex stimulation as the UCS was not very effective and thus he added a hypothalamic stimulation after the UCS. The behavioral CR in these experiments was the EMG activity in the contralateral forepaw of the rabbit. An abrupt jerk in the foreleg was observed as the behavioral CR showing extremely short latency (16 ms). It was observed that this CR was very similar to the unconditioned startle response. This refers to a facilitation effect of the CS pathway, that is, "local conditioned startle reaction" (LCSR), which can be considered obviously analogous to the alpha response (Voronin, 1976). The short latency eye-blink CR observed by Woody can be attributed to the same type of CRs.

3.3 Concluding remarks

The following could be a list of main conclusions based on evidence acquired in both invertebrate and vertebrate studies:

3.3.1 CS-alpha response properties

Repeated CS-alone presentations lead to habituation but the introduction of the UCS yields a sensitization effect (dishabituation).

A sensitization effect of the UCS appears as an increased alpha-responding (orienting), which might be considered as an indication of the associative alpha-learning at the first stage of learning providing that unpaired or random presentations of the CS and UCS do not yield this pairing specific effect. The nonspecific sensitizing effect of the UCS has been reported in control groups of some studies referred above (e.g., Carew, Walters, & Kandel, 1981b; Woody & Black-Cleworth, 1973; Brons & Woody, 1980). Sensitization effect has been observed also in the studies of a jaw-movement response (JMR): during unpaired control procedure the rabbits exhibited considerably increased responsiveness to the CS (Mitchell & Gormezano, 1970; Sheafor, 1975; Sheafor & Gormezano, 1972).

Increased alpha-responding might be considered as a prerequisite for true associative learning: there is some evidence that the CS can in turn sensitize the UCS-pathway. The latent learning in invertebrates (Walters, Carew, & Kandel, 1979, 1981; Carew, Walters, & Kandel, 1981) might indicate this effect. In vertebrates, Young, Cegavske and Thompson (1976) found that the excitability of motoneurons in the abducens nucleus (final common path for the nictitating membrane response) was enhanced by the sounding of a brief tone. Such an effect presupposes a pre-wired (but not necessary operative) connection between sensory and motor pathways. Woody and Brozek (1969) reported that the click-CS increased motoneuron sensitivity and Gormezano, Kehoe and Marshall (1983) observed that the CS augmented the amplitude of the ISI lenght. Doty (1961) had already earlier observed that the flexion system of the limb being conditioned (and that limb only) was greatly enhanced during the CS (direct stimulation of cortex) presentations almost 100 trials before the appearance of the first behavioral CR.

3.3.2 ISI and delayed CR learning

While the sensitization of alpha-responding may represent a necessary preliminary stage in the development of a true conditioned response, and thus of the sensitization of the UCS-pathway, some additional properties in the neural network are necessary for time interval learning (time-specifity, ISI-learning, or delayed CR concept). From the neuroanatomical point, a pre-existing convergence of neural systems representing the CS- and UCS-pathways at some level of the nervous system is a basic requirement for true associative learning. For an explanation of time interval learning, some intra- or interneuron timing mechanism should exist. As some empirical findings referred to above seem to show, the conditioned neural and later also the behavioral responses develop approximately at the temporal site of the UCS, and the nature of the CR seems to be closely connected to the properties of the UCS pathways. The learning of ISI in a trace conditioning paradigm (CS and UCS separated) seems to be a more demanding task than the learning of delayed CR (CS and UCS overlapping). This might indicate that an overlapping activation of the CS- and UCS-representation systems in the brain is needed for associative learning.

The mechanism for interval learning might be located in some specific structures or it might represent some endoneural property. The findings of Clark, McCormick, Lavond, Baxter, Gray and Thompson (1982) provide some kind of evidence for the structure effect, showing that cerebellar dendate nucleus lesions removed, in addition to the behavioral and neural conditioned response of the dendate nucleus, also the neural conditioned response model of the hippocampus. On the other hand, the experiment of Weisz, Solomon and Thompson (1980) showed that a bilateral lesion in hippocampus damaged the interval learning in trace but not in the delayed conditioning paradigm. Hence, it is possible that the hippocampus might include some "time interval generator". Berger (1984) suggests that the long-term potentiation (LTP) property of the hippocampus may be the basis for hippocampal neural plasticity during associative learning. He showed that LTP increased at the rate of learning during nictitating membrane conditioning in rabbits. Another possible mechanism for interval learning might be included in the internal properties of many neurons: self-oscillatory, burster-cell or pacemaker properties. As stated above, the single ganglion cells seemed to be unable to show delayed CR learning. However, the results of these studies showed that ganglion cells developed increasing oscillatory after-discharge spikes following the CS (Sinz, 1983). As von Baumgarten (1970) stated, oscillatory properties are a possible mechanism for interval learning. Spinal conditioning studies in vertebrates seemed to indicate that neither the spinal neural network obviously contains a necessary mechanism for time-interval learning (Patterson, 1980).

Endogenous pacemaker properties have been observed in motoneurons of the locust (Hoyle, 1980, 1982) and in sensory neurons of Aplysia responding to a sensitizing stimulus (Walters, Byrne, Carew, & Kandel, 1983). These neurons can learn to change their rhythmic spike generation according to the external stimuli so that they can become "locked" into a new rhythm of regularly repeated external stimuli, can respond after training to subthreshold stimuli and, more significantly, can produce few responses according to the learned interval after the actual stimuli have stopped (Voronin, 1971, 1976).

In experiments with regularly discharging neurons of a sea-hare (Aplysia) a single intracellular stimulation below the threshold for an action potential, paired with the following (ISI 6 s) stronger suprathreshold depolarization, could alone elicit a delayed action potential which was approximately equal to the used interstimulus interval (von Baumgarten, 1970). For example, the observed prolongation of the alpha response in Aplysia from three to five times compared to the original response duration might indicate such a endoneural mechanism (Hawkins, Castellucci, & Kandel, 1981).

3.3.3 UCS-pathway properties

Except for a general nonspecific sensitization effect, the UCS has its own specific response properties. These properties make it possible to follow the progress of the conditioning process by identifying the development of the true conditioned response as a different event from an appearance of an alpha-response to the CS. In addition, the sensitizing effect of the UCS may elicit pre-existing, earlier conditioned responses which, in certain experimental arrangements, might have been identified as "instrumental" responses. Hence the UCS can also act as a source of instrumental response evocation. Both invertebrate and vertebrate studies offer evidence of this sensitization function. The sensitizing power of the UCS is mainly based on its biological significance as an unconditioned type stimulus and a strong motivating content of the UCS decisively intensifies the development of associative learning, as the studies of Woody and Voronin showed.

3.4 The role of sensitization in classical and instrumental conditioning

Invertebrate studies seem to indicate, that the UCS is important in at least two ways. First, it elicits (according to the definition of the UCS) a definite UCR which usually, at least in more developed animals, is actually a complex of many responses. These responses represent either through evolution developed, inherited responses (such as food ingestion, swallowing, even salivation and different neurosecretatory responses), or during the ontogeny of each individual acquired, learned responses which may precede and thus also act as conditioned signals of a following UCS. The second, and equally important role of the UCS is its ability to produce a widespread sensitizing effect in many structures of the CNS.

The nonspecific sensitization can facilitate a multitude of different existing stimulus pathways in the CNS. This means that in a typical classical conditioning situation the UCS through its sensitizing power could increase the sensitivity to all stimuli in the temporal proximity of the UCS, including the possible CS-pathway which, instead of habituation to the repetitive CS presentations, increases its sensitivity (i.e., shows orienting or alpha-responding). The UCS thus sensitizes both learned and inherited pathways in its temporal proximity (this UCS effect does not obviously include any constraint for its effects on pre- or post-UCS events). As a result of this sensitizing effect, the animal is likely to produce such responses ("instrumental") which are specific to that UCS.

Hence, if an aversive UCS is used, it is highly probable that different "species-specific defense responses" (Bolles, 1972) can be observed. Correspondingly, the responses appearing in the proximity of the appetitive UCSs would be called "species-specific approach responses". Common to both these responses is that they are the first "instrumental" or "preparatory" response candidates to appear also in the interstimulus interval in classical conditioning. However, as should be evident, none of these responses are necessarily the new "real" conditioned response, which should be the result of the association of some CS and UCS and should show properties of the UCR system. Thus, the occurrence of an instrumental, as preparatory interpreted, response during ISI is not an indication of the learning of a new association.

A revised view of the conditioning process description based on identified biological structures might now be tentatively constructed. The neural manifestation of habituation can occur in a single cell of the CNS and is not necessarily dependent on the activity of other cells or pathways. Sensitization represents a more developed stage of the modifiability of the CNS. It is a process that can in an instant of time restore the sensitivity of habituated neurons and activate pre-existing (learned or inherited) pathways. The sensitization process represents a possible source for the occurrence of "instrumental" responses. The concept of instrumental conditioning thus needs some revision: instrumental responses are then by sensitization facilitated, pre-existing conditioned, or "unconditioned" responses. The increased or decreased frequency of instrumental responses (which is the usual measure of instrumental conditioning) is not necessarily an index of associative learning. It follows that instrumental conditioning as a separate operative associative mechanism does not actually exist; the factual operating associative mechanism can be described by a procedure included in classical conditioning, that is, the association of two temporally close events, of which the following event represents some biologically significant event.

Although sensitization might be considered as a solution for the problem of the response evocation of instrumental responses, the development of a new association through response-reinforcer association is the possible operating associative mechanism, as Mackintosh (1974) and Mackintosh and Dickinson (1979) have suggested, providing that the response (R) (the central representation of the R) can act similarly as a signal in a way similar to the CS in the classical paradigm. The measure of associative learning is not then the occurrence of the response R but a classical CR, the nature of which is mostly determined by the properties of the UCS-pathway.

There are also other possibilities for eliciting the response (R) in addition to the sensitization effect of the UCS. One way is to elicit the R "unconditionally", using a stimulus which acts like the UCS. This kind of CS (and hence the "unconditioned" R) was apparent in the experiments of Mpitsos and Collins (1975; referred above), for example, and Konorski (1967; leg flexion CS elicited by a shock). The "two-way" conditioning experiments of Asratyan (1980) in which a moderate UCS was used as the CS can be interpreted similarly: the UCS through its sensitization ability elicits species-specific responses.

True associative learning (classical conditioning) thus includes elements of elementary learning (habituation and sensitization) and of a more demanding property for learning: one stimulus (in addition to its own increasing sensitization owing to the UCS effect, that is, alpha response) acquires a unique ability to facilitate (elicit) the activity of another CNS-pathways (UCS-pathways). As the result of this process, the plasticity of the organism increases considerably. In practice, the conditioning process thus always includes the occurrence of other sensitized, learned responses, the form of which is largely dependent on the type of the UCS used in each situation.

The appearance of "instrumental" responses and the possible re-establishment of these pre-existing connections as a result of the sensitizing effect of the UCS represents no learning of any new responses. Thus, contrary to earlier definitions, instrumental conditioning should be regarded as a process close to sensitization process, and in this way as a representation of the more simple learning process, rather than true associative learning (classical conditioning). The fundamental assumption in two-process conditioning explanations, that CR similarity vs. dissimilarity with the UCR could differentiate instrumental (i.e., "preparatory") and classical CRs, appears then redundant. In instrumental conditioning the response R, attempted traditionally to define as the "CR", need not resemble UCR, because the learning of a new classical CR is a quite different process to that of elicitation of pre-existing CRs through the sensitization effect of the UCS. The UCS does not "reinforce" prior responses, but produces a set of responses (UCRs and pre-existing CRs) that can be associated with (and thus become elicited by) the preceding CS. The sensitization effect of the UCS can obviously be paralled with the suggestion of motivating (Bindra, 1972), "fear" generating (Mowrer 1947) or "species-specific defense responses" (Bolles, 1972) elicited by the UCS.

3.5 Summary

Invertebrate studies have demonstrated to a large exent (including anatomy, physiology and neurochemistry) some fundamental features of habituation, sensitization and elementary associative learning. These studies and single cell studies have shown that even a single neuron has necessary properties at least for habituation and sensitization (alpha-learning), and already a rather simple nervous network (as in invertebrates) is capable of real associative learning. On the other hand, if a traditional instrumental conditioning paradigm is thought to represent the learning of some new association, the occurrence of or the frequency of the response R is not a relevant measure of true associative learning; it reflects only sensitization. The arrangement of the "instrumental" conditioning paradigm may, however, include an operative associative mechanism similar to classical conditioning, but now, as the result of learning, instead of the frequency measurement of the R, a conditioned response which is determined according to the same rules as the CR in classical conditioning should be sought. While the role of sensitization in the development of "instrumental" and/or alpha-response seems to be established quite convincingly in invertebrate experiments, an interesting problem in the development of associative learning remains: how does a CS, after it has acquired conditioned alpha-response properties, act on the UCS-pathway in order to be able to elicit the CR? Is the effectiveness of the CS essential for the CR development?

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4 FIRST STUDY: BEHAVIORAL AND NEURAL CHARACTERISTICS OF SHORT-LATENCY AND LONG-LATENCY CONDITIONED RESPONSES IN THE CAT

Directed head movements to the conditioned stimulus (tone-CS to the left ear) were examined in six cats. An attempt was made to differentiate such orienting, "alpha" type response, from development of the long-latency conditioned response (CR) resembling the unconditioned response (UCR) to the unconditioned stimulus (UCS). The UCS was a brain stimulation to the lateral hypothalamus eliciting, in addition to orienting and approach behavior, a specific, rather stereotypical head movement. The specific direction of the unconditioned head movement was used for to distinguish it from the conditioned short-latency (alpha) head movement. Paired conditioning and randomly unpaired conditioning sessions (5 daily sessions each) were given in balanced order to each animal. Evoked neural recordings in the hippocampus and cingulate cortex supported behavioral findings of differentation. The time-amplitude course of evoked neural responses showed that an increase in negativity appeared as a short-latency response during the 150–450 interstimulus interval (ISI) period after the CS onset and an increase in positivity developed during the long-latency period (700–1000 ms ISI + 150–450 ms UCS period) on omitted UCS (CS-alone test) trials. The role of "alpha" conditioning and the nature and temporal relation of the "true" conditioned responses in associative learning are both discussed.

4.1 Introduction

Historically, there has been difficulty in achieving general agreement in the identification of a real learning-specific change in associative learning. In some cases a response similar to the original unconditioned response (alpha), or orienting response (OR), elicited by a conditioned stimulus (CS), has been defined as a conditioned response (CR). Such modifiability of the orienting (alpha) response probably represents associative learning, as the studies of the neurobiological mechanism of associative learning in invertebrates, for example (Hawkins & Kandel, 1984; Kandel & Schwartz, 1982) have suggested. In the present paper, the concept of alpha response has been used parallel with the concept of orienting response (Sokolov, 1975). The term "alpha-response" has been preferred when the unconditioned nature of this response has been emphasized without the connotation of any more specific characteristics (such as, for example, the orienting properties of that response).

The specifity of the conditioned alpha-response for paired treatment in these studies seems to be undeniable; unpaired controls do not show similar changes. The original concept of "true" associative learning (classical conditioning) defines conditioning as a process in which, after paired representations of the conditioned stimulus (CS) and unconditioned stimulus (UCS), the CS acquires a capacity to activate (in addition to an activation of its own pathway system, that is, alpha-responses) another pathway system, the UCS-pathway. As a result of such a convergent activation a conditioned response, possessing features (neural and/or overt behavioral manifestations) of the UCS-pathway system, should appear. This definition sets more demanding constraints for associative learning than for the development of an alpha response: the form and nature of the CR should display characteristics of the UCS-pathway system. However, the supposed underlying interaction of the neural CS and UCS pathway systems creates a problem. If the CS and UCS pathways were to be considered neuroanatomically totally separated systems, there would, of course, be no possibility for any convergent interaction. Thus, at least some neural (although initially, functionally non-operative) connections should pre-exist between these pathways. The existence of prewired converging connections in the development of the conditioned alpha-response is, in a way, self-evident: the CS elicits, as a function of novelty or intensity an orienting (alpha) response. In contrast, a pre-existing convergence of the CS- and UCS-pathways is less evident; the assumption of such convergence may appear rather uncomfortable for a traditional definition of "true" associative learning. However, some evidence of pre-wired neural connections between the CS- and UCS-pathways, again, in a case where the CR is similar to the original UCR, has been amassed. Young, Cegavske and Thompson (1976) observed such an effect in rabbits: a tone-CS could activate the final common path for a nictitating membrane response (abducens nucleus) before paired training. Further, Cohen (1982) found that an alpha response appeared to a light during CS-alone presentation in cardiac sympathetic postganglion neurons of a pigeon before paired training.

Thus, if a "prewiring" of neural connections is assumed also to form the basis of the "true" associative learning, then it is possible that the effect of paired training is not to form new connections, but rather to make some pre-existing connections between the CS- and UCS-pathways functionally operative. If so, then the distinction between the original alpha response (orienting response) and a conditioned short-latency CR (developing as a result of paired training) becomes difficult to make, especially if the latency of both responses is so short that the time-amplitude courses of both responses overlap. The question arises as to whether these two short-latency responses in fact represent only a modification of the same response; or whether they are of different origin. If the developing short-latency CR were rather different in form and nature compared to the original alpha-response, there would hardly be any problem, but thus far, observed short-latency CRs both in vertebrates and in invertebrates have usually been very similar to the original alpha-response. The development of a conditioned nose-twitch to the same click-CS which could also be conditioned to yield a eye-blink if a glabella tap UCS was used, might represent a case in which the specific nature of the short-latency conditioned response also appears similar to the original UCR (Woody, Yarowski, Owens, Black-Cleworth, & Crow, 1974). However, neuroanatomical studies have shown that the eye-blink and nose-twitch share the same muscles and thus represent overlapping response mechanisms.

While the form and nature of a short-latency CR do not alone provide a sufficient basis for making a distinction, a difference in the latency of the alpha and conditioned response might offer some clue as to the origin of the CR. In a study carried out by Woody and Brozek (1969), the neural response in facial nucleus (final common path for eye-blink response) of the cat showed that the glabella-tap UCS can elicit an unconditioned eye-blink in about 10 milliseconds; while a conditioned eye-blink to the tone-CS (click) had a latency of about 17 ms. Thus the CS seemed to need more time to reach the facial nucleus than the UCS, but the latency of the original alpha-response to the CS and developing conditioned short-latency response seemed to to overlap. Voronin, Gerstein, Kudryashov and Ioffe (1975) reported that a short latency CR which developed after paired training, resembled the original alpha response (startle response to a cortical stimulation CS) and appeared at the same latency (16 ms). The observed short-latency response indicated specificity for pairing while the original startle response habituated quickly on the CS-alone trials. Although the latency of the alpha-response was rather similar to the conditioned short-latency CR in the studies of Woody (1970), the results of nictitating membrane (NM) and eye-lid conditioning studies have shown longer latencies for the CR: observed minimum onset latency has been 58 ms and mean onset latency 80 ms (McCormick, Lavond, & Thompson, 1982).

In the case of a short latency CR (<50ms) there is no benefit to be gained from a test-trial (CS-alone) approach (omitted UCS trials), as there is with temporally separated long-latency CRs; because the alpha-response and conditioned alpha are either identical or overlap in time and form. Obviously the only way to confirm the associative nature of the learning of such short-latency CR is simply to try to exclude possible nonassociative explanations (i.e., general sensitization effect). The usual explicitly or randomly unpaired control procedures do this in principle, but the frequency of the UCSs and the temporal proximity of the CS and UCS ("additive" effect) do not correspond to the situation which exists in paired training. A lower rate in the incidence of the UCSs might yield a different habituation rate to the CS in unpaired control group. One possible improvement would be to double the frequency of the trials and thus of the UCSs so as to correspond to the number of UCSs in paired training. Further, the real temporal additive effect of the CS and UCS in paired training can be approached by using backward conditioning as a procedure for extinction (Patterson, Steinmetz, Beggs, & Romano 1982; Brons & Woody, 1980). Woody, Kim and Berthier (1983) showed, however, that a very similar extinction rate could be obtained both with a backward control and with the CS-alone procedures; and only the experimental group showed pairing specific changes in eye-blink responses. Accordingly, doubling the frequency of the UCS, or varying the interval of the CSs and UCSs during the unpaired control procedure does not seem to be critical (Berger & Thompson, 1978; Misulis & Durcovic, 1984).

In conclusion, the pairing specificity observed in the short latency CR should obviously be accepted as an indication of associative learning. Thus, if both short-latency (alpha) and long-latency ("true" CR) learning fulfill the same criteria for pairing specificity, and hence, associative learning, they still can be distinguished on the basis of their temporal features, and possibly by their localization to cortical or subcortical level. This assumption is partly supported by the observations of Woody, Yarowsky, Owens, Black-Cleworth and Crow (1974). The short latency eye-blink CR might be localized and identified primarly as a cortical association mechanism, because Woody and Brozek (1969) found that if the motor cortex of the cat was removed or made inoperative with a spreading depression technique, the short-latency eye-blink CR disappeared.

On the other hand in long-latency experiments the CR has been localized primarly at the brain stem or cerebellar level (McCormick, Lavond, & Thompson, 1983; McCormick & Thompson, 1984). Formally the developing long latency CR fulfills the original Pavlovian criterion: after paired training, the CS elicits a CR which resembles the original UCR. The nature and temporal properties (ISI-learning) of a long-latency CR thus provide a basis for differentiating the alpha and "true" CR. The long latency CRs in eye-lid and nictitating membrane experiments (Gormezano, Kehoe, & Marshall, 1983) indicate learning of the time interval (ISI): CRs follow the length of the interstimulus interval (ISI).

The behavioral conditioned response to the air-puff UCS in these studies, however, is rather similar to the alpha response (i.e., eye-blink) to the tone-CS. According to a prewiring hypothesis (e.g., Woody, 1982a, 1982b, Misulis & Durcovic, 1984) some similarity may be expected but the primary alpha response and the conditioned response need not necessarily be the same. The early classical conditioning experiments of Pavlov (1928) already showed that a dog could learn a salivation CR to the tone-CS. However, it is not easy to see why a primary alpha response to a tone-CS would be salivation, even if higher intensities were used. Thus, it may be concluded that even if the possibility of the prewiring of neural connections is allowed for, there might be differences in the original neuroanatomical closeness or remoteness of the CS and UCS pathway convergence. Depending on the nature of the CS and the UCS and the phase of the learning process, the developing short- and/or long-latency CR may appear almost identical, resemble each other, or be rather different in form and nature.

The learning of time interval (ISI), at least in vertebrates, represents an important and distinctive additional feature in associative learning and may be included in the definition of a "true" CR. Interval learning was not found in the earlier eye-blink studies of Brons and Woody (1980), in which they used a click-CS and glabella-tap UCS only. The eye-lid and nictitating membrane (NM) experiments (Gormezano, Kehoe, & Marshall, 1983; McCormick, Lavond, & Thompson, 1983) have, however, reliably indicated reliable interval learning capacity. Because a delay conditioning paradigm was used in these eye-lid and NM studies it might be suggested that the delay paradigm is superior to the trace conditioning used by Brons and Woody (1980). The experiments of Voronin (1976) involving a rather similar trace paradigm (click or flash CS and a cortical stimulation UCS) seem to support this conclusion. However, there are also examples of interval learning (i.e., long latency CR) of the NM response during a trace paradigm (e.g., Thompson et al., 1982). So, the trace vs. delay distinction obviously does not explain inability to demonstrate interval learning in eye-blink studies, at least exclusively.

In the later studies of Woody (Woody, Kim, & Berthier, 1983; Kim, Woody, & Berthier, 1983) when a hypothalamic rewarding brain stimulation was delivered after the glabella tap UCS as an additional (third) stimulus in a trace paradigm, long-latency CRs did appear; indicating interval learning. Thus it seemed that the original, motivationally rather weak effect of the glabella tap UCS became intensified and produced the long latency CR. Voronin (1976) using a direct cortical brain stimulation as the UCS, also found some signs of the learning of a time interval. Although Voronin (1976) used the same kind of trace paradigm as Woody (1982b), the UCS was probably motivationally more effective because it also elicited a gross leg movement as a behavioral UCR.

In any attempt to identify a short-latency CR and a long-latency CR a recording of the time-amplitude course of a response (neural or behavioral) during the ISI and also during the following UCS-period, especially on omitted UCS trials, appears to be important. The differentation of the specific CS- and UCS-pathway properties additionally at a behavioral level provides further cues for the verification of alpha- and conditioned responses as temporally separate events. The specification of the parameters of behavioral responses is also useful in the validation of the pairing-specific changes of corresponding neural responses.

These features of associative learning are studied in the present paper, which concerns the development of a specific behavioral response (head movement) in a typical classical conditioning paradigm. The head movement was recorded on a video-tape and the movement signal was measured with an accelerometer transducer during unpaired (control) and paired conditioning paradigms in which a tone-CS preceded a positive brain stimulation UCS. Evoked neural responses in cingulate cortex and hippocampus (subiculum, dendate gyrus, CA1 and CA3) were recorded simultaneously. The tone-CS was delivered directly to one (left) ear, thus eliciting an orienting head movement (alpha-response) to the left. The brain stimulation UCS, in turn, elicited as an unconditioned movement a head turn to the right, left or upwards, depending on the site of the stimulating electrode.

The development, maintenance and habituation of the neural and behavioral alpha response and on the other hand, the emergence of long-latency conditioned responses and their interaction were also studied in this paper. More specifically, differentation of a short latency alpha response from a possible long latency CR was attempted using the directionality and the latency of head movements as criteria.

The associative nature of the conditioned alpha response was studied by comparing the performance of each animal during paired and unpaired treatments. The test trials (CS alone), scattered within paired training, were intended to reveal possible behavioral and neural long-latency, "true" CRs. One of the main purposes of this paper was to determine also the time-amplitude course of an evoked response during the CS and UCS period. Multiple unit recordings in eye-lid and nictitating membrane studies in rabbits and cats have shown that a) the long-latency CR first begins to develop at the UCS period, later extending its onset latency towards the CS (on the ISI); and, b) the time-amplitude course of the neural (MUA) response precedes the appearance of the behavioral CR and resembles in form (in hippocampal and cerebellar recordings) the time-amplitude course of the developing CR (Thompson et al., 1984). Corresponding data for the time-amplitude features of evoked responses during omitted UCS trials in classical conditioning is scarce. In stimulus omission trials in a reaction time task Buchwald and Squires (1982) have found in cats a subset of the potentials which were originally produced to the regurally repeated task relevant tones (clicks with 1.5 s ISI). The observed omitted stimulus potentials in such a time conditioning type of situation suggests that similar changes can also be expected during test trials of classical conditioning. This assumption receives additional support from the findings of Voronin (1976) in time conditioning: neural unit responses were produced on the temporal sites of omitted responses.

Differing from earlier approaches, the time-amplitude course of evoked response during the brain stimulation UCS train (1024 ms) was analysed in the present study. Electrical brain stimulation was used as the UCS in this study because a baseline motivational state can be controlled and maintained and the animal does not need to perform any directed or consummatory movements to get the UCS. Each animal served as its own control so that two groups of animals received the paired (CC) and unpaired (CO) treatments in different orders. In the CC-CO order group, the unpaired sessions served as an extinction test indicating possible savings of associative learning after preceding CC sessions. In the CO-CC order group, the order of the treatments was reversed, and this group was expected to show, during unpaired sessions, initial orienting responses (OR) to the CS and UCS and then habituation. In addition, this treatment order should show whether pre-exposure to the CS or UCS facilitates (Mitchell & Gormezano, 1970) or retards (Berthier & Woody, 1984) subsequent paired learning.

Hippocampus and cingulate cortex activity were used in this study as neural correlates of the learning process. Earlier studies (e.g., Thompson et al., 1982) have indicated that a neural template of the CR can develop in the hippocampus before any behavioral CR has appeared. The time-amplitude course of the hippocampal response also shows learning of long-latency CRs, thus indicating interval (ISI) learning. The cingulate cortex was formerly considered as a relevant structure for associative learning and is included as one recording site also in this study. Gabriel, Miller, and Saltwick (1977), for example, have reported short-latency changes in cingulate cortex after paired training.

Earlier observations of occasional instrumental responses during ISIs (Sakurai & Hirano, 1983) and various recordings of "spontaneous" responses during the ITIs of paired and also of unpaired sessions, suggested that these response might represent a) a result of the sensitizing effect of the UCS "species-specific" appetitive or avoidance responses, and/or b) earlier learned associative CRs which are elicited by the environmental, "contextual" cues of the experimental settings. In the present experiment, the specific direction of the head movements to the CS and UCS were intended to reveal the presence of such spontaneous responses which then, in fact, might be considered rather elicited than emitted by the experimental set-up.

4.2 Methods

Subjects

The subjects were six adult cats weighing from 2.4 to 4.0 kg at the time of surgery.

Electrode implantation procedure

The electrodes for the slow potential recordings were made of Trimel insulated nichrome wire of 50 um diameter and the recording tip of the wire was cut transversly with scissors. The details of the electrode construction and implanation procedure are given elsewhere (Korhonen, 1981a, also Appendix I).

The electrodes were implanted during Mebunat anesthesia (40 mg/kg) in a stereotaxic instrument. The locations for the recording electrodes were: subiculum (A5.0, L1.5, H+6, and A5.0, L3.0, H+6), cingulate cortex (A7.0, L1.0, H+10), and dendate fascia (A5.0, L4.0, H+6.5), CA1 (A5.0, L5.0, H+8.5), CA3 (A5.0, L7.0, H+7.5) of the hippocampus.

The bipolar brain stimulation electrodes were made of teflon insulated, 250 um stainless steel wire and the tip of the electrode was exposed to approximately 200–400 um; the tips were separated from each other by approximately 500 um. The electrodes were aimed to four different locations in the lateral hypothalamus (A8.5, L2.5, H-4.5; A8.5, L3.0, H-4.0; A9.5, L3.0, H-3.0; A10.5, L2.5, H-4.5, according to the stereotaxic atlas of (Snider & Niemer, 1961). Two of the brain stimulation electrodes were on the left and two on the right side of the brain. The most effective electrode was used as a stimulating electrode. The indifferent reference electrode for monopolar recordings consisted of three interconnected skull screws. All electrodes were connected to an Amphenol 222-series round connector, which was cemented with screws and dental acrylic to the skull. A flexible, shielded cable connected the animal to amplifiers and stimulation source.

After the experiments the animals were given a lethal dose of Mebunat. The electrode assembly was left untouched and the brains were kept in formalin for a week before the sites of the electrodes were located by cutting slices along the electrode shafts (the tips of the electrodes were compared to the coordinates of the stereotaxic atlas).

Apparatus

During measurements the cats were in a ventilated, electrically shielded box (60 x 48 x 58 cm). The animals were monitored during the experiments through a video monitor and trials were recorded on a videotape for the later analysis of the movements. A miniature earphone was placed on a fixed stand in the dental acrylic of the skull and positioned at a distance of about 2.5–3.0 cm from the left ear of the cat. During trials the earphone delivered as a CS a tone of 1000 cps and 82 dB (A, measured at a distance of 2.5 cm).

The head movements were recorded with a three-dimensional movement transducer based on the magnetosensitive Hall-device (Honeywell, 92SS12-2). The details of this movement transducer construct are described in another paper (Korhonen, 1984, also Appendix II).

The apparatuses used in these experiments included a multichannel measurement system for recording both evoked responses and multiple-unit activity of several brain sites.

The recording equipment consisted of an assembly of eight integrated, low noise preamplifiers (Analog Devices, AD524) which were connected directly to the connector in the acrylic mass on the head of the cat (see Appendix III). The bandwidth of the preamplifiers (DC - 10 kHz) was further divided with filters into slow potential (0.2 - 100 Hz) and to multiple-unit activity (500 - 5000 Hz) bandwidths. Both slow potential and multiple-unit activities were recorded as separate channels, together with the movement signal and timing pulses onto a 14-channel instrumentation tape recorder (Racal Store 14) for a off-line analysis of the recordings in a laboratory computer (PDP 11/34).

The control of the whole experiment was carried out with a microcomputer (ABC-80) which delivered the discrete trials, randomized intervals and the sequences of CS- and UCS-stimuli in the control experiments, controlled the operation of the instrumentation tape recorder (Racal 14) and video-tape recorder, and also generated the isolated brain stimulation pulse trains (Figure 4.1).

The identification data generated by the microcomputer was displayed on the video-screen together with a time-graph display which was used to time the phases of head movements.

Procedure

After a recovery of at least one week, the effect of electrical brain stimulation on the lateral hypothalamus was tested in all of the cats. The stimulation parameters (bipolar pulses, duration 0.5 ms, frequency 100 cps, train duration 1024 ms) were adjusted so that the cats showed

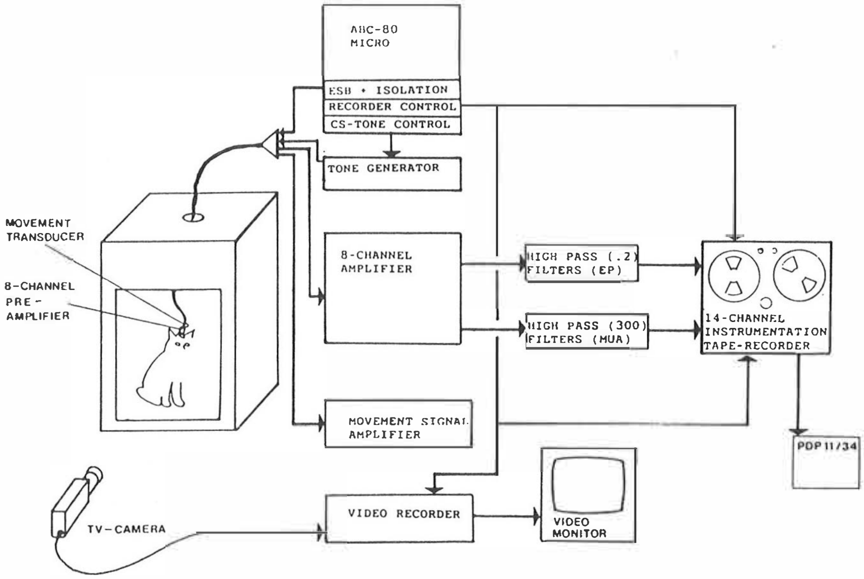


Figure 4.1 The measurement system for the recording of movement and evoked response.

slight orientation and/or approach movements during stimulation. An intracranial self-stimulation test was not used because the learning of bar-pressing movements might have yielded unwanted learned behavioral response sets as a part of the unconditioned brain stimulation effect.

Two groups of animals were used in these experiments. One group first received five paired conditioning sessions followed by five control sessions, and the other group experienced these treatments in reversed order. The control experiments consisted of 48 random CS and 48 UCS presentations. The number of stimuli per sessions, and the length of the sessions was twice as high as in conditioning sessions. The frequency of trials was doubled compared to the paired sessions (ITI was 20–40 s) in order to prevent habituation due to a lower frequency of stimuli. The trials during conditioning sessions utilized a 2048 ms tone-CS overlapping during the last 1024 ms with the brain stimulation UCS. The intertrial intervals varied randomly between 40 to 80 seconds. Each conditioning session consisted of 48 paired and 12 CS-alone (test) trials. Every fifth trial was a test-trial.

