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ORIGINAL INVESTIGATION

Attenuation of cocaine-induced conditioned locomotion is associated with altered expression of hippocampal glutamate receptors in mice lacking LPA1 receptors

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Abstract
Rationale Lysophosphatidic acid is a phospholipid mediator that modulates neurodevelopment and neurogenesis in the hippocampus through its actions on LPA1 receptors. Emerging evidences support LPA_1 as a mediator of learning and emotional behaviour. There are no studies addressing its role on behaviours associated to drug abuse.
Objectives We examined whether genetic deletion of LPA1 receptor in $maLPA_1$-null mice affected either cocaine-induced conditioned locomotion (CL) or behavioural sensitization (BS) induced by repeated cocaine exposure. We also analysed whether cocaine induced changes in the expression of functional markers of both dopamine- and glutamate-related genes in the striatum and the dorsal hippocampus.
Methods We monitored cocaine-induced CL and BS in both genotypes of mice. Striatal dopamine and hippocampal glutamate-related genes were measured by real-time quantitative PCR, Western blot, and immunohistochemistry.
Results $maLPA_1$-null mice exhibit an attenuated CL response after cocaine conditioning but a normal BS after repeated cocaine exposure. These behavioural changes were associated to alterations on the expression of metabotropic mGLUR3 glutamate receptors and on the actions of cocaine on the GLUR1 subunit of AMPA glutamate receptors in the hippocampus of $maLPA_1$ animals. Striatal dopaminergic markers (tyrosine hydroxylase, dopamine D1 receptor, and dopamine transporter DAT), were similar in both genotypes and were equally affected by cocaine exposure.
Conclusion The present results indicate that the lack of LPA1 receptor affect cocaine-induced conditioned locomotion but not behavioural sensitization. The findings suggest that LPA1 receptor may be necessary for a normal associative contextual learning associated to cocaine, probably through the modulation of hippocampal glutamatergic circuits.

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Keywords Lysophosphatidic acid · LPA 1 receptor · Cocaine · Addiction · Conditioned locomotion · Behavioural sensitization · Dopamine · Glutamate · Mouse

Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BS	Behavioural sensitization
CL	Conditioned locomotion
DAT	Dopamine transporter
LPA	Lysophosphatidic acid
mGluR	Metabotropic glutamate receptor
NAc	Accumbens nucleus
NMDA	*N*-methyl-D-aspartic acid
PCR	Polymerase chain reaction
TH	Tyroxine hydroxylase
wt	Wild-type

Introduction

Lysophosphatidic acid (LPA; monoacyl-*sn*-glycerol-3-phosphate) is a bioactive molecule to the family of phospholipids named to lysophospholipids that induces a variety of biological responses in different type of cells. LPA plays a functional role as mediators for different developmental events and adult physiology processes in the mammalians. Since more than 10 years ago, Chun & cols. started to identify the subfamily of lysophospholipid receptors (Chun et al. 1999; Hecht et al. 1996; Zhang et al. 1999), and now more than five of these receptors have been well characterised (Anliker and Chun 2004; Chun 2005; Ishii et al. 2004; Lee et al. 2006, 2007; Mutoh and Chun 2008; Noguchi et al. 2009). The LPA receptors are proteins that belong to the G protein-coupled receptor superfamily (Anliker and Chun 2004; Moolenaar 1999; Mutoh and Chun 2008). LPA activates multiple signal transduction pathways, some of them mediated by Rho-family GTPases (RhoA, Rac1, Cdc42) (Moolenaar et al. 2004) with possible implications in diverse processes as brain development, vascular remodelling, and tissue healing. Activation of these small GTPases helps to explain LPA effects on the control of the assembly of the actin cytoskeleton in response to extracellular changes. Some of these changes might have impact on synaptic remodelling and thus affect neuronal plasticity and memory processes.

Recent studies of our group have demonstrated that LPA can develop an essential role on neurogenesis in mouse brain. LPA_1-null mice ($maLPA_1$-null variant) showed a reduced ventricular zone, altered neuronal markers, and increased cell death that results in a loss of cortical cell density during the formation of the cerebral cortex (Estivill-Torrus et al. 2008). On related regions, such as hippocampal formation, the dentate gyrus showed defective in all processes from proliferation, differentiation to survival within the cerebral neurogenesis in adult mouse under normal and enriched environment housing conditions (Matas-Rico et al. 2008). Hippocampal adult-born neurons, specifically the subgranular zone of the dentate gyrus, appear to be necessary to synaptic plasticity, learning, and memory. Current behavioural results indicated that after evaluating functions of LPA_1 in sensorimotor activity, emotional and cognitive areas in adult mice, $maLPA_1$-null exhibited deficiencies in spatial memory retention and abnormal use of searching orientation strategies (Santin et al. 2009), defective working and reference memory independently of exploratory and emotional impairments attributed to hippocampal malfunction (Castilla-Ortega et al. 2010). Others results have shown an impairment of prepulse inhibition (PPI) response in LPA_1-null mice. LPA_1-null mice display a reduced ability to filter out irrelevant auditory stimulation, which may lead to the development of cognitive deficits (Harrison et al. 2003; Roberts et al. 2005) and schizophrenia-like psychoses (Desbonnet et al. 2009). Relationship between LPA1 receptor and its influence in neuropathological states could be related with the functions of LPA1 receptor in generating/controlling anxiety-like behaviours, and learning and memory alterations.

A relevant model to evaluate the contribution of LPA1 receptors to memory and learning is the analysis of the effects of drugs of abuse. Chronic exposure to drugs of abuse can induce long-term changes in neuroplasticity on several functional circuits. These changes can be revealed through behavioural analysis that include conditioned locomotion responses and behavioural sensitization. For instance, repeated chronic cocaine exposure is associated with the development of two different types of behavioural responses: conditioned locomotion (CL, the increase on basal locomotion in a context paired to cocaine administration) and behavioural sensitization (BS, the increase on the effect of cocaine on locomotion after repeated cocaine administration). These responses have been thought to be independent (Hotsenpiller and Wolf 2002; Tirelli et al. 2003), and they are related to dynamic modulation of dopamine and glutamate transmission in the basal ganglia, hippocampus, and limbic forebrain (Pierce and Kalivas 1997; Mohn et al. 2004; Rodríguez-Borrero et al. 2006; Chambers et al. 2010). Besides these classic transmitters, lipid mediators including lysophospholipids are gaining place as relevant signals contributing to the neuroadaptions associated to chronic drug exposure. The role of LPA1 receptors and their interactions with the glutamatergic and dopaminergic synaptic neurotransmission in the dynamic alterations associated to drug exposure remains to be determined. In the present work, we examined whether

genetic deletion of LPA1 receptors affects both cocaine-induced CL and BS as well as the functional expression of either dopamine-related or glutamate-related signalling genes. Results indicate that LPA1 receptor might be involved in the regulation of associative (hippocampus-dependent) but not implicit (striatum-dependent) learning such as cocaine-induced sensitization.

Material and methods

Animals

All procedures were carried out with wild-type (C57Bl/6; 25±5 g) and maLPA$_1$-null male mice (mixed background C57Bl/6×129SW; 25±5 g). The maLPA$_1$-null mice are a Málaga variant of LPA$_1$-null mouse colony (Estivill-Torrus et al. 2008), which arose spontaneously from the initially reported LPA$_1$-null mouse line (Contos et al. 2000) while crossing heterozygous foundational parents within their original mixed background. Currently, more than 14 maLPA$_1$-null generations have been obtained by backcrossing. Male mice were obtained from heterozygous×heterozygous/homozygous maLPA$_1$-null mating and genotyped for lpa1 deletion by PCR (Contos et al. 2000). All animals were maintained at the central vivarium of the University of Malaga. They were housed in clear plastic cages and maintained in a temperature- (20±2°C) and humidity (40±5%)-controlled room on a 12-h light/dark cycle with food and water ad libitum. The maintenance of the animals as well as the experimental procedures was in accordance with the European animal research laws (European Communities Council Directives 86/609/EU, 98/81/CEE, 2003/65/EC and Commission Recommendation 2007/526/EC).

Drug

Cocaine-HCl was obtained from Sigma-Aldrich (Madrid, Spain), dissolved in sterile 0.9% NaCl solution just before experimentation and administered subcutaneously (s.c.).

Behavioural studies

Open field test

The mazes used were four open field gray background of size 50×50×50 cm (Panlab, Barcelona, Spain). Animals were placed individually in the centre of the field after injection of drug or vehicle, and their behaviour were recorded and analysed for 30 min through videotracking system (Smart®, Panlab, Barcelona, Spain). The results were measured as total distance travelled (centimetre).

Cocaine dose–response curve

In order to determine the correct profile response to different doses of cocaine and to find the appropriate dose for a suitable conditioning process, we administered cocaine subcutaneously (s.c.) with a concentration of 0, 5, 10, and 20 mg/kg. Immediately after injection, animals were tested in the open field test and locomotion registered.

