



Research report

The effect of chronic fluoxetine on social isolation-induced changes on sucrose consumption, immobility behavior, and on serotonin and dopamine function in hippocampus and ventral striatum

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ABSTRACT

This study examined the effect of fluoxetine, a selective serotonin (5-HT) reuptake inhibitor, on isolation-induced changes on sucrose consumption and preference, spontaneous open-field activity, forced swimming behavior, and on tissue levels of 5-HT and dopamine (DA) in hippocampus and ventral striatum (VS). Male Sprague–Dawley rats were reared in social isolation or group housing from postnatal day 28. Thirty-two days later, half of the isolated animals were orally treated with fluoxetine (10 mg/kg/day) during the following 34 days. At the end of this period, behavior was assessed and afterward ex-vivo tissue samples were obtained. It was found that fluoxetine restored isolation-increased sucrose consumption and immobility behavior, without affecting locomotor activity, which appeared slightly increased in isolated groups both treated and untreated. In the hippocampus, isolation rearing depleted 5-HT contents and increased 3,4-dihydroxyphenylacetic acid (DOPAC) levels, as well as 5-HT and DA turnover. These neurochemical alterations were reversed by fluoxetine. In VS, treated and untreated isolated rats showed higher 5-HT levels than grouped congeners. Although fluoxetine did not affect 5-HT and DA contents in this region, it slightly reversed the alterations in the 5-HT and DA turnover observed in isolated rats. Overall, social isolation impaired incentive and escape motivated behaviors. At the neurochemical level, isolation rearing affected 5-HT rather than DA activity, and this differential effect was more noticeable in hippocampus than in VS. The chronic treatment with fluoxetine during the last month of rearing somewhat prevented these behavioral and neurochemical alterations. Our data suggest that isolation rearing is an appropriate procedure to model some developmental-related alterations underlying depression disorders.

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1. Introduction

Early life aversive experiences can represent a risk factor for the development of human depression [28]. In animal models, social isolation from weaning in rodents has been used to reproduce some developmental factors, which are thought to increase the risk for suffering depression during adolescence and adulthood [6,25,26]. Among the protracted effects of isolation rearing over brain and behavior [10], those regarding with the neurochemical adaptations of cortico-mesolimbic circuitries involved in shaping emotional behavior and stress-coping response [3,4,13,27,37] have received especial attention in the field of depression research.

The aim of the current study was to determinate the effect of fluoxetine, a selective serotonin reuptake inhibitor (SSRI), on behavioral and neurochemical deficits induced by isolation rearing in Sprague–Dawley rats. It has been found that animals with endogenous or directed serotonin (5-HT) depletion show enhanced alcohol, sucrose and saccharin intake [17,20,30,36]. Data from independent studies indicate that social isolation increases concomitantly sucrose, saccharin and ethanol consumption, while it reduces 5-HT contents enhancing 5-HT turnover in brain regions such as prefrontal cortex and hippocampus [2,3,5,16,19,34]. This evidence suggests that diminished 5-HT activity may be implicated in enhanced hedonic function in isolated rats. However, apart from widespread findings relating the mesolimbic dopaminergic system with alterations in reward-sensitivity in isolated animals [10,21,22], the relationship between 5-HT and hedonic function has been poorly explored in both standard and isolated reared rats. In contrast, serotonergic antidepressants have been found to reverse hedonic deficits in rats subjected to unpredictable chronic

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mild stress [38], but it is still unclear whether antidepressants can reverse isolation-induced alterations upon sensitivity to natural reward such as sucrose solution. Therefore, and based on our previous findings [3], in the current experiment, it was not only expected that isolated rats consumed more sucrose than grouped littermates but also that fluoxetine does reduce this effect.

The forced swimming test (FST), which is the most frequently used screening model for antidepressant effect, is selectively sensitive to clinical drugs with different monoamines targets, including the SSRI [7]. In this test, isolation rearing has seemed to be a predispositional factor that not only diminishes the drive to attempt the escape but also impairs the active behaviors involved in coping with an uncontrollable stress situation [4,5,6]. However, this effect depends on the length of rearing as it has been demonstrated elsewhere [4,5,18]. Thus, given that the rearing period in the current experiment is 1 month longer than the one we used previously, we suppose that immobility behavior in isolated rats would augment significantly relative to grouped rats. Since SSRIs reduce immobility by selectively increasing swimming behavior [7] it is expected that fluoxetine reverses isolation-increased immobility behavior in the FST.