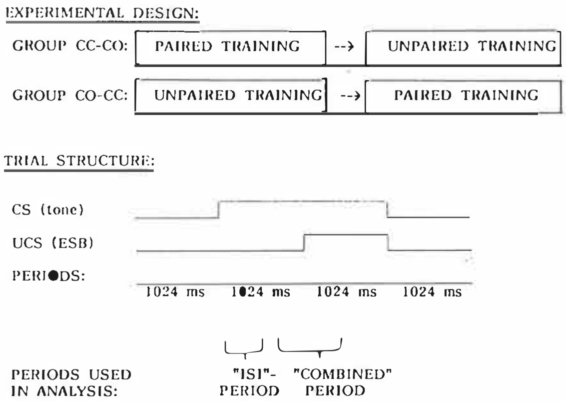


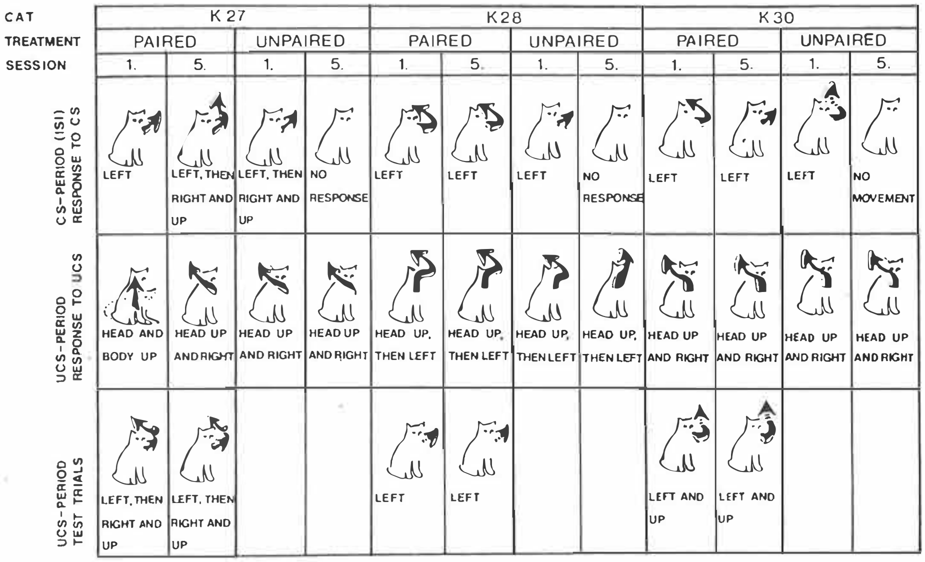
Figure 4.2 The experimental design and the structure of a trial. The ISI period consisted of the 150–450 ms period of the ISI and the Combined period of the 700–1000 ms of the ISI + 150–450 ms of the UCS period.

Data analysis

The recorded slow potential and movement signals were fed into the PDP 11/34 computer through a low pass filter (50 Hz) and digitized at the rate of 125 samples per second. The signal analysis was based on 4096 ms periods which included 1024 ms Pre-CS, 1024 ms CS and 1024 ms UCS, and 1024 ms post-UCS periods.

The movement signals were first rectified and then averaged over trials. A conditioning session was divided into four blocks of 15 trials (excluding the test trials) and then averaged. A separate average was formed of the test trials. In control sessions two averages were formed of the CS-alone trials (48) and UCS-alone trials (48).

Evoked responses were analysed in a similar way, separating the paired trials and test-trials. Within each trial, the alpha-response period (150–450 ms of ISI) and the conditioned response period (700–1000 ms ISI + 150–450 of the UCS-period, here referred to as "combined periods") were defined as critical periods for the development of the alpha-response and the "true" conditioned response respectively (Figure 4.2).



CAT	K 27				K28				K30			
TREATMENT	PAIRED		UNPAIRED		PAIRED		UNPAIRED		PAIRED		UNPAIRED	
SESSION	1.	5.	1.	5.	1.	5.	1.	5.	1.	5.	1.	5.
CS-PERIOD (ISI) RESPONSE TO CS	LEFT	LEFT, THEN RIGHT AND UP	LEFT, THEN RIGHT AND UP	NO RESPONSE	LEFT	LEFT	LEFT	NO RESPONSE	LEFT	LEFT	LEFT	NO MOVEMENT
UCS-PERIOD RESPONSE TO UCS	HEAD AND BODY UP	HEAD UP AND RIGHT	HEAD UP AND RIGHT	HEAD UP AND RIGHT	HEAD UP, THEN LEFT	HEAD UP, THEN LEFT	HEAD UP, THEN LEFT	HEAD UP, THEN LEFT	HEAD UP AND RIGHT	HEAD UP AND RIGHT	HEAD UP AND RIGHT	HEAD UP AND RIGHT
UCS-PERIOD TEST TRIALS	LEFT, THEN RIGHT AND UP	LEFT, THEN RIGHT AND UP			LEFT	LEFT			LEFT AND UP	LEFT AND UP		

Figure 4.3 The directions of head movements in the CC-CO group. The drawings are based on video-tape analyses. The top row shows head movements to the CS during the ISI (1024 ms). The next row shows unconditioned head movements to the brain stimulation UCS on paired trials. The bottom line shows head movements during the UCS period when the UCS was omitted (Test-trials).

4.3 Results

4.3.1 Video-tape analysis of head movements

In video recordings the direction of the head movement was analysed during the CS-period (i.e., ISI 1024 ms) and during the UCS-period (1024 ms).

CC-CO group

Unconditioned head movements to the CS and UCS and conditioned movements during test-trials are shown in Figures 4.3 and 4.4.

These figures show the details of a head movement within each analysed period: within the CS-period, during which the alpha response was supposed to occur; and within the UCS-period (true CR) during which the long latency, "true" CR was expected to appear. The second row in both

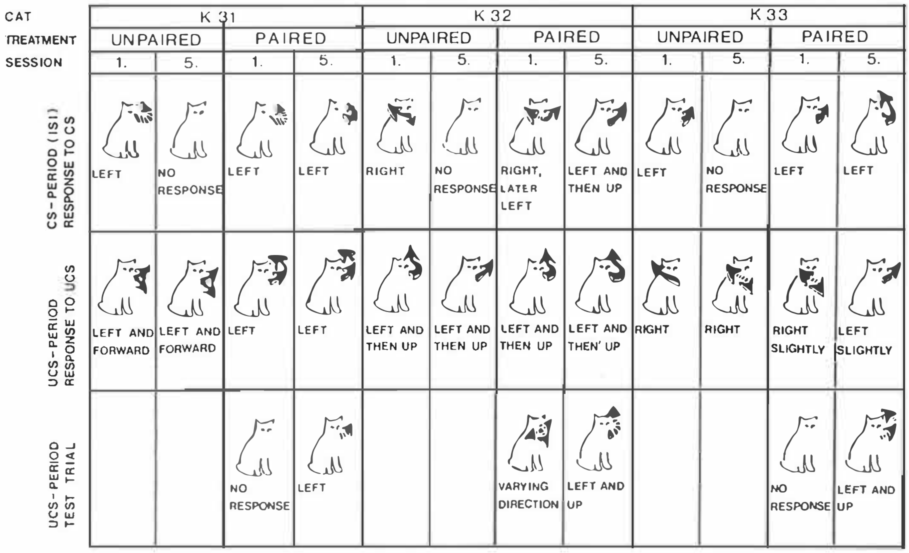


Figure 4.4 The directions of head movements in the CO-CC group. The drawings are based on video-tape analyses. The top row shows head movements to the CS during the ISI (1024 ms). The next row shows unconditioned head movements to the brain stimulation UCS on paired trials. The bottom line shows head movements during the UCS period when the UCS was omitted (Test-trials).

figures shows the shape of the unconditioned response during the UCS-period; and the third row indicates the form of the conditioned response during the same period when the UCS was omitted (test-trials). The cats K27, K28 and K30 belonged to the CC-CO group which first received the paired (CC) and then the unpaired (CO) treatment.

Cat K27. As a response to the CS, this cat turned its head towards the source of the tone (left) from the beginning of paired training. During the final training sessions the response to the CS appeared as a slightly modified head movement to the right and up as an additional component after the initial head movement to the left. During control sessions the head movement habituated.

To the UCS, a head movement to the right and up appeared and remained at the same level through the whole experiment (including unpaired control sessions).

During test trials, a head movement to the right and up gradually developed as a long latency CR. The frequency of these CRs, which appeared only during the UCS-period, was 2 responses out of the 12 possible trials (2/12) in the first paired session and 9/12 on the last paired session. On certain test trials occurring towards the end of the training period, this movement was occasionally observed to occur before the temporal site of the UCS during paired trials as well. Similarly, spontaneous head movements began to appear during the prestimulus period; during which the cat looked up to the ceiling of the cage. These responses were not initially recorded systematically, expect in a case of cat K28, which showed increasingly CR-like spontaneous responses during last paired sessions.

Cat K28. As a response to the CS, a head movement to the left appeared throughout the experiment. During control sessions this movement habituated.

As a response to the UCS, a head movement up and the to the left remained the same throughout the experiment; slight habituation appeared during late control sessions.

During test-trials, a slight head movement to the left occasionally occurred during the UCS-period. The frequency of CR was 2/12 on the first and 1/12 on the last paired session. Spontaneous head movements (a turn to the left) during ITIs already began to occur during the early sessions, and increased in frequency to the end of the experiment.

Cat K30. As a response to the CS, a head movement to the left and slighly up appeared in the training sessions habituating during following control sessions.

A head movement to the UCS was up and right during both paired training and control sessions. On some trials of the later paired sessions this head movement might begin slightly before the UCS.

Head movements to the left and then slightly up occurred occasionally during test-trials and the initial trials of the following first control session. The frequency of CR during the UCS period of the test trials was 2/12 in the first and 3/12 on the last paired session.

CO-CC group

A reversed order treatment was applied to cats K31, K32 and K33: they received first unpaired control sessions and thereafter the paired training sessions (Figure 4.4).

Cat K31. The response to the CS was a head movement to the left at the beginning of control sessions, later this movement habituated. At the beginning of following paired training, this cat began again to turn its head to the left with increasing intensity as training proceeded.

The response to the UCS at control sessions was a head turn to the left and forward; which became modified during paired training so that the forward component disappeared and head movement to the left became more intensive, and was accompanied by a body movement in the same direction. This body movement occasionally occurred later during paired training, before the UCS-period.

During test-trials a head and body movement to the left appeared. The frequency of the CR was 2/12 on the first and 6/12 on the last paired session.

Cat K32. As a response to the CS, varying head movements occurred during the first control sessions, which habituated during the last sessions. At the beginning of the paired training slightly varying movements (left, forward and right) again appeared at first; but were later modified to a head movement to the left and up.

As a response to the UCS, a head movement to the left and up remained throughout control and paired training sessions. The body and head movement up could occasionally occur before the UCS period during later paired sessions.

During the test-trials, a head movement to the left and up appeared with a frequency of 3/12 during the first and 6/12 during the last paired sessions of the UCS-period.

Cat K33. To the CS, a head movement to the left appeared during the first control session; but these movements habituated off. At the beginning of following paired training sessions only a slight movement to the left appeared.

The head movement to the UCS was to the right during control sessions and at the beginning of the paired training sessions which followed; but on the second session it began to change to the left, and remained like that to the end of training.

During test-trials, a head movement (continuing after the CS-response) to the left appeared at a frequency of 1/12 during the first, and 5/12 during the last paired session.

The response to the CS (alpha-response)

The alpha-response to the CS was in the direction of the tone source (i.e., to the left) for all the cats except K32. Cat K32 (control sessions first) initially showed varying head movements to the left, to the right and slightly up. During paired conditioning these head movements gradually changed, first as a slight turning to the left and at the end of the movement sequence, also upwards.

The response to the UCS and the responses during the UCS period (test-trials)

The unconditioned response to the brain stimulation UCS was either a horizontally directed movement to the left or right or a vertical movement upwards or some combination of these movements. The unconditioned response pattern remained quite steady throughout the experiment (and during control sessions). The brain stimulation-UCS was delivered either to the left or right side of the hypothalamus, and in most cats (K27, K28, K30, K31, K32) the direction of the unconditioned movement appeared opposite to the stimulated hemisphere. In cat K33 a head movement appeared in the direction of stimulated side of the brain in the first session; in the last session the direction was reversed to its opposite.

The responses occurring during the UCS-period of test-trials (CS-alone) during paired training (CC) indicated that as a conditioned movement pattern a response similar to the alpha-response appeared at the beginning of training. During the last sessions, the conditioned response changed if the direction of the original alpha-response to the CS and the unconditioned response pattern to the UCS were different. The response pattern of the UCS thus became a determinant of the CR.

In this experiment, the criterion for the identification of a "true" CR was based on,

a) the specific direction of the head movement

b) the appearance of this head movement within the UCS-period of the test-trials (CS-alone); thus indicating time-interval learning,

c) the frequency of the CR appearing during this period.

The direction criterion could not be used reliably in cats K28 and K31 because the shape and direction of the head movement appearing in the UCS-period was also rather similar to the alpha-response. However, time-interval learning could be established, and the frequency criterion also indicated learning effect.

The latency of the conditioned movement

The conditioned head movement in the test-trials of paired sessions occurred mostly during the UCS-period, but on some trials during the later training sessions the head movement might appear slightly before the UCS site. Such "anticipatory" movements occurred during the final paired sessions in cats K27, K30, K31, and K32.

Spontaneous responses during prestimulus periods and interstimulus intervals (ITIs)

The experiment was not designed to make a systematic recording of

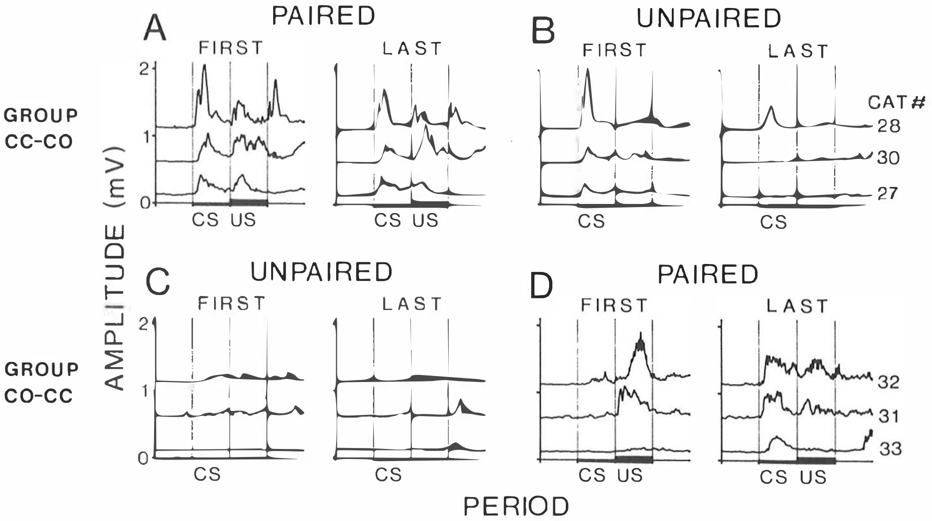


Figure 4.5 Averaged movement transducer recordings of head movements in the CC-CO group (top row) during paired (A) and CS-alone trials of unpaired control treatment (B) and in reversed order in the CO-CC group (bottom row) during unpaired (C) and paired treatments (D) over daily sessions.

spontaneous head movements. Spontaneous head movements resembling the unconditioned responses were detected during recordings in some cats (K27 and K28) during the prestimulus interval. Because the video-tape recordings were made automatically, and only a short prestimulus and trial periods were recorded, the amount and frequency of the spontaneous activity could not be defined at the beginning of the experiment. Only in a case of cat K28 was a recording of spontanous responses occurring during ITIs made during the final sessions. This recording revealed the occurrence of rather frequent and sharp head movements resembling the UCS-response pattern or alpha pattern (both to the left).

4.3.2 The head-movement transducer analysis

The shortest onset latency of the head movement from the beginning of the CS was measured in cat K28 (40–50 ms, see Figure 4.5).

The onset and peak latency varied in different cats: some cats showed sharp, short latency movements and some produced slowly rising responses.

The curves of the averaged movement signal of the head-movement

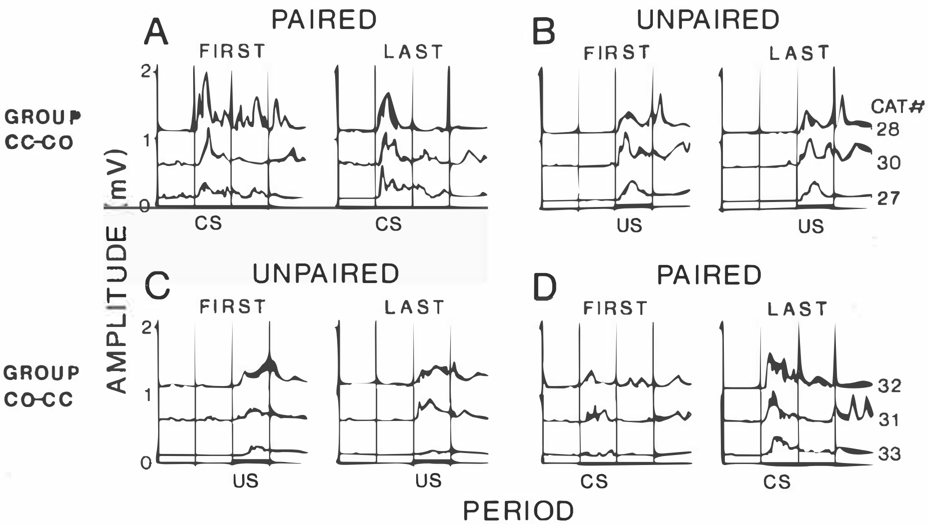


Figure 4.6 Movement transducer curves of the head movements as in Figure 4.5, but during Test-trials of paired sessions (A and D) and during UCS trials of unpaired (CO) sessions (B and C). A and B are for the CC-CO group, and C and D for the CO-CC group.

transducer are presented in Figures 4.5 and 4.6 in the same order as the treatments were delivered to each group (e.g., CC-CO and CO-CC). In Figure 4.5 the curves of the paired trials of the first and the last paired session (A), and the CS-alone trials of the first and the last unpaired session (B) of the CC-CO group are presented. Correspondingly, the movement signal curves for group CO-CC are given in C (unpaired sessions) and in D (paired sessions).

Alpha-response period: Group CC-CO

During the alpha-response period (150–450 ms ISI) of paired trials of the CC-sessions the amplitude decreased (habituated) slightly after the initial orienting phase (Figure 4.5). Further, at the beginning of each daily session the initial sensitization (orienting) phase appeared as a temporary increase in movement activity in the alpha-period of the first trials, habituating in later trials and increasing again during the last trials (Figure 4.7). During the CO-sessions the amplitude of the alpha-responses fell away to virtually nothing (habituated, Figure 4.5B).

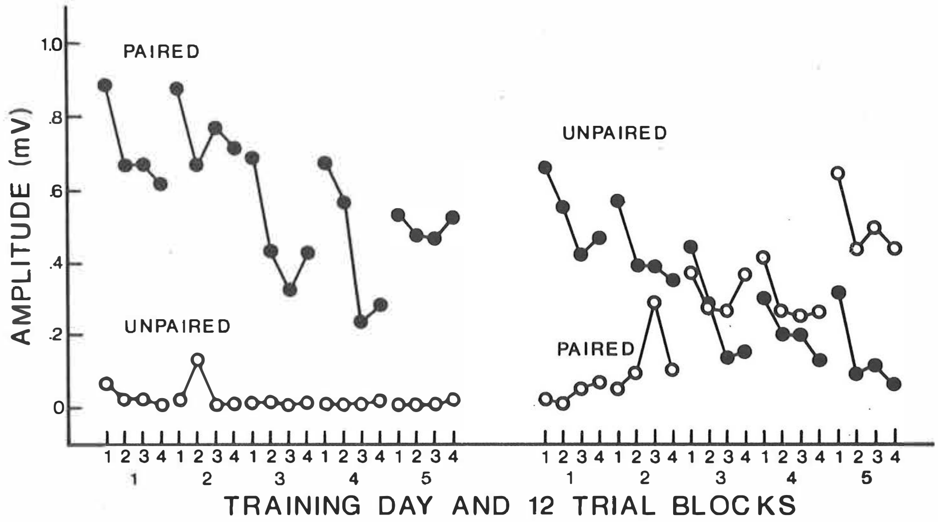


Figure 4.7 Changes in the intensity of the head movement in the CC-CO group (filled circles) and in the CO-CC group (open circles). This figure shows the initial sensitization within sessions during the first quarter (block = 12 trials) of a session. The CC-CO group shows stronger responses during the first block habituating considerably during later blocks. The CO-CC group acquired sensitization slowly during paired sessions.

Alpha-response period: Group CO-CC

In this group, the experiment began with unpaired control sessions during which the alpha-response habituated rapidly (in 10-20 trials, Figure 4.5C). During the following paired training (CC-sessions) the alpha-response amplitude recovered rather slowly: the initial orienting response to the CS did not appear until about the third paired session (Figure 4.5D, and 4.7).

Combined period: Group CC-CO

The UCR-amplitude changed slightly: some habituation could be observed during CC sessions (Figure 4.5A), but not during subsequent unpaired (CO) sessions (Figure 4.6B). Conditioned responses occurred in all cats during the test-trials (CS-alone) of paired sessions in the group CC-CO (Figure 4.6A). These cats also showed some transfer from paired training sessions to the first trials of the following unpaired control (CO) sessions but during the last paired sessions extinction was complete (Figure 4.5B).

Combined period: Group CO-CC

An interesting interaction between the CS and UCS can be observed in paired sessions of the CO-CC group (Figure 4.5D): first a strong sensitization to the UCS (compare 4.5 D to 4.6 C) which later slightly habituated while the amplitude of the alpha-response amplitude increased strongly. Thus the paired presentation of the CS and UCS after unpaired presentation seem to have a suppressive effect on the amplitude of the UCR. During the preceding unpaired sessions (Figure 4.6B) the amplitude of the UCR remained at about the same level over the sessions. Some conditioned responses occurred during the test-trials in this group.

The analysis of the movement transducer data

An average movement signal for both groups within sessions and over sessions for the short-latency (alpha) response are shown in Figures 4.7 and 4.8, respectively; and over sessions for the combined period (the long-latency response) in Figure 4.9.

The movement activity during paired sessions is presented in curves A and D; and during unpaired sessions, in curves B and C. The curves A and B represent data for the CC-CO group and curves C and D, for the CO-CC group.

The intensity of the short-latency head movement was greater during paired than the unpaired sessions: the analysis of variance (treatment order x treatment x sessions) indicated a significant difference, F(1,4)=58.64, p<.01 (The Geisser-Greenhouse conservative F-test was used in all analyses.) During the first phase of the experiment (both groups naive) the CC-CO and CO-CC groups (curves A and C in Figure 4.8) showed significant differences, F(1,4)=10.82, p<.05. The short-latency response to the CS habituated completely during the unpaired sessions of the CO-CC group (C). By contrast, the CC-CO group showed an abrupt increase in the intensity of the alpha-response (Figure 4.8A). During the following paired sessions, however, this level slightly decreased; showing significantly different acquisition in the CC-CO group compared to the paired sessions of the CO-CC group. A significant interaction between treatment and sessions supported this observation, F(1,4)=7.57, p<.05. The acquired increase in the intensity of the short-latency conditioned response during paired sessions of the CC-CO group habituated to a practically zero level during subsequent unpaired sessions (Figure 4.8.B): the difference between treatments (A and B curves in Figure 4.8) was significant, F(1,2)=578.04, p<.01.

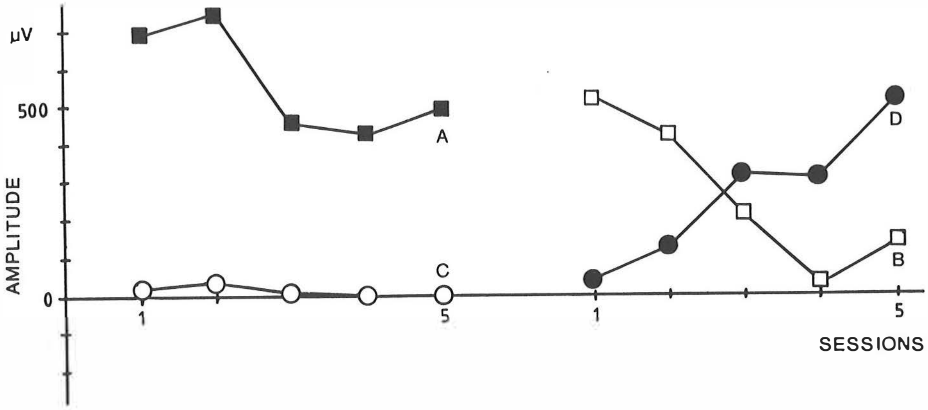


Figure 4.8 Changes in short-latency (150–450 ms ISI) head movements over sessions for the CC-CO group (A and B), and for the CO-CC group (C and D). A and D are for the paired training, and C and B are for the unpaired training.

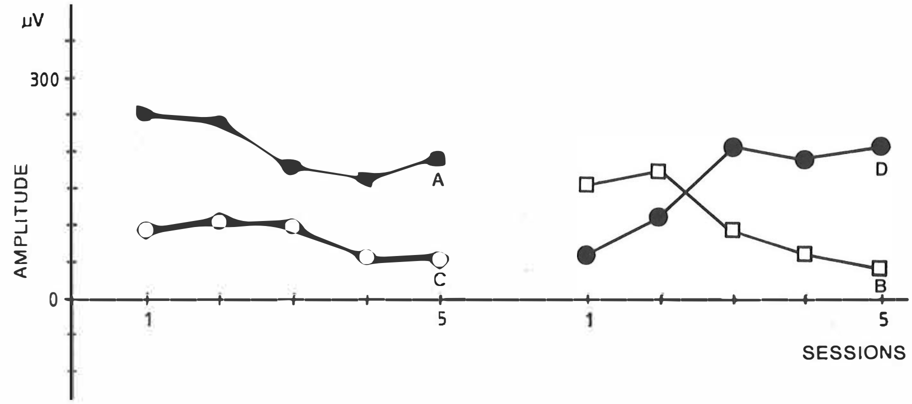


Figure 4.9 Changes in long-latency (Combined period) head movements during the Test-trials of paired sessions (A and D) and during the CS-alone trials of unpaired sessions (B and C). A and D are for the paired training, and C and B for the unpaired training

The long-latency conditioned response during the combined period showed much the same overall changes as the short-latency CR; although the amplitude of the long-latency CR was smaller. The groups CC-CO and CO-CC did not differ during the first phase of the experiment (A and C curves in Figure 4.9) but showed significant interaction during the second phase, F(2,7)=5.66, $p<.05$ (curves B vs. D): the CC-CO group habituated while the CO-CC group showed increasing acquisition.

4.3.3 The analysis of the evoked responses

The neural correlates (evoked responses) were recorded in different brain locations in order to compare changes in neural responses to behavioral responding. The verification of the location of the recording electrode sites showed that in the subiculum, CA1, and CA3 of the hippocampus and cingulate cortex comparable registrations could be found in at least two cats in each treatment group (see Table 4.1).

Table 4.1 Location of the recording electrodes for each cat

Recording site	K28	K30	K31	K32	K33
Presubiculum	X	X	X		X
Subiculum	X	X	X		X
Cingulate	X	X	X	X	
CA1	X	X	X	X	

The possibility of a movement artefact in evoked potential recordings was studied by comparing onset latencies in movements and evoked recordings. The shortest latency in head movement to the CS was in cat K28. The corresponding change in evoked response in CA1 in this cat is shown in Figure 4.10; indicating that evoked response began about 30-40 ms earlier than movement.

Alpha-response period

The results of the 150-450 ISI period showed that after the initial sensitizing effect (high level in negativity), a slight decrease usually appeared in paired training sessions of the CC-CC group in all recording sites, and that negativity might begin to increase again in last sessions. During the following unpaired control sessions this group showed extinction in

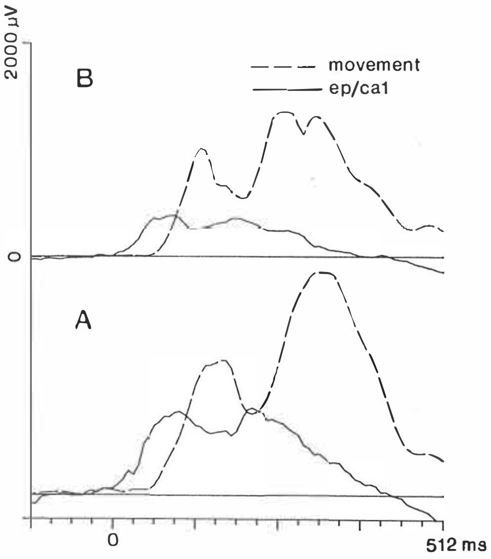


Figure 4.10 A comparison of onset latencies of the head movement and neural evoked responses in cat K28 to the tone-CS during the first (A) and last (5.) paired session.

negativity; approaching the zero potential level in the final sessions. The CO-CC group showed habituation during unpaired sessions while during the following paired sessions negativity began to increase.

A specificity for paired treatment was found in both groups (CC-CO and CO-CC). In the cingulate cortex, an analysis of variance (treatment order x treatment x sessions) showed that negativity was greater during paired conditioning, when averaged over treatment order, compared to the unpaired treatment. The main effect of the treatment was significant, $F(1,2)=10.05, p<.05$ (Figure 4.11).

Pairing specificity in the hippocampal CA1 was also verified. The analysis of variance (treatment order x treatment x sessions) indicated that the effect of paired treatment was significant compared to the unpaired treatment, $F(1,2)=19.16, p<.05$ (Figure 4.12).

Here again, greater negativity appeared during paired treatment.

In CA3, a significant difference between the paired and unpaired treatments was found only in the CC-CO group, $F(1,1)=182.05, p<.05$ (Figure 4.13); which again showed greater negativity during paired conditioning. During the second phase of the experiment, the CO-CC group showed in the CA3 an increase in negativity on paired sessions (D) while the CC-CO group showed extinction (B) on unpaired sessions

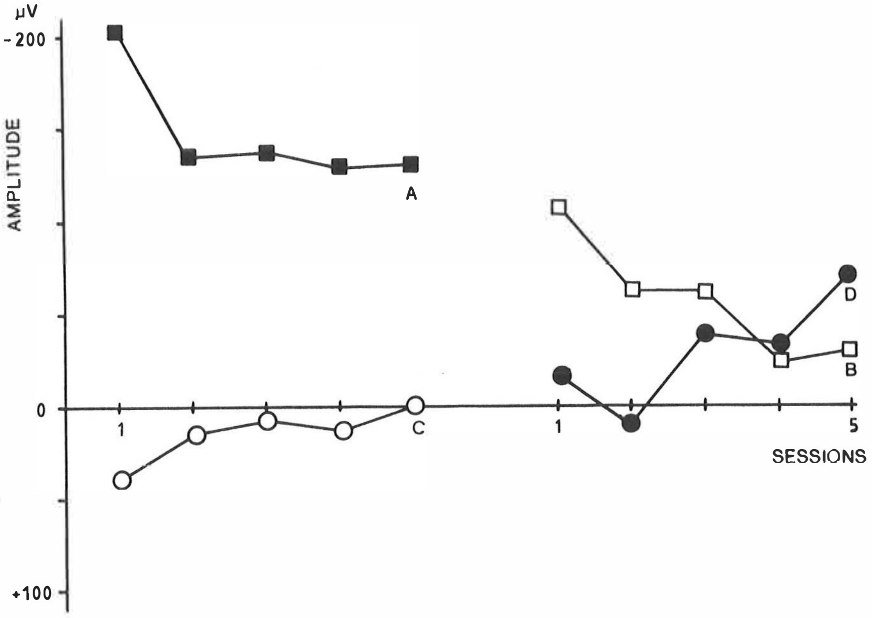


Figure 4.11 Short-latency evoked response session curves over paired sessions (A and D) and during unpaired sessions (B and C) for the CC-CO and CO-CC groups in cingulate cortex.

which was evident from the significant treatment x sessions interaction, F(2,4,)=7.44, $p<.05$.

If the change in the 150–450 period of the ISI is considered representing the pairing specific, short-latency CR, then these results show that the short-latency CR appeared as a negativity during the paired sessions in the cingulate cortex, CA1 and CA3 recordings (Figures 4.11, 4.12, and 4.13); while during the unpaired control sessions the negativity decreased, approaching zero level in all recording sites.

Combined period

The changes in the combined period (700–1000 ISI + 150–450 UCS) during the test trials of the paired sessions were studied in order to examine a possible difference in the potential level compared to the CS-alone trials of the unpaired control sessions. The observed changes in the combined period were opposite in polarity compared to the changes in the 150-450 ISI period. The initial positivity decreased to zero potential level during unpaired control sessions, but remained the same or slightly increased in paired sessions after initial habituation. A significant difference between

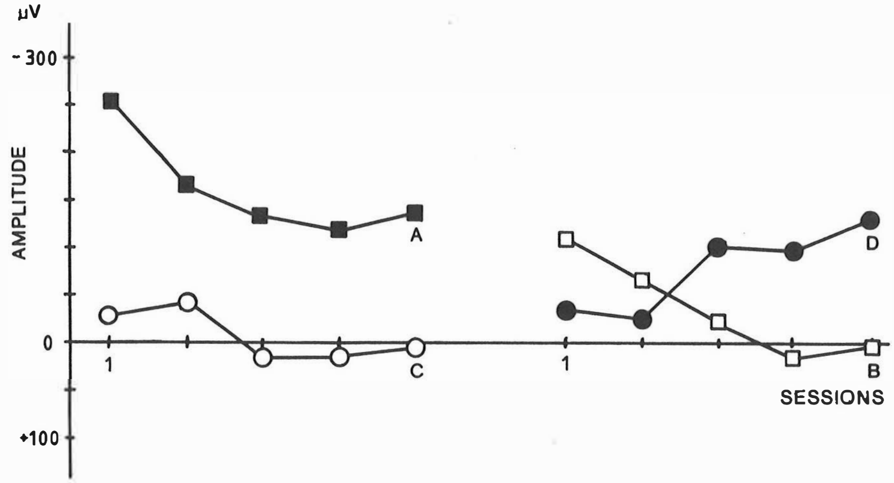


Figure 4.12 Short-latency evoked response session curves over paired sessions (A and D) and during unpaired sessions (B and C) for the CC-CO and CO-CC groups in CA1 of hippocampus.

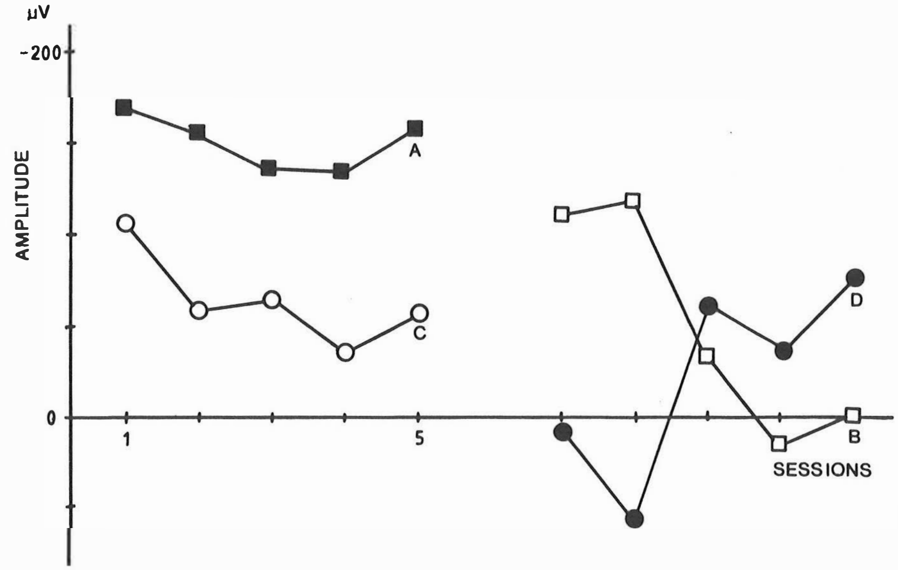


Figure 4.13 Short-latency evoked response session curves over paired sessions (A and D) and during unpaired sessions (B and C) for the CC-CO and CO-CC groups in CA3 of hippocampus.

the paired (A) and unpaired (C) treatments during the first phase of the experiment was found in the cingulate cortex, F(1,2)=49.95, $p<.05$ (Figure 4.14), and in CA3, F(1,2)=20.75, $p<.05$ (Figure 4.15).

