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Activation of 5-HT7 receptor by administration of its selective agonist, LP-211, modifies explorative-curiosity behavior in rats in two paradigms which differ in visuospatial parameters

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Summary

Aims: The serotonin 7 receptor (5-HT7R) subtype, coded by *Htr7* gene, is broadly expressed in the central nervous system (CNS) with clear involvement in behavioral functions such as learning/memory, regulation of mood, and circadian rhythms. In this study, we assessed effects of 5-HT7R stimulation by administration of its selective agonist, LP-211 (0.25 mg/kg i.p.), in adult Wistar-Han rats.

Methods: We used two different explorative-curiosity tests. Drug was administered either before one side-chamber familiarization (CF/V group) or immediately after it, to act on consolidation of familiarization (V/CF group).

Results: Exp. 1 for novelty seeking in black/white boxes (BWB), with door opening after 5 minutes in the familiar chamber, showed that (i) time spent in the novel environment (significantly higher than in familiar chamber for controls) is enhanced in V/CF group (potentiated recognition for a "visual" consolidation) and not different in CF/V group; (ii) activity and chamber transitions, made by CF/V rats, are significantly higher than for other groups (interference on recognition for a "spatial" acquisition). Exp. 2 for novelty preference in D- vs L-shaped chambers (D/L), with start from neutral center, gave different results: (i) time spent in the novel environment by CF/V group is significantly higher than other groups (potentiated "cognitive" acquisition); (ii) chamber transitions made by V/CF group are significantly higher than other groups (potentiated "emotional" consolidation).

Conclusion: These apparently conflicting results may reflect LP-211 effects on visual vs spatial memory (D/L apparatus has more pronounced hippocampal components than BWB). However, further experiments are needed to analyze more in depth the mechanisms involved.

KEYWORDS

cognitive memory, emotional memory, novelty preference, novelty seeking, synaptic plasticity

1 | INTRODUCTION

The serotonin 7 receptor (5-HT7R), broadly expressed in the central nervous system (CNS), as well as in periphery, is mainly located on GABA interneurons and glutamate terminals, in which it is coupled to Gs and G12 proteins. Its wide distribution in the CNS reflects the numerous roles played by this receptor, whereby a disregulation of 5-HT7R has been related to many neuropathological processes as well as cognitive and mood dysfunctions such as anxiety, schizophrenia, and depression.[1,2] Furthermore, in addition to its classical role as neurotransmitter, by activation of G12 protein coupled 5-HT7R, 5-hydroxytryptamine (5-HT) plays a significant role in regulating the

structure of the nervous system, acting as a trophic factor and hence increasing the neural network construction.[3] Accordingly, by administration of LP-211, a brain penetrant selective agonist, 5-HT7 receptor activation in a mouse model of Rett syndrome[4] led to an improvement in behavioral deficits and reversed the abnormal activation of proteins involved in the regulation of actin cytoskeleton dynamics (P21-activated kinases, cofilin, and the ribosomal protein S6).

LP-211 showed in different studies to be a suitable tool to elucidate the multiple functions of 5-HT7R in vivo.[5] Of particular interest to our study is the role of 5-HT7 receptor in learning and memory processing and its involvement in modulation of emotional and motivational processes.[6,7] In particular, 5-HT7Rs may modulate inhibitory control on the motivational drives arising from subcortical structures[8,9] therefore, as a whole, regulating impulsive behavior and sensation seeking, characteristics of many kinds of addiction and behavioral disorders[10]; at the same time, 5-HT7Rs consolidate visual and spatial items' memory.[11,12] We investigated, in a previous study, the modulation effects of LP-211 on risk proneness and novelty seeking behavior: Adult rats were subjected to a novelty preference test under acute stimulation and, subsequently, to a Probabilistic-Delivery Task (rPDT)[13-16] under subchronic stimulation. More specifically, rPDT showed that subjects receiving the 0.25 mg/kg LP-211 dose were more inclined to risk proneness than other groups. Novelty preference test showed that rats receiving LP-211 immediately after a first, short exposure to one chamber of the experimental apparatus spent (on the following day) a significantly higher time in the novel environment, in contrast to vehicle-treated rats who displayed the chance level. LP-211 treatment during consolidation of experience was thus able to allow them to get familiar with the chamber, afterward exhibiting a clear preference for novelty.[17]

