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ORIGINAL INVESTIGATION

Disruption of the US pre-exposure effect and latent inhibition in two-way active avoidance by systemic amphetamine in C57BL/6 mice

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Abstract
Rationale Pre-exposure to either one of the two to-be-associated stimuli alone is known to reduce the efficiency of the learning of their association when they are subsequently paired explicitly. In classical conditioning, pre-exposure to the conditioned stimulus (CS) gives rise to latent inhibition (LI); and pre-exposure to the unconditioned stimulus (US) results in the US pre-exposure effect (USPEE). Considerable evidence supports an important role of central dopamine in the regulation and modulation of LI; it has been suggested that the USPEE may be similarly controlled by dopamine, but this parallelism has only been directly demonstrated in the conditioned taste aversion paradigm.
Objective The present study tested this hypothesis by comparing the efficacy of systemic amphetamine treatment to affect the expression of LI and the USPEE in a two-way active avoidance paradigm.
Methods C57BL/6 male mice were tested in active avoidance using a tone CS and a foot-shock US. Twenty-four hours before, they were pre-exposed to 100 presentations of the CS or the US, or to the test apparatus only. Amphetamine (2.5 mg/kg) or saline was administered before stimulus pre-exposure and conditioned avoidance test, in which the mice learned to avoid the shock by shuttling in response to the tone.
Results Amphetamine disrupted both stimulus pre-exposure effects, thus, lending further support to the hypothesis that the USPEE is similar to LI in its sensitivity to dopamine receptor agonist. Hence, the USPEE paradigm may represent a valuable addition to the study of dopamine-sensitive processes of selective learning currently implicated in LI and Kamin blocking.

Keywords Active avoidance · Amphetamine · Associative learning · Blocking · Latent inhibition · Mice · US pre-exposure

Introduction

Associative learning is selective. One factor that critically determines the capacity of a stimulus to form an effective association during conditioning is its associative history. Selectivity in associative learning can be readily demonstrated when either one of the two stimuli in question is pre-exposed before their explicit pairing later in associative conditioning. In classical conditioning, pre-exposures to either the to-be-conditioned stimulus (CS) or unconditioned stimulus (US) before CS–US pairing can interfere with the subsequent development and/or expression of the conditioned response (CR). The decrement of the CR after non-reinforced CS pre-exposure is referred to as latent inhibition (LI; Lubow and Moore 1959), and that after US pre-exposure is referred to as the US pre-exposure effect (USPEE; Randich and LoLordo 1979). Both phenomena can be studied in numerous species, including human and rodent, and across a variety of associative conditioning procedures (Lubow 1989; Moser et al. 2000; Riley and Simpson 2001; Baker et al. 1981; Matzel et al. 1987; Claflin and Buffington 2006).

LI is commonly considered as a form of salience (or attentional) learning, reflecting the ability to ignore stimuli that historically predict no significant consequences. LI has been attributed to the development of selective attention

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away from the pre-exposed stimulus; that is, non-reinforced CS pre-exposure diminishes the perceived salience of the CS during conditioning (Mackintosh 1974, 1975; c.f. Lubow et al. 1981; Lubow 1989). Other suggestions favour the view that LI results from the acquisition of an association between the CS and the absence of a significant consequence during pre-exposure that later interferes with either the subsequent expression (Weiner 1990; Gray et al. 1991; Weiner 2003) or retrieval (Kraemer and Spear 1992; Bouton 1993) of the critical CS–US association. Some accounts also emphasize the role of contextual processing as a critical determinant of LI expression (Wagner 1981; Miller and Matzel 1988; Grahame et al. 1994; Lubow and Gewirtz 1995; McLaren and Mackintosh 2000; Hemsley 2005).

The USPEE can be, on associative grounds, understood as a form of Kamin blocking effect (Kamin 1969), whereby context–US association acquired during US pre-exposure interferes with the acquisition or expression of the CS–US association in the same context. Prior experience of the US is also expected to reduce the salience or surprise of the US such that subsequent CS–US association proceeds more slowly (Best and Domjan 1979; Domjan and Best 1980)—i.e. as a form of US habituation. Alternatively, the USPEE has been attributed to the development of US tolerance whereby repeated exposures to the US reduce the maximal associative strength held by the US (Batson and Best 1979, but see also De Brugada et al. 2005). Within the context of aversive instrumental learning, including conditioned active avoidance learning, pre-exposures to the shock US may also capture elements of learned helplessness, in which the emotional or motivational consequences after the experience of inescapable and uncontrollable shocks is emphasized (Overmier and Seligman 1967; Seligman and Maier 1967; Maier 1984). This can be demonstrated by the presence of escape failures in subsequence avoidance training. Accordingly, learning that one's action cannot alter the occurrence of aversive events, the animals are said to be in a generalized state of helplessness that can severely retard subsequent learning to avoid and even to escape from aversive events.

