DIFFERENCES IN THE BEHAVIOUR OF SPRAGUE–DAWLEY AND LEWIS RATS DURING REPEATED PASSIVE AVOIDANCE PROCEDURE: EFFECT OF AMPHETAMINE

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The present paper investigated the differences in passive avoidance learning between Sprague–Dawley and Lewis rats. After initial habituation (experimental Part 1), measured as latencies to enter the dark, preferable compartment, the effect of treatment with amphetamine (8 mg kg \(^{-1}\) b.w.), the retention performance compared with controls (saline) was tested in both rat strains in Parts 2–4. The intervals between Parts 2–4 were 24 or 49 days. Each experimental part consisted of testing lasting 6 days. On the 7th day the rats received drug treatment 1 h before the application of foot shock. The differences between rat strains were already detectable at the beginning of the study. During the repeated exposures of rats in Part 1, only Lewis rats, in contrast to Sprague–Dawley rats, exhibited the habituation. The repeated testing of rats in Parts 2–4, due to previous experience with an aversive stimulus, was considered as the retention test. In Parts 2–3 we observed only minor differences in the responses of both rat strains tested. Also no significant differences were observed between rat strains after amphetamine treatment that induced an amnesia-like effect in all retention trials. However, data shown in Part 4 revealed the largest differences between both strains. Control Lewis rats exhibited significantly higher retention responses than Sprague–Dawley rats. In the latter strain we observed no differences in avoidance latencies between controls and amphetamine treated rats. In Lewis rats the difference in avoidance performance between controls and amphetamine treated animals was highly significant due to their enhanced retention performance. In conclusion, the results presented in this study extend the known behavioural differences in tested rat strains to the passive avoidance procedure that, in addition, was performed for a total period of 4 months. Due to a known deficiency of hypothalamo-pituitary-adrenal axis activity in Lewis rats it can be hypothesized that the behavioural dissociation of this strain from Sprague–Dawley rats could be related to the different activity of this regulatory axis in the rat strains tested.

KEY WORDS: amphetamine, behaviour, habituation, passive avoidance, rat strains.

INTRODUCTION

Amphetamine (AMPH), an indirectly acting sympathomimetic drug with a potent psychostimulant action, belongs to a group of drugs with a high potential for abuse, and is one of the most frequently misused drugs. From clinical experience it is evident that there exists, in humans, an individual predisposition of humans to addiction. The exact mechanisms for the different vulnerability of some individuals to the reinforcing effects of drugs are not known. There are, however, indications that the stress-related activity of the hypothalamo-pituitary-adrenal (HPA) axis may play a role in the pathogenesis of addiction to psychostimulants [1–3].

In our previous studies we demonstrated that both AMPH and immobilization stress induced similar effects on some biochemical parameters in rats, e.g. the corticosterone and prolactin plasma levels or glutamic acid decarboxylase activity in various brain regions [4, 5]. Also, AMPH and immobilization stress in rats evoked similar changes in adenylyl cyclase activity both in myocardium and some brain structures [6–8]. In these studies we observed marked differences in responsiveness between Sprague–Dawley (S–D) and Lewis (LE) rats to both restraint stress and AMPH. These differences may be attributed to the well-documented deficit in HPA axis activity in the LE strain rats [9, 11, 12].

We deemed the investigation of the effects of AMPH on the behaviour of the S–D and LE rats to be of interest.
For our studies we have chosen the step-through passive avoidance procedure that represents a simple learning task with the use of an aversive stimulus (AS) that can also be considered as a stressor. Therefore, the different HPA axis reactivity in the two rat strains may become apparent in changed passive avoidance behaviour.

It has already been documented in several studies that a single dose of AMPH could produce long-lasting biochemical [13–15] and behavioural [13, 16–18] alterations. Therefore, we wished to estimate whether prolonged AMPH effects on passive avoidance behaviour could also be detected. For this reason, the procedure was repeated four times, and the individual experimental parts were separated by 1 month intervals.

