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Behavioral effects of the CRF_1 receptor antagonist R121919 in rats selectively bred for high and low activity in the swim test

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Summary This study assessed effects of a CRF_1 receptor antagonist, R121919, on the behavior of rats that have been selectively bred to exhibit very high or very low activity in a swim test. Following treatment with R121919 (10 mg/kg, s.c.) or vehicle, several types of behavior were examined including: (1) spontaneous ambulatory activity in a novel environment, (2) swim-test activity, (3), and responses in an elevated plus maze. The most pronounced effects were observed in the swim test. Although R121919 had little effect on the swim-test behavior of normal, non-selected rats, Swim High-active rats (SwHi), characterized by being very active and exhibiting pronounced struggling behavior in the swim test, showed increased activity (more struggling) after R121919; in contrast, Swim Low-active (SwLo) rats, characterized by being very inactive and exhibiting pronounced floating behavior in the swim test, showed decreased activity (more floating) after R121919. This effect was observed in both male and female rats. No differences between strains or the effects of R121919 were observed for spontaneous ambulation or in the elevated plus maze test.

1. Introduction

Preclinical laboratory animal studies remain one of our most important tools in behavioral neuropharmacology for identifying novel behaviorally active compounds. One of the classic methods for testing potential novel antidepressants is the forced swim test developed by Porsolt et al. (1978a,b). In the usual procedure for this paradigm, a rat is placed in a tank of water, removed after 15 min, and then returned to the tank the following day for a 5 min swim test. During the initial exposure to the swim tank, the rat engages in active responses in an attempt to escape from the tank, but these responses are unsuccessful in affecting escape and such active behavior diminishes. As a consequence when the animal is re-exposed to the tank (i.e., for the 5 min exposure), passive behavior (i.e., immobility or floating) is mark-

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edly increased. Administration of most current antidepressant drugs prior to the second exposure decreases the time spent engaged in this floating behavior.

We have used selective breeding to generate a strain of rats that exhibit very low activity in the swim test (designated the "Swim Low-active" or "SwLo" rat) as well as a strain that shows very high activity in the swim test (designated the "Swim High-active" or "SwHi" rat) (Weiss et al., 1998). SwLo rats show much less activity in the swim test than do randomly bred Sprague–Dawley rats whereas the SwHi rats show considerably more activity than do normal rats. Interestingly, although SwLo and SwHi show large differences in activity in the swim test, these two lines do not exhibit marked differences in ambulatory activity in the home cage when monitored across 24 h of the day, and SwLo rats have been found to exhibit more activity than SwHi rats in an open field; thus, the differences in the swim test are not attributable to gross differences in motor activity per se.

This paper evaluates the behavioral effects of the novel, putative antidepressant R121919, a corticotropin releasing factor type 1 receptor (CRF_1) antagonist, in these two rat strains. The goal was to determine if blockade of CRF_1 receptor signaling would produce different behavioral effects in rats that show very different behavioral propensities in a test widely used to assess antidepressant drug potential.

Corticotropin-releasing factor (CRF), a 41-amino acid neuropeptide, is generally considered to be the key mediator of the mammalian stress response (Owens and Nemeroff, 1991; Pelleymounter et al., 2002; Smagin and Dunn, 2000). The actions of CRF, and the related molecules urocortin, urocortin II, and urocortin III, are mediated primarily through the well characterized CRF receptors (CRF_1 and CRF_2) that are distributed throughout the brain in regions known to modulate the stress response (Primus et al., 1997; Smagin and Dunn, 2000; Van Pett et al., 2000). Reduction in CRFergic signal transduction by either pharmacological or gene mutation strategies has been shown to decrease anxiety and depression-like behaviors in animals (Bale et al., 2000; Kishimoto et al., 2000; Pelleymounter et al., 2002; Steckler and Holsboer, 1999; Timpl et al., 1998; Valdez et al., 2002). Based partly on these observations, CRF receptor antagonists have been postulated to possess anxiolytic and antidepressant actions (Steckler and Holsboer, 1999). R121919 has previously been found to exhibit anxiolytic effects in the defensive withdrawal paradigm with rats (Gutman et al., 2003; Heinrichs et al., 2002), and the results of a small open-label trial with R121919 in depressed patients also suggested that R121919 possesses antidepressant and anxiolytic properties (Zobel et al., 2000).

The effects of CRF are complex, influenced by both environmental and genetic factors. For example, CRF administered intracerebroventricularly has been shown to produce depression-like behaviors in monkeys, but only when the animals were housed together with peers (Strome et al., 2002). Studies in rodents also have found context-dependent effects; for example, CRF had activating effects when given to rodents that were in a familiar environment but produced anxiety-like "withdrawal" behaviors when given to animals that were in novel environments (Sutton et al., 1982). Perhaps most relevant to the present study, Keck et al. (2001) reported that anxiolytic effects of the CRF antagonist R121919 were only observed in rats that were highly anxious (i.e., had been selectively bred for this characteristic), whereas anxiety-related behaviors of rats that were not highly anxious were unaffected by the CRF_1 receptor antagonist.