Isolation rearing has been reported to reduce 5-HT content and release in hippocampus [2,19,27], whereas it increases them in ventral striatum (VS) (namely, nucleus accumbens) [12,21]. Overall, since both neurochemical alterations have been linked with behavioral deficit induced by isolation rearing, we hypothesized that increased sucrose intake and immobility behavior in isolated animals may be the indirect outcome of the depleted 5-HT system in hippocampus and enhanced 5-HT activity in VS, which could be prevented by the chronic treatment with fluoxetine. Furthermore, considering that isolation rearing also disrupts the mesolimbic dopaminergic system [10,11,21,22], and that the effect of serotonergic antidepressants has been partially attributed to their action on mesolimbic dopaminergic pathway [1,38], the dopamine (DA) concentration in VS and hippocampus was also assessed.

2. Methods

2.1. Animals and housing

At postnatal day (PND) 28 (after 1-week habituation to our colony room), 48 male Sprague–Dawley rats were housed either single (SI, $n=32$) or in groups of three (SC, $n=16$) exactly as we previously reported [5,6]. Animals were maintained in a temperature-controlled environment ($20.5 \pm 1.20^\circ\text{C}$) under 12-h light–dark cycle (lights turned on at 06:00 h). Food and water were freely available. All behavioral testing was conducted and videotaped during the night cycle (19:00–23:00 h). Experimental procedures were done in accordance to the guidelines of the Costa Rican Ministry of Science and Technology for the Care and Use of Laboratory Animals, and were approved by the Institutional Committee for Animal Care and Use of the University of Costa Rica.

2.2. Procedure and treatment

The groups were kept undisturbed under their respective housing conditions during 32 days, except for the routine bed changing done either once (for SI groups) or twice (for SC group) a week. At PND60, the SI group was further divided into two groups ($n=16$ each), the first group remained under social isolation, and the other one was orally (intra-gastric syringe) treated with fluoxetine hydrochloride (10 mg/kg dissolved in distilled water in a volume of 10 ml/kg; Raven, SJ, Costa Rica). During the following 34 days, the fluoxetine (FLX)-treated animals (SI-FLX) received a daily dose, whereas SI and SC groups only received vehicle (distilled water at 10 ml/kg). The last fluoxetine dose was administered 10 h before the second session of the FST (PND94). This fluoxetine dose was used because it has been demonstrated as being effective in the forced swimming and sucrose consumption tests [7,32]. Since fluoxetine administered during preweaning or immediately after weaning lead to disturbing and long-lasting effects on brain and behavior [23,29], we started the treatment 1 month after weaning when some isolation-induced behavioral and neurochemical alterations were already occurring [3,4,5].

2.3. Sucrose preference test

In our version of the sucrose preference test (SPT) [3,6], rats were individually housed during 48 h in standard cages where one bottle with 200 ml of 32% sucrose solution (w/v), another with 200 ml of tap water, and food ad libitum were available. After this period, sucrose (ml), water (ml) and food (g) were measured, and animals were returned to their previous housing conditions. Preference was calculated as follows: Preference % = [(sucrose consumption/sucrose + water consumption) \times 100]. The tests were carried out twice, at PNDs 90–91 and at 94–95. The latter test started approximately 1 h after the FST.

2.4. Open-field test

At PND92, spontaneous open-field activity was assessed as we previously reported [5]. Briefly, the testing room was dimly illuminated with one 25 W red bulb located 130 cm above the open-field floor. Each rat was placed into the center of a wood-made arena ($70 \times 70 \times 40$ cm divided in four equal squares) and during 10 min the number of lines crossed with the four paws and the number of rearings (standing on hind paws) were manually counted. The arena was cleaned between tests with a 90% alcohol solution.

2.5. FST

At PNDs 93–94, the FST was performed as we previously described [5]. Briefly, animals were exposed to a pretest for 15 min, 24 h prior to the 5-min swimming test. One single rat was placed into a Plexiglas cylinder (45 cm height, 31 cm diameter) filled with water ($25 \pm 0.5^\circ\text{C}$) to a depth of 30 cm. After each session, the rats were removed from water, dried with a towel, and placed in a warmed enclosure during 30 min before being returned to their housing cages. The water was changed between tests. The time spent immobile (floating posture including small movements necessary to keep the animal's head above the water), swimming (the movement, usually horizontal throughout the cylinder that also includes crossing into another quadrant), and climbing (vigorous upward-directed movements of the forepaws along the wall of the cylinder) was manually scored from the 5-min session (day 2), and was expressed as percent values [(seconds of each behavior/300 s) \times 100].

2.6. Ex-vivo monoamines concentration in hippocampus and VS

The neurochemical analysis was performed exactly as we previously reported [3,4,5]. At PND100, rats (8 by group) were decapitated, brains were quickly dissected on ice, and hippocampus and VS were bilaterally removed. Thereafter, each pooled sample (from both hemispheres) was analyzed for their content of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), DA and 3,4-dihydroxyphenylacetic acid (DOPAC), using high-performance liquid chromatography coupled with electrochemical detection (HPLC–EC). The substance concentration was expressed as nanograms per milligram of wet tissue weight. The turnover of 5-HT (5-HIAA/5-HT) and DA (DOPAC/DA) was also reported.

