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Stages of avoidance strategy formation in gerbils are correlated with dopaminergic transmission activity

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Abstract

This detailed analysis of behavior is aimed at the differentiation of the components of information processing during associative conditioning. In gerbils, the influences of various acquired non-avoidance strategies as pre-experience were studied during the learning of a standard avoidance task in the same shuttle-box. Identical cue stimuli, frequency-modulated tones as conditioned stimuli and electric footshocks as unconditioned stimuli, were used in various behavioral tasks. In addition to common parameters such as avoidance performance and reaction times, behavioral events such as the attention response and the orienting response were quantified. Thereby, components of shuttle-box learning such as signal detection and signal evaluation were found to be affected by pre-experience-dependent dynamics. Using a microdialysis technique during avoidance learning in the shuttle-box, we found that only strategy formation was correlated with high dopamine levels in medial prefrontal cortex. The increase in dopamine in medial prefrontal cortex may be an indicator of the involvement of working memory principles in signal evaluation stages of conditioning. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Dopamine; Microdialysis, In vivo; Prefrontal cortex; Shuttle-box; Working memory; (Gerbil)

1. Introduction

The acquisition of information by cognitive processes aims at a fast adaptation of individuals to their actual environment. Among such processes, associative conditioning reflects a behavioral context and its key stimuli (Rescorla, 1988). Using the simple shuttle-box learning task with gerbils, we were interested in analysing the components of associative avoidance conditioning, a type of behaviour which involves a conflict between an individual and its environment. Another focus of this work on avoidance conditioning was the re-evaluation of already stored long-term memories of pre-experiences. Continuous updating of the individual total experience thus becomes necessary during learning. This includes the comparison of stored information and recent short-term memory (Atkinson and Shiffrin, 1968), and is connected with decision-making for actions in the new context.

The learning studies described below include a search for physiological parameters of the brain which correlate with stages of the cognitive processes. The activity of the dopaminergic system in the presumably learning-relevant medial prefrontal cortex was investigated during avoidance conditioning of gerbils in the shuttle-box (Fig. 1).

The learning process was characterised by conditioned responses and reaction times as well as highly time-resolved behavioral events such as the attention response and the orienting response during the learning process. To better differentiate the information processing stages, we prolonged the learning of the avoidance strategy by inhibition. The same cue stimuli, frequency-modulated tones as conditioned stimuli and electric footshocks as unconditioned stimuli, were used in various behavioral paradigms during a non-avoidance pre-experience session. During the subsequent learning of a standard avoidance paradigm, the gerbil had to re-evaluate the conditioned stimulus that previously announced a “non-avoidable footshock” to learn

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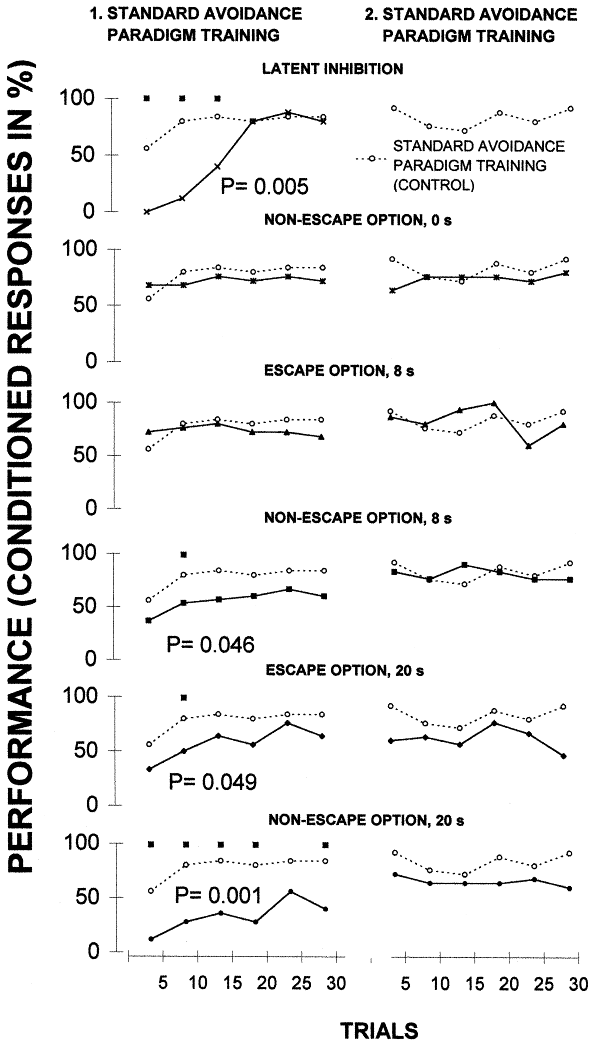

Fig. 1. Averaged learning curves during the standard avoidance paradigm training sessions of pre-experience groups (filled symbols) on day 1 and day 2, in comparison to standard avoidance paradigm control (open circles): latent inhibition (×), non-escape option, 0-s (*), escape option, 8-s (▲), non-escape option, 8-s (■), escape option, 20-s (◆), non-escape option, 20-s pre-experience groups (●) and the standard avoidance paradigm control group (○). In the analysis of conditioned responses, significant differences were found during the first training session between the profiles of non-escape option, 20-s ($P = 0.001$), escape option, 20-s ($P = 0.049$), non-escape option, 8-s ($P = 0.046$) animals in comparison to standard avoidance paradigm controls. The filled rectangles indicate the periods of significant differences between these groups (Least Significant Difference test, $P < 0.05$). Data are mean values.

a new meaning for the tone now that it announced an “avoidable footshock”. During standard avoidance paradigm training, the animals had to re-learn a context experience by re-evaluating the temporal relationships of the key stimuli.

In the avoidance learning paradigm of a shuttle-box, naive control gerbils initially respond to the unconditioned stimulus by escaping to the other shuttle-box compartment. During training, the gerbils adapt to the complete context, consisting of conditioned stimulus, unconditioned stimulus, and shuttle-box, by avoiding the unconditioned stimulus. Thereafter, the animals maintain a reliable avoidance strategy by changing shuttle-box compartment as soon as the start of the conditioned stimulus.

Feedback experience from trial to trial is necessary for the animal to learn the temporal relationship of conditioned stimulus and unconditioned stimulus, so it can change from the escape to the avoidance response. This implies a particular type of information processing, namely storage of the integrated experience of the past trials, with the option to compare the result with the partial experience of the actual training trial. This will influence and successively optimize the decision about which action to take during subsequent trials. We hypothesized that the working memory enables the short-term storage of information and allows context-adequate comparison with the consequence of decision-making in this associative learning process (Atkinson and Shiffrin, 1968; Baddeley, 1992, 1996; Richardson and Gratton, 1998; Platt and Glimcher, 1999). We assume that during acquisition of a behavioral strategy in the shuttle-box, working memory is required to accumulate the partial experiences from trial to trial to shape an increasingly adequate behavioral strategy (Rescorla and Wagner, 1972). Because working memory mechanisms are assumed to be involved during auditory avoidance strategy formation, we searched for a neural indicator of this involvement.

