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Effects of Stress on Drug-Induced Yawning: Constant Vs. Intermittent Stress

SERGIO TUFIK,[1] CLAUDIA DE LUCA NATHAN, BEATRICE NEUMANN, DÉBORA C. HIPÓLIDE, LETÍCIA L. LOBO, ROBERTA DE MEDEIROS, LANFRANCO R. P. TRONCONE, SANDRA BRAZ AND DEBORAH SUCHECKI

Department of Psychobiology Escola Paulista de Medicina, Rua Botucatu, 862, 1 andar São Paulo, SP 04023-062 Brazil

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TUFIK, S., C. DE LUCA NATHAN, B. NEUMANN, D. C. HIPÓLIDE, L. L. LOBO, R. DE MEDEIROS, L. R. P. TRONCONE, S. BRAZ AND D. SUCHECKI. *Effects of stress on drug-induced yawning: Constant vs. intermittent stress.* PHYSIOL BEHAV **58**(1) 181–184, 1995.—Experiment 1 tested whether chronic exposure to immobilization, foot shock or forced swimming would result in suppression of apomorphine-, pilocarpine-, and physostigmine-induced yawning. Immobilization caused suppression of yawning, whereas foot shock and swimming resulted in increased number of yawns. Since interstressor interval was long in the two latter stressors, animals could have recovered and the increase in yawning could be due to the last (acute) exposure to stress. In Experiment 2 we recorded the number of yawns induced by pilocarpine in animals exposed to 1 h of swimming or foot shock. No differences between control and acutely stressed animals were detected. These results suggest that yawning is differently altered by constant and intermittent stressors (i.e., diminished by constant and increased by intermittent stress).

Stress Yawning behavior Dopaminergic system Cholinergic system Rat

INTRODUCTION

FOR many years our group has been evaluating the behavioral and neuropharmacological consequences of paradoxical sleep (PS) deprivation (15,17,20,23–25). The single platform technique, developed by Jouvet et al. (13), induces an increase in both relative and absolute adrenal weights (19) and in plasma corticosterone levels (data not published), indicating its stressful nature. Moreover, several of the techniques employed for PS deprivation possess in their nature some sort or degree of stress (3,14). In an attempt to investigate the influence of stress on the effects of PS deprivation technique, Silva et al. (21) exposed rats to four different manipulations: PS deprivation, immobilization, forced swimming, and foot shock for 3 days. Following this period, apomorphine-induced aggressiveness was compared among the groups. Increased aggressiveness was observed in PS deprivation and foot shock groups.

Among the behaviors altered by PS deprivation, yawning is particularly interesting, because it can be elicited by several cholinergic (AChergic) agonists (26,27), by low doses of dopaminergic (DAergic) agonists (16), and polypeptides such as α-MSH and ACTH (12,15,29), suggesting there are different neurotransmitter systems involved in the modulation of this behavior.

Ninety-six h of PS deprivation result in an almost complete suppression of yawning induced by DAergic and AChergic agonists (25). Since activity of both neurotransmitter systems is also altered by stress (for a review see 5) we examined the effects of other widely used stressors on drug-induced yawning. Animals were chronically exposed to immobilization, forced swimming or inescapable shock (Experiment 1) or acutely submitted to forced swimming or inescapable shock (Experiment 2). Following stress exposure, yawning induced by DAergic and AChergic drugs was evaluated.

MATERIAL AND METHODS

Subjects

Male Wistar albino rats, from our own colony, aged 3–4 mo, weighing 250–270 g and placed three/cage were used. Animals were kept in a room with controlled temperature 25 ± 2°C and light/dark cycle (lights on at 07:00 h and off at 19:00 h). Purina lab chow and tap water were provided ad lib.

Drugs

Apomorphine hydrochloride, physostigmine sulfide, and pilocarpine hydrochloride (Sigma Chem. Co., USA) were prepared in distilled water, and injected in a volume 0.1 ml/100 g of body weight. Apomorphine (80 and 120 μg/kg) was administered SC; pilocarpine (1 and 4 mg/kg) and physostigmine (0.1 and 0.2 mg/kg) were injected IP.

Statistical Analysis

Data from Experiments 1 and 2 were analyzed in the same way. The results of each drug-induced yawning were analyzed separately,

[1] To whom requests for reprints should be addressed.