In CA3 recording of the CC-CO group, the positivity acquired during paired sessions (A) extinguished during the following unpaired sessions (B), F(1,1)=162.82, $p<.05$. The changes in the combined period of CA1 showed similar, although not statistically significant changes.

The results show that during the combined period, the polarity and changes in evoked responses were approximately a mirror image of the changes in the 150–450 period of the ISI (Figures 4.11, 4.12 and 4.13). The changes in the evoked responses during the combined period showed similar differences in different treatment order groups (CC-CO and CO-CC) as in the 150–450 ms ISI period. Again, in the CC-CO group, after an initial sensitization (increased positivity) to the CS during the first paired sessions, a slight habituation (decrease in positivity) appeared in cingulate, CA1, and CA3. During the last paired sessions positivity did not increase, but remained at about the level of earlier sessions or even slightly decreased. However, during the following unpaired sessions the positivity decreased, approaching the zero potential level in all recordings in the CC-CO group. Correspondingly, during unpaired sessions of the CO-CC group (C), the slight initial positivity (sensitization effect) habituated quickly, and the positivity changed in the direction of negative polarity. During the following paired sessions (D), in the CO-CC group, this decrease in positivity changed rather slowly (in the 2. or 3. session) to an increase in the last sessions.

4.3.4 The time-amplitude characteristics of evoked responses

Short-latency changes: alpha-response period

The features of the time-amplitude course (topography) of each evoked responses are shown in Figures 4.16–4.21; in which the curves of the paired conditioning trials, test-trials during the paired sessions, and CS-alone trials of the unpaired control sessions are presented as three-dimensional surfaces. In these figures, the features of the alpha-response period and the combined period can be seen as changes taking place over sessions. Depending on the order of the treatment (CC-CO or CO-CC group) a different type in development of the alpha-response appears. The negative response (150–450ms) to the paired representation of the CS and UCS in the CC-CO group is initially large, then habituates slightly and finally increases again during the last sessions. In the CO-CC group, the

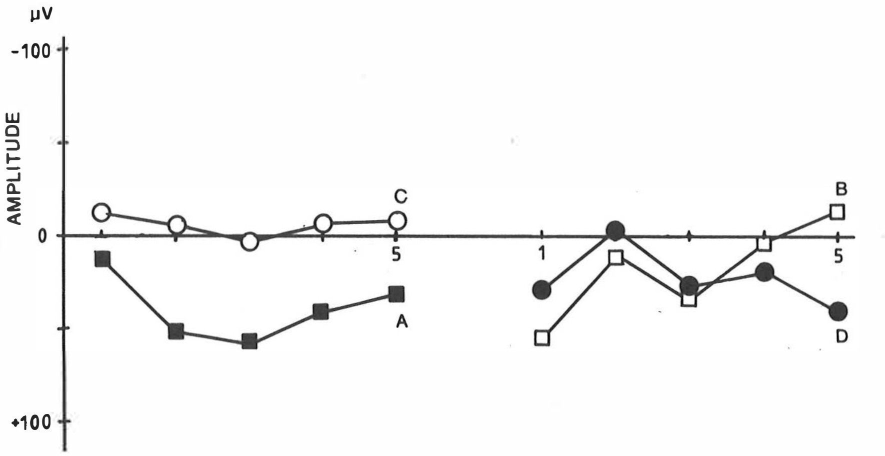


Figure 4.14 Long-latency evoked response (Combined period) in cingulate cortex.

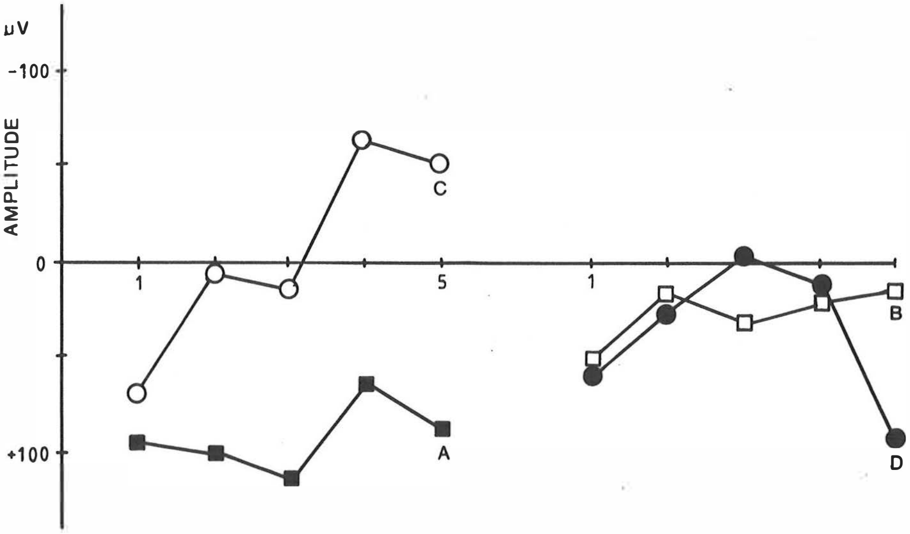


Figure 4.15 Long-latency evoked response (Combined period) in CA3 of hippocampus.

amplitude of the alpha-response increases only slowly after the habituation which takes place in the preceding unpaired control treatment.

Long-latency changes: conditioned response period

In an attempt to identify changes in the conditioned response (combined period) the test trials (every 5th in paired sessions) were averaged at each session (Figures 4.17 and 4.21). Some correspondence between the course of an evoked response during the unconditioned response period (UCS-period) and evoked response during the test trials of the same sessions was observed in cats K28, K30, K31, K32 and K33, especially in the cingulate cortex, although these responses appeared somewhat delayed. Cat K28 belonged to the CC-CO group; and thus the curves of the first unpaired trials (CO) show some savings in the time-amplitude course of the test-trials of the preceding paired training sessions in the short-latency response (Figure 4.18).

4.4 Discussion

In this study, both a short-latency alpha-response and a long-latency conditioned response were supposed to show nonassociative plastic properties (habituation and sensitization) within each "pathway"-system (CS and UCS) as such, and on the other hand, as a result of the their convergence, the development of a conditioned alpha response and of a "true" conditioned response, both of which could be identified according to their latencies and nature. The CS was selected so that it repeatedly elicited (before it habituated during unpaired sessions) an identifiable orienting alpha-response (head turn to the left), the occurrence of which could be identified according to its specific features also during the intertrial intervals, providing that such "spontaneous", "instrumental" responses, did indeed (begin to) appear.

4.4.1 Short-latency responses

The experiment reported in present paper showed an increase of the alpha-response (head movement to the tone-CS). Because the order of treatments was controlled, a differential development of the conditioned alpha-response could be observed. As a result, the paired-unpaired (CC-CO) order group showed rather strong sensitization during first paired trials: the sensitization (orienting) effect of a new CS and the new UCS obviously accumulated during the first trials eliciting strong head movements to the tone-CS. After the initial novelty of the CS had worn off, a slight decrease (habituation) was observed both in the behavioral

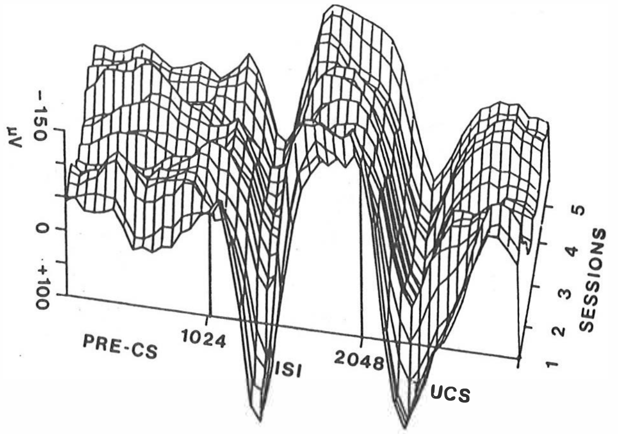


Figure 4.16 Changes in the time-amplitude course of evoked responses in cingulate cortex of cat K28 during paired sessions. The first block of the first session is the foremost curve (each curve is an average of 12 trials).

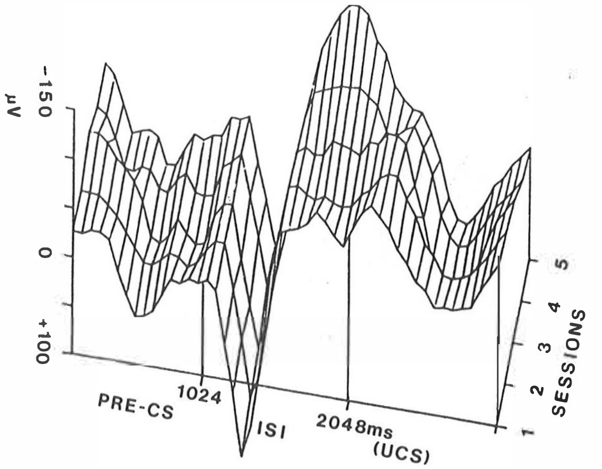


Figure 4.17 The time-amplitude course of evoked response in the cingulate cortex of cat K28 during the Test-trials (CS-alone) of paired sessions. Cat K28 belongs to the CC-CO group.

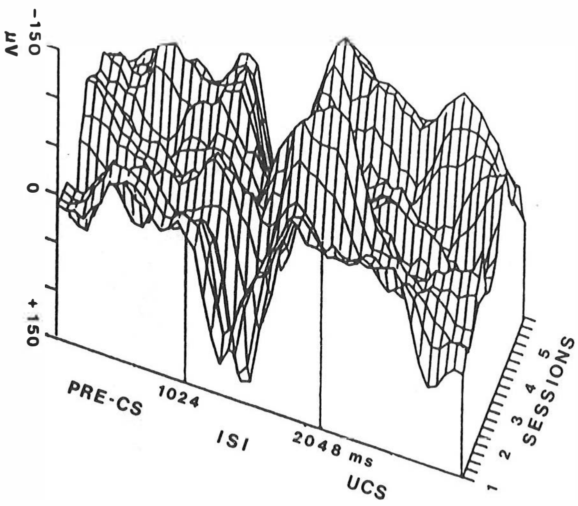


Figure 4.18 The time-amplitude course of evoked responses of the cingulate cortex of cat K28 during CS trials of unpaired (CO) sessions shows retention of acquired changes and subsequent extinction.

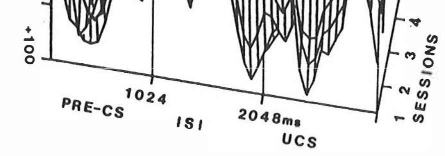


Figure 4.19 The time-amplitude course of evoked neural (cingulate cortex) response during unpaired sessions in cat K32 belonging to the CO-CC group. This cat first received unpaired sessions, and showed rapid habituation to the CS presentations.

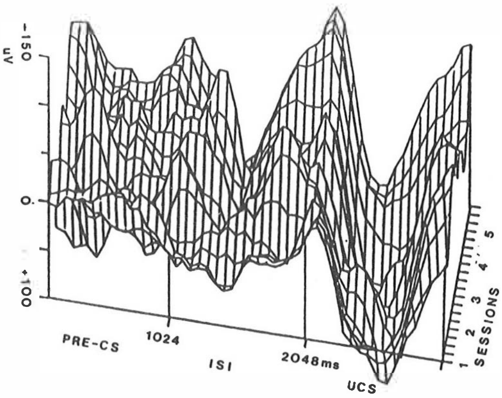


Figure 4.20 The time-amplitude course of cat K32 during paired trials of the paired (CC) sessions. The acquisition of a short-latency conditioned response to the tone-CS is slow in the CO-CC group compared to cat K28 (Figure 16), which belonged to the CC-CO group.

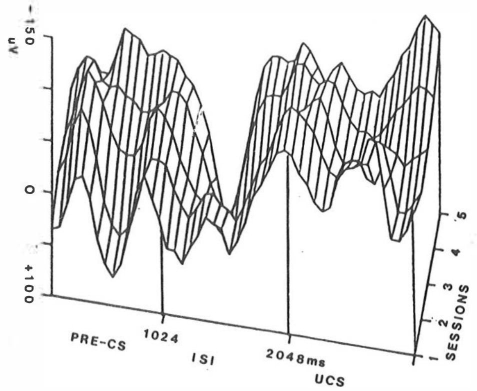


Figure 4.21 The time-amplitude course of evoked responses of the cat K32 during Test trials of paired (CC) sessions.

and also in neural responses. However, during the last sessions of the paired (CC) training, the associative effect of the paired presentation of the CS and UCS became obviously effective, and the amplitude of the alpha-response showed signs of an increase or at least remained at the previous level, while on the following unpaired (CO) sessions both the behavioral and neural responses extinguished to practically zero level. Sundberg (1974) has observed a similar correspondence. He found that the alpha-response to a brain stimulation CS decreased (habituated) after an initial orienting effect; while an avoidance (one-way) CR developed.

In the present study, the other experimental group (CO-CC group) received first the unpaired sessions and then paired training sessions. In this group, the initial orienting response to the CS was modest and habituated rather rapidly (during first 10–20 trials); compared to the strong and sustained alpha-response in the CC-CO group. More interesting was the development of the conditioned alpha-response during paired sessions following the unpaired control sessions. In behavioral responses, an increase to the UCS appeared first and thereafter the alpha-response showed signs of recovery (dishabituation); while the amplitude of the UCR slightly decreased (probably the orienting component habituated, Figure 4.5D)

4.4.2 Long-latency responses

Behavioral responses during the combined period showed that in three cats (K27, K30, K32) a component of the original UCR could be identified on CS-alone trials indicating time interval learning and "true" CR. Cat K28 did not show increases in the behavioral long-latency CR development. In cats K28 and K31 the UCR and the response to the tone-CS had similar directionality (to the left) and thus differentation of the alpha and CR on the basis of their nature was difficult. However, signs of ISI learning also appeared in these cats in movement transducer curves (Figures 4.5 and 4.6).

The effect of the UCS and CS in determining the directionality of the CR seems, according to these behavioral results, to be somewhat variable. Cat K33 showed that the original direction of the head movement to the UCS can become modified during learning. One explanation for this change might be that the intensity of brain stimulation applied to this cat was initially set too weak. Observations of the unconditioned evoked response amplitude and head movement transducer curves (Figures 4.5 and 4.6) during the UCS period support such an assumption.

The time-amplitude course of both the behavioral and neural responses in the present experiment seems to indicate that the form of the conditioned response, as well as the signs of time-interval learning can be used as necessary criteria in an attempt to differentiate changes occurring in a neural pathway system itself (CS- or UCS-pathway) from the changes in which one pathway system begins to activate another pathway system. In the earlier studies of multi-unit activity in rabbits and cats, the occurrence of the conditioned behavioral and neural responses has been shown closely to follow closely time-interval (ISI) between the CS and UCS; and the form of the conditioned response has been found to correspond to the form of the UCR (e.g., Thompson et al, 1982). However, in the nictitating membrane and eye-lid conditioning experiments, the time-amplitude course of the CR during the UCS-period has not usually been reported; although test-trials (CS-alone trials) have been performed. The appearance of the CR has been based on the observation that after learning, the specific time-amplitude course of a neural (or behavioral) response during the UCS-period, initially determined solely by the UCR, changes after learning so that the amplitude, onset latency, and certain specific features (e.g., bimodality) of this response curve appear in a form different from that observed in the original response. An exception is the study of Martin, Land and Thompson (1980) in which test-trials were also presented. These curves seem to show that the time-amplitude course of the neural CR also indicated time-interval learning as the earlier studies of Gormezano (e.g., Gormezano, Kehoe, & Marshall, 1983) had shown in behavioral NM-response development.

In the present study, observations of the properties of the time-amplitude course of evoked responses showed some correspondence between evoked responses of the omitted UCS-periods of the test trials and the UCRs of the paired conditioning trials. An appearance of the "true" CR located to the combined period (700+1000 ms during ISI period + 150–450 during UCS period) was found in most cats at behavioral and neural levels. Similar features appeared in the cingulate cortex of the cats K28, K31, K32 and K33, and in the CA1 of the cats K30, K31, and K33, and in CA3 in the cat K28. However, the complexity of an evoked response curve (differences in waveform and polarity) compared to a multi-unit recording (a frequency histogram curve) makes the critical, learning-specific changes difficult to identify.

Some earlier observations have shown that simultaneous measurement of the evoked response and multi-unit activity indicate that these two measures can closely correspond to the initial phases (components) of evoked responses (Rebert, 1976a). Although the change to a direction of positive polarity seem to accompany a decreased multi-unit activity (Rebert, 1977), the causal relationship between these measures is not direct, and there are differences in their specific dynamics. The continuation of evoked responses after the cessation of a cellular discharge suggest that these responses may differ in origin (Rebert, 1976b). The unit discharges are rapid bursts which cease abruptly, whereas evoked responses are prolonged and may reflect some combination of field potentials generated by neurons and their interaction with surrounding glial cells (Rebert, 1977). Thus the changes in evoked responses do not easily show signs of the time-amplitude course of the CR. In the multi-unit studies of nictitating membrane/eyelid conditioning, the unit activity increased in the proximity of the UCS. In evoked responses, a corresponding change can be either an increased or decreased positivity/negativity or some more complex fluctuation. Earlier conditioning studies of evoked responses have been based on the analysis of the ISI-period only, the test-trial approach has not been included, or used. Analysis of the ISI-period has shown changes in early or late evoked neural components or changes in overall negativity or positivity (John, 1967; Chiorini, 1969; Pinto-Hamuy, Bracchitta, & Lagarrigue, 1969; Rebert & Irwin, 1969; Irwin & Rebert, 1970; Rebert, 1972, 1976a, 1976b, 1977; Begleiter & Platz, 1969; Macar & Vitton, 1980; Pirch, Corbus, & Rigdon, 1983).

If time-interval learning is defined as an essential property of a "true" conditioned response (at least in vertebrate learning) then the analysis of the UCS-period of the test-trials (CS-alone) becomes important. If evoked changes are registrated only during the ISI period, the observation may partly reflect the subject's learning of a time-interval. For example, Pirch (1977a, 1977b) reported that surface-negativity occurred near the onset of the second stimulus, S2, but because the analysis covered the ISI only, the time-amplitude course during the UCS-period remained unclear. Some evidence of time-interval learning is shown in the study of Buchwald and Squires (1982). They presented rare (deviant) stimuli (probability was .15) randomly with frequent (standard) stimuli using a fixed ISI (1.5 s) paradigm. During additional sessions, stimulus omission was substituted for the rare stimulus. This arrangement is in a way comparable to a test-trial (UCS omitted) in classical conditioning, similarly indicating learning of a time-interval (fixed ISI in this case). The results of the Buchwald and Squires study (1982) showed that evoked responses also occurred on omitted trials, but the first (exogenous?) components were missing, and the whole response was delayed some tens of milliseconds compared with the response to a standard stimulus. An enhancement of positivity appeared between 200–500 ms, and although the time-amplitude course of the evoked response varied somewhat between cats it was replicable within cats. The results seemed to show that some subset of the potentials was produced by the stimulus omission.

In the present study, the evoked changes during the UCS-period of the test-trials showed some similarity within cats over trials and sessions (Figures 4.17 and 4.21). In addition, there appeared also some similarity with the UCR features also appeared in several cats and the occurrence of these changes during test-trials seemed to be delayed compared with the

UCR of the paired trials.

4.4.3 Classical-instrumental relationship

Another interesting finding in the present experiment was the appearance of "spontaneous" head movements during the intertrial intervals. The increasing frequency of these responses towards the end of the paired training can be interpreted as an indication of the sensitization effect of the UCS and probably also of the CS (after paired learning). These "spontanous" responses can be equated with instrumental responses: they are "emitted" rather than "elicited". Because such responses may also occur during ISIs (if ISI is long) they can act as a "CS" in a response-reinforcer type association (Mackintosh, 1974; Mackintosh & Dickinson, 1979). An example of a "controlled" emission of the instrumental response was the head movement of a rat towards the tone source in the study of Sakurai and Hirano (1983). Although the emitted head movement in their experiment served thereafter as an instrumental response for the following "reinforcement", the authors did not explicitly recognize the similarity of the observed "instrumental" learning (alpha-response – reinforcement) to the classical contingency (tone-CS – UCS). "Spontanous" responses have also been found in some other studies during the control sessions. Sheafor and Gormezano (1972), for example, reported increased spontaneous jaw movements in rabbits on unpaired control sessions; and Woody, Yarowsky, Owens, Black-Cleworth and Crow (1974) found an increase in spontaneous eye-blinking during ITIs of paired training.

In the present study, the source of spontaneous responses could be identified on the basis of the alpha- or conditioned responses (head movement in a specific direction). From the point of view of a naive observer (if he were not allowed to hear the tone-CS) the cats seemed simply to perform now and then instrumental head movements which then were "reinforced" with a positive brain stimulation UCS. Thus the nature of the association (CS-UCS or alpha response - UCS) seems to be rather parallel both in "instrumental" and classical conditioning: the main process involved in the brain is apparently an associative contingency between two neural representations (i.e., the tone and brain stimulation effects). The evidence presented in this study is, however, suggestive; more careful analysis of spontaneous movements appearing during ITIs is needed.

4.4.4 The characteristics of the UCR and CR

The results concerning evoked potentials, together with behavioral response data seem to indicate the development of a long-latency conditioned response at the UCS period (appearing as increased positivity in evoked activity) as a result of paired training. It appeared that the behavioral counterpart of this response also included some specific features of the unconditioned response system. Such long-latency conditioned responses, if compared to the original unconditioned responses, were weaker and did not occur on every test-trial (omitted UCS). It is possible that an increase in daily training sessions would have been more effective for the elicitation of more pronounced CRs. On the other hand, it is probable that the amplitude or frequency of long-latency CRs never attains the original unconditioned response values.

4.4.5 CS–UCS interaction

In addition to the interaction of the time-amplitude characteristics of evoked responses during the UCS and CS periods in this experiment, there was some evidence of interaction between the intensity of the behavioral responses to the CS and the UCS. This appeared in two ways: a) in the initially habituated group (CO-CC group) the first behavioral indication of paired learning was an increase in the behavioral UCR amplitude (see figure 4.5 D and 4.6 C) after which the amplitude in the alpha-response period began to increase, and b) while the amplitude of the alpha-response increased during paired training, the amplitude of the UCR seemed to decrease. Kimble and Ost (1961) have reported a similar interaction effect in their study of the conditioning of the human eye-lid.

An increase in the CS amplitude as a result of paired training is probably a necessary preliminary change to subsequent long-latency CR learning but what the role is of the reduction in the UCR amplitude as a result of paired presentations of CSs and UCSs appears more difficult to explain. It might be speculated that the increase in intensity of the alpha-response somehow modifies the subsequent UCR. This change in the UCR is obviously a learned effect, because in the present experiment some savings appeared in the first unpaired control sessions in the CC-CO group after paired training.

Kimble (1961) has earlier suggested that the CS has an increased (as a result of pairing) inhibitory effect on the UCR. Removing the CS should restore the magnitude of the UCR. This is also what seem to occur in behavioral responses of the CO-CC group also in this experiment. The change in the amplitude of the UCR during CO treatment in the CO-CC group could indicate that the initial slight suppression effect of the CS (although CSs and UCSs are unpaired) is possible. After the CS has habituated during later CO sessions, the amplitude of the UCR slightly increases in some recording sites.

In addition to the weakening of the assumed suppressive effect of the

CS on the UCR, the UCR itself goes through some degree of habituation. When paired training has begun in the CO-CC group, the CS again acquires its orienting power (conditioned alpha), and hence its possible suppressive effect on the UCR; which should now be maximal because of the temporal closeness of the CS and UCS. As a result of this increased effect of the CS, the amplitude of the behavioral UCR is also changes slightly. However, evoked responses do not behave in the present experiment entirely according to a suppression hypothesis: some recording sites show slight decreases, but some show increases in UCR amplitude.

Most probably these changes in UCR amplitude are a result either of dishabituation, that is, the sensitization effect of the UCS (increase) in the CO-CC group, or else a result in the CC-CO group of a of slight habituation of the UCR after an initial strong sensitization; due to the additive effect of paired presentation of the CS and UCS. What about the suppression hypothesis now? We may still suppose that the CS may have a modifying effect on the UCR through its suppressive power; but this effect is also contamined by the possible modifying effect of the UCR on the CS response, and hence also on the following UCR topography.

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5 SECOND STUDY: DIFFERENTATION OF CONDITIONED "ALPHA" (SHORT-LATENCY) AND DELAYED (LONG-LATENCY) CONDITIONED RESPONSES AT BEHAVIORAL AND NEURAL LEVEL

A differentation of short-latency (alpha) and long-latency (delayed) conditioned behavioral and evoked neural responses was attempted. Further, facilitation and retardation of these responses were studied in an experimental design in which 10 paired conditioning sessions either preceded (CC-CO group) or followed (CO-CC group) 10 randomly unpaired presentations of conditioned stimuli (CS) and unconditioned stimuli. A 2024 ms tone (1000 Hz) was delivered directly through a miniature earphone to the left ear, eliciting an orienting head movement ("alpha" response) to the left. The unconditioned stimulus (UCS) was a direct 1024 ms stimulation of the lateral hypothalamic area overlapping the CS (delayed paradigm) so that both stimuli terminated simultaneously. The unconditioned response (UCR) was approach behavior and stereotypic, unconditioned head movement to a definite direction in each cat. Results showed behavioral and neural differentation of the short-latency and long-latency conditioned response (CR). Additional zero-delay ("backward") conditioning sessions given to two cats after paired sessions supported the assumption that the short-latency conditioned alpha-response might be different in nature to the learning of a delayed conditioned response: previously conditioned short-latency CRs remained unchanged while long-latency (delayed) CRs disappeared. Paired presentation of the CS and UCS, begun at the beginning of the experiment (CC-CO group), yielded a pronounced facilitation of the short-latency and long-latency CRs; while if the unpaired treatment was given first (as in the CO-CC group), the result was initial habituation during unpaired sessions and subsequent inferior and retarded acquisition during the following 10 paired sessions. The results are interpreted to support the assumption of the role of sensitization in the separate modification of the properties of CS and UCS pathway systems and their interaction in the development of delayed "true" conditioned responses.

5.1 Introduction

Like numerous other studies in which the observed increase in the short-latency CR has been interpreted as representing associative learning (e.g., Voronin, 1976; Woody, 1982; Misulis & Durcovic, 1984) the earlier experiment (First Study) seemed to show that the pairing specifity appeared only as the result of paired training. The possibility of nonassociative learning (sensitization) was controlled by unpaired presentations of the CSs and UCSs. Even randomly unpaired presentation of the CS and UCS does not, however, completely control the contiquity (temporal proximity) of the stimuli. That is why some additional sessions were held in the present study, in which the temporal proximity of the CS and UCS was retained, but the order of presentation reversed. This "backward" or more precisely, zero delay paradigm was supposed to show whether the short-latency or long-latency CR either habituated or increased their amplitudes. Using a backward paradigm as an extinction procedure, a decrease in short-latency CRs was found in the earlier study of Woody (1970); but not in a later study in which the excitability in the cells of the motor cortex persisted even though the behavioral CR moved towards extinction (Brons & Woody, 1980).

The preexposure effect of the CS or UCS on subsequent paired training has been found in some studies (e.g., Reiss & Wagner, 1972; Mis & Moore, 1973; Solomon & Moore, 1975; Matsumura & Woody, 1982; Terry & Wagner, 1985; Saladin & Tait, 1986). The present experimental use of paired treatment and unpaired treatment in balanced order in the same animal also allowed a study to be made of the preexposure effect.

The earlier experiment (First Study) had shown that the long-latency CRs which appeared during test trials (CS-alone) were generally weaker in amplitude than short-latency CRs. In that experiment, long-latency conditioned behavioral responses could be rather easily identified on some trials but in evoked neural responses, the localization of the corresponding time-amplitude curve appeared more difficult, due to the differing and variable latency of these changes, and their weaker amplitude compared to the original unconditioned response. In the present study, an increase in the number of daily training sessions was supposed to show the nature of this change more conclusively.

5.2 Methods

Subjects

The subjects were 13 adult cats weighing from 3.2 to 4.5 kg at the time of surgery.

Electrode implantation procedure

The electrodes for the slow potential recordings were made of Trimel insulated nichrome wire of 50 um diameter and the recording tip of the wire was cut transversely with scissors. The details of the electrode construction and implanation procedure are given elsewhere (Korhonen, 1981a).

The electrodes were implanted during Mebunat anesthesia (40 mg/kg) in a stereotaxic instrument. The locations for the recording electrodes were: subiculum (A4.0, R4.0, H+7.0, and A6.0, R4.0, H+6.0), dendate fascia (A4.0, R5.0, H+7.0, and A6.0, R5.0, H+6.5) CA1 (A5.0, L5.0, H+8.5), CA3 (A5.0, L7.0, H+7.5) of the hippocampus.

The bipolar brain stimulation electrodes were made of teflon insulated, 250 um stainless steel wire, the tip of the electrode was exposed to approximately 200–400 um and the tips were separated approximately 500 um from each other. The electrodes were aimed at four different locations on the lateral hypothalamus (A8.5, L2.5, H-4.5; A8.5, L3.0, H-4.0; A9.5, L3.0, H-3.0; A10.5, L2.5, H-4.5, following the stereotaxic atlas of Snider & Niemer 1961). Behaviorally, the most effective electrode was used as a stimulating electrode. The indifferent reference electrode for monopolar recordings consisted of three interconnected skull screws. All electrodes were connected to an Amphenol 222-series round connector, which was cemented with dental acrylic to the skull. A flexible, shielded cable connected the animal to amplifiers and stimulation source.

After the experiments the animals were given a lethal dose of Mebunat. The electrode assembly was left untouched and the brains were kept in formalin for a week before the sites of the electrodes were located by cutting slices along the electrode shafts (all hippocampal electrodes were in a parallel row); and the location of the tip of the electrode was referred to the coordinates of the stereotaxic atlas (Snider & Niemer, 1961).

Apparatus

During measurements the cats were in a ventilated, electrically shielded box (60 x 48 x 58 cm). The animals were monitored during the experiments through a video monitor and trials were recorded on a videotape for later analysis of the movements. A miniature earphone was placed on a fixed stand in the dental acrylic of the skull and positioned at a distance of about 2.5–3.0 cm from the left ear of the cat. During trials the earphone delivered a tone-CS of 1000 cps and 82 dB (A, measured at a distance of 2.5 cm).

The head movements were recorded with a three-dimensional movement transducer based on the magnetosensitive Hall-device (Honeywell, 92SS12-2). Details of the construction of this movement transducer construct are given in another paper (Korhonen, 1984a).

The apparatus used in these experiments included a multichannel measurement system for recording both the evoked responses and the multiple-unit activity of several brain sites.

The recording equipment consisted of an assembly of eight integrated, low noise preamplifiers (Analog Devices, AD524) which were connected directly to the connector in the acrylic mass on the head of the cat. The bandwidth of preamplifiers (DC - 10 kHz) was further divided by filters to slow potential (0.2 - 50 Hz) and to multiple-unit activity (500 - 5000 Hz) bandwidths. The multiple unit data will be reported later. The preamplifier design has been described in another paper (Korhonen, 1984b). The slow potential and multiple-unit activities were recorded as separate channels, with a movement signal and timing pulses to a 14-channel instrumentation tape recorder (Racal Store 14) for a off-line analysis of the recordings by a laboratory computer (PDP 11/34).

The control of the whole experiment was carried out by a microcomputer (ABC-80) which delivered the discrete trials, the randomized trial intervals in the conditioning and control experiments, and the sequences of CS- and UCS- stimuli used in the control experiments; controlled the instrumentation tape recorder (Racal 14) and video-tape recorder actions, and also generated the isolated brain stimulation pulse trains. The idenfication data generated by the microcomputer was displayed on the video-screen, together with a time-graph bar display which was used for timing the phases of the head movements. All this alphanumeric information was superimposed on the video picture of the cat in the experimental cage.

Procedure

After a recovery period of at least one week, the effect of electrical brain stimulation on lateral hypothalamus was tested in all cats. The stimulation parameters (bipolar pulses, duration 0.5 msec, frequency 100 cps, train duration 1024 msec) were adjusted so that the cats showed slight orientation and/or approach movements during stimulation. An intracranial self-stimulation test was not used, because the learning of bar pressing movements might have yielded unwanted learned behavioral response sets as a part of the unconditioned brain stimulation effect.

Two groups of animals were used in these experiments. One group received ten paired conditioning sessions followed by ten control sessions, while the other group experienced these treatments in reverse order. The control experiments consisted of 96 random CS or UCS presentations. The number of stimuli per session, and the length of each session was about the same as it had been during the conditioning sessions. The frequency of the trials was doubled as compared to the paired sessions (ITI was 20–40 s) in order to prevent habituation due to a lower stimulus frequency.

The trials during conditioning sessions employed a 2048 msec tone-CS, overlapping during the last 1024 msec with the brain stimulation UCS. In backward conditioning sessions the tone-CS and the brain stimulation UCS overlapped during the first 1024 ms and started simultaneously. The intertrial intervals varied randomly between 40 to 80 seconds. Each conditioning session consisted of 48 paired and 12 CS-alone (test) trials. Every fifth trial was a test-trial. Unpaired session included approximately 48 CS and 48 UCS trials.

In two cats (K47 and K50) the experiment was continued after conditioning sessions in five additional backward or zero-delay conditioning sessions. The stimulus parameters were the same as in the previous treatments; but the tone-CS and brain stimulation UCS now started simultaneously. Every fift trial was, as before, a test trial (CS alone).

Data analysis

The recorded slow potential and movement signals were fed into the PDP 11/34 computer through a low pass filter (50 Hz) and digitized at the rate of 125 samples per second. The signal analysis was based on 4096 msec periods which included 1024 msec Pre-CS, 1024 msec CS and 1024 msec UCS, and 1024 ms post-UCS periods.

The movement signals were first rectified and then averaged over trials. A conditioning session was divided into four blocks of 12 trials (excluding the test trials) and then averaged. A separate average of 12 trials was made for test trials. In control sessions two averages were formed of CS-alone trials (48) and UCS-alone trials (48).

Evoked responses were analysed in a similar way, separating the paired trials and test-trials. Within each trial, the alpha-response period (128-328 ms of ISI) and the conditioned response period (700-1000 ms ISI + 0-300 ms of the UCS-period) were defined as critical periods for the development of the alpha-response and the "true" conditioned response, respectively.

In the statistical analysis an analysis of variance for repeated measures was used. Because the assumption of the symmetry of the variance-covariance matrix was not usually met in repeated measurement design (measurements are not independent), the conservative (Geisser-Greenhouse) F-test was used in the following tests of significance. In comparison of single averages, Tukey's HSD-test was used.

5.3 Results

5.3.1 Video tape analysis

Response to the CS during ISI: CC-CO group

For all cats in the CC-CO group, the head movement to the tone-CS was directed to the tone-source (i.e., left). The amplitude of this movement was large (>45 degree) from the beginning of paired training and increased as a result training to 90 degrees or more. An additional upward movement appeared during the final part of the head movement only in two cats, K39 and K48. These cats were the only ones in which a similar upward movement was also the last part of the unconditioned head movement to the brain stimulation.

