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Differential effect of buspirone and diazepam on negative contrast in one-way avoidance learning

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Abstract

The main aim of the present work was to investigate the effect of buspirone, a 5-HT_{1A} receptor agonist, on successive negative contrast in one-way avoidance learning. Successive negative contrast was induced by shifting rats from a large reward (30 s spent in the safe compartment) to a small reward (1 s). Acute administration of buspirone (0.25, 0.5, 0.75 and 1.0 mg/kg i.p.) did not attenuate the contrast effect, as opposed to that observed for diazepam (1 mg/kg i.p.). The highest dose of buspirone used, however, did interfere with the learning of the avoidance response itself. Chronic buspirone (20 days, 0.5 and 0.75 mg/kg i.p.) did not have any effect on successive negative contrast either. Overall, these results could suggest that the 5-HT_{1A} receptor is not involved in the negative contrast effect studied, quite different to that observed for the γ-aminobutyric acid (GABA) system. The findings are compared to results obtained with animal models selectively sensitive to some anxiolytic drugs, as are the so-called 'conflict models'.

Keywords: Animal model; Anxiety; Buspirone; Diazepam; One-way avoidance; Successive negative contrast

1. Introduction

The negative contrast effect was initially studied in behavioral appetitive tasks; rats that were shifted from a large to a small reward in a runway task generally decreased their running speed abruptly, and ran more slowly than animals that had had the lower level of reward throughout the task (Crespi, 1942). This kind of behaviour typically exhibited by shifted animals is referred to as successive negative contrast. A similar contrast effect was subsequently studied using a different task: in this case, rats that drank a 32% sucrose solution and were shifted to a 4% solution showed a robust successive negative contrast effect, drinking less than the unshifted control rats that drank only the low sucrose concentration (Flaherty, 1982).

A different successive negative contrast situation was recently characterized in aversive tasks involving one-way avoidance (Cándido et al., 1992). In one-way avoidance learning, rats are exposed to two markedly different compartments. In one, the 'danger compartment', rats receive a warning signal followed by a foot-shock; in the other, the 'safe compartment', the warning signal never appears. Rats placed in the danger compartment learn to run into the safe compartment when the warning signal is turned on, and so learn to avoid shock. Rats commonly learn this task very rapidly, and a high level of performance following acquisition is maintained over many trials (Mackintosh, 1974). Some authors have explained this performance on the basis not only of the drive properties of fear associated with the warning signal (Mowrer, 1947), but also on the assumption that the safe compartment is functionally equivalent to appetitive reinforcers; thus learning was enhanced, the more time was spent in that compartment (Cándido et al., 1984; Denny, 1971; Holloway and Baum, 1989). Based on the latter, a sudden reduction in the time spent in the safe com-

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partment should lead to a successive negative contrast effect similar to that described by Crespi (1942) for appetite-related tasks. We found that, when rats trained in a one-way avoidance task with a 30-s period in the safe compartment (30 s, pre-shift period) were switched to a 1-s safe time (post-shift period), their performance deteriorated in comparison with control subjects (unshifted rats that spent 1 s in the safe compartment throughout the task) (Cándido et al., 1992).

The successive negative contrast effect in appetitive or drinking behavior has been proposed as a useful approach to the study of anxiety. The importance of this approach is based on the fact that it is assumed to be a measure of an emotional process (see Flaherty, 1991), and has thus been widely used to test the action of anxiolytic drugs, including benzodiazepines and barbiturates (see Gray, 1977 and Flaherty, 1990). These substances generally attenuate or eliminate the behavioral effects associated with the negative contrast situation. This pharmacological action has also been observed with the successive negative contrast reported in one-way avoidance: diazepam eliminated the contrast effect in a dose-dependent manner, without affecting acquisition of the avoidance task itself (Morales et al., 1992). Diazepam exerts its effects via $GABA_A$ receptors, as shown by the finding that flumazenil (a benzodiazepine receptor antagonist; see Hunkeler et al., 1981) prevents the attenuating effect of this drug on the successive negative contrast effect in a dose-dependent manner, without affecting the avoidance task itself (Torres et al., 1994).