A number of recent studies have investigated the activity of CRF_1 receptor antagonists in swim-test models. The CRF receptor antagonist CP-154,526 was shown to be active in rat, but not mouse, forced swim-test models (Hodgson et al., 2007). The CRF_1 receptor antagonist SSR125543 administered at doses ranging from 3 to 30 mg/kg/day for 14 days significantly increased swimming in the Flinders Sensitive Line rats (Overstreet and Griebel, 2004). In this same study, the selective serotonin reuptake inhibitor (SSRI) fluoxetine (5 mg/kg, i.p.) and the tricyclic (TCA) antidepressant despiramine (5 mg/kg, i.p.) also produced significant increases in swimming behavior (Overstreet and Griebel, 2004). The CRF_1 receptor antagonists antalarmin, CP-154,526, LWH234 and R121919 have also been investigated in a single study compared to controls in the forced swim test (Jutkiewicz et al., 2005). Although all agents had some effect on reducing swim stress-induced HPA axis activation, only LWH234 produced significant changes in swim behavior.

The present study sought to determine if antagonizing CRF_1 receptor-mediated neurotransmission would have different effects in animals that had been specifically selected for very different levels of motor activity in a swim test. In addition, we further characterized the behavioral profile of these rats by examining effects in the elevated plus maze (EPM).

2. Methods

2.1. Animals

All animals were housed in standard 45 cm × 25 cm × 15 cm clear polycarbonate cages living directly on bedding in groups of two to three animals per cage. The cages were contained in enclosed laminar flow racks (Lab Products Inc., NJ, USA). Standard laboratory rat chow and water was available *ad libitum* for all animals. The colony room was maintained on a 12-h light:12-h dark cycle (lights on 07:00–19:00 h). No cage changes were performed on the day prior to any experimental manipulation.

Unless otherwise noted, all animals in these experiments were obtained from the "clean" immunological colony at the Emory West facility. These animals were the offspring of rats that had been caesarian derived from the 15th generation of the original SwHi/SwLo stains developed in our laboratory, with these particular rats subsequently maintained under virus-antigen-free conditions in the immunological colony. These "clean" SwHi/SwLo rats show similar swim scores to SwHi/SwLo rats maintained in the general colony. Also included in the studies described here are "control" rats that have been maintained in our colony since the inception of this breeding program; these have been bred randomly (i.e., bred without regard to swim-test scores) and are referred to as Swim-Non-selected rats (SwNs). Throughout the entire breeding cycle, brother–sister breeding was avoided in each generation. All of the experiments were approved by the Emory University Institutional Animal Care & Use Committee.

2.2. Drug treatment

The selective nonpeptide CRF_1 receptor antagonist, R121919, was generously supplied by Janssen Pharmaceutica (Beerse, Belgium). R121919 was dissolved in a 0.1 M sodium acetate buffer (Sigma, St. Louis, USA) adjusted to a pH of 4.5. R121919 solutions were prepared at a final concentration of 20 mg/ml and injected subcutaneously at a dose of 10 mg/kg. Previous work has indicated that this dosage results in occupancy of at least 80% of central nervous system (CNS) CRF_1 receptors during the time frame employed in these studies (Gutman et al., 2003).

2.3. Experiment 1

2.3.1. Activity test

In the first part of the study, three groups of male animals, SwHi ($n = 16$), SwLo ($n = 16$), and SwNs ($n = 16$) were tested, followed by testing in the second part of three groups of females from the same generation (SwHi, $n = 16$; SwLo, $n = 8$; SwNs, $n = 8$). Animals were 3–5 months of age at the time of testing; males weighed 400–500 g and females weighed 300–350 g. The experiment was conducted between 09:00 and 13:00 h.

Spontaneous ambulatory activity was measured for 20 min in a novel environment. On the day of testing, each animal was removed from its home cage, injected subcutaneously with either vehicle or the CRF_1 antagonist R121919 (10 mg/kg), and then immediately returned to its home cage. Behavioral testing took place beginning 45 min after injection. At this time, animals were transported to an adjacent testing room, and 5 min later were placed into a novel environment (a 40 cm × 45 cm clear plexiglas container) covered with bedding and containing 3 objects. Spontaneous locomotor activity was assessed by a photocell assembly (ANA 1219, Riverpoint Electronics) mounted in a frame; the frame contained an array of eight infrared light beams spaced equidistant from one another along the length of the cage. Movement of an animal within its cage resulted in interruptions of the light beams that were monitored electronically. The recording computer recognized any change in the pattern of light beams and responded by recording a new "sentence" in memory (a sentence described the status of the eight light beams, defining whether each was interrupted or uninterrupted). Ambulatory activity was distinguished from repetitive movements in the same location as follows: a unit (or count) of ambulatory activity was recorded if a newly generated sentence in memory included a change in the status of a beam that had not been altered in the previous 4 sentences; for this to occur required the animal to move into the field of a new beam. Spontaneous locomotion was assessed for 20 min and analyzed in 4 min periods.

