

Short Communication

Enhancement of Latent Inhibition by Chronic Mild Stress in Rats Submitted to Emotional Response Conditioning

Liana Lins Melo^{1,2}, Elenice A. de Moraes Ferrari¹, Nancy Airoidi Teixeira³, and Guy Sandner⁴

¹Laboratório de Sistemas Neurais e Comportamento, Departamento de Fisiologia e Biofísica, IB, Universidade Estadual de Campinas, Cidade Universitária Prof. Zeferino Vaz, 13083-970 Campinas, SP, Brasil; ²Unidade Acadêmica da Área de Ciências Biológicas e da Saúde, Universidade São Francisco, Av. São Francisco de Assis, 12916-900, Bragança Paulista, SP, Brasil; ³Departamento de Farmacologia, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Cidade Universitária Prof. Zeferino Vaz, 13083-970 Campinas, SP, Brasil; ⁴Laboratoire de Psychopathologie et de Psychopharmacologie de la Cognition, U 405 INSERM, Institut de Physiologie, Faculté de Médecine, Université Louis Pasteur, 11 rue Humann F-67085 Strasbourg Cédex, France

ABSTRACT

This work evaluated the influence of chronic mild stress on latent inhibition (LI) in rats, using a conditioned emotional response (CER) procedure. Rats were assigned to four groups: a non pre-exposed control group (NPC), a non pre-exposed stressed group (NPS), a pre-exposed control group (PC), and a pre-exposed stressed group (PS). Stressed animals were submitted to a chronic mild stress (CMS) regimen for three weeks. The off-baseline conditioned emotional response procedure had four phases: licking response training, tone-shock conditioning, retraining, and testing. Conditioning consisted of 2 tone (30 s) and shock (0.5 s) associations. Tone-shock conditioning evidenced by NPS and NPC groups suggests that stress did not interfere with the expression of a conditioned emotional response.

Pre-exposure was carried out using 6 tones (30 s) during 2 sessions before conditioning. Prior exposure to the tone resulted in a decrease in learning that was greater in stressed animals. The results indicate an increase in latent inhibition induced by chronic mild stress. Such LI potentiation after CMS may be related to dopamine (DA) neurotransmission reduction in the central nervous system.

KEYWORDS

tone pre-exposure; aversive learning; behavioral emotion; freezing; dopamine neurotransmission, suppression

INTRODUCTION

Latent inhibition (LI) refers to the procedure, non-reinforced pre-exposure to a stimulus, and to the behavioral outcome, characterized by a delay

Reprint requests to: Liana Lins Melo, Departamento de Fisiologia e Biofísica, IB, Universidade Estadual de Campinas, Cidade Universitária Prof. Zeferino Vaz, 13083-970 Campinas, SP, Brasil. Phone: 55 021 19 3788 62 05; e-mail: lianamelo@aol.com

in the establishment of a conditioned response to that stimulus. The term can also refer to the process underlying the delay in the establishment of a conditioned response. Initially considered within the framework of classical learning theory, LI has also been related to attention dysfunctions, such as those in the animal-model of schizophrenia, as well as in human patients with Parkinson and schizophrenia and in hyperactive children, (for reviews, see Gray et al., 1991; Lubow & Gewirtz, 1995; Lubow, 1997). Latent inhibition can be related to dopaminergic function, as indicated by several reports highlighting a critical role for the dopaminergic system. In fact, LI can be disrupted by the presence of the dopamine (DA) releasing amphetamine and enhanced by that of the dopamine blocking haloperidol (for review, see Weiner & Feldon, 1997). Both effects can be observed when such compounds are administered, either systemically or directly into the nucleus accumbens (Gray et al., 1995; Warburton et al., 1994; Weiner et al., 1987; Weiner et al., 1988, for review see Gray et al., 1997). In fact, this body of research provides a strong evidential basis for the conclusion that DA transmission in this nucleus plays a key role in regulating LI.

Abnormalities of DA transmission in the nucleus accumbens have been related to chronic mild stress (CMS) and the animal's decreased sensitivity to reward (Stamford et al., 1991; Willner, 1991; Papp et al., 1993). In fact, antidepressant-reversible reduced sensitivity to reward resulting from a chronic mild stress regimen has been investigated using various behavioral procedures, such as a reduced preference for sucrose solutions (Willner et al., 1987; Muscat et al., 1990), attenuation of place preference conditioning (Papp et al., 1991) and reduction of intracranial self-stimulation behavior (Moreau et al., 1994; Moreau et al., 1994; Moreau et al., 1992). Taken together, these findings suggest that the neural mechanisms underlying chronic mild

stress may share important similarities with those involved in the behavioral characteristics observed in patients showing psychopathologic symptoms. Therefore, chronic mild stress can provide a useful nonpharmacologic tool for interference in dopaminergic transmission and, consequently, the analysis of behavioral aspects related to emotion and cognition.

