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Loss of limbic system-associated membrane protein leads to reduced hippocampal mineralocorticoid receptor expression, impaired synaptic plasticity and spatial memory deficit

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Abstract

Background—The limbic system-associated membrane protein (LAMP) promotes development of neurons of limbic origin. We have previously shown that genetic deletion of LAMP results in heightened reactivity to novelty and reduced anxiety-like behaviors in mice. Here, we demonstrate a critical role of LAMP in hippocampal-dependent synaptic physiology and behavior.

Methods—We tested spatial memory performance, hippocampal synaptic plasticity and stress-related modalities in *Lsamp*$^{-/-}$ mice and their littermate controls.

Results—*Lsamp*$^{-/-}$ mice exhibit a pronounced deficit in spatial memory acquisition and poorly sustained CA1 long-term potentiation (LTP). We found reduced expression of mineralocorticoid receptor (MR) transcripts in the hippocampus, and reduction in the corticosterone-induced, MR-mediated non-genomic modulatory effects on CA1 synaptic transmission. Importantly, the impaired LTP in *Lsamp*$^{-/-}$ mice can be rescued by stress-like levels of corticosterone in a MR-dependent manner.

Conclusions—Our study reveals a novel functional relationship between a cell adhesion molecule enriched in developing limbic circuits, glucocorticoid receptors and cognitive functioning.

Keywords

limbic system-associated membrane protein; mineralocorticoid receptor; synaptic plasticity; spatial memory; stress

The limbic system-associated membrane protein (LAMP) is a neuronal surface glycoprotein expressed across mammalian species in limbic regions, areas subserving cognition, learning

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and memory, and autonomic behaviors (1–3). Regulation of LAMP expression during embryogenesis reflects neuronal specification, adoption of a limbic fate, and formation of proper circuits of the limbic pathway (4–6).

Currently, it remains largely unknown how LAMP affects limbic function in the postnatal brain. To address this question, we have recently generated a mouse line carrying a targeted disruption of the *Lsamp* locus and reported that *Lsamp*$^{-/-}$ mice are viable and showed no gross neuroanatomical or sensory and motor abnormalities (7). However, *Lsamp*$^{-/-}$ mice exhibited a heightened reactivity to novelty and mitigated anxiety-like behaviors (7). This underscores the possibility that loss of LAMP may cause circumscribed changes in the fine connectivity of limbic circuits that leads to a maladaptive response to novel environmental stressors, and is consistent with previous studies showing reduced levels of limbic *Lsamp* expression in rodents that exhibit dampened stress responsiveness and a “non-anxious” phenotype (8,9). Intriguingly, recent human genetic studies implicate allelic polymorphisms in *LSAMP* are associated with mood dysfunction, including panic disorder (10,11) and male suicide (12). These behavioral phenotypes prompted us to further examine potential alterations in functional outputs of the limbic system (i.e. hippocampal synaptic plasticity and spatial memory formation) and its stress-related modalities.

It has been well established that circulating stress hormones, such as endogenous corticosterone (CORT) in rodents, can be sensed by limbic structures, including the hippocampus, and shape limbic functional output and animal behavioral adaptation (13–16). CORT binds to two subtypes ligand-induced nuclear transcription factors, the high-affinity mineralocorticoid receptor (MR), which is restricted to the limbic areas; and the more ubiquitously distributed lower-affinity glucocorticoid receptor (GR), thus transcriptionally regulates responsive genes in the rodent hippocampus (15,17–19). Recently, a membrane-bound form of the MR has been identified and shown to mediate fast, non-genomic events at hippocampal synapses (20). These membrane MRs have been proposed to act as “stress sensors” by engaging the hippocampus in the behavioral responses to stress through amplifying the enhanced excitability induced by other stress hormones (21,22).

Given the fact that *Lsamp* and MR transcripts are particularly heavily expressed in the developing and postnatal hippocampus (6,16), we hypothesized a functional connection for LAMP and MR proteins in stress response and hippocampal-dependent synaptic physiology and behavior. Consequently, loss of LAMP may lead to functional abnormalities in the memory- and stress-regulating limbic circuitry, particularly the hippocampus, which could underlie the altered responsiveness to novelty and reduced anxiety-like behaviors.

Methods and Materials

Experimental Animals

The mouse line carrying a targeted disruption of the *Lsamp* locus has been described previously (7). All mice used in these studies are males (30–60 days for electrophysiology experiments and 90–180 days for behavioral tests) and were backcrossed for more than ten generations onto C57BL/6J background. *Lsamp*$^{-/-}$ and wild type (WT) mice were obtained by heterozygous breeding. Animal care and experimental protocols were approved by the Vanderbilt University Institutional Animal Care and Use Committee.

Morris water maze test

Standard Morris water maze test was performed to evaluate the spatial memory performances in mice, essentially as described previously (23,24) (Supplement 1). Briefly, mice were placed in a circular pool filled with opaque water in a room with prominent ambient visual cues, and were trained (four trials per day with an inter-trial interval of 1 h

for 7 consecutive days) to find a submerged platform. The latency to reach the platform, distance traveled, swim speed and time spent in each of four quadrants were obtained using an automated video tracking system (HSV image). A probe trial was conducted on day 8 in which the hidden platform was removed and the percent time spent in the target quadrant and the number of platform location crossings was recorded.