Other substances in addition to benzodiazepines and barbiturates have been used in studies of negative contrast. Buspirone, which acts via the serotonergic system, affects mainly (but not exclusively) $5\text{-}HT_{1A}$ receptors. This, in the case of receptors located on midbrain raphe cells, leads to a reduction in the firing rate of 5-HT neurons, thus reducing the release and the extracellular concentration of 5-HT in their terminal fields (mainly limbic structures) (Peroutka, 1985; Tunnicliff, 1991). Although buspirone appears to exert anxiolytic effects in humans (Goa and Ward, 1986; Napoliello and Domantay, 1991; Wheatley, 1982; but see also Deakin, 1993 and Sheehan et al., 1990), its effects in animal models sensitive to traditional anxiolytics are controversial, and depend to a large extent on the nature of the experimental task (Broekkamp and Jenck, 1989; Eison et al., 1986; Handley and McBlane, 1993; Traber and Glaser, 1987). Buspirone, regardless of whether acute or chronic administration was used, had no effect on successive negative contrast for those drinking responses described by Flaherty and colleagues in consummatory tasks, a finding that clearly contrasts with those reported for midazolam, another benzodiazepine tested by this group (Flaherty et al., 1990). The experiments described below were therefore designed to test the possible effect of buspirone on successive negative contrast in one-way avoidance learning, and to compare these results with the known effects of diazepam (Morales et al., 1992).

2. Materials and methods

2.1. Experiment 1

This experiment tested the effects of the acute administration of buspirone on successive negative contrast. The results were compared with those obtained in a group of animals receiving diazepam.

Animals

A total of 65 female Wistar rats, weighing 180–235 g, were used in this experiment. The rats were housed individually with food (Panlab, Spain) and tap water ad libitum. Room temperature was kept at about 20° C with lights on from 08:00 to 20:00 h. The entire experiment took place during the light phase, between 09:00 and 14:00 h.

Apparatus

A Letica one-way avoidance chamber was used. The avoidance chamber consisted of two equal compartments 27 cm long × 25 cm wide × 28 cm high, made of Plexiglas. The compartments were separated by a 0.5 cm thick partition 25 cm wide × 28 cm high, with a square 9 × 9 cm hole and a removable gate to allow movement between compartments. Both compartments thus had the same dimensions and were made of the same material, except that the danger compartment was fitted with a grid floor. The grid floor consisted of 19 stainless steel rods 4 mm in diameter and spaced 2 cm apart center to center, connected in series to a Letica LI-2900 module capable of delivering a continuous scrambled shock. The floor in both compartments was hinged to operate a microswitch when depressed; this allowed the apparatus, procedure and responses to be controlled by a PC-XT microcomputer. A speaker was placed in the middle of the lateral wall so that half was oriented to the danger compartment and the other half to the safe compartment. The warning signal was a 2000 Hz tone of 88 dB. The roof of the danger compartment consisted of a black glass panel, which was removed only to put the rat into the chamber. A rigid, non-transparent white plastic carrying box 24 cm long × 14 cm wide × 19 cm high was placed in the safe compartment in contact with the communication hole. This box was used as the safe compartment and to move the rat when the safe time was completed. The carrying box had a handle on top and no wall on the side in contact with the partition of the avoidance chamber and, therefore, with the communication hole

and gate. The floor, ceiling and walls of this box were made of the same material. An air extractor installed outside the avoidance chamber produced a background noise of 70 dB.

Procedure

On the day of the experiment, the rats were removed from their cages and lightly handled for about 1 min. They were then injected with various drugs, according to the different experimental conditions (see below) and returned to the cages for the next 30 min. Once this time had elapsed, the rats were placed in the avoidance chamber and allowed 5 min to explore both compartments without interference, in the presence of the background noise. Thereafter the communication gate was closed to shut the rat in the danger compartment, and the trials then began. Each trial consisted of a warning signal followed 5 s later by a 1-mA electric foot-shock. Both the warning signal and the shock continued until the animal moved into the safe compartment or until 30 s had elapsed. The gate between the two compartments was opened as soon as the warning signal sounded and closed when the rat entered the safe compartment. Time in the danger compartment before the start of the warning signal was the same for all experimental conditions (15 s). Once the safe time had been completed, the transportation box was lifted out of the apparatus and the rat was turned out into the danger compartment. This took from 1 to 2 s. The box was then replaced in the safe compartment of the avoidance chamber.