This study aimed to further assess the behavioral effects of an acute stimulation of 5-HT7R (by administration of LP-211) in adult rats through two slightly different experimental novelty paradigms. We performed both experiments dividing the subjects into three groups, receiving (i) two vehicle injections, or (ii) LP-211 immediately before, or (iii) immediately after the second familiarization session, respectively. Thus, we analyzed and compared the effects of LP-211, which were experienced during the course of visuospatial acquisition or rather during its consolidation. In the first experiment, we run a novelty seeking test by means of a black/white box (BWB), an apparatus composed of two environments having the same shape, which differ for the walls' color (black/white). In the BWB, the white side is usually slightly less preferred, hence any increase in time spent in this side may provide a measure of the exploratory drive toward an unknown place despite being slightly aversive, namely a measure of novelty seeking in mice and rats.[18,19] Time spent in the white side is expected to increase or decrease due to the balancing between effects of LP-211 on the rats' innate curiosity vs neophobia, respectively.[7,20] However, in this study, the contrast in color brightness between the two environments was intentionally reduced being based merely on faint visual elements. This was performed to make their recognition less immediate and slightly more complex, in order to give more emphasis to the putative LP-211 pharmacological effects on the acquisition and/or consolidation of such visual, nonspatial cues. To achieve this aim, we worked under dim light using an apparatus with two prevalently gray-walled chambers and distinguished only by end walls' color (still, black or white).

In the second experiment, we run a novelty preference test, using a different "spatial" apparatus, equipped with two differently shaped end chambers (D/L), having the same gray color: This setting has more pronounced hippocampal components than BWB.[21] The aim of this task is to test animals for free choice after recognition. As they are starting from a central neutral room, they have *first* to compare the known environment with the unknown one, *then* recognize both of them, and *finally* to make a preferential choice between the two. Note that when the starting point is the familiar side, as in BWB, elements recruited after door opening are rather attraction vs avoidance to the new chamber, with a motivational vs emotional and nearly no cognitive involvement.[20] In this case, due to the presence of clear spatial elements, the recognition of the two environments is, however, much simpler, and place preference is mostly due to drug-induced modulation of emotional rather than cognitive factors. Finally, in an additional group of rats, divided into three groups, we aimed to investigate the acute effect of LP-211 on anxious behavior in a dark-light box, a commonly used test to evaluate the anxious-like profile. The apparatus is composed of two very different rooms: a black chamber, covered by a roof to make it completely dark, and a white chamber, lit by a light source placed on its top.[22] This test is built on the innate aversion of rodents to brightly illuminated areas (if starting point is from dark side), or on alteration of the spontaneous exploratory behavior of rodents after response to stress, due to light exposure in a test environment (if starting point is from lit side).

2 | MATERIALS AND METHODS

All experimental procedures have been approved by the ISS animal welfare survey board on behalf of the Italian Ministry of Health (*formal license by G. Laviola, veterinary surveillance by G. Panzini*). Procedures were carried out in close agreement with the directive of the European Community Council (2010/63/EEC) and with the Italian law guidelines. We have tried to minimize animals' suffering and to use as few animals as possible, according to the principle of the 3Rs.

2.1 | Subjects, rearing, and testing conditions

All experiments were conducted with adult male (Wistar-Han Wild Type) rats, born in a colony from our facility (age > 120 days old; average weight 420 g). Animals were placed in triplets within Plexiglas cages (33 × 13 × 14 cm), in an air-conditioned room (T 21°± 1°C, relative humidity 60 ± 10%) with a 12-hour dark-light cycle (lights turned on at 8.00 PM). Water and food (Altromin-R, A. Rieper S.p.A., Vandoies, Italy) were available ad libitum.

All experiments were conducted inside the animal facility room to minimize the impact of transport to a novel testing room. In both the novelty seeking and the novelty preference tasks, the home-cages were carefully placed one by one on a cart adjacent to the experimental

apparatus. Animals were individually injected (i.p.), placed back in their home-cage for 15-20 minutes, and immediately placed in the apparatus. After exposure to the task, each subject received a second injection and was then gently replaced in its own home-cage. The triplet of rats residing in each cage was treated and tested at the same time. The dark-light task was conducted under the very same conditions, except that rats received a single injection 10 minutes before and did not receive injections after the task. Tests were conducted during the dark cycle between 9:30 and 14:30 PM.

2.2 | Novelty seeking task with LP-211

In this experiment, we used a pharmacological treatment with LP-211, a brain penetrant 5-HT7 selective agonist, by assessing its modulatory effects on various aspects of explorative-curiosity behavior. Rats (N = 21) received two administrations, one made 10 minutes before the start of the task and one immediately after the end of the task. We formed three experimental groups (N = 7 each):

1. control subjects, injected with vehicle (1% DMSO in saline solution) immediately before and right after the second exposition to the apparatus;
2. subjects injected with the drug immediately before and with vehicle right after the second exposition (Treatment Before, Effects During task: CF/V group);
3. subjects injected with vehicle immediately before and with the drug right after the second exposition (Treatment After, Effects Post task: V/CF group).

Animals were injected intraperitoneally with a dose of 0.25 mg/kg. We freshly prepared two different solutions: the LP211 drug dissolved in DMSO at 1% diluted in saline solution and the control vehicle (only DMSO at 1% diluted in saline solution). Animals were thus injected intraperitoneally with a volume of 1 mL/kg in proportion to weight (syringe: BD *plastipak* TM).

