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Differential effect of low doses of intracerebroventricular corticotropin-releasing factor in forced swimming test

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Abstract

In this work, we studied the effect of low doses of intracerebroventricular corticotropin-releasing factor (CRF) in six sessions of forced swimming test (FST). When CRF (0.01 and 0.1 μg) was administered pre-test, results showed that the 0.1-μg dose significantly increased swimming in SESSION2, SESSION3 and SESSION4, while the 0.01-μg dose proved ineffective. When CRF (0.1 and 0.03 μg) was administered post-test to evaluate retention of swimming response, the dose of 0.1 μg impaired retention, while the dose of 0.03 μg improved it, although these effects only reached significance in SESSION2. In an additional session (SESSION6), testing long-term retention of this swimming response, the 0.1-μg dose significantly impaired retention, whereas the 0.03-μg dose proved ineffective. A high dose of CRF (1 μg) was also included as a control of previous results [García-Lecumberri C, Ambrosio E. Role of corticotropin-releasing factor in forced swimming test. Eur J Pharmacol 1998;343:17–26]. In all the FST sessions, this high dose increased swimming when administered pre-test, while impairing retention when administered post-test. Preliminary data obtained with low doses of CRF suggest that a differential effect on retention of swimming response seems to exist depending on the dose, whereas a high dose of CRF clearly impairs retention. The role of CRF in learning and memory processes in FST is discussed.

Keywords: Corticotropin-releasing factor; Forced swimming test; Behavioral adaptation; Learning and memory processes; Stress; Coping behavior; Antidepressant drug screening

1. Introduction

Corticotropin-releasing factor (CRF) is a 41 amino acid polypeptide, regarded as the main regulating factor in pituitary ACTH and β-endorphin secretion [30]. CRF also seems to act as a neuromodulator in extrahypothalamic regions, since anatomical localization of CRF-immunoreactive neurons is not restricted to the neurosecretory system [29]. Intracerebral infusion of this peptide produces physiological and behavioral effects similar to those produced by stress, so CRF has therefore been suggested as a mediator in responses to stressful situations [8,24]. In addition, it has been proposed that this peptide plays a central role in the mediation of both activational and inhibitory behavioral responses involved in different strategies for coping with stressful stimuli [34]. Thus, several authors have shown the involvement of endogenous CRF in coping behaviors, which resulted in adaptive responses to stressful situations [12,16]. Recently, a CRF-like molecule has also been thought to act through the CRF receptors to mediate stress-induced behaviors, either alone or in concert with CRF [32].

CRF has also been shown to affect acquisition and/or retention of several learning paradigms. Overall, higher doses of CRF seem to impair these processes, and lower doses to improve them, although these effects also depend on the learning task employed. Hence, an intra-amygdala CRF (0.1 μg) microinjection administered after training in an inhibitory avoidance task improved retention both 24 h [21] and 1 week later [20]. In a similar task, it was found that CRF (0.05 and 0.1 μg) microinjections intra-locus coeruleus [5] and into the dentatus gyrus of the hippocampus [19] also improved retention 24 h later. Finally, intracerebroventricular administration of a CRF-binding protein ligand inhibitor (which results in increased endogenous CRF levels) dose-dependently facilitated retention in an

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inhibitory avoidance task and in an active avoidance paradigm in aged animals [14]. In addition, an intrahippocampal injection of antisense oligonucleotides directed against CRF has been shown to impair memory retention in an inhibitory avoidance task [35]. Likewise, intracerebroventricular CRF (0.1 μg), administered either 1 h before retest or 1 h after training, impaired retention in a similar task [27]. Others [18] have reported that intracerebroventricular CRF (0.05, 0.1 and 0.2 μg) failed to affect retention in a sucrose-motivated appetitive task, yet showed a memory-enhancing effect in a footshock-conditioned aversive task, suggesting involvement of the CRF system in the differential improvement of memory in rats exposed to aversive situations.