Deficits in LI can be reliably induced by the indirect dopamine agonist, amphetamine (Solomon and Staton 1982; Weiner et al. 1984, 1988; Gray et al. 1992b; Thornton et al. 1996) and are observed in subsets of schizophrenic patients (Baruch et al. 1988; Gray et al. 1992a). These observations have led to the application of LI to assess impairments in selective learning in relation to human psychosis and related behavioural traits and/or cognitive deficits in animals (Gray et al. 1991; Feldon and Weiner 1992; Weiner 1990, 2003; Lubow 2005). By contrast, despite of its relevance to selective learning, relatively few studies have focused on the neuropsychopharmacology of the USPEE, including its possible relation or application to schizophrenia research (Meyer et al. 2005, 2006a).

Recently, we have shown that LI and the USPEE are similarly abolished by systemic amphetamine (2.5 mg/kg, i.p.) in mice using a conditioned taste aversion (CTA) paradigm in which the animals learned to associate a flavored liquid with gastric malaise (Meyer et al. 2004). The present study is designed to test the generality of this parallelism by extension to another associative learning paradigm, namely active avoidance learning, in the same species. This is warranted because amphetamine is known to reduce liquid consumption in the CTA paradigm in both rats and mice (e.g. Russig et al. 2003; Meyer et al. 2004). Hence, the possibility that the expression of LI and USPEE was weakened as a result of the reduced consumption of the flavored CS during pre-exposure and conditioning, respectively, cannot be entirely excluded (Russig et al. 2003; Meyer et al. 2004). In contrast, the numbers of CS presentations in all phases of a conditioned avoidance experiment are strictly under the control of the experimenter. Furthermore, the active avoidance paradigm allows us to evaluate the expression of LI and the USPEE as learning progresses across successive trials, as opposed to the CTA conditioning procedure, in which the CR is typically measured in an extinction test (i.e. in the absence of any US after the CS) subsequent to a single CS–US pairing.

In this study, the effects of systemic amphetamine on the LI and the USPEE were examined in two independent experiments with identical pharmacological parameters (2.5 mg/kg, i.p.) adopted from our previous study using the CTA paradigm (Meyer et al. 2004). Amphetamine was administered before stimulus pre-exposure and conditioning, separated by 24 h. The behavioural parameters selected to produce the LI and the USPEE were selected on the basis of our previous demonstration that these effects could be disrupted by a prenatal manipulation designed to precipitate the emergence of psychotic-like behaviour, including enhanced sensitivity to systemic amphetamine challenge at 2.5 mg/kg in mice (see Meyer et al. 2005, 2006a,b).

Based on the previous psychopharmacological studies of amphetamine on LI expression in rats (Weiner et al. 1984, 1988; Solomon et al. 1981; Crider et al. 1982; Solomon and Staton 1982), we expect that the LI effect would be absent in the amphetamine-treated mice. A key objective in this study is to test whether the same amphetamine treatment regime would also lead to the attenuation or abolition of the USPEE—namely, the retardation in active avoidance performance seen in animals having been repeatedly pre-exposed to the shock US. A positive outcome in this direction would be in keeping with the finding reported by Meyer et al. (2004) in the CTA paradigm. However, our previous experience (e.g. Meyer et al. 2005, 2006a) also suggests that the US pre-exposure

procedure in this research would also lead to impairment in escape behaviour that would be indicative of behavioural or emotional change akin to learned helplessness. Hence, an attenuation of the USPEE by amphetamine may also be interpreted as the drug's ability to nullify the impact of US pre-exposures on the development of learned helplessness —a possibility that is also consistent with a dopaminergic modulation of the learned helplessness effect (Besson et al. 1998, 1999; Kram et al. 2002). The present experiment cannot decide between these two interpretations, and it is possible that they are equally valid at least within the present experimental design. Nevertheless, a negative outcome would be sufficient to falsify our hypothesis that the ability of amphetamine to disrupt the USPEE first demonstrated in the CTA paradigm can be readily extended to other associative learning paradigms according to the operational definition of the USPEE. The present study represents an important first attempt to examine the generality of the initial findings of Meyer et al. (2004).

Materials and methods

Subjects

The subjects were 81 naïve male C57BL/6 mice obtained from the specific pathogen-free facility at the Laboratory of Behavioural Neurobiology (Swiss Federal Institute of Technology Zurich). At the beginning of the experiment, the mice were about 8 weeks old and weighed approximately 28 g. All animals were maintained under ad lib food and water throughout the experiment. They were housed in a temperature- and humidity-controlled (22°C, approximately 55%) animal facility under a reversed light–dark cycle (lights on from 1900–0700 hrs). Behavioural testing was carried out during the dark phase of the cycle. All procedures described in the present study had been previously approved by the Cantonal Veterinarian's Office of Zurich and are in agreement with the Principles of Laboratory Animal Care (NIH publication no. 86-23, revised 1985).