2.2.1 | Experimental apparatus

The experimental apparatus used for the *novelty seeking* test is a BWB,[7] namely a Plexiglas box with smooth walls and floor (70 × 30 × 35 cm) composed of two different environments distinguished by the end walls' color. In detail, the rectangular maze is composed of four smooth walls: The walls on the longer sides are gray, whereas those on the short margins can be distinguished by color (black or white). In its center, thus at a distance of about 35 cm from the short margins of the maze, two compartments (30 × 35 cm) are realized by a wall in which there is a door with an easily removable panel, allowing the experimental subjects to pass (or not) from one compartment to the other, when required.

On both longer sides of the maze, there are two aluminum bars equipped with photocells connected by cables to a computer. The software in use is *Cage controller 1.27 for Dark Light for Rats and Mouse* (PRS Italy, Rome), that allows to score:

1. each subject's motor activity (beam interruptions per second) in either compartment;
2. time spent in every compartment (both forepaws and hindpaws in a same compartment);
3. transitions (number of times a subject crosses the door between the two compartments).

Data were automatically divided into six 300 seconds intervals (bins).

2.2.2 | Experimental protocol

This test was carried out under red dim light and required three consecutive days. The first day of training (habituation), they were placed in a single (black end wall) chamber of the apparatus with closed door for 30 minutes. The second day (familiarization + treatment), they were individually weighted and injected with the vehicle or the drug according to group. The triplet of rats residing in each cage was treated and tested at the same time, all the three treatment groups being assigned to individuals of the triplet. At the end of the 30-minute session, animals were placed back in their own cages. The third day (testing), we proceeded with the actual test session in drug-free status. Subjects are placed in the familiar environment, and the apparatus door is opened at the fifth minute, allowing them to discover and then free access to the second (white end wall) environment, still unknown. This procedure allows animals (provided they are able to recognize the starting one as "familiar") to discover the presence of an environmental novelty, leaving them to freely decide whether or not to explore it. The floors and walls of each chamber were cleaned between each animal with water and ethanol (1:1).

2.3 | Novelty preference task with LP-211

In this experiment, again, rats (N = 21) received two administrations, one made 10 minutes before the start of the task and one immediately after the end of the task. We formed three experimental groups (N = 7 each): control subjects, injected with vehicle; CF/V group; V/CF group (same as above).

2.3.1 | Experimental apparatus

The experimental apparatus used for the *novelty preference* test with shapes is a Plexiglas box composed of three different rooms, with smooth walls and floor (70 × 30 × 35 cm): Walls are gray and the middle starting chamber (10 × 30 × 3) gives access to two end chambers (30 × 30 × 35) that differ for shapes (D and L): The D-shape environment was a "familiar" room and the L-shape environment was the "new room." These rooms are separated with doors, which can be opened (or closed), allowing (or not) the experimental subject to pass from one room to the other.

Parameters (motor activity, time spent, transitions) were registered by photobeam sensors, as above.

2.3.2 | Experimental protocol

This test was carried out under red dim illumination and required three consecutive days. During the first day of training (habituation), the experimental subjects were put in the central room with one door opened toward the D-shaped end room: Rats can freely go to explore this D-shaped chamber and thereafter may consider it like a familiar one. The second day (familiarization + treatment), all subjects received two injections of LP-211 or vehicle according to groups described above: one 10 minutes before being placed into the familiar end side and one immediately after the 30-minute exposure. The triplet of rats residing in each cage was treated and tested at the same time, all the three treatment groups being assigned to individuals of the triplet. At the end of the 30-minute session, animals were placed back in their own cages. The third day (test), rats were put in the central room, with all doors open toward both D-shaped room (familiar) and L-shaped room (new environment), for all the 30-minute session duration. The floors and walls of each chamber were cleaned between each animal with water and ethanol (1:1).

2.4 | Dark-light task with LP-211

In this experiment, we tested 20 wild-type (Wistar-Han) rats. We divided each triplet of rats (living in the same cage) into three different groups: The first group did not receive injections (control group), the second group received an injection of LP-211 (drug group), and the third group received an injection of vehicle (vehicle group). Each injection was made 10 minutes before the start of the task. Animals were injected intraperitoneally with a dose of 0.25 mg/kg.

2.4.1 | Experimental apparatus

The experimental apparatus used for the *dark/light* task was a Plexiglas box, with smooth walls and floor (70 × 30 × 35 cm). The apparatus was composed of two different rooms: The starting room was the black side, which had a roof cover on its top, to make this environment completely dark; the light side had a white end wall and a LED light source, regulated to 100 lux on its top.

On both longer sides of apparatus, there were two aluminum bars equipped with photocells (as above) that allowed to score motor activity, time spent in every compartment and transitions.