The dopaminergic projections, from the ventral tegmental area A10 to the medial prefrontal cortex and other associative cortex areas, play an important role in cognitive functions (Sawaguchi et al., 1990a,b; Sawaguchi and Goldman-Rakic, 1991; Schultz et al, 1993; Sokolowski et al., 1994). For instance, working memory is impaired by dopamine depletion in medial prefrontal cortex (Brozoski et al., 1979; Sawaguchi and Goldman-Rakic, 1991; Sokolowski et al., 1994), and an impaired working memory can be improved by dopamine receptor agonists (Arnsten et al., 1994). Moreover, it was shown that an increase in activity of the dopaminergic system in medial prefrontal cortex was correlated with the retrieval of a working memory task (Watanabe et al., 1997). Presumably, an increase in extracellular dopamine through increased synaptic release reflects functioning of the working memory. Therefore, we now used microdialysis in medial prefrontal cortex followed by dopamine analysis by high performance liquid chromatography (HPLC) combined with behavioral measurements during avoidance learning by gerbils in the shuttle-box. In this way, not only group learning, but learning progress of individual animals could be followed and related to dopamine levels. It was of particular interest to find whether an increase in extracellular dopamine was correlated with the establishment or with retrieval of the avoidance strategy. We also aimed to correlate as precisely as possible the dopamine content in dialysates and individual behavior.

2. Materials and methods

2.1. Animals

Male gerbils (*Meriones unguiculatus*) weighing 80–100 g were kept in single cages (24 × 20 × 14 cm) with ambient illumination on a 12:12 h light/dark cycle with free access to pellets and water. Each animal was handled daily. All animal procedures were in accordance with National Institute of Health procedures for care and use of laboratory animals, and were approved by the ethics committee of the state of Sachsen-Anhalt (No. 53a-42502/2-059/59).

2.2. Behavioral tests

Gerbils ($n = 35$) were subjected, in a pre-experience session, to different learning paradigms in which inter-stimulus intervals between conditioned stimulus and unconditioned stimulus were varied. The animals thus could not avoid the unconditioned stimulus during the pre-experience session, but could either escape or not escape from the unconditioned stimulus. In another pre-experience session, the presentation of the conditioned stimulus tone alone was used for investigation of the latent inhibition. During the following standard avoidance paradigm training sessions, over two days during which the same tone was paired with a footshock, the animals had to re-evaluate the meaning of the conditioned stimulus.

The following behavioral parameters were evaluated by video analysis (Noldus, 1991) of behavior with continuous focal sampling (Rescorla, 1988): (1) avoidance performance measured in percent of conditioned responses to the conditioned stimuli, given as the mean of five trials each; (2) reaction time from conditioned stimulus start to change of compartment; (3) the first attention response after conditioned stimulus start in terms of movement arrest of animals as a sign of auditory signal detection; (4) the second and the subsequent attention responses as a sign of further information processing; (5) the orienting response as an indicator of the motor response independent of compartment change. The occurrence of the first attention response and second and subsequent attention responses, was analyzed up to 45 s from conditioned stimulus start of each trial averaged in blocks of 10 trials. The occurrence of orienting responses was also analyzed as frequency of occurrence during conditioned stimulus and during the inter-stimulus interval in the pre-experience session in 10 trial blocks.

Gerbils were divided into seven groups of five to eight animals each, and subjected to different behavioral paradigms as pre-experience. These paradigms varied as to the inter-stimulus interval between conditioned stimulus and unconditioned stimulus, on one hand and the escapability (escape paradigm) or inescapability (non-escape paradigm) of the unconditioned stimulus, on the other hand. Furthermore, in a latent inhibition group, only the conditioned stimulus was presented: the standard avoidance paradigm was presented (1) to naive controls ($n = 5$) or (2) as test for pre-experience groups. The conditioned stimulus tone (0.9–10 kHz, modulation frequency of 2.0 Hz, on average 60 dB SPL) was thus followed after 5 s by the unconditioned stimulus (0.6 mA pulses for a maximum of 15 s, scrambled via a grid floor). Tone and shock were interrupted when the gerbil moved into the other compartment. The pre-experience paradigms were as follows: (1) the non-escape paradigm in a pre-experience session without further delay (non-escape option, 0 s). Same parameters as in standard avoidance paradigm but the 7-s inescapable unconditioned stimulus continued even after the gerbil changed compartment ($n = 5$); (2) the escape paradigm in a pre-experience session with an 8-s inter-stimulus interval (escape option, 8 s). The 6-s conditioned stimulus tone and the 7-s unconditioned stimulus footshock were separated by an inter-stimulus interval of 8 s so that the gerbil could interrupt the unconditioned stimulus by changing shuttle-box compartment ($n = 5$); (3) the non-escape paradigm in a pre-experience session with an 8-s inter-stimulus interval (non-escape option, 8 s) ($n = 7$); (4) the escape paradigm in a pre-experience session with 20-s inter-stimulus interval (escape option, 20 s) ($n = 8$); (5) the non-escape paradigm in a pre-experience session with a 20-s inter-stimulus interval (non-escape option, 20 s) ($n = 5$); (6) for the latent inhibition paradigm, the conditioned stimulus was presented without an unconditioned stimulus ($n = 5$). The non-avoidance pre-experience paradigms 1–6 were followed by two standard avoidance paradigm sessions on the next two days. All animals in all sessions were trained with 30 trials and an inter-trial interval of 180 s.

2.3. Behavioral tests and in vivo microdialysis

A separate group of six animals was used for this experiment. Three days after a single habituation session in a shuttle-box (Coulbourn Instruments) which lasted 30 min without any stimulation, the animals were stereotaxically implanted (A-P 2 mm, M-L 0.8 mm, D-V 2.7 mm) under anesthesia [ketamine hydrochloride (Ketanest, Parke-Davis, Berlin, Germany)/xylazine hydrochloride (Rompun, Bayer, Leverkusen, Germany) in a proportion of 4:1 in 0.5 ml saline per animal] with a microdialysis probe into the right medial prefrontal cortex (CMA 12, 2 mm, Sweden). The probe was fixed with dental cement on the skull. All animals had resumed normal behavior including feeding by 2 h after surgery. After 26 and 50 h from implantation, the gerbils had a first and a second standard avoidance paradigm training with a combination of tone (conditioned stimulus) and footshock (unconditioned stimulus). After the experiments, the animals were killed with an injection of T61 (embutramine, mebezonium jodide, tetracaine hydrochloride, Hoechst, Wiesbaden, Germany), the brains removed, and sectioned. The sections were Nissl stained and used to verify the probe position histologically.

During behavioral experiments in a shuttle-box, the probe in medial prefrontal cortex of animals was perfused with Ringer (147 mM NaCl, 3 mM KCl, 2.3 mM $CaCl_2$, 1 mM $MgCl_2$ at a flow rate of 1.5 μl/min). Microtubings connected the probe to a counterbalancing arm with fluid swivel (Instech, Plymouth, PA) leading to a pump (CMA/100, Sweden). Three sequential 10-min samples were collected immediately before stimulation for calculation of individual baselines of measured dopamine taken as 100%. Six fractions were collected during the standard avoidance paradigm session with 30 trials plus one fraction after stimulation.

The following behavioral parameters from the video recording of animal performance were analyzed: (1) avoidance performance (conditioned responses), (2) reaction time, (3) the first attention response, and (4) the first orienting response. Conditioned response, attention response, and orienting response values were expressed in percent of conditioned stimulus presentations. Data are given as means of five trials each. Reaction times were averaged over five trials. Dopamine levels in dialysates were expressed as percentages of basal values for each animal and each training session (Stark et al., 1999). The combination of (1), (2), (3), and (4) was used to characterise the performance of individual gerbils during each training session (see Section 3). This information was used to divide the behavior of the six animals into three stages, low improvement of performance, strong improvement of performance, and constant high performance.