Cocaine conditioning, conditioned locomotion, and behavioural sensitization

Animals were exposed to a cocaine administration program with a dose of 20 mg/kg for five consecutive days (cocaine conditioning). In both genotypes, one half of the animals were treated with cocaine and the other half with vehicle. During the 5 days of cocaine administration, animals were exposed to the open field for 30 min when drug or vehicle was administered. After 5 days of drug consecutive administration, all mice rested 5 days without drugs. On the 11th day, we assessed both conditioned locomotion (CL) test by vehicle administration and behavioural sensitization (BS) test where the effect of a cocaine dose of 10 mg/kg was measured in the context paired with cocaine administration. CL and BS were evaluated using three different schedules. On the first, we tested serially CL and BS as described above (Fig. 2a). On the second, we used different set of animals for CL and BS (Fig. 3a). On the third (Fig. 4), we only evaluated BS in animals injected with saline and habituated to the open field until a stable pattern of locomotion is established (60 min after injection).

For both genotypes, we had three experimental groups: animals conditioned with cocaine (20 mg/kg) and administered with cocaine (10 mg/kg—cocaine sensitization), and animals conditioned with vehicle and treated with vehicle (vehicle control) or cocaine (10 mg/kg—acute cocaine).

Tyrosine hydroxylase immunohistochemistry

Eight adult mice (12 weeks old at the time of the experiment) were used for this study (four for each wild-type and maLPA$_1$-null group). The animals were deeply anesthetised and perfused transcardially with 0.1 M phosphate-buffered saline (PBS, pH 7.4), followed by ice-cold fixative solution (4% paraformaldehyde, 75 mM lysine, and 10 mM sodium metaperiodate in PBS). After 24 h in the fixative solution, all brains were cut into 40-μm-thick coronal sections with a microtome and stored in PBS supplemented with 30% sucrose and 0.1% sodium azide. After blocking the endogenous peroxidase activity, avidin, biotin, and biotin-binding proteins (Vector Labs kit), the brain sections were incubated with anti-TH antibodies (Sigma; T2928) at 1:3,000 dilution for 72 h at 4°C and processed by the avidin–biotin

method using biotinylated anti-rabbit IgG (Vector, 1:500) and ExtrAvidin–peroxidase conjugate (Sigma, 1:2,000). Immunoreaction product was visualised with 0.05% diaminobenzidine, 0.03% nickel ammonium sulphate, and 0.01% H_2O_2. Immunostaining was observed under a Nikon Eclipse 800 microscope, and images were acquired with a Nikon DXM1200, high resolution digital camera.

Analysis of mRNA expression

One hour after the end of behavioural experiments, all animals were sacrificed and their brains removed, frozen (−80°C), and dissected in coronal brain slices (2 mm thickness) with razor blades in a Mouse Brain Slicer Matrix (Zivic Instruments). The discrete brain regions were picked up using a scalpel (dorsal hippocampus) or a sample minicorer (striatum) by free hand dissection. Target brain regions were identified according to Paxinos and Franklin (2001): dorsal hippocampus from the bregma −1.22 to −3.52 mm and striatum from the bregma 1.54 to −0.46 mm.

Reverse transcription and real-time PCR

Real-time PCR was used to measure relative quantification of different receptors, transporters, and synthesis/degradation enzymes mRNA expression involved in the dopaminergic (TH synthesis enzymes, D1 receptor and DAT transporter) and glutamatergic (mGlu3 and mGlu5 receptors; NR1, NR2A, NR2B, NR2C subunits NMDA ionotropic receptor; GluR1, GluR2, GluR3, GluR4 subunits AMPA ionotropic receptor) neurotransmission. During all the process, material was clean and free of RNases.

Total RNA extraction from dorsal hippocampus and striatum was isolated using Trizol reagent (Gibco BRL Life Technologies, Baltimore, MD, USA) according to the manufacturer's instructions. Then the RNA was cleaned using RNeasy Mini Kit (Qiagen, Hilden, Germany). RNA concentration and purity was determined by measuring optical density at 260 and 280 nm using a spectrophotometer (Biotech Photometer, UV 1101, WPA). In all cases, RNA samples showed A260/280 ratios between 1.8 and 2.0. First-strand synthesis from each sample was carried out using random hexamer primer and M-MuLV reverse transcriptase (Roche Applied Science, Indianapolis, USA) according to manufacturer's instructions. Negative controls included reverse transcription reactions omitting reverse transcriptase. Resulting cDNAs were used as the template for real-time quantitative PCR with an iCycler system (Bio-Rad, Hercules, CA, USA) using the Quanti-Tect SYBR Green PCR kit (Qiagen, Hilden, Germany). Primers used for real-time PCR (accession no. from NCBI database) were shown in Table 1, and oligonucleotides were provided by Sigma-Proligo (Proligo France SAS, Paris, France).

Quantification was carried out according to standard curves run simultaneously as the samples with each reaction run in duplicate. The PCR product was separated in a 1% agarose gel electrophoresis to verify fragment size and the absence of contaminant fragments, quantified by measuring the absorbance at 260 nm, and serially diluted to 10^{-5} pg/ml. Several tenfold dilutions (10^{-1} to 10^{-5}) were checked for optimal cycling on the LightCycler, and three of them in an interval within which the samples fell were selected for standard curves.

Each reaction was run in duplicate and contained 2.5 μl of cDNA template, 8 μl of Master SYBR Green, 4.86 μl of PCR Ultra Pure Water, and 0.64 μl of primers in a final reaction volume of 15 μl. Cycling parameters were 95°C for 15 min to activate DNA polymerase, then 30–40 cycles of 94°C for 15 s, annealing temperature for 30 s (TH 60.2°C, D1 59.4°C, DAT 62°C, mGlu3 61.4°C, mGlu5 61.4°C, NR1 61.4°C, NR2A 61.4°C, NR2B 57.1°C, NR2C 57.1°C, GluR1 57.1°C, GluR2 55°C, GluR3 58.9°C, GluR4 57.1°C, and β actin 57.1°C) and a final extension step of 72°C for 30 s in which fluorescence was acquired. Melting curves analysis was performed to ensure that only a single product was amplified. Absolute values from each sample were normalised with regard to β actin (constitutive gene expression) used to reference standard. This internal standard was chosen based on first analyzing a panel of housekeeping genes that additionally included cyclophilin and SP1. By using the real-time quantitative PCR, we could measure the expression of different genes on selected brain regions (dorsal hippocampus and striatum) related to reward circuitry of treated animals, associated to the dopaminergic and glutamatergic system.

Western blotting

Animals were killed by decapitation, and the brain was immediately isolated, snap frozen in liquid nitrogen, and stored at −80°C until use. Lysates of mouse striatum and hippocampus were homogenised and incubated in RIPA buffer 1× (Thermo Scientific, Rockford, IL, USA) containing a proteinase and phosphatase inhibitor cocktail (sodium fluoride 50 mM, sodium orthovanadate 1 mM, sodium pyrophosphate 10 mM, β-glycerophosphate 10 mM, NaF 5 mM, $NaOV_4$ 100 μM, NaH_2PO_4 1 mM, aprotinin 80 μM, pepstatin A 2 mM, trypsin inhibitor 1 μM, phenylmethylsulfonyl fluoride 50 μM; Merck) for 2 h at 4°C and then centrifuged at 10,000×*g* for 15 min at 4°C.

Equivalent amounts of protein extract (35 μg) were separated by 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotted onto nitrocellulose membranes. We pre-incubated blots with a blocking buffer containing PBS, 0.1% Tween 20, and 2% albumin fraction V from bovine serum (Merck) at room

Table 1 Primers used for real-time PCR (accession no. from NCBI database) and oligonucleotides employed (Proligo France SAS, Paris, France)

		Gene ID	GenBank accession numbers	Forward sense primer	Reverse antisense primer	bp DNA fragment amplified
DA		*TH*	NM_009377.1	ccaaggaaagtgtcagagttgg	accctgcttgtattggaagg	150
		D1	NM_010076.1	ttctcctttcgcatcctcac	atgaccaagacagccaccaa	176
		DAT	AF_109391	acccgctgctgagtattttg	tacatagggcatggtagctgtg	193
GLU	mGluR	*mGluR3*	NM_181850.2	tgagtggtttcgtggtcttg	tgcttgcagaggactgagaa	153
		mGluR5	BC096533.1	aagaaggagaaccccaacca	ttcggagactggagagtttg	179
	NMDA	*NR1*	NM_008169	gtgcaagtgggcatctacaa	tgggcttgacatacacgaag	157
		NR2A	NM_008170	gtttgttggtgacggtgaga	aagaggtgctcccagatgaa	180
		NR2B	NM_008171	atgtggattgggaggatagg	tcgggctttgaggatacttg	249
		NR2C	NM_010350	ggaatggtatgatcggtgag	ccgtgaggcacattacaaac	225
	AMPA	*GluR1*	NM_008165.2 210	ttttctaggtgcggttgtgg	ttggagaactgggaaca	210
		GluR2	NM_001039195.1	aaggaggaaagggaaacgag	ccgaagtggaaaactgaacc	217
		GluR3	NM_016886.2	caacaccaaccagaacacca	atcggcatcagtgggaaa	229
		GluR4	NM_019691.3	ttggaatgggatggtaggag	taggaacaagaccacgctga	250