All rats were trained until they reached five consecutive avoidance responses in the pre-shift phase and eight consecutive avoidance responses in the post-shift phase. An avoidance response was considered to have taken place when the animal moved into the safe compartment within 5 s after onset of the warning signal. Once five consecutive avoidance responses had been achieved, the post-shift phase began. It consisted of trials conducted as during the pre-shift phase except that the time in the safe compartment was changed from 30 to 1 s for several groups of rats. The post-shift phase continued until rats reached eight consecutive avoidance responses. Both criteria were selected according to our earleir studies (Cándido et al., 1992; Morales et al., 1992).

Rats were randomly assigned to eight groups ($n = 8$, except in group 1-1/buspirone-1 where $n = 9$) (Table 1). Thus, group 30-1/vehicle (injected with vehicle) spent 30 s in the safe compartment during the pre-shift phase and 1 s in the post-shift phase. This safe time (1 s) remained constant throughout the experimental session for group 1-1/vehicle. Group 30-1/diazepam, on the other hand, received the same treatment as group 30-1/vehicle, except that animals in the former group were i.p. injected with diazepam (1 mg/kg). This group was used here as a positive control (see Flaherty et al., 1990).

The experimental groups treated with acute buspirone were group 30-1/buspirone-0.25, 30-1/buspirone-0.5, 30-1/buspirone-0.75 and 30-1/buspirone-1. All groups were treated as described for group 30-1/vehicle, except that buspirone was injected i.p. at a dose of 0.25, 0.5, 0.75 or 1.0 mg/kg, respectively (doses were chosen according to a previous report by Flaherty et al., 1990).

Finally, to study the possible influence of buspirone on the avoidance task employed, we used a group (1-1/buspirone-1) in which subjects received i.p. 1 mg/kg buspirone, but the times spent in the danger/safe compartments were the same as for group 1-1/vehicle (Table 1).

Dependent variable

The dependent variable used was the number of trials needed to reach the acquisition criteria in each phase. There were two criteria: number of trials to achieve five consecutive avoidance responses in the pre-shift phase and number of trials to achieve eight consecutive avoidance responses in the post-shift phase. Each criterion was taken as met on the first of each sequence of consecutive responses.

Statistical analysis

Values reported are means ± S.E.M. Scores for each criterion were analyzed with the Kruskal-Wallis test for

Table 1
Acute buspirone. Time spent (in seconds) in the safe and danger compartments during pre-shift and post-shift phases by each group, and treatment (buspirone, diazepam or vehicle) (time in danger compartment for all groups: 15 s)

Groups	n	Time (s) in the safe compartment during		Treatment (mg/kg i.p., −30 min)
		Pre-shift phase	Post-shift phase	
30-1/vehicle	8	30	1	Vehicle
1-1/vehicle	8	1	1	Vehicle
30-1/diazepam	8	30	1	Diazepam (1)
30-1/buspirone-0.25	8	30	1	Buspirone (0.25)
30-1/buspirone-0.5	8	30	1	Buspirone (0.5)
30-1/buspirone-0.75	8	30	1	Buspirone (0.75)
30-1/buspirone-1.0	8	30	1	Buspirone (1)
1-1/buspirone-1.0	9	1	1	Buspirone (1)

global significance. Comparisons between the different groups were done using the Mann-Whitney *U*-test.

Drugs

Buspirone (kindly donated by Bristol Myers, USA) was prepared in isotonic saline, used as vehicle solution. Diazepam (kindly donated by Productos Roche, Spain) was suspended in a 1% Tween-80 (Sigma, Spain) saline solution. Both drugs were administered i.p. The volume administered was 10 ml/kg.