2.3.2. Swim test

The swim test was conducted immediately after spontaneous locomotor activity testing was completed. The animal was removed from the locomotor apparatus and brought into an adjacent room where a set of "water wings" was affixed to the animal. These "water wings" consisted of a lightweight plastic bubble (made from "bubble pack" packing material), which was placed onto the midscapular area of the animal's back and held in place by a 1/2 in. wide strip of adhesive tape wrapped around the animal's sternum which held the bubble in place. This bubble does not restrict movement of the animal in any way while enabling all rats to float without sinking if they should cease movement in the swim tank. The animal was then transported to another adjacent room where the swim test was conducted. Animals entered the swim test approximately 80 min after receiving an injection of R121919 or vehicle.

The swim test was a modification of that initially described by Porsolt et al. (1978a), with the major modification being that deeper water is used in this case; the particular test used here has been previously described in considerable detail in an earlier publication (e.g. Weiss et al., 1998). The swim tank is a Plexiglas cylinder 65 cm tall and 30 cm in diameter filled with water (25 °C) to a height of 14 cm from the top. The room in which the test is conducted is darkened except for a 25 W light bulb illuminated 60 cm above the tank. The tank is surrounded by a black Plexiglas screen that serves to exclude any visual distractions outside the tank. With the floatation bubble in place, the animal was dropped into the water from approximately 20 cm above the surface of the water so that the entire body of the rat dips below the surface of the water on entry into the water. The animal remained in the swim tank for 15 min, after which the animal was removed, dried lightly with a towel, and returned to its home cage. During the 15 min swim test, the activity of the animal in the tank was recorded by videotape which was subsequently observed by an investigator who did not have knowledge of the experimental condition of the animal being scored. This investigator viewed the videotape and timed the duration of the two types of motor activity shown by the rat in the swim tank: (1) struggling, which was defined as vigorous movement of all paws with the forepaws breaking the surface of the water and (2) floating, which was defined as the animal remaining motionless with no movement of limbs. As reported previously (Weiss et al., 1998), reliability coefficients for the rating (timing) of these categories of behavior by different investigators have always been found to be above 0.90.

2.3.3. Elevated plus maze

For the elevated plus maze, two groups of male rats, SwHi, $n = 16$ and SwLo, $n = 12$ were tested. These animals were not the same as those used in Experiment 1, and these rats had not been exposed to the swim test prior to testing. The rats weighed approximately 300–350 g on their initial day of testing; the procedure was conducted between 09:00 and 13:00 h.

Another group of 40 non-selected Sprague–Dawley rats (SwNs; not SwHi or SwLo) were also tested on the elevated plus maze. Animals were injected subcutaneously with either vehicle or R121919 (10 mg/kg) in their home cage. The 40 non-selected Sprague–Dawley rats each received one of five doses of R121919 ($n = 8$ per dose); these doses were 0, 1.0, 3.16, 10.0 or 31.6 mg/kg subcutaneously. Sixty minutes after the injection, animals were transferred to an adjacent testing room for the test. The test was begun by placing the animal on the central platform of the maze facing into a closed arm. Animals remained on the plus maze for 5 min. Between testing of each animal, the maze was thoroughly cleaned with 70% ethanol. All sessions were videotaped for subsequent analysis.

The elevated plus maze consisted of two 50 cm enclosed arms (i.e., arms with 40 cm high walls at the sides), and two 50 cm open arms (i.e., arms with no walls at the sides). The four arms were arranged in a cross pattern extending out from a common 10 cm × 10 cm platform at the center where all arms met. The floor of the maze was mounted at a height of 50 cm above the floor of the room. Testing was conducted under low ambient lighting, and was approximately 75 lx. All testing on the elevated plus maze took place late in the light phase of the light/dark cycle (i.e., between 14:00 and 17:00 h) as we have found these conditions to produce the most consistent behavioral results in our laboratory.

Behavior on the elevated plus maze was analyzed by Ethovision Color Pro for Windows v2.3.19 (Noldus Information Technology, The Netherlands). This program assessed the total time that animals spent on the center platform and on open and closed arms, as well as the total distance moved.

2.3.4. Statistics

In all cases, data analysis was conducted by analysis of variance (ANOVA); the particular analysis used is described in the conjunction with each experiment. For post hoc comparisons of individual conditions, which were carried out when statistically significant main effects were found, Bonferroni *t*-tests were used, with statistically significant comparisons indicated in the text by noting a p value of <0.05.

3. Results

3.1. Locomotor activity

The results from measurements of spontaneous exploratory activity are shown in Fig. 1. Inspection of these data shows that whereas some differences were seen between the three rat strains, drug treatment had little effect on the spontaneous activity of the strains. A repeated measures ANOVA across time periods (five 4 min periods), with strain, gender, and drug treatment as other main factors, indicated there was a significant effect of time ($F[4,68] = 147.8$, $p < 0.001$), strain ($F[2,68] = 17.9$, $p < 0.001$), and gender ($F[1,68] = 10.3$, $p < 0.002$), but there was no significant effect of drug treatment ($F(1,68) = 0.04$, $p = 0.847$). The time × strain interaction was also significant ($F[8,130] = 6.2$, $p < 0.001$), apparently reflecting that SwHi rats differed from the other strains by being particularly active in the first 4 min period and then less active in subsequent time periods. Comparisons between the three individual strains (by Bonferroni *t*-tests) revealed that both SwHi and SwLo rats showed overall a significantly higher level of exploratory activity than did the SwNs rats (each comparison $p < 0.05$), but SwHi and SwLo rats did not significantly differ from each other.