2.7. Data analysis

Data were expressed as means \pm S.E.M. Group comparisons were carried out using unpaired or paired one-way variance analysis (ANOVA), when appropriate. Fisher-protected LSD test was utilized as a posthoc test for between-groups comparisons. An analysis of covariance (ANCOVA) of sucrose consumption and forced swimming behaviors including the body weight as covariate was also used. In all statistical analyses, significance was defined as $p < 0.05$.

3. Results

3.1. Sucrose consumption and preference

At PNDs 91 ($F_{(2,45)} = 8.52$, $p < 0.001$) and 95 ($F_{(2,45)} = 4.22$, $p < 0.02$), fluoxetine reduced significantly sucrose intake compared with the other groups (Fig. 1A). Relative to SC group, the SI group increased significantly the sucrose consumption at PND91 but not at PND95. In this latter test, the SC rats increased their consumption until a level in which the previous significant differences from SI rats disappeared (Fig. 1A). The repeated measures analysis revealed that this increase in the sucrose consumption from one test to another was significant in SC rats ($F_{(1,15)} = 8.30$, $p < 0.01$) but not in SI-FLX ($F_{(1,15)} = 2.72$, $p > 0.12$), nor in SI ($F_{(1,15)} = 0.40$, $p > 0.54$) animals, in which sucrose intake appeared only marginally increased after the FST (Fig. 1A). In both tests, the sucrose preference was lower in SI-FLX group than in the other groups (Fig. 1B), which did

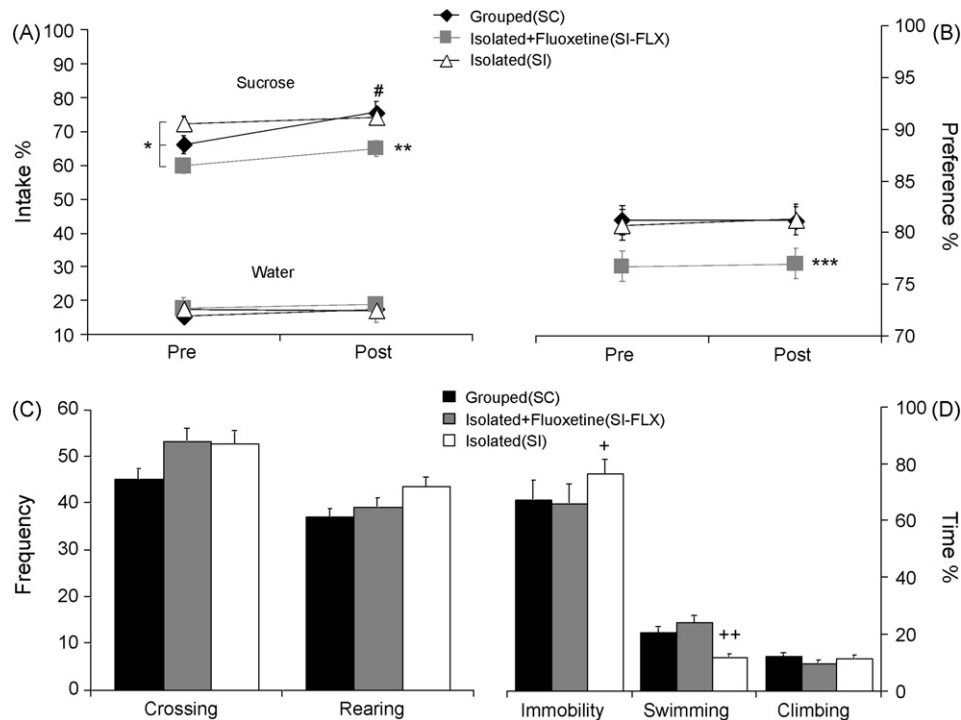


Fig. 1. (A) Sucrose and water consumption, and (B) sucrose preference percentages measured during the postnatal days (PNDs) 91–92 (pre) and 94–95 (post). In PNDs 93–94, the forced swimming test was carried out. The lines beside the symbols indicate significant differences among all groups: * $p < 0.001$. Isolated-fluoxetine (SI-FLX) group differed significantly from the others: ** $p < 0.02$. Grouped rats (SC) increased significantly their sucrose consumption from one test to another: # $p < 0.01$. Isolated-fluoxetine (SI-FLX) group differed significantly from the others: *** $p < 0.001$. (C) Open-field behavior assessed during PND 92. (D) Forced swimming test (PNDs 94–95). Data correspond to the 5-min session (day 2). Isolated group (SI) differed significantly from the others: + $p < 0.03$, ++ $p < 0.001$. For all tests (A–D) results are expressed as means \pm S.E.M. ($n = 16$ each).

