Research report

Impaired passive avoidance acquisition in Sprague–Dawley and Lewis rats after restraint and cold stress

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Abstract

The study examined the effects of restraint combined with cold water stress (IMO+C) on learning and memory of Sprague–Dawley (S–D) and Lewis (LE) rats in the passive avoidance task. The procedure started with 6 days of adaptation to the apparatus during which the recorded latencies to enter the dark compartment were used to assess the process of habituation. On the training day rats were exposed to IMO+C for 60 min and the stressor exposure terminated 1 h before the acquisition trial. Retention trials started 24 h later. To evaluate the possible long-term consequences of the acute and repeated stress presentation on the performance of the two strains with diverse activity of hypothalamic–pituitary–adrenal axis, this procedure was performed three times including stress application (Parts 1–3). Finally, an identical procedure was performed without stress (Part 4). An immediate behavioural effect of the stressor exposure was observed in an increase of latencies to enter the dark compartment before the shock delivery in rats of both strains; this enhancement approached significance after the second and third exposure to the stressor (Parts 2 and 3). Control animals of both strains acquired passive avoidance response after training in Parts 2–4. IMO+C produced significant impairment of this response irrespective of the strain. The three-time repeated exposure did not influence the ability to learn the task in the final procedure without stress. Differences in behaviour of S–D and LE rats were observed already during the first adaptation period. LE rats exhibited longer latencies upon the first exposure to the novel environment compared to S–D rats. Also only LE rats displayed habituation. In Part 4 marked strain differences in the latencies both before and after training were recorded. The results show that the repeated exposure to the IMO+C stressor proved to be a strong amnesic stimulus but without persisting consequences for the ability of rats to acquire the learning task. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Learning and memory; Lewis and Sprague–Dawley rats; Passive avoidance; Restraint and cold stress

1. Introduction

Repeated exposure to stress has been shown to induce an increase of behavioural effects of psychostimulants such as amphetamine and cocaine [1,28,30]. Also, data from animal models of drug self-administration indicate that stress can increase drug-seeking behaviour and reinstate drug-taking after prolonged drug-free periods [31]. These and other observations suggest that psychostimulants and stressors have similar neurobiological effects.

In our previous studies we have demonstrated that restraint stress induced effects comparable to those of amphetamine on several biochemical parameters in rats, e.g. the corticosterone and prolactin plasma levels, or glutamic acid decarboxylase and adenylyl cyclase activity in various brain regions [14–18]. We also found significant differences in responsiveness between Sprague–Dawley (S–D) and Lewis (LE) rats both to restraint stress and amphetamine [15,16]. These differences may be related to the well-documented deficit in hypothalamic–pituitary–adrenal (HPA) axis activity in the LE rats, found in comparison with several other strains including S–D [5–8,33,36]. Therefore, we used these two strains also in behavioural experiments designed to examine the effect of stress and psychostimulant drugs on learning and memory functions. In our previous experiments a relatively high dose of amphetamine (8 mg/kg) and exposure to restraint or restraint
combined with water immersion impaired learning and memory of rats in both active and passive avoidance paradigms. We also observed differences in learning and memory performance between S–D, LE and Wistar rats [11,12,21].

The present study was designed to examine the effects of the stressor consisting of restraint combined with water immersion on performance of LE and S–D rats in the passive avoidance learning task under similar conditions as employed in the experiments where learning impairment occurred after amphetamine [11]. In order to investigate the possible long-term effects of acute and repeated exposure to a strong stressor, the passive avoidance procedure was performed four times, with long time intervals between individual parts.

2. Materials and methods

2.1. Animals

In the study 12 male S–D and 13 male LE rats (Charles-River Laboratories, Sulzfeld, Germany) with average starting body weight 198 and 216 g, respectively, were used. Animals had free access to a standard pellet food and water. Rats were housed five per cage (42 × 26 cm²) and maintained on a standard 12 h light/12 h dark cycle, at a constant temperature (21 ± 1 °C) and relative humidity (50–70%). Training and testing were performed between 8:00 and 13:00 h. Treatment of animals was in accordance with the Declaration of Helsinki Guiding Principles on Care and Use of Animals [DHEW Publication, NHI 80-23].

