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Behavioral phenotype of maLPA$_1$-null mice: increased anxiety-like behavior and spatial memory deficits

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Lysophosphatidic acid (LPA) has emerged as a new regulatory molecule in the brain. Recently, some studies have shown a role for this molecule and its LPA$_1$ receptor in the regulation of plasticity and neurogenesis in the adult brain. However, no systematic studies have been conducted to investigate whether the LPA$_1$ receptor is involved in behavior. In this study, we studied the phenotype of maLPA$_1$-null mice, which bear a targeted deletion at the *lpa*$_1$ locus, in a battery of tests examining neurologic performance, habituation in exploratory behavior in response to low and mild anxiety environments and spatial memory. MaLPA$_1$-null mutants showed deficits in both olfaction and somesthesis, but not in retinal or auditory functions. Sensorimotor co-ordination was impaired only in the equilibrium and grasping reflexes. The mice also showed impairments in neuromuscular strength and analgesic response. No additional differences were observed in the rest of the tests used to study sensoriomotor orientation, limb reflexes and co-ordinated limb use. At behavioral level, maLPA$_1$-null mice showed an impaired exploration in the open field and increased anxiety-like response when exposed to the elevated plus maze. Furthermore, the mice exhibit impaired spatial memory retention and reduced use of spatial strategies in the Morris water maze. We propose that the LPA$_1$ receptor may play a major role in both spatial memory and response to anxiety-like conditions.

Keywords: Elevated plus maze, lysophosphatidic acid, maLPA1-null mice, neurologic screening, open field, water maze

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Lysophosphatidic acid (LPA, 1-acyl-2-*sn*-glycerol-3-phosphate) is a phospholipid that acts as an intercellular messenger and possesses growth factor-like activities. Lysophosphatidic acid affects a variety of cell functions, including cell proliferation, differentiation, survival and migration (Anliker & Chun 2004; Birgbauer & Chun 2006; Chun 2005, 2007; Moolenaar *et al*. 2004; Ye *et al*. 2002). The effects of LPA are mediated by a family of specific G protein-coupled receptors (GPCRs) (Anliker & Chun 2004; Bandoh *et al*. 2000; Fukushima *et al*. 2001; Ishii *et al*. 2004). Among these receptors, LPA$_1$ is a receptor coupled to Gi, Gq and $G_{12/13}$ family heterotrimeric G proteins; it has high affinity for LPA, and its downstream effectors are well characterized (Anliker & Chun 2004).

To date, few studies have addressed a possible role of the LPA$_1$ receptor in behavior. Harrison *et al*. (2003) and Roberts *et al*. (2005) reported prepulse inhibition impairment in LPA$_1$-null mice. These studies suggest that LPA, acting through the LPA$_1$ receptor, may mediate sensorimotor gating. LPA$_1$-null mice display a reduced ability to filter out irrelevant auditory stimulation, which may lead to the development of cognitive deficits. Despite these findings, no studies testing the involvement of LPA$_1$ in cognitive functions such as learning and memory have been reported, although Dash *et al*. (2004) did show enhancement of spatial memory in rats after post-training LPA microinjection in the hippocampus. Hippocampal LPA receptor subtypes therefore seem likely to play a role in adult cognitive function. However, the role of specific LPA receptors in adult animals remains to be established.

In the present study, we assessed the role of the LPA$_1$ receptor in sensorimotor, emotional and cognitive functions in adult mice. The study was performed in a maLPA$_1$-null mouse (Estivill-Torrús *et al*. 2008), a stable variant of the previously characterized LPA$_1$-null mutant (Contos *et al*. 2000). The maLPA$_1$-null variant was obtained during the

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propagation of LPA_1-null mice. These mice carry a targeted disruption in the *lpa*$_1$ gene. They show normal survival but display defective hippocampal neurogenesis, decreased levels of brain-derived neurotrophic factor (Matas-Rico *et al.* 2008) and altered cortical development (Estivill-Torrús *et al.* 2008). The brain alterations seen in maLPA_1-null mice are accompanied in adult animals by behavioral defects that affect their performance in neurological, emotional and memory tasks. Neurological impairments were observed in sensory functions (olfaction and somesthesis), limb reflexes and co-ordinated limb use (grasping reflex and equilibrium), as well as in neuromuscular strength. Although maLPA_1-null mice showed no impairment in either retinal or auditory functions, they exhibited impaired exploration in the open field (OF) and increased anxiety-like responses in the elevated plus maze (EPM) test. Finally, maLPA_1-null mice displayed impairments in spatial memory retention and abnormal use of searching strategies. These findings strongly suggest that the LPA_1 receptor is involved in both spatial memory and emotional behavior.

Materials and methods

Animals

The generation and characterization of maLPA_1-null mice have been described (Estivill-Torrús *et al.* 2008; Matas-Rico *et al.* 2008). The original null mice were obtained by targeted gene disruption using homologous recombination and Cre-mediated deletion on a 129X1/SvJ background. These animals were then backcrossed with C57BL/6J mice. Intercrosses of these mice, as well as with mice generated from one additional backcross (Contos *et al.* 2000), were begun immediately. An LPA_1-null mouse colony, termed maLPA_1-null from the *Málaga* variant of LPA_1-null, was spontaneously derived during the original colony expansion by crossing heterozygous foundation parents (maintained on the original hybrid C57BL/6J × 129X1/SvJ background). Intercrosses were performed with these mice and subsequently backcrossed for 15 generations with mice generated within this mixed background. MaLPA_1-null mice carrying the *lpa*$_1$ deletion were born at the expected Mendelian ratio, and they survived to adulthood. Targeted disruption of the *lpa*$_1$ gene was confirmed by genotyping (according to Contos *et al.* 2000); immunochemistry confirmed the absence of LPA_1 protein expression.

All experiments were conducted on age-matched male littermates from the following genotypes: wild-type [ma$lpa_1^{(+/+)}$], maLPA_1-null heterozygous [ma$lpa_1^{(+/-)}$] and homozygous [ma$lpa_1^{(-/-)}$] mice. All mice were approximately 3-months old at the start of behavioral testing. Mice were housed in groups of four on a 12-h light/dark cycle (lights on at 0700 h). Water and food were provided *ad libitum*. Experiments were conducted between 1000 and 1400 h. The different types of experiments were carried out on different groups of mice, such that no mouse participated in more than one phenotypic test. During behavioral testing, the experimenters were blind to the genotypes of the mice. All procedures were carried out in accordance with the European animal research laws (European Communities Council Directive 86/609/EEC and 2003/65/CE, and Commission Recommendation 2007/526/EC) as well as the Spanish National Guidelines for Animal Experimentation and the Use of Genetically Modified Organisms (Real Decreto 1205/2005 and 178/2004, and Ley 32/2007 and 9/2003).

Neurologic screening and auditory and retinal function

Neurologic assessment was performed in a testing room where the animals were previously habituated to the experimental conditions. All mice were taken from their home cages to the testing room and were kept there for 1 h before the neurological tests were carried out. To test sensorimotor orientation and co-ordinated limb and neurological function, the mice were subjected to a battery of tests taken from Marshall and Titelbaum (1974), modified by Bjorklund *et al.* (1980), and extended to additional reflexes by Bures *et al.* (1983). The following sensory reflexes were assessed: (1) somesthesis, in which a pin prick was applied to six sites on the lateral surface of the animal body, combining dorsal and ventral placements at rostral, middle and caudal levels; (2) whisker touch, in which a toothpick was brought close to the animal from the lower rear so as to avoid the visual field, and then lightly brushed against the vibrissae; (3) snout probe, in which a toothpick was gently rubbed against the snout of the mouse; (4) olfaction, where a small cotton swab dipped in ammonia solution was slowly brought close to the mouse's nose in a lateral-medial direction; (5) corneal reflex, in which the animal was restrained with a hand while the cornea was superficially stimulated with a fine, hair-tipped probe; (6) auditory startle, in which an unexpected, loud acoustic stimulus was applied and (7) head shaking, where the mouse was placed on a small, elevated platform and tested for reaction to a puff of air gently released through a narrow rubber tubing (internal diameter, 1 mm) to its pinna.

Limb reflexes and limb co-ordination were assessed using the following tests: (1) surface righting reflexes, in which the animal was placed on its back onto a flat surface, and the time for the animal to right itself was measured (2) forelimb suspension, where the mouse was grasped by one forepaw and suspended, and the latency time for the animal to grasp the hand with the free paw and use this to pull itself up onto the hand was recorded (failure criterion, 10 seconds); (3) grasping test, in which the mouse was hung by its tail and the forelimb palms were lightly touched with a stiff wire (diameter, 1 mm); (4) equilibrium tests, in which the mouse was placed facing downwards on a wire mesh platform tilted 30°, after which it was turned to face up the slope and then was finally placed on a horizontal wooden bar (diameter, 2 cm; length, 30 cm) suspended 50 cm above the floor, and its ability to stay on the bar was assessed; (5) placing reactions, where the mouse was restrained at the edge of the table and one foreleg or hindleg was displaced so that it hung over the edge.