temperature for 1 h. For protein detection, the blotted membrane lanes were incubated in the following primary antibodies and dilutions at room temperature overnight: monoclonal anti-tyrosine hydroxylase from mouse, diluted 1:1,000 (cat. no. T2928; SIGMA, Saint Louis, MI, USA); polyclonal anti-dopamine transporter from rabbit, diluted 1:1,000 (cat. no. AB1591P; Millipore, Billerica, MA, USA); monoclonal anti-NMDAR1 from rabbit, diluted 1:1,000 (cat. no. AB9864; Millipore), monoclonal anti-glutamate receptor type 3 from rabbit, diluted 1:1,000 (cat. no. 1731-1; Epitomics, Burlingame, CA, USA); polyclonal anti-phospho-GluR1 (Ser845) from rabbit, diluted 1:850 (cat. no. OPA1-4118; Thermo Scientific); monoclonal anti-β-actin from mouse, diluted 1:1,000 cat. no. A5316; SIGMA). After extensive washing in PBS containing 1% Tween 20 (PBS-T), a peroxidase-conjugated goat anti-rabbit antibody or goat anti-mouse antibody (Promega, Madison, WI) was added, both diluted at 1:10,000, for 1 h at room temperature. Peroxidase marker proteins with defined molecular weights (Full-Range Rainbow Molecular Weight Markers; cat. no. RPN800E, Amersham) were used for molecular weight determination in immunoblots. Membranes were then subjected to repeated washing in PBS-T and the specific protein bands visualised using the enhanced chemiluminescence technique (Western Blotting Luminol Reagent; cat. no. sc-2048, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and Auto-Biochemi Imaging System (LTF Labortechnik GmbH, Wasserburg/Bodensee, Germany).

Statistical analysis

Results are expressed as the mean±SEM (standard error of the mean) of at least ten determinations per experimental group. Statistical significance of behavioural data was assessed by one- and two-way ANOVA with/without repeated measures (RM-ANOVA), followed by post hoc Tukey test. Statistical significance of gene quantifications was analysed by two-way ANOVA. In all cases, we considered a statistically significant difference from chance at $*p<0.05$, $**p<0.01$, and $***p<0.001$. All data were analysed with statistical software SPSS-15 for Windows (Lead Technologies) and GraphPad Prism 4 (GraphPad Software, Inc.).

Results

Behavioural results

Dose–response effect of cocaine administration in maLPA$_1$-null mice

We carried out a cocaine dose–response curve in the open field for 30 min with wild-type and maLPA$_1$-null mice (Fig. 1). Cocaine differentially activated locomotion in both genotypes (treatment [$F(3,69)=48.128$, $p<0.001$] and genotype [$F(1,69)=5.976$, $p=0.017$] effect, two-way ANOVA). In both genotypes, the dose of 20 mg/kg produced the similar effects although maLPA$_1$-null mice had lower activity compared to wild-type group after a vehicle injection [$p=0.035$] (Fig. 1).

Cocaine conditioning in maLPA$_1$-null mice

Figure 2 depicts the experimental design (Fig. 2a) and the results on acquisition of sensitization (Fig. 2b), conditioned locomotion (Fig. 2c), and behavioural sensitization to cocaine (Fig. 2d). Based on cocaine dose–response studies, we selected the dose of 20 mg/kg for conditioning during

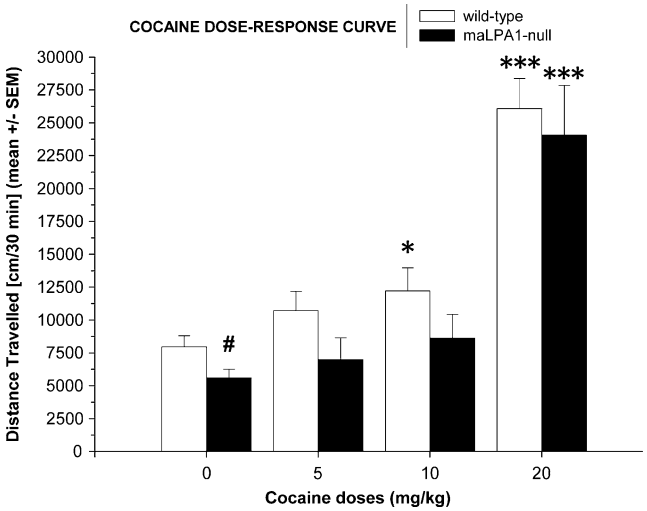


Fig. 1 Stimulation of locomotion induced by cocaine. Data represent mean distance travelled after three doses of cocaine by either wild-type and maLPA$_1$-null mice. *Error bars* indicate SEM. N=8–10 animals per group. ***p<0.001; *p<0.05 (control versus cocaine treatment); #p<0.05 maLPA$_1$-null vs. wild-type mice, Tukey's test

five consecutive days. Both genotypes had a similar acquisition of cocaine conditioning throughout the five consecutive injections (Fig. 2b) ([repeated measures (RM)-ANOVA: days effect, $F(4,164)=11.113$, $p<0.001$] for total distance travelled, [RM-ANOVA: days×treatment effect, $F(4,164)=11.436$, $p<0.001$] for treatment effect). We did not observe a significant difference between genotypes [RM-ANOVA: genotype effect, $F(1,41)=2.903$, $p=0.096$] nor treatment×genotype interaction [RM-ANOVA: interaction effect, $F(1,41)=1.442$, $p=0.237$].

Cocaine-conditioned locomotion and cocaine-induced sensitization in maLPA$_1$-null mice

Repeated injections of cocaine induced CL (treatment effect, $F(1,32)=7.822$, $p<0.05$, two-way ANOVA). Chronic cocaine pre-treated wild-type group exhibited a conditioned locomotor response in relation to the non-conditioned group [$p<0.05$] (Fig. 2c). However, cocaine-conditioned maLPA$_1$-null mice showed an attenuation of CL. These data suggest that the impairment of a CL response in maLPA$_1$-null mice has a memory deficit in the expression of contextual conditioning.

On the next day, we assessed BS with the same groups of animals used in the CL, but in this case, each chronic cocaine pre-treated group was injected with cocaine (10 mg/kg), and each basal locomotion group was injected with vehicle (vehicle control) or acute cocaine (10 mg/kg). We found that cocaine enhanced locomotion (treatment effect, $F(2,51)=28.608$, $p<0.001$, two-way ANOVA), with a similar response in both genotypes ($F(1,51)=0.870$, $p=0.425$, two-way ANOVA, ns) and no treatment×genotype interaction (interaction effect, $F(2,51)=0.870$, $p=0.425$, two-way ANOVA, ns). Thus, after priming injection, maLPA$_1$-null and wild-type mice showed a robust BS (Fig. 2d).

Cocaine-conditioned locomotion and -sensitization in maLPA$_1$-null mice independently evaluated

To further verify that previous exposure to the test in the CL study is not inducing a recovery of BS in maLPA$_1$ null mice, we tested again CL and BS in different set of animals. Additionally, mice were evaluated over longer period of time (90 min) and testing sessions were analysed by time bins (10 min) (see experimental design on Fig. 3a and sensitization acquisition Fig. 3b).

In the CL test, analysis of the main effects showed a significant effect of treatment [two-way ANOVA: treatment effect, $F(1,25)=97.783$, $p<0.001$], genotype [two-way ANOVA: genotype effect, $F(1,25)=18.012$, $p<0.001$] and interaction [two-way ANOVA: interaction effect, $F(1,25)=19.177$, $p<0.001$]. Chronic cocaine pre-treated wild-type group exhibited a normal conditioned locomotor response in relation to the non-conditioned control group [$p<0.001$] that remained for the entire 90 min test. However, although maLPA$_1$-null mice also showed conditioned locomotion (Fig. 3c), it was only restricted to the initial intervals of time (20 min) disappearing thereafter (Fig. 3d). From t=20 to t=90 min post field exposure maLPA$_1$ null mice had a similar behaviour than the non-conditioned animals. While the increased locomotion of cocaine-conditioned animals over saline-conditioned animals was of 83.4 %, that presented by maLPA$_1$ animals was of 65%. Moreover, total scores for CL in wild-type mice was 50.4% greater than that of maLPA$_1$.This attenuation of the response is in accordance with the previous experiment, although the initial CL observed was not present in the first experimental day. The variability on the response to novelty and conditioning conditions in maLPA$_1$ mice has been observed before, and we think it is related to a disturbance of emotional processes observed in these animals (Castilla-Ortega et al. 2010).