The aim of a further experiment was to obtain a better temporal resolution of the correlation between the dopamine content in dialysates and individual behavior. To this end, we collected dialysates from the right medial prefrontal cortex during the avoidance training session over 30 trials from trial to trial at 2-min intervals. The two requirements for this experiment were a fast-learning gerbil who acquired the avoidance strategy within the first training session and a measurable dopamine content in the 2-min dialysate fractions.

2.4. Dopamine analysis

Dopamine was analyzed with a HPLC apparatus (Shimadzu, Japan), flowsplitter (Acurate TM, LC Packings, The Netherlands) with a calibrator (CAL-100-0.3, LC Packings) and a reversed-phased-column (FUS-05-03-C18, LC Packings) with a guard column (flowrate 4.2 μl/min). For sample application to HPLC, a microautosampler (Famos, LC Packings) was used. and for detection, an electrochemical detector (Intro, Antec Leyden, The Netherlands) with microflowcell was used, all kept at 32.5°C. The mobile phase consisted of Na_2PO_4 (0.15 M), 1-octane sulfonic acid (7.3 mM), ethylenediaminetetraacetic acid (1 mM), pH 3.45, and 9% methanol.

The basal values for dopamine in dialysates were approximately at 0.50 ± 0.08 fmol/2.8 μl. The reliable detection limit was 0.1 fmol/sample.

2.5. Statistics

Effects of pre-experience (non-escape option, 0-s, escape option, 8-s, non-escape option, 8-s, escape option, 20-s, non-escape option, 20-s animals) on all parameters were compared to those in standard avoidance paradigm control group by a general linear model with repeated measurements (SPSS©). The significance of differences between the means of corresponding trials with different treatments was analyzed with the Least Significant Difference test.

The time profiles of conditioned response performance in combination with the dopamine levels of the strong improvement of performance and constant high performance stages were tested for differences by a general linear model with repeated measurements (SPSS©). The levels of significance are presented in Fig. 5. Low improvement of performance profiles were also different from strong improvement of performance profiles, but the small sample was not considered reliable enough to draw strong conclusions. Significance of differences between means of corresponding fractions at different dopamine performance stages was measured with the Newman–Keuls test.

3. Results

3.1. The inhibitory influence of a non-avoidance pre-experience on avoidance learning in the shuttle-box

Naive animals without previous experience in the shuttle-box showed a fast increase of performance to a stable level of 80% of conditioned responses in the first standard avoidance paradigm training session. Compared to the standard avoidance paradigm group, animals which were exposed to an inter-stimulus interval of 20 s with escapable and inescapable footshock (escape option, 20 s, and non-escape option, 20 s) and animals with an inter-stimulus interval of 8 s in combination with an inescapable unconditioned stimulus (non-escape option, 8 s) during the pre-experience session were inhibited during subsequent standard avoidance paradigm training, measured by conditioned responses. The non-escape option, 0-s group animals, which were previously exposed to the same temporal relationships as in standard avoidance paradigm training, but in combination with an inescapable footshock, as well as the escape option, 8-s group animals exposed to an inter-stimulus interval of 8 s in combination with an escape option from the unconditioned stimulus, during pre-experience did not differ from the standard avoidance paradigm control animals during subsequent standard avoidance paradigm training. The latent inhibition group showed inhibition, i.e., the start of the performance increase was delayed for approximately 10 trials, however the increase had the same timing as for the standard avoidance paradigm animals. From the beginning of the second standard avoidance paradigm session, all groups showed a very similar avoidance performance (Fig. 1).

The reaction times of the standard avoidance paradigm, non-escape option, 0-s and escape option, 8-s animals rapidly dropped below the 5-s limit during the first standard avoidance paradigm training session (Fig. 2). In contrast the reaction times of the escape option, 20-s, the non-escape option, 8-s and especially of the non-escape option, 20-s group animals remained initially between 5 and 6 s. These animals jumped mostly after the start of unconditioned stimulus, thus following, during parts of the first standard avoidance paradigm training an escape strategy, which was established during the pre-experience session. The reaction times of latent inhibition animals became shorter up to the fifteenth trial of the first standard avoidance paradigm session then dropped suddenly below the 5-s limit for the rest of the session.

All groups of gerbils showed the first attention response about 0.5 s after start of the tone from the beginning of any training and in all other trials (Data not shown.).

Forty-five seconds after trial start the number of attention responses of escape option, 8-s, escape option, 20-s and latent inhibition group animals was higher than those of the standard avoidance paradigm controls and decreased slowly over subsequent trials. This high level of the second and subsequent attention responses decreased slowly during the rest of the trial and more so in later trials. In contrast the non-escape option, 0-s, the non-escape option, 8-s, and the non-escape option, 20-s groups showed this high level for only a limited time immediately after trial start (Fig. 3).

The orienting response frequency of the non-escape option, 8-s, escape option, 20-s, and non-escape option, 20-s groups during conditioned stimulus was lower in the pre-experience session than in the standard avoidance paradigm group during the first training session. These animals did not initiate a motor response, presumably because of the delay between conditioned stimulus start and unconditioned stimulus end, i.e., the improbability of unconditioned stimulus occurring during conditioned stimulus (escape option, 20 s; non-escape option, 20 s). This effect was stronger when the unconditioned stimulus was inescapable (non-escape option, 8 s; non-escape option, 20 s). Also, the orienting response frequency of the latent inhibition group during conditioned stimulus in the pre-experience session was lower than that of standard avoidance paradigm controls (Fig. 4).

The orienting response frequency of the non-escape option, 0-s, escape option, 8-s, and non-escape option, 8-s groups during conditioned stimulus in the first standard avoidance paradigm session was higher than that of the standard avoidance paradigm group.

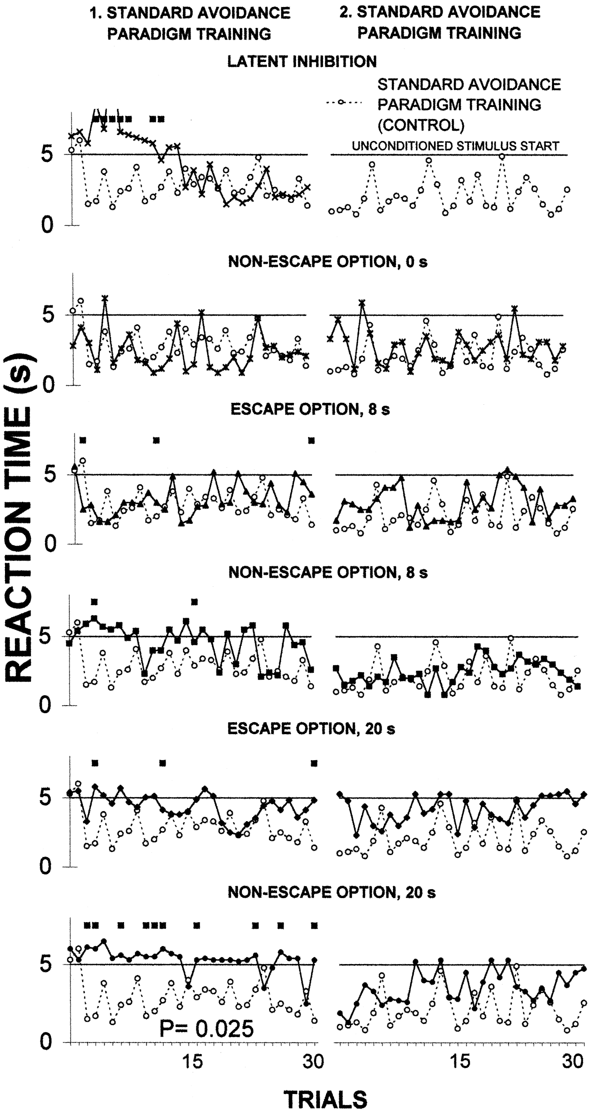

Fig. 2. Reaction times from conditioned stimulus start to change of shuttle-box compartment of the pre-experience groups (filled symbols) in comparison to standard avoidance paradigm control (open circles): latent inhibition (×), non-escape option, 0-s (*), escape option, 8-s (▲), non-escape option, 8-s (■), escape option, 20-s (◆), non-escape option, 20-s pre-experience groups (●) and the standard avoidance paradigm control group (○). In the reaction time analysis, significant differences were found during the first training session between the profiles of non-escape option, 20-s ($P = 0.025$) animals in comparison to the standard avoidance paradigm controls. The filled rectangles indicate the periods of significant differences between these groups (Least Significant Difference test, $P < 0.05$). Data are mean values.