In situ hybridization, immunocytochemistry and Western blot analysis

Levels of MR and GR mRNA transcripts were measured as described previously (25). Coronal brain sections (20μm) were mounted on slides, fixed in 4% paraformaldehyde, rinsed with 2 × SSC and dehydrated in ethanol series. Antisense cRNA probes (mouse, exon 2 coding region) for GR (26) and MR (15) were transcribed from linearized plasmids. *In situ* hybridization was performed using ^{35}S-UTP-labeled probes following standard protocols (see Supplement 1).

For immunocytochemistry analysis, E17 embryonic hippocampal neurons were dissociated and cultured essentially as previously described (27). At 14 days *in vitro* (DIV), neurons were fixed and stained with monoclonal antibody rMR1-18 that specifically recognizes the MR (28). Western blot experiments were conducted to assess the gross chemical contents of synaptic structure with specific antibodies. To examine the overall levels of MR as well as their subcellular distribution, we made several preparations to obtain cytosolic, purified crude hippocampal membranes and synaptosome fractions from wild type mice according to standard protocols using sucrose gradient ultra-centrifugation (29,30). The proteins obtained through subcellular fractionation were separated by SDS-PAGE and probed with monoclonal antibody rMR1-18 (for details, see Supplement 1).

Electrophysiology

Both extracellular field potentials and whole cell recordings were done in hippocampal slices prepared from 6–10 weeks old mice, as described previously (23,27) (see Supplement 1). Briefly, 400 μm coronal slices were cut and incubated in 95% O_2/5% CO_2-equilibrated ACSF (containing in mM: 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 1.2 $MgCl_2$, 2.0 $CaCl_2$, and 10 glucose, pH 7.3–7.4). Extracellular field excitatory postsynaptic potentials (fEPSP) were recorded in CA1 *stratum radiatum* using glass micropipettes filled with ACSF. Electrical stimulus (1–15V; 100 μs duration) was delivered to fibers in the *stratum radiatum* near CA3 region. Electrical signals were amplified using a differential amplifier (model 1800, A-M systems), filtered at 1 kHz and digitized at 10 kHz. Tetanus-induced LTP of fEPSP was elicited by two 100 Hz, 1-sec trains applied with a 20 sec interval.

Data analysis

Statistical analysis of data was carried out using GraphPad Prism 4.0 (San Diego, CA) and SPSS 17.0 (Chicago, IL) software, as described in Supplement 1.

Results

Impaired spatial memory performance in *Lsamp*$^{-/-}$ mice

To probe disturbances in learning and memory in *Lsamp*$^{-/-}$ mice, we examine the acquisition of drug conditioned place preference (CPP) and found that *Lsamp*$^{-/-}$ mice showed normal CPP (Figure S1 in Supplement 1). We then probed cognitive functioning by investigating the ability of *Lsamp*$^{-/-}$ mice to perform a conventional spatial memory task. We conducted Morris water maze (MWM) tests to evaluate spatial memory acquisition in both *Lsamp*$^{-/-}$ and WT mice. Mice were trained for 7 consecutive days to locate a submerged hidden platform using distal visual cues. Both WT (n = 9) and *Lsamp*$^{-/-}$ mice (n = 10) showed the expected learning curve and marked improvement in locating the platform

over the training period (Figure 1A). Notably, the *Lsamp*$^{-/-}$ mice showed a delayed learning acquisition curve, requiring significantly more time to locate the hidden platform. These animals exhibited longer latencies and sustained lesser spatial memory of the hidden platform location on days 6 and 7 as shown by the daily mean latency (± SEM, 4 trials per day) compared with the WT littermates (Figure 1A, $F(1,119) = 13.5$; $p < .01$ for the genotype effects, two-way ANOVA with repeated measures).

During the probe trial conducted at day 8, *Lsamp*$^{-/-}$ mice spent significantly less time searching in the target quadrant (TQ) where the hidden platform was placed during the training (Figure 1B, $F(1, 74) = 5.52$, $p < .01$, two-way ANOVA with repeated measures). This was paralleled by a significant decrease in the number of TQ platform crosses compared to WT mice (Figure 1C, $F(1, 74) = 4.28$, $p < .01$, two-way ANOVA with repeated measures). Additionally, there was no significant difference for the averaged number of non-TQ platform crosses in WT and *Lsamp*$^{-/-}$ mice (5.7 ± 0.9 and 6.2 ± 1.5 crosses, respectively, $p > .05$, *t* test); the averaged total number of quadrant crosses does not differ either (10.6 ± 1.6 and 10.9 ± 2.2 crosses, respectively, $p > .05$, *t* test). Although a trend towards more time spent in the TQ is apparent for *Lsamp*$^{-/-}$ mice, there was no overall significant difference for the time spent in each quadrant (Figure 1B, $F(3,74) = 1.89$, $p = .11$). Notably, no genotypic effect on the total distance traveled was detected (Figure 1D, $F(1,119) = 2.33$; $p > .05$; two-way ANOVA with repeated measures), indicating motor dysfunction is not likely a confounding factor. Together these findings indicate an inappropriate search strategy that could be attributed to a cognitive defect in *Lsamp*$^{-/-}$ mice.