The hypothesis that LI is disrupted by increased dopaminergic transmission suggests that it should be potentiated by a decrease in such transmission (Gray et al., 1997). Therefore, if CMS decreases transmission, it may enhance LI. This research was thus designed to investigate the effects of CMS on the LI phenomenon in rats using a conditioned emotional response (CER) paradigm based on behavioral suppression. A CER can result in the suppression of ongoing behaviors upon presentation of a sound previously paired with foot shocks. The conditioned function of the sound is assessed an evaluation of the suppression of the ongoing operant activity—as for example the suppression of drinking in a thirsty rat upon the presentation of a tone alone. Conditioned emotional response has been shown to be sensitive to latent inhibition (Sotty et al., 1996; Melo et al., 1997; Lacroix et al., 2000).

EXPERIMENTAL

Animals

Subjects were 36 male Wistar rats weighing 250-300 g purchased from Centro de Bioterismo - UNICAMP. The rats were housed 5 to a cage in a temperature-controlled room (25 C) under a 12/12 h light cycle (lights on at 07:00 h). Food and water were available *ad libitum* in the home cages until the beginning of the experimental procedure. Weight was checked daily throughout the experi-

ments. The rats were handled according to international recommendations for animal welfare and standards of the *Sociedade Brasileira de Neurociências e Comportamento*.

Experimental design

The subjects were randomly assigned to one of 4 groups: a non pre-exposed control group (NPC), a non pre-exposed stressed group (NPS), a pre-exposed control group (PC), and a pre-exposed stressed group (PS).

Chronic mild stress

The rats were submitted to a stress regimen lasting 21 d, during which they were subjected to 3 series of alternating periods of stress-inducing conditions. Each series consisted of various periods of confinement to small (18 cm x 8 cm x 7 cm) cages, a period of continuous overnight illumination, an overnight period of food and water deprivation immediately followed by 2 h of access to restricted food (resulting from the scattering of 18 precision pellets of 45 mg in the cage), 1 overnight period of water deprivation immediately followed by 1 h exposure to an empty bottle, and an overnight period of group housing in a soiled cage (100 mL water in the sawdust bedding). The rats were maintained on a reversed light/dark cycle from Friday evening to Monday morning (for details, see Moreau et al., 1994a; Moreau et al., 1994b).

Conditioning apparatus

The experimental chamber was a plexiglas box measuring 25 x 20 x 25 cm. The floor consisted of stainless steel rods connected to a grid-shock generator set to deliver 0.6 mA scrambled foot shocks of 0.5 s each when required. A licking spout, placed in the chamber through a hole in the

middle of a lateral wall, 5.5 cm above the grid floor and protruding 1.5 cm from the wall, provided the rat with the possibility of licking water. An outside loudspeaker placed 20 cm from the experimental chamber delivered a 30 s tone (2kHz, 85 dB A) when required. A personal computer was programmed to activate the loud speaker and the shock generator, as well as to record the number of licks during the experiments.

Conditioned emotional response procedure

Beginning on the 14th day of stress induction, the rats were submitted to an off-baseline conditioned response suppression consisting of the following 5 phases: *initial shaping*, *pre-exposure*, *conditioning*, *reshaping*, and *testing*. Water deprivation was introduced 20 h before the beginning of the experimental sessions.

Initial shaping. For 5 days, the rats were placed individually in the experimental chamber once a day for 20 min to establish a steady drinking-behavior baseline. The animals were then returned to the home cage and, after 1 h were allowed free access to water for 15 min.

Pre-exposure. For 2 consecutive days, the rats were placed in the same experimental chamber as that used in the initial training period. On each day, PC and PS rats were given 6 presentations of the tone (each one lasting 30 s), with a fixed inter-tone interval of 5 min. Rats belonging to the NPC and NPS groups were submitted to the same pre-exposure procedure, except that the loudspeaker was disconnected so that these rats were not pre-exposed to the tone.

Conditioning. On the next day, the rats were treated to two tone-shock pairings. The animals were placed in the experimental chamber and after 5 min, a tone was presented for 30 s, followed by a 0.5 s footshock. Five minutes later, the rats were exposed to a second tone-shock pairing and were left in the chamber for an additional five min.

Reshaping. The next day, after conditioning, the animals were submitted to a reshaping session to allow them to recuperate normal drinking behavior. The animals were placed in the experimental cage for 20 min and allowed to drink as much as desired, as in the initial training.

Testing. The day after the reshaping treatment, each rat was placed in the experimental chamber with free access to the waterspout. Immediately after 90 licks, the tone was presented. The tone switched off after the 100th lick or after 500s, whichever occurred first. The latencies for the completing licks 80-90 (A) and 90-100 (B) were recorded. There were three tone presentations during this session, with each one constituting a separate trial. This testing took place on the final day of the procedure of stress induction.