The rat forced swimming test (FST) is a non-escapable stressful situation and, as such, is widely used for screening potential antidepressant drugs [26]. However, it has also been suggested that the behavior displayed in the FST represents a learned adaptive response to this inescapable situation, and the involvement of learning and memory processes in such behavior [3,7,11,15,33]. We recently studied the role that CRF might play in the FST [9], using high doses of intracerebroventricular CRF following two different administration schedules: pre-test, as first stated [26], and post-test, to study the involvement of memory processes in this behavioral procedure [9]. We found that: (a) intracerebroventricular microinjection of CRF (0.5, 1 and 3 μg) administered pre-test, dose-dependently increased swimming; (b) the 1-μg dose of intracerebroventricular CRF proved ineffective when administered post-test to evaluate retention of swimming response; (c) the intracerebroventricular CRF antagonist alone (25 μg) produced an impairment in retention of this behavioral response when administered post-test, but proved ineffective when administered pre-test. These results showed a differential effect of CRF and the CRF antagonist depending on the administration schedule, and supported the idea that endogenous CRF is necessary for an adequate behavioral response in the FST. The present study sought to expand upon this prior work by examining the effects produced by low doses of intracerebroventricular CRF on this behavioral response in an experiment designed in two phases, using the two previously mentioned administration schedules.

2. Materials and methods

2.1. Subjects

Male Wistar rats (*Rattus norvegicus albinus*, Criffa, France), weighing 240–260 g at the start of the experiment, were housed in a room maintained at 20±2°C, with food and water constantly available, under a controlled light–dark cycle (light 08:00–20:00 hours), in facilities complying with the European Communities Council Directive of 24 November 1986 (86/609/EEC). In order to minimize the effects of non-specific stress, subjects were handled and acclimatized to the animal quarters for 1 week prior to any experimental procedure.

2.2. Surgery and verification of cannula placement

Rats were anesthetized intraperitoneally with a mixture of atropine (1 mg/ml, Palex), ketamine (40 mg/ml, Parke-Davis) and diazepam (5 mg/ml, Roche), and mounted in a Narishige stereotaxic instrument. Subjects were unilaterally implanted with a 23-gauge stainless-steel guide cannula 1 mm above the right lateral ventricle. The cannula was fixed to the skull with screws and dental cement. The implantation coordinates used were A/P, −0.8 mm from bregma; M/L, 1.5 mm; D/V, 3.5 mm from surface to skull [25]. Following surgery, a 30-gauge stylet was placed into the guide cannula and rats were allowed to recover for at least 1 week. Immediately after the experiments, rats received intracerebroventricular dye microinfusions and were sacrificed by decapitation. Cannula placement was verified by visual examination of slices made with a cryostat (Reichert-Jung, France), using a transmitted-light stand (bright/dark field). Only data from those rats with correct dye localization in the ventricular system were included in the data analysis. This verification was performed without knowledge of the behavioral response of each animal.

2.3. FST

Individual rats were forced to swim inside a Plexiglas cylinder (height: 60 cm, diameter: 19 cm) containing 19 cm of water at 25°C. Subjects were removed after 15 min in the cylinder (SESSION1) and allowed to dry. After 24 h, the subjects were returned to the cylinder and forced to swim for 5 min (SESSION2). This latter procedure was repeated every 24 h for 3 days more (SESSION3, SESSION4 and SESSION5). In the second phase of the experiment, we included an additional session (SESSION6) that was held 12 days after SESSION5, with no treatment between the two sessions, to test long-term retention of the behavioral response in the FST [22]. An automatic recording system (Panlab Animal Activity System, Panlab, Barcelona) was used to measure swimming, as described previously [9]. Swimming was represented primarily by struggling behavior (vigorous activity) because minimal changes due to swimming or floating could not be detected with the procedure used.

2.4. Intracerebroventricular microinjection procedure and drugs

Microinfusions were administered in a volume of 2 μl, using a 30-gauge injector connected to a Hamilton microsyringe (CR-700-20) by PE-20 tubing. The injector was left in place for 60 s to prevent backflow leakage and the stylet was then replaced. Rat/human CRF (Sigma; Spain) was

dissolved in artificial cerebrospinal fluid (CSF) and the pH adjusted to 7.4 by bubbling with CO_2. Solutions were divided into aliquots, frozen and stored. Peptide and control solutions were infused following two different administration schedules. In the first phase of the experiment, microinjections were administered 23.45, 5 and 1 h immediately before SESSION2, as previously reported [26]. Microinjections were given 1 h before the other sessions (SESSION3, SESSION4 and SESSION5). In the second phase, microinjections were given 1 h before SESSION1 and immediately after the other sessions (SESSION2, SESSION3 and SESSION4).