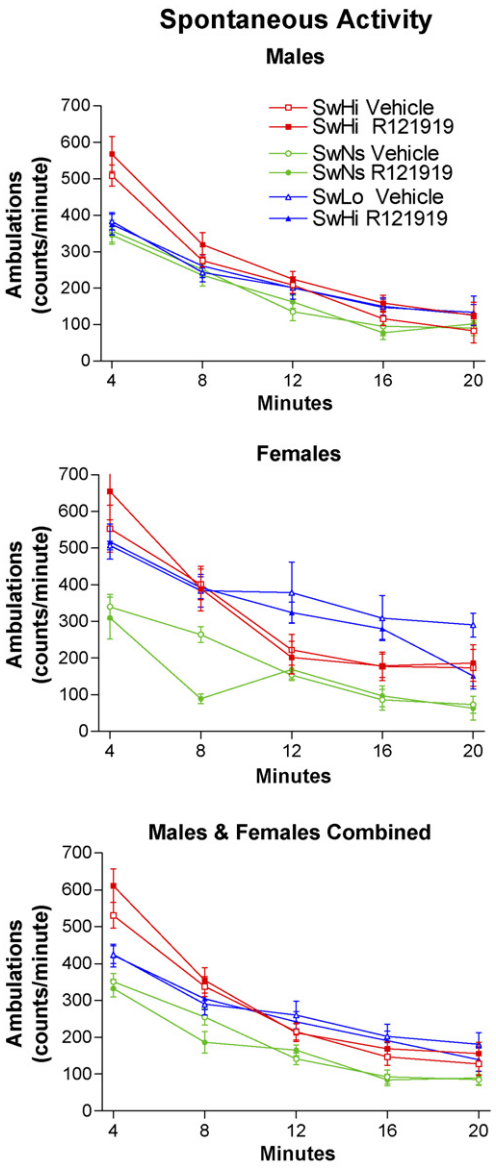

Figure 1 Spontaneous ambulatory activity of Swim High-active (SwHi), Swim Non-selected (SwNS), and Swim Low-active (SwLo) rats following injection of a CRF_1 receptor antagonist R121919 (10 mg/kg) or vehicle. Activity was recorded for 20 min beginning 50 min after injection. Shown are means (±standard errors) for the groups in 4 min bins; the value shown at each time point is for the 4 min period that precedes designation shown on the ordinate (thus, activity shown above "4" is mean activity seen in the 4 min ending at 4 min). Data are shown for males (at top), females (center), and both males and females combined (bottom). See text in Results section for description of any statistically significant differences between groups.

3.2. Swim test—struggling behavior

Struggling behavior observed in the swim test is shown on the left side of Fig. 2. As expected, SwHi rats spent much more time struggling than did SwNs and SwLo rats. As also can be seen on the left side of Fig. 2, R121919 increased the struggling behavior of SwHi rats but not of SwNs and SwLo rats. When time spent struggling was analyzed by a three-way ANOVA with gender, strain, and drug treatment as main factors, the analysis yielded significant main effects of strain ($F[2,68] = 259.9$, $p < 0.001$) and gender ($F[1,68] = 4.3$, $p = 0.041$), while the effect of drug treatment approached significance ($F[1,68] = 3.9$, $p = 0.052$). Most importantly, the strain × drug treatment interaction was highly significant