Apparatus

The apparatus consisted of four identical two-way shuttle boxes (model E10-15; Coulbourn Instruments, Allentown, PA, USA), each set in a ventilated sound- and light-attenuating shell (model E10-20). The internal dimensions of each shuttle box, as measured from the raised grid floor, were 35×17×21.5 cm. The box was separated into two identical compartments by an opaque partition wall. The two compartments were interconnected by an opening in the partition wall (6.7×7.7 cm), and there was no barrier on the floor level, thus, allowing the animal to traverse freely from one compartment to the other, which constituted a shuttle response. The grid floor was made of stainless steel rods, which were 0.4 cm in diameter and spaced 0.7 cm apart. The floor was mounted on a pivot such that it would be tilted slightly to the compartment where the animal was, and a switch of the animal's location from one compartment to the other was recorded as a shuttle. In addition, the grid floor was connected to a constant current shock generator (model E13-14; Coulbourn Instruments) that delivered the foot shock at 0.3 mA. Whenever a shock was delivered, it was administered to the entire floor of the shuttle box, i.e. to both compartments. The CS was a 2.9-kHz, 85-dB (A-scale) tone generated by a tone module (model E12-02; Coulbourn Instruments) placed behind the shuttle box on the floor of the shell. The boxes were illuminated during the experimental session by two diffuse light sources (1.1 W), each mounted 19 cm above the grid floor on the sidewall on each of the two compartments. The four shuttle boxes were connected via a universal interface to a PC running a programme written in Borland® Turbo Pascal developed in-house (P. Schmid, Laboratory of Behavioural Neurobiology, Swiss Federal Institute of Technology Zurich), which controlled the four boxes independently, and collected and stored all dependent variables.

Behavioural procedures

Experiment 1: The effect of amphetamine on LI (CS pre-exposure effect)

The experiment followed a 2×2 factorial design with the between-subjects factors pre-exposure (CS–PE vs nPE) and drug (Amph vs Sal). The numbers of subjects in each of the four treatment conditions were nPE/Sal=9, CS–PE/Sal=11, nPE/Amph=9, CS–PE/Amph=12. Animals belonging to the same litter were, as far as possible, assigned to different treatment conditions to minimize the potential confounds resulting from the litter effects (Zorrilla 1997). In addition, the individual shuttle boxes and the order of testing were counterbalanced within each experimental group. The experimental procedures consisted of two phases, separated by 24 h.

Pre-exposure Each animal was placed in the shuttle box. Animals in the CS–PE group received 100 presentations of the 5-s-tone CS with a variable inter-stimulus interval (mean of 40 s, ranging from 25 to 55 s). The mice in the nPE group were confined to the chamber for the same period of time without receiving any stimulus. The house-light remained on through the session.

Conditioning The animals were returned to the same shuttle boxes in which pre-exposure took place and underwent 100 avoidance trials presented at a variable inter-trial interval (ITI; mean of 40 s, ranging from 25 to 55 s), with the house-light on throughout the session. A trial began with the onset of the tone CS. If the animal shuttled within 5 s of CS onset, the tone CS was terminated and the animal did not receive any foot shock (i.e. an avoidance response). Avoidance failure was followed by the delivery of a foot shock (co-presented with the tone CS), and a shuttle response during foot shock terminated both the CS and the foot shock (i.e. an escape response). A foot shock (co-presented with the tone CS) could last for a maximum of 2 s, and if so, an escape failure was scored on such a trial. Any shuttle response during the ITI period had no programmed consequence: An ITI was defined as the time period between the end of one trial and the beginning of the next.

Experiment 2: The effect of amphetamine on the USPEE

Experiment 2 adopted a 2×2 (pre-exposure×drug) factorial design with an equivalent group size of 10 in each of the four experimental conditions: nPE/Sal, US–PE/Sal, nPE/Amph, and US–PE/Amph. Like in experiment 1, animals belonging to the same litter were assigned to different treatment conditions to the minimize potential confounds resulting from the litter effects (Zorrilla 1997). The individual shuttle boxes and the order of testing were also counterbalanced within each experimental group. As in experiment 1, each animal was run in the same shuttle box throughout the whole experiment.

The procedures followed that of experiment 1, with the pre-exposure and the conditioning conducted 24 h apart, except that on the pre-exposure day (day 1), animals in the US–PE group received 100 presentations of the US (foot shock; 0.3 mA, 2 s) instead of the tone CS. nPE subjects were confined to the chamber for the same period of time without receiving any stimulus. The conditioning procedure was identical to that described in experiment 1.