2.2. Experiment 2

The second experiment was designed to examine the possible long-term effect of buspirone on negative contrast, since it has been reported to be effective in humans only after at least 2 weeks of daily administration (Goa and Ward, 1986). Accordingly, we administered the drug for 20 days, and then tested its effect on successive negative contrast in one-way avoidance learning.

Animals

A total of 38 female Wistar rats, weighing 180–225 g, were used in this experiment. Housing and maintenance were as described in experiment 1. The entire experiment also took place during the light phase, between 09.00 and 14.00 h.

Apparatus

As described in experiment 1.

Procedure

The rats were randomly assigned to four groups, and for 20 days they received buspirone or vehicle daily via i.p. injection (Table 3). On the 20th day and 30 min after the last injection, they were subjected to the same experimental procedure as described in experiment 1. Two of the groups (30-1/chronic vehicle and 1-1/chronic vehicle) were given chronic injections of vehicle only, to test whether daily handling and/or continuous administration of the vehicle affected negative contrast. The other two experimental groups (30-1/chronic buspirone-0.5 and 30-1/chronic buspirone-0.75) received chronic i.p. injections of 0.5 mg/kg or 0.75 mg/kg of buspirone respectively, to test the possible effect of this drug on negative contrast.

Dependent variable

As in experiment 1.

Statistical analysis

As in experiment 1.

Drugs

As in experiment 1. In this case, however, diazepam was not used.

3. Results

3.1. Experiment 1

Overall analysis of the results showed statistically significant differences between groups in pre-shift, $H(7) = 34.58$, $P < 0.0001$, and post-shift phases, $H(7) = 23.60$, $P < 0.001$. The results are summarized in Table 2.

Effect of diazepam

The mean number of trials needed to reach the pre-shift and post-shift criteria in groups 30-1/vehicle, 1-1/vehicle and 30-1/diazepam is shown in Table 2. In the pre-shift phase, group 1-1/vehicle differed significantly from both group 30-1/vehicle ($U = 10.5$, $P < 0.02$) and group 30-1/diazepam ($U = 10$, $P < 0.02$). In the post-shift phase, significant differences were obtained between group 30-1/vehicle and groups 1-1/vehicle ($U = 5.5$, $P < 0.003$) and 30-1/diazepam ($U = 9$, $P < 0.01$), respectively, but not between group 30-1/diazepam and group 1-1/vehicle ($U = 31$, n.s.). These results replicate the successive negative contrast effect in one-way avoidance learning (Cándido et al., 1992), and the attenuation of this effect with the administration of diazepam (Morales et al., 1992) found earlier.

Effect of acute buspirone

The mean number of trials needed to reach the pre- and post-shift criteria in groups 30-1/vehicle, 30-1/buspirone-0.25, 30-1/buspirone-0.5 and 30-1/buspirone-0.75 is shown in Table 2. There were no differences between the buspirone-treated and control groups for the pre-shift or post-shift phase. Overall analysis of the results in these four groups showed no statistically significant differences between groups in pre-shift ($H(3) = 2.67$, ns) and post-shift phases ($H(3)$

Table 2
Acute buspirone. Mean number of trials (±S.E.M.) to reach the criteria of acquisition in the pre-shift and post-shift phases by each group

Groups	Pre-shift phase	Post-shift phase
30-1/vehicle	6.7 (1.5) [b]	18.8 (3.2) [b]
1-1/vehicle	15.1 (2.5) [a]	6.1 (1.3) [a]
30-1/diazepam	6.5 (1.6) [NS,b]	7.2 (2.5) [a,ns]
30-1/buspirone-0.25	8.2 (0.9) [NS]	15.9 (3.3) [NS]
30-1/buspirone-0.5	7.7 (1.4) [NS]	20.7 (6.1) [NS]
30-1/buspirone-0.75	6.7 (0.9) [NS]	22.1 (3.9) [NS]
30-1/buspirone-1.0	17.9 (3.3) [a]	36.5 (10.3) [NS]
1-1/buspirone-1.0	34.4 (6.8) [a,b]	10.0 (3.5) [a,ns]

[a] Significant differences when compared with group 30-1/vehicle. [NS] Non-significant differences when compared with group 30-1/vehicle. [b] Significant differences when compared with group 1-1/vehicle. [ns] Non-significant differences when compared with group 1-1/vehicle).