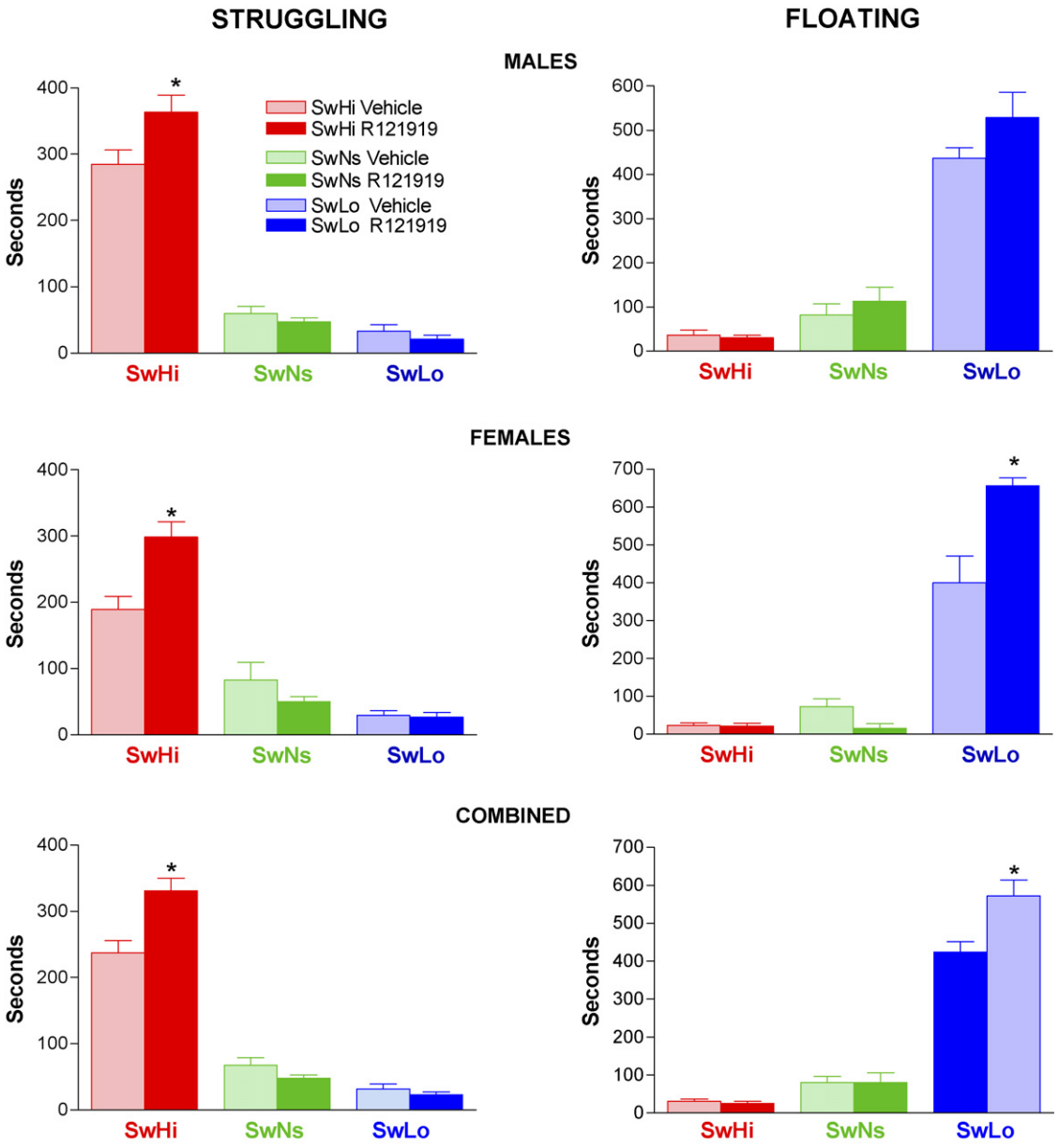

Figure 2 Swim test activity of Swim High-active (SwHi), Swim Non-selected (SwNS), and Swim Low-active (SwLo) rats approximately 80 min following s.c. injection of a CRF_1 antagonist R121919 (10 mg/kg) or vehicle. Swim test was conducted immediately after spontaneous activity shown in Fig. 1. Struggling behavior is depicted on the left; floating behavior is in the right graphs. Shown are means (±standard errors) for struggling activity and floating behavior in the 15 min swim test; the value shown at each time point is for the 5 min period that precedes designation shown on the ordinate (thus, activity shown above "5" is mean activity seen in the 5 min ending at 5 min) *significantly different (at least $p < 0.05$) from vehicle-injected animals of the same strain.

($F[2,68] = 13.0$, $p < 0.001$), thereby indicating that treatment with R121919 affected struggling behavior of the three rat strains differently. The ANOVA also yielded a significant gender × strain interaction ($F[2,68] = 9.0$, $p < 0.001$). Analysis of simple main effects (analyzed in separate two-way ANOVAs [strain × treatment] for males and females) confirmed that, in the SwHi rats treatment with R121919 produced a highly significant change in struggling behavior (i.e., an increase in struggling) of both males ($F[1,42] = 13.4$, $p < 0.001$) and females ($F[1,26] = 20.0$, $p < 0.001$). R121919 did not produce a statistically significant change in struggling of either males or females in SwLo and SwNS rats (see Fig. 2). This finding was also the same when animals were combined without differentiating by gender (see bottom part of Fig. 2—effect of treatment in SwHi rats: $F[1,74] = 10.7$, $p < 0.001$; in SwLo and SwNs: nonsignificant). However, close inspection of the data suggested that R121919 in fact tended to *decrease* struggling behavior of both SwNs and SwLo rats, an effect that may have been masked in the overall analysis by inclusion of SwHi rats. When a three-way analysis (gender, strain, and drug treatment) was conducted including only the SwNs and SwLo rats,

the effect of drug treatment, which decreased struggling in these strains, approached significance ($F[1,40] = 3.8$, $p < 0.06$). Thus, whereas R121919 significantly increased struggling of SwHi rats, this drug had an opposite effect on struggling behavior of SwNs and SwLo rats that approached statistical significance.

