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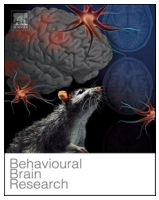

Short communication

Social avoidance and altered hypothalamic-pituitary-adrenal axis in a mouse model of anxious depression: The role of LPA_1 receptor

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ABSTRACT

Anxious depression is a prevalent disease with devastating consequences. Despite the lack of knowledge about the neurobiological basis of this subtype of depression, recently our group has identified a relationship between the LPA_1 receptor, one of the six characterized G protein-coupled receptors (LPA_{1-6}) for lysophosphatidic acid, with a mixed depressive-anxiety phenotype. Dysfunctional social behaviors, which have been related to increased activation of the hypothalamus-pituitary-adrenal (HPA) axis, are key symptoms of depression and are even more prominent in patients with comorbid anxiety and depressive disorders. Social behavior and HPA functioning were assessed in animals lacking the LPA_1 receptor. For these purposes, we first examined social behaviors in wild-type and LPA_1 receptor-null mice. In addition, a dexamethasone (DEX) suppression test was carried out. $maLPA_1$-null mice exhibited social avoidance, a blunted response to DEX administration and an impaired circadian rhythm of corticosterone levels, which are features that are consistently dysregulated in many mental illnesses including anxious depression. Here, we have strengthened the previous experimental evidence for $maLPA_1$-null mice to represent a good animal model of anxious depression, providing an opportunity to explore new therapeutic targets for the treatment of mood disorders, particularly this subtype of depression.

1. Introduction

Depression is a major health concern in modern societies. Major depressive disorder is a heterogeneous illness with various subtypes. One of these subtypes is depression disorder with comorbid anxiety, also known as anxious depression, that involves more disability, more resistance to treatment, greater risk of suicide and is associated with more severe psychological, physical and social impairments than either condition alone [1]. The association between depression and impaired social functioning is well recognized. Social withdrawal and lack of social support are key features involved in both the onset and maintenance of the depressive state and can be a relevant target for intervention [2]. Dysfunctional social behaviors are even more prominent in patients with comorbid anxiety and depressive disorders than either disease alone [3].

From a neurobiological point of view, social avoidance behavior has been related to increased activation of the hypothalamus-pituitary-adrenal (HPA) axis [4]. In this sense, relatively high HPA-axis activity has been related to increases in behavioral inhibition and increased social avoidance. In fact, there is a direct link between increased cortisol stress-responsiveness and social avoidance behavior [4]. Because HPA-axis dysfunction is a well-known biological marker of depression, its function in depressed patients has been extensively studied using a sensitive pharmacological challenge test, the dexamethasone (DEX) suppression test (DST) [5]. Although there are conflicting results, DEX nonsuppression has been linked to depression. Most likely, the explanation for the conflict lies in the existence of different subtypes of this disease. Patients with anxious depression show DST nonsuppression