Response to the CS during ISI: CO-CC group

In the CO-CC group, head movements to the tone-CS habituated rapidly during the 20–30 trials of unpaired (CO) sessions, and remained at that level throughout the remaining control sessions.

In this group the head movement to the tone-CS developed slowly during the following paired (CC) conditioning sessions. The earliest head movements (to the left) to the tone-CS appeared after about 10–20 paired trials, and the amplitude of this movement gradually increased as training proceeded. One cat (K45) showed exceptional development in the direction of the head movement: an initial head turn to the left finally changed (at about 6. and 7. paired sessions) to a head movement to the right. The direction of the unconditioned head movement in this cat had been to the right in all sessions.

The unconditioned head movement and responses during the UCS-period of the test-trials

The unconditioned head movement to the brain stimulation UCS was either a horizontally directed movement to the left or right or a vertical movement up and forwards or some combination of these movements. A typical combination of head movements appeared in each animal and remained stereotypical over subsequent sessions. Only a very slight change in the intensity of the movement was observable after unpaired training (i.e., habituation) or after paired training (sensitization).

The brain stimulation reward was applied either on the left or right hemisphere of the lateral hypothalamus and the observed unconditioned head movement was in most cases a contralateral movement to the stimulation side but not in every cat. A closer study of the UCS period of the test trials indicated that a long-latency behavioral conditioned response developed in most cats (group CC-CO: K35, K37, K38, K39, K40, and group CO-CC: K42, K43, K45, K47 and K50).

The indentification of the long-latency CR on omitted UCS-trials was based a) on the latency of the movement (>700 ms from the beginning of the CS), and b) on specific behavioral features of the movement (similarity with the UCR). These criteria were rather easy to apply on those cats in which the unconditioned and thus the expected long-latency CR was contralateral to the short latency (alpha) response. Cats K35, K37, K38, K39, K40, and K47 showed learning of a long-latency CR which was a head movement to the right appearing during the UCS period of the test trials (UCS omitted); while the short latency response to the tone CS was a head movement to the left. The identification of the long-latency component in those cats in which the alpha CR and long-latency CR had the same direction was more difficult. The latency of the CR was then used as the main criterion and in the CC-CO group the CS alone trials in the following unpaired control sessions could be used as additional evidence of the CR component: it habituated earlier (in 10–20 trials) than the alpha response to the tone CS.

Correspondingly, the acquisition of the habituated alpha response in the CO-CC group after unpaired control sessions was quicker (first signs of the alpha response appeared in the first 10–20 paired trials) than the long-latency CR during Test-trials (earliest signs appeared after 30–40 paired trials). An interesting detail was the relative direction of the head movement which was conspicuous in cat K47. A typical differentation at the level of behavioral responses was: first a head movement to the tone-CS (left), and then a head movement to the direction of the omitted UCS (i.e., right on CS-alone trials). A reversed sequence of these behavioral responses occasionally occurred, however, if the head of the cat was already turned to the left at the beginning of the tone-CS or the cat was making a turn to that direction: the tone-CS might then elicit a head movement first to the right and then after a delay (about the ISI) a head movement to the left!

All cats developed a CR which had a similar direction to that originally elicited by the brain stimulation UCS; and in eight cats the direction of this long-latency CR was thus different from the movement elicited by the tone-CS (i.e., left). The frequency of long-latency CRs is given in the Table 5.1. The number of CS-alone test trials within each session was twelve, and hence the frequencies of the CRs are related to this maximum.

Spontaneous responses during the intertrial intervals (ITI)

The occurrence of spontaneous behavioral responses was observed in some cats appearing during intertrial intervals (ITI) as the paired training proceeded. Spontanous head movements began to occur after one or two paired sessions and appeared as a head movement to the left (i.e.,

Table 5.1 The number and average percentage of CRs during 12 Test-trials (CS-alone) of the last (10.) paired session.

Group CC-CO		Group CO-CC	
Cat	No. of CRs	Cat	No. of CRs
K35	5	K42	3
K37	3	K43	8
K38	11	K45	3
K39	12	K46	1
K40	10	K47	12
K48	4	K50	4
K52	2		
	Average % 56		Average % 43

in the direction of the tone-CS). Such responses were at first rare, but increased on later sessions so that in cat K47, for example, the frequency of these movements was around 3–5 head turns on average during the ITI. The last five ITIs of each session were recorded on video tape, and these observations indicated that cats K35, K48, K42, K47, K50, and K52 showed spontaneous responses. In some cats these responses were repeated short head turns to the left and this position was then maintained until the next trial.

5.3.2 The head movement transducer analysis

The shortest onset latency of the head movement was about 45 ms and the longest was about 150 ms. The onset latency seemed to decrease from the usual initial value of 120-130 ms to 50-80 ms as paired training proceeded (see Figure 5.1).

At the same time, the amplitude of the short latency head movement increased, and the movement became more vigorous (Figure 5.2). Averaged over sessions, the CC-CO group showed shorter onset latencies (x=63.9 ms) than the CO-CC group (x=104.2 ms). The difference between these groups approached statistical significance ($p>.07$). The decrease in onset latency over paired training sessions (Figure 5.1) appeared significant (ANOVA for repeated measurements, $F(9,10)=6.47$, $p<.001$). The averaged head movement curves of different cats of the CC-CO and CO-CC

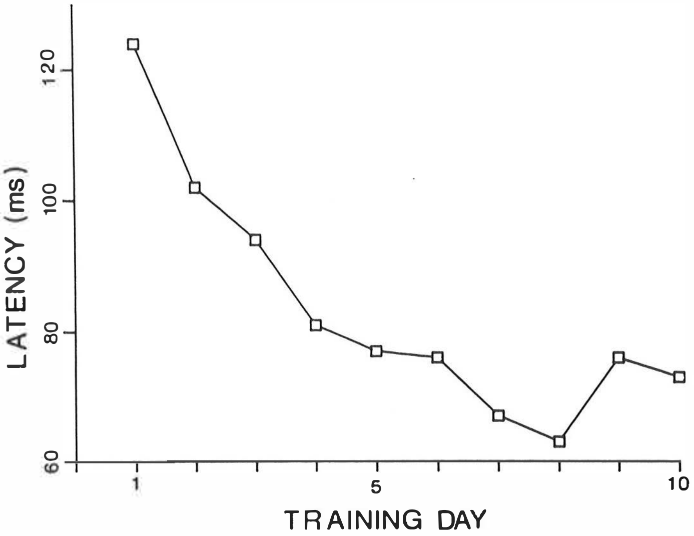

Figure 5.1 An average change in the latency of the short-latency head movement over paired training (CC) sessions averaged over treatment order (n=12).

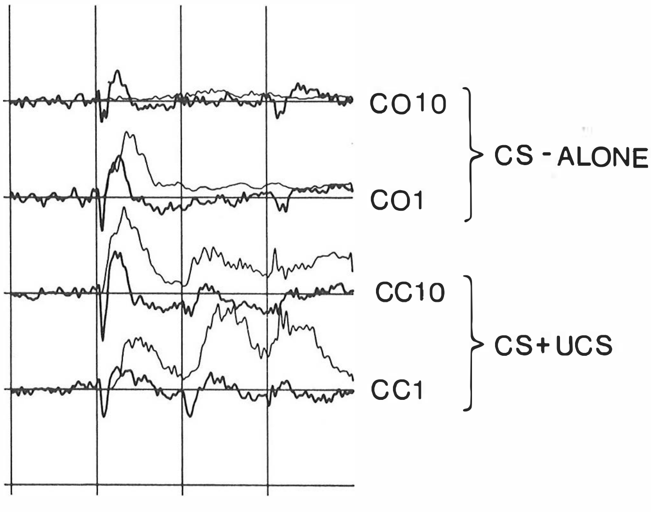

Figure 5.2 The changes in onset latencies of head movements and evoked neural response of dendate fascia in the cat K48 during the first (CC1) and last (CC10) paired and during the CS-alone trials of the first (CO1) and last (CO10) unpaired session. This cat belonged to the CC-CO group and shows some savings of the CR on the first unpaired sessions. Thin line is the movement transducer curve and thick line is evoked response curve. The time interval between vertical lines is 1024 ms.

groups, from the first to the last paired (CC) and unpaired (CO) session (sessions 1, 3, 5, 7, and 10), are shown in Figures 5.3 and 5.4.

Habituation

Long-term habituation has previously been described in invertebrates. In this study, the development of a long-term habituation was verified in a vertebrate preparation: habituation during unpaired treatment in the CO-CC group, and savings of the short-latency CR over CO sessions following CC sessions was observed (see Figures 5.3 and 5.4). The use of balanced-order groups allowed a comparison of extinction after paired training in the CC-CO group to the habituation of initially unpaired CSs and UCSs in the CO-CC group. The habituation of the UCSs, contrary to that of the CSs, appeared only slight in some cats.

Alpha response period

During the alpha response period (ISI) of paired trials in the CC-CO group (Figure 5.5) the head movement rapidly reached a high level (during the 1. session) retaining this level during later paired sessions (A).

The significant interaction of treatment and session effects for this group, F(9,45)=8.11, $p<.05$, was due to extinction during unpaired sessions (B). The comparison of single paired vs. unpaired sessions showed that the difference was significant ($p<.05$) from 3. session to the last session (Tukey's HSD-test was used for the comparison of session averages). The comparison between session averages indicated a habituation effect during unpaired sessions (B): the last five unpaired sessions showed a significant ($p<.05$) decrease in movement activity compared to the first unpaired session. Like the CC-CO group, the CO-CC group showed a significant difference between unpaired (C) and paired treatments (D): F(1,5)=19.03, $p<.01$. The difference between the CC-CO group (A) and the CC-CO group (C) during the first phase (i.e., the first 10 paired vs. unpaired sessions) of the experiment was significant F(1,10)=44.67, $p<.001$; indicating that the amplitude was higher in the paired group.

The interaction of the curves (B and D) of the second phase of the experiment is due to habituation in the CC-CO group while the group CO-CC shows significant acquisition, F(4,35)=12.91, $p<.001$. The comparison of individual paired vs. unpaired sessions (B vs. D) of the second phase of the experiment showed that the first session, and seventh, eight and ninth sessions, respectively, were different from each other ($p<.05$); showing a decrease in the CC-CO group during unpaired training and an increase in the CO-CC group during paired training. An increase from the first paired session in the alpha-response during paired sessions of the CO-CC

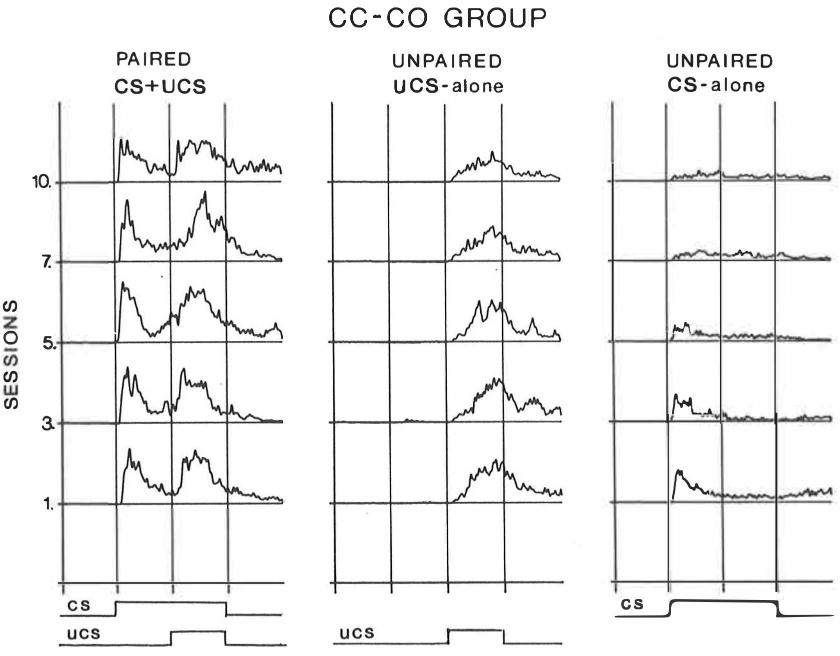

Figure 5.3 Averaged head movement curves of the 1., 3., 5., 7., and 10. session are depicted for the cat 37 belonging to the CC-CO group. The time interval between vertical lines is 1024 ms.

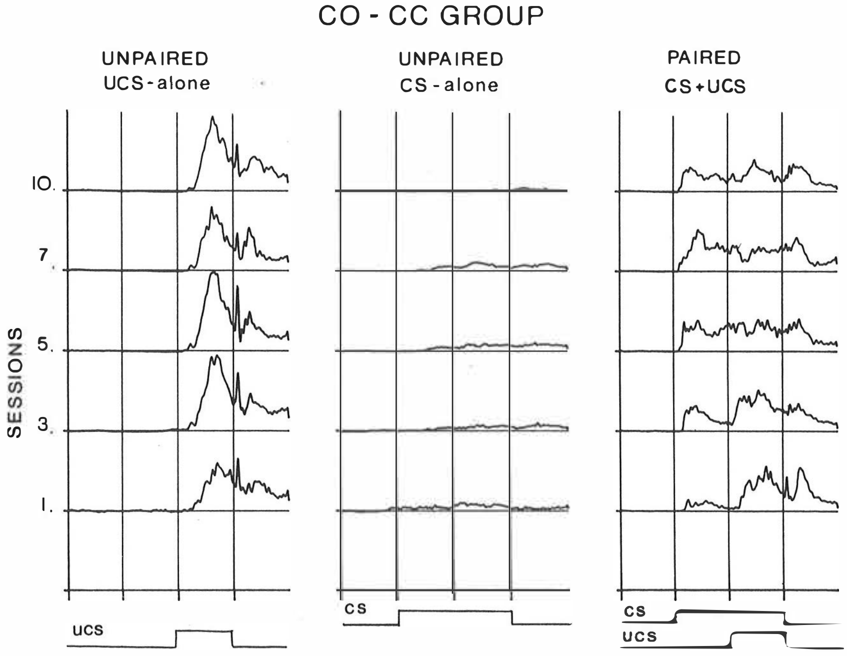

Figure 5.4 Averaged head movement curves of the 1., 3., 5., 7., and 10. session are depicted for the cat 47 belonging to the group CO-CC. The time interval between vertical lines is 1024 ms.

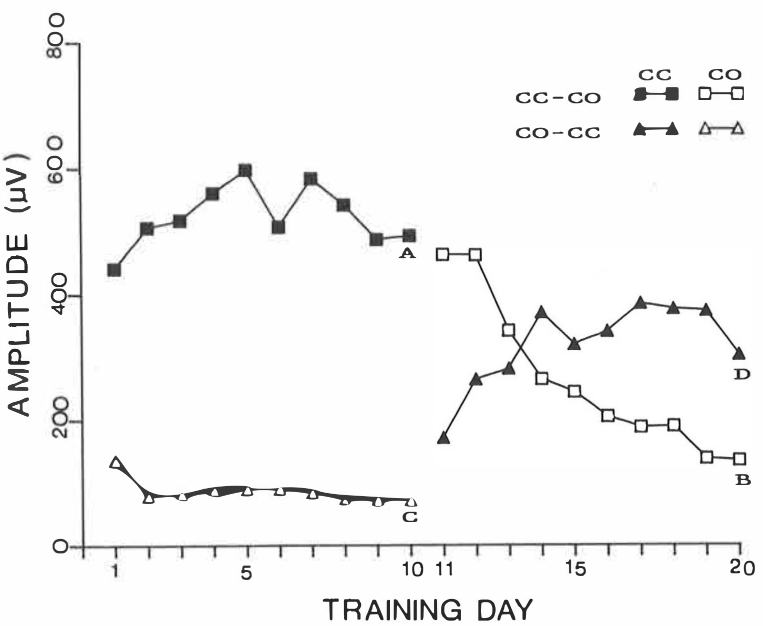

Figure 5.5 The changes in the short-latency CR of the head movement during paired (A and D), and unpaired (B and C) sessions of the CC-CO and CO-CC groups, respectively.

group (D) was significant ($p<.05$) compared to the last sessions (6., 7., 8., and 9.).

The retardation effect, appearing as the slow acquisition rate of the CO-CC group during paired training after unpaired sessions appeared as a significant interaction of treatment and sessions $F(4,35)=3.81$, $p<.05$ when an ANOVA was computed for the paired sessions of the CC-CO and CO-CC groups (A vs. D).

Conditioned response period

In the movement analysis, the integrated area of the UCS period (= 1024 ms) of the CS-alone trials of paired and unpaired sessions were compared. During the first phase of the experiment (conditioning in the CC-CO and unpaired treatment in the CO-CC group), the interaction between treatment and sessions was significant, $F(4,36)=2.84$, $p<.05$, indicating an increase in the long-latency conditioned response in the CC-CO group (A) while the CO-CC group showed no acquisition during unpaired control sessions (C) (Figure 5.6).

Thus, the long-latency CR showed progressive increase in the acquisition of pairing specificity, while the short-latency (alpha) response showed

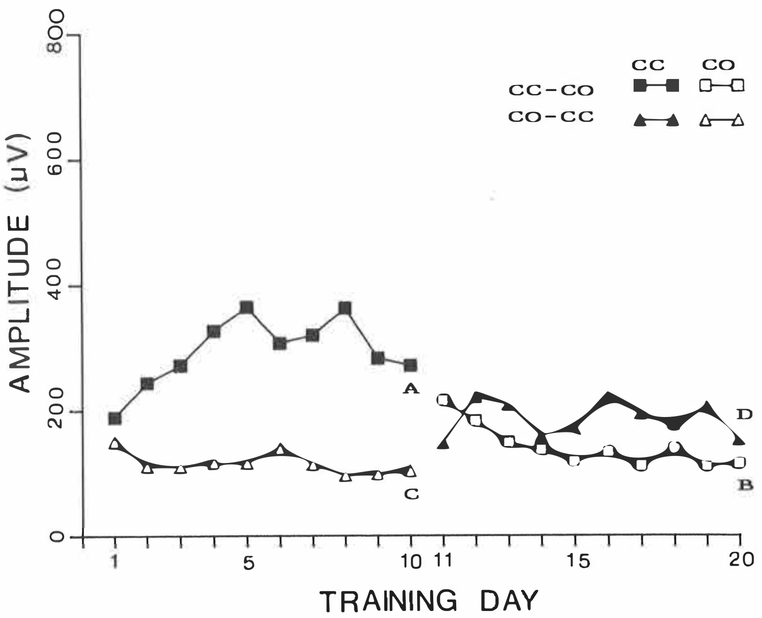

Figure 5.6 The changes in the long-latency CR of the head movement during paired (A and D), and unpaired (B and C) sessions of the CC-CO and CO-CC groups, respectively.

rapid initial acquisition, retaining this level over the paired sessions. The comparison of session averages of the paired long-latency responses showed a significant ($p<.05$) increase in acquisition from the second session on. The habituation effect of the conditioned long-latency CR in the CC-CO group during subsequent unpaired sessions (B) was not statistically significant ($p>.08$), although Figure 5.6 indicates an overall decrease in the long-latency responses which finally reached the same level as those of the CO-CC group during unpaired sessions (C). It is important to note that the behavioral unconditioned responses were similar in intensity in both treatment order groups (see Figure 5.7) during unpaired control (CO) sessions.

The comparison of the long-latency conditioned head movement changes to changes in the conditioned alpha response (Figures 5.5 and 5.6) shows that both responses undergo similar development at different stages of training; but the magnitude of the long-latency CRs is lower troughout the experiment. The CC-CO group shows a strong additive effect when the CS and UCS are paired from the beginning of paired training, in contrast to the paired training phase of the CO-CC group; which indicates that after unpaired treatment it is difficult to attain the same level in conditioned responses (both in alpha and long-latency CRs) as that shown by the

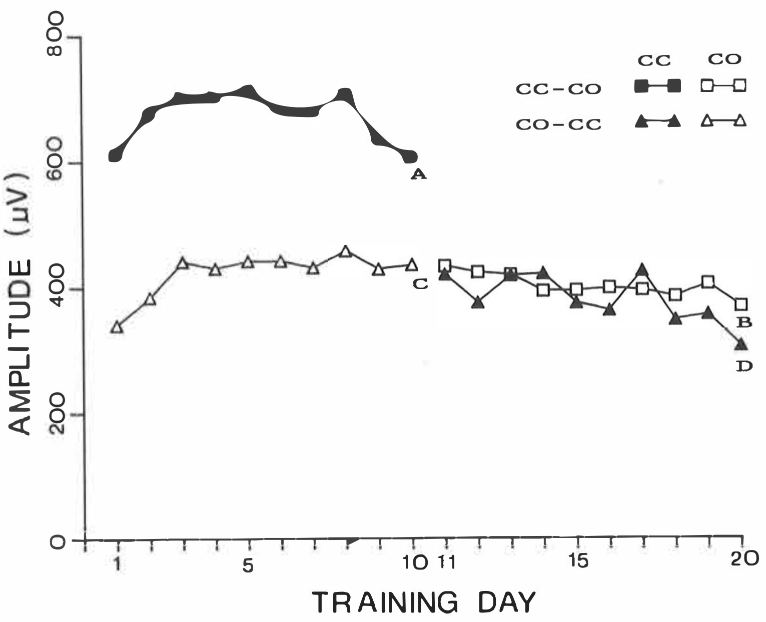

Figure 5.7 The changes in the intensity of the unconditioned head movement to the brain stimulation UCS during paired (A and D), and unpaired (B and C) sessions.

CC-CO group right from the beginning of the experiment.

Analysis of the unconditioned head movements during the UCS period of paired sessions in the CC-CO group (A) reveals an interesting feature: the level of the UCR is significantly higher in this group when compared to the corresponding UCRs of the following unpaired sessions (B), F(1,10)=15.66, p<.05, or of the paired sessions (D) of the CO-CC group, F(1,10)=8.66, p<.05, Figure 5.7. The behavioral UCR of the CC-CO group rapidly declines from the high level it acquired during paired training, habituating to the same level as the unpaired UCRs of the CO-CC group showing no significant difference. Unlike the CC-CO group, the CO-CC group is unable, after unpaired treatment (CO), to show such additive effect to the UCS. This interaction of the treatment (CO or CC) and group (i.e., the treatment order, CC-CO or CO-CC) was significant; F(1,10)=14.61, p<.01. These results might indicate an additive effect (sensitization) of the CS on the the temporally close UCS on paired trials and, on the other hand, the retardation effect of the unpaired sessions on the subsequent paired sessions in the CO-CC group. Similarly, the high level of the alpha responses in the CC-CO group might indicate a mutual nonassociative sensitization effect of the UCS- and CS pathways (see discussion).

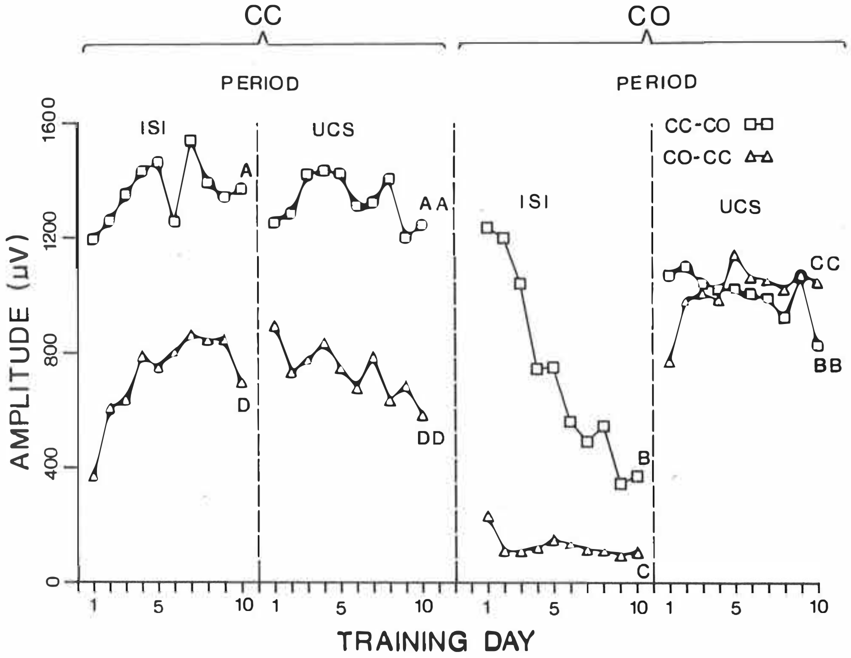

Figure 5.8 The changes in the maximum amplitude of the head movement response during paired (CC) and unpaired (CO) sessions during ISI- and UCS-periods over daily sessions. Note a difference between the CC-CO and CO-CC groups during paired trials especially on the UCS-period because during the UCS-periods of unpaired sessions both treatment groups show similar intensity in unconditioned head movement.

Another interesting feature of the increase in amplitude of the unconditioned head movement with time was that in the CO-CC group, the UCR during paired training seemed to decrease slightly, while the short-latency response to the CS increased. Such "seesaw" effect was further studied using maximum response amplitudes of the head movement responses during the ISI and UCS periods. The maximum amplitude was supposed to be more indicative of the intensity of the peak value of the head movement than the average integrated area measurement used above. The results of the maximum amplitude changes are depicted in Figure 5.8.

The maximum amplitude analysis seemed to yield overall results very similar to those obtained for the integrated average response analysis. Although Figure 5.8 shows a certain amount of "see-saw" effect between the CS and UCS periods (D vs. DD) of paired sessions in the CO-CC group, the interaction was not statistically significant.

5.4 Neural evoked responses

Evoked responses were recorded in this experiment in different hippocampal locations. Verification of the actual sites of the electrodes indicated that in three locations (subiculum, dendate fascia and CA1 of hippocampus) a sufficient number of recording sites could be found for the analyses (Table 5.2).

Table 5.2 Location of the recording electrodes for each cat.

Recording site	K35	K37	K48	K52	K42	K45	K46	K47
Subiculum	X	X	X	X	X	X	XX	
Dendate	XX	XX	X		XX	X	X	X
CA1	XX	XX	X		XX	X	X	X

The analysed periods were almost identical to those examined in the earlier (First) study: 128-328 ms of the CS-period (ISI) for the alpha response and 700-1328 ms for the long-latency CR analysis (CS-alone trials of paired sessions).

The latency of the head movement and evoked response

The latencies of the head movement and evoked response curves were studied in order to find out whether the head movement could represent some source of artefact influencing the evoked recordings. The effect of the movement artefact on the recording cable and preamplifier system (on the head of the cat) was tested before the experiments using a resistor network as a dummy subject. These tests showed that the differential input preamplifiers could reject even intense movement effects on recordings. Some examples of actual recordings made during different phases of the experiments seem to confirm this observation. Figure 5.2 shows that in the cat K48, for example, (group CC-CO) the relationship of these latencies is changing during the training. In the first paired session, the latency of the head movement is longer than during the last (10.) paired session, as the analysis of the latency of the head movements indicated above. Because the latency of the evoked response is not, however, similarly changing, the onset latencies of the head movement and evoked response differ about 100 ms in the first session; but not during the last session during which these latencies seem to be rather overlapping. Additional evidence of the technical independence of the head movement and evoked recordings comes from the same cat (K48) during the unpaired control sessions: while the short latency conditioned behavioral response (head movement) habituated completely on the tenth unpaired session, the evoked response retained its amplitude almost unchanged (Figure 5.2). Further, the possibility of the movement artefact in evoked recordings can be tested for by comparison of the latencies of the head movements (Figures 5.3 and 5.4) and evoked responses (Figures 5.9 and 5.10) during the unconditioned response. These figures indicate a considerable difference in the time amplitude courses of these curves.

Short-latency changes in evoked responses

The development of conditioned short-latency neural responses in subiculum, dendate fascia, and CA1 is shown in Figures 5.11, 5.12, and 5.13. The specifity of neural changes to the paired treatment compared to the unpaired (CO) treatment appeared in both groups. Both in the CC-CO and CO-CC groups, an increase in negativity was observed in all recording sites during the 128–328 ms ISI of paired training analysed; and a decrease (habituation) during the unpaired sessions. Accordingly, analysis of variance indicated a significant treatment x sessions interaction in subiculum; F(1,4)=10.81, p<.05; in the dendate fascia: F(1,6)=6.40, p<.05; and in the CA1: F(1,8)=8.15, p<.05.

The difference between paired and unpaired treatments becomes clearer if the paired treatment of the CC-CO group (A) is compared with the unpaired treatment of the CO-CC group (C). This first phase of the experiment shows a rapid shift in negativity in paired training (A) in the CC-CO group and correspondingly, a low level of response during unpaired treatment (C) in the CO-CC group, F(1,8)=42.78, p<.001 in CA1 (Figure 5.13). The interaction of an increase in acquisition (A) and a decrease (B) was significant only in subicular recordings, F(1,4)=4.23, p<.05 (Figure 5.11). The comparison of individual paired vs. unpaired sessions (Tukey's HSD-test) showed a significant (<.01) difference between sessions 6, 7, 8 and 10.

The significant treatment and session interaction effect in all analysed recording sites (subiculum: F(1,4)=6.19, p<.05; dendate fascia; F(1,6)=7.72, p<.01, and CA1: F(1,8)=14.99, p<.001) during the second phase of the experiment was due to an extinction in the CC-CO group (B) and to a slow acquisition rate in the CO-CC group (D). The comparison of the first and second sessions to sessions 9 and 10 showed a significant extinction effect in all recording sites.

A mutual interaction between the CS and the UCS

Like to the movement recordings, the paired presentation of the CS and

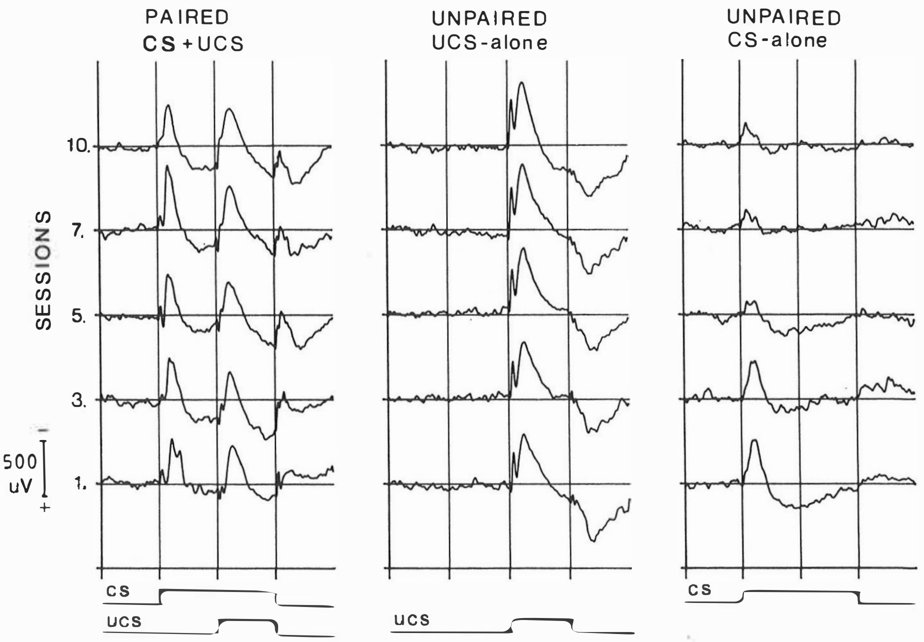

Figure 5.9 Evoked response changes of the cat K37 in CA1 of hippocampus during 1., 3., 5., 7., and 10. session. The CS-alone trials of unpaired sessions show savings from preceding paired sessions in the CC-CO group.

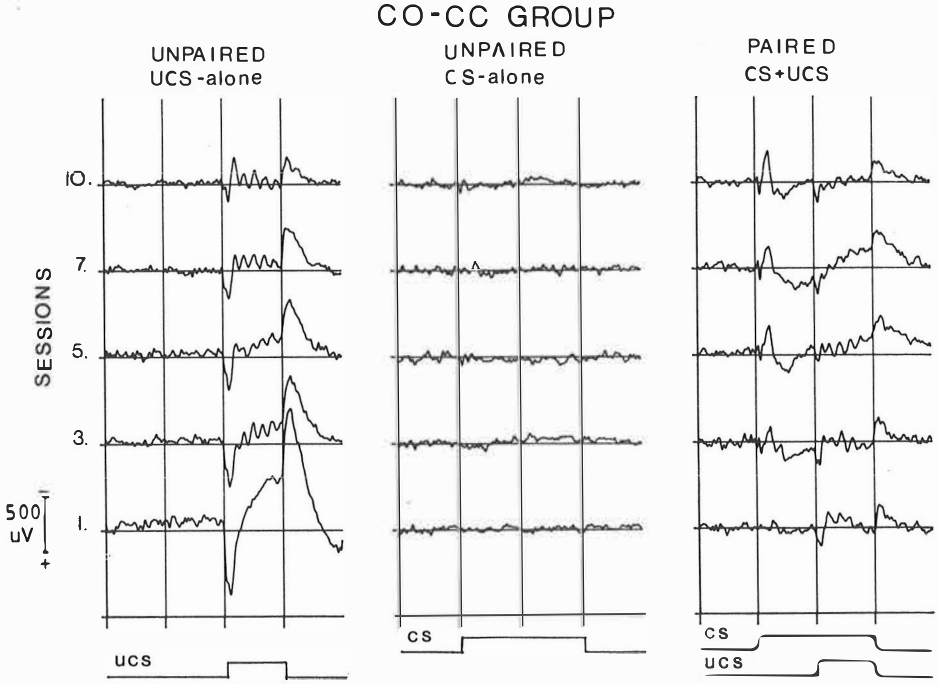

Figure 5.10 Evoked response changes of the cat K47 in CA1 of hippocampus during 1., 3., 5., 7., and 10. session. The CS-alone trials of unpaired sessions show in the CO-CC group rapid habituation from the first unpaired session.

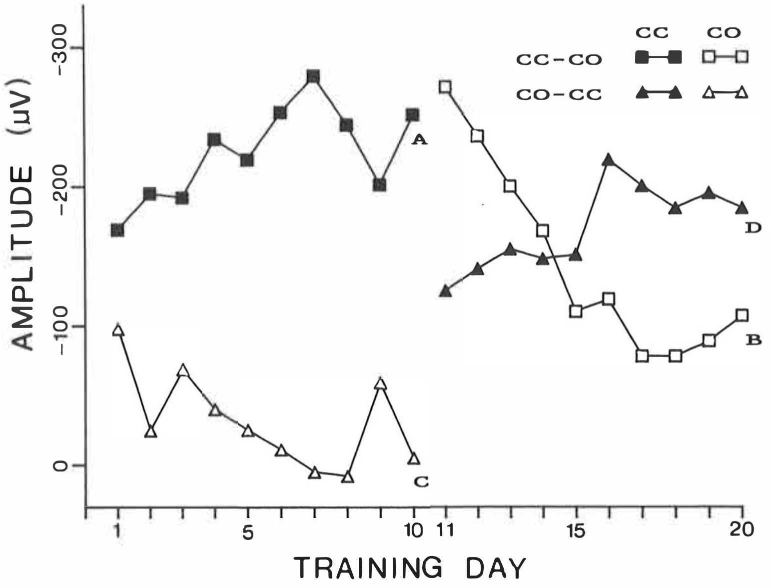

Figure 5.11 The changes in the short-latency CR in subiculum during paired (A and D) and unpaired (B and C) sessions in the CC-CO and CO-CC groups, respectively.