Drug administration

D-amphetamine sulfate (Sigma-Aldrich, Switzerland) was dissolved in sterile 0.9% NaCl solution to achieve the required dosage of 2.5 mg/kg (calculated as the salt) with an injection volume of 5 ml/kg. This dose is identical to the one we have previously shown to abolish LI and the USPEE in the CTA paradigm (Meyer et al. 2004). It is higher than the typical dose (1–1.5 mg/kg, i.p.) that reliably disrupt LI in rats using the lick-conditioned suppression paradigm (Weiner et al. 1984, 1988). Control subjects received saline solution with the same injection volume. All solutions were freshly prepared on the required days and administered via the intraperitoneal (i.p.) route 30 min before testing on both the pre-exposure and conditioning days in experiments 1 and 2. The pre-exposure session lasted for approximately 70–75 min, and the conditioning session 90 min.

Data analysis

Data of the two experiments were separately analyzed, and so were the data derived from the pre-exposure and conditioning phases of each experiment. In the pre-exposure phase, the total number of spontaneous shuttles in-between the two compartments was taken as a measure of spontaneous locomotor activity and analysed using 2×2 (drug×pre-exposure) analysis of variance (ANOVA). In the conditioning phase, two measures of avoidance performance were obtained: the number of avoidance responses and the number of escape failures. In addition, the number of spontaneous shuttles during the ITIs was taken to gauge the acute effect of amphetamine on locomotor activity. All measures were expressed in blocks of 20 trials, and then subjected to a square-root transformation (to better conform to the assumptions of parametric ANOVA) before a three-way ANOVA with the between-subject factors drug (Amph vs Sal) and pre-exposure condition (nPE vs CS–PE or US–PE), and the within-subject factor of 20-trial blocks. A priori restricted analyses were conducted to examine the statistical presence of LI and the USPEE in either the amphetamine- or saline-treated animals. All statistical analyses were carried out using the statistical software SPSS for Windows (version 13) implemented on a PC running the Windows XP operating system.

Results

Experiment 1: the effect of amphetamine on LI

Pre-exposure day

Amphetamine treatment enhanced spontaneous locomotor activity, and this effect was equivalently observed in both (non-pre-exposed) nPE and (CS-pre-exposed) CS–PE subjects relative to their respective saline-treated controls. A 2×2 (drug×pre-exposure) completely randomized ANOVA design of the total number of shuttles yielded only a significant main effect of drug [$F(1,38)=44.44$, $p<0.001$]. The mean (±SEM) total shuttles in the four groups were nPE/Sal=84±11, CS–PE/Sal=77±8, nPE/Amph=250±41, and CS–PE/Amph=271±31. CS pre-exposure did not appear to affect the frequency of spontaneous shuttles.

Conditioning day

Avoidance response The number of avoidance responses increased across blocks, and this was evident in all four groups with a highly significant main effect of blocks [F(4.148)=47.94, $p<0.001$] (see Fig. 1a). Overall, amphetamine led to a deviation (in blocks 1 and 2) from the monotonic increase of avoidance performance seen in the saline animals. This led to the emergence of a drug×blocks interaction [F(4,148)=3.86, $p<0.005$] that is solely attributed to the quadratic trend [F(1,37)=11.56, $p=0.002$]. LI was clearly evident in the saline-treated animals throughout the session with the nPE/Sal mice performing consistently better than CSPE/Sal mice, and an overall presence of LI was supported by the main effect of preexposure [F(1,37)=4.81, $p<0.05$]. The LI effect, however,