not differ between one another. As is shown in Fig. 1B, despite the mean differences were quite similar between PNDs 91 ($F_{(2,45)} = 2.64$, $p > 0.08$) and 95 ($F_{(2,45)} = 3.35$, $p < 0.04$), the significance was only reached at the second test. The water consumption did not differ among groups during the first ($F_{(2,45)} = 0.85$, $p > 0.43$) and second test ($F_{(2,45)} = 0.60$, $p > 0.55$), and nor did vary significantly from one test to another within any group (Fig. 1A). However, in SI-FLX ($F_{(1,15)} = 0.34$, $p > 0.57$) and SC animals ($F_{(1,15)} = 2.74$, $p > 0.12$) water intake increased slightly in the second test, whereas in SI rats, it tended to decrease ($F_{(1,15)} = 0.06$, $p > 0.82$). The food consumption during the sucrose tests (PND91: $F_{(2,45)} = 0.10$, $p > 0.90$; PND95: $F_{(2,45)} = 0.78$, $p > 0.46$) did not vary among groups (data not shown). However, the body weight measured at PND91 was significantly lower in SC rats ($F_{(2,45)} = 3.94$, $p < 0.03$) compared with SI-FLX and SI animals, which did not differ between them (SC = 426.2 ± 7.2 ; SI-FLX = 473.8 ± 6.9 ; SI = 482.8 ± 9.2). The statistic subtraction of the body weight effect over the between-groups comparison of sucrose consumption (ANCOVA analysis) did not affect the significant differences previously detected between SI-FLX and the other conditions at PNDs91 ($F_{(2,44)} = 5.49$, $p < 0.007$) and 95 ($F_{(2,44)} = 4.93$, $p < 0.01$) (data not shown).

3.2. Open-field behavior

Spontaneous open-field activity did not change significantly following isolation rearing or fluoxetine treatment (Fig. 1C). However, locomotion ($F_{(2,45)} = 1.42$, $p > 0.25$) was lower in SC group compared with SI-FLX and SI groups, which did not differ between one another. Although not significant ($F_{(2,45)} = 1.12$, $p > 0.34$), the SC animals showed the lowest and SI rats the highest rearing frequency (Fig. 1C).

3.3. Forced swimming behavior

As is shown in Fig. 1D, fluoxetine reduced immobility behavior in SI rats to a level in which this group (SI-FLX) reached the SC values ($F_{(2,45)} = 3.94$, $p < 0.03$). The SI rats showed the highest immobility time differing not only from SI-FLX rats but also from SC littermates. Regarding the swimming time, the SI-FLX and SC groups differed significantly from SI one ($F_{(2,45)} = 8.82$, $p < 0.001$), showing the SI-FLX rats the highest and SI animals the lowest levels (Fig. 1D). Climbing behavior did not differ among groups ($F_{(2,45)} = 0.37$, $p > 0.69$), but SI-FLX rats showed the lowest and SC animals the highest values (Fig. 1D). The ANCOVA analysis of immobility ($F_{(2,44)} = 3.29$, $p < 0.05$) and swimming ($F_{(2,44)} = 7.80$, $p < 0.001$) behaviors with body weight as covariate revealed that the significant differences previously detected among groups remained almost the same (data not shown).

3.4. 5-HT and DA concentration in hippocampus and VS

In the hippocampus (Fig. 2A), significant differences in the 5-HT concentration among all groups were found ($F_{(2,21)} = 18.17$, $p < 0.0001$). SI-FLX rats showed the highest, SC the mid-range, and SI group the lowest 5-HT levels. The 5-HIAA concentration (Fig. 2A) was significantly lower in SC group than in others ($F_{(2,21)} = 7.79$, $p < 0.003$). The SI-FLX and SI groups did not differ between each other, but the latter one showed the highest 5-HIAA levels. The 5-HT turnover (Fig. 2A) was significantly higher in SI animals compared with SI-FLX and SC rats ($F_{(2,21)} = 3.66$, $p < 0.04$). These latter groups did not differ between each other but SC animals showed the lowest ratios.