3.2. The influence of the avoidance strategy learning on extracellullar dopamine in medial prefrontal cortex of gerbils

Recombining individual behavioral parameters and events allowed the avoidance learning of the six animals of this group to be divided post hoc into three stages. (1) Low improvement of performance ($n = 2$): in two of six cases, the animals showed, in the low improvement of performance phase, a steep increase of attention responses up to

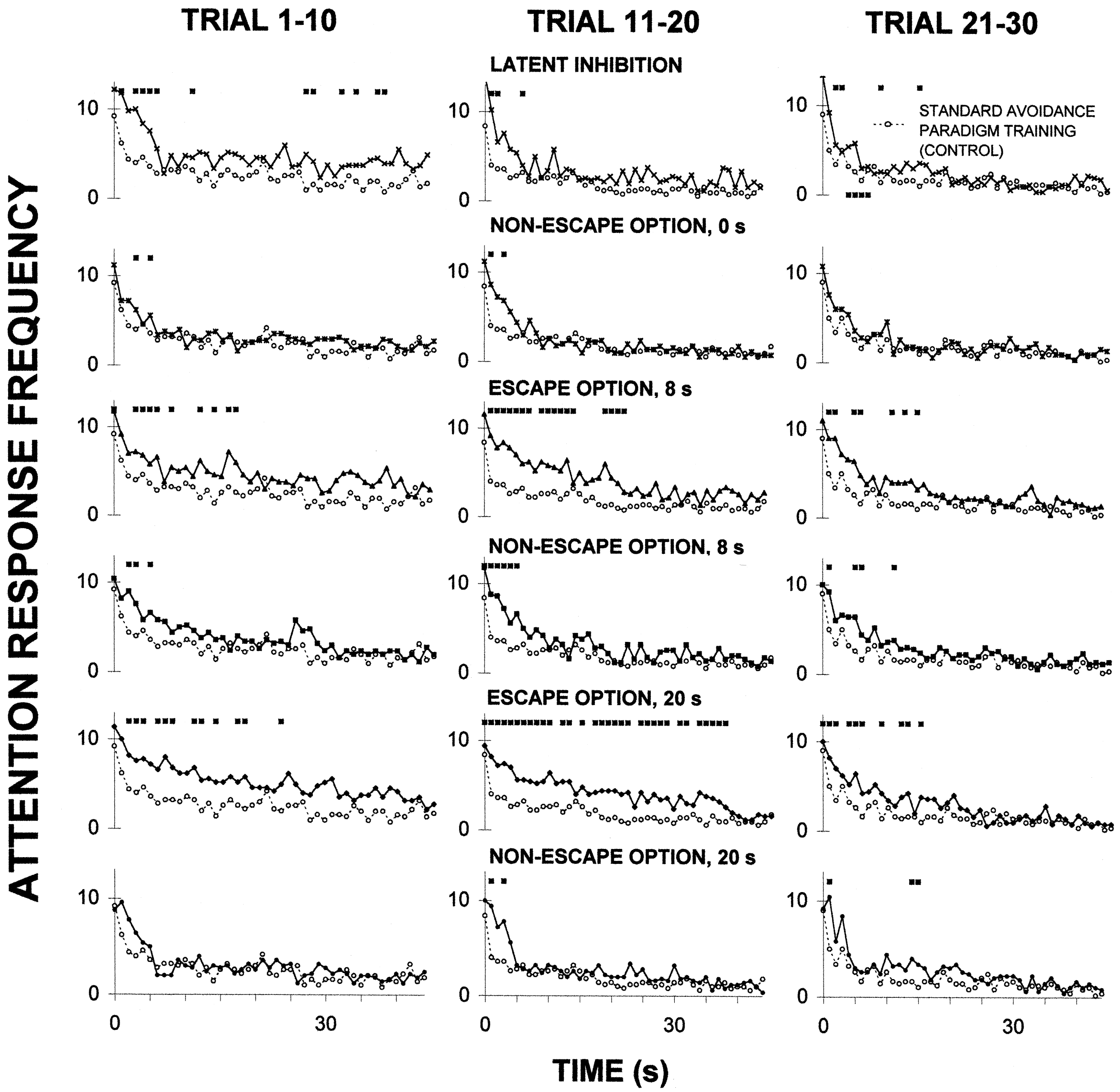

Fig. 3. Averaged frequency of attention responses between 0 and 45 s after trial-start shown separately for trial intervals 1–10, 11–20, and 21–30. Data are given for pre-experience groups (filled symbols) in comparison to the standard avoidance paradigm controls (open circles): latent inhibition (×), non-escape option, 0-s (*), escape option, 8-s (▲), non-escape option, 8-s (■), escape option, 20-s (◆), non-escape option, 20-s pre-experience groups (●) and the standard avoidance paradigm control group (○) during the first standard avoidance paradigm training session. The filled rectangles indicate the periods of significant differences between these groups (Least Significant Difference test, $P < 0.05$). Data are mean values.

100% from initial low levels during the first standard avoidance paradigm session. The increase of conditioned responses from zero level to less than 20% was slow and accompanied by shortening of the reaction time to the footshock, with only rare avoidance of the shock. The likelihood of the first attention response appearance increased over the trials of this session. In comparison, the appearance of the first orienting response was inconsistent. This suggests that the conditioned stimulus became a meaningful stimulus, but that the conditioned stimulus-dependent avoidance strategy was undeveloped (Fig. 5A–D). There was no increased activity of the dopaminergic system at this stage (Fig. 5E). (2) Strong improvement of performance ($n = 6$): in six cases, for animals during the first or the second standard avoidance paradigm session, respectively, the percentage of attention responses was already higher than 75% at the beginning of training and increased further to 100%. The start of the first orienting response appearance was irregular, but was 50% and increased to nearly 100% at the end of the session. Thus, we concluded that the gerbils took into account the temporal relations of conditioned stimulus and unconditioned stimulus. From about trial 10 onward, the reaction times decreased to critical values around 5 s, i.e., jumping occurred frequently before unconditioned stimulus presentation, leading to footshock avoidance. At this stage, compared with the low improvement of performance stage, there was a stronger increase in performance from initially 13–47%