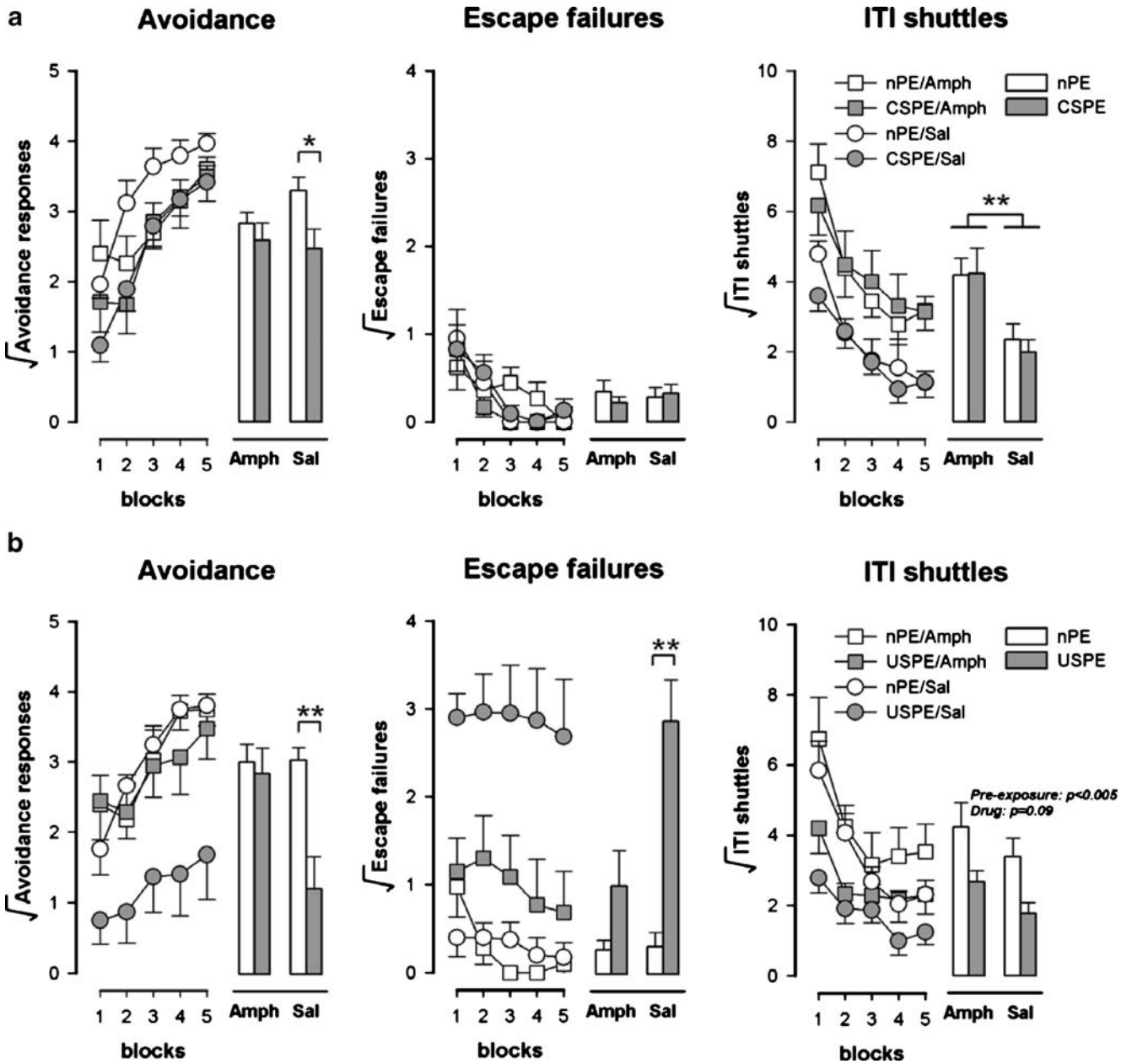

Fig. 1 Effects of amphetamine on latent inhibition (**a** experiment 1) and the US pre-exposure effect (**b** experiment 2) in two-way active avoidance learning. Three dependent measures (illustrated from *left to right*) are number of avoidance responses, escape failures, and the frequency of spontaneous shuttles recorded during ITIs. All measures are expressed in units of 20-trial blocks and square-root transformed. The histogram accompanying each graph depicts the mean value of the corresponding variable averaged across blocks to illustrate the relevant between-groups comparisons, including the presence of LI or the USPEE in saline- and amphetamine-treated mice. *Error bars* refer to ±SEM. The *single asterisk* and the *double astrisks* denote a significant difference of $p<0.05$ and $p<0.001$, respectively

appeared attenuated in the amphetamine-treated animals, with a tendency observed only in the first two blocks. Despite the lack of a significant interaction between drug and pre-exposure [F(1,37)=1.47, p=0.23], a priori restricted ANOVAs revealed a significant effect of pre-exposure only in the saline-treated animals [F(1,18)=5.13, p<0.05] but not in the amphetamine-treated mice [F<1].

Escape failure There was a general decrease in the number of escape failures as a function of blocks in all four groups [F(4,148)=18.24, p<0.001] (see Fig. 1a). No other main effects or interaction terms attained statistical significance.

ITI shuttles The number of spontaneous shuttles decreased across blocks at a comparable rate in all four groups, leading to a highly significant blocks effect [F(4,148)= 50.03, p<0.001]. Amphetamine treatment led to an elevation in the number of spontaneous shuttles [F(1,37)=13.46, p<0.001], and this effect was equivalently seen in both nPE and CS–PE subjects. No other main effects or interaction terms attained statistical significance.

To further ascertain whether ITI shuttles might nonetheless affect the measures of avoidance and escape failure differentially so as to account for any between groups difference in these measures, additional covariance analysis (ANCOVA) using ITI shuttles as the covariate was conducted. Although the covariate attained significance [F(1,36)=7.95, p<0.01], the main effect of pre-exposure remained statistically significant [F(1,36)=5.09, p<0.05]. This suggested that the covariance between the ITI shuttles and the avoidance response cannot account for the pre-exposure effect revealed in the latter measure. An ANCOVA of escape failures with the ITI shuttle as the covariate failed to yield any significant effect indicative of group differences—consistent with the ANOVA results described above.