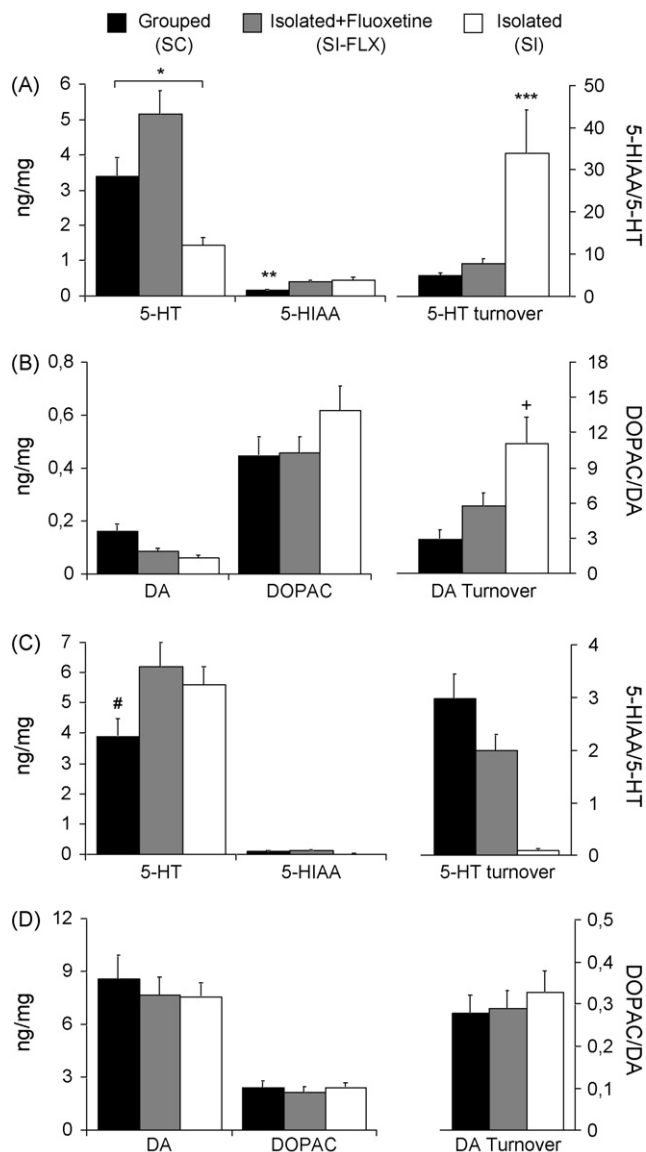


Fig. 2. The concentration of 5-HT, 5-HIAA, DA, and DOPAC and the DA and 5-HT turnover in hippocampus (A and B) and ventral striatum (C and D). Brain samples were obtained at postnatal day 100. Results are expressed in nanograms per milligram (ng/mg) of wet tissue weight as means \pm S.E.M. ($n=8$ rats each). 5-HT turnover = (5-HIAA/5-HT). DA turnover = (DOPAC/DA). The lines above the bars indicate significant differences among all groups: * $p < 0.0001$. The grouped animals (SC) differed significantly from the others: ** $p < 0.003$. The isolated group (SI) differed significantly from the grouped ones (SC): + $p < 0.04$. The grouped rats (SC) differed significantly from the others: # $p < 0.0001$.

On the other hand, the concentration of DA ($F_{(2,21)} = 1.45$, $p > 0.26$) in hippocampus was not different among groups (Fig. 2B), but both SI-FLX and SI groups showed lower levels than SC group. On DOPAC contents significant differences were not found ($F_{(2,21)} = 1.10$, $p > 0.35$), but SI rats showed the highest amount, whereas SI-FLX and SC groups did not differ between one another (Fig. 2B). The DA turnover ($F_{(2,21)} = 3.86$, $p < 0.04$) was significantly higher in SI rats compared with SC but not with SI-FLX rats (Fig. 2B). In this latter group, the DA turnover appeared marginally restored by fluoxetine, showing a ratio much close to the SC animals (Fig. 2B).

In VS (Fig. 2C), the 5-HT concentration was significantly lower in SC rats ($F_{(2,21)} = 15.24$, $p < 0.0001$) compared with SI-FLX and SI groups, which showed quite similar values between them. There

were no differences on 5-HIAA concentration ($F_{(2,21)} = 0.70$, $p > 0.51$) but SI group showed the lowest and SI-FLX rats the highest concentrations (Fig. 2C). The 5-HT turnover did differ among groups ($F_{(2,21)} = 0.54$, $p > 0.59$). However, the SC group showed a higher turnover than the other groups. In SI-FLX rats, there was a trend towards increase with a mean turnover closer to the levels of SC group than to the levels of SI group, which showed the lowest 5-HT ratio (Fig. 2C). Finally, the DA ($F_{(2,21)} = 1.19$, $p > 0.31$) and DOPAC ($F_{(2,21)} = 0.48$, $p > 0.62$) contents (Fig. 2D), and the DA turnover ($F_{(2,21)} = 1.51$, $p > 0.23$) did not differ significantly among groups (Fig. 2D).