The deficit in each orientation, limb use and neurological test was rated on a three-point scale: 0, absent; 1, weak or 2, strong. Use of this battery of tests allowed us to determine whether the maLPA_1-null mutation affected a particular brain region, interfered with a specific function or affected the central nervous system (CNS) as a whole (Bures *et al.* 1983). Ten ma$lpa_1^{(+/+)}$ mice, eight ma$lpa_1^{(+/-)}$ mice and eight ma$lpa_1^{(-/-)}$ mice were used to assess neurologic functions. Data were analyzed by a non-parametric Kruskal–Wallis test to assess the variance of the neurological test between different groups. Subsequently, appropriate paired comparisons were carried out using a Mann–Whitney *U*-test. A value of $P < 0.05$ was considered statistically significant. Additionally, the deficits in neurological test were presented as a percentage of incidences for each treatment.

The neurologic screening was completed using the hang wire and the tail flick tests to test, respectively, neuromuscular strength and analgesic response. In the hang wire test, the mouse was placed on a wire cage lid and the lid was gently moved back and forth, enabling the mouse to grip the wire. The lid was then turned upside down at a height of 15 cm above the surface of the bedding material; mice can easily fall from this height and land on their feet without injury. Latency to fall onto the bedding was recorded, with a cut-off time of 60 seconds. Eight ma$lpa_1^{(+/+)}$ mice, nine ma$lpa_1^{(+/-)}$ mice and 10 ma$lpa_1^{(-/-)}$ mice were used to assess neuromuscular strength. The tail flick test was performed using a water tail flick test. The mouse was restrained for tip tail immersion into a 52 ± 0.5°C water bath. The amount of time until the rodent flicked or moved its tail was recorded as the latency time. Three trials (T1–T3), spaced 20 min apart, were conducted with each animal. To avoid tissue damage, animals were never exposed to pain stimuli for more than 8 seconds. Thirteen ma$lpa_1^{(+/+)}$ mice, eight ma$lpa_1^{(+/-)}$ mice and 14 ma$lpa_1^{(-/-)}$ mice were used to study the analgesic response. In both tests, data were analyzed by one-way analysis of variance (ANOVA) followed by *post hoc* comparisons (Fisher's test).

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To determine whether auditory or retinal function was altered in the absence of *lpa*$_1$ expression, auditory brainstem responses (ABRs) and electroretinograms (ERGs) were obtained from ma*lpa*$_1^{(+/+)}$ and ma*lpa*$_1^{(-/-)}$ mice. Auditory brainstem responses were measured in response to clicks presented at a rate of 30 bursts/second. The mice were anesthetized with ketamine (100 mg/kg) and xylazine (4 mg/kg) by intraperitoneal injection, and the ABR tests were performed in a small sound-attenuating chamber. Analysis was performed on ma*lpa*$_1^{(+/+)}$ and ma*lpa*$_1^{(-/-)}$ mice using 11 mice per genotype. Auditory brainstem responses were recorded with subcutaneous platinum needle electrodes placed at the vertex (non-inverting input), right-side mastoid prominence (inverted input) and tail. Electroencephalographic (EEG) activity was amplified and then fed into an analog-to-digital converter [AD1, Tucker–Davis (TDT)]. Each averaged response was based on 300–500 repetitions of the stimulus recorded over 10-ms epochs. Auditory brainstem response waveforms were recorded in 5- to 10-dB steps decreasing incrementally from the maximum amplitude of 90 dB SPL. The ABR threshold was defined as the stimulus level that evoked a peak-to-peak voltage two SDs above mean background activity (Cediel *et al.* 2006; Ngan & May 2001; http://www.eumorphia.org/EMPReSS/). Auditory brainstem response data were expressed as mean ± SEM and were statistically analyzed by *t*-test.

Electroretinographic recordings were made from four ma*lpa*$_1^{(+/+)}$ and four ma*lpa*$_1^{(-/-)}$ mice. Before recording, animals were adapted to the dark overnight; then they were anesthetized and their pupils dilated with a topical drop of 1% tropicamide (Colircusí Tropicamida; Alcon Cusí, SA, El Masnou, Barcelona, Spain). To optimize electrical recording, 2% methocel (Ciba Vision AG, Hetlingen, Switzerland) was added to each eye immediately before placing the corneal electrode. The non-registered eye was covered with an opaque contact lens. Animals were placed in a Faraday cage, and experiments were conducted in absolute darkness. Bipolar recording was performed between an Ag:AgCl electrode fixed on a corneal lens and a reference electrode located on the head skin; ground electrodes were located on the tail and nose. Scotopic flash ERGs were recorded from each eye in response to light stimuli that consisted of light-emitting diodes (LED-white light) centered on the visual axis and located 5 mm away from the cornea. Light stimuli were presented for 5 ms at five increasing intensities ranging from 10^{-3} to 10^{1} cd-s/m^2. The interval between light flashes was 10 seconds, and four to eight consecutive recordings were averaged for each light presentation. The ERG signals were amplified, band-pass filtered between 0.3 and 1000 Hz and digitized at 10 kHz with a data acquisition board (Power Laboratory 4ST; AD Instruments Pty. Ltd., Oxfordshire, UK). Recordings were analyzed off-line by an investigator blinded to the experimental treatment of the animal (Mayor-Torroglosa *et al.* 2005).

Activity and habituation in the OF and EPM

To identify differences in exploratory/motor activity, reactivity to novel or anxiety-inducing environments and habituation, we used the OF and the EPM. In order to adapt the animals to the experimental conditions, each mouse was manipulated by hand for 5 min/day for a week before testing. All mice were taken from their home cages into the testing room and kept in the room for 1 h before behavioral testing.

The OF apparatus used in this experiment was a square, brightly illuminated (500 lux) wooden arena with dimensions of 50 × 50 × 38 cm. Each animal was placed in the center of the apparatus, and its behavior was monitored for a total of 5 min using a real-time video-tracking system (SMART 2.5, Panlab, Barcelona, Spain). Following the recording of (novelty) behavior, each individual's behavior was again recorded 24 h later (familiarity) to evaluate the effects of reactivity to novelty and habituation mechanisms. For data analysis, the OF was divided into two concentric rectangles: an outer zone, 8.3 cm in from the walls, and an inner zone, 8.3 cm in from the outer zone. The distance moved and the percentage of time spent in the center of the OF were taken as indices of exploratory activity and anxiety-like behavior, respectively. Behavior in the OF was recorded for nine ma*lpa*$_1^{(+/+)}$ mice, 12 ma*lpa*$_1^{(+/-)}$ mice and 12 ma*lpa*$_1^{(-/-)}$ mice. Significant differences in the percentage of time and distance moved were determined by two-way ANOVA with one repeated measure (novelty vs. familiarity). Simple main effects were performed after significant interaction, and Fisher's *post hoc* comparisons were used when appropriate. In order to control for possible differences in baseline activity in the three genotypes (Bothe *et al.* 2004), we calculated the habituation activity change score [day 2 activity/day 1 + day 2 activities)]. Comparisons among groups were performed using one-way ANOVA followed by Fisher's *post hoc* tests.

Unconditioned anxiety-like behaviors were assessed using an EPM consisting of two open arms (30 × 5 cm), two enclosed arms (30 × 5 cm, with end and side walls 15 cm high), and a connecting central platform (5 × 5 cm). The maze was raised to a height 38.5 cm above the floor and illuminated (100 lux) from the top. Each mouse was placed in the intersection of the four arms of the maze and allowed to explore freely for 5 min (novelty). After 24 h, the mouse was again placed into the maze for 5 min (familiarity). During this test, mice were monitored using a real-time video-tracking system (SMART 2.5, Panlab). An arm entry was defined as a mouse entering an arm of the maze with all four legs. General activity/exploration was evaluated using the total number of entries into the arms. Anxiety was assessed by comparing activity in the open vs. closed arms using the following index: time spent in open arms/(time spent in open arms + time spent in closed arms) (Malleret *et al.* 1999). Low values indicate high anxiety-like behavior levels, and high values indicate low anxiety-like behavior levels. In this experiment, 8 ma*lpa*$_1^{(+/+)}$ mice, 10 ma*lpa*$_1^{(+/-)}$ mice and 10 ma*lpa*$_1^{(-/-)}$ mice were used. Data were analyzed by two-way ANOVA with one repeated measure (novelty vs. familiarity), followed by *post hoc* comparisons using Fisher's test.