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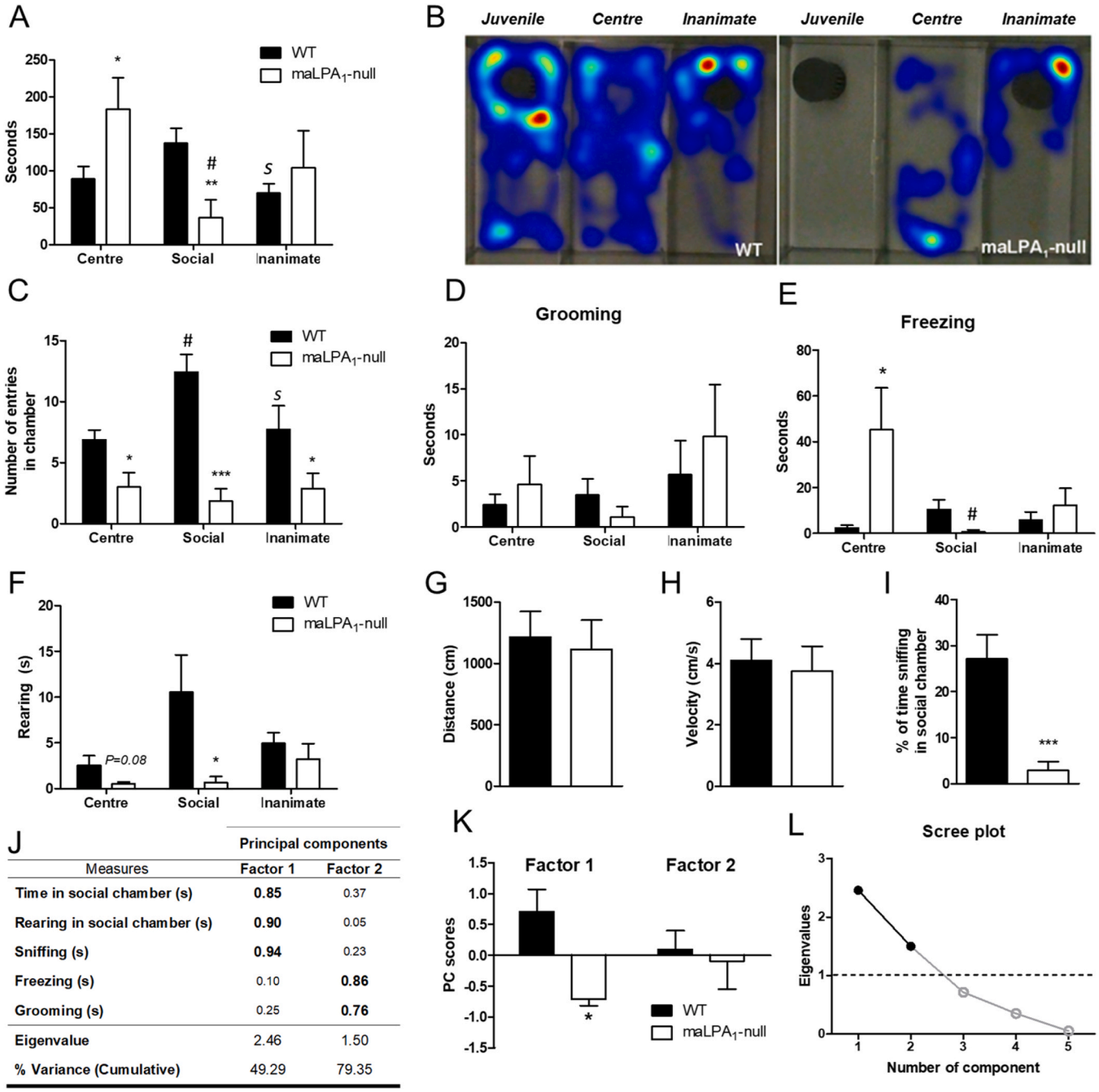
rates that indicate a dysregulation in the HPA-axis [6].

Recently, our group has been the first to propose a possible relationship between the LPA_1 receptor, one of the six G protein-coupled receptors through which lysophosphatidic acid (LPA, 1-acyl-2-sn-glycerol-3-phosphate) acts, and the mixed depressive-anxiety phenotype, which may shed light on the unknown neurobiological basis of this subtype of depression [7,8]. In fact, based on the validity criteria and considering the results of accumulated data with studies using animals lacking the LPA_1 receptor, maLPA$_1$-null mice have been proposed as an animal model of the mixed depressive-anxiety phenotype [7]. Moreover, absence of the LPA_1 receptor induces exaggerated endocrine responses to emotional stimuli [8,9], causes hypoactivity and impairs adaptation



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	Principal components	
Measures	Factor 1	Factor 2
Time in social chamber (s)	**0.85**	0.37
Rearing in social chamber (s)	**0.90**	0.05
Sniffing (s)	**0.94**	0.23
Freezing (s)	0.10	**0.86**
Grooming (s)	0.25	**0.76**
Eigenvalue	2.46	1.50
% Variance (Cumulative)	49.29	79.35