4. Discussion

The major aim of the present study was to investigate whether the serotonergic antidepressant fluoxetine could reverse the social isolation-induced changes on incentive and escape-motivated behaviors, and on 5-HT and DA function in hippocampus and VS. As we expected, fluoxetine counteracted the behavioral alterations induced by isolation rearing. At the neurochemical level, isolation rearing affected 5-HT rather than DA activity, and this differential effect was more noticeable in hippocampus than in VS. The chronic treatment with fluoxetine during the last month of rearing somewhat prevented these alterations.

4.1. Sucrose consumption and preference

In agreement with previous reports [16,34], our results not only show that isolation rearing increases sucrose intake but also support the evidence about the enhanced incentive motivation and reward-sensitivity observed as consequence of this housing condition [10,21,22]. Higher sucrose consumption in isolated rats has been recently reported at a time point (PND94) similar to the one we used here [3], suggesting that sensitivity to a natural reward is consistently affected by isolation during a specific developmental window. After the FST (PND95), all groups increased their sucrose intake, but uniquely in the grouped rats this increase was so much high that previous differences from the social isolated ones disappeared. This indicates, as previously shown [3], that an acute stress situation like the FST increases the intake of a 32% sucrose solution uniquely in those animals which have not been prestressed (i.e., grouped rats). It may be thought, therefore, that whether acute (FST) or chronic stressors (isolation rearing) are applied, sucrose intake tends to increase at this specific concentration. However, FST stress did not produce an additive effect over the isolation stress. Namely, the isolated animals, which showed the highest sucrose intake in both tests did not increase their consumption after the FST. This may be due to the fact that these animals either reached the palatability threshold for sucrose or were satiated.

On the other hand, the chronic administration of fluoxetine during the last month of rearing reduced sucrose intake in isolated rats even under the levels of grouped congeners. This effect was also observed after the FST (PND95), where the last fluoxetine dose was administered 10 h before the start of the sucrose test. Again, fluoxetine-treated rats showed lower intake than grouped and isolated animals. Fluoxetine also diminished sucrose preference in both tests, but this reduction was only significant at PND95. Even though isolated rats always showed the highest sucrose intake, preference in these animals did not differ from the grouped rats at any test. Water and food consumption was not different among groups, indicating first, that fluoxetine did not induce a generalized deficit in ingestive behavior, and second, that reduced preference was primarily due to a net decrease in sucrose intake rather than to an overall decrease in fluid intake. Moreover, both isolated groups

showed similar body weight, suggesting that reduction in sucrose intake induced by fluoxetine was not a consequence of reduction in body weight. The latter was also supported by the results of ANCOVA analyses, in which body weight was included as covariate in the comparison of sucrose consumption among groups.

In our knowledge, this is the first study demonstrating that fluoxetine reverses the increased sucrose intake in isolated rats. There is evidence that fluoxetine administered during 15 days (7.5 mg/kg/day) also reverses the increased (32%) and decreased (1%) sucrose intake induced by the chronic treatment with interferon- α in non-isolated rats [32]. Animals subjected to unpredictable chronic mild stress have also shown a progressive reduction in sucrose consumption and preference for a 1% solution [17,38]. This decreased intake is restored by chronic but not by acute treatment with antidepressants, including fluoxetine [38]. Therefore, previous and current findings indicate first, that sucrose intake varies considerably as a function of concentration and stress regime, and second, that fluoxetine is able to reverse both, decreased and increased intakes of 1% and 32% sucrose solutions, respectively. Furthermore, the effect of fluoxetine over sucrose intake and preference described here suggests that 5-HT system is somehow involved in regulating motivated behavior towards an appetitive incentive. In support to this argument, animals with 5-HT depletion has shown enhanced alcohol, sucrose and saccharin intake, [17,20,30,36], as it has also been reported in isolated rats, including current data [2,16,19]. Thus, it is not surprising that augmenting the 5-HT central tone reversed the high sucrose intake observed in isolated animals, which showed depleted hippocampal 5-HT concentration as well.