3.3. Swim test—floating behavior

Floating behavior observed in the swim test is shown on the right side of Fig. 2. As expected, SwLo rats spent much more time floating than did SwNs and SwHi rats. R121919 increased this predominant behavior of SwLo rats but did not affect the floating of SwHi and SwNs rats. When floating was analyzed by a three-way ANOVA with gender, strain, and drug treatment as main factors, the analysis yielded significant main effects of strain ($F(2,68) = 279.5$, $p < 0.001$) and drug treatment ($F[1,68] = 8.5$, $p < 0.005$), while the effect of gender was not significant ($F[1,68] = 0.1$, NS). Most importantly, the strain $\times$ drug treatment interaction was highly significant ($F[2,68] = 11.0$, $p < 0.001$), indicating that R121919 treatment affected the floating behavior of the three rat strains quite differently. Analysis of simple main effects (analyzed in separate two-way ANOVAs [strain $\times$ - treatment] for males and females) confirmed that, in SwLo rats but not in SwHI and SwNS rats, treatment with R121919 produced a significant increase in floating behavior of both males ($F[1,42] = 4.5$, $p < 0.04$) and females ($F[1,26] = 44.5$, $p < 0.001$). This was also true if animals were combined without differentiating by gender (see bottom part of Fig. 2—effect of treatment in SwLo rats: $F[1,74] = 20.5$, $p < 0.001$; in SwLo and SwNs, NS). Thus, R121919 significantly increased floating of SwLo rats but did not affect floating behavior of SwNs and SwHi rats.

3.4. Elevated plus maze

The influence of R121919 in SwHi and SwLo rats is shown on the left side of Fig. 3. These data reveal no appreciable effects of R121919 on the elevated plus behavior of either SwHi or SwLo rats. Both the percentage of time spent on open arms (top) and the total distance rats moved on the maze (bottom) were analyzed by 2 $\times$ 2 ANOVA. For the analysis of time spent on the open arms, there was no significant effect of strain ($F[1,26] = 0.3$, $p = 0.618$) or drug treatment ($F[1,26] = 2.1$, $p = 0.163$), and the strain $\times$ drug treatment interaction also was not significant ($F[1,26] = 0.2$, $p = 0.698$). For the analysis of total distance moved on the elevated plus maze (see bottom left), there was no significant effect of strain ($F[1,26] = 0.04$, $p = 0.843$) and the strain $\times$ drug treatment interaction was not significant ($F[1,26] = 0.2$, $p = 0.648$). The effect of drug treatment, however, approached significance ($F[1,26] = 3.2$, $p = 0.087$).

In a separate group of non-selected male Sprague—Dawley rats, the influence of R121919 was tested on performance on the elevated plus maze by conducting a dose-response study. These data are shown on the right side of Fig. 3. Both measures shown were analyzed by a one-way ANOVA with drug dose as the main effect variable. In these ANOVAs, dose of R121919 did not have a significant effect on either the percentage of time spent on the open arms ($F[4,35] = 0.6$, $p = 0.717$) or the total distance animals moved on the elevated plus maze ($F[4,35] = 0.4$, $p = 0.801$).

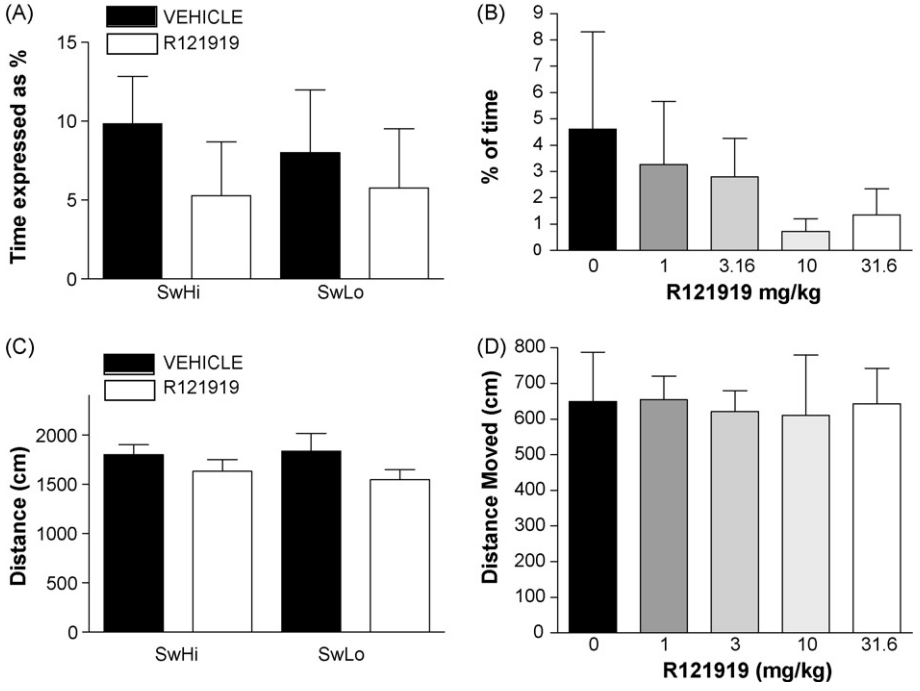

Figure 3 Measurement of behavior on an elevated plus maze for Swim High-active (SwHi) and Swim Low-active (SwLo) rats injected s.c. with a CRF antagonist R121919 (10 mg/kg) or vehicle (at left), and for non-selected (normal) rats injected with ascending doses of the CRF antagonist R121919. At top is shown the percent time spent on the open arm, and at bottom is shown the total distance moved on the maze. Means ($\pm$standard errors) are shown.