Lsamp$^{-/-}$ mice exhibit impaired synaptic plasticity in the hippocampus

As a physiological correlate to the behavioral findings, we conducted electrophysiological assessment at the Schaffer collateral-CA1 synapses. fEPSPs in the CA1 *stratumradiatum* layer were recorded in response to electric stimulation of the Schaffer collateral pathway. An input-output curve was constructed for fEPSP slope as a function of input fiber volley. Two-way ANOVA analysis with repeated measures revealed no significant difference for genotypic effects (Figure 2A, $F(1, 19) = 1.21$, $p = .2$), indicating basal synaptic transmission is normal in *Lsamp*$^{-/-}$ mice. In comparison, when paired pulse stimulation was used to assess potential alternations in presynaptic functions, *Lsamp*$^{-/-}$ slices showed a significantly reduction in the paired pulse ratio at 60 ms inter-pulse interval (Figure 2B, P2/P1 percent ratio: *Lsamp*$^{-/-}$, 138 ± 4.1, WT, 149 ± 4.2; repeated measures ANOVA/Bonferroni *post hoc* test, $p < .05$), suggesting that loss of LAMP may affect presynaptic mechanisms of synaptic transmission.

We next examined whether loss of LAMP could result in changes of CA1 LTP, a standard form of plasticity and a putative cellular model for learning and memory (31–33). We recorded LTP in two sets of experiments in which evoked fEPSP and whole cell EPSC (eEPSC) were measured separately. We found that LTP of fEPSP in *Lsamp*$^{-/-}$ slices was significantly reduced compared with that in WT slices (Figure 2C). This reduction of LTP is particularly prominent at 30 min after LTP induction by two trains of tetanic stimuli (averaged mean fEPSP slopes during 50–60 min post tetanus: WT, 142 ± 3.6%; *Lsamp*$^{-/-}$, 123 ± 4.1%, $t(19) = 8.4$, $p < .01$; $n = 10$). We next conducted whole cell recording from CA1 neurons and tested LTP of eEPSC induced by theta-burst stimulus, which is considered a more physiological stimulation protocol. We found that while the early phase of LTP was largely unaltered, there was a consistent reduction in the late phase LTP (averaged mean eEPSC percentage during 30–40 min post tetanus: WT, 149 ± 2.6%; *Lsamp*$^{-/-}$, 127 ± 6.1%; $t(15) = 7.3$, $p < .001$; $n = 8$, Figure 2D). These data suggest that loss of LAMP results in altered synaptic transmission and impaired plasticity in adult hippocampus.

Lsamp deletion results in altered MR-mediated non-genomic modulation on synaptic transmission in CA1

Given the fact that MR and GR are known to mediate stress responses and to differentially affect synaptic plasticity, memory, and behavior (16,34–36), we hypothesized that changes of MR/GR mediated signaling may contribute to the altered synaptic plasticity in CA1. We first examined levels of total MR and GR protein in homogenized hippocampal tissues using Western blots and found no significant difference between *Lsamp*$^{-/-}$ mice and their littermate controls (n = 4, data not shown). We next tested levels of MR/GR mRNA transcripts in WT and *Lsamp*$^{-/-}$ mice using *in situ* hybridization. Strong hybridization signals for both MR and GR transcripts were localized to the hippocampus proper and dentate gyrus in adult mice (8–12 weeks of age, n = 6, Figure 3A). Densitometry analysis revealed that the low affinity GR transcripts were not changed in the *Lsamp*$^{-/-}$ animals compared with those from their WT littermates in the hippocampus (for CA1, $t(11)=1.22$, $p = .85$). In contrast, there was a significant reduction of MR transcripts in all subfields of *Lsamp*$^{-/-}$ hippocampus (Figure 3A; for CA1, $t(11)=3.98$, $p < .01$). This MR-specific difference was revealed as early as postnatal day 6 (Figure 3B, $t(11)= 3.27$, $p < .01$), suggesting a developmental origin due to the lack of LAMP.

The altered expression of MR, but not GR, posed a potentially novel mechanism of altered hippocampus-dependent memory and synaptic plasticity in the *Lsamp*$^{-/-}$ mice. While MR acts primarily as a nuclear transcriptional regulator upon ligand binding, recent studies have revealed a fast, non-genomic action that is associated with a putative membrane-bound MR at synaptic sites (20,37,38). We therefore first tested whether this presynaptic MR component could be resolved using immunocytochemical labeling in cultured low-density hippocampal neurons. We found that as expected, the majority MR signals exists in the nucleus, cytosol, and to a lesser extend in the primary dendritic locations. However, very low MR signal exists that shows colocalization with the presynaptic marker synapsin I (Figure 3C). We next tried to detect MR in the synaptic compartments of hippocampal tissues using subcellular fractioning followed by Western blots. We found that MR protein can be detected in cytosolic, crude membrane and purified synaptosome fractions from WT hippocampus (Figure 3D). Therefore, although MRs associated with *synaptic* membrane could not be resolved, the fact that MR exists in the purified synaptosomes suggests that a pool of MR is physically situated in modulating synaptic function, and is consistent with the notion of synaptic membrane-associated MRs (20).