2.4.2 | Experimental protocol

This experiment was carried out in 1 day, in which all animals were tested. Each animal was injected 10 minutes before the test, and after placed gently in the dark side, with the door already open giving access to the white lit side, so that they had the opportunity to decide whether or not to enter and to explore the other room. The triplet of rats residing in each cage was treated and tested at the same time. The test had the total duration of 30 minutes, divided automatically into 300-second intervals.

2.5 | Statistics

Data (displayed as mean ± SEM) were analyzed using StatView II (Abacus Concepts, CA, USA) and were processed by analysis of variance (ANOVA). For novelty seeking and novelty preference tasks, analysis was carried out by a *split-plot* model with a three-level treatment (vehicle, drug before, and drug after) per repeated-measure (six time bins). Dark-light testing implied a vehicle vs drug two-way design. Level of significance was set at $P < 0.05$. Multiple post hoc comparisons were run with the Tukey HSD test.

3 | RESULTS

Both the Novelty Seeking and Novelty Preference tasks allowed to analyze and compare three key variables (locomotor activity, time spent in a novel environment, and number of transitions), in subjects that were divided into three groups, receiving either vehicle only or LP-211, either before or after the familiarization session.

3.1 | Novelty seeking task with LP-211

3.1.1 | Activity rate

While the interaction between time and the pharmacological treatment with LP-211 is not meaningful (time*treatment $F_{8,72} = 1.588$, $P = 0.1434$), post hoc analysis displays a significantly greater locomotor activity ($P < 0.05$) in those subjects who had experienced the drug effect during exposure for familiarization with the environment (CF/V Group). This, compared both to subjects who had experienced the drug effect immediately afterward, during the mnestic consolidation (V/CF Group), and to the control subjects (Vehicle Group). This profile is observed during each time interval of the task except the second (10-15 minutes) interval, in which there is a significant locomotor activity increase in the V/CF subjects (Figure 1A).

3.1.2 | Time spent in the novel environment

The interaction between time and the pharmacological treatment is not significant (time*treatment $F_{8,68} = 1.481$, $P = 0.1804$). Post hoc analysis shows a significant difference, between groups, for time spent in the novel environment, during the whole task, as a function of the drug administration: Time spent in the novel environment (which is significantly higher than in familiar chamber for controls, as expected) is enhanced in V/CF group ($P < 0.05$). On the contrary, time spent by the CF/V subjects in the novel room does not differ from the chance level (Figure 1B).

3.1.3 | Transitions

About number of transitions between the two rooms, the interaction between time and drug administration group is significant (treatment*time, $F_{8,72} = 2.015$, $P = 0.056$). Those transitions made by CF/V subjects result significantly higher than those made by V/CF