2.2. Treatment

During the exposure to restraint stress combined with water immersion (IMO + C), rats were immobilized by fixing the front and hind legs of the rat with adhesive plaster with mull inside to a plaster with mull inside to a

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During the exposure to restraint stress combined with water immersion (IMO + C), rats were immobilized by fixing the front and hind legs of the rat with adhesive plaster with mull inside to avoid pain; then the animal was restrained in a snug-fitting vertical wire-mesh. This mesh was bent to conform to the size of individual animal and a bandage fixed this shape of mesh. The restrained rats were immersed in the water bath (22 °C) in such a way that the upper 1/4 of the rat was outside of water [13]. After stressor exposure for 60 min rats were returned to the home cage.

2.3. Apparatus

A shuttle-cage (Coulbourn Instruments Inc., PA) consists of two communicating compartments of equal size (26 × 26 cm²), separated by a sliding door (8 × 8 cm²). The starting compartment was illuminated and the shock compartment was dark. A stainless steel bar floor was used for delivery of scrambled constant current. WinLinc software was used for designing passive avoidance testing and to process experimental data.

2.4. Procedure

One pre-training trial was performed daily on 6 consecutive days (pre-acquisition phase—PreAcq). The rat was placed in the illuminated starting compartment for 50 s. After this interval the sliding door was raised and the latency to enter the dark compartment was recorded. On the seventh day rats of each strain were divided at random into controls (7 S–D and 7 LE) and subjects exposed to IMO + C (5 S–D and 6 LE). The stressor exposure terminated 60 min before training. Control animals received no treatment.

During the acquisition trial the subjects were placed in the illuminated compartment of the apparatus as in the previous sessions and the latency to enter the shock compartment was recorded. The door was then closed and the aversive stimulus (AS) consisting of a footshock (0.3 mA, 1 s) was delivered. The rat was removed from the dark compartment 1 min after termination of the footshock.

Retention tests started 24 h after the acquisition trial. Each animal was again placed in the illuminated compartment and the latency to enter the dark box (retention latency) was recorded with a 180 s ceiling. Retention trial was repeated for further 8 days (post-acquisition phase), thus completing Part 1 of the study. During the following 2 weeks rats were left undisturbed.

Part 2 started on day 31 of the study. It consisted again of 6 sessions preceding acquisition trial (PreAcq); on the 7th day a shock trial followed, combined with the same treatment as in Part 1. Retention testing lasted 9 days. Similar procedure was then performed in Part 3 that started on day 61. In these experiments the rats exposed to stressor did not enter the dark chamber within 180 s and had to be moved through the entrance to the shock compartment by the experimenter. Part 4 started on day 122 and differed from the preceding parts by the absence of stressor exposure. The design of the study is summarised in Fig. 1.

2.5. Data collection and analysis

Data were analysed non-parametrically (due to the small number of animals in the groups and the cut-off time of latency performance). First, to compare the difference within groups during both all PreAcq phases (always df 5) and all retention phases (always df 8) the Friedman analysis of variance (ANOVA) followed by Dunn’s method was used. Second, to compare the differences between the control and IMO + C groups within a given strain the Mann—Whitney U-test (always df 1) was used. So, a comparison of latencies measured in (a) the first PreAcq trial (i.e. Day 1, 31, 61 and 116),
performed (T). One hour later the acquisition trial (Acq) was performed. On Day 7, 37 and 67 the rats were exposed to IMO continued for eight subsequent days (Retention). Individual parts were separated by 14 days (Pause). Part 4 started 39 days after the end of the previous retention testing; animals were not exposed to IMO+C.

(b) the first retention trial (i.e. Day 8, 38, 68 and 123), and (c) immediately before the footshock delivery (i.e. Day 7, 37, 67 and 122) were done. Third, to compare the difference between the strains within a particular group the average latency values obtained over all nine retention trials were calculated and then analysed using the Mann–Whitney U-test. The criterion for statistical significance was $P < 0.05$ (two-tailed).

3. Results

Fig. 2 summarises the results from passive avoidance testing obtained during experimental Parts 1–4 in S–D and LE rats.