We next conducted electrophysiological measurements to assess the non-genomic, MR-mediated synaptic functions in WT and *Lsamp*$^{-/-}$ hippocampal slices. Using a CORT concentration selective for prominent membrane MR occupancy (100 nM) (20) (Supplement 1), we measured the effects of CORT on spontaneous mEPSCs in CA1 neurons. We found that while bath application of CORT did not affect mEPSC amplitude and kinetics, it increased mEPSC frequency within 5 min in both WT and *Lsamp*$^{-/-}$ CA1 neurons, but to a differential degree (Figure 4A). There was an average 89 ± 12% of increase of mEPSC frequency in WT neurons (n = 9), which is significantly higher than that obtained from *Lsamp*$^{-/-}$ neurons (33 ± 8% increase, $t(20) = 4.35$, $p < .001$). Cumulative plot of the inter-event intervals from these cells revealed that CORT acts by shifting the plot to the left; yet this occurred to a lesser extent in *Lsamp*$^{-/-}$ neurons (Figure 4B; effect of CORT treatment, $p = .004$ for WT neurons, and $p = .03$ for *Lsamp*$^{-/-}$ neurons, Kolmogorov-Smirnov test). Importantly, the facilitatory effects of CORT on mEPSC were completely blocked by the MR-specific antagonist spironolactone (100 nM) in both WT and *Lsamp*$^{-/-}$ mice, confirming that the responses were indeed MR-mediated (Figure 4B, $p > .05$, for both WT or *Lsamp*$^{-/-}$ cells, compared between spironolactone treated and un-treated groups in the presence of CORT; Kolmogorov-Smirnov test). Therefore, consistent with reduced levels of

the MR transcripts, CORT-induced fast non-genomic facilitation of mEPSC frequency is significantly diminished in *Lsamp*$^{-/-}$ hippocampal neurons.

Enhanced MR signaling rescues the LTP deficit in *Lsamp*$^{-/-}$ mice

Given the established genomic roles of MR in hippocampal plasticity (14,16,34,39) and the fact that *Lsamp*$^{-/-}$ mice showed impaired LTP and reduced MR transcript expression, we hypothesized that reduced MR-mediated genomic signaling could partially underlie the mechanisms of impaired LTP in the hippocampus from *Lsamp*$^{-/-}$ animals. Consequently, stimulation of MR signaling might partially rescue the LTP deficits. We therefore perfused *Lsamp*$^{-/-}$ slices with 100 nM CORT for 20 min in the absence or presence of 100 nM spironolactone before eEPSC-LTP induction using the same theta burst protocol. Indeed, compared with vehicle perfusion, CORT application significantly enhanced LTP and largely abolished the LTP deficit observed in *Lsamp*$^{-/-}$ slices (Figure 5A, $F(2,147) = 11.6$, $p < .01$ for treatment effects). Importantly, this effect of CORT was abolished when slices were co-perfused with spironolactone (Figure 5A; $F(2,147) = 2.13$, $p = .12$ compared with vehicle treatment, two way ANOVA/Bonferroni post hoc tests). These data indicate that increased stimulation of MR-mediated genomic signaling at least partially rescues the LTP deficits in *Lsamp*$^{-/-}$ slices.

Because CORT at concentration tested (100 nM) is not selective for MRs and is known to impair CA1 LTP in WT animals (39,40), we tested effects of CORT on CA1 LTP in the WT littermates. Consistent with previous reports (34,41,42) and in striking contrast with *Lsamp*$^{-/-}$ animals, CORT application significantly reduced the amplitude of LTP in WT slices (Figure 5B, $F(2,131) = 22.3$, $p < .001$ for treatment effects). Furthermore, this effect is not blocked by 100 nM spironolactone (Figure 5B; $F(2,131) = 2.24$, $p = .12$, compared with vehicle treatment, two-way ANOVA). Therefore, we conclude that the impaired synaptic plasticity in *Lsamp*$^{-/-}$ mice involves a mechanism related to reduced levels of MR-mediated genomic signaling events.

Discussion

LAMP serves as an important mediator for axonal growth and patterning during limbic development (4–6,43). We have recently reported that *Lsamp*$^{-/-}$ mice exhibit heightened reactivity to novelty and reduced anxiety-like behaviors without disruption of basic sensory or motor behaviors, indicative of subtle changes related to the limbic functional throughput (7). The present study provides further evidence for the role of LAMP in cognitive functions by showing that genetic disruption of the *Lsamp* locus alters the readout of limbic circuitry in the adult brain. Specifically, *Lsamp*$^{-/-}$ mice exhibited deficits in spatial memory and hippocampal synaptic plasticity. *Lsamp*$^{-/-}$ mice had a profound deficit in MWM test, which measures hippocampus-dependent spatial memory acquisition (44). This impaired spatial learning is consistent with the electrophysiological finding that *Lsamp*$^{-/-}$ hippocampal slices exhibit normal basal synaptic transmission but impaired LTP, a cellular model for learning and memory behavior (31,32). Although a heightened response to novel, stressful situations was observed in our previous study (7) and could affect the platform search strategy, the lack of changes in prepulse inhibition and hyperactivity, combined with normal sensory and motor function in *Lsamp*$^{-/-}$ mice (7), makes it unlikely that the MWM deficits observed in this study are due to the issue of response to novelty. Furthermore, the mutant mice display a typical learning curve during training but manifest specific differences in target quadrant percentages and platform crossings in the probe trials. Overall, these behavioral results suggest that *Lsamp*$^{-/-}$ mice are capable of learning, but show significant defects in spatial memory acquisition and retention.

