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Effects of Hippocampal LIMK Inhibition on Memory Acquisition, Consolidation, Retrieval, Reconsolidation, and Extinction

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Abstract Long-lasting changes in dendritic spines provide a physical correlate for memory formation and persistence. LIM kinase (LIMK) plays a critical role in orchestrating dendritic actin dynamics during memory processing, since it is the convergent downstream target of both the Rac1/PAK and RhoA/ROCK pathways that in turn induce cofilin phosphorylation and prevent depolymerization of actin filaments. Here, using a potent LIMK inhibitor (BMS-5), we investigated the role of LIMK activity in the dorsal hippocampus during contextual fear memory in rats. We first found that post-training administration of BMS-5 impaired memory consolidation in a dose-dependent manner. Inhibiting LIMK before training also disrupted memory acquisition. We then demonstrated that hippocampal LIMK activity seems to be critical for memory retrieval and reconsolidation, since both processes were impaired by BMS-5 treatment. Contextual fear memory extinction, however, was not sensitive to the same treatment. In conclusion, our findings demonstrate that hippocampal LIMK activity plays an important role in memory acquisition, consolidation, retrieval, and reconsolidation during contextual fear conditioning.

Keywords Dorsal hippocampus · BMS-5 · Memory · Rats

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Introduction

Dendritic spines are small actin-rich protrusions from neuronal dendrites that comprise post-synaptic sites and receive the majority of excitatory synaptic inputs in the brain [1–5]. Modifications in dendritic spine numbers and morphology are highly dynamic and support several forms of synaptic plasticity, including learning and memory [4, 6–8].

NMDA receptor (NMDAR) activation and Ca^{2+} influx are the two main events that occur at the hippocampal synapses following the induction of long-term potentiation (LTP) and memory acquisition [9–11]. Shortly after a series of essential processes including cytoskeleton rearrangement, AMPA receptor (AMPAR) trafficking and protein synthesis occur in order to induce the establishment of long-term memory (LTM). Importantly, the modulation and stabilization of excitatory transmission—which involves the insertion of glutamate receptors into the post-synaptic membrane—that follow LTP induction or learning are controlled by actin dynamics and changes in dendritic spines [7, 12–14].

Several studies have demonstrated a close relationship between the increase in spine number and spine size after LTP induction [1, 15–17]. In fact, dendritic structural changes followed by LTP (spine enlargement) are induced by cofilin translocation in response to an NMDAR-dependent activity [18]. On the other hand, a decrease in the same dendritic spine

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parameters underlies long-term depression (LTD), which is the main mechanism underpinning spine shrinkage [19, 20].

Currently, the pathways involving the Rho family of small GTPases, such as RhoA, Rac1, and Cdc42, have been carefully studied together with actin dynamics and memory processing [21–24]. Inhibition of the Rho-associated kinase (ROCK) in the lateral amygdala impairs the formation of long- but not short-term fear memory [25].

The p21-activated kinase (PAK), another critical regulator of actin remodeling, which is activated downstream of Rac1 and Cdc42 signaling [26], has been suggested to be crucial in dendritic spine/synapse loss and memory deficits associated with Alzheimer's disease [27, 28]. Recently, the pharmacological activation of Rac1 has been shown to enhance fear extinction memory [29]. Additionally, inhibition of Rac1 in the dorsal hippocampus was able to disrupt contextual fear memory reconsolidation [30].

Both the Rac1/PAK and RhoA/ROCK signaling pathways activate the LIM kinase (LIMK), which is involved in actin dynamics and synaptic plasticity [31, 32]. When activated, LIMK induces actin polymerization through the phosphorylation of cofilin. Specifically, in its unphosphorylated state, cofilin depolymerizes actin, but its depolymerizing activity is inhibited when it is phosphorylated by LIMK [23, 33–35]. Indeed, Rac1/PAK- and RhoA/ROCK-induced cofilin reorganization of actin can be blocked by LIMK inhibition [36, 37]. LIMK knockout mice present markedly diminished levels of phosphorylated cofilin and exhibit several abnormalities in dendritic spine morphology and synaptic transmission [20, 38]. Moreover, they show changes in the LTP profile [38] as well as memory deficits in the Morris water maze and cue fear conditioning [20, 38]. Although this interesting study shed new light on the role of LIMK in memory [20, 38], it is possible that other proteins can compensate for the absence of LIMK in these transgenic mice. Indeed, testicular protein kinase (TESK) can also inactivate cofilin [39]. Also, these LIMK knockout animals present behavioral abnormalities that go beyond mnemonic processes, such as motor changes [40]. Moreover, since these animals do not express LIMK throughout their lives, it is not possible to determine whether the memory effects are related to memory acquisition, consolidation, and/or retrieval deficits. Additionally, we have recently suggested that LTP de-potentiation during memory forgetting rely on spine shrinkage controlled by LIMK activity [41].