Table 3
Chronic buspirone. Time spent (in seconds) in the safe and danger compartments during pre-shift and post-shift phases by each group, and treatment (buspirone or vehicle) (time in danger compartment for all groups: 15 s)

Groups	n	Time (s) in the safe compartment during		Treatment (mg/kg/day × 20 days i.p.)
		Pre-shift phase	Post-shift phase	
30-1/chronic vehicle	9	30	1	Vehicle
1-1/chronic vehicle	10	1	1	Vehicle
30-1/chronic buspirone-0.5	10	30	1	Buspirone (0.5)
30-1/chronic buspirone-0.75	9	30	1	Buspirone (0.75)

= 1.81, ns). However, significant differences were now found when groups 30-1/vehicle, 30-1/buspirone-0.25, 30-1/buspirone-0.5, 30-1/buspirone-0.75 and 30-1/buspirone-1 were compared in the pre-shift phase ($H(4) = 13.139$, $P < 0.01$), but not in the post-shift phase ($H(4) = 2.988$, ns). These results show that buspirone (0.25, 0.5 and 0.75 mg/kg) did not affect successive negative contrast in one-way avoidance learning, in evident contrast with the results after diazepam injection. The findings with animals injected with 1 mg buspirone (30-1/buspirone-1) call for separate analysis. As Table 2 shows, rats treated with the latter dose of drug appeared to need more trials in both phases to reach the learning criterion, in comparison with the controls. We found statistically significant differences between groups 30-1/buspirone-1 and 30-1/vehicle in the pre-shift phase ($U = 7.5$, $P < 0.007$), but not in the post-shift phase ($U = 23$, ns). These data suggest that buspirone (1 mg/kg) interferes with the acquisition phase of one-way avoidance learning, but not with the contrast effect itself, which was not attenuated by any of the doses of buspirone tested.

To more precisely characterize the effect of 1 mg buspirone, we used a new group of rats (1-1/buspirone-1) in an experimental procedure identical to that described for group 1-1/vehicle, except that the rats received buspirone at an i.p. dose of 1 mg/kg. By comparing the results for these groups, we hoped to determine whether this dose of buspirone affected the acquisition or maintenance of one-way avoidance learning, as the results reported above suggested. The results are shown in Table 2. There were significant differences between the two groups in the pre-shift ($U = 15.5$, $P < 0.03$), but not in the post-shift phase ($U = 31$, ns). Consequently, buspirone at a dose of 1 mg/kg apparently interferes only with the acquisition phase of avoidance learning, but not with maintenance of the response once it has been learned. This finding was also supported by comparisons between groups 30-1/vehicle and 30-1/buspirone-1, and between groups 1-1/vehicle and 1-1/buspirone-1, which clearly showed that the contrast effect was not modified by buspirone. The effect of this drug was thus apparently limited to increasing the number of trials needed to reach the criteria in both pre- and post-shift phases.

Table 4
Chronic buspirone. Mean number of trials (±S.E.M.) to reach the criteria of acquisition in the pre-shift and post-shift phases by each group

Groups	Pre-shift phase	Post-shift phase
30-1/chronic vehicle	7.5 (0.9) [b]	13.5 (3.5) [b]
1-1/chronic vehicle	14.6 (2.9) [a]	3.6 (1.1) [a]
30-1/chronic buspirone-0.5	10.3 (2.2) [NS]	14.3 (3.5) [NS]
30-1/chronic buspirone-0.75	10.0 (2.0) [NS]	20.8 (5.9) [NS]

[a] Significant differences when compared with group 30-1/chronic vehicle. [NS] Non-significant differences when compared with group 30-1/chronic vehicle. [b] Significant differences when compared with group 1-1/chronic vehicle. [ns] Non-significant differences when compared with group 1-1/chronic vehicle).