4.2. Forced swimming and open-field behavior

On the FST, the isolated rats showed the highest immobility time and the lowest levels of active behaviors (i.e., swimming and climbing), suggesting that isolation rearing acted as a predisposition factor in producing either a failure of persistence in escape-directed behavior or an acceleration in the development of a passive behavior that disengages animals from active forms of coping [4,7]. The differences in forced swimming behavior observed between treated and untreated isolated rats and between both and grouped ones seemed not to be due to the differences in body weight, as it was revealed by the ANCOVA analysis. In Sprague–Dawley rats, we previously found increased immobility time following an isolation period slightly longer than we used here [5], similar was also described in this strain in an earlier report [18]. In contrast, after a shorter rearing period (around 1 month), no differences were detected between isolated and grouped rats, despite that isolated ones showed the highest immobility time and lowest levels of active behaviors [4]. Current and previous findings support the standpoint that depressive-like behavior in Sprague–Dawley rats increases proportionately to the isolation duration [3,4,18]. On the other hand, fluoxetine reversed immobility behavior in isolated rats due to a special increase in swimming behavior, coinciding with the well-known effect of serotonergic antidepressants on this FST behavior [7]. The noradrenergic antidepressant desipramine (20 mg/kg/day) has been reported to reverse the isolation-increased immobility behavior in Sprague–Dawley rats [18] suggesting that antidepressant drugs irrespective to their specific cellular targets are able to restore isolation-induced behavioral deficits in the FST. However, in Fawn-Hooded and Wistar isolated rats, either longer rearing periods or desipramine treatment have produced marginal or contradictory effects, respectively [15,39]. This suggests that the strain used may become a critical issue to model depressive-like behavior in isolated rats with this paradigm.

Regarding spontaneous open-field activity, both isolated groups showed almost identical levels, confirming that reduced immobility in isolated-treated rats was not a consequence of a generalized psychomotor excitation provoked by fluoxetine. Locomotion and rearing tended to be higher in treated and untreated isolated rats compared with grouped ones, but the extent of these differences was too small to be statistically significant. Similar findings have been reported in Sprague–Dawley rats [5,35] supporting the notion that hyperlocomotor activity is not a generalized consequence of isolation rearing. In this strain, rather than in others, there seems to be a strict developmental window to observe this effect [4,5].

4.3. 5-HT, 5-HIAA, and 5-HT turnover in hippocampus and VS

Isolation rearing reduced 5-HT contents, and increased 5-HIAA amount, and 5-HT turnover in hippocampus. Thus, it may be thought that isolation stress could demand a high 5-HT utilization which could not be compensated by an increase in its biosynthesis leading to lower 5-HT contents and enhancing 5-HIAA levels and 5-HT turnover. Decreased hippocampal 5-HT concentration, and attenuated 5-HT release in response to KCl, imipramine, parachloroamphetamine, novelty, and stress have been detected following isolation rearing [2,19,27]. This suggests that disrupted presynaptic 5-HT activity (release and synthesis) in hippocampus is a consistent neurochemical trait of social isolation. In support to the latter, it was found that isolation rearing increases binding and mRNA expression of the 5-HT_{1A} receptor in hippocampus [8,33]. Since this receptor is most densely located at postsynaptic level in this region in normal rats [1,7,24], its upregulation could be considered as a protracted adaptation in response to deficits in hippocampal presynaptic 5-HT activity induced by social isolation. The fact that isolation rearing reduces 5-HT fibers innervating hippocampus from raphe nuclei [13,37] may contribute to explain the lowered 5-HT concentration detected here in hippocampus. However, it is worth mentioning that the effect of isolation rearing on 5-HT function may vary according to the length of the rearing period and the brain region analyzed. For example, an elevated turnover with no differences in 5-HT concentration was detected in hippocampus and prefrontal cortex of Sprague–Dawley rats which were isolated during approximately 1 or 2 months, respectively [4,5].

On the other hand, the current results showed that 1 month of fluoxetine administered during last month of housing was enough to counteract the effect of isolation rearing on 5-HT levels and turnover. Others SSRIs have also reversed isolation-increased 5-HIAA amount and 5-HT turnover in hippocampus of rats and mice [25,31]. Our data suggest that antidepressant could normalize presynaptic activity and postsynaptic neurotransmitter requirements, stabilizing therefore the 5-HT tone, which could be ultimately involved in the restoration of behavioral deficits observed on sucrose intake and immobility behavior in isolated rats.

In VS, both isolated groups showed significantly higher 5-HT contents than grouped rats. These results are in agreement with other studies showing that both 5-HT postmortem and release levels are increased in nucleus accumbens following isolation rearing [12,21]. Although isolated rats showed here the lowest 5-HIAA amount and 5-HT turnover, no significant differences were found on these parameters among groups, in accordance with previous reports [10,21]. Increased 5-HT function in VS (namely, nucleus accumbens) following isolation rearing [12,26] has been addressed as a physiological adaptation to stress or secondary to changes in other neurotransmitter, such as dopamine [27], and has been linked with the behavioral deficits it induced [10], including enhanced