Our study has revealed a novel, selective and secondary effect of *Lsamp* genetic deletion on molecular elements of the central stress response, which could potentially account for the altered responses to environmental stressors observed previously (7). We focused our studies on the central steroid receptors in *Lsamp*$^{-/-}$ mice for several reasons: first, recent data collected from our laboratory revealed a more rapid rise of plasma CORT levels in response to a novel environmental stressor in *Lsamp*$^{-/-}$ mice, but peaking at the same levels as WT mice (Catania and Levitt, unpublished observations); second, the fact that MR transcripts are selectively expressed in the limbic structures, and show anatomical overlap with LAMP. Both MR and LAMP exist in CA1 synapses and are associated with functional membrane microdomains (6,20,45,46); third, in the light of impaired LTP observed in *Lsamp*$^{-/-}$ animals, both MR and GR are known to differentially affect synaptic plasticity in CA1, and the high affinity MR is capable of modulating CA1 synaptic function through both genomic and non-genomic pathways (20,37,38,40). Lastly, limbic MR deficiency in rodents shares several structural and behavioral features with *Lsamp*$^{-/-}$ mice. For example, *Lsamp*$^{-/-}$ mice exhibit normal motor development, acoustic startle and pre-pulse inhibition (7), which is similar to the normal motor and gated-acoustic reflex responses reported for mice carrying a deletion of limbic MR (16). Limbic MR deletion results in alteration of mossy fiber distribution, impaired water maze and radial maze tasks and hyperactivity toward a novel object (16), which are in striking similarity with *Lsamp*$^{-/-}$ animals exhibiting deficits of LTP and spatial memory, and heightened reactivity in novel situations (6,7, this study). Conversely, selective over-expression of limbic MR enhances new memory consolidation and retrieval, and reduces CORT-mediated anxiety behavior, further substantiating the involvement of MR in both learning and plasticity and stress responses (36,47). These lines of evidence support a strong functional relationship between MR and LAMP. Given the early postnatal reduction of MR transcripts in *Lsamp*$^{-/-}$ mice, this interaction may emerge developmentally. It is notable that since we failed to observe global changes in hippocampal MR proteins, it remains speculative that MR levels at functional subcellular domains may change in *Lsamp*$^{-/-}$ mice. Further analysis is needed to ascertain the specific mechanisms through which MR and LAMP relate to each other.

It has been demonstrated that elevated levels of corticosteroid hormones or exposure to stress impairs LTP and facilitates long-term depression in the hippocampus through differential engagement of MR- or GR-mediated genomic events, which is presumably achieved through receptor nuclear translocation and balanced regulation of genes involved in adaptive stress responses (34,41,48). We have found that *Lsamp*$^{-/-}$ mice exhibit significantly reduced MR transcripts in the hippocampus, whereas the level of GR transcripts was unaltered. Although with the currently available antibodies we were unable to detect changes in total MR protein levels in hippocampal membrane and crude synaptosomal preparations in *Lsamp*$^{-/-}$ mice, it is possible that only MR at distinct functional microdomains of the synapse is affected by loss of LAMP. This is important because such alterations, in combination with lower transcript levels, are likely to also cause a disruption of MR/GR balance, a concept stating that optimal functioning and normal ratio of both receptors are required for maintenance of behavioral homeostasis (22). This scenario is perhaps best reflected by the opposing effects of CORT on CA1 LTP in *Lsamp*$^{-/-}$ and WT mice, that pharmacological activation of MR points to a prominent effect of MR that unconventionally overrides GR activation. This shift in MR/GR balance could explain the failure of high levels of CORT to elicit the normally-observed GR-mediated impairment of LTP, thus resulting in prolonged and prominent MR activation in *Lsamp*$^{-/-}$ mice. Furthermore, reduced MR expression levels and altered membrane MR-mediated events may also impact the formation of MR/GR heterodimers and their related co-factors, thus changing the transcription of certain genes involved in stress recovery processes (22). Therefore, it is possible that *Lsamp*$^{-/-}$ mice might be spared from the impairing effects of stress-like levels of CORT on LTP via enhanced MR-mediated genomic events. It is

noteworthy that this phenomenon is not unprecedented. Similar effects of high CORT on LTP have been observed previously in animals that received low amount of maternal care in early-life, and expressed lower levels of hippocampal MRs (49).