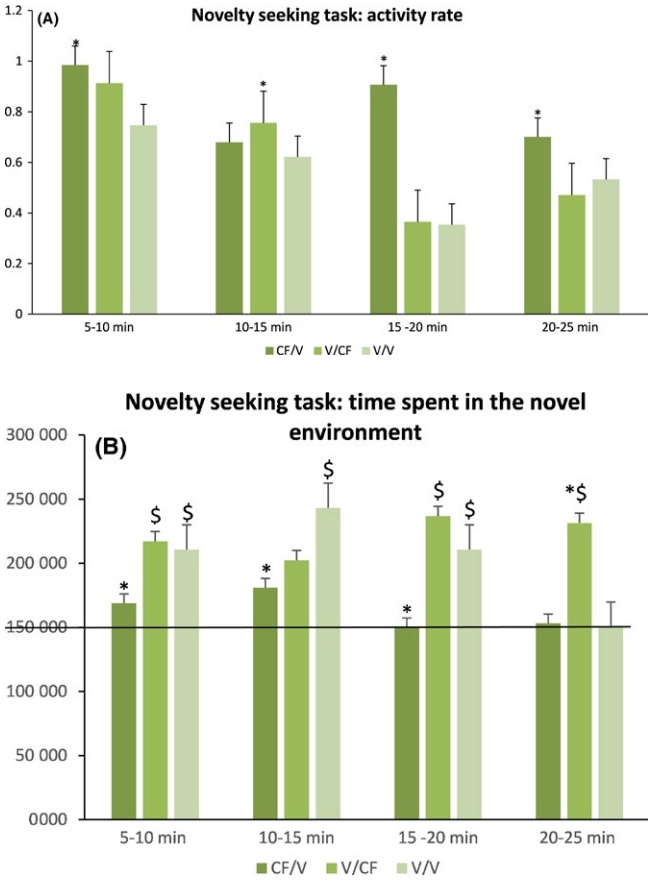

FIGURE 1 (A) Novelty seeking task with LP-211: activity rate. Mean activity rate measured as number of beam line crossing per 5-min period (vehicle n = 7; CF/V n = 7; V/CF n = 7). Wistar rats were assessed for novelty seeking behavior after treatment with LP-211 administered immediately before (CF/V) or immediately after (V/CF) the previous training session. Post hoc analysis displays a significantly greater locomotor activity in those subjects who had experienced the drug effect during the exposure to familiarization with the environment (CF/V group). $^{*}P < 0.01$. (B) Novelty seeking task with LP-211: time spent in the novel environment. Time (s) spent in the novel chamber during a 30-min test session, measured into six 300-s intervals (vehicle n = 7; CF/V n = 7; V/CF n = 7). Rats are the same as in panel "A" Time spent by the V/CF subjects, as well as control subjects, is higher than chance level. On the contrary, time spent by the CF/V subjects in the novel room does not differ from the chance level. $^{*}P = 0.01$ compared to vehicle group; $^{\$}P < 0.01$ compared to chance level of 150 s

subjects, compared to the vehicle injected subjects. Overall, this profile is similar to that of activity rate (data not shown).

3.2 | Novelty preference task with LP-211

3.2.1 | Activity rate

Despite ANOVA analysis for activity rate did not show a significance ($F_{10,85} = 1.182$; $P = 0.3145$), in the post hoc analysis the Tukey threshold was 0.45 ($df = 85$; $K = 6$). About activity rate, the pharmacological treatment did not seem to show any effect as all three groups had a similar behavior: During the first 10 minutes, all subjects had a peak of excitation due to exploration in the novel environment; after 10 minutes, they showed a steady decrease of arousal about the novel environment (data not shown).

3.2.2 | Time spent in the novel environment

ANOVA for the time spent in the novel room did not show a significant *value* ($F_{10,85} = 0.367$; $P = 0.9574$); the Tukey threshold was 43.5 ($df = 85$; $K = 6$). About time spent in the novel chamber, the only group showing a difference was the CF/V one: CF/V subjects spent more time in the novel room and they did not lose interest during the whole test. In other words, they did not show the usual habituation, due to progressive gain of information and were elevated compared to the other groups. There was no difference between V/CF and control groups which both apparently show scarce interest to novelty, due to the short habituation (Figure 2A).

3.2.3 | Transitions

Analysis about transitions into the novel environment shows a significant trend ($P = 0.065$) indicating effects of pharmacological treatment as a function of time ($F_{10,85} = 1.841$). The Tukey threshold was 1.14 ($df = 85$; $K = 6$). V/CF group shows a significantly higher number of transitions during the whole test, compared to other groups. V/CF subjects did recognize the novel chamber (see above, time spent) but, for some reason, they displayed an increased back-and-forth (more than twofold than controls). As a possibility, we propose that these animals might show a conflicting profile: On the emotional side, they may become anxious regarding the experience of novelty. They are still attracted by it but, at the same time, feel it necessary to come back often in the familiar zone (Figure 2B).

3.3 | Dark-light task with LP-211

3.3.1 | Activity rate

Our data did show a quite relevance between the three groups of animals. Despite the ANOVA did not have a significant trend (treatment*time, F10,80 = 0.600; $P = 0.8098$), we did the post hoc analysis with Tukey's ($df = 80$; $K = 6$) which is protected against false positives. Results showed that there were differences only during the first 10 minutes: at 5 minutes after the start, vehicle group's activity rate was higher compared to controls and treated rats; at 10 minutes, vehicle group's activity rate was lower than the others. During the remaining time, activity rate did not show any difference between the three groups of animals (Figure 3A).

3.3.2 | Time spent in light side

The interaction between time and the pharmacological treatment was not significant (treatment*time, $F_{10,80} = 1.080$; $P = 0.3872$). The post hoc analysis conducted by Tukey's ($df = 80$; $K = 6$) did not show