In a separated group of animals, we measured the BS response after a cocaine priming (10 mg/kg) and their corresponding acute cocaine controls (10 mg/kg). Our results revealed that the groups who had been cocaine sensitised showed an increased locomotor activity compared to those who had received a cocaine dose for the first time [two-way ANOVA: treatment effect, $F(1,44)=33.400$, $p<0.001$, Fig. 3e], regardless of genotype [two-way ANOVA: genotype effect, $F(1,44)=11.392$, $p=0.01$] and interaction [two-way ANOVA: interaction effect, $F(1,44)=1.865$, $p=0.179$]. Cocaine priming injection induced behavioural

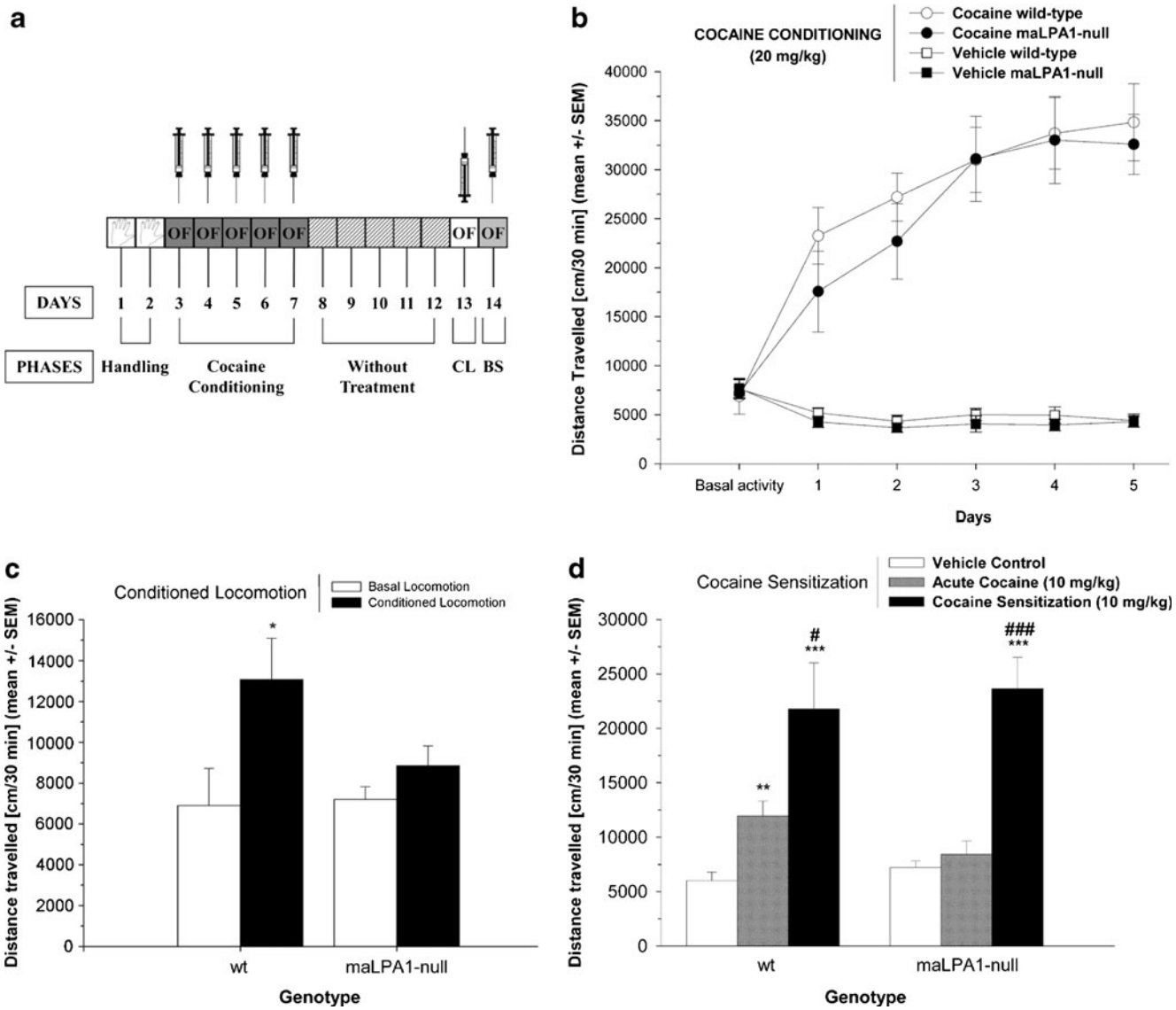
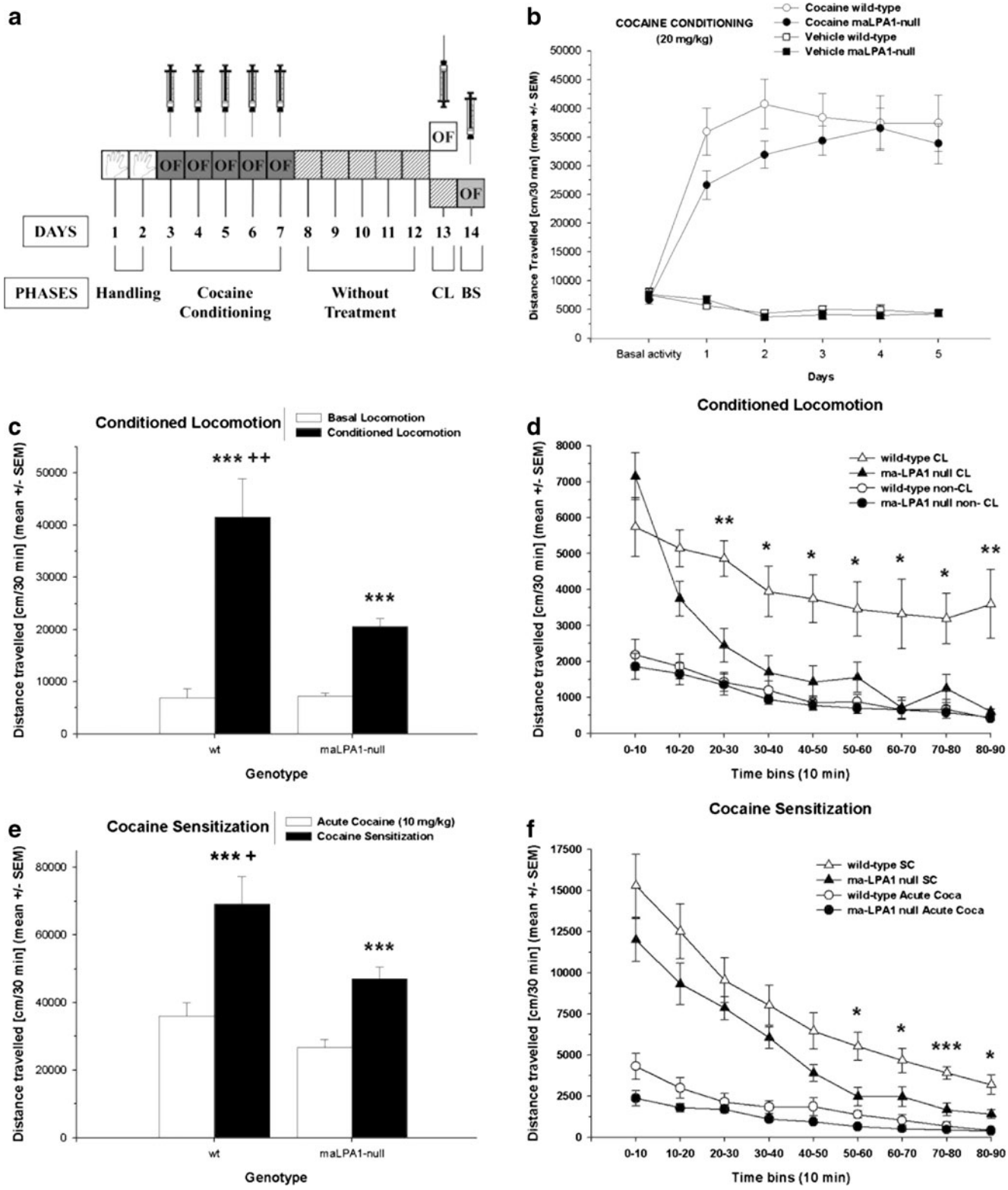


Fig. 2 Effects of cocaine on conditioned locomotion and behavioural sensitization measured serially in the same group of either wild-type or maLPA$_1$-null mice. **a** Scheme for cocaine sensitization treatment and serial tests for conditioned locomotion and behavioural sensitization. **b** Chronic cocaine treatment (20 mg/kg) significantly enhanced locomotor activity in open field during 5 training days regardless of genotype compared to vehicle groups. **c** Conditioned locomotion was observed in cocaine-exposed wild-type group of animals, but not in maLPA$_1$-null mice. **d** A cocaine-priming injection (10 mg/kg) elicited robust reinstatement of locomotor response in both genotypes showing behavioural sensitization. Data represent mean distance travelled±SEM. ***$p<0.001$ and **$p<0.01$, *$p<0.05$ compared to vehicle control, #$p<0.05$ and ###$p<0.001$ compared to acute cocaine (10 mg/kg)

sensitization compared with acute cocaine controls [$p<0.001$] in both genotypes, but this response was lower in maLPA$_1$-null mice [$p<0.01$] (Fig. 3e). This robust cocaine-induced sensitization was also observed between time bins from 0 to 50 min when the effect of sensitization is equally stronger in both genotypes (Fig. 3f). At the last minutes of the BS session (50–90 min), we found a significant difference in locomotor activity between wild-type and maLPA$_1$-null mice [$p<0.05$; $p<0.001$], but these differences could be due to a residual effect of contextual associative memory rather than as a result of cocaine-induced sensitization by itself. Global scores for BS over acute cocaine were similarly enhanced in both sensitised genotypes (47.9% for wild-type and 43.4% for maLPA$_1$).