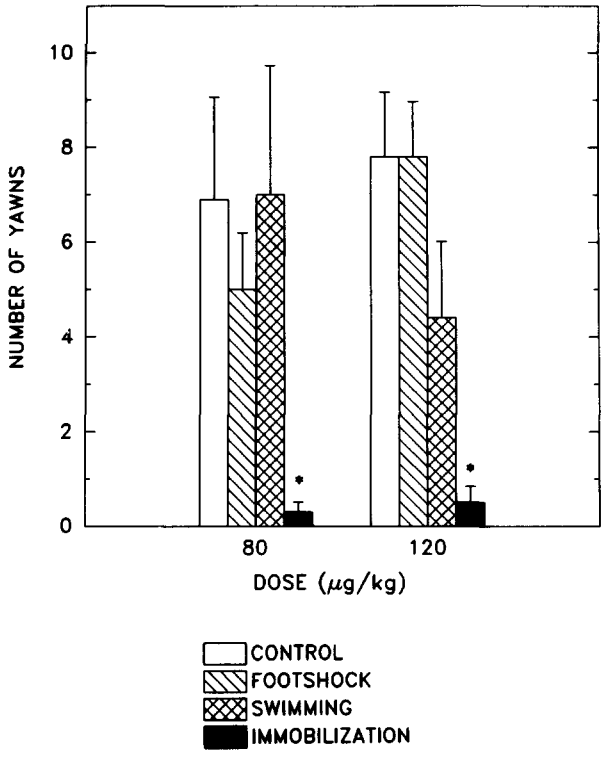

FIG. 1. Apomorphine-induced yawning in animals chronically submitted to different types of stress. Values represent mean ± SEM of 10 animals/group. *$p < 0.02$; different from all other groups.

by dose, by a nonparametric one-way analysis of variance, Kruskal–Wallis Test. Post hoc comparisons were made by Mann–Whitney *U* Test with the level of significance set at $p < 0.05$.

EXPERIMENT 1

Stress Procedure

Animals were assigned to one of the following groups:

1. Control. Animals were kept in their home cages, in groups of three, in the animal room, throughout the study.
2. Footshock. Animals were placed individually in acrylic boxes (14 × 25 × 28 cm), provided with electrified grid floor, through which shocks were delivered. The shock intensity was 2 mA, 0.1 s duration, with intervals of about 2 s. Animals were exposed to the shocks twice daily. Each session lasted 1 h.
3. Immobilization. Animals were placed in plastic restraining cylinders (21 cm long, 6 cm in diameter). Both extremities of the cylinder were closed by ventilated doors. Twice daily the rats were removed from the restrainers, and for 1 h, were allowed feeding and drinking in their home cages.
4. Forced swimming. Rats were placed in tanks containing water (23 cm in height) at 20°C, twice a day, for 1 h. After swimming session was over, the animals were dried with paper-towel and returned to their home cages.

Stress procedures were carried out for 4 consecutive days. During the intervals between stress sessions (foot shock and swimming) animals were placed in groups of three in their home cages in the animal room.

Testing Procedure

Testing began immediately after the end of the last stress session on the 4th day. Each testing session was composed of animals from all stress modalities. Only one drug was administered per day; thus, 80 animals (20 from each stress group, 2 doses per drug) were tested each day. After injection, animals were placed, individually, in wire-mesh cages and number of yawns was recorded for 30 min. Testing period took place between 14:00 and 15:30 h.

RESULTS

The results of apomorphine-induced yawning are presented in Fig. 1. A significant difference was obtained for both doses: $H = 12.7$; $p < 0.006$ (80 μg/kg), and $H = 18.977$; $p < 0.001$ (120μg/kg). Mann–Whitney *U* Test showed that yawning induced by either dose of apomorphine was suppressed only following immobilization ($p < 0.02$).

Figure 2 shows the results with 1 and 4 mg/kg of pilocarpine. ANOVA revealed differences among groups for both doses: $H = 11.27$; $p < 0.01$ (1 mg/kg), and $H = 22.91$; $p < 0.001$. Pairwise comparisons showed that for both doses of pilocarpine immobilization resulted in fewer yawns compared to control ($p < 0.02$) and foot shock ($p < 0.002$) groups. In addition, this latter group presented augmented pilocarpine-induced yawning, compared to both control ($p < 0.02$) and swimming ($p < 0.002$) groups.

Figure 3 presents the results of physostigmine-induced yawning (0.1 mg/kg: $H = 12.799$; $p < 0.01$; 0.2 mg/kg: $H = 23.245$; $p < 0.01$). Once again immobilization resulted in suppression of yawning induced by physostigmine. No further differences were found among groups.