Fig. 1. Assessment of Social behavior revealed dysfunctional social behavior in the absence of the LPA_1 receptor. (A) The amount of time (the mean ± S.E.M) or (B) a representative picture of time mice spent in each of the three chambers revealed social avoidance in the null mice. (C) Data are corroborated by the number of entries into each chamber. (D) Duration (s) of grooming, (E) freezing and (F) rearing in each compartment. (G) Percentage of time in relation to the total time spent in the 'social compartment' by each genotype sniffing the juvenile mice, rearing, grooming or freezing. (H) The distance traveled and (I) the velocity measured during the three compartments test revealed no differences between the genotypes. (J) Principal component analysis of social behavior measures. Interpretable factor loadings are in bold. The results revealed evidence both for a factor relating to social behavior and for another factor that relates to exploratory behavior. (K) Variables with negative scores are inversely related to the factor; therefore, absence of the LPA_1 receptor is negatively related with social behavior. Rotation method: Varimax with Kaiser normalization. Rotation converged in 3 iterations. KMO = 0.51; $\chi^2 = 34.69$; $p < 0.001$. (L) Scree plot of the principal components analysis. Data are represented as the mean ± standard error of the mean (S.E.M). *$p < 0.05$, * *$p < 0.01$ and * **$p < 0.001$ significant differences between genotypes. #$p < 0.05$ significant difference with the central compartment. *s* $p < 0.05$ significant difference with social compartment. n = 7 wild-type mice and 7 maLPA$_1$-null mice.

of the HPA-axis after chronic stress [10], which are factors that have also been strongly correlated with depression [11]. While suggestive of a dysfunctional HPA-axis, this issue has not yet been systematically evaluated in maLPA$_1$-null mice. Thereby, the aims of this study were to elucidate the involvement of the LPA$_1$ receptor in two separate depression related characteristics: HPA-axis functioning and in social behavior. For this purpose, social behavior was evaluated using the three compartments test in wild-type and maLPA$_1$-null mice. Moreover, in both genotypes, DST was carried out in order to measure the corticosterone response.

2. Methods

2.1. Animals

For this study we used the Malaga variant of null mice for the LPA$_1$ receptor, termed as "maLPA$_1$-null" [12], derived from the original colony of Contos et al. [13]. Except for 1-month-old juvenile mice used for the Social Preference Test, 3-month-old male mice were used. For the Social Preference Test 14 mice were used. An additional group of 15 animals were subjected to the DST (Figs. 1 and 2).

Animals were housed in groups of 4 in a cycle of 12 h light/dark (lights on at 07:00 h) with water and food available ad libitum. The experiments were carried out between 9:00 and 15:00 h.

The procedures were approved by the Ethics Committee of the University of Malaga (CEUMA: 1-2015-A, 08-7-15-273) and complied with the European Animal Research Laws (EU Directive 2010/63/EU, EU, 90/219 / EEC and Regulation [EC] Nº 194/2003) and the Spanish Guidelines on Animal Experimentation and Use of Genetically Modified Organisms (Royal Decrees: 53/2013, 178/2004; Laws 32/2007, 9/2003 and Decree 320/2010).

2.2. Social preference test

The social preference test was performed in a rectangular box formed by three chambers (LE894 Panlab Harvard Apparatus, Spain). The left and right compartments were equipped with wire cups that contained either an unfamiliar male juvenile mouse (the 'social compartment') or an unfamiliar object. The time spent in each chamber, locomotion (distance travelled [cm]) and velocity (cm/s) were registered using a video tracking system (Ethovision XT, Noldus, Wageningen, The Netherlands), whereas ethological parameters (time sniffing the juvenile [social target], freezing, rearing or grooming) were scored by two trained observers who were blind to the genotype of the animals.

2.3. Dexamethasone suppression test and corticosterone assay

For plasma corticosterone level determination, two samples of blood were collected from the lateral tail vein at 09:00 and 15:00 h. Immediately after the first blood collection, mice were administered IP vehicle (saline) or 0.1 mg/kg DEX (Sigma Aldrich, D0720000).

The tubes containing the blood samples in presence of EDTA were centrifuged, and the supernatant was stored at − 80ºC. Plasma corticosterone levels were determined in duplicate using a commercially available Enzyme Immunoassay Kit (sensitivity ca. 27.0 pg/ml) following the manufacturer's instructions (Assay Designs/Stressgen, Ann Arbor, Michigan, USA).