Consistent with previous reports on the MR-mediated non-genomic roles (20,37,38), our study supports a role for MR in facilitating mEPSC frequency at the Schaffer collateral-CA1 synapse through a fast pre-synaptic mechanism. We found that the level of MR transcripts is associated with differential enhancement of mEPSC frequency following MR activation. These results, combined with the fact that enhanced MR-mediated signaling overcomes the LTP deficits observed in *Lsamp*$^{-/-}$ mice, underscore the functional diversity of MR signaling in regulating hippocampal synaptic physiology and behavior through both genomic and non-genomic processes. Additionally, the data presented here suggest that developmental alterations in limbic circuits, even subtle, may lead to impaired 'stress sensing' capacity and behavioral outcome. Such *Lsamp* deficiency-induced defect is likely to bear significant consequences on neuronal excitability in situation of stress, especially during the appraisal of novel situations, thus affecting behavioral responses even in the presence of sufficient endocrine signals (i.e. heightened CORT levels). The present data suggest that the 'stress sensing' ability of *Lsamp*$^{-/-}$ mice is dampened, a finding that is in agreement with previous reports showing altered behavioral reactivity and 'fearless' phenotype upon exposure to novel (stressful/anxiety-evoking) environment in *Lsamp*$^{-/-}$ mice (7,8) and *Lsamp* low-expressor rats (9). Perhaps more intriguingly, considering recent reports on the genetic association of *LSAMP* with human psychiatric conditions such as mood and anxiety conditions (11), panic disorder (10), male suicide behavior (12), and schizophrenia (50), future studies focusing on CNS steroid receptors and 'stress sensing' mechanisms could be potentially illuminating in deciphering the etiology of these disorders. Therefore, although a physical molecular link between LAMP and MR remains to be established, we have revealed a novel functional connection between these two proteins of limbic origin that may cooperate developmentally in shaping key behavioral outputs such as emotional regulation and learning and memory.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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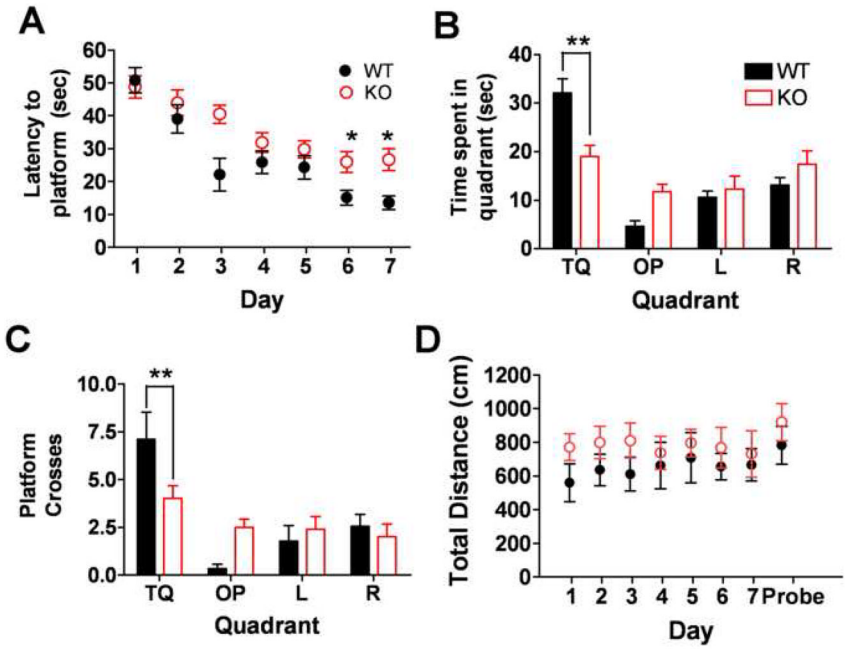

Figure 1.

Lsamp$^{-/-}$ mice exhibit impaired spatial learning as revealed by MWM task. **A.** Wild type (WT) littermate control mice (n = 9) take significantly less time to learn the location of the hidden platform as shown by the daily mean latency (± SEM) compared to *Lsamp*$^{-/-}$ (KO) mice (● n = 10, $p < 0.01$ for genotype effects, two-way ANOVA) and achieve better spatial memory performance of the task on days 6 and 7 (* $p < 0.05$, repeated measures two-way ANOVA/Bonferroni *post hoc* tests). **B.** *Lsamp*$^{-/-}$ mice spend significantly less time in the target quadrant (TQ, ** $p < 0.01$, repeated measures two-way ANOVA/Bonferroni *post hoc* tests) during the probe trial. **C.** WT mice exhibit a significant increase in the number of TQ platform crosses compared to *Lsamp*$^{-/-}$ mice. (** $p < 0.01$; Quadrants: TQ, target; OP, opposite; L, left; R, right). **D.** No overall significance was observed on the swimming distance between *Lsamp*$^{-/-}$ and WT mice ($p > 0.05$ for genotype effect, repeated measures two-way ANOVA).

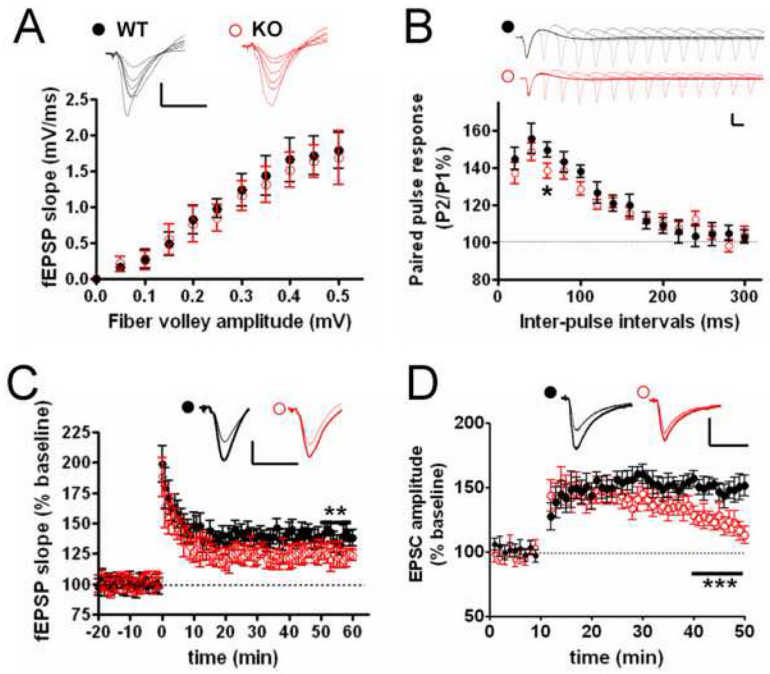

Figure 2.