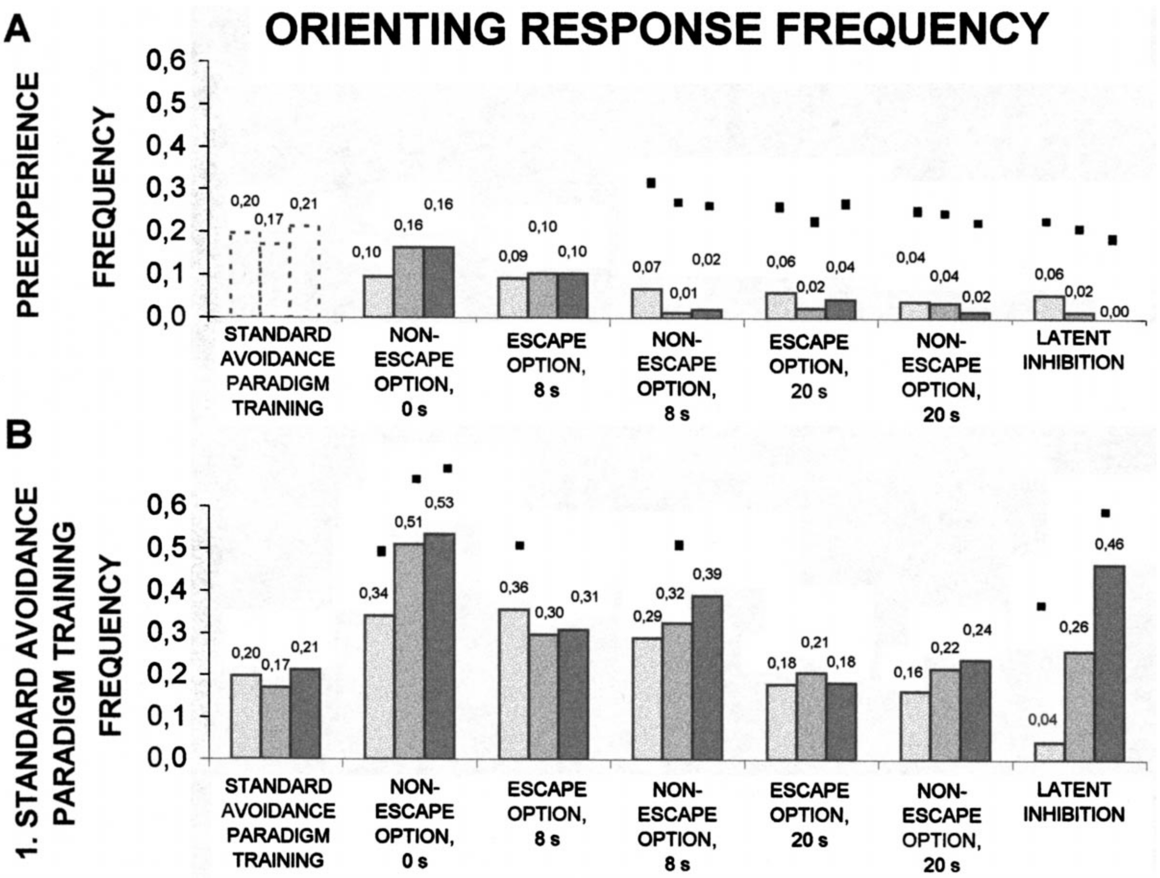

Fig. 4. Averaged frequencies of orienting responses during the exclusive conditioned stimulus presentation shown for trial intervals 1–10, 11–20, and 21–30 during pre-experience session (A) and during the first avoidance training session (B). Data are given for pre-experience groups (filled symbols) in comparison to the standard avoidance paradigm controls (open circles): latent inhibition (×), non-escape option, 0-s (*), escape option, 8-s (▲), non-escape option, 8-s (■), escape option, 20-s (♦), non-escape option, 20-s pre-experience groups (●) and the standard avoidance paradigm control group (○) during the first standard avoidance paradigm training session. The filled rectangles indicate the periods of significant differences between these groups (Least Significant Difference test, $P < 0.05$). Data are mean values.

at the end of sessions. This increase was presumably related to formation of the avoidance strategy. This could be derived from these four gerbils at the strong improvement of performance stage at the first training session which showed a constant high performance at the second training session (Fig. 5A–D). During this strong improvement of performance stage, the extracellular dopamine level increased steadily and significantly up to the middle of the training session and dropped before the session ended (Fig. 5E). (3) Constant high performance ($n = 4$): in four cases, the gerbils had a first attention response and first orienting response of 100% and the avoidance performance at a stable level of more than 60% of conditioned responses during the second standard avoidance paradigm session. The means of reaction times of these animals decreased to below the 5-s limit shortly after the start of the training session, i.e., the established strategy was already transferred to the conditioned stimulus (Fig. 5A–D). The constant high performance stage, therefore, mainly involves recall of the learned strategy. The dopamine levels did not increase during this stage (Fig. 5E).

Comparing the time courses of behavioral measures with time courses of the relative dopamine values at the low improvement of performance, strong improvement of performance and constant high performance stage revealed that only during the strongest improvement of performance during the strong improvement of performance stage (i.e., during acquisition of the avoidance strategy) was there initially a strong increase of extracellular dopamine in medial prefrontal cortex which was followed by a decrease. The dopamine profiles of the constant high performance stage were significantly different from the strong improvement of performance profiles (SPSS©, a general linear model: $P < 0.05$) (Fig. 5D,E).

Another experiment aimed to obtain a better temporal resolution of the correlation between the dopamine content in dialysates and individual behavior (Fig. 6A,B). Due to the very fast learning, the different performance stages were compressed and merged into one another within one standard avoidance paradigm session. Nevertheless, the following distinctions could be made. From the beginning to about trial 9 of the training session (defined as low