2.4. Statistical analysis

Data are expressed as the mean ± standard error of the mean (S.E.M.). Comparisons between groups (wild-type mice vs maLPA$_1$-null mice) in social behavior variables (time spent in each chamber, sniffing, rearing, freezing and grooming) were analyzed by Student́s t-test for independent samples together with effect size Cohen's d. Moreover, we also performed t-test for dependent samples to compare the time spent for each group in the different compartments of the maze (centre, social and inanimate). Power analysis (1-β) was also performed to control the probability of Type 1 error in our analysis. Furthermore, to determine the relationship between behavioral variables in the social chamber and to reduce them to a smaller set of dimensions that could underlie the genotype effects [14], a principal components analysis (PCA) was carried out. PCA was tested for sampling adequacy by the Bartlett sphericity and the Kaiser-Meyer-Olking (KMO) tests and followed by a varimax orthogonal rotation, which ensures that the extracted factors are independent of one another. The resulting factors with an eigenvalue > 1 were selected, and the contribution of each variable to a factor (i.e., 'factor loading') was considered significant when it was more than 0.60 in absolute value.

For corticosterone measures, an ANCOVA using the basal CORT levels (measured at 9:00 h) as the covariable was utilized, thus controlling the different basal levels between genotypes.

Only probabilities ≤ 0.05 were considered significant. For the benefit of clarity and brevity, only relevant results of these statistical analyses are reported.

3. Results

3.1. Social behavioral results

The data obtained in the social preference test are shown in Fig. 1. The statistical analysis showed that wild-type animals spent more time in the 'social compartment' in comparison to the amount of time spent in the 'inanimate compartment'. However, the animals lacking the LPA$_1$ receptor spent significantly less time in the 'social compartment' (t_{12} =−2.75, p = 0.017, (1-β)= 0.84, d= 0.15) and more time in the 'central compartment' (t_{12} =3.22, p = 0.007, (1-β)= 0.87, d= 0.17; Fig. 1A and B).

In addition, maLPA$_1$-null mice had significantly fewer entries in the 'social compartment' than those of the wild-type animals (t_{12} =−5.99,

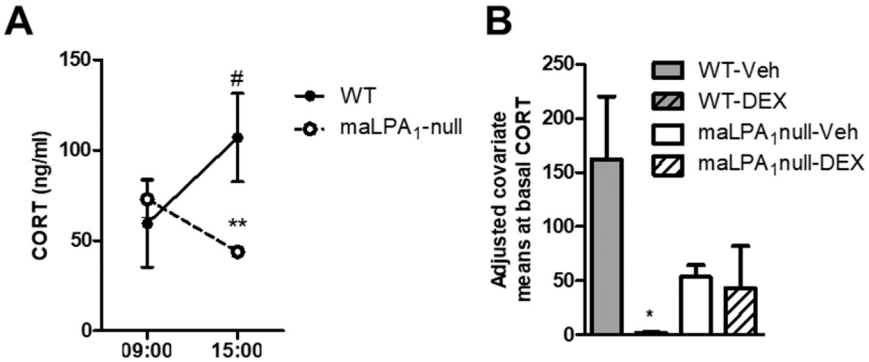

Fig. 2. HPA-axis response to dexamethasone is altered in mice lacking the LPA$_1$ receptor. CORT release from 9:00–15:00 in both genotypes indicated an inverted pattern in the maLPA$_1$-null mice in comparison with that in the wild-type mice (A). After controlling for the baseline values of CORT, the data indicated that maLPA$_1$-null mice exhibited nonsuppression of CORT levels at 6 h after dexamethasone administration (B). Data are represented as the mean ± standard error of the mean (SEM). * $p \leq 0.05$ and * *$p \leq 0.01$ compared to the wild-type mice-Veh; # $p < 0.05$ compared to the wild-type mice-DEX. N: wild-type mice-Veh= 5; wild-type mice-DEX= 5; maLPA$_1$-null mice-Veh= 5; maLPA$_1$-null mice-DEX= 5).