Experiment 2: the effect of amphetamine on the USPEE

Pre-exposure day

Amphetamine treatment enhanced spontaneous locomotor activity on the pre-exposure day, and this effect was more pronounced in the amphetamine-treated nPE subjects as compared to the amphetamine-treated US–PE animals. Across the two pre-exposure conditions, no differences in spontaneous locomotor activity were detected in the saline-treated animals. The mean (±SEM) total shuttles in the four groups were nPE/Sal=93±12, US–PE/Sal=93±15, nPE/Amph=347±52, and US–PE/Amph=154±19.

These impressions were supported by a 2×2 (drug×pre-exposure) ANOVA of the total number of shuttles that yielded a main effect of drug [F(1,36)=29.10, p<0.001], pre-exposure [F(1,36)=10.77, p<0.01], and their interaction [F(1,36)=10.88, p<0.01].

Conditioning day

Avoidance response The number of avoidance responses generally increased over blocks, indicating the acquisition of avoidance learning in all groups (Fig. 1b). The main effect of blocks was highly significant [F(4,144)= 21.67, p<0.001]. Like in experiment 1, amphetamine treatment tended to distort the monotonic trend of acquisition evident in the saline animals. Again, this tendency was observed regardless of pre-exposure condition and attributed solely to blocks 1 to 2 (c.f. Fig. 1a). However, this effect failed to attain statistical significance here [drug×blocks, F(4,144)=2.56, p=0.066].

Notably, US pre-exposure reduced performance substantially in the saline-treated animals constituting the USPEE. By contrast, the USPEE was hardly detectable in the amphetamine-treated animals. The abolition of the USPEE by amphetamine was confirmed by the significant interaction between drug and pre-exposure [F(1,36)=6.29, p<0.05] that was accompanied by a significant main effect of pre-exposure [F(1,36)=9.14, p<0.005] and of drug [F(1,36)= 5.85, p<0.05]. A priori restricted analyses strengthened this conclusion that yielded a main pre-exposure effect only in the saline animals [F(1,18)=14.05, p=0.001], but not in the amphetamine animals [F<1].

Escape failure The USPEE was also evident in this measure, and this further illustrated that, although amphetamine clearly attenuated the USPEE, a residual effect was still somewhat evident (Fig. 1b). Besides the general main effect of blocks [F(4,144)=3.33, p<0.05], the main effects of pre-exposure [F(1,36)=25.63, p<0.001], drug [F(1,36)= 8.73, p=0.001], and their interaction [F(1,36)=8.02, p< 0.01] attained statistical significance, complementing the impressions derived from the avoidance measure. Again, a priori restricted analyses supported the conclusion of a disruption of the USPEE by amphetamine, with a main pre-exposure effect only detectable in the saline animals [F (1,18)=26.41, p=0.001], but not in the amphetamine animals [F(1,18)=3.03, p=0.10].

ITI shuttles Similar to experiment 1, spontaneous shuttles during ITIs generally decreased as a function of blocks (Fig. 1b) and resulted in a main-effect block [F(5,144)= 44.31, p<0.001]. US pre-exposure reduced the number of ITIs shuttles that was most pronounced in the first two blocks. These impressions were supported by the main

effect of pre-exposure [$F(1,36)=10.41$, $p<0.005$], as well as its interaction with blocks [$F(4,144)=4.17$, $p<0.005$]. Although the effect of amphetamine on ITI shuttles was visible graphically, which is in keeping with that seen in experiment 1, it failed to attain statistical significance in this experiment [$F(1,36)=3.03$, $p=0.09$].

Again, we conducted ANCOVA using ITI shuttles as the covariate on the measures of avoidance response and escape failure. The results suggested that ITI shuttles did not significantly co-vary with either of the two measures, and the pattern of statistical outcomes remained similar to those described above based on the ANOVAs of the two measures.

Discussion

The present study confirmed the efficacy of systemic amphetamine to disrupt the USPEE in the two-way active avoidance paradigm, and thereby extending our previous results obtained using the CTA paradigm (Meyer et al. 2004). The drug regime was essentially identical in both studies: Amphetamine at 2.5 mg/kg was administered on both pre-exposure and conditioning days. The same treatment also led to the disruption of the LI effect, although the form of LI disruption seen in this study in the active avoidance paradigm was distinct from that seen in the CTA experiment (Meyer et al. 2004), and it differed from the pattern seen commonly in rats (e.g., Solomon and Staton 1982; Weiner et al. 1984, 1988).

The nature of the disruption of the USPEE by amphetamine

In this paper, the USPEE was readily demonstrable in the measure of conditioned avoidance as well as escape failure: Pre-exposures to the US retarded subsequent acquisition of the conditioned avoidance response and led to a pronounced increase in the number of escape failures. Amphetamine abolished the USPEE as indexed by avoidance learning, and greatly attenuated the USPEE as seen in the escape behaviour. In both measures, the disruption of the USPEE in this study was, therefore, exclusively attributed to the drug's effect in the US–PE condition. This conforms precisely to the pattern of results previously obtained in a taste-conditioned aversion paradigm in which amphetamine treatment before pre-exposure and conditioning prevented the deleterious effect of US pre-exposure on subsequent CS–US learning (Meyer et al. 2004).