Spatial memory in the water maze

To study spatial memory, we conducted place navigation in the Morris water maze using 9 ma*lpa*$_1^{(+/+)}$ mice, 10 ma*lpa*$_1^{(+/-)}$ mice and 10 ma*lpa*$_1^{(-/-)}$ mice. Animals were adapted to the experimental conditions for 1 week before behavioral testing. All mice were taken from their home cages into the testing room and kept in the room for 1 h before the behavioral test. Mice were trained in a circular pool (diameter, 150 cm) filled with water (24–26°C) and made opaque with non-toxic white paint. The goal platform (diameter, 11 cm) could be placed anywhere in the pool at a distance of 30 cm from the pool edge. The platform was submerged 1 cm beneath the surface of the water. The pool was placed in an experimental room furnished with several place-fixed extra-maze cues. The pool remained immobile in the room throughout the experimental period. A real-time video-tracking system (SMART 2.5, Panlab) was used to record the animal's movements in the pool.

The experimental procedure was conducted over 4 days of spatial training, followed by 1 day of reversal training. One day before training, all mice were habituated to the experimental conditions, swimming in the pool without the escape platform for 1 min. This trial was used for checking whether the mice showed any preference or lack of preference for any of the four quadrants that would be used later in the spatial learning task (supporting information Fig. S1). In addition, the habituation trial was analyzed to study the exploratory behavior of the mice. The pool was divided into three concentric circles (outer, middle and inner zones); the time spent and distance traveled by the mice in each zone, as well as the distance traveled and the mean velocity in the pool, were obtained. Spatial learning training was conducted on four consecutive days (days 1–4) with three trials per day; the intertrial interval (ITI) was 15 min. For data analysis, the pool was divided into four quadrants (A–D). The mice were able to escape from the water using a submerged platform that was placed in the center of quadrant B, where it remained throughout the experiment. The mice were introduced into the pool from one of the four release positions in quadrant A, B, C or D. The trial ended when the animal found the platform. When a mouse did not find the platform within 60 seconds, the experimenter showed the animal the platform location, where it remained for 10 seconds. After this period, the mouse was returned to its cage for 15 min, after which it was introduced into the pool again. To test behavioral flexibility, on day 5 the platform was moved to the opposite quadrant (quadrant A), where it remained for three trials, with an ITI of 15 min

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(reversal learning task). The first 30 seconds of the first reversal trial were used to conduct a trial to probe spatial retention. This period of time was used because none of the mice were able to find the novel platform location during the first 30 seconds of the training. To analyze the spatial training and the reversal task, escape latencies, distance swum and velocity were recorded for each trial and were collapsed into a block of three trials per training day. The percentage of time spent swimming in the three concentric zones of the pool was calculated for the spatial learning phase, to evaluate thigmotaxic behavior (i.e. peripheral pool time) and its possible influence in spatial learning. Finally, the probe trial was analyzed by recording the percentage of time spent in the trained (A) and non-trained quadrants (B, C and D). Data were analyzed using two-way ANOVA with repeated measures (habituation, spatial learning and probe trial) and one-way ANOVA (reversal task). In the habituation trial, a one-way ANOVA of both time spent and distance moved in each of the three zones was performed when genotype by zone interaction was reported. In this case, the Bonferroni procedure was adopted to control the overall level of significance. In the probe trial, a single ANOVA of time in the training quadrant was conducted when the genotype by quadrant interaction was reported. Fisher's *post hoc* comparisons were used when appropriate.

To analyze the search strategies used by the mice in the pool, two independent investigators blinded to mouse genotype determined a predominant search strategy for each trial of the last day of the spatial training (day 4). The search paths of each mouse in each trial were plotted using SMART 2.5 image software and were categorized into one of the following mutually exclusive search strategies (Brody & Holzman 2006): spatial strategies, involving spatial direct, spatial indirect and focal correct quadrant strategies; systematic but non-spatial strategies, involving scanning, random and focal incorrect target strategies; and strategies involving repetitive looping paths, i.e. chaining, peripheral looping and circling strategies. The use of each search strategy was presented as a percentage of incidences in each trial performed during the last training day (day 4). Paired comparisons were carried out using a Mann–Whitney *U*-test.

In order to study the possible influence of exploratory impairments (i.e. increased thigmotaxic behavior) discovered during the habituation trial on spatial learning performance and search strategies, Pearson correlations were calculated for each group comparing the time spent in the outer zone during the habituation and time spent in the target quadrant during the probe test. In addition, the degree of association between thigmotaxic behavior during habituation and search strategy was also calculated for each group, using the point biserial correlation coefficient (r_{pb}).

Finally, to establish whether the water maze deficit reflects a non-specific, sensorimotor or motivational performance deficit, various groups of mice were trained in a visual-cued task. In this study, 10 ma$lpa_1^{(+/+)}$ mice, 6 ma$lpa_1^{(+/-)}$ mice and 6 ma$lpa_1^{(-/-)}$ mice were used. Mice were trained in the water maze, adapted to the experimental procedure, and received a habituation trial as described above. Twenty-four hours after completing the habituation trial, the animals began training in the visual-cued task; this training lasted for 3 days. Mice were trained to locate a visible, grey-colored platform that rested 2 cm above the water surface. The platform was moved to a new location each trial. The visual-cued task consisted of four trials, each starting from one of the four release points, with an ITI of 5 min. Mice were allowed to rest on the platform for 10 seconds. Data were analyzed using a two-way ANOVA with repeated measures (genotype × training days), followed by *post hoc* comparisons when appropriate (Fisher's test).

Results

Neurological abnormalities and preserved auditory and retinal function in maLPA1-null mice

Kruskal–Wallis analysis showed that there was significant variance in somesthesis ($H = 7,5$; $df = 2$; $P < 0.05$), olfaction ($H = 8,699$; $df = 2$; $P < 0.05$), grasping ($H = 7,7$; $df = 2$; $P < 0.05$) and equilibrium ($H = 7,82$; $df = 2$; $P < 0.05$) tests between ma$lpa_1^{(+/+)}$ and maLPA$_1$-null mice. Paired comparisons using Mann–Whitney *U*-tests revealed that the absence of LPA$_1$ receptor resulted in a significant impairment in somesthesis ($U = 15$; $P < 0.05$), olfaction ($U = 49$; $P < 0.05$), grasping ($U = 56$; $P < 0.05$) and equilibrium ($U = 58.5$; $P < 0.05$) (Table 1). In contrast, the remaining sensory, limb reflex and limb co-ordination tests did not reveal any performance differences among the three groups ($P > 0.05$).

Neuromuscular strength analysis, assessed by the hang wire test, showed a significant effect of genotype ($F_{2,24} = 16.92$; $P < 0.001$). The absence of LPA$_1$ receptor was associated with shorter latencies to fall compared with ma$lpa_1^{(+/+)}$ and ma$lpa_1^{(+/-)}$ mice ($P < 0.05$; Table 1). In the tail flick test, the three groups of mice exhibited different responses to pain ($F_{2,32} = 4.48$; $P < 0.001$). The latency time of the pain response in ma$lpa_1^{(-/-)}$ mice was significantly longer than that of ma$lpa_1^{(+/+)}$ and ma$lpa_1^{(+/-)}$ mice ($P < 0.05$; Table 1); no significant differences were found between ma$lpa_1^{(+/+)}$ and ma$lpa_1^{(+/-)}$ mice ($P > 0.05$).

Auditory brainstem response profiles showed similar responses in both wild-type and maLPA$_1$-null animals after stimulation. Animals of both genotypes showed a similar, five-peak wave pattern (Fig. 1a) and similar click-ABR thresholds, 59.1 ± 5.1 and 67.27 ± 3.32 dB SPL, respectively ($t_{21} = 1.303$; $P > 0.05$) (Fig. 1b). Thus, the two groups of mice did not differ in their inter-peak latencies (I-II: $t_{19} = -0.042$; II-III: $t_{19} = -1.44$; III-IV: $t_{19} = 0.50$; IV-V: $t_{18} = -0.6$; I-III: $t_{19} = -1.325$; III-V: $t_{18} = -0.264$; $P > 0.05$; Fig. 1c), corroborating the absence of defective auditory response in maLPA$_1$ null mice.