Data analysis. Drinking suppression ratios were calculated as $A/(A+B)$ for each rat. A suppression ratio approaching 0.0 indicates complete suppression (i.e., robust conditioning to the tone), whereas a suppression ratio of 0.5 indicates a failure to learn about the tone-shock relationship. The suppression ratios were analyzed using a three-way mixed design analysis of variance

(ANOVA, Statview Software), with the between-subject factors being latent inhibition (two levels: pre-exposed or not) and stress treatment (two levels: stress or no-stress), whereas the within-subject factor was the sequence of the test (three levels: one for each measurement).

RESULTS

The data show greater suppression ratios for the pre-exposed stressed animals than for the pre-exposed non-stressed group and for both non pre-exposed groups (Fig.1). The results show that there was a decrease in expression of CER induced by the chronic mild stress in the pre-exposed condition. The ANOVA showed a pre-exposure effect ($F_{1,64} = 81.48, P < 0.0001$), indicating that latent inhibition was obtained under these experimental conditions. The ANOVA also showed an effect for treatment ($F_{1,64} = 5.23, P < 0.05$), which might be related to the decrease in conditioning of pre-exposed and stressed animals. The interaction between the effect of pre-exposure and the stress

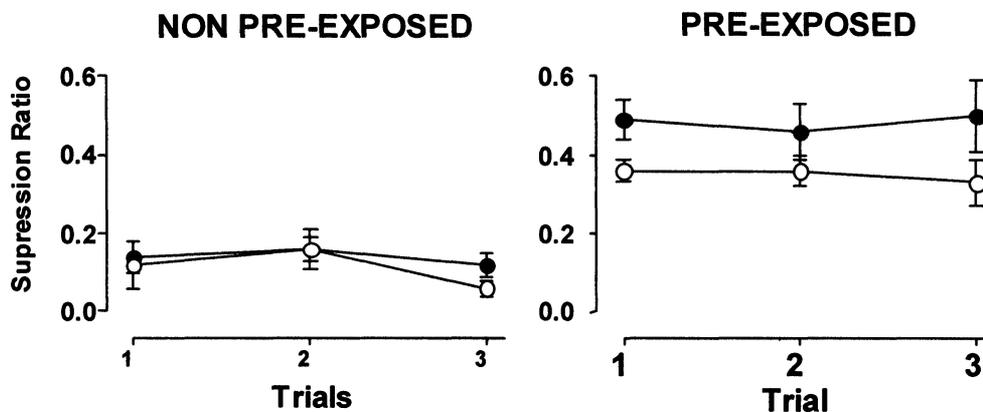


Fig. 1: Effect of chronic mild stress on the acquisition of a conditioned emotional response in rats non pre-exposed (left) and pre-exposed to the conditioned stimulus (right). Mean suppression ratio \pm s.e.m. is plotted as a function of three successive trials. (●) chronically stressed; and (○) non-stressed rats.

treatment was not significant, although close to threshold for significance ($F_{1,64} = 3.70$, $P = 0.06$). The measurements for the three trials did not differ significantly ($F_{2,64} = 0.17$, NS).

DISCUSSION

The present study induced the learning of a conditioned emotional response in two groups of rats, one submitted to intermittent unpredictable mild stress for a prolonged period and another comprising control non-stressed rats. The data show that both groups of rats exhibited equivalent suppression ratios, suggesting that the stress procedure did not interfere with learning a conditioned emotional response. The study also demonstrated that rats pre-exposed to the tone show higher suppression ratios than do non pre-exposed rats, confirming the acquisition of LI. The observation that the decrease in learning produced by prior exposure to the tone was higher in stressed animals confirmed the initial hypothesis, namely, that the induction of chronic mild stress enhances the LI process because the animals involved under-went a sharp decrease in the expression of CER. The results can be interpreted as evidence that stress was responsible for the decrease in the effect induced by the pre-exposure to the CS. In fact, the CMS had an even more detrimental impact after learning had already been affected by pre-exposure to the CS. Probably the increased impact was responsible for the marginally significant interaction of the effects of CMS and pre-exposure in the conditioning.

Despite the demonstration of LI in both animals and humans, the factors affecting this phenomenon remain to be clarified. Although LI can be largely explained by the competition between the information acquired during pre-exposure and the associations formed during the phase of acquisition (Bouton, 1993), its modulation

requires additional analysis (Lubow & Gewirtz, 1995). The data presented here suggest that CMS may be one of the variables that interact with stimulus associations during pre-exposure, acquisition, or both.