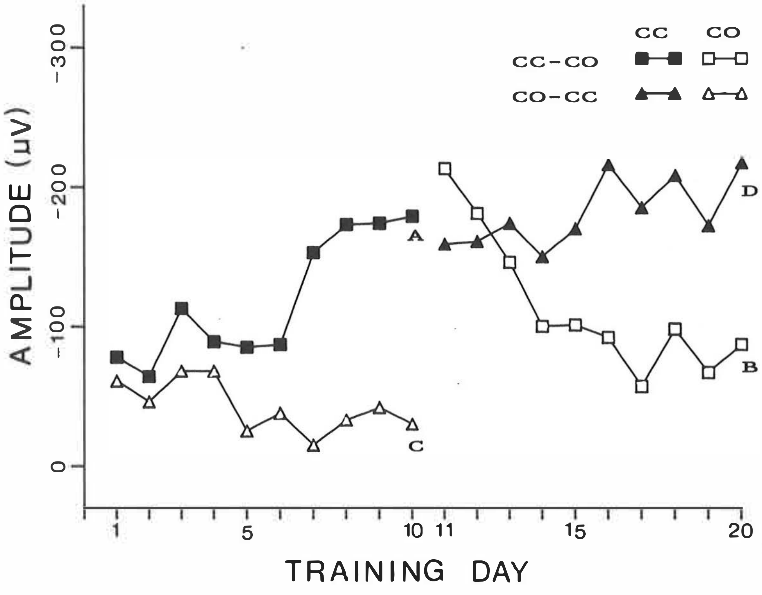

Figure 5.12 The changes in the short-alatency CR in dendate fascia during paired (A and D) and unpaired (B and C) sessions in the CC-CO and CO-CC groups, respectively.

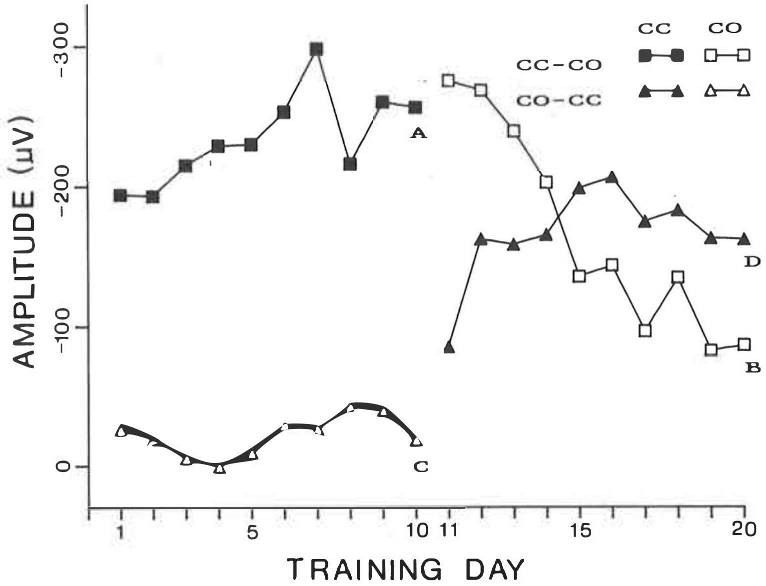

Figure 5.13 The changes in the short-latency CR in CA1 of hippocampus during paired (A and D) and unpaired (B and C) sessions in the CC-CO and CO-CC groups, respectively.

UCS from the beginning of the experiment (CC-CO group) seemed to produce an effect different from that which resulted when paired training followed unpaired control sessions. This difference (A-D) appeared significant in the subiculum, F(1,4)=3.76, p<.05, and may reflect either some additive mutual interaction of the CS and UCS orienting responses in the CC-CO group, which rapidly habituates on following unpaired sessions, and/or some retardation effect of the UCS on the CS amplitude in the CO-CC group during paired sessions. The movement curve showed immediate increase to a high level of responding during paired sessions in the CC-CO group, while the acquisition of the short-latency neural CR in the CC-CO group increased more slowly over the sessions. In evoked responses dendate fascia showed exceptional development in the CC-CO group: during the paired training the negativity increased only slowly over the sessions.

In evoked responses, the CS seemed to have a depressive effect on the intensity of the UCR in both groups. In the CC-CO group, the max-min amplitude of the UCR of the paired sessions is depressed by the CS, while during unpaired sessions following the paired the UCS-alone curves show some "release" of the depressive effect of the CS, that is, the amplitude increases considerably (see Figures 5.14, 5.15, and 5.16, AA vs. BB and DD vs. CC).

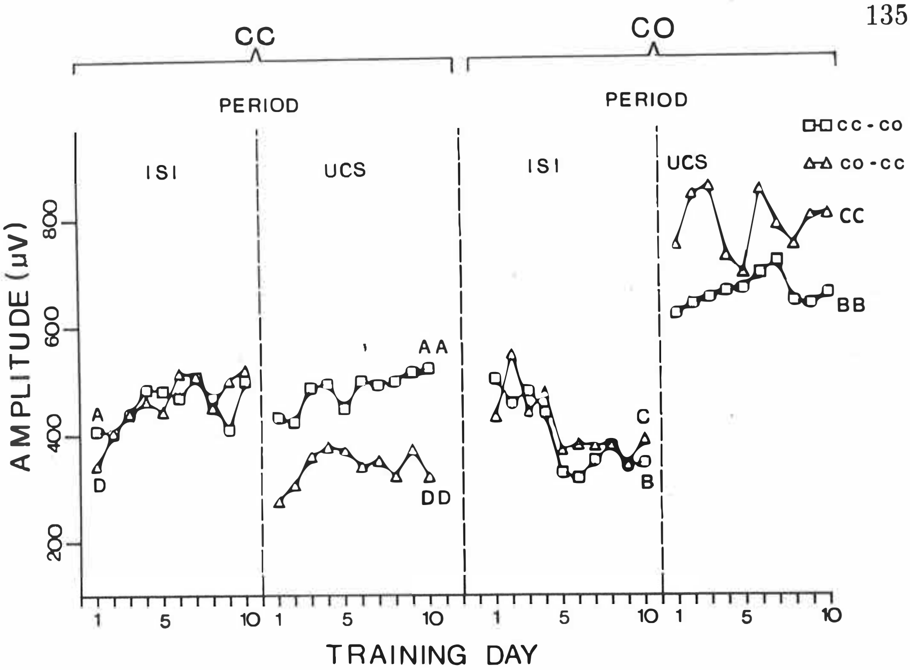

Figure 5.14 The maximum amplitude curves of evoked responses during the ISI of the paired .(A and D) and unpaired (B and C) sessions and of the UCS-period of paired (AA and DD) and unpaired (BB and CC) sessions in subiculum.

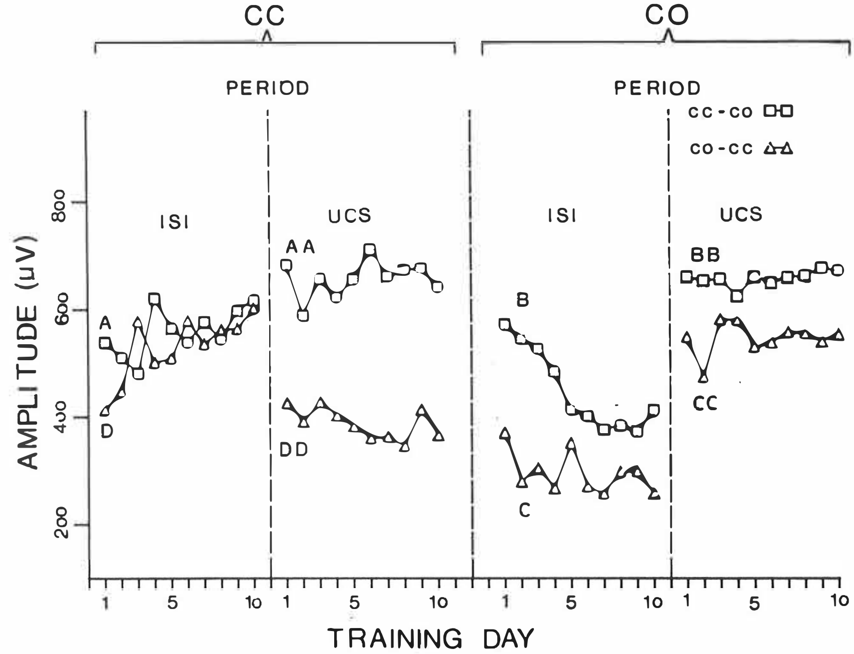

Figure 5.15 The maximum amplitude curves of evoked responses during the ISI of the paired (A and D) and unpaired (B and C) sessions and of the UCS-period of paired (AA and DD) and unpaired (BB and CC) sessions in dendate fascia.

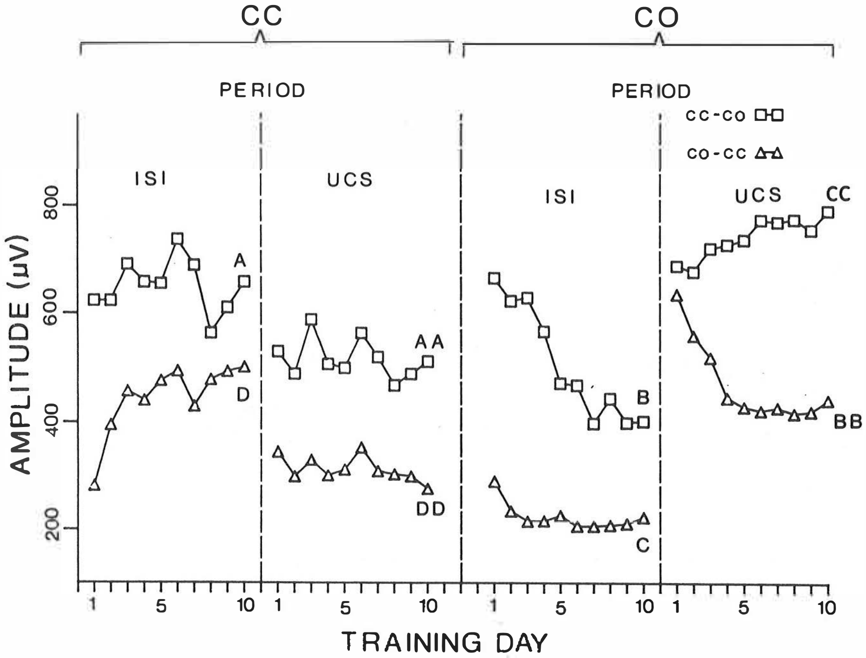

Figure 5.16 The maximum amplitude curves of evoked responses during the ISI of the paired (A and D) and unpaired (B and C) sessions and of the UCS-period of paired (AA and DD) and unpaired (BB and CC) sessions.

In the CO-CC group, the original max-min amplitude to the UCR during unpaired sessions is at about the same level as on the UCS-alone trials of the CC-CO group, but becomes depressed during subsequent paired sessions. Statistical analysis showed that retardation between the UCS periods of unpaired vs. paired treatment was significant ($p<.05$) in dendate fascia and CA1 (CC vs. DD in Figure 5.15). A significant "release" effect was found in CA1 (AA vs. BB, Figure 5.16). Some mutual interaction of the CS and UCS effects in the evoked responses was supported by a finding that a decrease in the amplitude of the UCR appeared to be somehow related to the increase in the amplitude of the short-latency CR taking place in the CO-CC group as a result of paired training. Such a "seesaw" hypothesis was studied in evoked responses as the same manner as in behavioral responses (see Figure 5.8), but the analysis of variance (D vs. DD) did not reveal significant interaction (see Figures 5.14, 5.15, and 5.16).

Long-latency conditioned evoked responses

Contrasting with an increased negativity in the evoked short-latency CR, the long-latency change appeared as an increased positivity during the combined 700-1024 ms ISI and 0-300 ms UCS period of the test-trials. The analysis of variance (order x treatment x sessions) showed that in the long-latency CR too, the specifity for paired treatment compared to unpaired treatment developed in both groups in the subiculum, F(1,4)=45.06, p<.01; in the dendate fascia, F(1,6)=31.81, p<.001; and in the CA1, F(1,8)=23.71, p<.001 (see Figures 5.17, 5.18, and 5.19).

Like the development of the short-latency CR, the comparison of the first phase of the experiment showed a significant difference between the paired treatment (A) of the CC-CO group and the unpaired treatment (C) of the CO-CC group (subiculum: F(1,4)=224.37, p<.001; dendate fascia: F(1,6)=16.69, p<.01; CA1: F(1,8)=11.75, p<.01).

The analysis of the second phase of the experiment showed that the long-latency CR in the CC-CO group (B) extinguished; while in the CO-CC group (D), positivity increased slightly over sessions (significant treatment x sessions interaction) in the dendate fascia, F(3,18)=3.67, p<.05; and in the CA1, F(3,36)=3.49, p<.05). The comparison of individual session averages of paired treatment (B) showed significant (p<.05) extinction in the CA1 during the last (6., 7., 8., 9., and 10.) sessions, compared to the first session. Contrary to the movement recordings, an abrupt increase in positivity developed from unpaired (C) to paired treatment (D) in the CO-CC group in the subiculum, F(1,2)=178.71, p<.01; in the dendate fascia, F(1,3)=17.74, p<.05; and in CA1, F(1,4)=28.26, p<.01.

Backward conditioning

Two cats of the CO-CC group were further trained for five days in order to study the effect of a "backward" conditioning paradigm on extinction of learned changes. Because the original paired conditioning paradigm was based on a delay paradigm, the presentation of the CS and UCS so that they started simultaneously represented a zero delay paradigm with temporally overlapping UCS and CS. This arrangement was supposed to yield the temporal contiguity of the CS and UCS similar to the forward contingency (CS–UCS); thus providing an equal nonassociative sensitization effect.

The video-tape analysis showed that on paired UCS-CS trials the unconditioned response to the brain stimulation dominated the direction of the head movement, so that the behavioral response on the UCS+CS trials was very similar to the UCR on the preceding forward paired training (CC) sessions. The general appearance of this response remained unchanged from the 1. backward (BW) session to the last (5.) BW session. The CS-alone test trials (every 5. trial) showed initial savings (during the 1. BW session) from the preceding paired training, exhibiting very similar head movements to the CS as those observed in the forward

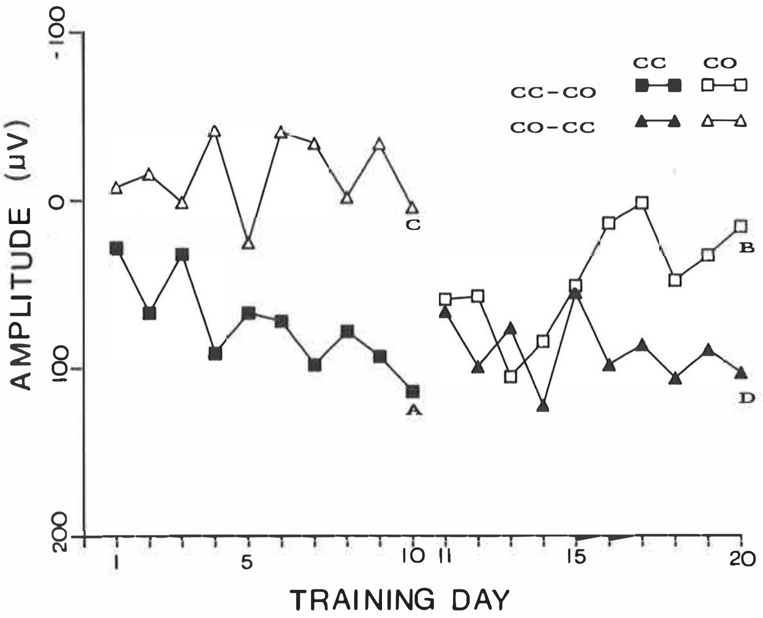

Figure 5.17 The long-latency CR during paired (A and D) and unpaired (B and C) sessions of the CC-CO and CO-CC groups in subiculum.

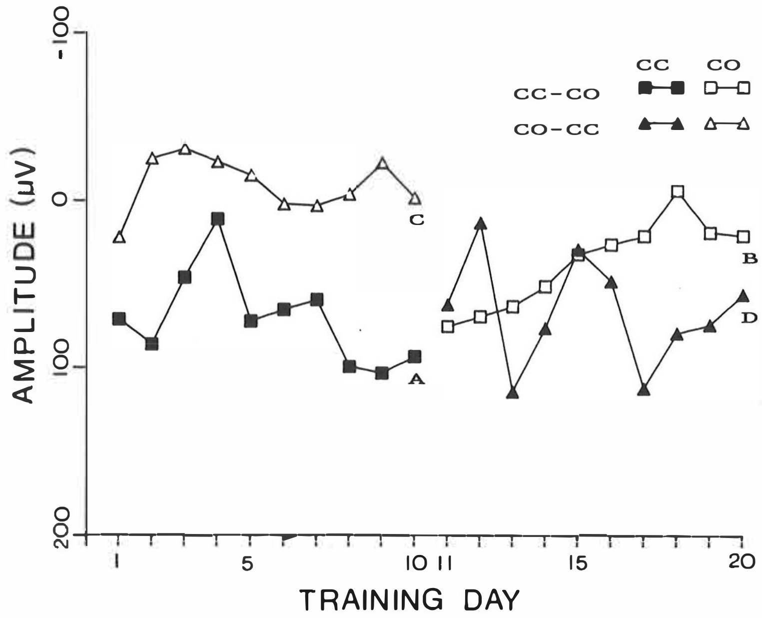

Figure 5.18 The long-latency CR during paired (A and D) and unpaired (B and C) sessions of the CC-CO and CO-CC groups in dentate fascia.

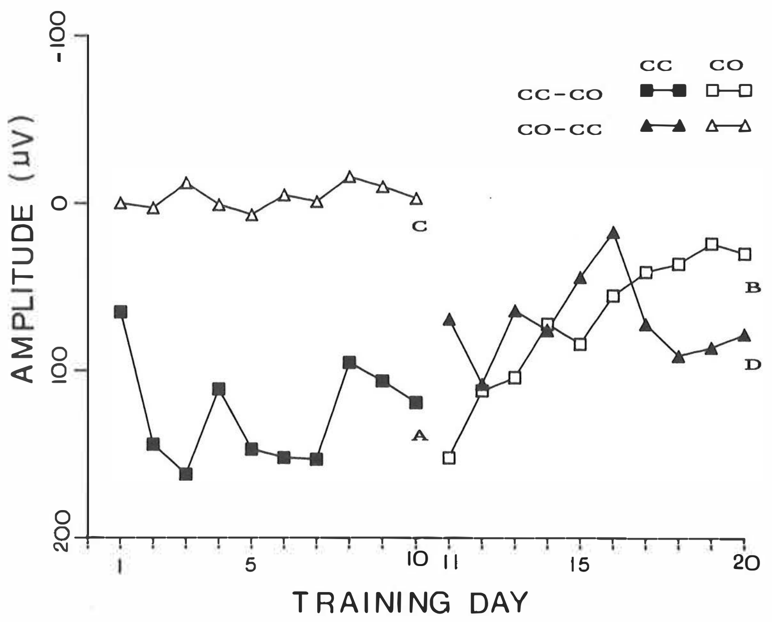

Figure 5.19 The long-latency CR during paired (A and D) and unpaired (B and C) sessions of the CC-CO and CO-CC groups in CA1.

paired sessions: cat K47 turned its head in the direction of the tone source (left) as a response to the CS, while the UCR to the brain stimulation UCS (and UCS+CS on BW sessions) was a head turn to the right. Such short-latency response to the CS-alone presentations habituated slightly on the second and third BW sessions, but then began to increase again, so that on the fifth session it was of the same magnitude as on the last paired (CC) session before BW treatment. The second cat, K50, showed similar changes, except that in this cat the direction of the head movement to the UCS originally had the same direction as the head movement to the tone-CS (i.e., left). However, the short-latency response on the CS-alone trials of BW sessions appeared indistinguishable from the responses on preceding paired (CC) session both in its topography and amplitude.

While the short-latency response seemed to retains its intensity on BW sessions in these cats, the long-latency conditioned behavioral response disappeared. This change could be reliably identified in cat K47 because the direction of the head movement was opposite to the short-latency CR.

The movement transducer curves confirmed the behavioral observations. The movement signal on the UCS+CS trials showed a change of amplitude with time similar to that which had characterized the preceding UCS-alone trials of the CO sessions; and practically no habituation occurred.

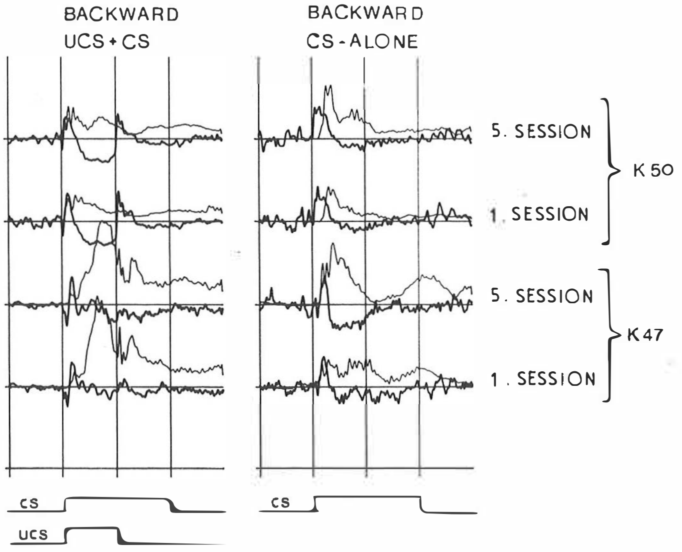

Figure 5.20 Evoked and head movement responses during the first and fifth backward (BW) conditioning sessions in cats K47 and K50 (CC-CO-BW group). Evoked responses in cat K50 are from dendate fascia and in the cat 47 from CA1.

The evoked neural responses also showed similar topography and amplitude on the UCS+CS trials as on the UCS-alone trials of preceding CO sessions. On the CS-alone trials of the BW sessions, the evoked responses maintained about the same level as in the last CC sessions. By contrast, the long-latency component disappeared from the CS-alone curves of the movement signals and the evoked curves on these trials also showed some decrease (Figure 5.20).

5.5 Discussion

5.5.1 The nature of the conditioned response: can short-latency and long-latency CRs be differentiated?

Video-analyses showed that in addition to the initial head movement towards the tone source (i.e., left), some modification of this short-latency response appeared in some cats: after paired training the movement also included some components of the UCR, for example, head movement to the left and up in cats K39 and K48 (unconditioned head movement up); or a more radical modification, as in cat K45; which changed the direction of its alpha response from the initial movement to the left to a head movement to the right on the 6th and 7th paired session (the unconditioned head movement to the UCS was a head turn to the right).

These examples suggest a possibility of a close connection between the CS- and UCS-pathway systems. On the other hand, the behavioral analysis also indicated that a differentation of the short-latency and long-latency CRs was possible. In addition to being a latency criterion, the direction of the long-latency CR can be considered as an index of the nature of this CR. This was most obvious in cats which showed a development of a long-latency movement contralateral to the short-latency response. Such dissociation of the alpha and long-latency CRs was now verified for the first time under controlled conditions, and in an arrangement in which both qualitative and quantified behavioral responses together with the neural aspects of conditioned response development were investigated in the same experimental design.

The analysis of backward conditioning sessions in cats K47 and K50 also suggested the different associative nature of the short-latency and long-latency CR: only the long-latency CRs disappeared as a result of the reversal of the experimental conditions from classical conditioning to backward conditioning.

5.5.2 Associative learning and specifty for pairing

Both the behavioral and neural short-latency and long-latency responses indicated significant pairing specificity as compared to the unpaired treatments. Thus, a conclusion favoring associative learning seems inescapable: the paired presentation of the CS and UCS yielded a different result from that obtained for the unpaired controls. However, before any final conclusion of the associative nature of this learning can be drawn, the effect of the temporal proximity of the CS and UCS for learning should be studied.

In the present study, the contingency (definite order) of the CS and UCS was controlled by using randomly assigned CS and UCS presentations during unpaired treatment. One problem, however, which remains is the role of the temporal proximity of the CSs and UCSs (contiguity effect) during paired treatment. During unpaired sessions, although the CSs and UCSs may both sometimes occur in a right contingency (i.e., CS–UCS) or in a backward contingency (UCS–CS), the time intervals (ITI) separating CSs and UCSs are considerable longer than during paired trials. To ensure that this factor, too, is controlled, it is necessary to carry out reversed-order trials with similar interstimulus-interval parameters.

The present experiment included additional backward training sessions after the CO-CC treatments. Only two cats were available for this treatment and thus the statistical conclusions remained open. The backward results suggested, however, that a) the behavioral or evoked neural short-latency responses to the tone during the CS-alone test trials did not habituate; they might even increase and, b) the UCR (head turn to the right) covered the short-latency response (head turn left) trials in cat K47, and c) the long-latency CR acquired during the preceding paired training (in the CO-CC-BW group) habituated. Thus it seems as if the temporal contiguity was a necessary and sufficient condition for an increase in the short-latency CR during paired sessions. Hence, the nature of the short-latency changes might be regarded as nonassociative (sensitization).

By contrast, the long-latency CR also indicated sensitivity to a contingency of stimuli; suggesting associative learning. The "true" associative nature of the long-latency CRs is also supported by behavioral observations: different head movements appeared to the CS and UCS, and the developing long-latency CR showed a similar specific direction of the head movement as the UCR.

Previous studies have shown that a pairing specific increase in the amplitude of a short-latency cortical evoked response to a click CS extinguished when a backward paradigm was applied after paired training (Woody, 1970; Woody & Brozek, 1969). A later study (Brons & Woody, 1980) showed, however, that while a short-latency cortical (sensori-motor) conditioned response did not show extinction during subsequent backward sessions, the behavioral (eye-blink) CR showed marked depression on such trials.

In a more recent study (Kim, Woody, & Berthier, 1983), a hypothalamic rewarding brain stimulation (HS) was added to the design so that the HS followed the CS-UCS pair 240 ms. In contrast to earlier studies, this experiment showed the development of a long-latency (>200 ms) CR. At the same time the initial short-latency CRs to the CS diminuated during paired training, and eventually 98% of the CRs were long-latency responses. The backward presentation of the hypothalamic stimulation (HS-CS-UCS or HS-UCS-CS sequencies) increased the amplitude of the unconditioned response (indicating nonspecific sensitization), and also, initially, the amplitude of the eye-blink to the CS (thus showing alpha-responses to the CS before paired training). During forward paired training, the short-latency CR became smaller while the long-latency CR (>200 ms) became a dominant response. The peak-amplitude of the developing long-latency CR occurred between the UCS and subsequent HS (i.e., peak latency varied between 340–580 ms) and this was also present on extinction trials following the paired training (Kim, Woody, & Berthier, 1983). Thus, these studies indicated that in addition to a accelerating effect of the HS on paired learning, learning of a long-latency eye-blink CR was possible.

Voronin, Gerstein, Kudryashov and Ioffe (1975), also using brain stimulation in later studies, confirmed the acceleration effect of the HS, but mainly on the short-latency CR. The UCS was directly applied on the motor cortex, eliciting a short-latency unconditioned foreleg movement in rabbits. The CS was either a flash or click which acquired a capacity to elicit a very short-latency (12–16 ms) leg movement UCR. However, Voronin also reported long-latency CRs appearing at moments corresponding to the omission of the UCS (CS-alone trials).

In the present study, reliable pairing-specific learning of a short-latency and long-latency CR was found in both behavioral and neural responses. The development of a long-latency CR was slower, and the magnitude of the response remained smaller, especially with evoked responses. No significant diminution of the short-latency CR was found; while the long-latency CR developed, although some signs of an interaction of the alpha-reponse and unconditioned response appeared.

The backward conditioning sessions showed that on the other hand, the associative nature of the acquired short-latency CR is not self-evident. Because the backward paradigm controls the temporal proximity effect of the CS and UCS, the nonassociative sensitization during forward and backward conditioning paradigm can be considered to be rather similar. The comparison of the CS-alone trials of the unpaired (CO) sessions to the CS-alone trials of the 5. backward sessions (Figure 5.20) shows the difference: during the unpaired sessions both short-latency and long-latency responses disappeared, but during backward training, the short-latency responses to the CS did not extinguish, while the long-latency CR disappeared.

5.5.3 A retardation effect of the unpaired sessions on subsequent paired learning

In this study, the balanced order of presentation of the treatments (CC-CO and CO-CC groups) also made it possible to study the effect of unpaired presentations of the CSs and UCSs on subsequent paired learning. Some earlier studies have reported depressive or facilitatory effects of the preexposure of the CS and/or UCS on behavioral CR but in the present study, these effects are studied in a controlled sequence and also as changes in neural (EP) responses. Previous behavioral studies have suggested that some degree of retardation can be most probably expected as an effect of the pre-exposure of the CS (Berthier & Woody, 1984; Lubow & Moore, 1959), or of the UCS (e.g., Mis & Moore, 1973; Siegel & Domjan, 1971). It has been suggested that the retardation effect of the pre-exposure can be explained as a result of a) the learning of incompatible conditioned responses during pre-exposure, which may then retard the learning of a new association to the same stimuli, or b) adaptation or habituation to the CS during the pre-exposure.

The present study indicated that the unpaired presentation of CSs and UCSs had a retardation effect on subsequent paired learning. Because the CO-CC group showed marked habituation of the initial orienting response to the CS on unpaired sessions, the possibility that the CS might have acquired some incompatible association during unpaired treatment appears improbable. Possible explanation might be, either that the long-term habituation of the alpha response (and thus slow recovery) or that the unpaired UCSs might have acquired some incompatible associations during unpaired sessions. This suggestion was supported by the finding that the amplitude of the UCR during subsequent paired training was lower on the first session (this was also observed in First study) indicating a probable blocking effect when the CS is paired with the UCS after their unpaired presentations.

A nonspecific facilitatory effect of UCS pre-exposure on subsequent learning was not found; in contrast to, for example, Kim, Woody and Berthier (1983), who reported that the initial pre-exposure of the HS sensitized both responses to the click CS and glabella-tap UCS on subsequent paired training.

5.5.4 Additive orienting effects in the CC-CO group?

The CC-CO group seemed to show higher response levels during paired training, both in behavioral and neural measures than the CO-CC group during paired training. This might be due to some additive effect resulting from the temporal closeness of the CS and UCS without the blocking effects found in the CO-CC group. One possible explanation is that the overlapping of the continous train of tone-CS (1000 Hz) with the stimulation train of the lateral hypothalamus had some interaction effect. Generally, the CO-CC group in this study was not able to reach the same level in behavioral short-latency CRs, at least on ten paired sessions, as the CC-CO group showed from the very beginning. The different performance of the CO-CC and CC-CO groups should at least exclude the possibility of a purely mechanical additive effect; because the stimulus overlap was identical in both groups.

If the UCS periods of paired sessions in the CC-CO group are compared to the corresponding periods of paired sessions in the CO-CC group, a difference in baseline is obvious. In behavioral responses a sudden decrease in the UCS period in the paired sessions can be observed during subsequent unpaired UCS-alone sessions. In evoked responses, the UCS-alone responses during CO sessions in both groups are rather similar: they are generally at a higher level than during paired sessions. Thus some release (in the CC-CO group) or suppression (in the CO-CC group) takes place when the order of the treatments is changed from paired to unpaired or vice-versa.

What kind of mechanism can be imagined, which would explain the difference which appears as a result of different treatment order? In the CC-CO group it could be easily suggested that some additive orienting effect of the paired CS and UCS presentations evokes a high-level behavioral or neural response both to the CS and UCS and on later sessions, the associative learning process over sessions probably compensates for the initial orienting response effect. It is more difficult to explain the lower level of the UCS period during unpaired sessions in the CO-CC group although the paired CS-UCS presentations are identical to those of the CC-CO group. On the other hand, as Figures 5.11, 5.12, and 5.13 show, the UCS-alone neural response levels in both groups are a) higher on unpaired than paired sessions and b) at about the same level in both groups on unpaired sessions. It follows that the difference between AA and DD cannot be a) due to a difference in the UCR amplitudes between these groups during unpaired sessions or, b) due to a habituation of the UCR; because no habituation can be dedected on the unpaired UCS-alone trials.

One remaining possibility is that on unpaired sessions, the habituated CS of the CO-CC group somehow affects the UCR during the following paired presentation. The additive effect on paired sessions in the CC-CO group could be understood as heterosynaptic facilitation (Harvey, Gormezano, & Cool-Hauser, 1985) resulting from a) a facilitation between the UCS and the CS and b) an activity-dependent neuromodulation (Hawkins & Kandel, 1984; Byrne, 1985) type of effect between the CS and UCS. But why does the CO-CC group not show such facilitation? Moreover, it shows that the habituated CS can have a more suppressive effect than a novel CS on the following UCR.

There is, however, another way to solve this problem: the habituated CS in the CO-CC group represents long-term habituation which has a suppressive trace effect, that is, its effect is exerted over a period covering at least the duration of the CS (2048 ms in this experiment) and possibly due to some irreveversible long-term changes in neuron chemistry, the UCS is no longer capable of reaching the same effect on these neurons as in the CC-CO group. Another explanation might be that the UCS has acquired, on unpaired sessions, some association to environmental stimuli; and thus the paired presentation of the CS and UCS might show some kind of blocking effect on a new contingency. Then the higher level of the CS and UCS periods on paired sessions in the CC-CO group than in the CO-CC group might be interpreted to result from the lack of any retarding or blocking contingencies in this group. However, if some contingency between environmental stimuli and UCS were formed during unpaired sessions in the CO-CC group, then such suppression should also be present during paired sessions, that is, the amplitude of the UCR compared to the UCS-alone trials of unpaired sessions should further decrease during paired sessions. As Figures 5.8, 5.12, 5.13, and 5.14 show, such changes did not appear either in behavioral or neural UCRs. A probable explanation to this dilemma might be that a) the temporal proximity (or overlapping) of the CS and UCS results in a decrease in the UCR because the CS "dissipates" the energy of neural cells ("trace" effect). In the CO-CC group, the CS has undergone long-term habituation and thus lost its orienting power which in the CC-CO group presumably facilitates the subsequent paired UCR. Thus both the CC-CO and CO-CC groups are sensitive to the suppressive effect of paired and overlapping presentation of the CS and UCS (appearing as a decrease in the UCR amplitude), but only the CC-CO group can compensate this suppression effect because the novel CS and UCS yield strong and mutual facilitation (orienting responses).

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6 THIRD STUDY: INTERACTION BETWEEN THE TIME-AMPLITUDE COURSE OF THE UNCONDITIONED RESPONSE AND THE CONDITIONED SHORT-LATENCY (ALPHA) RESPONSE

According to some initial observations in preliminary studies and in the present studies (First and Second studies), an interaction between the time-amplitude course of the unconditioned response (UCR) and the developing conditioned response to the CS is proposed. Changes in the topography of the conditioned stimulus period were studied as a function of the UCR topography in both behavioral (head movements) and neural evoked responses. Three alternative explanations were examined: first, the observed change can reflect a general, increased orienting effect of the CS, second, a strong stimulus such as an UCS might disclose some basic "maximum orienting response", specific to each brain site, and third, the UCS can have a real specific influence on the CS-pathway system; it might leave some "trace" on the response topography of the CS. The results presented here supported the "trace"-hypothesis although the acquired evidence must be considered rather tentative than conclusive.

6.1 Introduction

The results of earlier preliminary experiments (Korhonen & Penttonen, 1981a, 1981b) had suggestively indicated that the development of the time-amplitude course of the evoked potentials during the conditioned stimulus (CS) period (interstimulus interval, ISI) could exhibit certain overall features similar to the time-amplitude course of the unconditioned stimulus (UCS) period.

The hypothesis formulated according to these initial findings was that the effect of an unconditioned stimulus might appear in evoked responses so that after paired training, the time-amplitude course of the CS includes some "traces" of the UCR. The problem then becomes whether we can prove that the UCS has modified the sequence of neural events during the CS period. The hypothesis of an interaction of the UCR and CS periods was examined in this paper using the experimental data collected in earlier and present studies.