As outlined in the Introduction, there are several theoretical accounts of the USPEE (see review by Riley and Simpson 2001). One view emphasizes the parallel between LI and the USPEE, and postulates that mechanisms underlying the reduced associability of the CS in LI may be similarly operative in reducing the associability of the US (Best and Domjan 1979; Domjan and Best 1980). Another view posits that the USPEE, as a form of Kamin blocking effect in which context–US association acquired during pre-exposure, blocks the acquisition of the target CS–US association that takes place in the same context. It has been shown that this offers a satisfactory account for the USPEE at least in the CTA paradigm (De Brugada et al. 2003, 2004, 2005). Hence, the ability of amphetamine to disrupt the USPEE within the CTA paradigm may be readily anticipated by the drug's known effect on LI (e.g., Weiner et al. 1988; Moser et al. 2000) and on the Kamin blocking effect (Crider et al. 1982; Jones et al. 1997; O'Tuathaigh et al. 2003). However, it is not certain that the effect of US pre-exposure on avoidance learning can be solely accounted for by the Kamin blocking effect. Unpublished data from our laboratory indicated that a clear USPEE persisted when pre-exposure to the US was conducted in another context (Chang, 2005, unpublished Ph.D. thesis, Swiss Federal Institute of Technology Zurich). Moreover, the account based on Kamin blocking cannot predict the expression of the USPEE in the measure of escape failure that is commonly attributed to an effect on motivation reflecting a state of learned helplessness (Seligman and Maier 1967; Seligman et al. 1975; Maier 1984). The learned helplessness hypothesis developed by Seligman and colleagues, thus, offers a non-associative and context-independent account of the USPEE. The effect of US pre-exposure on escape failure was severely reduced by amphetamine, and this may suggest an action of the drug beyond LI-related mechanism and Kamin blocking.

The critical issue is whether the USPEE phenomenon seen in active avoidance can be solely explicable by selective learning similar to Kamin blocking and the LI effect through processes that weaken the associability of the US (and thereby the formation of the target CS–US association and/or the generation/strength of the CR), or by learned helplessness and related processes that affects motivation, or perhaps, a combination of these different processes. To delineate the contribution of these separate processes, additional experiments would be required, perhaps, with the use of fewer number of pre-exposures to the US. The use of active conditioned avoidance in this study introduces these confounds that are not as critical as in our former study when the CTA paradigm was employed because learned helplessness is unlikely to operate there. Evidence that amphetamine nullified the USPEE here by disrupting the development of learned helplessness is provided by the measure of escape failure. The increase number of escape failures induced by the US pre-exposure procedure can only be readily explained by the learned helplessness hypothesis, but not in terms of Kamin block-

ing or other attentional accounts of the USPEE. This interpretation would suggest that the apparent similarity between the abolition of the USPEE in CTA and conditioned active avoidance is mediated by two distinct effects of amphetamine that is unique to each paradigm. This admittedly less parsimonious accommodate would seem necessary to accommodate for the data obtained from different experimental designs.

Learned helplessness

To the best of our knowledge, the effects of systemic amphetamine on the classical learned helplessness paradigm with the triadic design have not been investigated. Instead, the learned helplessness procedure has been widely used for the screening of antidepressant drugs (for a review, see Maier 1984; Willner 1984; Vollmayr and Henn 2001; Cryan et al. 2002). Notably, a critical role of central dopamine in the control and modulation of inescapable shock-induced escape deficits in the learned helplessness paradigm has been identified (Muscat et al. 1992; Besson et al. 1998, 1999; Takamori et al. 2001; Kram et al. 2002; Millan et al. 2004). Specifically, it has been demonstrated that the reversal of shock-induced deficits in avoidance and/or escape learning is associated with an increase in the functional responsiveness of central dopaminergic systems (Besson et al. 1998; Takamori et al. 2001). This agrees with the observation that dopamine function is enhanced after chronic antidepressant treatment (see Willner 1983). It is also in keeping with our results that amphetamine pre-treatment reduced the impact of US pre-exposures on subsequent avoidance learning, and that similar dopaminergic mechanisms are involved in the cognitive and emotive consequences after repeated exposures to inescapable shocks that are common to the learned helplessness and the USPEE procedure employed here. The USPEE paradigm, thus, appears to provide an interesting link between the learned helplessness and the LI paradigms as applied to the study of depression and schizophrenia, respectively.