Electroretinogram analysis of visual function revealed no defective processing in mice lacking LPA$_1$ receptor. In 1-month-old maLPA$_1$-null mice, average dark-adapted ERG waveforms and amplitudes were similar to those seen in wild-type animals; no substantial differences were observed for either *a*- or *b*-wave amplitudes over the stimulus intensity range used (Fig. 1d). For example, at 0.3 cd-s/m^2, the wild-type *a*-wave amplitude was 205.7 ± 28.5 μV, and the null value was 251.4 ± 17.1 μV; the wild-type *b*-wave amplitude was 477.1 ± 8.5 μV, and the null value was 480.0 ± 48.5 μV($n = 4$). In addition, both *a*-wave and *b*-wave dark-adapted thresholds were normal for both groups of mice. Thus, the absence of LPA$_1$ receptor did not appear to affect the retinal pathway.

maLPA$_1$-null mice show impaired activity in the OF and increased anxiety-like behavior in the EPM under novelty conditions

In the OF test, the two-way ANOVA [genotype × trial (novelty vs. familiarity)] revealed significant effects of genotype ($F_{2,30} = 5.29$, $P < 0.01$), trial ($F_{1,30} = 38.08$, $P < 0.001$) and interaction ($F_{2,30} = 5.14$, $P < 0.01$) in the total distance traveled (Fig. 2a). Simple main effects analysis showed that the three genotypes traveled different distances in the OF only during the first trial ($F_{2,60} = 8.18$, $P < 0.01$).

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Table 1: Neurological screening of ma$lpa_1^{(+/+)}$, ma$lpa_1^{(+/-)}$ and ma$lpa_1^{(-/-)}$ mice

(**A**) **Somesthesis**			
Genotype	Absent deficit (0), %	Weak deficit (1), %	Strong deficit (2), %
ma$lpa_1^{(+/+)}$	100	0	0
ma$lpa_1^{(+/-)}$	58.8	35.29	6
ma$lpa_1^{(-/-)}$	33.33	66.66	0
(**B**) **Grasping**			
Genotype	Absent deficit (0), %	Weak deficit (1), %	Strong deficit (2), %
ma$lpa_1^{(+/+)}$	100	0	0
ma$lpa_1^{(+/-)}$	58.8	41.12	0
ma$lpa_1^{(-/-)}$	73.33	26.66	0
(**C**) **Equilibrium**			
Genotype	Absent deficit (0) , %	Weak deficit (1) , %	Strong deficit (2) , %
ma$lpa_1^{(+/+)}$	100	0	0
ma$lpa_1^{(+/-)}$	82	12	6
ma$lpa_1^{(-/-)}$	50	43	7
(**D**) **Olfaction**			
Genotype	Absent deficit (0), %	Weak deficit (1) , %	Strong deficit (2) , %
ma$lpa_1^{(+/+)}$	100	0	0
ma$lpa_1^{(+/-)}$	70	24.5	5.5
ma$lpa_1^{(-/-)}$	46.66	46.66	6.68
(**E**) **Tail flick and hangwire tests**			
Genotype	Tail flick test (seconds)		Hangwire test (seconds)
ma$lpa_1^{(+/+)}$	2.0 ± 0.15		51.1 ± 4.5
ma$lpa_1^{(+/-)}$	1.9 ± 0.32		46.8 ± 4.3
ma$lpa_1^{(-/-)}$	2.6 ± 0.17*		18.3 ± 4.7*

A–D, data are expressed as percentage of mice; E, data are expressed as mean ± SEM escape latencies.
*$P < 0.05$, ma$lpa_1^{(-/-)}$ vs. ma$lpa_1^{(+/+)}$ and ma$lpa_1^{(+/-)}$.

Post hoc comparisons showed that both ma$lpa_1^{(+/+)}$ and ma$lpa_1^{(+/-)}$ mice traveled longer distances than ma$lpa_1^{(-/-)}$ mice ($P < 0.05$). In addition, ma$lpa_1^{(+/+)}$ and ma$lpa_1^{(+/-)}$ mice traveled a shorter distance during the second trial than they did during the first ($F_{1,8} = 9.7$, $P < 0.01$ and $F_{1,8} = 58.34$, $P < 0.01$, respectively). However, ma$lpa_1^{(-/-)}$ mice showed the same exploration of the OF in both conditions ($F_{1,8} = 2.07$, $P > 0.05$). Furthermore, ma$lpa_1^{(-/-)}$ mice displayed significantly lower intersession activity levels than did ma$lpa_1^{(+/+)}$ mice when activity change scores were analyzed [($F_{2,30} = 4.59$, $P < 0.05$); (ma$lpa_1^{(+/+)}$: 0.35 ± 0.032; ma$lpa_1^{(+/-)}$: 0.41 ± 0.012; ma$lpa_1^{(-/-)}$: 0.47 ± 0.035)].

If the abnormal activity levels in the maLPA$_1$-null mice are indicative of anxiety-like behavior, one would expect clear differences in the percentage of distance traveled in the center among the three genotypes. However, the two-way ANOVA [genotype × trial (novelty vs. familiarity)] did not show any significant general effects of genotype ($F_{2,30} = 0.13$, $P > 0.05$) or interaction ($F_{2,30} = 1.309$, $P > 0.05$) (Fig. 2b). However, two-way ANOVA revealed significant differences between the two trials (novel vs. familiar context; $F_{1,30} = 14.09$, $P < 0.001$), suggesting that all the mice, regardless of genotype, spent a smaller percentage of time in the center during the second trial (familiar context) than during the first (novel context). Likewise, similar

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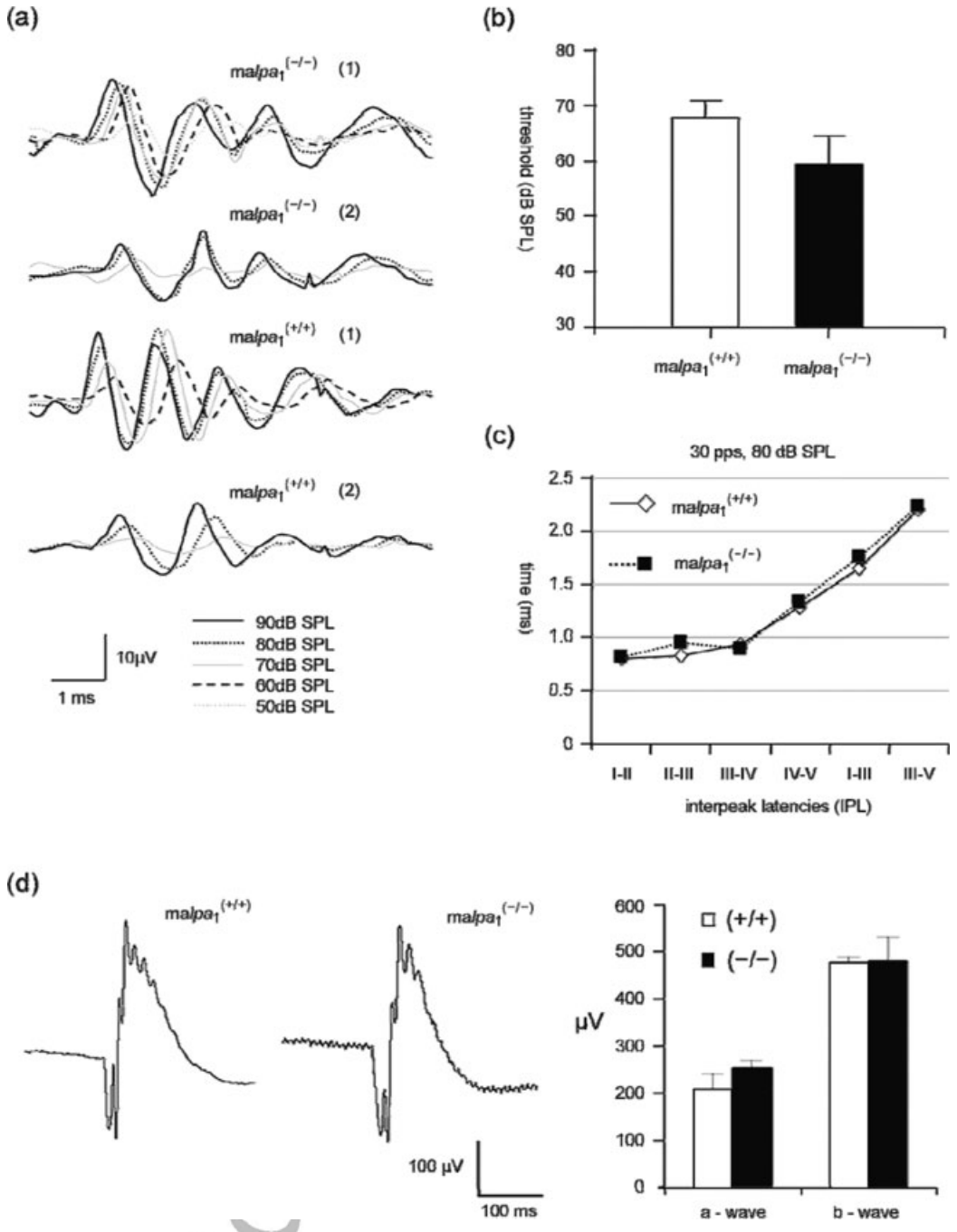