AMPH was administered in the first three acquisition trials, always 1 h before AS delivery. This time interval and the selected dose were used to imitate the design of our biochemical experiments in which the similarities of immobilization stress and AMPH effects were observed [19]. The final part of the study was performed after a time period of 49 days in order to check for possible long-lasting behavioural consequences of both the repeated passive avoidance procedures with the foot-shock delivery and the effects of AMPH.

MATERIALS AND METHODS

Animals

Thirteen male Sprague–Dawley (S–D) and 14 male Lewis rats (LE) (Charles-River Laboratories, Sulzfeld, Germany), with starting body weights 185–220 g, were used. Animals had free access to a standard pellet food and water. Rats were housed in a box for storage of SPF animals (FluFrance, Vissous, France) with controlled light regime (12L:12D cycle), temperature (21 ± 1°C), relative humidity (50–70%) and air pressure. Behavioural tests were performed from 8 a.m. to 1 p.m. Treatment of animals was in accordance with the Declaration of Helsinki Guiding Principles on Care and Use of Animals (DHEW Publication, NHI 80-23).

Apparatus

The shuttle-cage (Coulbourn Instruments Inc., Pennsylvania, USA) consists of two communicating compartments of equal size (26 cm × 26 cm) that are separated by a sliding door (8 cm × 8 cm). One compartment is illuminated, the other one (shock chamber) is dark. A stainless steel bar floor is used for application of foot-shock.

Step-through passive avoidance

The study started with six consecutive trials during which the animals must have reached the following criterion: latencies to enter the dark compartment shorter than 30 s. In each trial the rat was placed in the illuminated compartment for 50 s. After this interval the sliding door was raised so that the rat could pass through the entrance to the dark compartment. Rats that failed to enter within 180 s were gently moved into the dark chamber by the experimenter, and this value was considered as cut-off latency. On the 7th day rats of each strain (n = 13 for S–D, n = 14 for LE) were divided at random into two groups; one group received i.p. injection of saline, 1 ml kg⁻¹ of b.w., the other one amphetamine, 8 mg kg⁻¹ of b.w. (amphetamine sulphate, Sigma, USA). One hour later, an acquisition trial was performed: when the animal entered the dark compartment, the door was closed and a 0.3 mA foot-shock, lasting 1 s, was applied through the grid floor; this completed Part 1 of the study.

After 24 days, Part 2 commenced. It consisted of six sessions identical to those of Part 1, ending again with a shock trial combined with saline or AMPH administration. The procedure was then repeated in Part 3, starting on day 61, and was finished in Part 4, starting on day 116.

Data collection and analysis

Experimental data were recorded by WinLinc software (Coulbourn Instruments Inc., Pennsylvania, USA) that enables us to both design experimental protocols for passive avoidance testing and to process experimental data.

All statistical tests were conducted using the Systat 9 software (SPSS, Inc., Chicago, Illinois, USA). Data were analysed non-parametrically. First, to compare the differences during training days the Friedman analysis of variance (ANOVA) was used. Second, to compare the differences between saline and AMPH groups during the 1st and during the 6th day the Mann–Whitney test was used. Third, to compare the differences within groups between the 6th (last) day of each part of the study and the 1st day of the subsequent part the Wilcoxon matched-pairs signed ranks test was used. The criterion for statistical significance was P < 0.05 (two-tailed).

RESULTS

Figure 1 summarizes the obtained latencies (in seconds) of two rat strains in the passive avoidance task. Out of 27 rats used in the study three animals died during the experiment: one S–D and two LE rats, all treated with AMPH.

Part 1: There were clear differences in behaviour of the two strains. First, LE rats showed higher initial (1st day) latencies than S–D rats (χ² = 10.26, d.f. 1, P = 0.001) and lower latencies on the 6th day (χ² = 3.83, d.f. 1, P = 0.005). Also, a significant difference between the 1st and 6th day latencies was found in LE rats only (z = 3.30, d.f. 1, P = 0.001). These findings indicate that only LE rats displayed habituation to the novel environment assessed by the speed of passing to the dark compartment. This was confirmed by an overall analysis of repeated measurements, which showed a significant difference in LE (F = 39.47, d.f. 5, P < 0.0001) but not in S–D rats (F = 10.24, d.f. 5, P = 0.069).
Fig. 1. Comparison of the behaviour of Sprague-Dawley (S–D) and Lewis (LE) rats in the passive avoidance situation; the effect of amphetamine treatment. Each part (1–4) consists of 6 testing days. In Parts 1–3 AMPH or saline (T = treatment) were administered on the 7th day, and the aversive stimulus (AS) followed 60 min later. Data on latencies are expressed as the mean ± SEM values. Statistical significances are given in the results.