3.2. Experiment 2

The results are summarized in Table 4 . Analysis of the results in groups 30-1/chronic vehicle and 1-1/chronic vehicle showed again that negative contrast occurred in the one-way avoidance task. During the pre-shift phase, rats in group 1-1/chronic vehicle needed more trials to reach the acquisition criterion than rats in groups 30-1/chronic vehicle ($U = 12$, $P < 0.03$). This effect was clearly reversed in the post-shift phase ($U = 21$, $P < 0.05$) (Table 4). Thus daily handling during a 20-day period does not appear to have affected the contrast effect.

We found no significant differences, in either of the phases, between groups given chronic injections of buspirone (30-1/chronic buspirone-0.5 and 30-1/chronic buspirone-0.75) and the contrast control group (30-1/chronic vehicle) (Table 4). This finding was further evidence that buspirone had no effect on successive negative contrast in one-way avoidance learning, an observation reported in a previous study using a different contrast situation (Flaherty et al., 1990).

4. Discussion

Our results show, firstly, that the acute administration of buspirone (0.25, 0.5, 0.75 or 1.0 mg/kg) does not attenuate the effects of successive negative contrast

in one-way avoidance learning, a finding clearly different from the attenuation found after the administration of diazepam (1 mg/kg). Secondly, the chronic administration of buspirone (20 days, 0.5 or 0.75 mg/kg) also had no effect on successive negative contrast. These findings suggest that the 5-HT_{1A} receptor system (the major system involved in the central action of buspirone; see, for example, Peroutka, 1985 and Tunnicliff, 1991) does not participate in the negative contrast effect in one-way avoidance. Overall, our results are similar to those obtained for the contrast effect in drinking behaviour; a study by Flaherty and colleagues showed that several serotonergic substances (including buspirone) had no effect on successive negative contrast, whereas benzodiazepines clearly attenuated the response to contrast (Flaherty et al., 1990).

Several experiments have shown that the effect of successive negative contrast in one-way avoidance is sensitive to the action of drugs such as diazepam (Morales et al., 1992; Torres et al., 1994). It could be asked whether the negative contrast was accompanied by a state of anxiety, in line with the models proposed by other authors (see Gray, 1982, for review). From this standpoint, the sudden decrease in the amount of reinforcement may lead to the appearance of a state of frustration (frustrative non-reward), which may give rise to responses characteristic of increased arousal and/or anxiety level (Amsel, 1958). In the present context, such changes would affect performance of the avoidance response, thus giving rise to the negative contrast effect. In theory, diazepam affects this emotional state, attenuating and/or preventing the appearance of negative contrast (Flaherty, 1991; Morales et al., 1992). Similarly, a reduction in the brain concentrations of serotonin would be expected to induce a response similar to that described after the administration of benzodiazepines. Buspirone interferes with serotonergic activity in brain regions that project on the limbic system (VanderMaelen et al., 1986), and induces responses very similar to those caused by benzodiazepines in several animal models, regardless of whether the peripheral (see Eison et al., 1986, for example) or intracerebral route of injection (see Carli et al., 1989 and Kostowski et al., 1989, for example) is used. However, the experimental evidence based on effects of drugs (including buspirone) that reduce the brain action of serotonin in animal models sensitive to the action of benzodiazepines and barbiturates is not entirely unequivocal (see Handley and McBlane, 1993, for a recent review). This was especially evident in the so-called 'conflict paradigms', in which some authors have failed to observe any effect of buspirone (Barrett and Gleeson, 1991; Broekkamp and Jenck, 1989; Martin et al., 1993; Soubrié, 1989; Sanger, 1990; see Treit, 1991, for a possible explanation for the inconsistent effects of 5-HT_{1A} receptor agonists in some behavioural models). Some authors have gone so far as to suggest that a reduction in the central nervous system concentrations of serotonin is not accompanied by an anxiolytic-like response, but rather by a general increase in the organism's level of impulsivity which, in certain behavioral tests, might be misinterpreted as anxiolysis (Soubrié, 1986,1989). Thus the effect of successive negative contrast in one-way avoidance, although highly sensitive to diazepam, appears not to be very sensitive to buspirone (as also has been found in conflict situations). This would tentatively rule out a role for the 5-HT_{1A} receptor system in modulating the contrast effect, although it would be worth investigating the action of substances that act more specifically on this system (e.g., 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT)). Other 5-HT receptors may also be involved; for example, the 5-HT_3 receptor has recently been related with anxiolytic-like responses in different animal models (excluding, once again, conflict situations) (Barnes et al., 1992).