Figure 1: Auditory and retinal function in mice lacking LPA_1 receptor. (a) Representative auditory brainstem responses (ABR) recordings for two wild-type [ma$lpa_1^{(+/+)}$] and two maLPA$_1$-null [ma$lpa_1^{(-/-)}$] mice. Typical waveforms comprising four or five peaks are distinguishable in a time period of about 8 ms following stimulation and similar for both genotypes. Test for mice used 10-dB steps down from the maximum amplitude of 90 dB SPL. (b) Average ABR thresholds for click stimulus of ma$lpa_1^{(+/+)}$ and ma$lpa_1^{(-/-)}$ mice. Data presented as mean ± SEM. No significant differences were observed between the two groups ($n = 11$; $P < 0.01$). (c) Graph showing the determination of 30 pps 80 dB SPL click-ABR inter-peak latencies, in milliseconds, for one wild-type [ma$lpa_1^{(+/+)}$] and one maLPA$_1$-null [ma$lpa_1^{(-/-)}$] mice. Statistical analysis (*T*-test; $n = 11$) showed no differences in latencies attributable to absence of LPA_1 receptor. (d) Representative dark-adapted ERG tracings for one wild-type [*malpa*$_1^{(+/+)}$] and maLPA$_1$-null [ma$lpa_1^{(-/-)}$] mice at 0.3 cd-s/m^2 stimulus intensity and corresponding mean (±SEM) amplitudes of the *a*- and *b*-waves evoked.

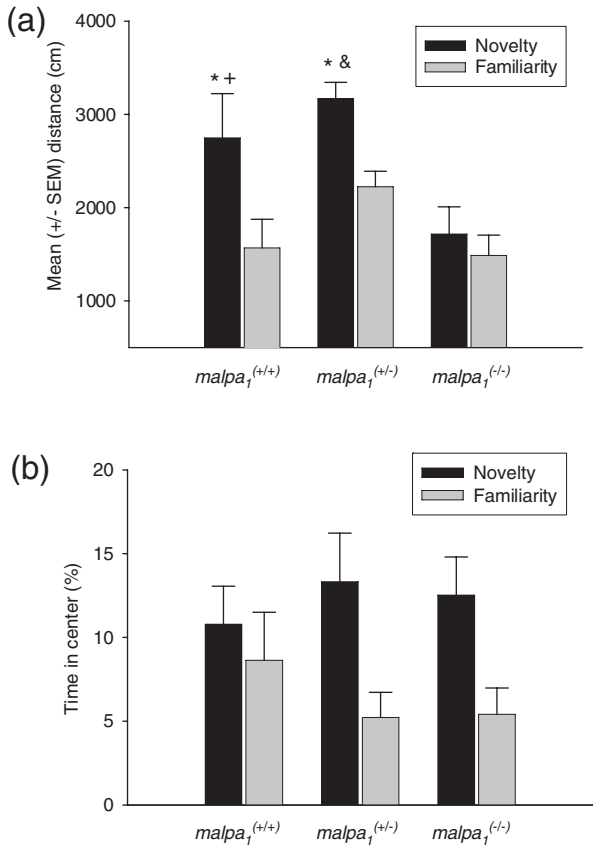

Figure 2: Open field exploration in mice lacking LPA$_1$ receptor. (a) Data represent mean (±SEM) distance moved in the OF. ma*lpa*$_1^{(+/+)}$ and ma*lpa*$_1^{(+/-)}$ showed decreased motor activity (distance traveled) during the second trial (familiarity) compared with the first trial. However, ma*lpa*$_1^{(-/-)}$ genotype showed the same activity in both trials. In addition, ma*lpa*$_1^{(-/-)}$ mice traveled less distance than the other two genotypes only during the first trial (novelty). *$P < 0.01$ (novelty vs. familiarity); $^+$ $P < 0.05$ [ma*lpa*$_1^{(+/-)}$ vs. ma*lpa*$_1^{(-/-)}$]. (b) Data represent mean percentage of time (±SEM) spent in the center of the OF. The three genotypes spent less percentage of time in the center zone during the second trial (familiarity) than during the first trial (novelty; $P < 0.05$).

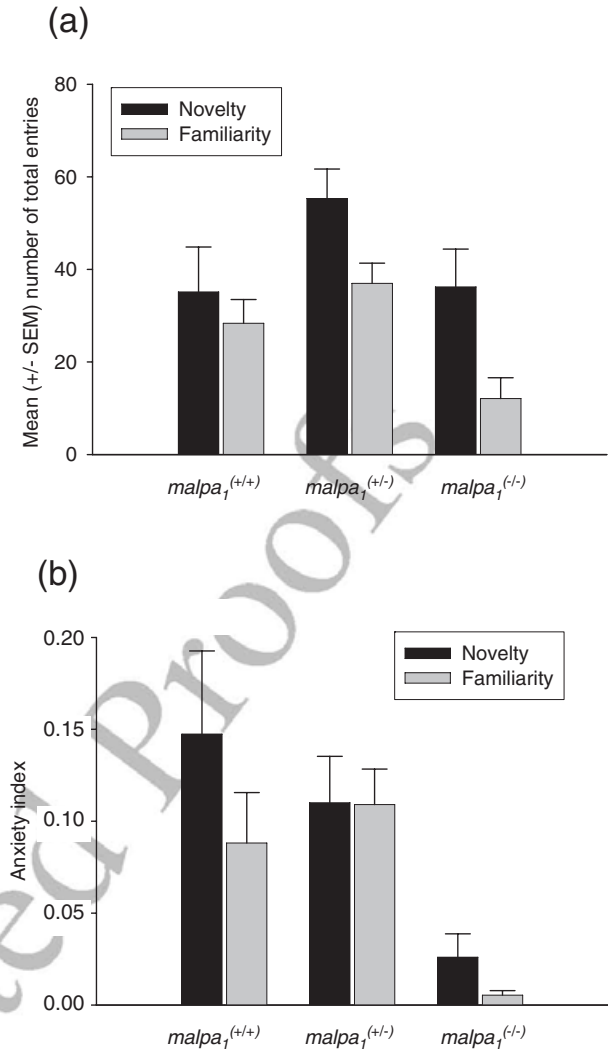

Figure 3: Increased anxiety-like behavior in mice lacking LPA$_1$ receptor in the elevated plus maze. (a) Data represent mean (±SEM) number of total transitions in the EPM. All the genotypes showed decreased motor activity during the second trial (familiarity) compared with the first trial (novelty) ($P < 0.05$). In addition, ma*lpa*$_1^{(-/-)}$ genotype exhibited less exploratory activity than the other two genotypes ($P < 0.05$). (b) Data represent anxiety index calculated in the EPM. ma*lpa*$_1^{(+/+)}$ and ma*lpa*$_1^{(+/-)}$ exhibited less anxiety-like behavior during both first and second trials (novelty vs. familiarity) than ma*lpa*$_1^{(-/-)}$.

activity levels were evident for the activity change scores in all the genotypes [($F_{2,30} = 1.77$, $P > 0.05$); (ma*lpa*$_1^{(+/+)}$: 0.41 ± 0.061; ma*lpa*$_1^{(+/-)}$: 0.30 ± 0.043; ma*lpa*$_1^{(-/-)}$: 0.27 ± 0.056)]. These results do not support the interpretation that enhanced anxiety-like behavior in maLPA$_1$-null mice is the reason for impaired exploration in this genotype.

In the EPM, two-way ANOVA conducted on the total number of entries in the arms revealed a significant general effect of genotype ($F_{2,25} = 3.98$, $P < 0.05$) and trial (novelty vs. familiarity) ($F_{2,25} = 21.51$, $P < 0.001$; Fig. 3a), indicating that, in EPM as in OF, mice of all three genotypes showed less exploration during the second trial than during the first trial. *Post hoc* comparisons showed that ma*lpa*$_1^{(-/-)}$ genotype mice exhibited less exploration in both trials than the other two genotypes ($P < 0.05$).

The two-way ANOVA conducted on the anxiety index revealed a significant effect of genotype ($F_{2,25} = 7.12$, $P < 0.01$) and trial (novelty vs. familiarity) ($F_{2,25} = 5.38$, $P < 0.05$; Fig. 3b). *Post hoc* comparisons showed that the maLPA$_1$-null mice exhibited more anxious-like behavior than the other two genotypes ($P < 0.05$).