4. Discussion

The goal of this paper was to determine the behavioral effects of CRF_1 receptor antagonists in animals bred for high and low activity in a swim test. Interestingly, R121919 did not simply increase or decrease activity in the swim test; instead, the drug increased the dominant behavioral tendency of the type of animal that received the drug. Thus, in SwHi rats the drug increased their highly active behavior (struggling behavior), while having no significant effect on struggling behavior of either SwLo or SwNs rats (in which the drug actually tended to decrease struggling behavior). In SwLo rats, the drug increased their inactive responding (floating behavior), while having no significant effect on floating behavior of either SwHi or SwNs rats. As shown in Fig. 1, the influence described here was clearly observed in both male and female rats of the different rat lines. It is also of interest that in non-selected rats (SwNs), which as a group have no dominant behavioral response in the swim test, R121919 had minimal effects on their swim-test behavior. In a comprehensive review of the effects of compounds that have been tested in the forced swim test (Cryan et al., 2005), there is no report of a single compound that could produce both an increase in struggling behavior and also an increase in immobility (or floating) behavior under either different conditions or in different types of animals. In the brief test of spontaneous exploratory activity carried out prior to the swim test, and on the elevated plus maze, R121919 had no significant effect on the behavior of rats of any line.

The activity of CRF_1 receptor antagonists in various adaptations of the forced swim test has been highly variable. It should be noted, however, that differences in implementation of the classic Porsolt swim-test procedure including depth and temperature of the water, number of test days, dose and route of administration may account for some, but likely not all, of the observed differences. In a study by Hodgson et al. (2007), both rats and mice were exposed to the swim test for 15 min on day one. After the first swim exposure on day one as well as immediately before a 6 min swim on testing day two, animals received a dose of the CRF_1 receptor antagonist CP-154,526 (3—30 mg/kg, i.p.). In this experiment, 30 mg/kg of CP-154,526 (but not 3 or 10 mg/kg) reduced immobility in the swim test in rats but not mice. A second study investigated the effects of the CRF_1 receptor antagonists CP-154,526, its methyl analog antalarmin, LWH234, and the compound used in this present study, R121919, in rats at doses ranging from 3 to 30 mg/kg (Hodgson et al., 2007). Similar to the first study, animals were exposed to a 15 min swim test on day 1, but received an intraperitoneal injection with a CRF_1 receptor antagonist or vehicle 23.5, 5, and 1 h before being exposed to a 5 min swim test on day two. A somewhat more sensitive scoring method was used in this study in which both climbing, struggling, and immobility were scored in 5 s increments during the 5 min swim test (Detke et al., 1995). This method has shown increased sensitivity in detecting SSRIs, which despite currently being the most widely prescribed class of antidepressants, have failed to produce consistent results in the Porsolt swim test. In this study, only LWH234 produced significant effects in the swim test, though decreasing immobility and increasing swimming were observed only at the highest dose tested (30 mg/kg/day). In contrast to the study by Hodgson et al. (2007), CP-154,526 was not active in this experiment, although both positive controls (desipramine and fluoxetine) decreased immobility in this experiment. Similar to our results in the SwNS rats, R121919 was also not effective in this study (Jutkiewicz et al., 2005).

Griebel et al. (2002) have also reported antidepressant-like effects of both antalarmin (3—30 mg/kg) and SSR125543 (30 mg/kg, p.o.), a CRF_1 receptor antagonist, in the forced swim test. More recently, Overstreet and Griebel (2004) have also investigated the effects of chronic administration (14 days, i.p.) of SSR125543 in the Flinders Sensitive Rat line. Similar to our SwLo animals, the Flinders Sensitive Line rat shows more immobility on the forced swim test relative to its control counterpart, the Flinders Resistant Line (Overstreet, 1993, 2002). Chronic administration with the CRF_1 receptor antagonist SSR125543 (3—30 mg/kg, i.p.) for 14 days significantly increased swimming in the Flinders Sensitive Line. It should be noted that rats were only subjected to a single 5 min swim 22 h after the 14th day of drug administration.