Loss of LAMP impairs CA1 synaptic plasticity. **A.** Input-output curves for WT and *Lsamp*$^{-/-}$ (KO) slices revealed no significant difference in basal synaptic transmission (two-way ANOVA, $p = 0.2$ for genotype effect). **B.** Paired pulse responses of fEPSPs at inter-pulse intervals ranging from 20–300ms. Reduced paired pulse ratio was observed in *Lsamp*$^{-/-}$ slices at 60 ms inter-pulse interval (* $p < 0.05$, repeated measures ANOVA). **C.** Impaired LTP of fEPSP in CA1 synapses in *Lsamp*$^{-/-}$ slices. The amplitude of potentiation is significantly lower after 30 min post-LTP induction compared with that from WT slices (** $p < 0.01$, *t* test). **D.** LTP of theta burst-evoked whole cell EPSCs is also significantly reduced in *Lsamp*$^{-/-}$ neurons (*** $p < 0.001$, *t* test).

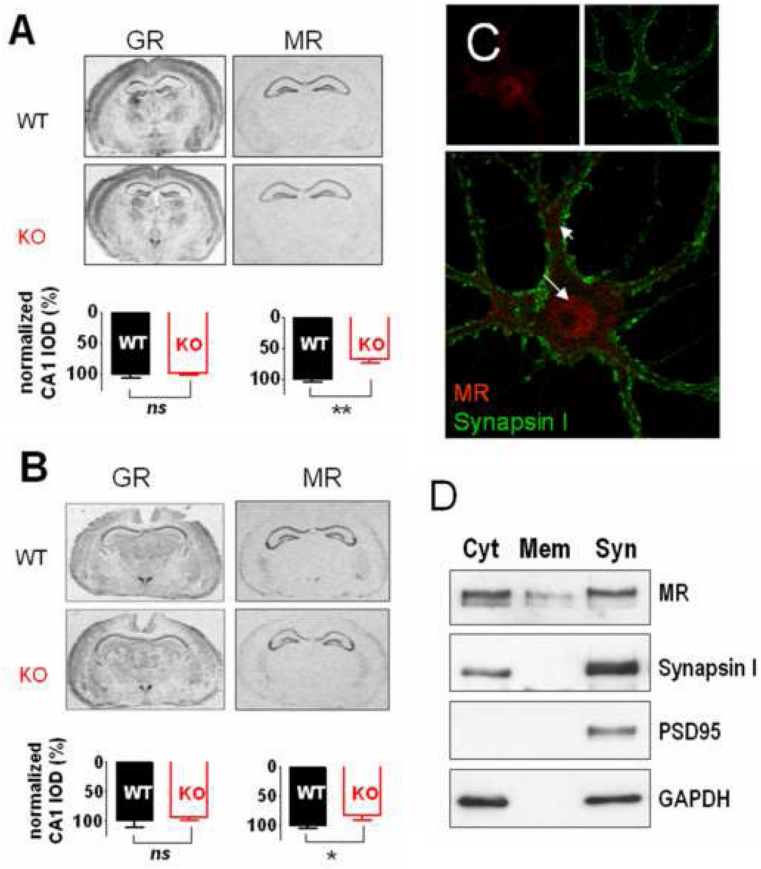

Figure 3.

Reduced MR transcripts and MR-induced non-genomic modulation of synaptic transmission in *Lsamp*$^{-/-}$ mice. **A.** *In situ* hybridization of GR and MR mRNA transcripts in adult (8–12 week) WT and *Lsamp*$^{-/-}$ (KO) mice. Quantification of CA1 hybridization signal revealed a significant difference in MR (** $p < 0.01$, *t* test) but not in GR (*ns*, $p > 0.05$). **B.** Quantitative differences in MR *in situ* hybridization signals are revealed in early postnatal stages (P6). While the IOD of GR transcripts in CA1 area is not changed, there is a significant reduction of MR transcript signal in CA1 in postnatal day 6 *Lsamp*$^{-/-}$ mice (*$p < 0.05$; non-paired *t* test, $n = 3$). **C.** Potential subcellular MR distribution in low density cultures of hippocampal neurons (DIV 21). MR immunoreactivity is localized primarily in the nucleus (*arrow*) and proximal dendrites (*arrowhead*); no visible signal was detected in the presynaptic area marked by synapsin I (*green* puncta). **D.** Subcellular fractionation reveals strong MR immunoreactivity in the hippocampal cytosol (*Cyt*), but also can be detected in the purified membrane (*Mem*) and synaptosomal (*Syn*) fractions (20 μg protein each lane). Absence of signal for synapsin I, PSD95 and GAPDH indicates that MR immunoreactivity detected in the purified hippocampal membrane was not due to contamination.