2.5. Experimental procedures

The present experiment was designed in two phases to test the effects of low doses of intracerebroventricular CRF on swimming, when administered following two administration schedules, pre-test and post-test. For this purpose, in the first phase of the experiment, 35 male rats were randomly assigned to three groups. Subjects were infused intracerebroventricularly with 0, 0.01 and 0.1 μg of CRF before the test. In the second phase, 40 male rats were randomly assigned to three groups. Subjects were infused intracerebroventricularly with 0, 0.03 and 0.1 μg of CRF. As the dose of 0.01 μg failed to produce any effect in the first phase of the experiment, we chose a threefold higher dose of intracerebroventricular CRF that had been shown to affect retention of an inhibitory avoidance task [31]. To better evaluate the CRF effect, swimming in SESSION1 was measured in two ways, namely, during the first 5 min and throughout the entire session (15 min). Here, we included an additional session (SESSION6), conducted 12 days after SESSION5 with no treatment in the intervening period, to test long-term retention of this behavioral response. To enable comparison with our previous results [9], a group treated with a high dose of CRF (1 μg) was also included in the two phases of the experiment.

2.6. Statistical analysis

Swimming (number of impulses) per session was compared by using a repeated measure analysis of variance (ANOVA). 'Treatment' was the between-subjects' factor and 'session' was the within-subjects' factor. In the first phase of the experiment, the data were statistically processed as follows: swimming in SESSION2, SESSION3, SESSION4 and SESSION5 was expressed as the percentage of change in relation to the first 5 min of SESSION1 (baseline value), as previously reported [7]. In the second phase of the experiment, SESSION1 could not be used as a baseline value since it was affected by treatment. After significant ANOVA, post hoc comparisons were run on individual means, using the Duncan Multiple Range Test.

3. Results

Fig. 1 shows the effect of different doses of intracerebroventricular CRF on swimming in several sessions of the FST, when administered before the test in the first phase of the experiment. Statistical analysis revealed a significant effect of 'treatment', $F(3, 19) = 8.94$, $P = .001$, and 'session', $F(3, 57) = 10.56$, $P < .001$. The 'treatment' × 'session' interaction was not statistically significant. Our data showed that doses of 0.1 and 1 μg of intracerebroventricular CRF produced significant increases in swimming throughout all sessions of the FST, except in the last session with 0.1 μg of CRF. The lowest dose of CRF (0.01 μg) proved ineffective.

Fig. 2A, B and C shows the effect of different doses of intracerebroventricular CRF on swimming during several sessions of the FST, when administered after the test in the second phase of the experiment. Fig. 2A shows intracerebroventricular CRF effect on swimming in the first 5 min and throughout the entire 15 min of SESSION1. Fig. 2B shows intracerebroventricular CRF effect on swimming during 5 min of SESSION2 to SESSION5. Statistical analysis revealed a significant effect of 'treatment', $F(3, 26) = 13.27$, $P < .001$), 'session', $F(5, 130) = 23.35$, $P < .001$, and a 'treatment' × 'session' interaction, $F(15, 130) = 2.41$, $P < .01$). Our results show that low doses of intracerebroventricular CRF (0.1 and 0.03 μg) failed to produce any effect in SESSION1. However, when we tested the high dose of CRF (1 μg) for comparison with previous results, we found a similar and significant increase in swimming during