The first experiment reported here is based on the material presented in the earlier paper (Korhonen & Penttonen, 1981a). The second and third part of this paper are based on the experimental material presented in the First and Second study of this thesis.

6.2 Experiment 1

In this experiment, the brain stimulation train duration (550 ms) was shorter than in later studies (First and Second study); and the delayed conditioning paradigm consisted of a 1500 ms tone and light CS overlapping with the UCS during the last 550 ms. Because the studies reported on this thesis indicated similar signs of a correspondence between the UCR and CS periods, the difference between the length of the ISI (980 ms) and the UCS (550 ms) in the earlier experiment (Korhonen & Penttonen, 1981a,b), compared to 1024ms ISI and 1024ms UCS in present studies, was supposed to be advantageous for the identification of some specific feature, a "trace", of the suggested UCS effect on the CS period.

Methods

Evoked neural responses were recorded in four cats from the cingulate cortex and hippocampus during training sessions carried out according to the classical conditioning paradigm. The experiment consisted of three unpaired control sessions followed by eight paired sessions during which a 1500 ms light and tone CS were presented together with a brain stimulation UCS (550 ms and overlapping the last part of the CS). The details of the methods used are described in Korhonen and Penttonen (1981a).

Results

The time-amplitude course of the UCS period (550 ms) and the CS period (ISI) are shown in Figure 6.1, in which an average curve (45 trials) of the UCS period of the first paired session is taken as a reference; and this part of the curve (thick line) has been moved 980 ms left from its original position and superimposed on the CS period (thin line) of the 5. paired session of the same cat and the same recording electrode so that the onsets of both stimuli coincide. The recordings of four different electrodes on four cats (K73, K74, K75, and K76) are shown in this figure.

The evoked response curves are selected only on the basis of their specific topographical features, not their anatomical or functional significance.

The main purpose of this comparison was to find out whether the time-amplitude course of the initial UCS-period had any interaction with the developing CS and, second, whether the 550 ms brain stimulation UCS

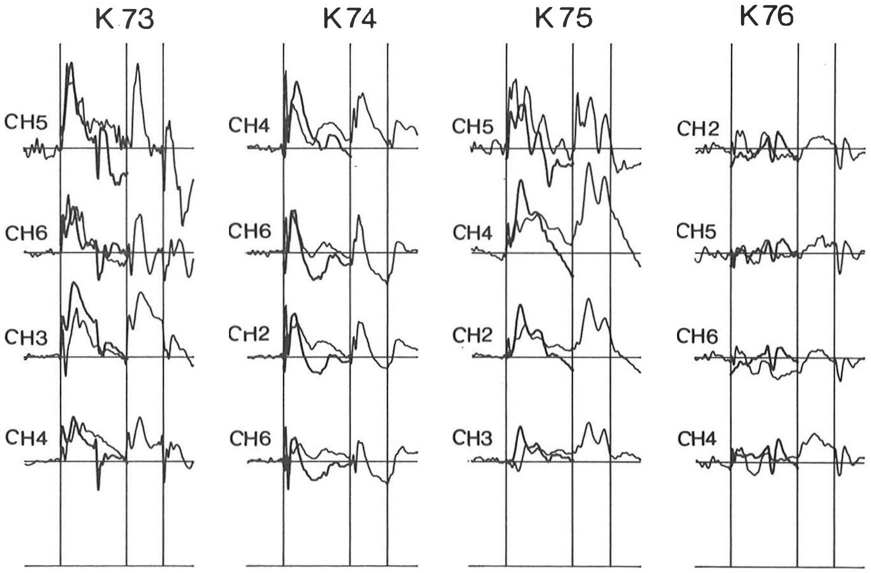

Figure 6.1 Averaged (45 trials) evoked response curves (thin line) of different recording sites in cats K73, K74, K75, and K76 of the fifth paired session are presented together with the average curve of first paired session (thick line). This curve has been moved left 980 ms from its original position and superimposed on the ISI-period so that the onset of the tone-CS and the onset of the UCS coincide. The first vertical line represents the beginning of the CS and the second vertical line the beginning of the UCS.

had left any specific identifiable "trace" of its own topography which then could be interpreted as a modifying effect of the UCS on the ISI. Because the offset of the UCS elicited another evoked response it was supposed that it could leave an identifiable mark on the corresponding time point of the CS period, that is, at about a latency of 550 ms after the CS onset. The UCS period of the first paired session was taken as a model because the UCS alone trials of the preceding control sessions were not available for this study.

Apparent similarities between the time-amplitude courses of the UCS of the first paired session and the CS period of the fifth paired session can be observed for all cats (Figure 6.1). The time-amplitude courses of both traces coincide in their main details, although differences in the delays of the later evoked components can be found in all cats. This possibility, that the neural response may have the same underlying form in all cases is not immediately obvious; since each individual cat, and the different recording sites within each animal, reveal rather dissimilar time-

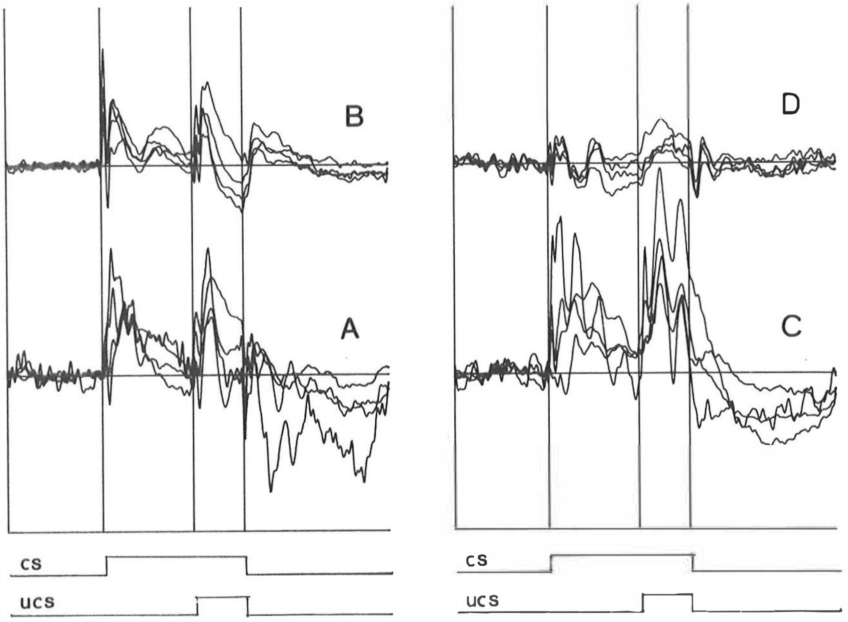

Figure 6.2 Averaged evoked responses of four different recording sites during fifth paired session of cats K73 (A), K74 (B), K75 (C), and K76 (D) are superimposed to show the common point of a change at about the latency of 450-600 ms from the CS onset.

amplitude curves; see for example, cat K75. But the results indicate that there is at least some specifity of the form of the neural response within every brain structure examined.

This finding does not justify the assumption of a causal influence of the UCS effect on the CS-period. Rather it could be argued that changes in the CS-period after paired learning are similar to an original orienting response to any novel or sensitizing stimulus. Some specific trace of the time-amplitude course of the UCS-period should be identified in the CS-period (ISI) if a definite causality between these events is to be shown. A closer examination of the curves presented in Figure 6.2 show suggestive signs of such correspondence. This figure shows that in the case of all cats there are recordings in which some signs of the UCS offset occurring after the corresponding time delay (i.e., 550 ms after the CS onset) can be detected. The latency of this point of change is not constant, but varies slightly (from 450 ms to 600 ms). A more detailed description of the nature of this variability is given in Figure 6.3, in which an example of the changes undergone by the CS period over five consecutive daily paired sessions is shown and compared, as in Figure 6.1, to the time-amplitude course of the UCS period of the first paired session (thick line).

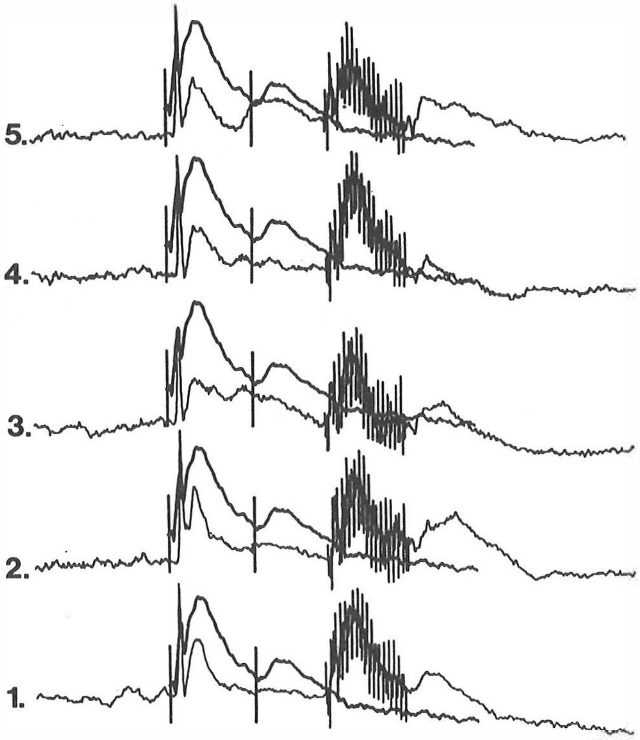

Figure 6.3 The development of a "trace" coinciding with the time-amplitude characteristics of the UCR is shown over five consecutive daily paired sessions. The curve of first paired session (thick line) is moved left 980 ms in time as in Figure 6.1. The first vertical line represents the beginning of the CS and the second vertical line the beginning of the UCS.

The changes in evoked responses in the selected four recording sites in each cat are shown in Figures 6.4, 6.5, 6.6, and 6.7. In the first column (A), the CS-alone trials of the 1. unpaired session (thin line) and 3. unpaired (thick line) control session curves are superimposed. The curves indicate only slight habituation over three unpaired control sessions. The main topography of the evoked responses during the CS period remains largely unchanged over three control sessions. In column B the 1. paired (thin line) and 5. paired (thick line) sessions are superimposed and these curves show that, a) the most prominent change in the evoked response occurs in the late components (>150 ms) and, b) the response during the UCS period shows high stability in its topography over sessions (except for cat K76). Column C includes a comparison of the last (3.) unpaired control session (thin line) and the last (5.) paired session (thick line) curves and thus shows the maximum increase in evoked responses compared to the habituated "baseline" level of the last control session.

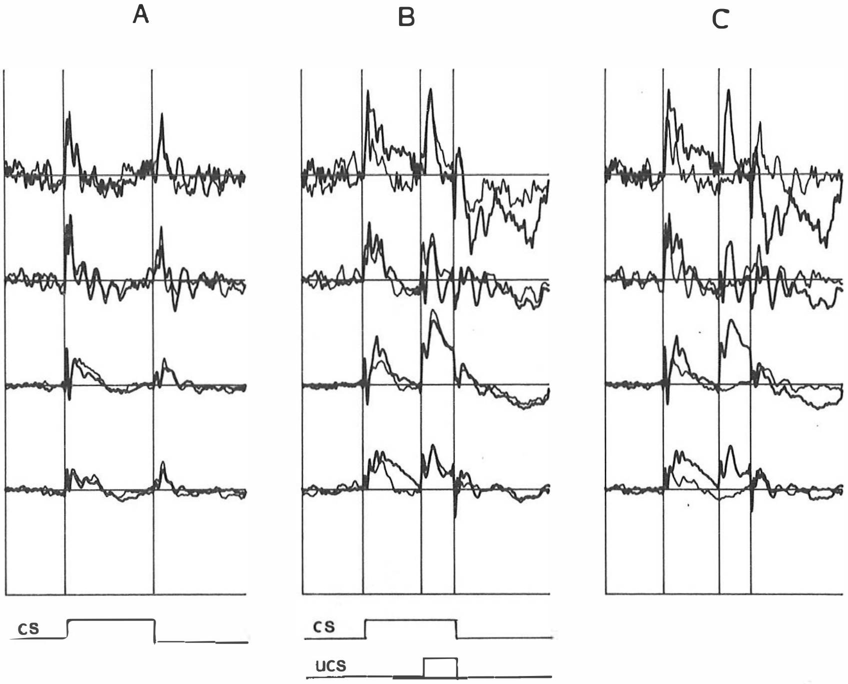

Figure 6.4 Neural evoked responses in four different recording sites of cat K73. Column A shows the averaged evoked response curves of first (thin line) and third (thick line) CS-alone trials during unpaired sessions. Column B shows the averaged response curves of first (thin line) and fifth (thick line) paired sessions. In column C, the last (3.) unpaired session average curve (thin line) is compared to the fifth paired session average curve (thick line).

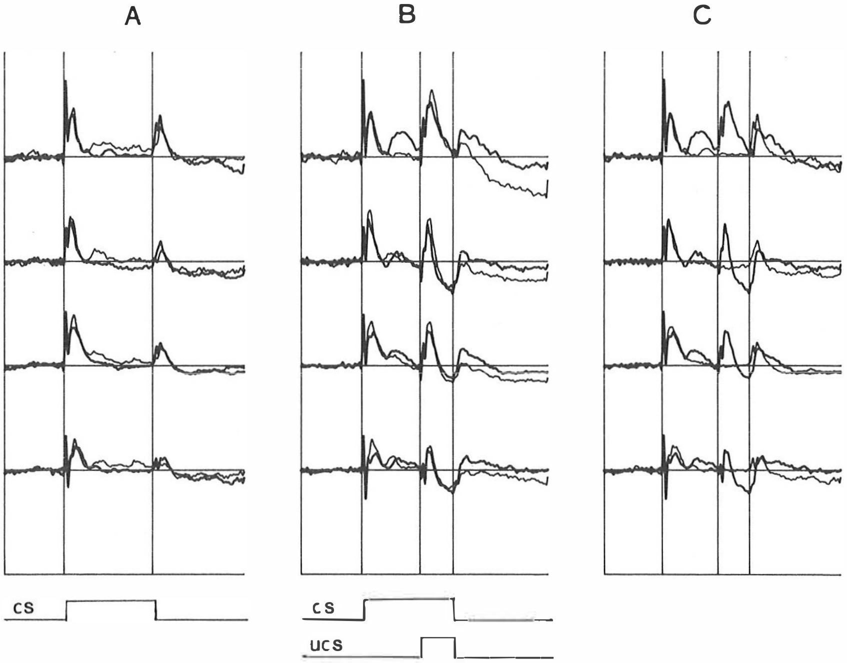

Figure 6.5 Neural evoked responses in four different recording sites of cat 74. See figure 6.4 for an explanation of the details.

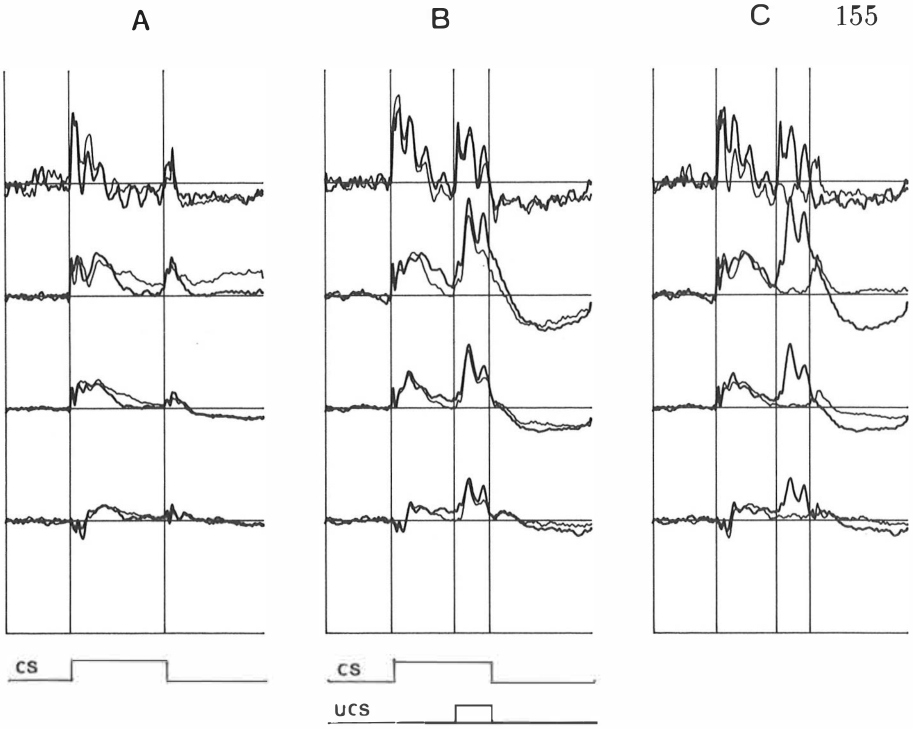

Figure 6.6 Neural evoked responses in four different recording sites of cat K75. See figure 6.4 for an explanation of the details.

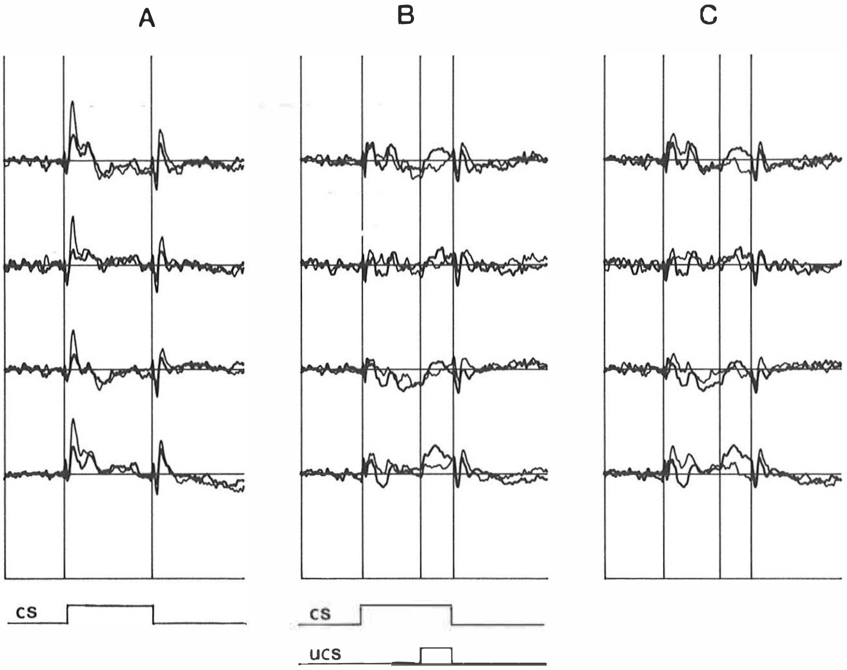

Figure 6.7 Neural evoked responses in four different recording sites of cat K76. See figure 6.4 for an explanation of the details.

Discussion

The results of this experiment suggested some similarity between the UCR and CS periods (ISI). This similarity appeared a) in certain common characteristics of the time-amplitude courses in evoked neural response components, which show the largest changes after about 100 ms from the CS onset, and b) in the specific sign of a "trace" occurring on the 980 ms ISI period and presumably reflecting the properties of the shorter (550 ms) UCS stimulation train.

In this early study there were various shortcomings in the experimental design which were corrected in later experiments. In retrospect, the lack of the behavioral response recordings (on video and from movement transducer) appeared as the most serious defect. Further, the UCS-alone template of unpaired sessions, as recorded in later studies, was not available for this analysis.

6.3 Experiment 2

In this experiment (i.e., First study of the present thesis), the length of the UCS brain stimulation train was increased from 550 ms to 1024 ms because it was suspected that the short UCS stimulation train had some unwanted effect on the time-amplitude course of the evoked response during the CS-period (which appeared useful, as the first experiment of this paper showed). Further, the durations of the UCS periods were equalized so as to allow the same time for the development of the response in all cases.

This change in the design of the experiment was also considered important for a differentation of short- and long-latency CRs. In the present experiment, the UCS-alone trials of the last unpaired control sessions were thought to represent a "base level" of the UCR topography because the initial orienting effects to the UCS were habituated in the group which had received first unpaired and then paired treatment (the CO-CC group). Correspondingly in the group which had received first paired and then unpaired treatment (the CC-CO group), possible learned modifications of the UCR after paired sessions had disappeared as a result of extinction; and hence the shape of the UCR was thought to approach a "basic" form.

Methods

The details of the methods used in this experiment are described in the First study of this thesis. The session average curve of the UCS-period of the UCS-alone trials involving the fifth unpaired control was designed to represent the features of the UCR without the initial orienting (as with the CO-CC group) or learned effects (as in the CC-CO group) and this curve was used as a model for purposes of comparison.

Results

Evoked response curves and movement recordings appearing during the UCS period were compared to the time-amplitude course of the evoked and movement responses observed during the CS-period of paired conditioning sessions. The comparison of single trial curves and also of session average curves seemed to suggest some overall resemblance between these curves. A preliminary survey showed that especially in the CO-CC group, the average shape of the UCS-period of the UCS-alone trials of the unpaired control sessions might represent, or correspond to the final form of the evoked and movement response curve observed during the CS-period of the subsequent paired sessions (especially during the last session). The correspondence of these periods, in both recorded evoked response and movements, is shown in Figures 6.8, 6.9, 6.10 and 6.11.

A correlation coefficient between sampled (128) points was defined for these periods (see Figures 6.12 and 6.13). A similar correlation was also calculated between the UCS-periods of the last (5.) unpaired and the last (5.) paired session in order to compare the stability of the unconditioned response topography.

Because the correlation between the UCS-period of unpaired sessions and the CS-period of paired sessions appeared to be quite high ($r>.800$) in some recording sites, this raised the question of whether the observed time-amplitude course merely indicated some general topography, appearing in all channels equally. The specificity of the characteristics of the response curve was examined by comparing specific features and latencies of the curves of different recording sites in the same cat. Figures 6.12 and 6.13 show the curves used for this comparison, and also the correlation coefficients between the UCS-period of one channel and CS-period of another channel in the same animal. In each cat the two channels were selected so as to represent the greatest differences in time-amplitude courses. The correlations between different recordings showed negative or only small positive coefficients compared to the notably high correlations observed within each recording; thus indicating channel specifity in each cat.

These correlations suggested some interaction between the UCS- and CS-periods during associative learning. The consistency of this change over consecutive training sessions, especially in the CO-CC group, was observed because in this group the initial responses to the CS after unpaired control sessions appeared to develop rather slowly (Figures 6.9 and 6.11). The specifity for pairing of this similarity was subsequently

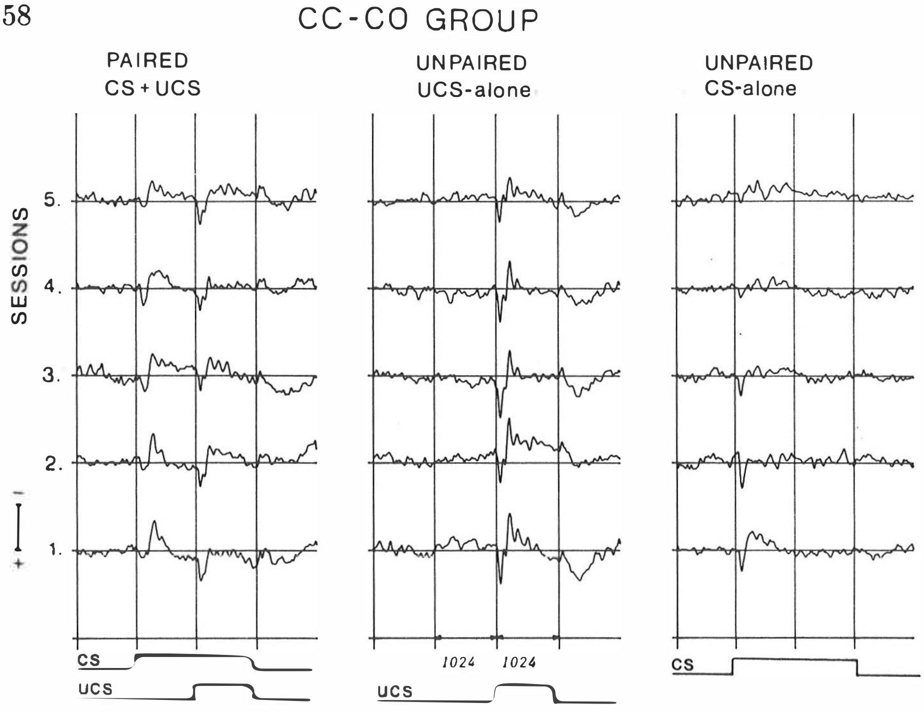

Figure 6.8 Evoked response curves of five sessions during averaged paired, unpaired UCS-alone and unpaired CS-alone subiculum trials of cat K30 from the CC-CO group.

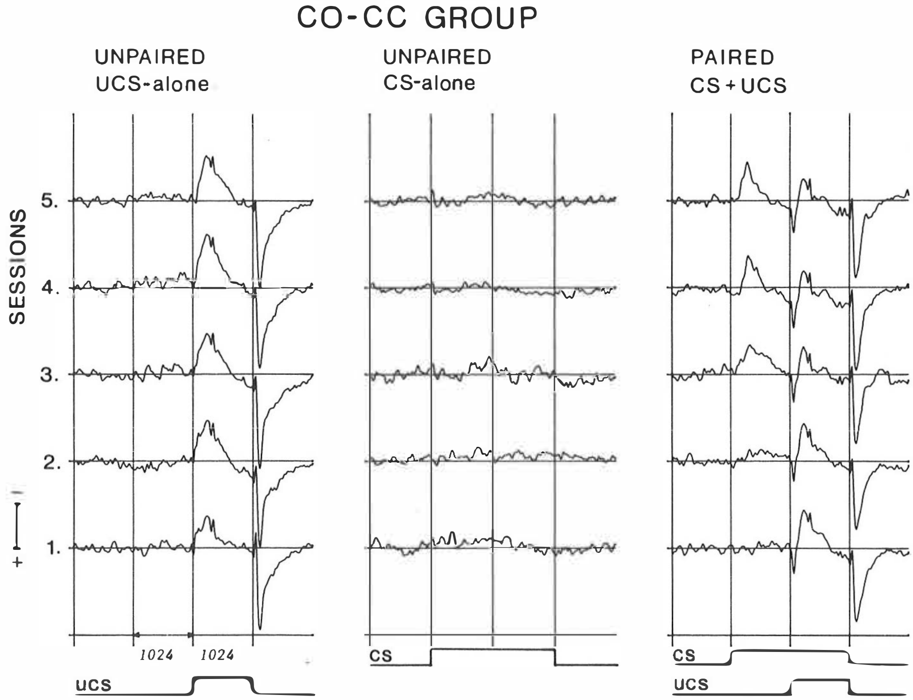

Figure 6.9 Evoked response curves of five sessions during averaged paired, unpaired UCS-alone and unpaired CS-alone CA1 trials of cat K31 from the CO-CC group.

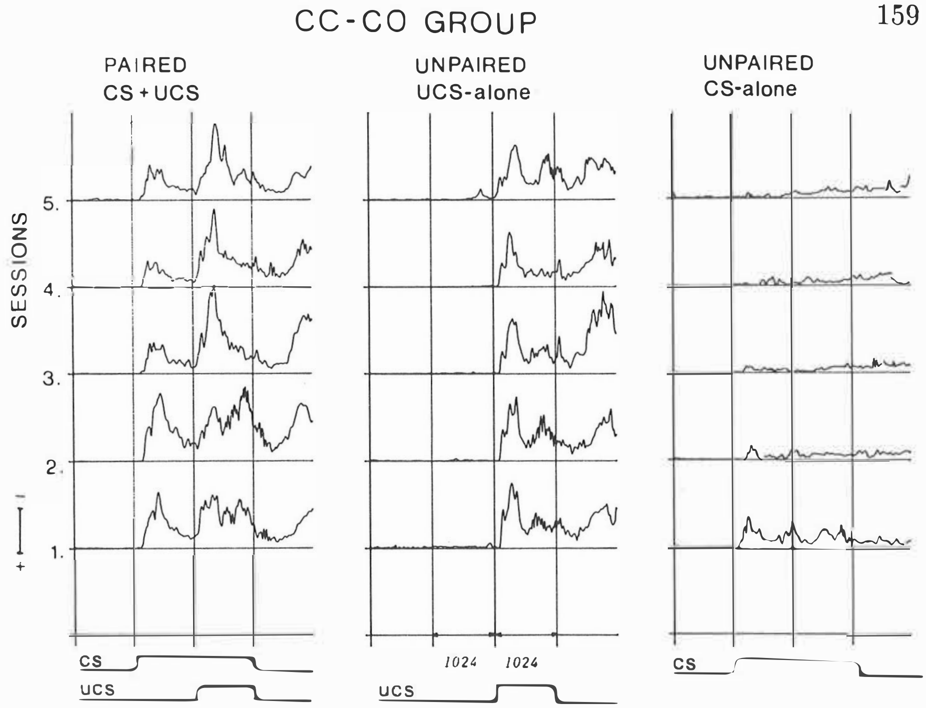

Figure 6.10 Head movement curves of five sessions during averaged paired, unpaired UCS-alone, and unpaired CS-alone trials are shown for cat K30.

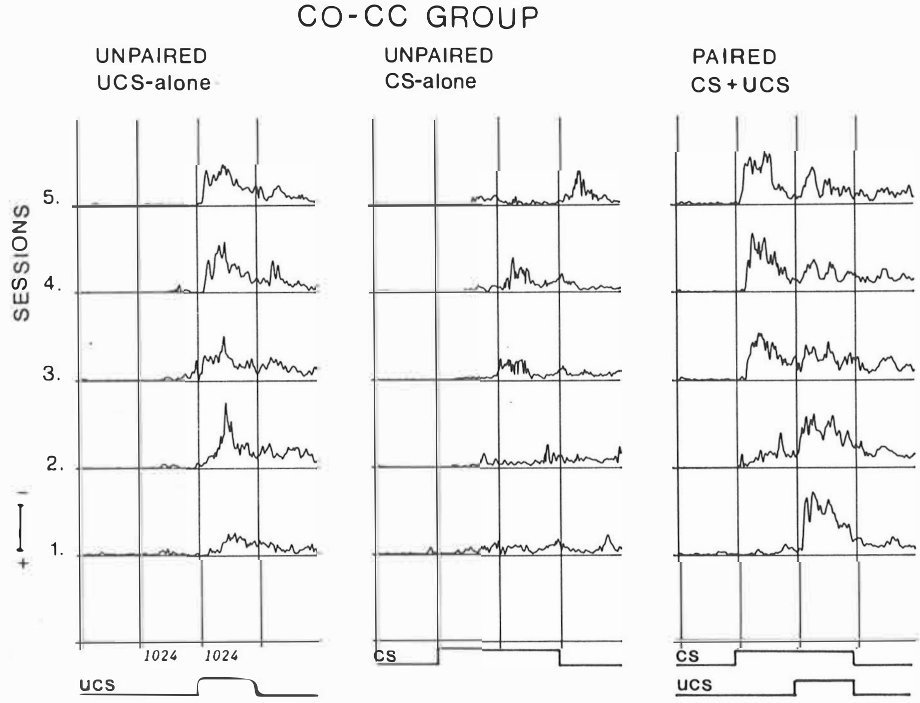

Figure 6.11 Head movement curves of five sessions during averaged paired, unpaired UCS-alone, and unpaired CS-alone trials are shown for cat K31.

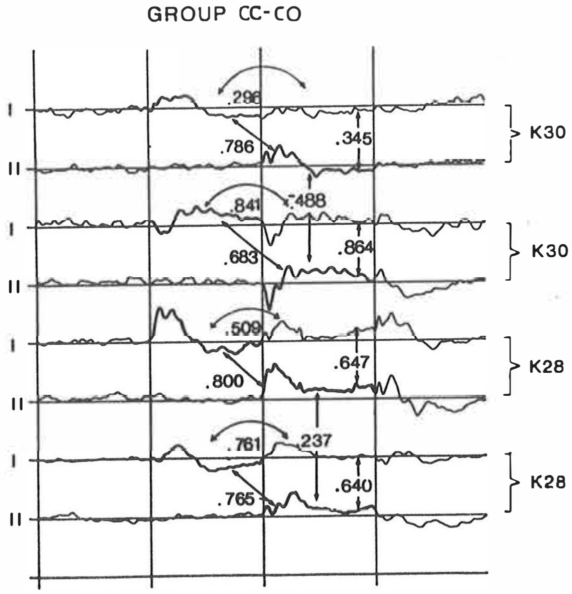

Figure 6.12 An averaged UCS-period of fifth unpaired UCS-alone trials (II) is correlated with the averaged ISI-period of fifth paired session (I) in the CC-CO group. Correlations are also calculated between the UCS-periods of fifth unpaired UCS trials ands fifth paired trials within the same recording site. A similar correlation over different recording sites of the same cat is also calculated to show that the time-amplitude course of the evoked response is not some general stereotypical form appearing in all recording sites in identical form. A correlation between the ISI-period and UCS-period of fifth paired session is also shown for purposes of comparison with the correlation between the UCS-alone trials of fifth unpaired session and the ISI-period of fifth paired session. The first vertical line represents the beginning of the CS and the second vertical line the beginning of the UCS.

supported by the features of the time-amplitude course observed during the CS-period of unpaired sessions: unpaired presentations of UCSs did not have a similar effect on the evoked response of the CS-period as that observed during paired treatment. The response to the CS merely showed habituation over control sessions.

The changes in correlation coefficient over paired and unpaired sessions, averaged over animals for different recording sites, are shown in Figures 6.14, 6.15, and 6.16. The UCS-alone period of the fifth unpaired session is also used here as a reference. These figures show that the CS period of the CO-CC group has low positive or even negative correlations during unpaired sessions (C); while the UCS-alone trials of these sessions show high correlations to the reference session (from .75 to .95) in cingulate cortex, subiculum and CA1 recordings indicating high stability of the

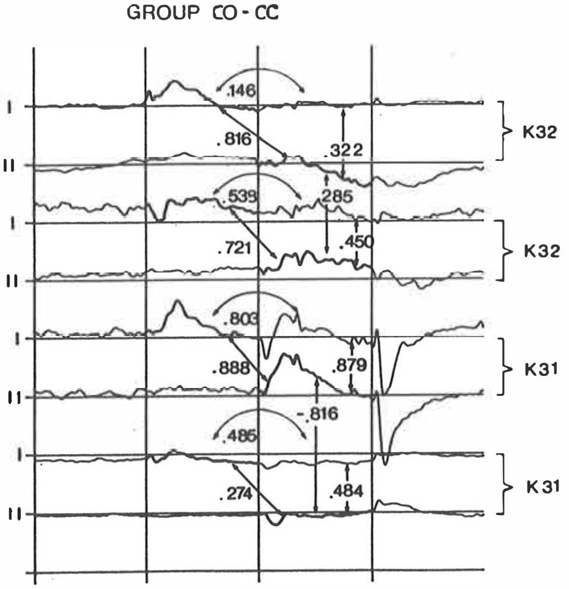

Figure 6.13 An averaged UCS-period of fifth unpaired UCS-alone trials (II) is correlated with the averaged ISI-period of fifth paired session (I) in the CO-CC group. Correlations are also calculated between the UCS-periods of fifth unpaired UCS trials ands fifth paired trials within the same recording site. A similar correlation over different recording sites of the same cat is also calculated to show that the time-amplitude course of the evoked response is not some general stereotypical form appearing in all recording sites in identical form. A correlation between the ISI-period and UCS-period of fifth paired session is also shown for purposes of comparison with the correlation between the UCS-alone trials of fifth unpaired session and the ISI-period of fifth paired session.

unconditioned response over sessions. The low correlation values of the unpaired CS-period (C) are probably due not to a difference in topography, but to a decrease in the amplitude of the response after habituation. The CO-CC group shows, however, a rapid increase in correlations during the CS-periods of the subsequent paired sessions (D): on the last paired session the correlations were about .70 for cingulate, .85 for CA1, and .50 for CA3.