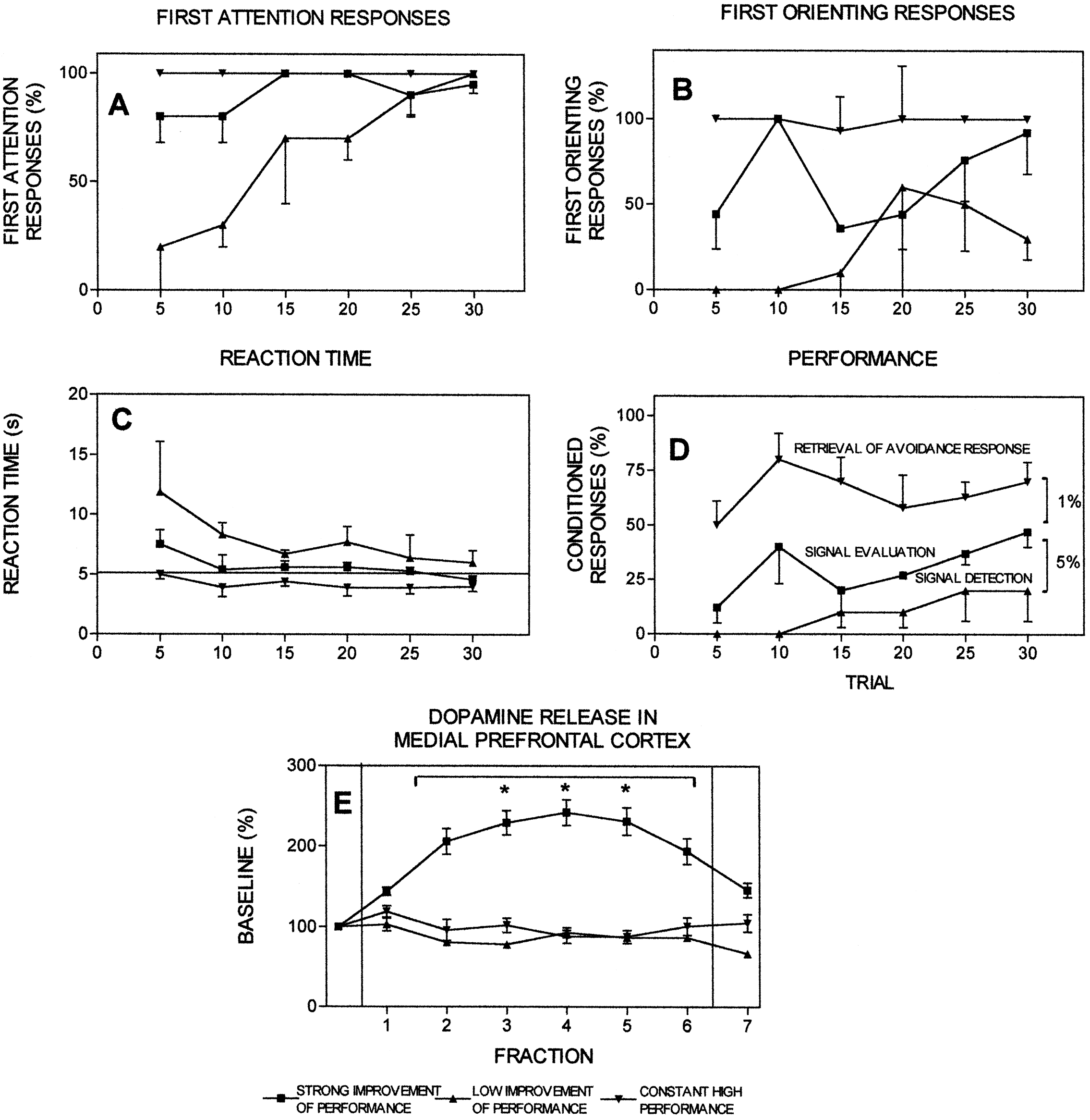

Fig. 5. Averaged first attention response profiles (A), first orienting response profiles (B), reaction time profiles (C), and performance profiles (conditioned responses) (D) of animal groups during stages of low improvement of performance, of strong improvement of performance, and of constant high performance. A data point is the mean of five trials with all animals of a group ± SEM. In the conditioned response percent analysis, solid brackets indicate significant differences found within the stimulation period between the groups of stages (SPSS©, a general linear model). Temporal profiles of relative content of dopamine in brain dialysates during corresponding low improvement of performance, strong improvement of performance, and constant high performance stages of animals (E). Data are mean values ± SEM. Profiles represent 10-min samples including five trials each during the stimulation period (between vertical lines). Dopamine values are related to the average of the three 10-min samples before stimulation, defined as 100% baseline. The first six values represent the stimulation period and the last value, the unstimulated recovery period. In the dopamine analysis, the solid bracket indicates significant differences between strong improvement of performance stage and the constant high performance stage (SPSS©, a general linear model: $P < 0.05$) found within the stimulation period. Significant differences between corresponding fractions of strong improvement of performance and constant high performance stage are indicated by asterisks (Newman–Keuls Test, $P < 0.05$).

improvement of performance stage), the reaction times became shorter and the first attention responses were present, but not yet reliably related to conditioned stimulus start. Conditioned responses did not occur. The dopamine content, thus, did not reach a maximum during this initial stage of learning. During the strong improvement of performance stage, the first attention response occurred shortly after the conditioned stimulus start and distinct the first orienting responses were measurable from trial 16. At the end of this stage up to trial 24, the frequency of conditioned responses was already high. The dopamine content of dialysates increased further and reached its maximum at the end of this strong improvement of performance stage. We assume that by about trial 24, the gerbil had acquired the behavioral strategy and thereafter could retrieve the avoidance behavior. The first attention response was closely

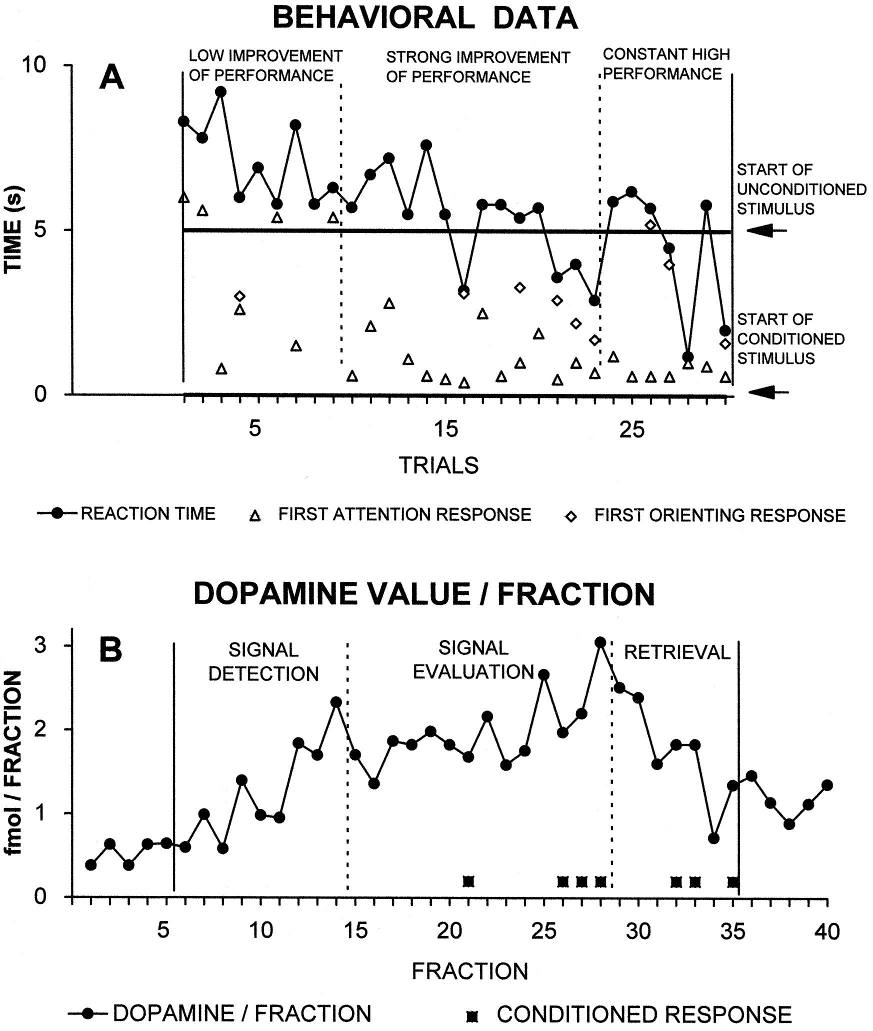

Fig. 6. Individual learning with all stages during one session: profiles of reaction time, of the first attention response, and of the first orienting response versus time from conditioned stimulus start of an animal during low improvement of performance, strong improvement of performance, and constant high performance stages (A). Temporal profile of dopamine content in brain dialysates during corresponding stages of signal detection, signal evaluation, and retrieval of the avoidance strategy (B). This profile represents 2-min samples for each trial of the stimulation period (between vertical full lines). The first five samples and the last five samples represent dopamine values before and after the stimulation period.

connected to the conditioned stimulus start. In this case, the first orienting response could not always be differentiated from movement of the animal. For four trials, this gerbil received an unconditioned stimulus, and changed compartment so quickly, that the first orienting response was not detected. We assume that the gerbil mastered the avoidance strategy in principle, especially as the dopamine content in dialysates started to decrease (Fig. 6A,B).

4. Discussion

Development of the avoidance performance was inhibited in the escape option, 20-s, non-escape option, 8-s, and non-escape option, 20-s pre-experience groups during subsequent standard avoidance paradigm training. The tendency to inhibition of the formation of an avoidance strategy was increased both with the increase of the inter-stimulus interval (the non-escape option, 8-s compared with the non-escape option, 20-s group) and with the inescapability of the unconditioned stimulus in pre-experience sessions (compare the escape option, 20-s with the non-escape option, 20-s group) (Fig. 1).