Finally, we analysed whether habituation to the field may affect the expression of BS. To this end, animals conditioned either with saline or cocaine 20 mg/kg were injected with saline and habituated to the field for 60 min. Then, they were injected with cocaine (10 mg/kg). Results are depicted in Fig. 4. Open-field habituated animals of both genotypes exhibited a clear response to cocaine ($F(3,63)=33.641$; $p<0.001$), which was clearly different



in between groups conditioned with saline with respect to those conditioned with cocaine that exhibited a marked cocaine-induced sensitization. There were no differences in between genotypes ($F(1,63)=0.583$; $p=0.448$) nor specific interactions genotype×cocaine in the BS test ($F(3,63)=0.141$; $p=0.935$).

◀ **Fig. 3** Effects of cocaine on conditioned locomotion and behavioural sensitization measured in different groups of either wild-type or maLPA$_1$-null mice. **a** Scheme for cocaine sensitization treatment and tests for conditioned locomotion and behavioural sensitization. **b** Chronic cocaine treatment (20 mg/kg) significantly enhanced locomotor activity in open field during 5 training days regardless of genotype compared to vehicle groups. **c** Wild-type and maLPA$_1$-null mice pre-treated with cocaine showed a conditioned locomotor response compared to their control groups, but this response was significantly lower in maLPA$_1$-null compared with wild-type mice. Statistical significance is indicated by ***p<0.001 compared to control groups and ++p<0.01 compared to conditioned wild-type mice. **d** CL session in time bins (10 min), the analysis showed a CL deficit in maLPA$_1$-null (20–90 min). Statistical significance is indicated by *p<0.05 and **p<0.01 compared to conditioned maLPA$_1$-null mice. **e** Cocaine priming injection (10 mg/kg) induced a robust BS in both genotypes showing BS. Statistical significance is indicated by ***p<0.001 compared to acute cocaine (10 mg/kg) and +p<0.05 compared to maLPA$_1$-null mice cocaine sensitised. **f** BS session in time bins (10 min), the analysis did not show differences in sensitization between genotypes, except at the last minutes of the session (50–90 min). Statistical significance is indicated by *p<0.05 and ***p<0.001 compared to sensitised maLPA$_1$-null mice. *Error bars* indicate SEM

Immunohistochemical, Western blotting, and genomic results

In order to gain insights on the potential mechanisms implicated on the effects of LPA1 receptor deletion on CL, we explored the integrity of dopaminergic transmission on the striatum and the expression of glutamatergic receptors in the dorsal hippocampus. Main data and statistical analysis are given in Tables 2, 3, 4, 5, 6, and 7 and Figs. 5, 6, and 7.

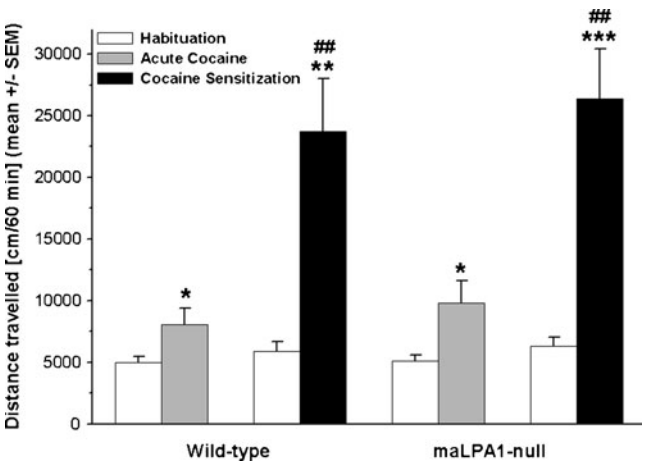


Fig. 4 Lack of effects of a previous habituation to the open field (60 min of exposure) on acute cocaine (10 mg/kg) induced behavioural sensitization measured in both wild-type and maLPA$_1$-null mice injected for 5 days with either saline or cocaine 20 mg/kg. See details in the text. Both genotypes exhibited a similar enhanced locomotion after cocaine with respect to that measured along 60 min of habituation (*open columns*). This psychostimulant effect was greater on animals that were injected previously for 5 days with cocaine (*black columns*, sensitised animals) than on those that received 5 days of saline (*gray columns*). Statistical significance is indicated by *p<0.05, **p<0.01, and ***p<0.001 cocaine vs. habituated locomotion. Both genotypes exhibited sensitised responses ##p<0.01 and ###p<0.001 repeated cocaine vs. repeated saline. *Error bars* indicate SEM

Effects of LPA1 receptor deletion and cocaine treatment on TH, DAT, and dopamine D1 receptor

Since cocaine-induced sensitization depends on integrity of dopaminergic transmission on the basal ganglia (Pierce and Kalivas 1997), we analysed whether maLPA$_1$ null mice exhibited a normal dopaminergic innervation at both neuroanatomical and biochemical levels. Table 2 shows that mRNA levels of the TH, DAT, and D1 receptor were similar in both wild-type and maLPA$_1$ mice. The mRNA of these genes was not affected by chronic cocaine treatment (statistical analysis shown on Table 3). Cocaine treatment was very close to produce a significant reduction on both genotypes.

In order to get further information on anatomical distribution of TH and on the expression of the protein for TH and DAT, we performed immunohistochemistry and Western blot analysis. Figure 5 shows that TH distribution has a similar pattern in both genotypes and that chronic cocaine reduced TH equally in both genotypes (Table 4 for statistical analysis). This is consistent with the normal behavioural response in terms of sensitization after chronic exposure.

Effects of LPA1 receptor deletion and cocaine treatment on glutamate receptors in dorsal hippocampus

Since we observed that maLPA$_1$-null mice displayed a blunted CL, we suspected that it may be derived of a contextual memory deficit. This deficit may be related to an alteration of hippocampal function in LPA$_1$ null mice that may implicate an altered glutamate neurotransmission. In order to evaluate this hypothesis, we assessed the mRNA expression of glutamate receptors (metabotropic glutamate receptors mGluR5 and mGluR3, ionotropic glutamate *N*-methyl D-aspartate (NMDA) receptor subunits NR1, NR2A, NR2B, and NR2C, and ionotropic glutamate α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunits GluR1, GluR2, GluR3, and GluR4), followed by confirmation by Western blot. Data are presented in Figs. 6 and 7 and on Tables 5, 6, and 7.

Metabotropic glutamate receptors

LPA$_1$ null mice exhibited a normal level of expression of glutamate receptors mRNA levels on dorsal hippocampus (Tables 5 and Fig. 5) with the exception of a significant increase in mGluR3 receptor [two-way ANOVA: genotype

Table 2 Data are presented as mean±SEM of quantitative real-time PCR analysis of TH, D1, and DAT mRNA expression normalised to the levels of β-actin mRNA in striatum

			TH, D1, and DAT mRNA expression (mean±SEM) in the dorsal striatum of ma-LPA$_1$ null mice by quantitative real-time PCR (target gene/beta-actin)		
			TH	D1	DAT
Genotype	Wild-type	Vehicle	0.005±0.0006	0.118±0.0128	0.011±0.0018
		Cocaine	0.005±0.0003	0.102±0.0050	0.007±0.0007
	maLPA$_1$-null	Vehicle	0.005±0.0004	0.109±0.0064	0.011±0.0025
		Cocaine	0.005±0.0002	0.106±0.0051	0.009±0.0009

effect: mGluR3, $F(1,12)=7.807$, $p=0.0162$] (Fig. 6a). Cocaine treatment did not affect the expression levels of mGluR5 and mGluR3 (Table 6).

Ionotropic NMDA receptor

Cocaine conditioning induced a significant decrease on mRNA expression of NR1 subunit (Fig. 6b; Table 6), regardless of genotype [two-way ANOVA, genotype effect: $F(1,14)=0.0420$, $p=0.8405$]. The remaining subunits of the glutamate ionotropic NMDA receptor (NR2A-B-C) did not change after chronic cocaine pre-treatment independently of genotype (Tables 5 and 6).

Ionotropic AMPA receptor

Of the four subunits of the glutamate ionotropic AMPA receptor analysed, only GluR3 was affected as results of chronic cocaine treatment in both genotypes (Tables 5 and 6; Fig. 6c). Cocaine treatment decreased GluR1 expression in wild-type but not on maLPA$_1$ null mice. The remaining subunits were affected neither by genotype no by cocaine treatment (Tables 5 and 6).