The conclusion that the change in the time-amplitude course of the CS-period is a result of the paired treatment is thus supported: the CS period begins to show a waveform similar to that of the UCR. When the CS-period correlations of the CC-CO group (A) during the first paired conditioning phase of the experiment are compared to the first CS period of the unpaired phase of the CO-CC group (C), the difference due to treatment appears significant: in cingulate cortex $F(1,2)=523.22$, $p<.01$, and in CA1, $F(1,2)=38.84$, $p<.05$. The CS-periods of the CC-CO group (A) indicate that this group shows from the beginning of paired training

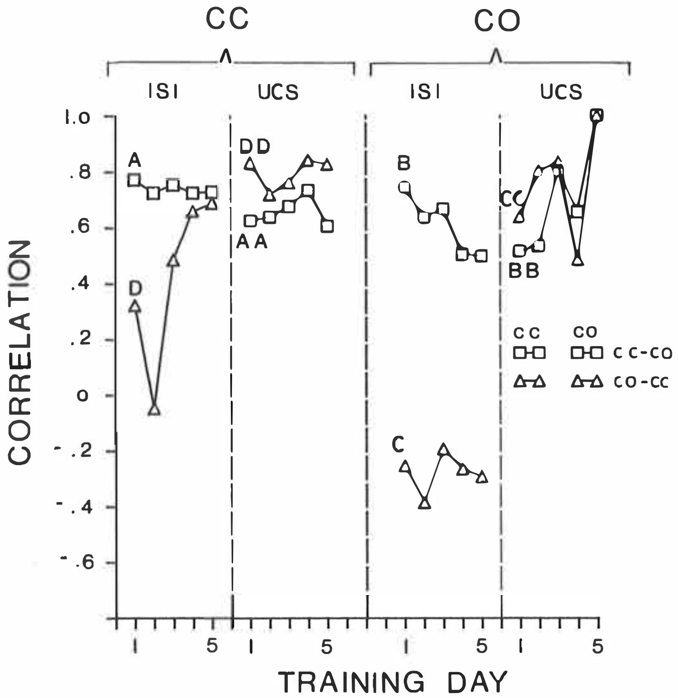

Figure 6.14 The changes in correlation coefficients over five paired sessions of the ISI-periods (A, B, C, and D) and of the UCS-periods (AA, BB, CC, and DD) of the CC-CO group (open squares) and of the CO-CC group (open triangles) are shown. The reference to which these correlations were calculated was the average of the UCS-alone trials of fifth unpaired session. In this figure, the changes in correlation coefficients in cingulate cortex are shown.

a time-amplitude course similar to the reference UCS curve of the last unpaired session (the difference between A and BB was not significant) while the CO-CC group seems to acquire the refence form gradually (D). Thus the increase or high level in correlation between the CS-period and reference curve appears only as a result of paired presentations of the CS and UCS. This conclusion is further supported by observation of the extinction occurring in the CC-CO group during the following unpaired sessions (B). The analysis of variance (order x treatment x sessions) showed significant treatment x sessions interaction F(1,2)=32.83, $p<.05$ (the Geisser-Greenhouse conservative F-test was used in all analyses reported in this paper) in correlations in cingulate recordings: correlations increased on paired and decreased on unpaired sessions (see Figures 6.12, 6.13, and 6.14).

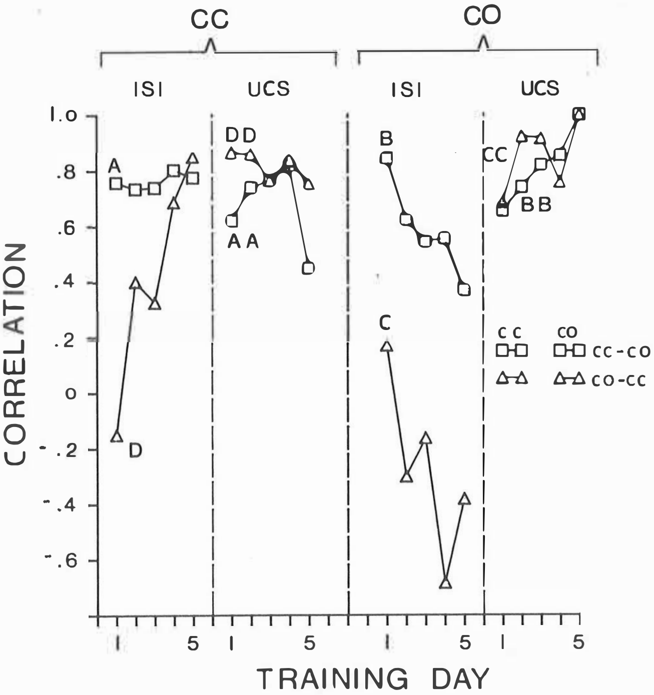

Figure 6.15 The changes in correlation coefficients in CA1. See figure 6.4 for an explanation of the details.

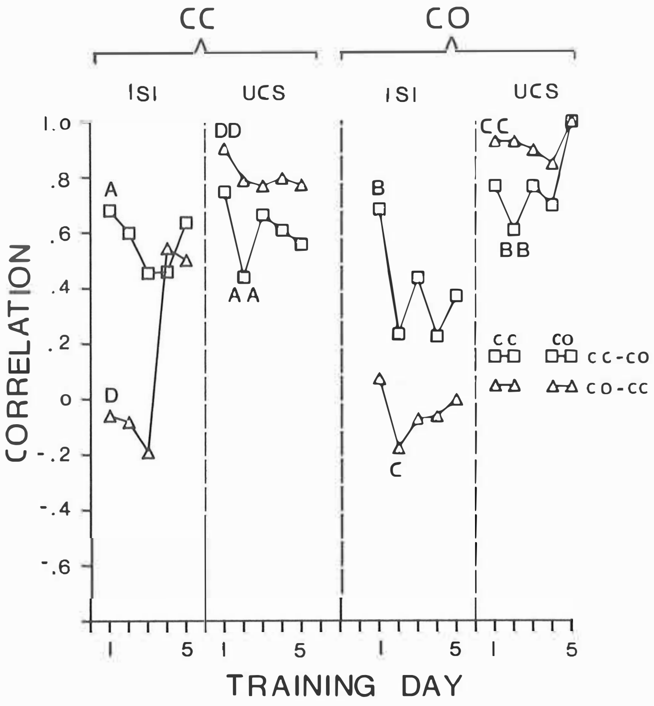

Figure 6.16 The changes in correlation coefficients in CA3. See figure 6.4 for an explanation of the details.

Discussion

These preliminary analyses of the specific features of the time-amplitude course during the CS-period of paired training seem to suggest that a) the time-amplitude characteristics of the unconditioned response during the whole UCS-period seem to have some specific effect on or at least, a correlation with the characteristics of the CS-period of paired sessions, and b) this effect is something more than some general characteristic of evoked responses, which might appear in similar form in all recordings.

Such specific interaction between the time-amplitude courses of the UCS and CS-periods in evoked response CS periods has not, to our knowledge, been reported in earlier studies. Hori, Toyohara and Yoshii (1970) reported in their conditioning study of neural unit activity that most neurons, after they had been successfully conditioned, responded to the CS with a similar pattern as to the UCS. However, they do not present any more specific details of this relationship.

If the interaction between the UCS- and CS-periods is a reality, one might ask whether this similarity is anything more than an indication of some typical response pattern produced by the recorded brain site, and always appearing in the same stereotypical form (provided that the eliciting stimulus is strong or novel). While it is possible that the observed interaction can accounted for in these terms, it is also possible that the time-amplitude course of the CS-period gradually takes on a form which also includes a specific "trace" effect, originating from the UCR.

6.4 Experiment 3

The results of the second study showed that correlations between the time-amplitude courses of the UCS- and CS-periods were high after paired learning. Although these results seemed to suggest some specific mutual interaction between these periods, the causal relationship remained unproven. If some "trace" of the specific influence of the UCS on the CS period could be identified also the causal relationship would also be confirmed.

The first experiment provided evidence supporting the existence of such specific influence. The present experiment, which involved backward conditioning, might be expected to provide further evidence for the existence of such a "trace" influence; because under this arrangement the temporal relationship of the CS and UCS was reversed (UCS-CS) so as to yield a time-amplitude course different from that which characterized the forward-paired trials (CS-UCS). In this group, a specific time-amplitude course of the CS-period had already been acquired during the preceding paired sessions and the possible change in this pattern during the subsequent backward conditioning could be interpreted as support for a "trace" hypothesis.

In addition, compared to the second study, this experiment involved more training sessions and new recording sites (e.g., subiculum and dendate fascia of the hippocampus).

Methods

The details of the methods used in this experiment are described in the Second study of the present thesis.

Results

The time-amplitude course of the UCR period of the last (10.) unpaired session was selected as a "template", which then was correlated to the CS- and UCS-periods of all paired and unpaired sessions. The correlations were calculated using the original sampling points of each period (128 samples).

In evoked responses, some similarity of the time-amplitude course of the CS period (ISI) to the original UCR topography was observed after paired training. Such similarity appeared in the CC-CO group from the beginning of paired training. In the subiculum and CA1, correlations of the paired sessions (A) of the CC-CO group and unpaired sessions (C) of the CO-CC group in the first phase of the experiment showed significant differences in the CS period; $F(1,4)=31.54$, $p<.01$, and $F(1,8)=25.68$, $p<.001$. In dendate fascia, the CS period of the paired sessions (A) exceptionally increased slowly over sessions. Figures 6.17, 6.18, and 6.19 indicate that in all recording sites the time-amplitude course of the CS period of paired sessions approached the topography of the UCR curves: the correlations observed on the last paired session (A and D) were high (from +.7 to +.9) while the correlation of the UCR to the CS period of the CS-alone trials of unpaired control sessions (B and C) was low (from +.2 to +.4); or negative on some sessions.

In the CO-CC group, the change in correlation from the unpaired training level (from C to D) was significant in dendate fascia, $F(1,3)=24.41$, $p<.05$, in subiculum, $F(1,2)=29.49$, $p<.05$, and in CA1, $F(1,4)=31.10$, $p<.01$.

Backward conditioning was studied in two cats. The UCS-CS trials were compared to the CS-alone trials of the last paired sessions (CC10) and to the CS-alone trials of the last backward conditioning session in order to find some "trace" of a different topography and hence some modificatory effect of the backward treatment. This comparison is shown in Figures 6.20 and 6.21. It suggests that the UCS-CS presentation did indeed have some modifying effect on the CS-alone response during backward treatment.

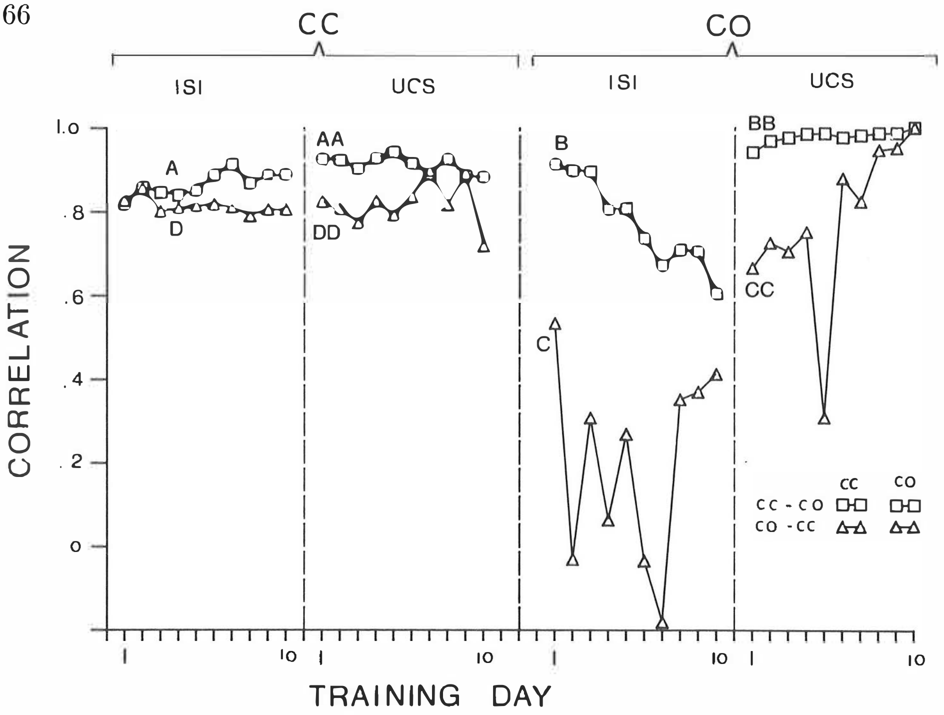

Figure 6.17 The changes in correlation coefficients in subiculum. See figure 6.4 for an explanation of the details.

The correlations were calculated between these curves, and they show that the similarity between UCS-CS and CS-alone trials of the fifth backward session is higher (r=.775 for cat K50 and .573 for K47) than the similarity between the CS-alone trials of the fifth backward sessions and CS-alone trials of the last paired (CC) session (r=.423 for K50 and .376 for K47). The details of the time-amplitude courses of the CS-alone curves of the last forward paired (CC) and last backward-paired (BW) session indicate a conspicuous difference occurring at the latency of 100-600 ms after the CS onset: the CS-alone curve of the fifth BW sessions has taken on a new form related more closely to the UCS-CS curve of the fifth BW sessions than to the 10. CC session curve.

Discussion

The results of this study confirmed the findings of the second study: the time-amplitude course of the CS-period of paired sessions begins to show the same characteristics as the UCR. This study also yielded some additional support for the assumption of the specific role of the UCR in this change. The response during the CS-period showed some modification in its topography after the backward UCS-CS trials were run: the CS-alone trials of the backward sessions began to show greater similarity to

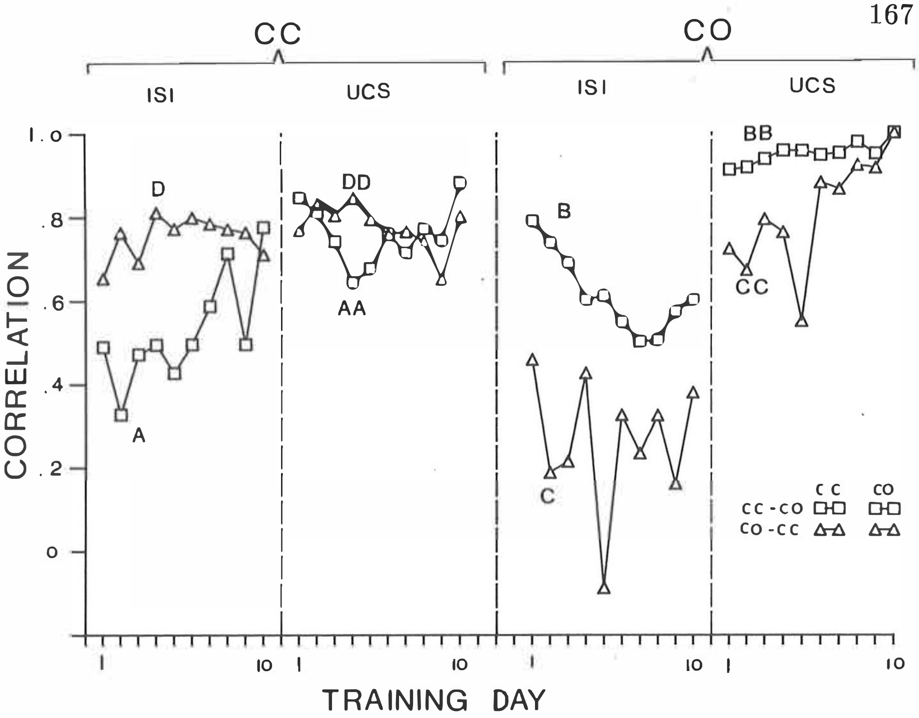

Figure 6.18 The changes in correlation coefficients in dendate fascia. See figure 6.4 for an explanation of the details.

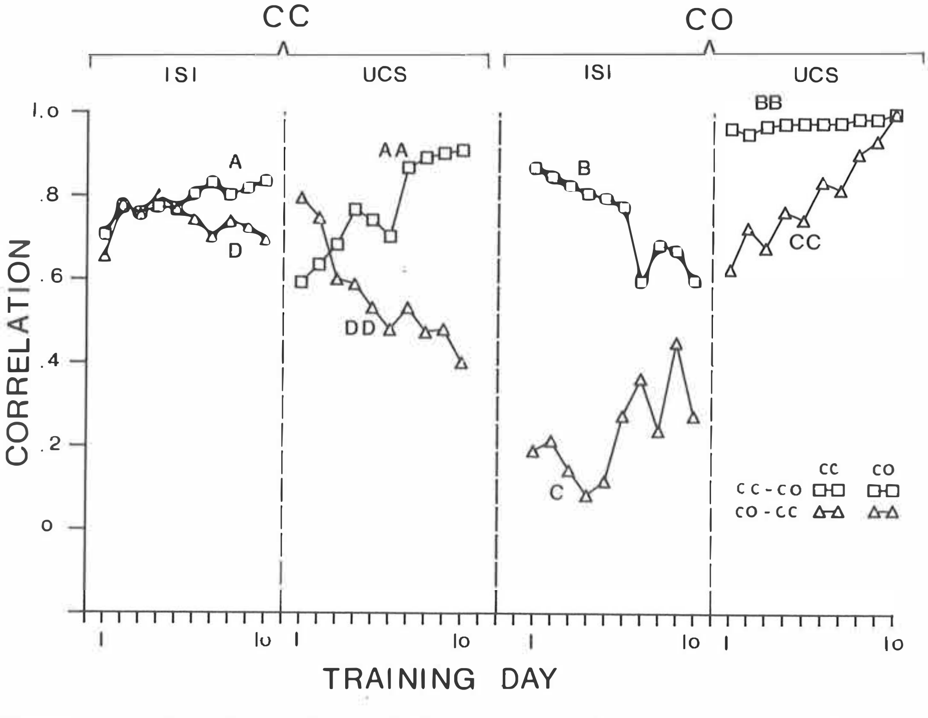

Figure 6.19 The changes in correlation coefficients in CA1. See figure 6.4 for an explanation of the details.

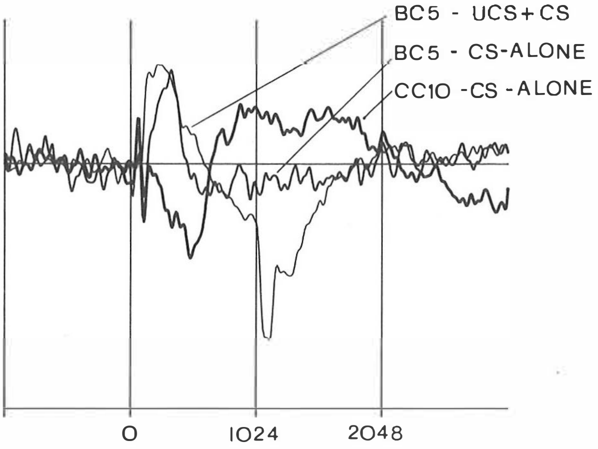

Figure 6.20 The modificatory effect of the different UCS (UCS+CS) provided by backward conditioning (BW) sessions is shown in the CA1 recording of cat K47. For purposes of comparison, a curve representing the average of the CS-alone trials of the last (10.) paired session (CC10-CS-alone) (thick line) is superimposed on the last paired backward-conditioning session (BC5-UCS+CS) curve (thin line), and on the last (5.) backward CS-alone (BC5-CS-alone) curve (intermediate line). The CS-alone curve of the fifth BC session in both cats indicates greater similarity to the paired BC5-UCS-CS trials than to the preceding paired CC10-CS-alone trials.

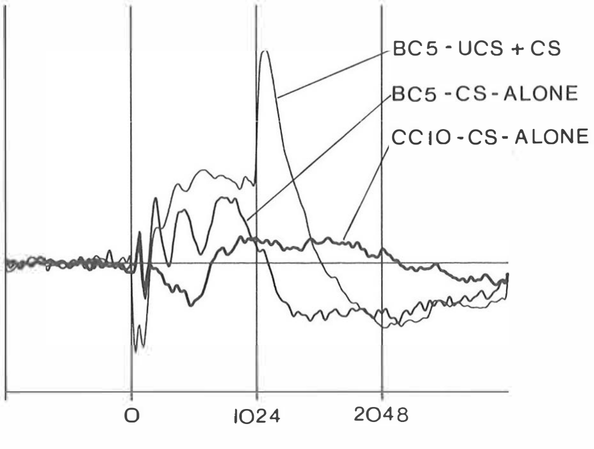

Figure 6.21 The modificatory effect of the different UCS (UCS+CS) provided by backward conditioning (BW) sessions is shown in dendate fascia recording of cat K50. For purposes of comparison, a curve representing the average of the CS-alone trials of the last (10.) paired session (CC10-CS-alone) (thick line) is superimposed on the last paired backward-conditioning session (BC5-UCS+CS) curve (thin line), and on the last (5.) backward CS-alone (BC5-CS-alone) curve (intermediate line). The CS-alone curve of the fifth BC session in both cats indicates greater similarity to the paired BC5-UCS-CS trials than to the preceding paired CC10-CS-alone trials.

the UCS-CS trials than to the CS-alone trials of the preceding paired sessions.

6.5 General discussion

If it is assumed that there is some interaction between the time-amplitude courses of the UCR and the response to the CS during paired training, there are at least three alternative explanations for the phenomenon.

First, the observed increase in similarity may reflect only a general, increased orienting effect of the CS acquired during the paired training. A pairing-specific increase in the amplitude of the evoked short-latency (alpha) response was verified in the second and third study of this thesis. However, a closer examination of the observed changes showed that the time-amplitude course of the evoked short-latency responses seemed to be rather different in different recording sites. Thus the hypothesis of some general form to the response to the CS was not supported.

A second alternative explanation might be that within each recorded brain site some typical "maximum" response of the neuronal assembly to a strong or significant stimulus was recorded. According to such "maximum orienting response" hypothesis, a strong stimulus such as an UCS should disclose the essential time-amplitude course of the neural evoked response in each recording site. And the CS, too, after paired training, can be supposed to acquire a similar capacity to elicit responses (ORs); which thus would resemble the UCR in their form.

Although the results of the second and third experiment showed that the maximum-OR hypothesis might explain short-latency part of the CS response, later components of the CS-period may be more sensitive to a modifying influence of the UCS. Hence, a specific sign or "trace" of the influence of the UCS was suggested as a third explanation. Some support, although not conclusive, for the "trace" hypothesis was found in the first and third experiments. Deviations in the length of the brain stimulation UCS in the first study, and a change in the UCS effect in the backward conditioning sessions of the third experiment seemed to provide evidence for a specific modifying effect on the later components of the CS-period.

What would be the consequences to the interpretation of associative learning, if the "trace" hypothesis were accepted? Associative learning might then be described as a process in which the nonassociative sensitizing effect of the UCS is necessary in the first phase of learning in order to promote an increase in orienting response to the CS. In this initial phase, the time-amplitude course of the CS predominantly shows the characteristics of the orienting responses to the CS. If the paired training is continued, the UCS may partially modify this response. In the studies presented here this modifying effect of the UCS also appeared in some cats behaviorally: the head movement to the tone-CS (left) might change during paired training to a head turn to the right if the head turning UCR was also to the right.

In evoked neural responses, the time-amplitude course of the CS-period can be considered as a time-series of neural events in the neuronal population of the recorded brain site. The CS "triggers" this series of neural events and probably the intensity of the CS (i.e., its orienting power) determines how long-lasting a "trace" it can elicit. The specific nature of this trace depends both on properties of the CS and on the properties of the UCS. Because an unconditioned response to the UCS can be recorded in the same neuronal population which shows a response to the CS, it is probable that both stimuli can exert some modifying effect on those neurons. Even though such modifying influence may be present, it does not necessarily mean that the plasticity in these brain regions will occur. However, the brain locations studied here were structures in which neuronal plasticity has been observed in many studies (Berger & Thompson, 1978; Deadwyler, West, & Robinson, 1981; Weisz, Clark, & Thompson, 1984). The results of the present study suggest that these structures play a part in associative learning because they show specific modifications in their function, that is, they are sensitive and adaptive to the effects of both the CS and UCS. The role of the UCS as a determinant of the time-amplitude course of the neural response to the CS is in accordance with the traditional concept of behavioral classical conditioning: after paired training the CS begins to evoke features of responses which were originally elicited only by the UCS.

The nictitating membrane studies (e.g., Berger & Thompson, 1978) have shown that a delayed conditioned neural response pattern develops as a results of paired training and this pattern (occurring at the temporal site of the UCR) can be seen to have taken on features similar to those the UCR originally showed. Thus these studies have shown that brain systems can develop a delayed temporal model of a trace triggered by the CS. However, no specific change in the time-amplitude course of the CS-period (ISI) has been shown in these experiments, except habituation or latent facilitation to the CS; as Young, Cegavske and Thompson (1976) reported. One explanation for the lack of such observations might be that neural multiple unit recordings are not such sensitive indicators of a learned change as neural evoked responses. On the other hand, the complex form of evoked responses makes it more difficult to interpret at least the late components of such a change.

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7 GENERAL DISCUSSION AND CONCLUSIONS

One basic idea behind these experiments was that an attempt might be made to study a short-latency orienting response ("alpha" response) elicited by a conditioned stimulus (CS) and a long-latency conditioned response (delayed, "true" CR) manifestations as different, although related phenomena. The assumption was that the properties of these two neural pathway systems (so far they can be considered as "pathways") should show, on one other hand, some plasticity as independent pathways (habituation and sensitization); while on the other hand, as converging pathway systems they could be expected to show, a) mutual interaction appearing as sensitization of the CS response when CSs and UCSs are presented in unpaired sequencies, or b) "true" CR (CS begins to activate the UCS system) when the CS and UCS are presented in paired and contingent sequence.

In the present experiments, the choice of a CS which repeatedly elicited an identifiable orienting response (alpha response) during the first 10–30 trials before habituation in unpaired sessions and without habituation on paired sessions, was useful for the identification of the alpha response whenever it appeared during conditioning (and also during intertrial intervals). This property of the CS was also assumed to be advantageous for a study of the source of "spontaneous" responses usually present in traditional instrumental conditioning arrangements. In principle, an analogous associative process was assumed to explain learning in both paradigms (stimulus-reinforcement or response-reinforcement).

In traditional instrumental arrangements the source of instrumental responses, for example environmental stimuli, has been ignored, only the response R being recognized and its frequency counted. In the present thesis, the response R (i.e., alpha or orienting response) is deliberately elicited by a directional tone-CS to the left ear in order to bring the "instrumental" response under experimental control. In spite of such elicitation, the analogy to an instrumental arrangement remains obvious: if an uninformed observer were allowed to follow these experiments without hearing the tone-CS, she/he would only see that the cats from the beginning of the experiment were "spontaneously" now and then turning their heads to the left and that these responses were reinforced with a brain stimulation reward which produced a visible unconditioned response. After paired training the observer would report that the cats produced vigorous instrumental head movements in order to get the brain stimulation rewards. A gradual increase in the frequency of these instrumental responses might also have been reported if the cat had belonged to the experimental group which had first experienced unpaired treatment (i.e., the alpha responses were habituated). Additional support for the functional, "instrumental" similarity of the elicited alpha-response to the traditional instrumental response emerged in the later stages of paired conditioning: some cats began to "emit" spontaneous responses during intertrial intervals as well.

The brain stimulation technique was chosen because direct stimulation of the brain confirms minimal habituation over trials and second, the behavioral response to the brain stimulation UCS appears as a stereotypical series of movements thus providing favorable conditions for the idenfication of the time-amplitude course of this response. One further essential benefit to be derived from the use of the brain stimulation UCS is, of course, its reliable unconditioned effect and in addition, as the results of studies of evoked neural responses in the present papers revealed, the typical time-amplitude course of neural responses appeared relatively unchanged over evoked trials.

One aspect of this study was the comparison of paired and unpaired presentations of the CSs and UCSs. A second aspect was that balancing the order of presentation of treatments allowed the animals to act as their own controls and in addition, yielded material which would reveal any possible retardation or facilitation effect of the preexposure of unpaired CSs and UCSs and the rate of extinction on subsequent unpaired sessions.

7.1 Major findings of the present studies

Invertebrate studies have demonstrated habituation and sensitization: here, both these phenomena have been shown in a vertebrate. In the present studies, nonassociative sensitization appeared at the beginning of unpaired training in the CO-CC group as an increase in the short-latency (alpha) response to the CS, habituating on later unpaired sessions. Short-term habituation was observed as a decrease in response amplitude within each session and long-term habituation appeared as a decrease in the size of responses over sessions (see Figure 4.7). Dishabituation (sensitization) was found during the first few paired training sessions in the experimental group which had first experienced habituation during preceding unpaired presentations of the CSs and UCSs. During later sessions associative alpha conditioning manifested itself as a steady increase in the amplitude of the short-latency responses. Correspondingly, the group which had received first paired and then unpaired treatment first showed an increase in the short-latency orienting response (probably manifesting sensitization), retaining this high level or even increasing it (probably indicating associative alpha conditioning), and showing extinction (habituation) of the acquired short-latency response during subsequent unpaired sessions.

In these studies, a long-latency CR was also expected to develop as a result of paired training, and the distinction between the short-latency (alpha) and long-latency conditioned responses was based a) on a latency difference between these responses (the long-latency was expected to show interval learning) and, b) on the different behavioral response topography (long-latency head turn left, right or up). The results showed that the short-latency and long-latency CRs could be differentiated both in behavioral and evoked neural responses. However, identification of the specific long-latency changes in evoked neural responses appeared more difficult than observation of the corresponding responses has been in multiunit neural activity studies (e.g., Thompson et al., 1983). The complexity of the time-amplitude course of an evoked response makes it difficult to exactly define the critical change in neural evoked potentials on omitted UCS trials. The experiments reported here suggest that an essential feature is an increase in late positivity compared to an increase in negativity during the alpha-response period. Some observations of the more specific characteristics of the long-latency CR were provided in the First study: the form of the evoked response included similar features as had elicited by the UCR.

Some interaction between the CS and the UCS, in addition to an increase in the short-latency (alpha) response, was also found. It seemed that the short-latency CR developed earlier in the trials than the long-latency CRs (measured here on test-trials), for example, in cat K47. Second, another interaction effect between the CS and UCS was observed to occur as a result of the different order of presentation of treatments (paired and unpaired). The group which received unpaired treatment first seemed to suffer from the retarding influence of the unpaired presentations of CSs and UCSs, while the other group, on the other hand, showed some additive, facilitating effects in the amplitude of behavioral and neural responses when the experiment was started directly with the paired treatment. Thirdly, yet another interaction between neural slow potential responses between the CS and UCS was found when the time-amplitude course of the unconditioned response and the time-amplitude course of the CS-period (ISI) after paired training were compared: the CS-period began to show overall time-amplitude characteristics similar to those shown by the

UCR. As far as we know, this is the first time that such "trace" effect has been reported in evoked response studies. Multiunit activity studies have previously indicated that the developing long-latency CR seems to have the same time-amplitude course as the UCR and that a neural "model" develops many trials earlier, before any behavioral response has appeared. The multiunit studies show, however, only the long-latency response topography appearing at about the time the UCS occurred (on omitted trials) but there are only casual observations of the alpha response (i.e., from the CS-period).

7.2 Some issues

7.2.1 Is alpha-conditioning "true" associative learning?

In many studies, an increase in the short-latency orienting response to the CS (alpha response) has been accepted as a CR. This assumption has usually been based on the fact that compared to the unpaired presentation of CSs and UCSs only the forward paired presentation of these stimuli has yielded an effect regarded as conditioning, that is, an increase in the amplitude of the alpha response.

Whether conditioned alpha learning should be considered as true associative learning has been subject to dispute (see Misulis & Durcovic, 1984; Woody, 1982a). This problem comprises two main issues. First, the nature of the conditioned alpha response is usually identical with the orienting response to the CS and thus does not as such represent the learning of any new response, as the original Pavlovian definition of classical conditioning assumes. Second, the latency of this response is usually very short (<50ms), which about corresponds to the minimum conduction time needed for a sensory stimulation (CS) to arrive at the cortex and produce some behavioral orienting response. For example, studies of the conditioned eye-blink in cats by Woody (1982b) showed that the latency for an eye-blink was about 20 ms after a click-CS for glabella tap UCS and about 30-40 ms if an UCS eliciting nose-twitch was used. Thus the form and latency of the short-latency conditioned alpha response strongly suggest a) the presence of prewired neural connections between the neural representation of the CS and the conditioned eyeblink and b) an absence of feedback loops to lower brain structures. On the other hand, there are some observations of short latency conditioned responses in which a different CR has also been conditioned to the same CS. Woody (1982b) showed that an eye-blink CR or a nose-twitch CR developed to a click-CS if a glabella tap UCS to the nose or an air-puff UCS to the nose were applied, respectively. Both CRs showed about the same onset latency. Thus it would appear that the nature of the CR was determined by the specific nature of the UCS, so that one Pavlovian criterion was fullfilled. The apparently different nature of the CRs in the Woody studies is not, however, indisputable: target musculature for both conditioned responses have some overlap (Woody, 1982b).

Although a prewiring hypothesis in associative learning sets some constraints on the nature of conditioned responses, it does not necessarily represent an overwhelming barrier to an explanation in terms of associative learning in case where the alpha response and a long-latency CR are different. It is probable that neural connections between different "pathway" systems are manifold and the use of specific converging connections is obviously the mechanism which principally defines the nature of the developing response. Thus it seems safe and useful to assume that the development of associative learning presupposes a) the presence of prewired, although not necessarily functionally operative, connections between brain systems involved in conditioning (i.e., CS- and UCS-pathways), and b) the development of specific neural changes in the converging points of these connections at many levels of the brain simultanously. Recent studies in inverbrates have yielded substantial evidence for these basic assumptions and have shown that the neural mechanisms for habituation, sensitization and associative learning are closely related in the nervous system.

The invertebrate and also vertebrate studies have suggested that the conditioned alpha response should be accepted as an instance of pairing specifity and hence classical conditioning. Providing that the problem of similarity between the OR and the developing conditioned alpha response is bypassed, and assuming that associative alpha learning has been demonstrated there remains, however, one other problem: the learning of a time interval, that is, the delayed CR concept. In many cases the delayed CR and the specific form of the developing long-latency CR (similarity to the UCR) seem to be connected to eachother. If the interstimulus interval is very short, differentation of the short-latency (alpha) CR and "true" CR becomes difficult.

Neuroanatomically, the difference of a short-latency alpha CR and long-latency CR might be hypothetized to represent a different anatomical localization for associative change in the CNS. The short-latency CRs might be assumed to represent cortical associative changes and long-latency CRs might indicate the involvement of other, more remote structures (as brain stem or cerebellum) and the presence of some intra- or intercellular neural mechanism for interval learning. Cortical associative learning might, moreover, represent some neural self-organizing process (Creutzfeldt, 1976; Kohonen, 1984) within and between adjacent projection areas of cortex; rather than a capacity to learn time intervals and long-latency conditioned responses which are closely related to the elicitation of behavioral conditioned responses. Some support for the "adjacent" vs. "remote" connection hypothesis in the development of associative learning can be found from recent findings in which the specific lesions, ablations, or stimulation of lower brain structures (brain stem, cerebellum) have selectively abolished the learned, conditioned response without any disturbance in an unconditioned response (Lincoln, McCormick, & Thompson, 1982; McCormick, Guyer, & Thompson, 1982; McCormick & Thompson, 1984; Solomon, Lewis, LoTurco, Steinmetz, & Thompson, 1986). Correspondingly, lesions in the neocortex can abolish a short-latency CR (Woody, Yarowski, Owens, Black-Cleworth, & Crow, 1974).