Considering the time courses of shortening of the reaction times of different pre-experience groups during the first standard avoidance paradigm session, the less inhibited escape option, 20-s, and non-escape option, 8-s groups showed conditioned responses more frequently than did the non-escape option, 20-s groups. The strongly inhibited non-escape option, 20-s animals showed a mix of escape and avoidance behavior for some time, before they achieved a stable avoidance strategy. However, compared with that of the escape option, 20-s, and non-escape option, 8-s groups, replacement of the escape strategy was less time consuming. Obviously, for the non-escape option, 0-s, and the escape option, 8-s animals, the temporal meaning of the tone from the pre-experience corresponded closely to the conditioned stimulus announcing unconditioned stimulus during standard avoidance paradigm training, but with the new option of avoiding the unconditioned stimulus (Fig. 2). Fundamental re-evaluation of the conditioned stimulus content was not necessary for these groups. The latent inhibition of the latent inhibition group was the indicator of a time-consuming re-evaluation of the meaning of the conditioned stimulus from the pre-experience session, namely, that conditioned stimulus did not announce any subsequent event in the shuttle-box context, and thus the tone was not relevant to the animal's own behavior.

The short latency of the first attention response after conditioned stimulus start showed that this behavioral event was externally elicited by conditioned stimulus. Therefore, we conclude that the animals of all groups detected the tone stimulus instantly as a relevant signal, although with different interpretations, depending on the actual behavioral situation. This result confirmed that during the latent inhibition pre-experience session, a behavioral strategy was formed which could not be manifested by common parameters such as conditioned response or reaction time due to the absence of an unconditioned stimulus.

The increase of the second and subsequent attention responses in the pre-experience groups during standard avoidance paradigm could indicate enhanced information processing. Presumably, the second and subsequent attention response events reflected prolonged attention trains. During this stage of avoidance learning, the conditioned stimulus acquired a new meaning concerning the modified temporal relations between cue stimuli. The escape task groups (escape option, 8 s, and escape option, 20 s) showed prolonged periods of increased second and subsequent attention response frequencies that persisted into the inter-trial interval, independent of their reaction times or conditioned response performance with a tendency of shortening of these periods during ongoing standard avoidance paradigm training. Compared to this, the non-escape option groups (non-escape option, 8 s, and non-escape option, 20 s) exhibited increased second and subsequent attention response frequencies only during the short interval of conditioned stimulus presentation. A speculative interpretation is that, as the non-escape option animals had a reliable experience of the inescapable unconditioned stimulus during the pre-experience, there was no need for prolonged attention. The observation of increased second and subsequent attention response frequencies within the inter-trial interval may reflect the need for sufficiently long inter-trial intervals to control the learning process (Fig. 3).

The observation, that the first attention response generally remained the same and that second and subsequent attention responses increased in the animals with pre-experience excludes a loss of attention to the conditioned stimulus caused by pre-experience (Lubow et al., 1976).

The decrease of orienting response frequency of latent inhibition, non-escape option, 8-s, escape option, 20-s, and non-escape option, 20-s groups during the pre-experience session was interpreted as development of an adequate behavioral strategy; i.e., no motor response was initiated because of the improbability of unconditioned stimulus during conditioned stimulus (Mackintosh, 1973; Baker and Mackintosh, 1979) (Fig. 4).

The occurrence of the orienting response during the first standard avoidance paradigm session indicated that the gerbils re-evaluated the temporal relationships learned from the pre-experience training when they changed to the new temporal relationships of the avoidance conditioning. However, the finalization of the motor response in these animals was inhibited to different extents. The inhibited animals presumably waited for the unconditioned stimulus start, as indicated by the delay of avoidance performance development (Fig. 4). They presumably needed time to re-evaluate the difference between the escape strategy they acquired during the pre-experience training and the option of an adequate avoidance strategy during actual standard avoidance paradigm training. In comparison to the standard avoidance paradigm group, the orienting response frequency was enhanced in the non-escape option, 0-s, escape option, 8-s, and non-escape option, 8-s groups. We speculated that these animals had a less reliable pre-experience due to the relatively short inter-stimulus interval in combination with the non-avoidability of the shock. The same happened after the re-evaluation of conditioned stimulus meaning for an enhanced information processing. The latent inhibition-animals showed a comparatively low orienting response frequency during the inhibited first 10 trials. These animals had to recognize the causal relationship between conditioned stimulus and unconditioned stimulus. The strong increase of the orienting response frequency during further standard avoidance paradigm training was presumably caused by the information processing enhanced for establishment of the avoidance strategy.

No general suppression of motor responses as an indicator of an inhibition caused by an inescapable unconditioned stimulus during standard avoidance paradigm training as seen with the learned helplessness model (Maier and Seligman, 1976; Seligman et al., 1968, 1971; Jackson et al., 1980) was shown by our data. The inhibited groups initially used the unconditioned stimulus start as a trigger for compartment change and did not generally have a suppressed motor response.

We conclude from the results that (1) the efficacy of developing an escape strategy in a shuttle-box during pre-experience and therefore, the extent of inhibition (i.e., the time-consuming re-evaluation of the meaning of the conditioned stimulus), in a subsequent standard avoidance paradigm training, depended on the difference in duration of the inter-stimulus intervals between the two training sessions. Inhibition was reinforced by the inescapable shock as feature of the unconditioned stimulus. The inescapable shock as unconditioned stimulus alone without an inter-stimulus interval (non-escape option, 0 s) during pre-experience training did not lead to inhibition in a subsequent standard avoidance paradigm training. The results provide evidence for the temporal coding hypothesis (Barnet et al., 1991; Barnet and Miller, 1996) which claims that the temporal relationships of cue stimuli are part of the associative content of conditioned stimulus and determine the type of the conditioned response. Furthermore, the results provide evidence that the animals coded a complete behavioral strategy on the basis of the content of conditioned stimulus. The extent of inhibition during acquisition of an avoidance behavior is the expression of a time-consuming re-evaluation of the meaning of the conditioned stimulus.

We also conclude that the first attention response, the second and subsequent attention response, and the orienting response events, reflect different components of information processing. In combination with the commonly used conditioned response performance and reaction times, they can indicate various components during avoidance strategy formation (Fig. 7).

4.1. Signal detection

The first attention response was conditioned stimulus start triggered and reflects the general assumption that the tone at the beginning of the first session in the shuttle-box is transformed instantly into a signal to announce the unconditioned stimulus.

The repeated second and subsequent attention responses may indicate reinforced information processing. The unconditioned stimulus start triggered a second and subsequent attention response frequency peak during the initial part of the avoidance training. The orienting response, as indicator of initiation of the motor response, was predominantly unconditioned stimulus start triggered (Fig. 7). The animal was forming a further association, namely, the possibility of escaping from unconditioned stimulus after conditioned stimulus announcement by changing compartment in the shuttle-box.

We concluded from the behavioral measurements, during standard avoidance paradigm training on two consecutive days, and parallel analysis of dopamine levels in medial prefrontal cortex that the post hoc defined stage of low improvement of performance corresponded to signal detection as a stage of avoidance learning. The levels of extracellular dopamine in medial prefrontal cortex were not influenced during the low improvement of performance stage (Fig. 5).