Western blot analysis

Taking in consideration that alterations in mRNA expression are not always followed by effects on protein translation and function, we examined the protein levels of NR1 and GLUR3 subunits and the amount of phosphor-GLUR1 that has been specifically linked to sensitization. Both, GLUR3 and NR1 were decreased by cocaine (Table 7; Fig. 7) although the effect on the GLUR3 was only observed in maLPA$_1$ null mice. Analysis of phosphorylated forms of GluR1 (Ser845 phospho-GluR1) did not revealed an effect of treatment despite a non-significant decrease ($p=0.1$) in cocaine-treated wild-type animals.

Discussion

The present study was planned to study whether targeted deletion of LPA$_1$ is involved in cocaine-induced conditioned locomotion and sensitization. These two different modalities of learning involve different circuits of the cortico-limbic-striatal interplay. Our results strongly suggest that LPA$_1$-mediated signalling is relevant for the expression of contextual CL induced by cocaine, but not BS after repeated exposure. The impairment on the CL response exhibited by maLPA$_1$-null mice could indicate a failure to learn correctly the association between properties of an environment (open field) and cocaine-rewarding properties, or a failure to sustain the expression of the conditioned response because of emotional interferences, since maLPA$_1$ exhibits alterations in emotional processing (Castilla-Ortega et al. 2010). In this work, we demonstrated that the memory deficit in the expression of cocaine-CL may be associated to alterations in glutamatergic receptors in the dorsal

Table 3 Statistical analysis of either TH, D1, or DAT mRNA expression in the striatum (two-way ANOVA did not show differences among genotypes (wild-type vs. maLPA$_1$-null mice), treatments (vehicle vs. chronic cocaine) and interaction in the expression of these mRNAS)

	ANOVA results: statistical analysis of TH, D1, and DAT mRNA expression in the dorsal striatum of ma-LPA$_1$ null mice by quantitative real-time PCR (target gene/beta-actin)		
	TH	D1	DAT
Genotype	$F[1,28]=0.119$; $p=0.732$	$F[1,28]=0.123$; $p=0.727$	$F[1,28]=0.421$; $p=0.5213$
Treatment	$F[1,28]=0.635$; $p=0.432$	$F[1,28]=1.787$; $p=0.192$	$F[1,28]=3.394$; $p=0.076$
Interaction	$F[1,28]=0.154$; $p=0.697$	$F[1,28]=0.8367$; $p=0.368$	$F[1,28]=0.1597$; $p=0.692$

Table 4 Statistical analysis of the expression of both TH and DAT protein in the striatum (two-way ANOVA did not show differences among genotypes (wild-type vs. maLPA$_1$-null mice), treatments (vehicle vs. chronic cocaine), and interaction in the expression of these proteins)

	ANOVA results: statistical analysis of TH and DAT protein expression in the dorsal striatum of ma-LPA$_1$ null mice by Western blotting (target protein/beta-actin)	
	TH	DAT
Genotype	$F[1,12]=1.42$; $p=0.2568$	$F[1,12]=0.08$; $p=0.7862$
Treatment	$F[1,12]=35.32$; $p<0.0001$	$F[1,12]=0.10$; $p=0.7532$
Interaction	$F[1,12]=0.41$; $p=0.5355$	$F[1,12]=0$; $p=0.9667$
	Cocaine effect	

hippocampus in the absence of LPA$_1$. In contrast, maLPA$_1$-null mice developed a robust acquisition of the cocaine-conditioning and BS response after cocaine priming injection (10 mg/kg), without differences in respect to wild-type control mice. This dissociation between cue-induced reward-seeking behaviour and behaviours directly producing contingent reward reflects the involvement of different functional neural circuits (Grimm and See 2000; Vorel et al. 2001). Moreover, recent studies have clearly confirmed this hypothesis, showing how CL and BS may be dissociated in mice (Hotsenpiller and Wolf 2002; Tirelli et al. 2003).

Mesocorticolimbic dopaminergic system plays an important function in cocaine-induced BS (Pierce and Kalivas 1997). In our case, we neither found maLPA null mice-selective changes in BS nor in underlying biochemical measures. Thus, the mRNA levels of the enzyme for dopamine synthesis TH, the dopamine D1 receptor, and the dopamine transporter DAT were affected in the basal ganglia. TH immunoreactivity in striatum, NAc, substantia nigra, and hippocampus was normal in both genotypes studied. Moreover, the decrease in TH content in the dorsal striatum induced by cocaine was equal in both genotypes. In our results, it seems that striatal dopaminergic system is not affected neither by the lack of LPA1 receptor nor the CL task, although it showed some changes in TH protein. This indicates that both the normal BS response to cocaine and the underlying molecular mechanisms are preserved in maLPA$_1$ null mice.

Dorsal hippocampus plays an essential role in the recall for contextual memories of cocaine-induced reward (Meyers et al. 2003). Specifically, hippocampal dentate gyrus seems essential for generating and expressing contextual memories of fear and cocaine drug-induced reward in a conditioned place preference (CPP) test (Hernandez-Rabaza et al. 2008) as well as participates in both conditioned locomotion and behavioural sensitization (Pierce and Kalivas 1997; Mohn et al. 2004; Rodríguez-Borrero et al. 2006; Chambers et al. 2010). Behavioural results reported here are consistent with previous works that demonstrated an essential role of the hippocampus in self-administration reinstatement by contextual cues (Fuchs et al. 2005) and CPP test (Meyers et al. 2006). Nowadays, we know that glutamatergic signalling in the brain reward circuitry exerts powerful control over drug-seeking behaviour. Re-exposure to an environment previously paired with cocaine-reward produced an enhancement in the levels of GluR1 phosphorylation (Ser845 phospho-GluR1) in dorsal hippocampus evaluated in the CPP probe (Tropea et al. 2008). We did not observe such enhancement but a tendency to a decrease in GLUR1 phosphorylation in the dorsal hippocampus as results of exposure to cocaine-paired environment. However, we did find specific genotypic changes in glutamate receptors. They were restricted to

Table 5 Data are presented as mean±SEM of mRNA expression normalised to the levels of β-actin mRNA of both metabotropic (mGluR) and ionotropic (iGluRs: NMDA-Rs and AMPA-Rs) glutamatergic receptors in dorsal hippocampus

			mRNA expression of remaining glutamatergic receptors in dorsal hippocampus by quantitative real-time PCR (target gene/beta-actin)					
			mGluR	NMDA-Rs			AMPA-Rs	
			mGluR5	NR2A	NR2B	NR2C	GluR2	GluR4
Genotype	Wild-type	Vehicle	0.041±0.0045	0.034±.0033	0.027±0.0033	0.008±0.0017	0.167±0.0311	0.009±0.0020
		Cocaine	0.042±0.0056	0.029±0.0037	0.019±0.0011	0.007±0.0014	0.152±0.0186	0.008±0.0009
	maLPA$_1$-null	Vehicle	0.056±0.0073	0.035±0.0037	0.022±0.0037	0.009±0.0026	0.17±0.0106	0.008±0.0005
		Cocaine	0.043±0.0042	0.027±0.0037	0.022±0.0020	0.005±0.0008	0.150±0.0078	0.007±0.0010

Table 6 Statistical analysis of metabotropic (mGluRs) and ionotropic (iGluRs: NMDA-Rs and AMPA-Rs) glutamatergic in dorsal hippocampus (two-way ANOVA showed differences among genotypes in mGluR3, treatments in NR1 and GluR3, and interaction in GluR1 by using the real-time quantitative PCR)

ANOVA results: statistical analysis of glutamatergic receptors mRNA expression in dorsal hippocampus of ma-LPA$_1$ null mice by quantitative real-time PCR (target gene/beta-actin)											
	mGluRs		NMDA-Rs				AMPA-Rs				
	mGluR3	mGluR5	NR1	NR2A	NR2B	NR2C	GluR1	GluR2	GluR3	GluR4
Genotype	$F(1,12)=7.807$, $p<0.0162$	$F(1,15)=1.582$, $p<0.2277$	$F(1,14)=0.0420$, $p<0.8405$	$F(1,13)=0.0695$, $p<0.7962$	$F(1,12)=0.0323$, $p<0.8604$	$F(1,16)=0.0944$, $p<0.7625$	$F(1,15)=3.289$, $p<0.0898$	$F(1,12)=0.0006$, $p<0.9797$	$F(1,15)=0.1549$, $p<0.6994$	$F(1,12)=0.3562$, $p<0.5617$
Treatment	$F(1,12)=0.6455$, $p<0.4373$	$F(1,15)=0.9013$, $p<0.3575$	$F(1,14)=4.722$, $p<0.0474$	$F(1,13)=3.720$, $p<0.0759$	$F(1,12)=2.389$, $p<0.1481$	$F(1,16)=1.252$, $p<0.2797$	$F(1,15)=1.317$, $p<0.2692$	$F(1,12)=0.8238$, $p<0.3819$	$F(1,15)=5.786$, $p<0.0295$	$F(1,12)=0.3282$, $p<0.5773$
Interaction	$F(1,12)=2.941$, $p<0.1121$	$F(1,15)=1.272$, $p<0.2771$	$F(1,14)=0.0016$, $p<0.9679$	$F(1,13)=0.0849$, $p<0.7753$	$F(1,12)=2.068$, $p<0.1760$	$F(1,16)=0.3958$, $p<0.5382$	$F(1,15)=5.323$, $p<0.0357$	$F(1,12)=0.01681$, $p<0.8990$	$F(1,15)=0.0937$, $p<0.7637$	$F(1,12)=0.04964$, $p<0.8274$
	Genotype cocaine effect		Cocaine effect				ma-LPA$_1$ cocaine effect		Cocaine effect	

both the metabotropic mGLUR3 receptor and to the GLUR3 subunit of the AMPA receptor.