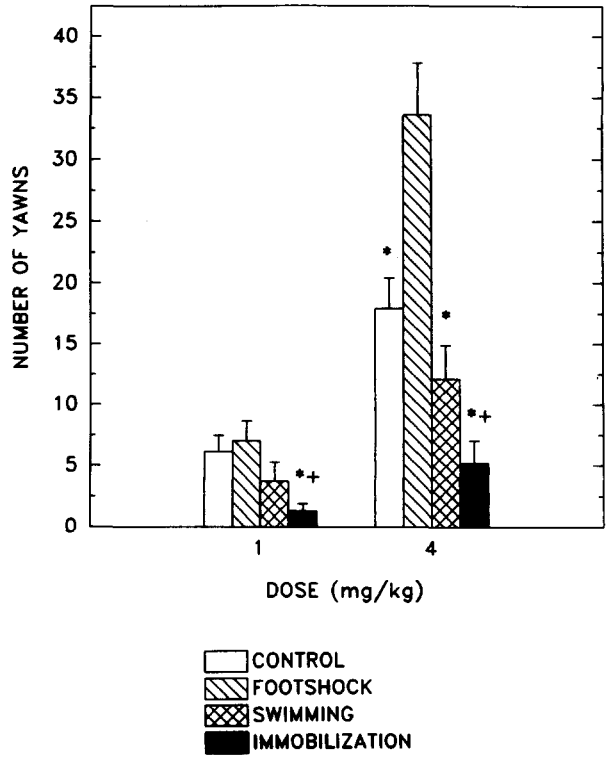

FIG. 2. Yawning induced by pilocarpine in animals submitted to different stressors over a period of 96 hr. Values are presented as mean ± SEM of 10 animals/group. *$p < 0.02$; different from foot shock + $p < 0.02$; different from control.

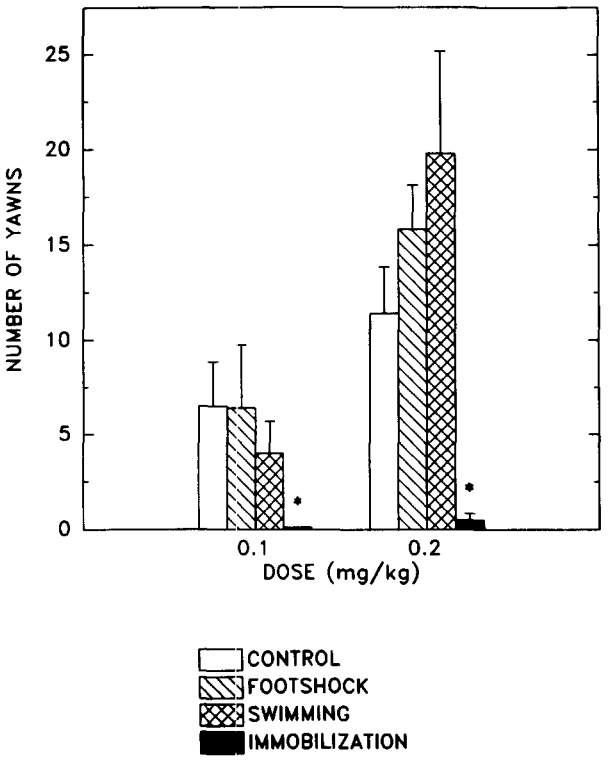

FIG. 3. Physostigmine-induced yawning following 96 h of stress. Values are presented as mean ± SEM of 10 animals/group. *$p < 0.05$; different from all other groups.

EXPERIMENT 2

The results of Experiment 1 showed a consistent suppression of drug-induced yawning behavior following immobilization, while foot shock resulted in increased pilocarpine-induced yawning and swimming caused no change of behavior. Animals subjected to immobilization are constantly exposed to the stressor, except for 2 periods of 1 h in which the animals are allowed food and water in their home cages. On the other hand, foot shock and swimming are intermittent stressors, to which animals are exposed twice daily for 1 h, spending the rest of the 24 h in their home cages. It is possible, therefore, that the results obtained with foot shock and swimming were a consequence of the last exposure to stress. To test this possibility animals were acutely exposed to foot shock or swimming. Pilocarpine was administered in different time intervals after stress and number of yawns was recorded.

Stress and Testing Procedures

Different groups of animals were exposed to foot shock or swimming during 1 h. Stressful stimuli were applied in the same way as in the previous experiment. Control animals, kept in groups of 3 in the home cages, were tested in parallel. Stress sessions started at different times of the day. Thus, one triad of groups (control, foot shock and swimming) was manipulated from 08:00 to 09:00 h. The second triad was exposed to stress from 10:00 to 11:00 h, and the last one from 12:00 to 13:00 h. All animals were tested at the same time of the day: from 14:00 to 15:30 h. Therefore the interval between stress onset and testing varied among triads of groups: 2, 4, and 6 h. Following the allotted period of time, animals were injected with 2 mg/kg of pilocarpine, IP, and placed individually in wire-mesh cages and number of yawns was recorded for 30 min. The experiment was run in duplicate and values were collapsed for statistical analysis.