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Figure 4: Spatial learning in mice lacking LPA_1 receptor. Mice of all genotypes learned to locate the hidden platform position, as shown by decreasing escape latencies (a) and distance moved (b) in the acquisition phase (days 1–4). The reversal phase analysis (day 5) showed no differences among the three genotypes either in escape latency (a) or in distance moved (b) in the Morris water maze ($P > 0.05$). Analysis of velocity (c) revealed that ma$lpa_1^{(+/+)}$ and ma$lpa_1^{(+/-)}$ mice increased velocity over the training days ($P < 0.05$), whereas LPA_1-null mice failed to increase velocity through the spatial training ($P > 0.05$). (d) The percentage of searching time in three different zones in the water maze (inner, middle and outer zones) showed that the three genotypes spent more time in the middle than in the other two zones during the spatial training ($P < 0.05$). No differences were seen among the genotypes ($P > 0.05$). (e) Data represent mean (±SEM) of percentage of total time spent in each quadrant during the probe test in the water maze. ma$lpa_1^{(-/-)}$ and ma$lpa_1^{(+/-)}$ mice genotypes were not able to remember the location of the platform in the target quadrant (B). *$P < 0.05$; ma$lpa_1^{(+/+)}$ vs. ma$lpa_1^{(+/-)}$ and ma$lpa_1^{(-/-)}$.

Impaired spatial memory retention in maLPA$_1$-null mice

The Morris water maze was used to test spatial memory training. The habituation trial analysis showed differences among the three genotypes in the velocity of swimming ($F_{2,26} = 4.44$, $P < 0.05$). In addition, ma$lpa_1^{(-/-)}$ mice exhibited lower velocity than ma$lpa_1^{(+/+)}$ mice ($P < 0.05$), but not than ma$lpa_1^{(+/-)}$ mice ($P > 0.05$) (Table 2). The two-way ANOVA conducted on the time spent in the three zones of the water maze showed significant differences among the three zones ($F_{2,54} = 142.34$, $P < 0.001$). *Post hoc* comparisons revealed that the mice spent more time in the outer zone than in the other two zones ($P < 0.05$) and more in the middle zone than in the inner zone ($P < 0.05$). In addition, interaction between genotype and zone was observed ($F_{4,54} = 4.82$, $P < 0.05$). The analysis of the time spent in the three zones of the pool revealed genotype differences in the time spent in the outer zone ($F_{2,26} = 4.85$, $P < 0.0167$) and in the middle zone ($F_{2,26} = 5.042$, $P < 0.0167$), but not in the inner zone ($F_{2,26} = 2.694$, $P > 0.0167$). *Post hoc* comparisons showed that ma$lpa_1^{(-/-)}$ mice spent less time in both the outer and middle zones than did ma$lpa_1^{(+/+)}$ mice ($P < 0.05$), but not ma$lpa_1^{(+/-)}$ mice ($P > 0.05$) (Table 2). With respect to the distance moved in the three zones, the two-way ANOVA showed significant effects of genotype ($F_{2,26} = 4.04$, $P < 0.05$), zone ($F_{2,54} = 98.54$, $P < 0.001$) and interaction ($F_{4,54} = 2.607$, $P < 0.05$). The analysis of the main effects, using *post hoc* comparisons, showed that ma$lpa_1^{(-/-)}$ mice swam shorter distances than ma$lpa_1^{(+/+)}$ mice ($P < 0.05$), but not than ma$lpa_1^{(+/-)}$ mice ($P > 0.05$). In addition, the mice swam longer distances in the outer zone than in the other two zones ($P < 0.05$), and longer in the middle zone than in the inner zone ($P < 0.05$) (Table 2). The analysis of the distance moved in each of the three zones of the pool revealed genotype differences in the middle ($F_{2,26} = 5.666$, $P < 0.0167$) but not in the other two zones [inner zone: ($F_{2,26} = 4.41$, $P > 0.0167$); outer zone ($F_{2,26} = 0.063$, $P > 0.0167$)]. *Post hoc* comparisons revealed that ma$lpa_1^{(-/-)}$ mice swam a shorter distance in the middle zone than ma$lpa_1^{(+/+)}$ mice ($P < 0.05$), but not than ma$lpa_1^{(+/-)}$ mice ($P > 0.05$; Table 2).

The analysis of spatial learning revealed a spatial memory impairment in ma$lpa_1^{(-/-)}$ mice. Two-way ANOVA using repeated measures over the training days did not reveal a significant main genotype effect in either escape latencies ($F_{2,26} = 2.48$, $P > 0.05$) or in the distance moved ($F_{2,26} = 0.77$, $P > 0.05$; Fig. 4a,b, respectively). However, in all groups, day of training was found to affect escape latencies ($F_{3,78} = 22.61$, $P < 0.001$) and the distance moved ($F_{3,78} = 17.34$, $P < 0.001$). *Post hoc* comparisons showed that mice of all genotypes were able to learn the location of the hidden platform, as revealed in the reduction of escape latencies during the spatial training phase (first four days of testing) ($P < 0.05$). The swimming velocity of the ma$lpa_1^{(-/-)}$ mice did not increase during the training, in contrast to the increase in swimming velocity observed in ma$lpa_1^{(+/+)}$ and ma$lpa_1^{(+/-)}$ mice (Fig. 4c). The two-way ANOVA revealed an interaction effect (genotype × training days; $F_{6,78} = 2.27$, $P < 0.05$). Simple main effects analysis

Table 2: Habituation of ma$lpa_1^{(+/+)}$, ma$lpa_1^{(+/-)}$ and ma$lpa_1^{(-/-)}$ mice in the Morris water maze

Zone	Variable	ma$lpa_1^{(+/+)}$	ma$lpa_1^{(+/-)}$	ma$lpa_1^{(-/-)}$
Outer	Time (seconds)	34.34 ± 2.26	41.57 ± 3.71	47.91 ± 3.34*
	Distance (cm)	586.54 ± 36.45	614.12 ± 67.59	603.93 ± 56.81
Middle	Time (seconds)	20.48 ± 1.45	14.26 ± 2.85	10.52 ± 2.39*
	Distance (cm)	455.05 ± 51.21	326.23 ± 73.10	186.25 ± 50.71*
Inner	Time (seconds)	4.92 ± 1.09	2.75 ± 0.75	2.10 ± 0.82
	Distance (cm)	104.53 ± 24.54	50.21 ± 12	32.93 ± 14.43
Total arena	Velocity (cm/second)	19.17 ± 1.36	16.56 ± 1.65	13.59 ± 1.07*
	Distance (cm)	1156.13 ± 84.12	990.57 ± 100.92	823.12 ± 65.36*

Data are expressed as mean ± SEM. *$P < 0.05$, ma$lpa_1^{(+/+)}$ vs. ma$lpa_1^{(-/-)}$.

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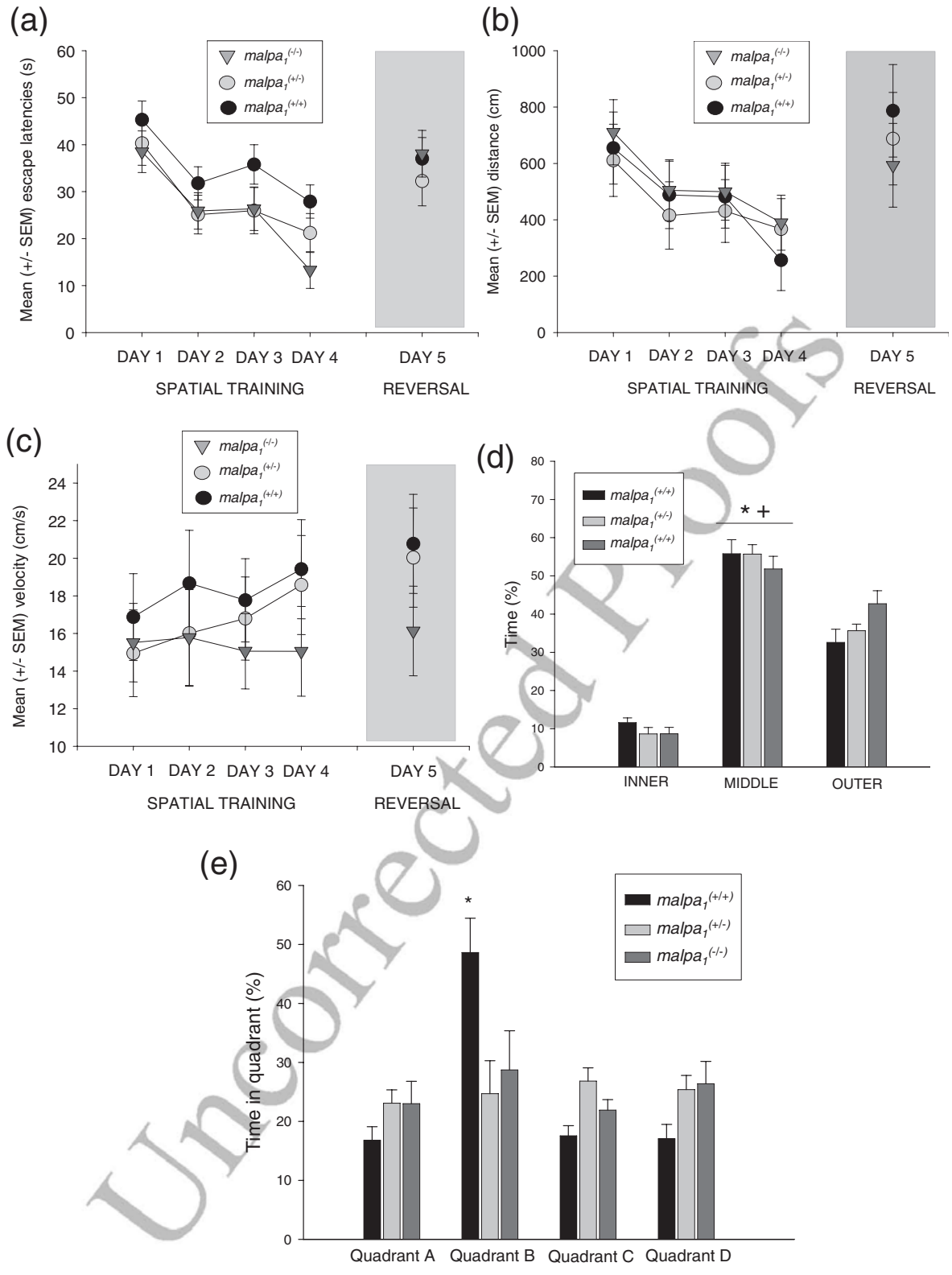