Concerning the AMPA receptors, our results indicate that the GLUR3 subunit is differentially regulated by cocaine in the hippocampus of maLPA$_1$ null mice with respect to control mice, indicating that the AMPA receptor may be related to the LPA1 receptor-dependent genotype differences in CL observed in the present study. However, the nature of the alterations found differs from that reported by previous studies. In the NAc, cell surface AMPA receptor (GluR1) increased after cocaine withdrawal, but it is internalised after cocaine challenge. This internalisation is accompanied by alterations in the activation of mitogen-activated protein kinases (c-Jun N-terminal kinase "JNK", and p38; extracellular signal-regulated kinase "ERK", in withdrawal and cocaine challenge, respectively) (Boudreau et al. 2007; Kourrich et al. 2007). But while we found that mRNA levels of both GLUR1 and GLUR3 diminished after chronic cocaine pre-treatment, those studies revealed that there was an increased association between GluR1 and GluR3 subunits in cocaine-sensitised rats suggesting a sensitization-specific modulation on those AMPA subnits (Boudreau et al. 2007). Therefore, immunoblotting revealed that most AMPARs in the NAc of naive rats are either GluR1/2 or GluR2/3, whereas <10% are homomeric GluR1 or GluR1/3, and this is reversed by cocaine treatment leading to BS. The differences with present results may be based on the different species (rat) and different brain area (nucleus accumbens) used in the experimental design, so further studies are needed to understand the role of these AMPA receptors on cocaine sensitization and conditioned locomotion on maLPA$_1$ null mice. In any case, there are glutamatergic projections from the hippocampus to the NAc, which regulate DA transmission in this structure. Specifically, the ventral hippocampal glutamatergic neurons project to the NAc shell region, whereas the dorsal hippocampus sends glutamatergic projections to the NAc core region. Whether the changes in GLUR3 expression observed in the hippocampus of sensitised maLPA$_1$ null mice are the basis of the lack of CL through the regulation of NAc physiology remains to be determined.

NMDA receptor-mediated glutamate transmission is required for neuronal plasticity and brain reward in cocaine-addiction mechanism. In the present study, we showed a decrease on gene expression of NR1 after chronic cocaine pre-treatment but not of different subunits of the glutamate ionotropic NMDA receptor (NR2A-B-C), independently of genotype. These results are consistent with current literature because NR1 knockdown mice has showed an attenuation of sensitization and CPP response induced by cocaine (Ramsey et al. 2008), but this fact could be influenced by the accurate location at which NMDA receptors are decisive and appears not to be limited to

Table 7 Statistical analysis of the expression of NMDA NR1, AMPA phosphor-GLUR1, and AMPA GLUR3 proteins in the dorsal hippocampus (two-way ANOVA show differences in between genotypes for GLUR3 receptors and for cocaine versus vehicle treatment for NR1 and GLUR3 receptors)

	ANOVA results: statistical analysis of NMDAR1, phospho-GluR1, and GluR3 protein expression in the hippocampus of ma-LPA$_1$ null mice by Western blotting (target protein/beta-actin)		
	NMDAR1	Phospho-GluR1	GluR3
Genotype	F[1,12]=0.24; p=0.634	F[1,12]=0.59; p=0.4589	F[1,12]=5.88; p=0.032
Treatment	F[1,12]=8.05; p=0.015	F[1,12]=2.71; p=0.1254	F[1,12]=5.19; p=0.0418
Interaction	F[1,12]=0.01; p=0.933	F[1,12]=3.08; p=0.104	F[1,12]=3.15; p=0.101
	Cocaine effect		Genotype effect
			Cocaine effect

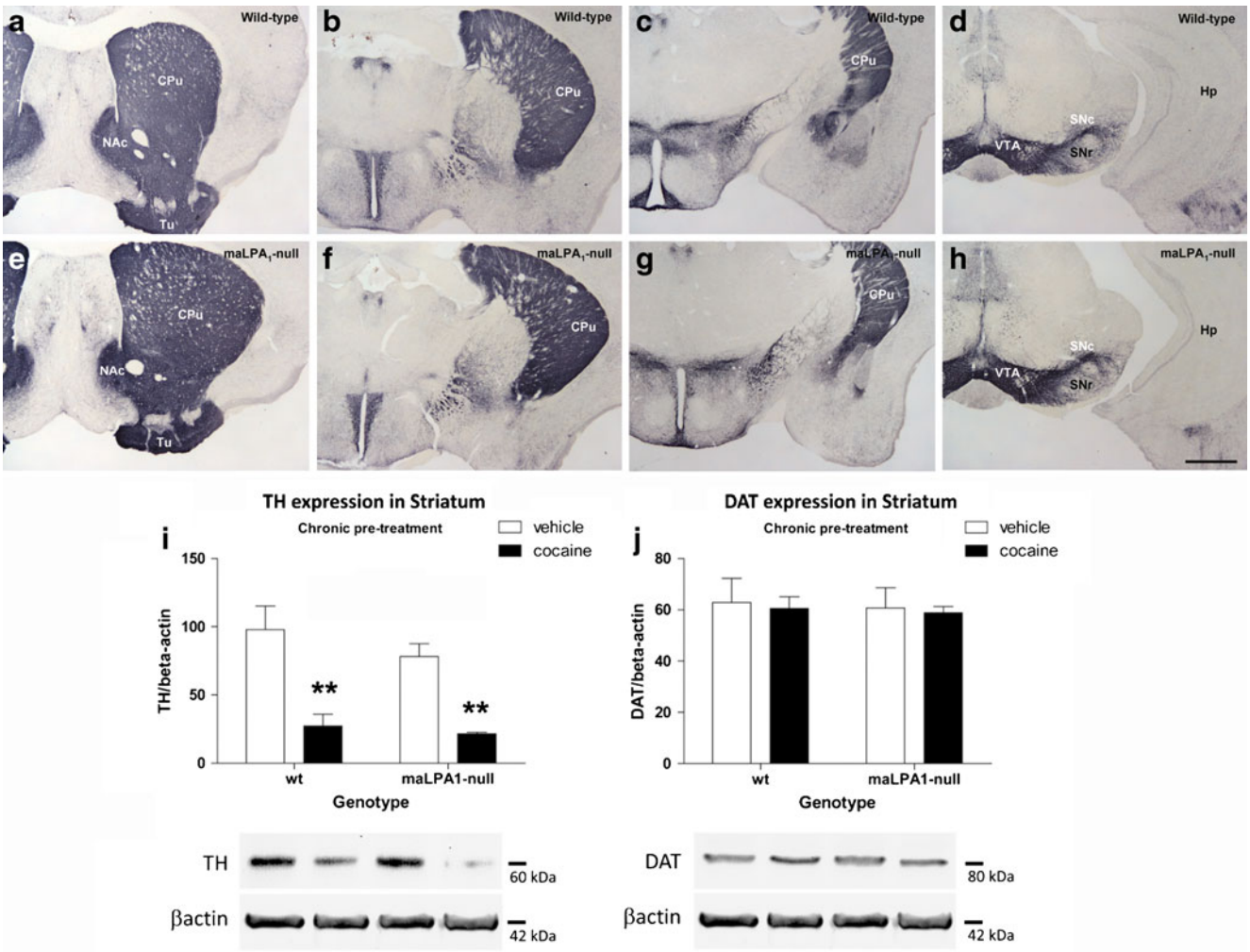


Fig. 5 **a** TH-positive cells at different coronal brain slices using TH immunohistochemistry in wild-type and maLPA$_1$-null mice. TH immunoreactivity expression did not show qualitative differences between both genotypes in caudate putamen (CPu), nucleus accumbens (NAc), substantia nigra pars reticulata (SNr), substantia nigra pars compacta (SNc), hippocampus (Hp), and olfactory tubercle (Tu). Approximate distances from bregma are level A +0.62 mm, level B −5.8 mm, level C −1.58 mm, and level D −3.40 mm. *Scale bar*=100 μm. **b** Quantitative analysis of protein levels in the striatum after chronic pre-treatment with vehicle and cocaine in wild-type and maLPA$_1$-null mice. Analysis of protein levels of TH (AA) and DAT (BB) using Western blot analysis. Protein levels were normalised to beta-actin and values are shown as mean optical density±SEM. TH protein levels shows a significant difference due to factor treatment, but not in DAT protein levels