sucrose intake [3]. Nevertheless, 5-HT levels in VS contrast with those detected in hippocampus suggesting a differential effect of isolation rearing on 5-HT neurons and projections in the brain as it has been suggested elsewhere [2,19,27]. In contrast, fluoxetine did not affect 5-HT contents but marginally restored the isolation-reduced 5-HT turnover. The 5-HT projections of VS arise principally from raphe nuclei [1,24]. Since serotonergic antidepressants are known to attenuate the firing rate of 5-HT neurons in the raphe nuclei [9], in this experiment, fluoxetine could reduce the presynaptic 5-HT activity lowering its turnover without altering the biosynthesis. The lacking effect of fluoxetine on 5-HT concentration in VS supports the evidence that there are diverse 5-HT regulatory mechanisms along the brain, possibly attributed to a differential distribution and density of 5-HT transporters and receptors [1,24]. In support of the latter, it was found that anpirtoline, an agonist of 5-HT_{1B} receptor, reverses selectively the social isolation-reduced 5-HT turnover in striatum. This effect not only differs from those observed in other brain regions but also from those produced by the SSRI citalopram [31].

4.4. DA, DOPAC, and DA turnover in hippocampus and VS

Regarding DA contents in hippocampus, isolation rearing increased significantly DA turnover and marginally DOPAC levels, without altering DA amount, which appeared slightly diminished in both isolated groups. Disruption in hippocampal neurochemistry has been widely described at several cellular and molecular levels [10,13], but alterations in hippocampal DA function following isolation rearing have been less investigated. However, a recent report also showed that DOPAC and DA turnover are augmented in hippocampus of isolated rats [25], suggesting that enhanced dopaminergic activity observed in other brain regions following isolation rearing could be extended to the hippocampus. In contrast, fluoxetine restored the enhanced DOPAC and DA turnover levels without affecting DA contents, in a similar manner as produced by the SSRI Fluvoxamine [25]. The reduced innervation of 5-HT fibers in hippocampus induced by isolation rearing [13,37] could augment the DA activity in this brain region due to an attenuation of the inhibitory regulation of 5-HT over DA neurons [25]. Furthermore, the SSRIs have shown to attenuate the firing rate of DA neurons in the ventral tegmental area (VTA) [9], the main source of dopaminergic fibers of hippocampus [24]. Therefore, in the current experiment fluoxetine could reduce DA activity not only due to a local upregulation of 5-HT transmission but also by a reduction in the firing rate of hippocampal dopaminergic inputs.

Similar to previous studies, we did not find differences in striatal DA concentration between isolated and grouped rats [3,21]. It has been suggested that presynaptic DA activity is enhanced in the VS of isolated rats [12,22], but several works concur to show that this is not always reflected at the biosynthesis level (i.e., tissue amount) [10]. Fluoxetine did not affect any neurochemical parameter in this region, but it seemed to restore marginally the increased DA turnover observed in isolated rats. At the behavioral level, the effect of fluoxetine on sucrose consumption could be attributed to an interaction between 5-HT and DA transmission into VS [38], through the putative action of 5-HT_{2C} receptors. These receptors are located in γ -aminobutyric acid (GABA) and DA cells in VTA [1], they inhibit dopaminergic transmission in VS via constitutive activity [24] and can be stimulated indirectly by fluoxetine [1]. It is known that sucrose intake and DA influence one another, such that sucrose consumption increases DA release in VS and DA agonist administered into this region increases sucrose intake [14,40]. Therefore, in the current experiment fluoxetine could counteract the isolation-increased sucrose consumption and DA turnover due to a downregulation of DA activity in VS by stimulating 5-HT_{2C}

receptors of VTA. However, this assumption deserves to be fully investigated.

5. Conclusions

In summary, the present study demonstrated that isolation rearing induces alterations on natural reward-sensitivity and depressive-like behavior. At the neurochemical level, isolation rearing produced differential effects on hippocampal and VS 5-HT and DA activity. The administration of fluoxetine (10 mg/kg/day) during the last month of the isolation period counteracted these behavioral and neurochemical effects. Since fluoxetine acts principally increasing the 5-HT availability in the synaptic cleft through inhibiting the 5-HT transporter, the upregulation of the 5-HT transmission seems to be involved in the restoration of behavioral deficits induced by isolation rearing on incentive and escape motivated behaviors. These behavioral effects could be addressed not only as the outcome of an increased and decreased 5-HT transmission in VS and hippocampus, respectively, but also as a secondary consequence of a minor disruption in the mesolimbic dopaminergic activity. Overall, the present results support the viewpoint that isolation rearing is a useful procedure to model some developmental risk factors underlying depressive disorders. Further investigations with this model using different antidepressants, doses, and administration regimens including additional control groups (i.e., group-housed treated rats) are required to clarify the likely role of monoaminergic systems in determining the behavioral and neurochemical outcomes showed here.

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