The present study did not provide an answer to the question of adjacent or remote connections but it gave rather conclusive evidence of the differentation of the short-latency change from the long-latency, "true" CR on the grounds of their different nature and latency. Thus interval learning may well be the specific feature of true associative learning which constitutes the essential distinguishing feature between alpha and delayed CR. It is also probable that interval learning is present only in complex nervous systems; thus far, such interval learning has not been reported in invertebrates. Whether or not alpha learning is associative, it is probably the case that the CS must first acquire significance, "power", in order to provide a substantial trace which in turn may connect temporally separated sequences of events. Thus, using the traditional terminology of conditioning: an initially "neutral" CS must first become a conditioned stimulus, that is, show conditioned orienting response development.

7.2.2 What is the role of a neural "trace" concept in associative learning?

The concept of stimulus trace in learning was originally introduced by Pavlov (1927). Hull (1943) suggested that each stimulus forms a molar trace in the nervous system, that is, an effect which actually prolongs the influence of a discontinuous stimulus after its offset. The trace concept was formulated as a "bridge" between the interstimulus interval of the CS and UCS. Some later attempts to explain associative learning have also used such a concept, for example, Sutton and Barto (1981).

Some recent findings regarding the cellular and neuronal mechanisms of associative learning have given rise to new, more empirically oriented views. Based on the data acquired in invertebrate studies, Walters and Byrne (1983) using a concept of "activity dependent neuromodulation" have attempted to explain classical conditioning in relation to sensitization at the cellular level. Byrne (1985) suggested that a temporal specifity of associative learning is achieved if the electrical activity of the CS-pathway is able to amplify the sensitizing or reinforcing effects of the UCS on the CS-pathway. In order to retain the order of the CS and UCS (CS before UCS) it must be assumed that the incoming CS can initiate biochemical changes in a cell which then become amplified by the following modulating effect of the UCS. Byrne (1985) suggested that adenylate cyclase might represent this process. This suggestion is plausible if it is assumed that as a result of the pairing of the CS and UCS, the CS becomes capable (i.e. it is amplified) of increasing cAMP as the UCS originally did. Because the sequential order of the CS and UCS and some minimum ISI between them can be considered as a prerequisite for associative learning, then a) the CS must have a long-lasting effect ("trace") in order to overlap in time with the following UCS modulatory effect, and on the other hand, b) if the UCS modulatory effect has begun in the cell before the CS (as in simultanous and backward conditioning), it should somehow be capable of cancelling the amplification of the CS input.

Kandel and Schwartz (1982), and Walters and Byrne (1983) have suggested that the changes in neural cells during classical conditioning occur in presynaptic terminals. Alkon (1984), on the other hand, has proposed that this critical event can be identified as a post-synaptic change. Whether this plasticity can be localized to pre- or post-synaptic level is not a critical issue for the concept of associative learning; the most point important is that both theories are based on the assumption of a convergence of the CS- and UCS-pathway systems at a cellular level. These theories also include the assumption of an interaction of spatially close CS and UCS inputs in the presynaptic terminal or postsynaptic dendritic area, and so are able to explain associative convergence as a localized, restricted change in the neural network (see Alkon, 1984).

Originally, the trace concept was introduced to account for a prolonged stimulus effect in the nervous system. What would be the consequences if the assumptions of prewiring and convergence of the CS and UCS pathways were accomodated to this concept? If the trace concept is extended to a description of time series of temporally close, causally-connected events and their traces in a neural network, then the time-amplitude course of a response to a stimulus event depicts averaged plasticity resulting from all previous experiencies to closely related stimuli.

The effect of the CS is most conspicuous when the CS is novel, that is, the nervous system has not yet developed a model of it. The initial orienting response to the CS habituates rapidly if there is no "booster" to re-create its significance. Sensitization can be achieved as a result of nonassociative learning (i.e., the CS and UCS are not paired) or through associative learning (classical conditioning) in which a change in the neural network remains rather permanent (memory function). As the empirical findings suggest, a more durable change results if the CS and BUCS are presented temporally close and in this order. The effect of the UCS appears essential: the UCS can bring about cellular processes which can also amplify the cellular response to the CS. If a trace concept is used, the original orienting time-amplitude course of the neural response to the CS changes, and may now (in addition to nonassociative sensitization) be partially modified by the UCS, which may also add events to a time series initially started by the CS. As a result, the original trace to the CS is prolonged: the CS can, after paired learning, elicit a trace which is modified and extended so that the CR can be observed on omitted UCS trials as a new "member" in this time series. Some support for this view was obtained in the present experiments: on omitted UCS (test) trials both behavioral and neural response appeared at the site of the UCS. In evoked response studies such trace is not so unambiguous as it has been in most multiunit activity studies.

In addition to signs of interval learning, a modification of the neural trace was found in which the time-amplitude course of the response to the CS (ISI) became partially modified. A probable participation of the UCS in the modification of the CS trace was also found at behavioral response level: some cats changed the direction of their head movement to the CS after paired training in the direction of the UCR. Although some specific signs of such interaction was found, the evidence for the modificatory effect of the UCR on the CS period was suggestive rather than conclusive in these experiments. If such interaction, however, is present, it might be speculated that in a neural network, a novel stimulus can alter old traces as a function of their unconditioned significance and temporal order and proximity. Providing that a necessary state for sensitization has been achieved in the nervous system, the CS or any stimulus closely related to the CS (e.g., environmental stimuli) may act as a "triggering" event which can start a series of such modified neural events, that is, traces.

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TIIVISTELMÄ: VÄLITTÖMÄT JA VIIVÄSTETYT HERMOSTOLLISET JA KÄYTTÄYTYMISVASTEET KLASSISEN EHDOLLISTAMISEN AIKANA KISSALLA

Väitöskirja muodostuu kahdesta teoreettisesta esityksestä, kolmesta tutkimusraportista ja yleisten päätelmien osasta, sekä liitteenä eräitä kokeiden instrumentointia koskevia teknisiä raportteja.

Teoreettisessa osassa on pyritty yhdistää tämänhetkinen tieto oppimisen hermostollista perusprosesseista ehdollistamisen peruskäsitteisiin. Ensimmäisessä esityksessä ("Sensitization and associative learning") tarkastellaan herkistymisen (sensitization) merkitystä ehdollistumisessa ja erityisesti sen mahdollista merkitystä usein "spontaaneiksi" katsottujen instrumentaalisten vasteiden (responses) tuottajana. Toisessa teoreettisessa esityksessä ("Neurobiological processes, neural responses and associative learning") ehdollistumista tarkastellaan hermostollisten toimintojen näkökulmasta etsien yhteistä selityspohjaa niille neurobiologisille perusilmiöille (habituaatio, sensitisaatio ja assosiatiivinen oppiminen), joiden tutkimuksessa selkärankaisilla ja erityisesti selkärangattomilla on viime vuosina edistytty merkittävästi. Nämä tutkimukset ovat tuoneet yhä selvemmin esille hermojärjestelmän ennaltakytkettyjen yhteyksien (prewiring) merkityksen myös vaativimmassa assosiatiivisen oppimisen muodossa, klassisessa ehdollistumisessa. Assosiatiivisen oppimisen voisi siten katsoa edustavan mahdollisesti vain eräänlaista valikoitua olemassaolevien — vaikkakin toiminnallisesti käyttämättömien — esikytkentöjen vahvistamista ja käyttöönottoa. Hermoston tasolla tämä tapahtunee joko/tai pre- tai postsynaptisina neurokemiallisina ja osittain solukalvojen rakenteellisina muutoksina.

Väitöskirjan kokeellisessa osassa (First, Second and Third study) yritettiin toisaalta selvittää, mikä on herkistymisen merkitys klassisessa ehdollistumisessa (assosiatiivinen vs. ei-assosiatiivinen oppiminen) ja toisaalta, voidaanko tarkoilla käyttäytymisen ja hermoston toiminnan samanaikaisilla mittauksilla selventää assosiatiivisen oppimisen lainalaisuuksia.

Ensimmäisessä tutkimuksessa (First study) selvitettiin, onko mahdol-

lista erottaa klassisessa ehdollistamisessa välitön, lyhytviiveinen ("alpha") vaste viivästetystä, pitkäviiveisestä (delayed) vasteesta. Tässä kokeessa (samoinkuin toisessa kokeessa) ehdollinen ärsyke (conditioned stimulus, CS) oli valittu siten, että se sai aikaan helposti tunnistettavan orientoitumisreaktion (pään liike vasempaan korvaan annetun äänen suuntaan). Ehdottomana ärsykkeenä käytettiin lateraaliseen hypotalamukseen annettua sähköistä aivoärsytystä, jonka todettiin tuottavan lähestymiskäyttäytymistä ja aiheuttavan selvän käyttäytymisvasteen (vakioinen päänliike tiettyyn suuntaan). Ehdollisen ja ehdottoman ärsykkeen välinen aika (interstimulus interval, ISI, 1024 ms) oli riittävän pitkä, jotta käyttäytymisvasteita voitiin havainnoida luotettavasti ja tarkasti videonauhoituksista. Päivittäinen koekerta (session) sisälsi 60 yrityskertaa (trial), joista 48 oli ehdollistamistrialia ja 12 (joka 5.) oli ns. testi-triali, jonka aikana esitettiin ainoastaan CS ja tutkittiin puuttuvan UCS:n esittämisajankohdalla mahdollisesti esiintyviä vasteita. Kukin eläin kävi läpi ehdollistamiskertojen (CC) ja satunnaisessa järjestyksessä tulevien, CS:iä ja UCS:iä sisältävien kontrollikertojen (CO) sarjat tasapainoitetussa järjestyksessä (CC-CO ja CO-CC ryhmät). Perättäisia koekertoja oli 5–10 yhtä käsittelytapaa kohti.

Tulokset osoittivat, että pään liikkeessä sekä välitön että viivästetty vaste kasvoivat ehdollistamiskertojen aikana ja sammuivat kontrollikertojen aikana. Niissä tapauksissa, joissa käyttäytymisvaste UCS:ään (ehdoton vaste, UCR) oli erisuuntainen päänliike kuin UCS:n tuottama käyttäytymisvaste, CS:n aiheuttaman välittömän vasteen lisäksi esiintyi viivästettyjä vasteita myös testitrialeilla ja niiden lukumäärä kasvoi ehdollistamiskertojen tuloksena, ja b) viivästettyjen vasteiden käyttäytymistasoiset ja hermostolliset aika-amplitudipiirteet sisälsivät samoja erityispiirteitä kuin UCR:llä oli alkujaan. Välittömien ja viivästettyjen vasteiden lisäksi havaittiin eräillä kissoilla "spontaanien", "instrumentaalisten" pään liikkeiden lisääntymistä ehdollistamiskertojen myötä myös trialien välisinä aikoina. Pään liikkeiden voimakkuuden muutoksia tutkittiin kiihtyvyysanturin avulla, jonka antamat tulokset tukivat käyttäytymisanalyysia: ehdollistamiskäsittelyt kasvattivat merkitsevästi sekä välittömien että viivästettyjen vasteiden (testi-trialeilta mitattuina) amplitudia verrattuna kontrollikäsittelyyn ja osoittivat myös näiden vastetyyppien eriytymistä. Käsittelyjärjestys vaikutti oppimiseen siten, että suoraan ehdollistamisella (CC) aloittanut CC-CO ryhmä osoitti nopeaa ja voimakasta lisäystä sekä välittömässä että viivästetyssä vasteessa, kun taas ensin satunnaisten CS:ien ja UCS:ien kontrollikerrat kokenut CO-CC ryhmä oppi hitaasti seuraavilla ehdollistamiskerroilla.

Hermostollisten herätepotentiaalien mittaukset tuottivat samankaltaisia, käsittelyjärjestykselle ominaisia tuloksia. Herätepotentiaalien vastemuutokset ilmenivät ehdollistamisen tuloksena lisääntyneenä negatiivisuutena välittömässä vasteessa (150–450 ms ISI jaksolla) ja lisääntyneenä positiivisuutena viivästetyn vasteen aika-alueella. Yleensäkin saadut tulokset näyttävät osoittavan, että välitön ja viivästetty vaste voidaan erottaa toisistaan, kun käytetään sekä vasteen alkamisajankohtaa että sen erityispiirteitä tarkastelun perusteina. Herätepotentiaalit eivät ole tulkinnallisesti yhtä yksiselitteisiä kuin hermosolujen laukeamistaajuuden muutokset, joilla on voitu osoittaa luotettavasti viivästettyjen vasteiden kehittyminen UCS-jakson aikana. Nyt tehdyissä kokeissa havaittiin myös herätepotentiaaleissa vastaavia muutoksia testi-trialeilla.

Toisessa tutkimuksessa (Second study) pyrittiin varmentamaan ensimmäisen tutkimuksen havaintoja välittömästä ja viivästetystä vasteesta ja niiden erottelusta. Koekertojen määrä kaksinkertaistettiin ja lisäksi mukana oli ylimääräisiä nolla-viive koekertoja ("backward") näiden aikana kehittyvien vasteiden assosiatiivisten ominaisuuksien selvittämiseksi. Tämänkin tutkimuksen tulokset osoittivat sekä käyttäytymis- että hermostovasteiden eriytymistä välittömään ja viivästettyyn osaan. Kuten edellisessä kokeessa, havaittiin myös nyt CC-CO ryhmällä nopea ehdollistuminen, joka tulkittiin yhdistyneeksi CS:n ja UCS:n orientoitumisvaikutukseksi kokeen alkuvaiheessa ja päällekkäiseksi assosiatiiviseksi oppimiseksi ehdollistamisen loppuvaiheessa. Välittömän vasteen luonnetta (assosiatiivinen vs. ei-assosiatiivinen) selvitettiin vertaamalla nolla-viive ehdollistamisen tuloksia tätä menettelyä edeltäneen klassisen ehdollistamisen aikana kehittyneisiin opittuihin muutoksiin: välittömät vasteet näyttivät säilyvän takaperoisen ehdollistamisen aikana, kun taas viivästetyt vasteet katosivat. Tämän tulkittiin osoittavan välittömän, orientoitumistyyppisen vasteen olevan ehkä enemmän herkistymisen tulosta, kun taas viivästetyn vasteen oletettiin edellyttävän mahdollisesti lisäksi hermostollisten CS- ja UCS-"ratajärjestelmien" ehdollistamisen tuloksena kasvavaa vuorovaikutusta: CS alkaa oman lisääntyneen orientoitumisvaikutuksensa lisäksi ja ehkä juuri sen avulla aktivoida myös toista, toiminnallisesti ja ajallisesti lähelle sijoittunut hermostotoimintojen verkkoa, UCS-järjestelmää.

Alustavia havaintoja eräistä tälläisista mahdollisista ehdollisen ja ehdottoman ärsykkeen tuottamien hermostollisten muutosten vuorovaikutuksista, "jäljistä" ("trace") on esitetty kolmannessa tutkimusraportissa (Third study). Siinä ehdotetaan, että UCS:llä olisi yleisen herkistävän vaikutuksen lisäksi myös aivan erityinen laadullinen vaikutus opitun vasteen muotoon. Tätä oletusta tukevat havainnot siitä, että CS:ään tuotetun, opitun herätepotentiaalivasteen erityiset aika-amplitudipiirteet (ISI:n aikana) korreloivat alkuperäisen ehdottoman vasteen erityspiirteisiin; UCS näyttää jättävän "jäljen" opittuun välittömään vasteeseen.

Yleisessä tulosten johtopäätösosassa pohditaan erityisesti välittömän (alpha) oppimisen assosiatiivisuuden luonnetta ja ehdotetaan, että alpha-oppiminen saattaisi edustaa sekä ei-assosiatiivista että assosiatiivista oppimista eräin rajoituksin (käyttäytymisvasteen samankaltaisuus alkuperäisen orientoitumisreaktion kanssa ja viivästetyn vasteen puuttuminen), ja että alpha-oppiminen mahdollisesti edustaisi neurobiologisena ilmiönä alkuvaihetta viivästetylle aikavälioppimiselle. Lisäksi pohditaan oletettujen CS- ja UCS-ratajärjestelmien yhdysvaikutuksen merkitystä assosiatiiviselle oppimiselle varhaisempien "jälki"-hypoteesien ja viimeisimpien hermoverkoista saatujen tietojen (mm. esilangoitus) valossa.

APPENDIX I: A FAST IMPLANTATION SYSTEM FOR CHRONIC MULTIPLE-UNIT ELECTRODES

The implantation of several electrodes in the same animal and the recording of multiple-unit activity during implantation via these electrodes created a need to develop a fast implantation procedure. The electrodes in this system are made of 25-75 um insulated stainless steel wire which is covered with thin hypodermic needles of fixed length. The fast implantation is based on the use of a stereotaxic dental drill assembly in which the hole for the electrode can be drilled directly according to the coordinates of the desired location. The implanted electrode remains in its position without cementing because of the friction between the electrode shaft and the hole.

An implantation system was developed for the study of neural responses (multiple-unit discharges and slow potential changes) in different brain sites of cats making it possible to implant several electrodes in a short time while monitoring neural activity during operation.

The implantation methods used earlier (Buchwald, 1973; Olds, 1973; John, 1973; Orona, Foster, & Lamberth, 1980) have required excessive time for the fixing of the electrode with dental cement and then waiting for the hardening of the cement before the electrode carrier can be released. Another problem has arisen from the need to implant several electrodes in a small area; conventional implantation procedures and equipment (electrode carriers) require more working space around the carrier.

In order to meet the requirements of high electrode density and minimum implantation time, a new procedure was developed. This method is based on the use of hypodermic needles of fixed diameter (0.41 mm, dental needles, 27G x 1 3/8") as a support for electrode wires. These tubes are long enough (45 mm) to allow cutting them to a fixed length which makes it possible to calibrate each electrode in advance. The structure of the electrode carrier (composed of the handpiece of the dental drilling machine fitted to the stereotaxic electrode carrier arm) is shown in Figure 9.1. The details of the adapter, which is fitted to the jaws of the handpiece instead of the dental drill is shown in Figure 9.1.

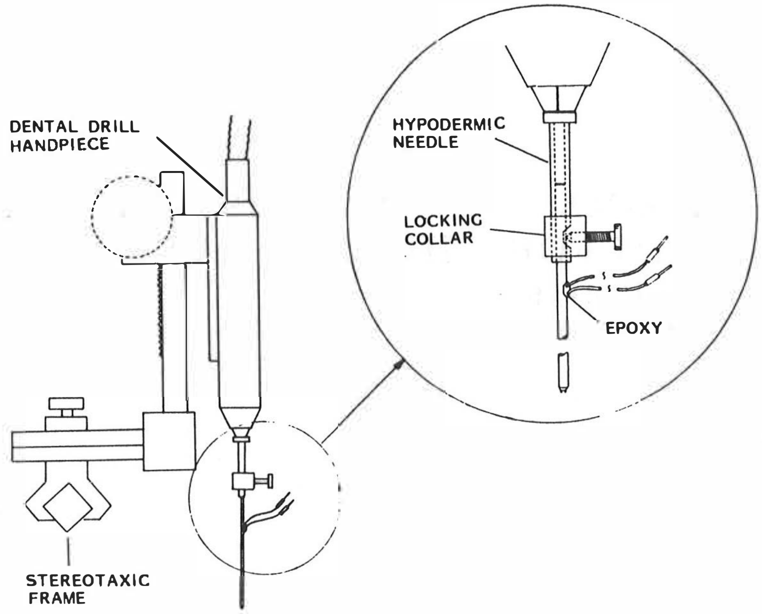

Figure 9.1 The details of the electrode adapter.

The implantation of an electrode is carried out in the following order. First, the hole for the electrode is drilled directly into the coordinates of the desired site using a drill attached to the electrode carrier. This drill is made from a ball-head dental drill by grinding the tip of it in a lathe to a diameter of about 0.43 mm. After the appropriate opening into the skull has been made, the drill is changed to the electrode adapter (with electrode attached) and the electrode is lowered step by step into the target coordinates while monitoring the neural activity with the oscilloscope. After the desired point is reached, the lock of the electrode adapter can be released and the electrode carrier pulled up. The electrode remains steady because of the friction between the hole and the electrode shaft. The tolerance must be adjusted so that the electrode moves tightly in the hole, but not so tight as to prevent withdrawal, if necessary (see Figure 9.2).

The preparation of electrodes is shown in Figure 9.3. The hypodermic needles are cut to the desired length (i.e., 32 mm is the proper length for cats). The side hole is opened using a small dental grinding disc. Because the depth coordinates vary for different electrode sites, the approximate

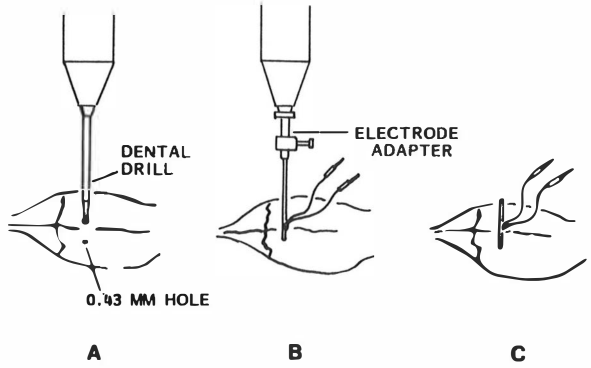

Figure 9.2 The implantation procedure. **A.** Drilling the precision hole for the electrode shaft. **B.** The implantation of the electrode using the electrode adapter. **C.** The implanted electrode remains in its position by means of the friction between the electrode shaft and the hole.

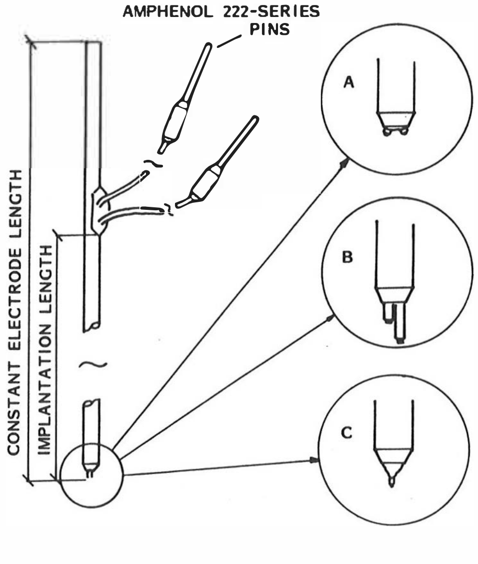

Figure 9.3 The details of different electrode constructions.

height of the side opening of each electrode is determined in advance. In practice some standard classes of electrodes are used, for example, 7, 9, 11, 13, 15, 17, 19, 22, 25 mm long electrodes. In order to prevent damage to the electrode wire insulation, the edges of the holes must be refined carefully. The wire(s) can be fitted into the tube after the upper part of the tube is bent about 30 degrees to allow the pushing of wire(s) directly through the tube. After threading the wire(s) the tube is bent back and the shaft of the electrode is straightened. The electrode wire can be fixed with epoxy or cyanoacrylate cement. The miniature connector pins (Amphenol 222-series) can be soldered to wires using a special stainless steel solder and soldering fluid (e.g., Castolin 157). The electrodes are then washed in warm water and floated in sterile solution for an hour. The electrodes can then be stored in the covers of dental needles to be ready for later use.

After the implantation of all electrodes, a thick layer of dental cement is moulded around the base of the electrodes for support. The electrode shafts are then cut down to the correct size. Each electrode is connected each in turn to the miniature connector plug (Amphenol 222-12N31). The whole assembly can then be embedded in dental acrylic.

Using the implantation system described above, it has been possible to implant 10 electrodes in about an hour and a half and simultaneously search for the desired neural activity through the monitor. Another benefit of the system has been that the electrodes with their connector pins can be prepared to the desired length before implantation.

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APPENDIX II: HALL-EFFECT ACCELEROMETER FOR RECORDING HEAD MOVEMENTS OF LABORATORY ANIMALS

The measurement of specific movements in laboratory animals during conditioning experiments requires small devices which can sense even slight and slow movements, preferrably in all three spatial dimensions. The available commercial devices are usually rather heavy and insensitive for slow accelerations and, on the other hand, the compact transducers are also expensive.

Some self-made constructions based upon phonocartridge pick-up stylus design for the unidimensional recording of movements have been put forward in biomedical literature (e.g., Mundl & Malmo,1979).

The three-dimensional version of apparatus of this kind using phonocartridge type design would become rather bulky. Some of the compact commercial three-dimensional accelerometers are based upon sophisticated piezo-electric constructions allowing reasonable compact size for the transcducer.

One way to solve the problem of detecting three-dimensional movement is to put a ferromagnetic fragment of metal in a small cube (15 x 15 mm) and to detect the accelerations of the metal fragment by a magnetosensitive Hall-device. A thin coil-spring from a wrist-watch was used in this construction to keep a small ferromagnetic grain (about 5 mm diameter) in the middle of a cube (see Figure 10.1).

A suitable ferromagnetic fragment was found in the bottom of a chessman in a small travel chess set. The cube was filled with thin oil to damp the extra oscillatory movements of the magnet. After filling the cube with oil it was sealed with epoxy and the Hall device (Honeywell, 92SS12-2) glued to the side of the cube. The best position for a Hall device (if only one device is used) on the surface of the cube can be found by comparing the acceleration curves of each dimension on an oscilloscope, and after approximate matching curves have been pinpointed the Hall-circuit can be permanently attached.

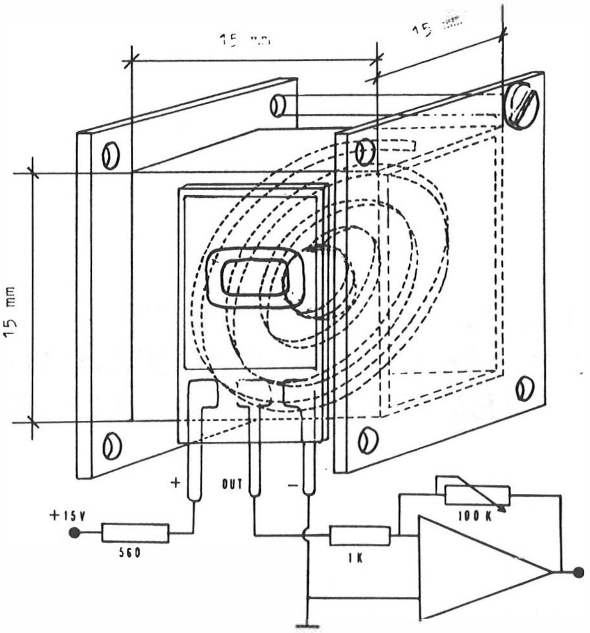

Figure 10.1 The construction of the Hall-accelerometer. Any low noise operational amplifier, e.g., LM11, will be found suitable.

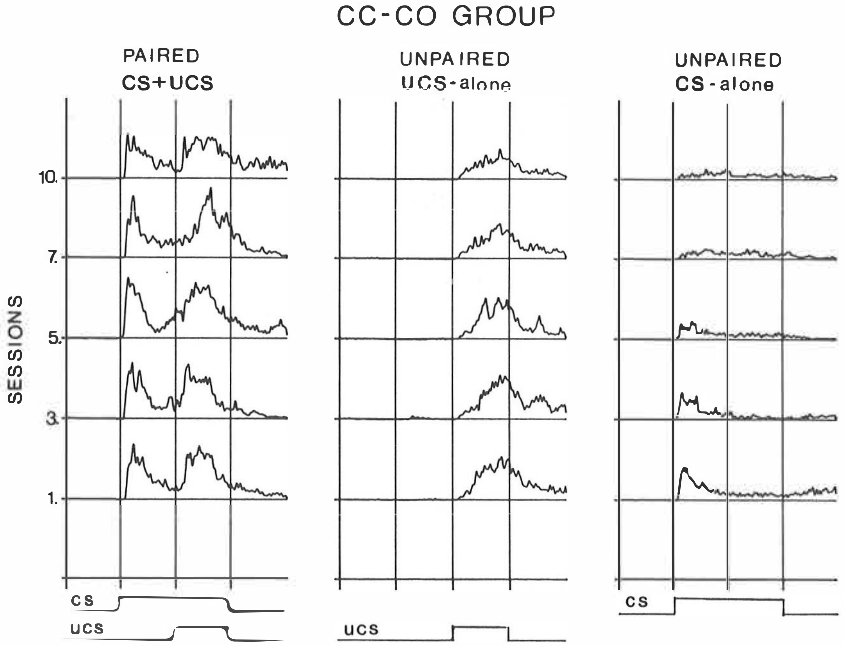

Figure 10.2 An example of the averaged head movement signals in the cat during classical conditioning (CC) and unpaired (CO) treatments at different experimental stages (daily sessions).

The Hall-device used in this construction also includes an amplifier with a linear output related to the distance of the magnetic fragment of metal from the transducer. The Hall transducer needs a voltage source of 9V. The electrical recording circuit is also presented in Figure 10.1.

An example of the rectified average curve at different phases of conditioning is given in Figure 10.2. These curves are recordings of classical conditioning experiments with cats in which the development of an orienting head movement elicited by a tone-CS to one ear and the form of the unconditioned head movement to positive brain stimulation and possible emergence of the conditioned head movement were studied. Figure 10.2 shows the movement signals during paired and unpaired treatments. These experiments also included simultaneous recording of brain potentials and the fast response time of the movement transducer permitted a comparison of the latencies of neural changes and movements. The accelerometer is fixed to the preamplifier assembly which is connected directly to a corresponding connector plug on the head of the cat.

Reference

Mundl, W. J., & Malmo, H. P. (1979). An accelerometer for recording head movement of laboratory animals. *Physiology & Behavior,* 23, 391-393.

APPENDIX III: MULTICHANNEL LOW-NOISE INTEGRATED DIFFERENTIAL PREAMPLIFIER HEAD GEAR FOR MULTIPLE-UNIT AND EVOKED RESPONSE RECORDINGS

Recent rapid development in monolithic instrumentation amplifier circuits has made these devices useful for many bioelectrical measurements. A low noise level and high input impedance are typical in new bipolar amplifier circuits.

The amplifier circuit applied here is a pin-programmable (gain 1 to 1000X) low noise instrumentation amplifier made by Analog Devices (AD524). The input impedance of this ampilifier is 1 gigaohm and practical noise level (peak to peak) in our application is <10 uV (DC to 5000Hz).

Manufacturer's specifications for AD524

Input offset drift 0.5 uV/C
Input bias current +-10 nA
Input offset voltage 50 uV
Gain bandwidth product 25 MHz
Power supply range +-6V to +-18V
CMR 100 dB (G=10)

The mechanical construction of the preamplifier assembly is presented in Figure 11.1. The assembly consists of four small circuit boards connected with strip pin connectors (Augat) to the base and top plate.

Both the recording and stimulating signals are carried along the printed circuit boards so that no separate wiring is needed and the assembly can be easily opened for preparations or changes in construction. The printed circuit wiring is shown in Figure 11.2. In this application, the gain is programmed with a jumper to 1, 10, or 100 X.

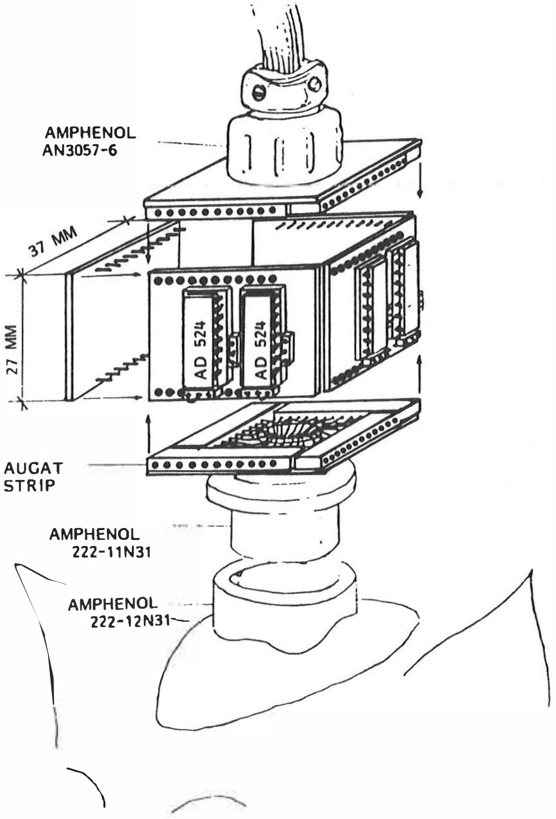

Figure 11.1 The mechanical construction of the detachable preamplifier assembly.

The wires for the electrical brain stimulation are also arranged to go through the printed circuit board. This minimizes the induction of stimulating pulse trains to the recording channels and provides an easy access to the details of the assembly. The detachable structure of the top- and base-plates also makes it possible to use different types of external connector and cable system with the same amplifier circuit cards. The general wiring diagram of the amplifiers and brain stimulation lines is shown in Figure 11.3.

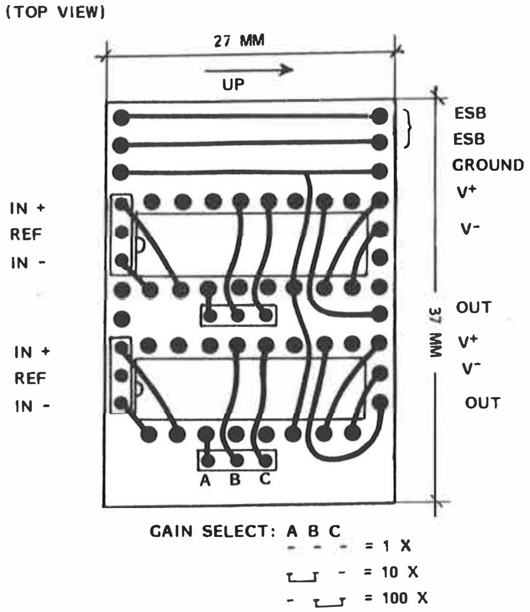

Figure 11.2 The wiring diagram of the printed circuit board.

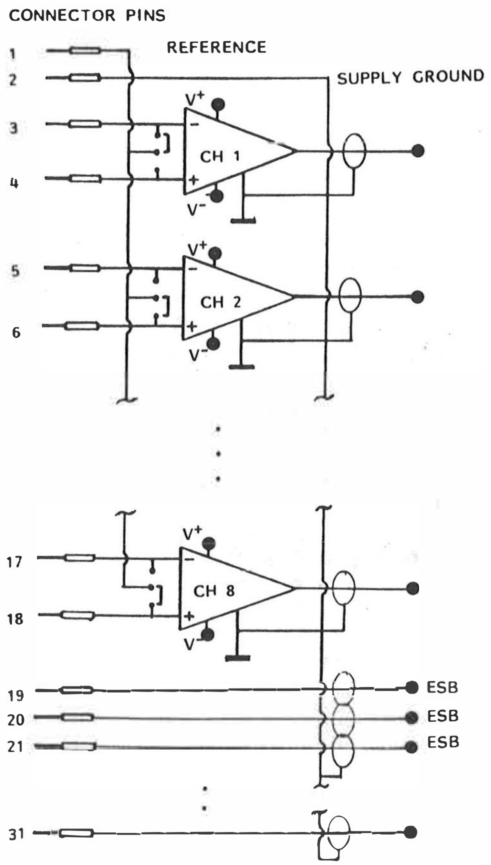

Figure 11.3 General wiring diagram.