3.1. Part 1

On the first exposure to the apparatus, LE rats displayed a significantly longer mean latency compared to S–D ones ($n = 13$ and $n = 12$, respectively, $x^2 = 9.5$, $P = 0.002$). A significant decrease in the latency during the PreAcq phase was found in LE rats ($x^2 = 35.4$, $P < 0.001$) but not in S–D ones ($x^2 = 9.8$, $P = 0.08$). On the first retention day, a significantly prolonged latency had the control S–D rats as compared to IMO+C ones ($x^2 = 7.8$, $P = 0.005$); no difference was found in LE rats ($x^2 = 0.1$, $P = 0.72$). Further, there was a significant decrease in the latency during the retention phase in IMO+C treated LE rats ($x^2 = 19.4$, $P = 0.013$) but not in the control LE rats ($x^2 = 4.8$, $P = 0.8$) as well as in S–D rats (for control group: $x^2 = 12.0$, $P = 0.2$; for IMO+C group: $x^2 = 10.9$, $P = 0.2$). Finally, the control S–D rats had significantly higher average latency as compared to that in LE ones ($x^2 = 4.5$, $P = 0.03$); no difference was found in IMO+C rats between S–D and LE strains ($x^2 = 0.0$, $P = 1.0$).

3.2. Part 2

In the first day of the second PreAcq phase no difference between the control and IMO+C groups was found in both LE ($x^2 = 0.7$, $P = 0.4$) and S–D ($x^2 = 1.9$, $P = 0.2$) strain. Repeated measurements in LE rats during the PreAcq phase revealed a significant decrease in the latency in IMO+C rats ($x^2 = 17.0$, $P = 0.005$) but not in the controls ($x^2 = 4.7$, $P = 0.5$); in the S–D strain, a significant decrease was found in the controls ($x^2 = 14.5$, $P = 0.01$) but not in IMO+C rats ($x^2 = 7.5$, $P = 0.2$). On the first retention trial, the controls of both LE and S–D strains had significantly prolonged latencies as compared to IMO+C ones (for LE: $x^2 = 9.1$, $P = 0.003$; for S–D: $x^2 = 6.3$, $P = 0.004$). The Friedman analysis revealed a significant decrease in the latency during the retention phase in the control group ($x^2 = 21.3$, $P = 0.006$) but not in IMO+C group ($x^2 = 7.0$, $P = 0.5$) of LE rats; on the contrary, in S–D rats a significant decrease in the latencies was found in IMO+C group ($x^2 = 17.7$, $P = 0.03$) but not in the control group ($x^2 = 13.8$, $P = 0.09$). As to the average latency calculated in the retention phase no difference between the strains was found within particular groups (for controls: $x^2 = 0.8$, $P = 0.4$; for IMO+C: $x^2 = 0.0$, $P = 1.0$).

3.3. Part 3

There was a significant difference on the first PreAcq day between the controls and the IMO+C rats in both LE ($x^2 = 7.8$, $P = 0.008$) and S–D ($x^2 = 5.2$, $P = 0.02$) strains. During the PreAcq phase, no significant change in the latency was found (for control S–D group: $x^2 = 6.0$, $P = 0.3$; for IMO+C S–D group: $x^2 = 10.7$, $P = 0.06$; for control LE group: $x^2 = 10.5$, $P = 0.06$; for IMO+C LE group: $x^2 = 4.5$, $P = 0.5$). On the first retention trial a significant difference between groups was revealed (for LE rats: $x^2 = 9.1$, $P = 0.003$; for S–D rats: $x^2 = 5.6$, $P = 0.02$). Specifically, the latency of control groups was significantly longer than that of IMO+C groups. The overall analysis of repeated measurements during the retention phase showed a significant decline in the latency of the controls in both strains (for LE rats: $x^2 = 23.6$, $P = 0.003$; for S–D rats: $x^2 = 21.3$, $P = 0.006$) but there was no change in the latency of both IMO+C treated animals (for LE rats: $x^2 = 8.7$, $P = 0.4$; for S–D rats: $x^2 = 12.2$, $P = 0.14$). Finally, the control LE rats had significantly higher average latency than S–D ones ($x^2 = 3.9$, $P = 0.04$); no difference between both strains was found in IMO+C treated rats ($x^2 = 0.8$, $P = 0.4$).
3.4. Part 4