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showed that the three genotypes exhibited significantly different velocity during day 4 ($F_{2,104} = 3.35$, $P < 0.05$). *Post hoc* comparisons revealed that ma$lpa_1^{(-/-)}$ mice were slower than the other two genotypes ($P < 0.05$) (Fig. 4c).

During the reversal phase, no differences were observed among the three genotypes on either escape latencies ($F_{2,26} = 0.26$, $P > 0.05$) or distances swum ($F_{2,26} = 1.15$, $P > 0.05$) (Fig. 4a,b). In contrast, the three genotypes exhibited different swimming velocity during this phase ($F_{2,26} = 3.46$, $P < 0.05$). *Post hoc* comparisons revealed that ma$lpa_1^{(+/+)}$ mice were faster than ma$lpa_1^{(-/-)}$ mice ($P < 0.05$) (Fig. 4c).

The analysis of the percentage of time spent during the spatial learning in the outer, middle and inner zones of the water maze showed that mice of all three genotypes exhibited a strong overall preference for the middle zone of the water maze, where the platform was located, spending less time in the outer and inner zones ($F_{2,50} = 148.16$, $P < 0.001$; LSD: outer vs. middle and inner; middle vs. inner ($P < 0.05$)) (Fig. 4d). Taken together, these results indicate that the absence of LPA_1 receptor is not associated with enhanced thigmotaxis during spatial learning.

To test spatial memory retention (Fig. 4e), a probe trial was conducted during the first 30 seconds of the first reversal trial. The two-way ANOVA (genotype × quadrant) showed a strong effect of quadrant ($F_{3,78} = 5.69$, $P < 0.01$) and interaction ($F_{6,78} = 3.74$, $P < 0.01$; Fig. 4). Single ANOVA of time in the training quadrant showed a significant effect of genotype ($F_{2,26} = 4.31$, $P < 0.05$). *Post hoc* comparisons showed that ma$lpa_1^{(+/+)}$ mice spent more time in the training quadrant than did ma$lpa_1^{(-/-)}$ and ma$lpa_1^{(+/-)}$ mice ($P < 0.05$).

The alterations in behavior in the water maze of ma$lpa_1^{(-/-)}$ mice were not associated with any sensorimotor or motivational deficits (Fig. S2). Mice of all three genotypes correctly performed a visual-cued task, and there was no difference in the visual-cued task performance [escape latencies: ($F_{2,19} = 3.42$; $P > 0.05$) or in the distance swum ($F_{2,19} = 2.11$, $P > 0.05$)]. Differences across the training days in both escape latencies ($F_{2,38} = 28.81$, $P < 0.001$) and distance swum ($F_{2,38} = 14.6$, $P < 0.01$) were found, showing that all the mice were able to reduce their escape latencies and distance swum between day 1 and the following days of the study ($P < 0.05$). No significant interaction effect was observed on either escape latencies ($F_{4,38} = 1.30$, $P > 0.05$) or distance swum ($F_{4,38} = 0.30$, $P > 0.05$). However, as was shown during the spatial learning, the mice belonging to the ma$lpa_1^{(-/-)}$ genotype exhibited a general reduction in their velocity [($F_{2,19} = 5.06$, $P < 0.01$; LSD: ma$lpa_1^{(-/-)}$ vs. ma$lpa_1^{(+/-)}$ and ma$lpa_1^{(+/+)}$ ($P < 0.05$)].

The analysis of strategy choice throughout the three trials during the last training day (day 4) revealed that the groups used different strategies in the water maze (Table 3). The absence of LPA_1 receptor affected the search strategy in the Morris water maze such that ma$lpa_1^{(-/-)}$ and ma$lpa_1^{(+/-)}$ mice used fewer spatial strategies than ma$lpa_1^{(+/+)}$ mice (31%, 33% and 56%, respectively). Test monitoring showed that deletion of the lpa_1 gene changed the preferences of the mice in favor of non-spatial systematic strategies [ma$lpa_1^{(+/+)}$, 33%; ma$lpa_1^{(+/-)}$, 56%; ma$lpa_1^{(-/-)}$, 46%] and repetitive looping [ma$lpa_1^{(+/+)}$, 11%; ma$lpa_1^{(+/-)}$, 11%; ma$lpa_1^{(-/-)}$, 23%]. Paired comparisons showed that ma$lpa_1^{(+/+)}$ mice displayed significantly more spatial strategies than ma$lpa_1^{(+/-)}$ and ma$lpa_1^{(-/-)}$ mice (U = 310; $P < 0.05$; and U = 285; $P < 0.05$, respectively).

Finally, correlational analysis showed that neither time spent in the target quadrant during the probe test nor search strategy correlated with time spent in the outer zone during habituation for any genotype. The results for time target quadrant/time outer zone are as follows: ma$lpa_1^{(+/+)}$, $r = -0.59$ ($t_7 = -1.237$; $P > 0.05$); ma$lpa_1^{(+/-)}$, $r = -0.35$ ($t_7 = -0.796$; $P > 0.05$); ma$lpa_1^{(-/-)}$, $r = 0.04$ ($t_8 = 0.1154$; $P > 0.05$). The results for search strategy/time outer zone are as follows: ma$lpa_1^{(+/+)}$, $r_{pb} = 0.16$ ($t_7 = 0.46$; $P > 0.05$); ma$lpa_1^{(+/-)}$, $r_{pb} = -0.21$ ($t_7 = -0.5089$; $P > 0.05$); ma$lpa_1^{(-/-)}$, $r_{pb} = 0.23$ ($t_8 = 0.714$; $P > 0.05$). These data suggest that the increased thigmotaxis reported in the maLPA$_1$-null mice during the habituation trial is not associated with either impaired spatial memory retention or use of inappropriate search strategies.

Discussion

The neurological and behavioral phenotypes of maLPA$_1$-null mice documented in this study strongly suggests that the LPA_1 receptor is involved in several CNS-dependent functions. However, it is unclear whether the effects in adult mice are directly mediated by the receptor or are instead because of developmental abnormalities. Several reports have indicated a critical role of LPA and LPA_1 receptors on normal brain development (Anliker & Chun 2004; Choi *et al.* 2008; Chun 2005; Estivill-Torrús *et al.* 2008); this may account for some of the neurological and behavioral impairments observed in the maLPA$_1$-null mice. Nevertheless, previous observations suggest that the effects of LPA_1 on many cerebral processes may be context dependent and may occur during both development and adult life (Matas-Rico *et al.* 2008).