Part 1—habituation of untreated rats (open symbols with solid lines; \(n = 13\) for S–D, \(n = 14\) for LE). Parts 2–4—retention testing (controls—open symbols with dashed lines; \(n = 7\); amphetamine treated—filled symbols with solid lines; \(n = 5–6\)).
The behaviour of rats in pre-shock trials of Part 2–4 can be considered as reflecting delayed retention of both the exposure to the apparatus during the first six sessions, and the subsequent shock trial.

Part 2: The increase in latencies of the 1st day compared with the 6th day of Part 1 was only marginal, reaching significance in LE rats treated with AMPH \((z = 2.02, \text{d.f.} 1, P = 0.043)\). A significant difference between the 1st and 6th day latencies of Part 2 was found in the control S–D group \((z = 2.37, \text{d.f.} 1, P = 0.018)\), and the AMPH group of LE strain \((z = 2.20, \text{d.f.} 1, P = 0.028)\). In agreement with these results, an overall analysis of repeated measurements revealed a significant decline in latencies in control S–D rats \((F = 14.49, \text{d.f.} 5, P = 0.013)\) and in AMPH group of LE rats \((F = 15.95, \text{d.f.} 5, P = 0.007)\). There was no difference between AMPH and control groups in either strain.

Part 3: Following the second shock delivery the latencies of the 1st day increased in comparison with the 6th day of Part 2 in control S–D \((z = 2.37, \text{d.f.} 1, P = 0.018)\) and control LE rats \((z = 2.37, \text{d.f.} 1, P = 0.018)\) but not in AMPH treated animals \((z = 1.48, \text{d.f.} 1, P = 0.14)\), for LE: \(z = 1.83, \text{d.f.} 1, P = 0.068\). The overall analysis of repeated testing showed no significant difference between the groups \((F = 6.04, P = 0.30)\) for S–D AMPH: \(F = 8.17, P = 0.15\) for LE saline: \(F = 10.49, P = 0.06\) for LE AMPH: \(F = 8.02, P = 0.16\) always d.f. 5). Nevertheless, the comparison of latencies measured on the 1st and 6th day revealed a significant difference in LE saline rats \((z = 2.20, \text{d.f.} 1, P = 0.028)\). As for the difference between the control and AMPH groups, significantly longer latencies were found in LE rats during the 1st day \((z = 4.81, \text{d.f.} 1, P = 0.028)\) but not in S–D rats \((z = 2.37, \text{d.f.} 1, P = 0.12)\). No significant difference between the controls and AMPH treated rats during the 6th day was found in either S–D or LE animals.

Part 4: Some further differences between the strains were observed. First, control LE rats exhibited significantly longer latencies on the 1st day as compared with those measured during the 6th day of Part 3 \((z = 2.37, \text{d.f.} 1, P = 0.018)\). Second, the difference between the values of LE and S–D saline treated rats observed on the 1st day was close to significance \((z = 3.76, \text{d.f.} 1, P = 0.0053)\). No difference was found in AMPH treated animals \((z = 0.70, \text{d.f.} 1, P = 0.40)\). However, the average value in control animals obtained over all 6 days was significantly higher in LE rats \((z = 4.46, \text{d.f.} 1, P = 0.035)\). Third, a difference between the control and AMPH groups was found in LE rats only, both on the 1st day \((z = 7.28, \text{d.f.} 1, P = 0.007)\) and on the 6th day \((z = 4.88, \text{d.f.} 1, P = 0.027)\). These differences in latencies remained stable both in the control and AMPH treated animals over Part 4 (for S–D saline: \(F = 3.10, P = 0.68\); for S–D AMPH: \(F = 0.49, P = 0.99\); for LE saline: \(F = 4.22, P = 0.52\); for LE AMPH: \(F = 5.54, P = 0.35\); always d.f. 5).