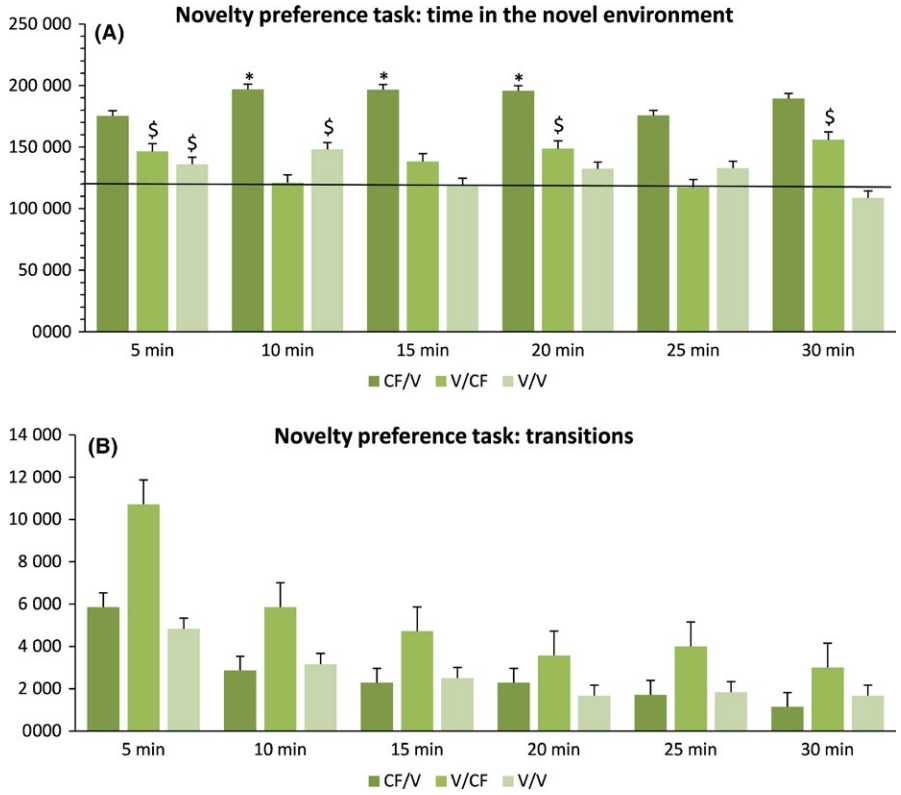

FIGURE 2 (A) Novelty preference task with LP-211: time spent in the novel environment. Time (s) spent in the novel chamber during a 30-min test session, measured into six 300-s intervals (vehicle n = 7; CF/V n = 7; V/CF n = 7). Wistar rats were assessed for novelty preference behavior after treatment with LP-211 administered immediately before (CF/V) or immediately after (V/CF) the previous training session. Post hoc analysis shows that CF/V subjects spent more time in the novel room and they did not lose interest during the whole test. There was no difference between V/CF and control groups, which show only slight interest to novelty. $^{*}P = 0.01$ compared to vehicle group; $^{\$}P < 0.01$ compared to chance level of 120 s. (B) Novelty preference task with LP-211: transitions. Number of times a subject crosses the door between the central and the novel compartments during a 30-min test session, measured into six 300-s intervals (vehicle n = 7; CF/V n = 7; V/CF n = 7). Rats are the same as in panel "A". Transition into the novel environment shows a significant trend ($P = 0.065$): the V/CF group shows a significantly higher number of transitions during the whole test compared to other groups

a significant difference about time spent in lit environment by control and vehicle subjects during nearly the whole task. There was only one difference in that, at 20 minutes, time spent by the drug treated rats in the light side were slightly increased over other groups, denoting slight anxiolytic effects (Figure 3B).

3.3.3 | Transitions

About number of transitions between the two rooms, the interaction is not significant (treatment*time, $F_{10,80} = 0.612$; $P = 0.7994$). Post hoc analysis ($df = 80$; $K = 6$) shows a little difference during the first 5 minutes of the test, because the vehicle group seems to have a bit more transitions between the two rooms, a profile similar to activity rate and denoting arousal. During the remaining time, all three groups show a similar trend of habituation without any difference (data not shown).

4 | DISCUSSION

In the present study, we deeply investigated the effects of the activation of 5-HT7 receptor on exploration of novelty subsequent to collecting and consolidation of memory for a familiar environment, focusing on both emotional and cognitive components involved, through the use of two experimental paradigms which differ in visuospatial parameters. We found that activation of 5-HT7Rs by the administration of LP-211 had a significant effect on time spent in an unknown environment, increasing or decreasing it depending on the specific paradigm in use. As a whole, LP-211 exhibited a modulatory effect on motivational drives and improved both visuospatial and emotional consolidation. On the contrary, in a third experiment, it showed a very slight anxiolytic effect in a dark-light task.

In the first experiment, a novelty seeking task in black/white box, the apparatus is made up of two same-shaped chambers which differ in their end walls' colors (black or white). Subjects are placed for a while in one of the two chambers, already familiar as they previously explored it at least twice, and they are then allowed to discover the novel environment.[14,20] This method usually places a special emphasis on the emotional component, connected to the rats' innate fear of the clear and (slightly more) bright environments. However, we intentionally kept slight this component, as the two chambers are prevalently gray and distinguished by their end walls' colors (one white, one black) that, in low light conditions, are rather similar to a dark vs