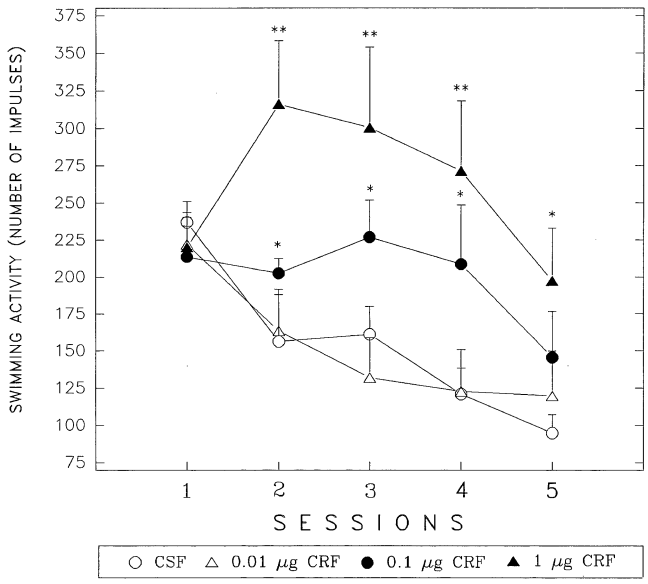

Fig. 1. Effects of different doses of intracerebroventricular CRF on swimming in several sessions of the FST, when administered pre-test in the first phase of the experiment. Data represent mean swimming (±S.E.M.) in each session, as measured by the number of impulses. The treatment groups were as follows: CSF ($n = 5$), 0.01 μg ($n = 5$), 0.1 μg ($n = 7$) and 1 μg ($n = 6$). Duncan test: $*P < .05$, $**P < .01$ vs. CSF group.