DISCUSSION

Differences in behaviour of the two rat strains, S–D vs LE, could be observed during the first 6 days of the experiment. In the initial part of the study the latency to enter the dark, and therefore preferable, compartment in days preceding the acquisition trial was considered to represent a simple but usable measure of the animal’s adaptation to the apparatus, and was used to express habituation abilities of the studied rat strains. In general, this habituation potency is termed as the simplest form of learning and memory [20–22]. LE rats showed significantly longer initial latencies than S–D rats and also only exhibited habituation, i.e. a gradual decline in latencies over the 6 days. Differences in behaviour in various experimental situations have been reported between LE rats and several other rat strains [23–26]; also in tests studying exploratory behaviour in a novel environment. For example, in the open field procedure LE rats were more active in the periphery of the arena than Fischer rats and they also spent less time in grooming and produced fewer faecal boli [9, 10].

Considering the learning of the passive avoidance task, control animals of both strains behaved in a more or less similar way during the retention testing of Parts 2 and 3. The intensity of the used foot-shock was comparatively mild, so that retention testing may disclose even less pronounced differences in the learning ability of the two strains. Another factor that may contribute to the sensitivity of the experimental paradigm in the dissociation of learning and memory processes between the strains is the long time periods between the acquisition- and retention-trials. As a consequence, the first retention test (Part 2) showed a not very marked increase in avoidance of the shock-compartment in either strain. Only following the second acquisition trial was there significant increase in retention latencies (Part 3) to a similar extent in both strains. A striking difference between the strains was observed in the final part of the experiment. Compared with S–D strain, LE rats exhibited much longer latencies without any indication of adaptation throughout the testing period. We assume that this behaviour could be explained in terms of firm and enduring memory traces. In contrast, S–D rats showed similar avoidance behaviour in these tests as in the preceding retention trials.

AMPH affected the behaviour of the two strains in a similar way. There were only minor differences in retention testing of AMPH groups between S–D and LE rats in Part 2. A similar picture emerged during Part 3 trials where marked dissociation between control and AMPH groups was caused by an increase of avoidance latencies in control animals. However, this dissociation only reached a significant level in LE rats. The behaviour of S–D and LE rats dissociated strongly in Part 4. While in S–D rats the difference between controls and the drug treated group almost disappeared, in LE rats the difference increased due to very high values of latencies in con-
trol animals. These results confirm the observations that LE rats, compared to several other strains—in the present study with S–D strain—behaved in a different way in several experimental situations [10–13]. Our findings extend these differences to the passive avoidance learning task. To what extent the behavioural modifications could be related to the known deficits of HPA axis activity remains to be investigated. Also the question of a possible relation of the habituation shown by LE rats in the first part of the study and the high level of avoidance latencies in the third, and especially in the final part, cannot be answered on the basis of data achieved in the present experiment.

In general, the present results indicate that AMPH treatment induced an efficient and lasting amnesia-like effect. There are several explanations for the amnesia-inducing action. A comparatively high dose of AMPH induces stereotyped behaviour [3] that can affect several factors important for learning and retention of the passive avoidance situation. Among the likely ways in which learning the task could have been influenced are sensory motor disturbances precluding the correct use of visual and spatial cues, changed sensitivity and responsiveness to foot-shock, modified arousal or attention, and perseverative stereotyped movements. A more direct effect on memory traces or the possibility of a state-dependent learning should also be considered. Further experiments are needed to investigate the mechanisms responsible for the amnesia-like effect of AMPH when administered in a single dose before the acquisition trials.

In our previous biochemical studies we observed some similarities between the effects of restraint stress and AMPH treatment [4–8]. In addition, in a behavioural study (unpublished observation) performed under identical experimental conditions as the present one, immobilization stress produced similar amnesia-like action as reported here for AMPH. As a consequence, for the interpretation of our results, we could consider the possibility that immobilization stress and AMPH may share some of the mechanisms participating in producing amnesia-like effects in the passive avoidance learning test.

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