Our results show that the acute administration of buspirone at a dose of 1 mg/kg does not modify the contrast effect per se, although it significantly increased the number of trials needed to acquire the avoidance response. We conclude that buspirone interferes with this type of learning only during the acquisition phase, but not during maintenance. These findings can be explained in several ways. (a) The drug may have interfered with the capacity for spatial learning; this would have repercussions on the acquisition of the avoidance response, which clearly involves a spatial component. In this connection, it has been reported (Rowan et al., 1990) that buspirone at a dose of 2 mg/kg reduced the ability to learn in a water maze, and that this effect was more marked during the acquisition trials. (b) An increase in the subject's level of impulsivity might give rise to the appearance of behaviors incompatible with the response required for the avoidance task. This notion has been extensively dealt with by, among others, Soubrié (1986) who suggests that the decrease in brain concentrations of serotonin is accompanied mainly by increases in impulsivity, with repercussions on the performance of learning tasks. In any case, experimental studies have demonstrated a complex interaction between the central concentrations of serotonin and the learning of avoidance tasks (see Ögren, 1985, for review). (c) Buspirone may affect the dopaminergic system, thus the dose we used may have interfered slightly with the motor system, hindering acquisition of the avoidance response. In this connection, some degree of uptake of buspirone has been noted in brain regions rich in dopamine receptors (Kaulen et al., 1985), where buspirone may interfere with receptor functioning (Riblet et al., 1982; Wood et al., 1983). (d) Moderate to high doses of buspirone induce the so-called 'serotonin syndrome', which also

might be responsible for the behaviour observed (Sternbach, 1991). Regardless of how these findings are explained, it clearly appears that, above a certain dose (1 mg/kg in our case), learning of the avoidance response begins to deteriorate, an effect that was clearly seen at a dose of 2 mg/kg (we tested doses up to 10 mg/kg, data not shown). This interference was not seen in the present study at doses below 1 mg/kg, which were similar to those used in an earlier study of negative contrast (Flaherty et al., 1990).

During the chronic administration of buspirone, the rats were handled for 20 days. This type of daily handling may profoundly affect behavior and brain biochemistry. Handling similar to the type used in this experiment, during a 7-day period, led to an anxiolytic-like response in the elevated plus-maze test resembling that observed with anxiolytic drugs (Andrews and File, 1993). The handling procedure may even interfere with the anxiolytic effect of benzodiazepines in different behavioral tests (Boix et al., 1988; Brett and Pratt, 1990). In addition, this type of handling modifies serotonin concentrations in brain regions such as the hippocampus (File et al., 1990). In the present study, however, daily handling did not affect the characteristics of negative contrast.

In summary, neither the acute nor the chronic administration of buspirone modified the successive negative contrast effect in one-way avoidance tasks, a finding that contrasts with the effects of benzodiazepines such as diazepam. Our results reproduce the findings obtained using other different contrast situations (the drinking test of Flaherty and colleagues) as well as those results recorded in other behavioral paradigms unrelated to negative contrast (e.g., models of conflict). Overall, these results make successive negative contrast, as characterized in the present study, a useful tool to investigate the effects of anxiolytic substances, as our findings may help to elucidate the mechanisms of the central action of these drugs. However, further studies currently in progress in our laboratory should document the suitability of this model.

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