Avoidance learning after amphetamine treatment

Here, the effects of amphetamine on avoidance learning as such (i.e., in the nPE animals) and on LI appear to differ from the data derived from rats. Notably, amphetamine did not generally enhance avoidance learning here, although it was obviously effective in promoting spontaneous shuttles during ITIs: It was most clearly and consistently seen in experiment 1, and a non-significant trend was still detectable in experiment 2 ($p<0.09$). If the motor-enhancing effect of amphetamine was to lead to improved avoidance performance, this was only seen in the first block of testing—this was evident in both experiments, in the comparison between nPE/Amph and nPE/Sal mice (see Fig. 1a,b). In experiment 1, amphetamine even impaired avoidance learning from block 2 onwards in the nPE subjects. Indeed, this selective effect of amphetamine on the nPE (but not in the PE) condition was responsible for the overall absence of LI in the amphetamine-treated animals in experiment 1. This is in sharp contrast to an equivalent study in rats in which *d*,*l*-amphetamine (1.5 mg/kg, i.p., administered before both pre-exposure and conditioning) facilitated acquisition of avoidance responses in all treatment groups irrespective of the pre-exposure condition and abolished LI at the same time (Weiner et al. 1988).

Although this discrepancy between rats and mice does not undermine the interpretation of our novel finding here concerning amphetamine's effect on the USPEE, we attempted an analysis pooling the nPE subjects from the two experiments to examine the overall effect on the drug on avoidance learning in mice. This yielded no difference between experiments, but only an interaction between drug and blocks [$F(4,136)=5.18$, $p<0.001$] that is solely attributed to a quadratic trend [$F(1,34)=11.44$, $p<0.002$]. It reflects that there is evidence for enhanced avoidance in the first block, but subsequently, the effect of amphetamine reversed and tended to impair avoidance performance. We do not have an explanation for this particular pattern of results, and the extent to which this may represent a species difference would require further evaluation in other mouse strains (e.g., Anismann 1976), as well as in additional dose-response analysis.

It follows that the pattern of LI disruption demonstrated in this study differs from that seen in our previous report using the CTA paradigm (Meyer et al. 2004), yet not completely so. First, Meyer et al. (2004) indicated that the abolition of CTA LI by amphetamine in mice was associated with bidirectional effects of the drug: Amphetamine enhanced conditioning in the CS–PE condition, but weakened conditioning in the nPE condition. This pattern of results has also been observed in rats (Ellenbroek et al. 1997; Russig et al. 2003). Second, there is increasing evidence that depending on the specific paradigms, the same treatment (drugs, selective brain lesions, or environmental manipulations) can result in differing patterns of LI modulation (Reilly et al. 1993; Purves et al. 1995; Gallo and Candido 1995; Buhusi et al. 1998; Schmajuk et al. 2000, 2001; Oswald et al. 2002; Schmajuk 2005; Meyer et al. 2006b; Pothuizen et al. 2006). Our data (in this paper and in Meyer et al. 2004) further indicate that this impression is not unprecedented in either rats or mice. This is also in keeping with the suggestion that distinct psychological or neural mechanisms may be involved in the regulation and modulation of LI depending on the

specific conditioning procedure used (Schmajuk et al. 2000; Schmajuk 2005). It is, therefore, important to investigate the sensitivity of LI (and by extension, the USPEE) across different associative paradigms with respect to any specific treatment (e.g., Pothuizen et al. 2006). With this approach, we are able to extend our previous finding of amphetamine on LI and the USPEE from the CTA paradigm (Meyer et al. 2004) to the present active conditioned avoidance paradigm.

Conclusion

The LI effect as well as the USPEE was absent in the amphetamine-treated mice, although the pattern of disruption differed between the two experiments. This, nonetheless, lends support to the possibility that the USPEE studied in this research may be, similar to LI, under the critical influence of the dopaminergic system. Specifically for the USPEE, amphetamine essentially abolished the pre-exposure effect significantly without affecting avoidance performance in the nPE control. Furthermore, given that the expression of the USPEE in the avoidance paradigm in this study is indicative of learned helplessness, the interpretation that amphetamine can attenuate the learned helplessness effect remains a distinct possibility; and this is consistent with suggestions of a dopaminergic modulation in its development (Besson et al. 1998, 1999; Kram et al. 2002).

This should encourage further investigations with dopamine receptor antagonists, including neuroleptic drugs, which are known to affect LI (see Weiner 1990; Moser et al. 2000; Weiner 2003). Dopaminergic transmission is involved in multiple behavioural controls including cognition, affect, motivation, reward, and attention (e.g., Mogenson et al. 1980; Wise and Rompre 1989; Gray 1998; Tzschentke 2001; Nieoullon 2002; Everitt and Robbins 2005); and its dysfunction has been implicated in a number of psychiatric disorders (Carlsson et al. 1999; Laruelle et al. 2003). Thus, neuropsychopharmacological parallelism, as well as divergence between the USPEE and LI, is of equal relevance towards a better understanding of their respective neural substrates and their potential application as animal model for specific psychiatric symptoms or diseases.

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All experiments reported in this paper comply fully with the current laws of Switzerland where they were performed.

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