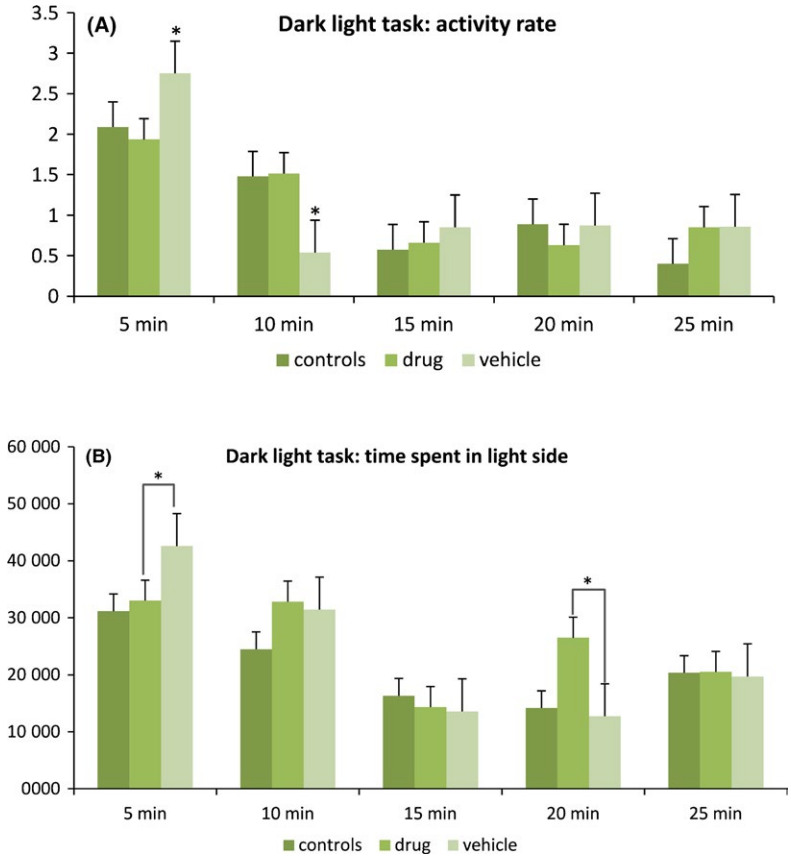

FIGURE 3 (A) Dark-light task with LP-211: activity rate. Mean activity rate measured as number of beam line crossing per 5-min period. Wistar rats, assessed for anxiety-like behavior, were divided into three different groups; the first did not receive injections (control group), the second received an injection of LP-211 (drug group), and the third received an injection of vehicle (vehicle group). There was a difference during the first 10 min: at 5 min after the start, vehicle group's activity rate was higher while, at 10 min, vehicle group's activity rate was lower, than both controls- and drug-treated rats. (B) Dark-light task with LP-211: time spent in the light side. Time (s) spent in the lit chamber during a 25-min test session, measured into five 300-s intervals. Rats are the same as in panel "A". At 20 min, time spent by the drug treated rats in the light side was slightly increased over other group. In both panels, $^*P < 0.01$

light grayscale. The cognitive component, as far as explicit memory for spatial shape is concerned, is therefore missing: Motivational components such as curiosity vs neophobia are recruited.[14,20] Furthermore, in these conditions, the recognition of the familiar environment over the unknown one is based on visual cues, and the novelty seeking was enhanced for those subjects who better understood the visual difference. In the second method, a novelty preference task was carried out through an apparatus whose chambers differ by shape (D/L) but not by color: Since the presence of clear spatial elements makes the recognition of the two environments much simpler and immediate, we can evaluate the pure motivational component of the memory instead. Being devoid of cognitive difficulty components, levels of the novelty preference is principally due to emotional factors (ie attractive vs avoidant drive) possibly related to the familiar room, likely associated to feelings such as "comfortable" or "boring" (respectively). Now starting from a central neutral environment, subjects are allowed to visit both the end chambers, hence being able to make first a discrimination and then a preferential choice.[7] Comparing results from these two methods allowed us to evaluate differential effects of LP-211 on the involved components, namely motivation rather than emotional memory, which may affect the experimental subjects' explorative behavior.

In the first group (CF/V), the familiarization was experienced by subjects during the ongoing course of drug effects, so that LP-211, by activating the 5-HT7R serotonin receptors in the hippocampus and PFC, could modulate the destiny of sensory inputs gained while exploring the environment. Indeed, animals in this familiarization stage get inputs of two kinds: (i) spatial informations (eg shape of the environment), contributing to the hippocampus-depending memory acquisition; and (ii) visual informations (eg color and brightness of the environment), still presenting a cognitive significance, but also connected to the most instinctual and ancestral components, eliciting attraction or avoidance in turn, controlled by PFC. In the first experiment, carried out in an apparatus with same-shaped chambers, there were not spatial cues; colors, in dim light conditions, had no marked brightness differences, being rather similar to a more vs less dark on a grayscale. In these conditions, CF/V subjects seemed like frantic and failed to distinguish the known from the novel environment: The time spent in the novel environment was comparable to the chance level, and activity as well as the number of transitions between the two environments were significantly higher than controls. It may indicate an LP-211 interference on the emotional evaluations and/or a potentiation of the spatial inputs' acquisition. As such, being both rooms rectangular in shape, CF/V subjects had no cue whatsoever to distinguish between the two same-shaped chambers. In the second experiment, carried out in the apparatus with different-shaped chambers, the recognition of the known environment was easier than for the BWB, and indeed CF/V subjects showed a significantly higher novelty preference: Probably, an enhancement of the motivational drive was caused by the drug, because the familiar room become more intensely so, possibly related to potentiated acquisition and storage of spatial elements.