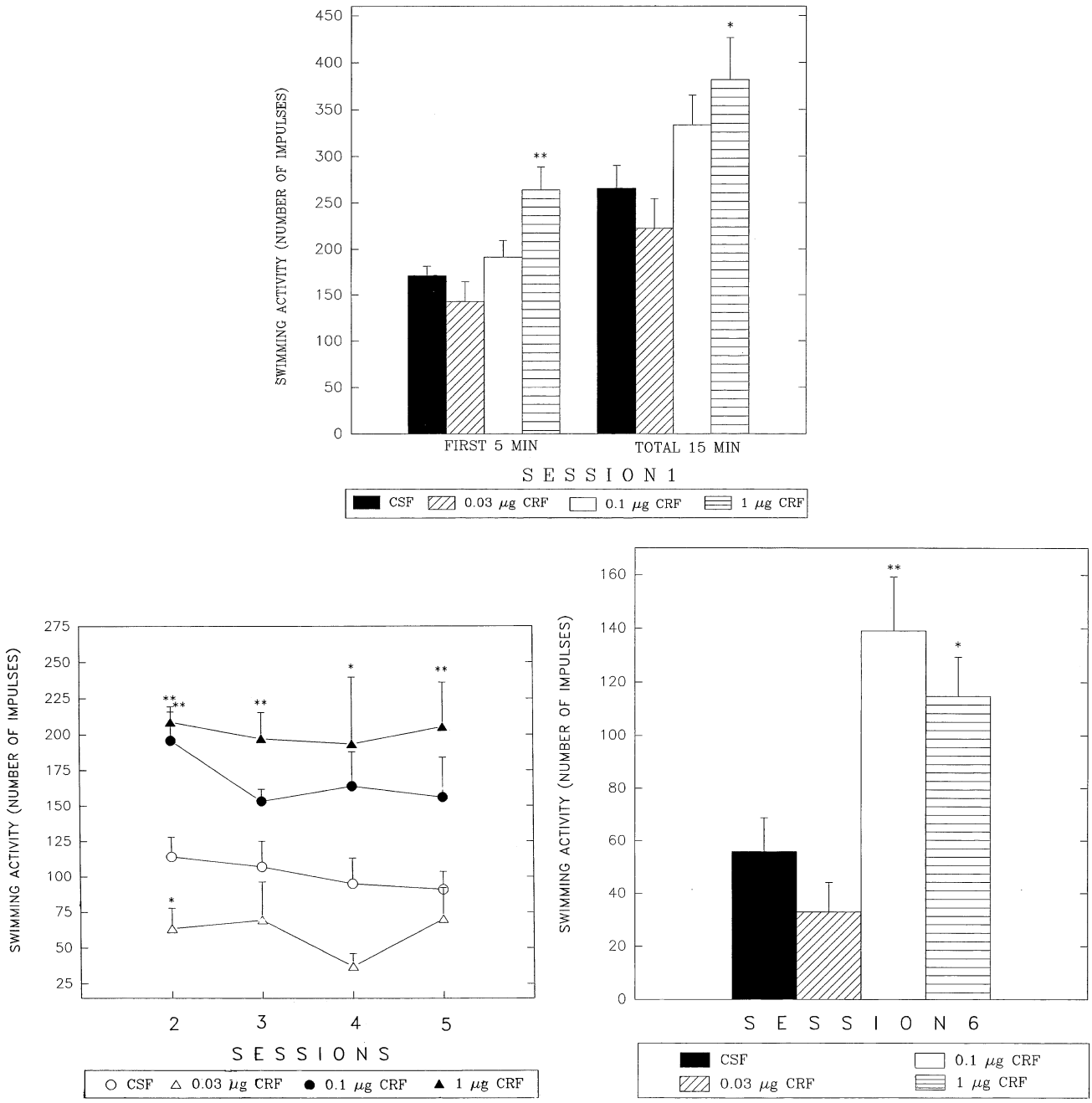

Fig. 2. (A) Effect of different doses of intracerebroventricular CRF on swimming in the first 5 min and throughout the entire 15 min of SESSION1 of the FST, when administered according to a different schedule in the second phase of the experiment. (B) Effect of different doses of intracerebroventricular CRF on swimming during 5 min of SESSION2 to SESSION5 of the FST, when administered post-test in the second phase of the experiment. (C) Effect of different doses of intracerebroventricular CRF on swimming in an additional session (SESSION6) of the FST, held without any intervening treatment 12 days after SESSION5, to test long-term retention of this behavioral response. Data represent mean swimming (±S.E.M.) in each session, as measured by the number of impulses. The treatment groups were as follows: CSF ($n=9$), 0.03 μg ($n=6$), 0.1 μg ($n=8$) and 1 μg ($n=7$). Duncan test: * $P<.05$, ** $P<.01$ vs. CSF group.

the first 5 min ($P<.01$) and the entire 15 min ($P<.05$) of this session. In the other sessions, the dose of 0.1 μg of intracerebroventricular CRF produced an increase in swimming that reached significance in SESSION2 ($P<.01$), while the high dose of CRF (1 μg) maintained statistically significant increases in all the sessions of the FST. On the contrary, the lowest dose of CRF (0.03 μg) produced a decrease in swimming in all sessions, though this only reached significance in SESSION2 ($P<.05$).

Fig. 2C shows the effect of different doses of intracerebroventricular CRF on swimming in an additional session (SESSION6) of the FST, conducted without any intervening treatment 12 days after SESSION5, to test long-term retention of this behavioral response. Data obtained in this last

session showed that, whereas statistically significant increases in swimming were induced by 0.1 μg ($P < .01$) and 1 μg ($P < .05$) doses of CRF, decreases produced by the lowest dose of CRF (0.03 μg) failed to attain significance.

4. Discussion

Results obtained in this study show that low doses of intracerebroventricular CRF are effective in the FST, but this effectiveness depends on the administration schedule used. In the first phase of the experiment, a low dose of CRF (0.1 μg, but not 0.01 μg) produced significant increases in swimming throughout the sessions when it was administered before the test. This CRF effect on swimming may be explained by a dose-dependent increase in behavioral activation, as has been previously suggested [4,9], probably due to the contiguity between intracerebroventricular CRF microinjection and behavioral testing. However, these CRF-induced increases in swimming were progressively lower during the sessions, suggesting a possible behavioral adaptation to consecutive intracerebroventricular CRF microinjections, as shown by other authors [1,17].