On the first PreAcq day, a significantly higher latency was found in the control LE rats as compared to IMO/C27/C treated ones ($\chi^2 = 6.0, P = 0.03$); no difference was found in S–D rats ($\chi^2 = 0.7, P = 0.4$). During the PreAcq phase, no change in the latency was found in both S–D strain (for controls: $\chi^2 = 3.1, P = 0.7$; for IMO+C: $\chi^2 = 8.2, P = 0.1$) and LE strain (for controls: $\chi^2 = 4.2, P = 0.5$; for IMO+C: $\chi^2 = 0.8, P = 0.9$). No
difference between groups of both strains was found on the first retention trial (for LE: \( \chi^2 = 0.9, P = 0.4 \); for S–D: \( \chi^2 = 1.9, P = 0.2 \)). During the retention phase the latency remained stable and very high in both groups of S–D strain (for controls: \( \chi^2 = 13.7, P = 0.09 \); for IMO+C: \( \chi^2 = 7.5, P = 0.5 \); a significant decrease of the latency was found in LE rats originally subjected to IMO+C (\( \chi^2 = 20.0, P = 0.01 \)) but not in the controls (\( \chi^2 = 1.3, P = 1.0 \)). While the control LE rats had a significantly higher average latency than the control S–D ones (\( \chi^2 = 4.0, P = 0.04 \)), no difference was found between strains in IMO+C rats (\( \chi^2 = 0.5, P = 0.5 \)).

In Part 4 also a significant difference between groups was found in LE strain (\( \chi^2 = 8.2, P = 0.004 \)) but not in S–D strain (\( \chi^2 = 0.8, P = 0.4 \)).
3.5. IMO+C effects on latencies to enter the shock compartment before the footshock delivery

Fig. 3 shows the effect of IMO+C on latencies to enter the shock compartment before the footshock delivery. A comparison of these latencies between the controls and stressed rats measured in Part 1, did not reveal any difference (for S–D: \(x^2 = 0.17, P = 0.7\); for LE: \(x^2 = 2.1, P = 0.2\)). However, the stressed animals exhibited significantly longer latencies compared to
controls in Part 2 (for S–D: $x^2 = 7.2, P = 0.007$; for LE: $x^2 = 9.1, P = 0.003$) and in Part 3 (for S–D: $x^2 = 10.0, P = 0.002$; for LE: $x^2 = 6.6, P = 0.01$).

4. Discussion

The present findings on impaired passive avoidance learning in S–D and LE rats after IMO+C can be summarized as follows. First, IMO+C administered before the acquisition trial impaired passive avoidance response learning to the degree that can be considered as amnesia. Second, most differences between the strains recorded in control animals were abolished by the amnestic effect of the stressor. Third, the three-time repeated procedure with stressor application did not impair the ability of rats to learn the task in the fourth set of experiments performed without treatment. In addition, the latencies exhibited by the stressed animals in the acquisition trial before the AS application increased stepwise from the first to the third stressor exposure.

The avoidance latencies of all control animals increased significantly after the second and the following acquisition trials. IMO+C produced strong and lasting impairment of passive avoidance performance in both S–D and LE rats. Impaired learning and memory in stressed animals have been demonstrated by many studies despite the heterogeneity of restraint and/or cold stress and of the used learning task (e.g. [3,26,27,34]). However, also facilitation of learning and memory has been reported [23,37]. In search for hormones and other endogenous substances involved in stress-induced influences on learning and memory formation, attention has been focused on HPA axis activity. For example, passive avoidance performance in rats has been shown to depend upon optimal levels of ACTH, corticosteroids and adrenomedullar catecholamines; vasopressin and oxytocin influence inhibitory learning as well [2,19,20,35]. Epinephrine, the primary product of adrenal medulla, has been demonstrated to be a potent modulator of memory in the passive avoidance paradigm. Higher than optimal dose of epinephrine may produce retrograde amnesia [24]. Corticotropin releasing hormone influences behaviour in various experimental situations [4,33,36]. Influence of IMO+C (and amphetamine) on some of these parameters was estimated in our biochemical studies, as mentioned in Section 1, but because the experimental conditions were not identical with those occurring during the passive avoidance procedure used, the findings cannot be directly related to the behavioural experiments.