About the V/CF subjects, the input acquisition took place under normal drug-free condition, whereas the memory consolidation took place under LP-211 effect. In the present study, immediately after the familiarization in a BWB apparatus (namely during the memory

consolidation), 5-HT7R activation (through LP-211) caused in rats a significant increase in the time spent in the novel environment, compared to control and CF/V groups. As we said, dim light condition and same-shaped chambers made the two environments a bit hard to recognize except for the V/CF subjects: This finding may well be interpreted by suggesting a potentiated visual (and/or emotional) consolidation. The concept of "familiar" room, from the original cognitive perspective, added an emotional perspective: potentiated consolidation of visual cues entailed fixing of the visual elements into enhanced knowledge about the room. That chamber become not only "known" but also hence "boring," giving the motivational drive for the enhanced seeking in the novel room. In the second experiment, carried out in a D/L apparatus, as the spatial difference between the known and the novel environment was much easier to remember, the novelty preference was again essentially due to emotional factors. In this task, the V/CF subjects' novelty preference was comparable to the controls' one, whereas V/CF transitions between the environments were oddly elevated. Therefore, given the same level of cognitive recognition based on easier shape memory, data may reveal that rats had good reasons to come back more often to the familiar room. The explanation of this behavioral peculiarity could imply an enhancement of the emotional consolidation, which gave to the concept of "familiar" this time the meaning of "comfortable." In other words, even if animals were attracted by novelty, they needed to go back often to the known environment likely due to a positive feeling consolidated to it. Therefore, we hypothesized that (through the administration of LP-211 prior to consolidation of "familiar" data) the activation of 5-HT7 receptors could strengthen the consolidation for "emotional components" of memory, thus giving more prominence to the "sense of security" experienced by subjects in the familiar room.

The hippocampus plays a pivotal role, both in humans and rats, in the systematic organization of explicit memory, whereas the prefrontal cortex (PFC) exerts an active control on memory organization during its encoding and retrieval, filtering the input stimuli depending on their relevance in that specific context.[23,24] In an earlier study, the LP-211 acute administration resulted in a clear activation of orbital prefrontal cortex and hippocampus, consistent with the broad presence of the 5-HT7 receptor in these areas, strengthening the assumption that this receptor is involved in learning and memory processes.[9] In addition, several authors elucidated its role in memory processing,[8,25-27] adding many confirmations on the functional role of 5-HT7R in processes implemented by hippocampus and PFC. Memory consolidation is the process by which faint memory traces are converted in long term memories. In order for this to be achieved, the synaptic connections have to be remodeled through protein synthesis; some of these proteins are directly involved in synaptic transmission, others play a regulatory or structural role.[28] Synaptic plasticity and, hence, memory consolidation, have been associated with dendritic spines morphological changes, mechanism regulated by signaling pathways that engage the neurotransmitter serotonin (5-hydroxy-tryptamine [5-HT]) and the extracellular matrix (ECM).[29,30] In particular, a causal link between 5-HT7 receptor activation and the MMP-9-dependent dendritic spine morphogenesis, involving hyaluronan receptor CD44 and GTPase Cdc42, was observed.[3] According to these assumptions, LP-211 administration in adult rats resulted in a significantly increase of the dendritic spines' number and density in the proencefalic neurons[31]; in adolescent rats, it induced plastic re-arrangements of the proencefalic loops, causing behavioral changes that continue in adult life.[27] Our results seem to be consistent with the above recent findings, since animals receiving LP-211 showed an overall gain for the acquisition and the consolidation of visuospatial and emotional memory, resulting in alterations of behavioral parameters.

In particular, activation of 5-HT7 receptors seems to favor exploration by: (i) enhancing visual consolidation, (ii) improving the ability to discriminate a familiar environment, and (iii) consequently allowing rats to display curiosity for an unknown chamber. Furthermore, when there is little room to improve acquisition and storage of spatial elements, LP-211 may still be resulting in an increased motivation to explore, as it seems to change the modality for perception of "familiar" items: this, possibly by acting on hippocampal-PFC crosstalk and strengthening the consolidation of emotional components of memory. However, according to the results of the dark/light task, LP-211 in itself seems to have just a transient and very slight acute pharmacological effect on the anxious-like behavior.

To sum up, LP-211 may reveal a useful tool to act over emotional processing of visual stimuli, and as such may show some overlap with other serotonergic drugs.[32] In conclusion, although further studies are necessary also with the help of novel drugs like LP-211, the role of 5-HT7 receptor in emotional and motivational regulations as well as in processing of memory is becoming increasingly better understood.

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CONFLICT OF INTEREST

There is no conflict of interest to disclose.

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