RESULTS

Table 1 shows the results of Experiment 2. No differences among groups were observed, regardless the time interval between stress onset and testing. Comparison among control groups, however, revealed a difference between control 2 h and control 6 h ($p < 0.05$).

DISCUSSION

The results of the present study show that immobilization was the only stressor that consistently suppressed drug-induced yawning. Chronic foot shock and swimming, on contrary, promoted an increase or no change in number of yawns. This effect was not due to the last stress session, as shown by results of Experiment 2, in which acute exposure to the latter stressors did not result in change of yawning frequency.

Evidence from our and other laboratories suggests that constant chronic stress suppresses drug-induced yawning, regardless the modality of stress. Thus, PS deprivation induced by the single platform technique suppresses apomorphine, pilocarpine-, and physostigmine-induced yawning (17–19,25), suggesting this manipulation renders both DAergic presynaptic and AChergic postsynaptic receptors subsensitive to their agonists. Nunes Jr. et al. (18,19) PS deprived rats using the multiple platform technique (in this case, 10 animals are placed on top of 18 platforms, 6 cm wide, placed 10 cm apart, inside a large water tank, which dimensions are 125 × 45 × 36 cm, thus enabling the animals to jump from one platform to another), therefore preventing immobilization and social isolation. Yet, the authors replicated the effects of the single platform technique-induced deprivation on yawning behavior. In addition, Bourson and Moser (2) reported suppression of apomorphine- and physostigmine-induced yawning in animals isolated for 7 days. Based on the above mentioned results it is possible to hypothesize that to suppress drug-induced yawning, animals must be exposed to stress in a constant fashion. To reinforce this notion, in the present study, chronic foot shock and swimming were employed intermittently during 96 h and resulted in increased or no change in number of yawns. It appears as though existence of interstressor intervals differentially modulates neuronal and neuroendocrine functions (4).

Yawning is believed to be mediated by a balance between DAergic and AChergic neurons (28). The former, most likely the nigrostriatal pathway, exerts an inhibitory action on the latter, most likely striatal AChergic neurons (1,22). Thus, yawning is elicited by low doses of apomorphine (probably acting at the presynaptic receptor level) and AChergic drugs that act at the postsynaptic receptor level (17,27). It has been demonstrated that

TABLE 1

NUMBER OF YAWNS INDUCED BY 2 mg/kg OF PILOCARPINE IN ANIMALS ACUTELY EXPOSED TO FOOTSHOCK OR SWIMMING AND TESTED 2, 4 OR 6 h AFTER THE STRESSOR

	Time Interval		
Group	2 h	4 h	6 h
Control	8.5 ± 1.6	10.6 ± 1.6	14.4 ± 2.0*
Footshock	12.8 ± 2.2	14.6 ± 2.8	13.2 ± 1.9
Swimming	13.0 ± 1.7	15.4 ± 2.2	14.0 ± 1.4

Values are presented as mean ± SEM of 20 animals/group/time interval. * $p < 0.05$; compared to control 2 h.

both acute and chronic intermittent stress result in increase of ACh release and of high affinity choline uptake by presynaptic membranes of the septo-hippocampal system (9,10), a system believed to be involved in yawning behavior (27,30). However, augmented binding of muscarinic AChergic receptors can be observed only after chronic intermittent stress (6,7,9). A down-regulation of M2 muscarinic receptors was demonstrated following 96 h of PS deprivation by the multiple platform technique (20). Acute stress, and even acute treatment with ACTH and corticosterone do not produce changes in receptor binding (8,10), although increased sensitivity, but not number, of muscarinic receptors is reported at 1 and 48 h following a 2 h immobilization period in some brain regions (11). Overall, the data suggest that changes in receptor sensitivity require longer exposure to stress (10) and/or longer time span between stress onset and functional evaluation of these receptors, since we did not find changes in yawning 6 h following 1 h of either foot shock or swimming (Exp. 2). We cannot ignore, however, that failure in showing statistical differences in Exp. 2 could be due to differences in control groups among time intervals. Within the framework of the present experiment is not possible to explain such differences.

In conclusion, the results of the present study indicate yawning can be altered in a stressor-specific manner. Immobilization, the only stressor to which animals were constantly exposed, was also the only one that resulted in yawning suppression. An opposite effect was observed following stressors in which long intersession intervals were present.

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