The diverse HPA activity of S–D and LE rats may participate in the behavioural differences observed in the present study. Generally, LE rats displayed a smaller HPA reaction to a wide range of stressors compared to several other rat strains including S–D rats. [5–8,33,36]. We could observe differences in behaviour already during the first 6 days of the study when adaptation to the new environment took place and the declining latencies to enter the preferable dark compartment may thus reflect the habituation ability of the rats [9,22]. LE rats exhibited significantly longer latencies on the first exposure to the apparatus compared to S–D rats and also only LE rats displayed habituation. Further differences between the strains were registered between the stressed and control groups within the same strain; e.g. the latency of S–D control animals was longer compared to that of IMO+C group in the first retention test of Part 1, whereas no differences between groups occurred in LE strain. More marked differences between strains were recorded during the latter parts of the study. In Part 3 the avoidance latency of control LE animals remained higher during retention testing than those of S–D rats. Higher avoidance response in LE rats emerged also in Part 4: the latencies registered during the PreAcq sessions were higher in LE controls compared to IMO+C group, whereas no such difference was displayed by S–D strain. Finally, the control LE rats exhibited higher average latencies than S–D rats during the final retention testing. The results suggest that a tendency towards higher avoidance response appeared in LE rats during the later phases of the study. This behaviour may reflect a more cautious strategy adopted by this strain in coping with experimental situation. The question, if a more enduring memory of the AS participated in a stronger avoidance behaviour, remains to be further examined. Alternatively, the persisting avoidance response may indicate the absence of processes facilitating extinction of behaviour that is no longer relevant; corticoid hormones have been suggested to operate in adaptive behaviour that is most relevant to the situation [20]. Yet another feature of LE rat’s behaviour may be responsible for the observed difference in comparison with S–D animals. LE rats have been shown to display more avoidance of the aversive stimuli in models for testing anxiety in animals, like elevated plus maze or open field, in comparison with several other strains [29]. The three-time repeated footshock stressor applied in our study may thus have elicited apprehension not only of the shock compartment, but also of the apparatus as a whole, which in turn could result in a transient freezing reaction (fear to initiate the movement) [25]. However, there have been several contradictory findings of LE rat’s behaviour reported in relation to the anxiety, mostly in comparison with Fisher 344 strain [33,36]. Obviously, the type and duration of the stressor as well as of the testing conditions play a decisive role in the relative responsiveness of the different strains. In the present experiments the intensity of the IMO+C
stressor was apparently sufficient to remove most of diversity between the used strains.

Despite the strong amnestic effect of IMO+CS, the stressor-exposed rats of both strains displayed a progressively increasing avoidance response after being transferred to the lighted compartment of the apparatus during the acquisition trial. The first exposure to IMO+CS did not produce a discernible alteration of behaviour in animals during the acquisition trial. However, in the second training, rats of both strains did not enter the dark chamber as readily as during the first retention or second training, rats of both strains did not enter the dark chamber during training further increased after the third IMO+CS; the latencies reached the 180 s ceiling and animals had to be moved into the dark chamber by the experimenter. The increasing inhibitory response during the training session is in sharp contrast with the amnesia recorded in subsequent retention testing. Locomotor impairment of stressed animals was unlikely to have been a major cause of the enhanced avoidance latency: there were no visible behavioural alteration observed and, in addition, it is unlikely that the physical insult would grow from the first to the third stressor exposure when the interval between them was 1 month. As a more plausible interpretation appears to be the occurrence of state-dependent learning. This hypothesis suggests that internal state of the subject elicited by a drug or, presumably, by endogenous substances released by the stressor, should be similar during the acquisition and retention trials. Different internal state at training and testing has been considered to be responsible for retrieval failure manifested as memory loss [10,32]. According to this notion we may hypothesise that the presence of interoceptive contextual cues evoked by IMO+CS during acquisition precluded the retrieval of the avoidance response because the internal state of the organism during testing did not match that occurring at training. This may explain why significant avoidance response was seen only during the second and third acquisition trial when animals were under the influence of the stressor, but not during retention testing that started 24 h later.

Part 4 was performed in order to examine whether the previous repeated exposures to stressor would influence the behaviour of rats in the passive avoidance procedure. However, although IMO+CS proved to be a strong amnestic stimulus, it was without consequences for the ability of rats to acquire the learning task.

The present results confirmed that differences between S–D and LE rats previously found in our biochemical and behavioural studies can be extended to passive avoidance learning task. Also, the combined restraint and cold stressor induced a significant amnestic effect comparable to that produced by amphetamine in a similar experimental situation [11]. These results offer possibility to investigate the interaction of stress and psychoactive drugs in affecting cognitive functions.

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