The observed neurological deficits in the maLPA$_1$-null mice may affect behavioral performance involving both motor and cognitive functions. However, the deletion of LPA_1 receptor does not appear to induce severe neurological deficits. Visual and auditory functions are not impaired, and when sensory reflexes were assessed, only mild deficits in somesthesis and olfaction were observed. Results from somatosensory tests, including the tail flick test, may be important when studies with painful stimulation are performed. However, withdrawal from pain is probably not involved in the behaviors we evaluated in our study. Weak olfaction deficits reported in the maLPA$_1$-null mice may be involved in exploratory and spatial tasks because olfactive cues are used when animals explore the environment (Lavenex & Schenk 1997; Rossier & Schenk 2003). Nevertheless, no deficits were observed in

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Table 3: Search strategies in the Morris water maze of ma*lpa*$_1^{(+/+)}$, ma*lpa*$_1^{(+/-)}$ and ma*lpa*$_1^{(-/-)}$ mice

Strategy		ma*lpa*$_1^{(+/+)}$, %	ma*lpa*$_1^{(+/-)}$, %	ma*lpa*$_1^{(-/-)}$, %
	Spatial	56	33	31
	Non-spatial, systematic	33	56	46
	Repetitive looping	11	11	23

Data are expressed as the percentage of mice.

exploration by maLPA$_1$-null mice during either NOR or EPM tasks, suggesting that the impairments reported in those tasks are not because of impaired olfaction. Our evaluation of limb reflexes and co-ordination showed that knockout mice were able to achieve a good level of co-ordination and placing. In addition, only a minor deficit was observed in grasping and equilibrium tests; maLPA$_1$-null mice displayed an adequate response and only showed a reduced ability (time) to maintain equilibrium. The deficit observed in the maLPA$_1$-null mice that is most likely to have influenced the behavioral tasks used in our study is related to muscular weakness. Tasks based on exploration, such as OF and the water maze, may be influenced by muscular weakness; variables such as distance traveled, escape latency and velocity can be severely reduced. Although overall speed was altered, distance and escape latencies were not significantly impaired in maLPA$_1$-null mice during spatial training in the water maze. Likely, hypolocomotion during the first exposure to the OF may result from the muscular weakness of these animals. In order to prevent or minimize bias in determining the cognitive and emotional deficits of maLPA$_1$-null mice in those tasks, we used some variables partially independent of these alterations as indicative of emotional and cognitive impairments, such as the percentage of time that the mice spent in a region of the maze.

The findings obtained in the OF showed a reduced exploratory reactivity of maLPA$_1$-null mice to a novel environment in comparison with the other two genotypes. This overall decrease in activity might simply result from disturbances in motor functions, leading to the observation of a floor effect in overall activity; it might also involve changes in emotional variables. However, the exploration of the OF in the novelty condition is not associated with anxiety-like behavior. In fact, locomotor activity in the center in response to novelty was similar among the three genotypes, suggesting no significant genotype differences in anxiety-like behavior in response to the novel environment. Thus, the hypolocomotion of maLPA$_1$-null mice in the OF is more likely to be because of their above-mentioned motor impairments.

The role of the LPA$_1$ receptor in the habituation of activity in the OF is suggested by the activity levels during the second trial, especially when the activity change scores were compared. It is also important to note that the abnormal intertrial habituation in maLPA$_1$-null mice probably cannot be explained by enhanced anxiety-like behavior, because no significant differences exist among the genotypes in both the percentage of time spent in the center of the OF. However, because the different activity levels on trial 1 of testing call into question the interpretation of the results as habituation deficit, these data must be interpreted cautiously. Further studies are required to clarify whether the low activity levels in the maLPA$_1$-null mice during the first trial may explain the impairment reported here.

In contrast to the OF results, on first exposure to the EPM, the three genotypes exhibited a preference for the closed arms in the absence of significant differences in the total arm entry score, a pattern previously seen in other genotypes (Holmes *et al*. 2000). Notwithstanding, the low activity displayed by maLPA$_1$-null mice in open arms indicates enhanced anxious-like behavior under novelty conditions, which is specific to this genotype. It is well known that prior exposure to the EPM alters baseline behavior on re-exposure to the test (Dawson *et al*. 1994; Holmes *et al*. 2000), and that mice and rats with increased reactivity to the novelty did not reduce open arm exploration during retest (Ballaz *et al*. 2007; Holmes *et al*. 2000). In our experiment, when mice were re-exposed to the EPM 24 h after trial 1, all three genotypes exhibited a normal intertrial reduction in exploration of the EPM. The fact that the retest profile was not affected by the increased anxiety-like behavior observed in the maLPA$_1$-null mice suggests the involvement of different mechanisms in the two behavioral processes. It is noteworthy that both impaired exploration in the OF and enhanced anxiety-like behavior in the EPM when maLPA$_1$-null mice were tested cannot be attributed to a general

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locomotion impairment or to the motor and sensory deficits reported in these knockout mice. Results reported in the EPM may on occasion contradict those obtained in the OF; although these tasks are based on novelty exploration and emotional response to environmental challenges, the results for each depend strongly on the test conditions (Belzung & Griebel 2001). Therefore, different levels of stress and emotion triggered by the tasks likely explain the differences in the results of these tests in the two conditions studied.

Finally, our findings indicate that the absence of LPA_1 receptor impairs spatial memory. In the water maze, maLPA$_1$-null mice exhibited impaired spatial retention during the probe test and increased propensity to adopt inappropriate search strategies (i.e. non-spatial strategies) during the last training day. Nevertheless, deletion of the *lpa*$_1$ gene did not cause a general spatial learning deficit because all genotypes showed similar learning curves, improving their performance across the spatial training. Reduced overall speed may result from the motor alterations observed during neurological tests. The impairment in speed in the maLPA$_1$-null mice coincides with the longer latency scores observed on days 3 and 4, suggesting the influence of speed on this variable. However, no significant differences in escape latencies were observed among the genotypes during spatial training on these same days. It is therefore likely that the absence of differences among the genotypes in their escape latencies is because of the mild impairment in speed of the mice used in our study. Escape latency impairment has been reported in mice with stronger reduction of overall speed than we have seen in our study (Stein *et al*. 2006). In contrast, poor performance during the probe trial and the use of inappropriate search strategies have also been observed in ma*lpa*$_1^{(+/-)}$ mice, but without speed impairments, arguing against the influence of speed deficits in the behavioral impairments reported here.

The cognitive nature of the impairment in maLPA$_1$-null mice can also be questioned on the grounds that anxiety enhancement and abnormal motor behavior increase thigmotaxic behavior in the water maze (Petrosini *et al*. 1996; Rodgers 1997). In fact, during habituation of maLPA$_1$-null mice, increased peripheral exploration is consistent with the anxious-like behavior enhancement previously shown in these animals. Thigmotaxis has been used as an index of anxiety-like behavior that may interfere with the normal acquisition of a spatial learning task (Champagne *et al*. 2002; Whishaw 1995). However, enhanced thigmotaxis in maLPA$_1$-null mice does not seem to be the reason for the deficits reported here. During the habituation trial, animals of all three genotypes displayed a tendency to swim in the peripheral zone. Thigmotaxis is the normal behavior when rodents are exposed for the first time to the water maze; it is replaced by more accurate strategies when animals are repeatedly trained in this task (Whishaw 1995). The maLPA$_1$-null mice used in this study exhibited the normal pattern of significant thigmotaxic behavior during the habituation trial. During spatial training in the water maze, this stronger preference was replaced in maLPA$_1$-null mice as well as in the other two genotypes by search strategies more centered in the middle zone of the water maze. Thus, taken together, our data suggest that enhanced thigmotaxis in maLPA$_1$-null mice during the habituation trial may be because of anxiety-like behavior enhancement or motor impairment, but the fact that reduction of this behavior across the spatial training occurred in the way expected for normal animals argues against its influence on the impairments that we observed in the water maze. In relation to this finding, thigmotaxic behavior in the maLPA$_1$-null mice during the habituation trial does not correlate with either searching strategies or performance during the probe test.

The absence of deficits in the visual-cued task argues against the involvement of sensorimotor or motivational alterations in the performance of the ma*lpa*$_1$-null mice in the water maze. Our data support the notion that the cognitive deficits observed in these mice are not because of increased emotionality, sensorimotor or motivational deficits.

In summary, our data show that the LPA_1 receptor has a role in generating or controlling anxiety-like behavior as well as in cognitive processes such as spatial memory. These results support a role for LPA signaling via LPA_1 receptors in major neuropsychiatric and cognitive disorders, a hypothesis that merits study in humans.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1: Quadrant preference during the habituation trial in the water maze. No differences in preference (or lack of preference) during the habituation trial were observed among the three genotypes

Figure S2: Visual-cued task in the water maze. All genotypes were able to learn a visual-cued task as shown by decreasing escape latencies (a) and distance moved (b) during the training (days 1–3). No differences either in the escape latencies (a) or distance moved (b) in the water maze were observed among the three genotypes ($P > 0.05$). However, the analysis of velocity (c) revealed that ma$lpa_1^{(-/-)}$ mice were slower than the other two genotypes in the visual-cued task ($P < 0.05$)

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