$p < 0.001$, (1-β)= 1, d= 0.32; Fig. 1C).

Regarding the ethological behaviors, mice lacking the LPA_1 receptor remained in the freezing state in the 'central compartment' for a longer time ($t_{12} = -2.41$, $p = 0.032$, (1-β)= 0.60, d= 0.13; Fig. 1E), while they spent significantly less time rearing in the 'social compartment' ($t_{12} = -2.39$, $p = 0.034$, (1-β)= 0.61, d= 0.13) and relatively less time (in relation to the total time in the 'social compartment') rearing ($t_{12} = -2.43$, $p = 0.032$, (1-β)= 0.69, d= 0.13) and sniffing ($t_{12} = -4.33$, $p < 0.001$, (1-β)= 0.97, d= 0.23; Fig. 1F and G). Importantly, we did not observe any differences between groups in either the velocity or the distance measurements (Fig. 1H and I).

PCA extraction resulted in Factor 1 consisting of time (0.85), rearing (0.90) and sniffing (0.94) in the 'social chamber' and Factor 2 consisting of freezing (0.86) and grooming (0.76) in the 'social chamber' (KMO=0.51; χ^2=34.69; $p < 0.001$; Fig. 1J). Additionally, mice lacking the LPA_1 receptor had significantly lower PC scores in Factor 1 compared to those of the wild-type mice ($t_{12} = -3.47$, $p = 0.005$, (1-β)= 0.95, d= 0,19; Fig. 1K).

3.2. CORT response to the dexamethasone suppression test

The normal pattern of daytime release of CORT was inverted in the maLPA$_1$-null mice, which displayed higher levels at 9:00 h and lower levels at 15:00 h in comparison with the levels of the wild-type mice (Fig. 2A). Related to DST, the ANCOVA analysis revealed a significant interaction in the analysis of covariance between genotype and treatment ($F_{[1,15]}$=5.39; $p < 0.05$; Fig. 2B), showing nonsuppression by maLPA$_1$-null mice in the DST.

4. Discussion

Social behavior is fundamental for emotional regulation [15]. Conversely, social isolation can have negative consequences on an individual's physical, cognitive and mental health [16]. In fact, loss of social contact has a significant impact on cardiovascular health and life expectancy, cognitive impairment and mood [17–20]. Dysfunctional social behaviors are particularly prominent in patients with comorbid anxiety and depressive disorders than either disease alone (Saris et al., 2017). Understanding the neurobiological mechanisms responsible for social isolation, which in turn are related to the development of several psychological diseases, may have important implications for the treatment or prevention. For this reason, we have further characterized the involvement of the LPA1 receptor in social behavior. This receptor has been linked to problems in emotional regulation [9,21] and the development of a mixed depressive-anxiety phenotype [7,8].

On the other hand, social behavioral disturbances have been associated with dysregulation of the HPA-axis [4,22]. In fact, there is an association between relatively high HPA-axis activity and social avoidance, indicating the importance of an intact and functional HPA-axis for exhibiting successful social behaviors [4]. Different data suggest the participation of the LPA1 receptor in the regulation of the HPA axis. Thus, on the one hand, exogenous administration of LPA induces a reduction of CORT levels at baseline but potentiates CORT release following chronic stressor application [23]. On the other hand, animals lacking the LPA1 receptor show signs of dysregulation of the HPA axis [9,10]. However, this axis has not been specifically evaluated in maLPA$_1$-null animals. The dexamethasone suppression test is commonly used to evaluate the dysregulation of the HPA axis particularly in depressive patients [24,25] and may be a useful tool to study the viability of the HPA axis in animal models [26,27].

Considering these data and with the aim of better characterizing the null animal as an animal model of depression, we have studied on the one hand the social behavior and on the other hand the response of the HPA axis to the application of an exogenous glucocorticoid in both wild type and maLPA$_1$-null mice.