When we changed the administration schedule in the second phase of the experiment, we found that the dose of 0.1 μg of intracerebroventricular CRF led to significantly increased swimming only in SESSION2. Due to the fact that swimming in this session was evaluated 24 h after intracerebroventricular administration of CRF, we understand that CRF affected swimming by acting on the retention process of this behavioral response. As we have previously mentioned (see Introduction), it has been proposed that behavior in the FST is a learned response in which memory processes are involved [3,7,11,15,33]. In addition, it has been suggested that stress responsive hormones could play a modulator role in these memory processes [33]. Accordingly, administration of anisomycin, an antibiotic that impairs memory, produced an increase in swimming in SESSION2 by impairing retention of this behavioral response 24 h later [7]. Likewise, in the present work, intracerebroventricular microinfusion of 0.1 μg of CRF might produce an impairment in retention that resulted in increased swimming. Similar results were obtained in the last additional session (SESSION6, 12 days after SESSION5, with no intervening treatment) with this same dose of CRF, again suggesting a CRF-induced impairment of retention and the involvement of memory processes in this behavioral response. In contrast to the results found with the dose of 0.1 μg, a lower dose of CRF (0.03 μg) produced a decrease in swimming that could be interpreted as an improvement in retention 24 h later (SESSION2). However, this improvement was not statistically maintained in the other sessions. What is more, the lowest dose of CRF proved ineffective in long-term retention of this behavioral response.

We previously found that certain levels of endogenous CRF are necessary to produce an adequate behavioral response in the FST (i.e. decreased swimming during the sessions) [9]. Thus, we showed that the lack of endogenous CRF, due to the intracerebroventricular administration of CRF antagonist alpha-helical CRF-(9–41), produced an impairment in long-term retention that resulted in increased swimming. It is possible that 0.03 μg of CRF could be too low a dose to maintain the improvement in retention shown in SESSION2 throughout all the sessions, especially if behavioral adaptation to intracerebroventricular CRF occurred. In contrast, the dose of 0.1 μg of CRF may lead to a degree of hyperactivity in the CRF system, which might then interfere with learning and retention of stress-related behaviors. In this regard, it has recently been shown that hyperemotionality produced by central overexpression of CRF in transgenic mice resulted in impaired learning and retention in several spatial tasks, an impairment which was then normalized by central administration of the CRF antagonist alpha-helical CRF-(9–41) [13,28]. Altogether, these data suggest that an adequate level of CRF might be necessary to improve behavioral response in the FST, while a lack or an excess of CRF could impair this response.

A high dose of CRF (1 μg), microinjected following both administration schedules, before and after the test, was included in this work as a control of previous results [9]. This dose produced similar behavioral activation in the present and in the previous study when it was administered before the test. However, it affected retention only in the present study and not in the previous one, when administered after the test. It is possible that these differences may be related to endogenous CRF levels. It has been suggested that swim stress could affect the endogenous CRF system by increasing CRF mRNA [10] or by altering CRF receptors [6]. In addition, it has been proposed that intracerebroventricular CRF might initiate a sequence of events that would lead to a long-lasting release of endogenous CRF [23]. Moreover, seasonal rhythms in hypothalamic CRF content have been reported with maximal levels in rats in spring [2]. Since the present work was conducted during that period, the CRF effect on retention produced by the dose of 1 μg found in the present study could be due to a greater release of endogenous CRF after intracerebroventricular CRF microinjection.

Data from this work suggest that the effectiveness of low doses of intracerebroventricular CRF on swimming is different, depending on the administration schedule used. Low doses of CRF seem to modulate swimming by increasing behavioral activation when administered pre-test and by affecting (impairing or improving) the retention process of this behavioral response when administered post-test. However, a distinct threshold of intracerebroventricular CRF dose seems to be necessary to differentially affect retention and to produce behavioral activation in consecutive sessions of the FST. Thus, in the first phase of the experiment, the dose of 0.1 μg produced an increase in swimming behavior

by increasing behavioral activation throughout the sessions. In the second phase of the experiment, the dose of 0.1 μg of CRF produced an increase in swimming behavior by an impairment in retention that was maintained long term. On the contrary, the dose of 0.03 μg produced a decrease in swimming behavior by an improvement in retention that could not be maintained long term.

To summarize, preliminary data obtained with low doses of CRF suggest that a differential effect on retention of swimming response seems to exist depending on the dose, whereas a high dose of CRF clearly impairs retention. More studies will need to be done to confirm that the trend shown by these low doses on retention is consistently significant. Since the FST is widely used to screen potential antidepressant drugs, the results of the present work involving memory processes should be taken into account in pharmacological studies with this behavioral test.

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