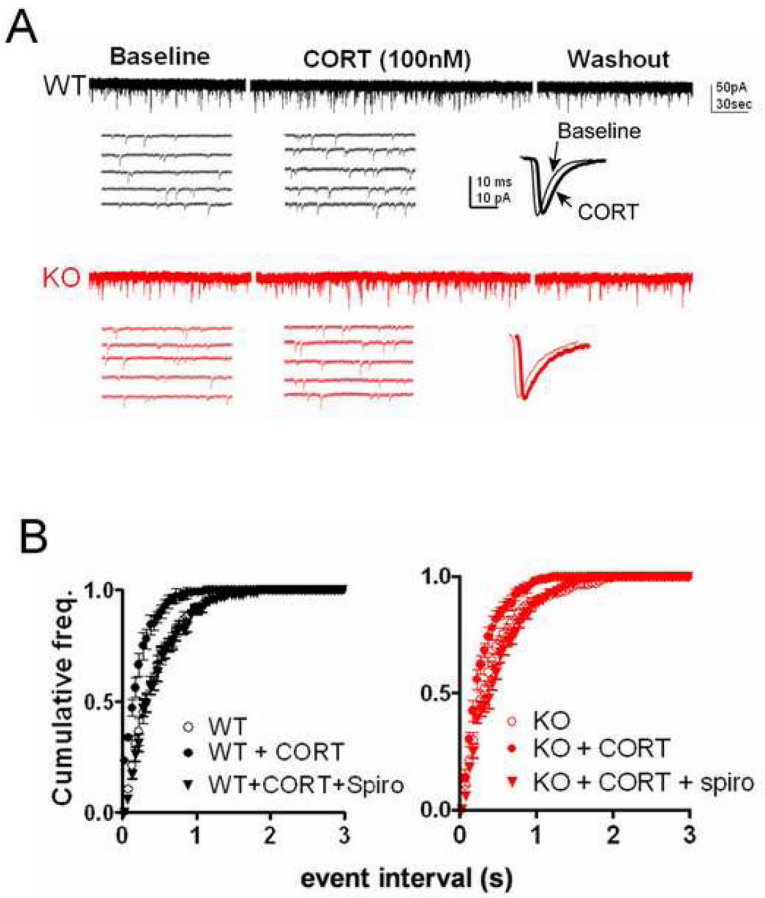

Figure 4.

Differential CORT-induced increase of synaptic activity in *Lsamp*$^{-/-}$ animals. **A.** CORT (100 nM) rapidly and reversibly increases mEPSCs frequency in CA1 neurons in WT and in *Lsamp*$^{-/-}$ animals but to a differential degree. No change of mEPSC amplitudes and their rise (τ_1) and decay (τ_2) time constants is observed following CORT application (τ_1: baseline, 1.34 ± 0.08 ms; CORT, 1.37 ± 0.06 ms. τ_2: baseline, 6.20 ± 0.23 ms; 6.15 ± 0.18 ms). **B.** CORT perfusion shifts the cumulative plot of the mEPSC inter-event intervals leftward in both genotypes but in a quantitatively different manner (effect of CORT treatment, p = 0.004 for WT neurons, and p = 0.03 for *Lsamp*$^{-/-}$ neurons, non-parametric Kolmogorov-Smirnov z test for two samples). This effect is blocked by co-application of 100 nM MR specific antagonist spironolactone (spiro).

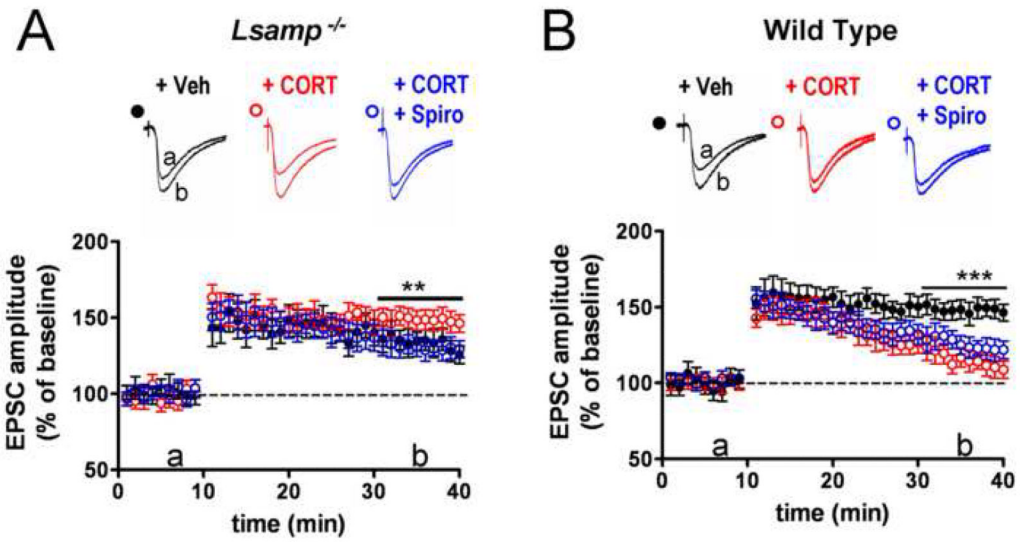

Figure 5.

Rescue of LTP deficits in *Lsamp*$^{-/-}$ (KO) hippocampal slices by elevated MR signaling. **A.** A 20-min perfusion (10 min plotted) with 100 nM CORT in the *Lsamp*$^{-/-}$ slices prior to LTP induction rescues the late-phase LTP (** p<0.001, compared with CORT and vehicle groups, repeated measures ANOVA/Bonferroni *post hoc* test). CORT was present throughout the entire recording duration. Co-application of CORT with MR specific antagonist spironolactone (spiro, 100nM) failed to rescue LTP deficits observed in *Lsamp*$^{-/-}$ slices. **B.** Contrasting responses to CORT application in WT slices. Compared with vehicle treatment, CORT dramatically reduced LTP amplitude (*** $p < 0.001$, repeated measures ANOVA/Bonferroni *post hoc* test). This effect is not antagonized by MR antagonist spironolactone.