The present study has allowed us to know the involvement of the LPA_1 receptor in social behavior. Thus, while wild-type mice showed a tendency to socialize, the lack of the LPA_1 receptor was associated with increased social avoidance tendencies during social exposure. Since no differences in either the velocity or distance travelled were observed between the groups, social avoidance may not be attributed to abnormalities in locomotor activity. Hence, the substantial decrease in vertical activity, a validated indicator in rodent depression [28] and anxiety [29] models, along with a reduced time spent sniffing the juvenile animal, was associated with a lower score in the sociability factor in PCA analyses. This result seems to suggest that the absence of the LPA_1 receptor led to impaired social behaviors [30].

Regarding the endocrinological factors, DST revealed a blunted response in null mice to an exogenous corticosteroid administration. Moreover, the circadian rhythm of corticosterone, a domain that is consistently dysregulated in many mental illnesses including anxious depression [31], seems to be disturbed in maLPA$_1$-null mice. The dysregulation of the HPA axis evidenced by DST could be related to alterations in social behavior in maLPA$_1$-null animals. However, further studies are needed to verify this association and to provide enough detail regarding CORT levels over time to allow adequate assessment of circadian changes in CORT levels in the absence of the LPA_1 receptor.

Over the past decade, it has been shown that the LPA_1 receptor can be targeted by antidepressants, such as tricyclic, tetracyclic, desipramine and fluoxetine [7], causing ERK1/2 stimulation and cellular responses [32]. The LPA_1 receptor could also be involved in catecholamine secretion [33]. For instance, gintonin, a ligand for LPA receptors, has anti-inflammatory effects against neurodegenerative conditions like Alzheimer's, Parkinson's, anxiety, and depression [34]. Research suggests that gintonin's release of catecholamine, acetylcholine, and glutamate in the brain may alleviate depression-like effects, providing insights for potential use in managing neurodegenerative conditions. Regarding the anti-inflammatory effects, recently Nagata et al. (2023) [35] reported that LPA treatment prevents microglial activation, suppresses IL-1β and TNF-α expression in the hippocampus, improving depression-like behaviors. Interestingly, another recent study found that amitriptyline, a typical tricyclic, mediates antidepressant effects such as normalizing the CORT-induced decrease in sucrose preference by binding to LPA_1 receptor [36]. Thus, the use of LPA_1 receptor agonists could potentially be a new family of antidepressants with anxiolytic profiles, having a buffering effect on HPA axis and stress hormones release. However, to date no drugs targeting LPA receptors have been approved by any regulatory agency.

Overall, the results presented here have provided, for the first time, data for the involvement of the LPA_1 receptor in social behavior, implicating the absence of the LPA_1 receptor in social avoidance. Moreover, altered HPA-axis functioning and possibly circadian rhythms, factors involved in the etiology of depression and especially the anxious depression subtype, have been verified in maLPA$_1$-null mice. However, we also want to mention certain limitations of our study. On the one hand, the sample size is small, so it has been necessary to statistically control that the observed differences are not due to Type 1 errors. Thus, the resulting effect sizes could be considered as small and medium. On the other hand, it would have been very interesting to include females in our study, because it would have enhanced the extrapolation and interpretation of our results. In conclusion, these results extend the previous evidence on the participation of the LPA_1-receptor in the development of depression-like features and expanding the opportunity for developing drugs to target the LPA_1 receptor that may be useful for the treatment of mood disorder.

CRediT authorship contribution statement

Conceptualization: **C.P., F.RdF., G.E.-T., L.J.S.** and **R.D.M.-F.**; Data curation: **A.N.-Q., F.G.-S., P.S.-P.** and **R.D.M.-F.**; Formal analysis: **C.P., P.S.-P.** and **R.D.M.-F.**; Funding acquisition: **C.P.** and **G.E.-T.**; Investigation: **A.N.-Q., C.P., F.G.-S.** and **R.D.M.-F.**; Methodology: **P.S.-P** and

R.D.M.-F.; Supervision: **C.P., F.RdF.** and **L.J.S.**; Writing – original draft: **C.P., F.G.-S.** and **R.D.M.-F.**; Writing – review & editing: **C.P., F.G.-S., P. S.-P.** and **R.D.M.-F.**

Declaration of Competing Interest

Authors declare no conflicts of interest. All authors have agreed with the submission in its present form

Data availability

Data will be made available on request.

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