4.2. Signal evaluation

Conditioned stimulus start was closely followed by the first attention response; however, attention response frequency decreased during conditioned stimulus. The unconditioned stimulus start was still followed by a decreasing second and subsequent attention response peak in the course of the training session. During this stage, the animal started a motor response before the unconditioned stimulus start, as indicated by the increasing orienting response-frequency. Execution of a motor response before start of the unconditioned stimulus was the prerequisite of the first avoidance experience (conditioned response) (Fig. 7). Through ongoing information processing during training, the conditioned stimulus content will acquire trial-by-trial context-adequate meaning (Rescorla and Wagner, 1972; Rescorla, 1988) by associating the precise temporal relationship (Barnet et al., 1991; Barnet and Miller, 1996; Davis et al., 1989) of the shuttle-box cue stimuli tone and footshock (Bischof et al., 2000).

Obviously, during signal evaluation, the animals acquired the most important information for the formation of the shuttle-box avoidance strategy, i.e., they learned to avoid the punishment (by the unconditioned stimulus) by

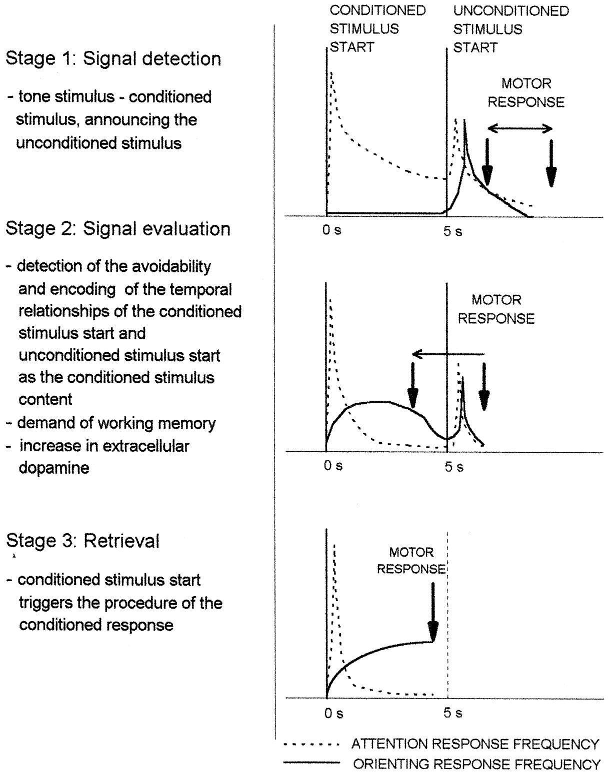

Fig. 7. Different stages of the avoidance strategy formation in the shuttle-box, immediately after conditioned stimulus and unconditioned stimulus start. The first attention response frequency peak indicates that the tone stimulus became a signal. The second attention response frequency peak is followed by an orienting response frequency peak and by the motor response in a broad time-window. (1) Signal detection: after the start of standard avoidance paradigm session, two attention response frequency peaks were observed. (2) Signal evaluation: in comparison to the stage 1 orienting response, events became more frequent between conditioned stimulus start and unconditioned stimulus start, i.e., the animal is processing the temporal relationships of conditioned stimulus start and unconditioned stimulus start. The motor response was shortened over the 5-s limit of unconditioned stimulus start. The probability of conditioned response events was increased by recognition of the avoidance strategy. (3) Retrieval: the animal has encoded the meaning of the avoidance strategy on conditioned stimulus. Immediately after conditioned stimulus start and the attention response frequency peak, the animals performed the conditioned response.

changing compartments within the period between the conditioned stimulus start and unconditioned stimulus start. We assume that, during this acquisition stage of avoidance, the previously accumulated experience was repeatedly compared and combined with continuing feedback experiences of positive or negative trials (conditioned response = avoidance or non-conditioned response with punishment = escape) (Sternberg, 1969) leading to "memory-guided performance" (Goldman-Rakic, 1992). We conclude that, from a theoretical point of view, working memory mechanisms were necessary for comparison and association of events and facts in order to determine their temporal and causal relationships within the context or environment (Rescorla, 1988; Baddeley, 1993; Eysenck et al., 1997). Calculating relations (associations at a higher cognitive level) according to the working memory model of Baddeley (1992, 1993, 1996) is the responsibility of the so-called central executive.

The strong improvement of performance stage derived from the behavioral events during two standard avoidance paradigm sessions corresponded to the stage of signal evaluation. During the strong improvement of performance stage, i.e., during acquisition of the avoidance strategy, there was first a marked increase of extracellular dopamine in medial prefrontal cortex followed by a decrease (Fig. 5E). The dopamine profiles of the constant high performance stage were significantly different from those of the strong improvement of performance profiles (SPSS©, a general linear model: $P < 0.05$).

An increase of extracellular dopamine in medial prefrontal cortex had been found during strategy formation in an operant discrimination task (Yamamura et al., 1994; Izaki et al., 1998). Based on the present results, this could be an indicator that working memory principles are involved in certain stages of conditioning. The dopamine system of the medial prefrontal cortex in primates is strongly involved in cognitive functions as tested in working memory tasks (Goldman-Rakic, 1992; Schultz, 1992; Watanabe et al., 1997). Working memory, which is based on short-term storage of information, is vulnerable to dopamine loss (Goldman-Rakic, 1992).

The hypothesis of working memory implies that cognitive information is temporarily kept so that it can be referred to after input of information (Atkinson and Shiffrin, 1968; Baddeley, 1992, 1993, 1996). It is assumed that this system is used for the performance of many cognitively demanding processes involved in comprehension and problem solving (Eysenck et al., 1997).

4.3. *Retrieval*

During retrieval, the first attention response still occurred soon after conditioned stimulus start. The second and subsequent attention responses, as an indicator of enforced information processing during conditioned stimulus, decreased further. The orienting response events were immediately followed by execution of the motor response before unconditioned stimulus start, resulting in a conditioned response (Fig. 7). The complete conditioned stimulus encoded avoidance strategy is retrieved after conditioned stimulus start.

The constant high performance stage as defined post hoc, using behavioral parameters, corresponded to the stage of retrieval. Dopamine levels were not increased during this stage. The results showed a significant difference in extracellular dopamine between acquisition and retrieval of a shuttle-box avoidance strategy in medial prefrontal cortex of the gerbils (Fig. 5). During the retrieval of the established shuttle-box avoidance strategy, the dopaminergic system was not activated (Stark et al., 1999) and, therefore, working memory principles were presumably not required. This assumption is supported by the finding (Watanabe et al., 1997) that in monkey medial prefrontal cortex, dopamine levels increased during a working memory task and not during a sensory-guided reference memory control task.

Therefore, conscious reproduction of events and facts for comparison and subsequent decision-making drives working memory mechanisms in medial prefrontal cortex, whereas the retrieval of learned procedures from the memory, elicited by an external conditioned stimulus does not require these mechanisms.

Furthermore, we found a correlation between dopamine content of dialysates and individual behavior. Signal detection, signal evaluation and retrieval of the behavioral strategy all occurred within one training session. It could thus be demonstrated that the key phase of shuttle-box learning, i.e., the recognition of temporal relationship of start of conditioned stimulus tone and the unconditioned stimulus footshock, is accompanied by the greatest dopamine increase in medial prefrontal cortex.

It has been proposed that the activity of the dopaminergic system is increased only by appetitive stimuli (Hernandez and Hoebel, 1990; Schultz, 1992; Feenstra and Botterblom, 1996). This does not contradict our present results, since the successful avoidance of an aversive reinforcement by feedback experience, i.e., strategy development with the help of working memory principles in medial prefrontal cortex may be considered by the animals as a "reward", similar to an appetitive stimulus (see also Koob, 1992; Young, 1993; Stark and Scheich, 1997).

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