We can reasonably assume that LI potentiation after CMS can be related to a reduction in central nervous system DA neurotransmission. Such abnormalities in DA transmission in the nucleus accumbens have also been related to chronic mild stress and to the decrease in sensitivity to reward that can be observed in stressed animals (Stamford et al., 1991; Willner, 1991; Papp et al., 1993). In such cases, the hypothesis relating LI, attention, and dopaminergic modulation can offer insight into the interpretation of the data, suggesting that exposure to CMS can result in alterations in neural activity that affect LI in rats. If suggestions that the enhancement of LI depends on a blockage of dopaminergic transmission, especially in the nucleus accumbens, are considered, then the reduction of DA release triggered by CMS should give rise to the same effect as does the systemic or micro-injection of haloperidol into this structure. In fact, direct evidence of LI potentiation dependent on DA blockage has been demonstrated after intra-accumbens haloperidol microinjection in pre-exposed rats (Gray et al., 1997).

Another possibility is that chronic stress decreases the sensitivity of postsynaptic dopaminergic receptors in the nucleus accumbens (Papp et al., 1993), as well as decreasing central dopaminergic neurotransmission (Zebrowska-Lupina et al., 1992). The chronic administration of anti-depressant drugs increases the responsiveness of dopaminergic D2 receptors in the nucleus accumbens, and this effect can mediate the therapeutic effect of tricyclic antidepressants observed in animals submitted to CMS (Muscat et al., 1990; Moreau et al., 1994a; Moreau et al., 1994b). Possibly, the decreased sensitivity of postsynaptic dopaminergic receptors in the nucleus

accumbens (Papp et al., 1993) and in central dopaminergic neurotransmission might be related to the failure to cope that generally develops under chronic stress situations. When coping is not possible, some other emotional response takes place, possibly behavioral depression (Puglisi-Allegra et al., 1991; Puglisi-Allegra et al., 1990). In contrast to the protocol of other studies, that of the present study scheduled the CMS so that it was present both before and during the experimental LI investigation. This schedule might have been responsible for the chronic and enduring alterations of the underlying neurotransmission mechanisms that can mediate the LI enhancement by the CMS.

The data presented here can be useful for the understanding of both schizophrenic and depressive disturbances. On one hand, the development of LI can result from the dysfunctional processes that are operative in schizophrenia (Weiner, 1990; Lubow, 1989). On the other hand, the exposure to chronic stressful situations is generally related to the symptoms and processes characteristics of depression (Willner, 1991; Willner et al., 1992; Moreau et al., 1994a; Moreau et al., 1994b). Our study is unique in combining these issues in a single experimental design. Moreover, further studies investigating the possible effects of antidepressant and antipsychotic treatments in animals submitted to both LI and CMS can contribute to a better understanding of the processes underlying LI and various psychopathological disturbances.

Anhedonia present in both pathologies has also been related to dopaminergic dysfunction. Although the present study was not concerned with the measurement of anhedonia in stressed rats, the data suggest that exposure to the combination of two aversive experiences (CMS and CER) can alter behavior in relation to pleasant consequences and sensitivity to reinforcement. Moreover, pharmacological studies using both antipsychotic and anti-depressant drugs can provide

insight into the complex interactions involved in the relevant psychopathologies.

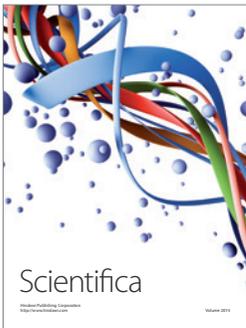
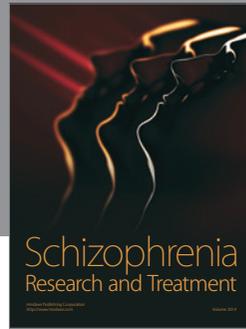
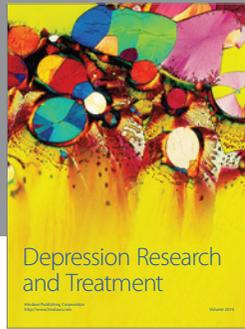
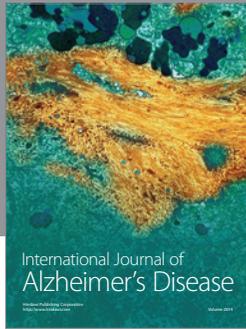
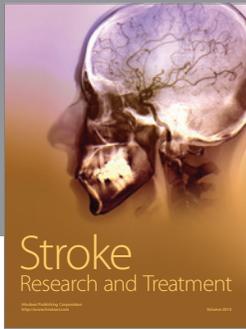
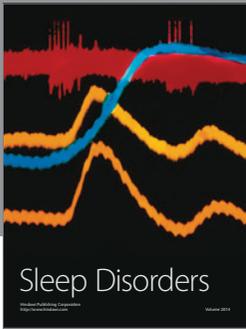
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