What explanation can be made for our results? To begin with, it should be noted that a single dose of R121919 was used in these studies (i.e., 10 mg/kg), so an issue that needs to be addressed is whether this dose effectively blocked CRF_1 receptors. The dose used was selected based on binding studies examining how particular doses of R121919 affected in vivo binding of CRF in brain.(Gutman et al., 2003). These results showed that binding of CRF to brain tissue was significantly reduced at doses beginning at 5 mg/kg, and was reduced by 80—90% (the maximum obtainable) by doses of 10 mg/kg or above. Because 10 mg/kg produced the maximal occupancy of CRF receptors, this dose, which we have used in other studies (Gutman et al., 2003), was chosen for the present studies as well. Funk et al. (2007) also found R121919 significantly reduced ethanol self-administration in ethanol-dependent rats; a dose response was noted in this study with nearly identical behavioral responses (i.e., reduction in bar pressing for ethanol) at both 10 and 20 mg/kg doses of R121919; this dose was also in the middle of the dosing range used in the Jutkiewicz (2005) study where similar behavioral results were observed at both 10 and 30 mg/kg R121919 doses.

As stated above, the focus of this study was on the effect of R121919 would have on the extremes of swim-test activity for which the SwHi and SwLo rats had been selectively bred. Antagonism of CRF_1 receptors accentuated the dominant behavioral tendency of both the SwHi and SwLo rats, causing the highly active SwHi rats to show even more activity in the swim test (increased struggling) than they do normally, while causing the markedly inactive SwLo rats to show even less activity in the swim test (increased floating) than they do normally. Such findings suggest that blocking CRF_1 receptors removed an inhibitory influence on systems subserving strong behavioral propensities. Therefore, these data suggest that normal stimulation of CRF_1 receptors in SwHi and SwLo rats acts to modulate, or inhibit, the strong behavioral tendencies shown by these rats in the swim test, and does this irrespective of whether the result is an increase or decrease in motor activity.

Insofar as the most widely known influence of CRF on behavior is its effect on anxiety, fear, and stress responses (e.g. Dunn and Berridge, 1990; Owens and Nemeroff, 1991; Takahashi, 2001), might the behavioral effects described

above be explained by an influence of R121919 on the emotionality or anxiety level of the rats? Antagonism of CRF_1 receptors has usually been interpreted to produce decreased anxiety, fear or stress; therefore, the effects of R121919 could be explained if reduced fear/anxiety/stress would produce the behavioral changes seen in drug-treated SwHi and SwLo rats. However, Weiss et al. (1998) reported that swim-test behavior of SwHi and SwLo rats was not associated with any consistent difference in measures thought to reflect emotionality or anxiety, such as open field activity and defecation. Also, as reported in the present results, SwHi and SwLo rats did not differ on the elevated plus maze. Further, SwHi and SwLo have been found to show little difference in pituitary—adrenal axis reactivity in response to a stressor (Weiss et al., 1998; West et al., 1993). In summary, there is no direct evidence to suggest that a potential decrease in anxiety, fear, or stress by R121919 might affect the swim-test behavior of SwHi and SwLo rats in the manner that was observed.

What the present results highlight is that the influence of CRF on behavior can depend on the characteristics of particular animals. Blockade of CRF_1 receptors with R121919 affected swim-test activity of SwHi and SwLo rats quite differently, and the effects observed in each of these two rat lines differed from what was observed in non-selected (i.e., normal, randomly bred) rats. That behavioral changes produced by CRF receptor antagonism depends on pre-existing propensities of an animal has been reported previously (Keck et al., 2001). Keck et al. (2001) reported that CRF_1 receptor blockade by R121919 reduced anxiety (as measured on an elevated plus maze) only in rats that were bred to be highly anxious on the elevated plus maze (HAB); animals bred to be low-anxious were unaffected by this manipulation (LAB rats). Of interest, when this same group tested these same strains of rats in an acoustic startle test, the strain bred for low anxiety in the elevated plus maze (LAB) actually had *higher* baseline startle. When this same cohort of animals was tested in a social defeat paradigm, HAB rats spent more time freezing and emitting more ultrasound vocalization calls during the social defeat than their LAB counterparts which spent more time rearing and grooming. Although the LAB rats showed less signs of behavioral activation during the social defeat, they actually secreted adrenocorticotropin and corticosterone at higher rates than did their HAB counterparts (Frank et al., 2006).

The present results extend and broaden this observation. What is particularly noteworthy in the present study is that not only did CRF antagonism affect swim-test behavior of SwHi and SwLo rats differently, but the behavior affected does not appear to be intimately connected with anxiety or fear or stress—existing data indicates that the marked difference in swim-test activity of SwHi and SwLo rats is not an expression of differences in anxiety/fear/stress responses. Rather than affecting fear or anxiety, the apparent influence of CRF on the swim-test behavior of SwHi and SwLo rats, as revealed by blockade of CRF_1 receptors, was to inhibit, or modulate strong behavioral propensities of the SwHi and SwLo animals. These results suggest that in certain animals (in this case, SwHi and SwLo rats), endogenous CRF acting through CRF_1 receptors does not promote a particular behavioral response (such as anxiety) but, instead, can primarily influence behavior by affecting CNS systems that modulate strong behavioral tendencies. The present results therefore suggest that endogenous CRF influences a wider range of functions than may have been previously thought.

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Conflict of interest

None declared.

Disclosure

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