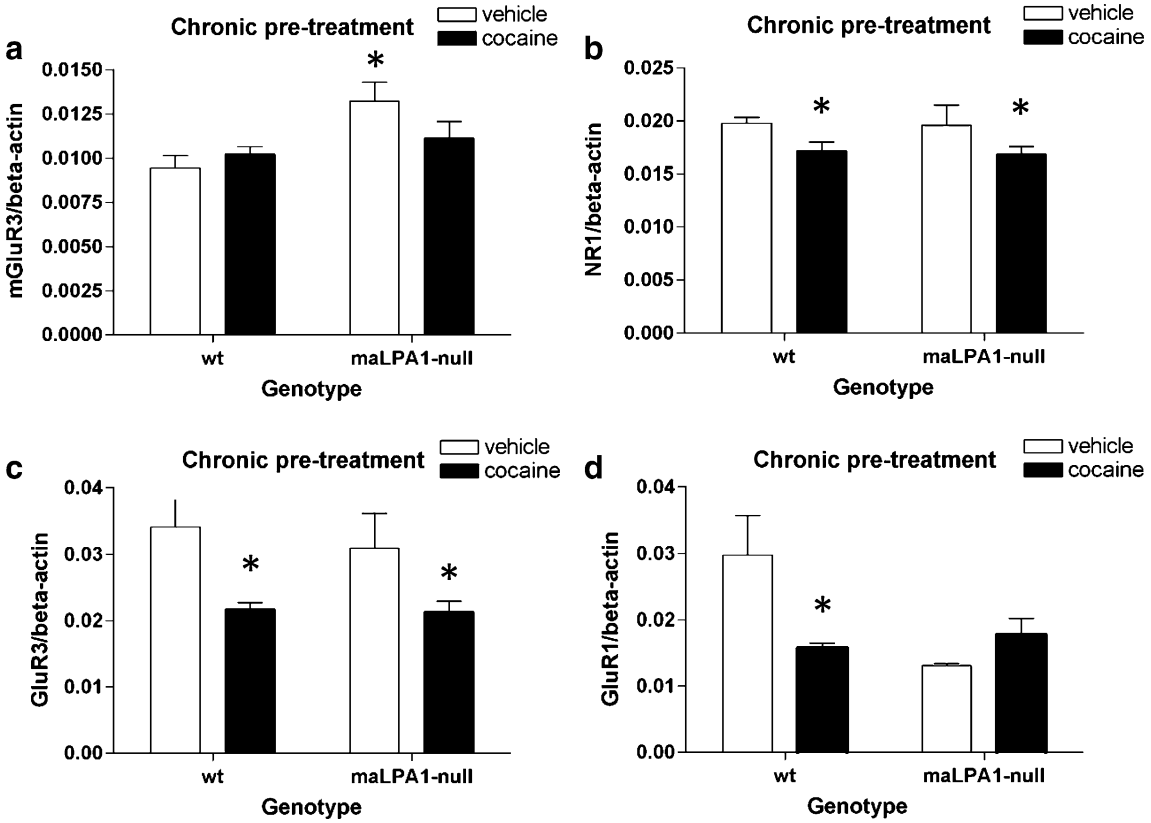


Fig. 6 **a**–**d** Quantitative real-time PCR analysis of mGluR3, NR1, GluR3, and GluR1 mRNA expression normalised to the levels of β-actin mRNA in hippocampus. Each value corresponds to mRNA levels in wild-type and maLPA$_1$-null mice after chronic pre-treatment (conditioning with vehicle or cocaine for 5 days following CL). maLPA$_1$-null mice showed an enhancement on mGluR3 expression depends on genotype factor (**a**), while that chronic pre-treatment induced a decrease on NR1 and GluR3 expression in both genotypes (**b**–**c**). Furthermore, we found differences on GluR1 expression by interaction effect of treatment in maLPA$_1$-null mice (**d**). Statistical significance is indicated by *$p<0.05$. *Error bars* indicate SEM

dopamine neurons themselves. Mice with specific inactivation of NR1 in dopaminergic neurons showed no alteration of short-term sensitization but a decreased long-term sensitization (Engblom et al. 2008; Zweifel et al. 2008).

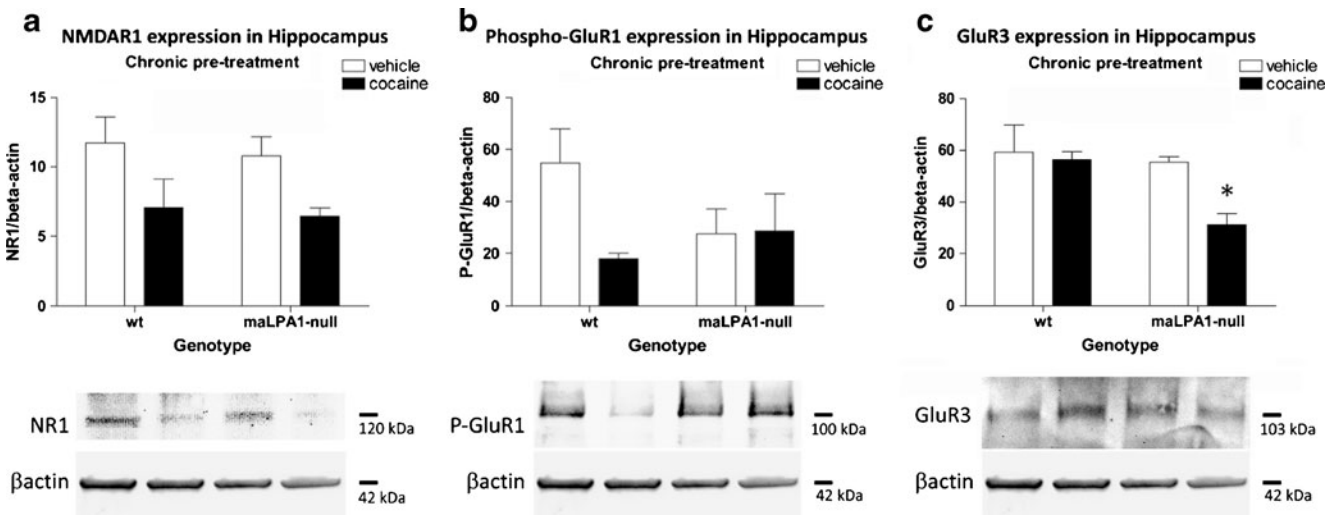


Fig. 7 **a** Quantitative analysis of protein levels in the dorsal hippocampus after chronic pre-treatment with vehicle and cocaine in wild-type and maLPA$_1$-null mice. Analysis of protein levels of NR1 (AA), GluR3 (BB), and Ser845 P-GluR1 (CC) using Western blot analysis. Protein levels were normalised to beta-actin as arbitrary units of optical density (mean±SEM)

The fact that in both genotypes (maLPA$_1$ null mice and wild type), both sensitization and NR1 response of cocaine were identical, further support the role of this receptor in sensitization and separate it from CL responses.

Finally, the only genotypic change in glutamate receptors that appeared in maLPA$_1$ null mice and that was not differentially regulated by cocaine is the mGLUR3 receptor. Altered CL is associated with a significant dysregulation of mGLUR3 mRNA levels in dorsal hippocampus of maLPA$_1$-null mice whose meaning requires further research. Current studies have demonstrated that functional upregulation of mGluR2/3 and downregulation of mGLUR5 could be factors in the transition to dependence in cocaine self-administration with monkeys (Adewale et al. 2006; Bauzo et al. 2009) and rats (Peters and Kalivas 2006). mGLUR2/3 acting as autoreceptors regulate Glu release, and it may be possible that altered glutamate release may be underlying the persistence in locomotion that cocaine-exposed animals display when they are exposed to cocaine-paired environments. And by controlling this glutamate release, mGLUR2/3 may be a relevant participant in the establishment of cocaine-induced CL. Previous work have demonstrated that chronic cocaine injections modulated glutamate release through alteration in mGLUR2/3 function in the NAc (Xi et al. 2002). Furthermore, the effect of a potent mGlu2/3 receptor agonist (LY379268) can reduce the reinstatement of drug seeking induced by stimuli conditioned to cocaine, although it was relatively ineffective in antagonizing the reinforcing effects of cocaine during self-administration (Baptista et al. 2004). Others results have showed effects opposite to those induced by pharmacological stimulation of group II mGluRs. Knockout mice lacking the mGluR2 subtype exhibited enhanced cocaine-sensitization and CPP compared with wild-type animals (Morishima et al. 2005). Many of these results only focus on the effects of cocaine in NAc, but the role of these receptors in the contextual/hippocampal expression of cocaine's reinforcing is still largely unknown.

In conclusion, the impairment of cocaine-induced CL showed by maLPA$_1$-null mice cannot be explained by alterations of the expression of genes related to dopaminergic transmission on striatal system. However, the alterations of metabotropic glutamatergic receptor, mGluR3, in maLPA$_1$-null mice and AMPA receptors (GLUR3) after chronic cocaine pre-treatment in dorsal hippocampus could explain these disturbances. Thus, we propose that LPA1 receptors may act as functional modulator of conditioned responses to cocaine through its regulation of glutamatergic transmission.

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