Considering (1) the existence of a striking association between synaptic plasticity and dendritic spine remodeling through actin dynamics; (2) actin dynamics are regulated by the Rac1/PAK and RhoA/ROCK pathways; and (3) LIMK is the common convergent downstream enzyme of both pathways: in the present study, we evaluated the effects of hippocampal inhibition of LIMK on memory acquisition, consolidation, retrieval, reconsolidation, and extinction in the contextual fear conditioning in rats.

Methods

Animals

Male Wistar rats (age 2–3 months, weight 290–350 g) from our breeding colony were used. Animals were housed in plastic cages, four to five per cage, with water and food available ad libitum under a 12/12 h light/dark cycle (lights on at 7 a.m.) in a constant temperature of 24 ± 1 °C. All experiments were performed in accordance to the national animal care legislation and guidelines (Brazilian Law 11.794/2008) and approved by the Ethics Committee of the Federal University of Rio Grande do Sul.

Stereotaxic Surgery and Cannula Placement

Rats were deeply anesthetized by an i.p. injection of ketamine/xylazine (75 and 10 mg/kg, respectively) and bilaterally implanted in the brain targeting the dorsal hippocampus, with 27-gauge guide cannula. Coordinates for hippocampus cannulation were anterioposterior (AP) −4.0 mm (from bregma), laterolateral (LL) ±3.0 mm, and dorsoventral (DV) −1.6 mm, positioned just 1.0 mm above the CA1 area of the dorsal hippocampus [42]. Before the behavioral tests, animals were allowed a recovery period of 5–7 days. Following the behavioral experiments, subjects were sacrificed and their brains were dissected and preserved in 10% formaldehyde to verify for cannula position. Only animals with correct cannula placements were included in the statistical analysis.

Drugs and Administration

BMS-5 (Tocris), a potent non-specific LIM kinase inhibitor, was prepared in a vehicle solution (1% DMSO in sterile isotonic saline). At the time of infusion, a 30-gauge infusion needle was fitted into a guide cannula, with its tip protruding 1.0 mm beyond the guide cannula end and aimed at the pyramidal cell layer of CA1 of the dorsal hippocampus. A volume of 1 μl of BMS-5 (20 and 200 μM) or vehicle (DMSO 1%) was bilaterally infused in a time of 90 s. The doses of BMS-5 were based on its IC50 value (provide by the manufacturer) and in vitro studies [43, 44].

Open-Field Test

The OF chamber consisted of a 50-cm high, 60 × 40-cm plywood box with a linoleum floor divided into 12 equal rectangles or "sectors." Animals were exposed for 5 min, and the number of crossings between sectors was registered. The number of crossings was considered a measure of motor performance.

Measurement of Pain Threshold

Animals were placed individually into the conditioning chamber. After a 3 min exposure, animals received a series of 1 s electric foot shocks, starting at an intensity of 0.1 mA. The intensity was increased by 0.1 mA every 30 s between successive stimuli until the rats showed the first sign of pain (jumping or vocalizing), and the corresponding value was defined as the pain threshold.

Contextual Fear Conditioning

The conditioning chamber (context) consisted of an illuminated Plexiglas box (25.0 × 25.0-cm grid of parallel 0.1-cm caliber stainless steel bars spaced 1.0 cm apart). The illumination inside the room was ~400–800 lx. In the conditioning session (training), rats were placed in the chamber for 3 min for habituation and then received two 2 s foot shocks, of 0.5 mA, separated by a 30 s interval. Before returning to their home cages, animals were kept in the conditioning environment for an additional 30 s.

Reactivation session Depending on the experiment performed, subjects were reexposed to the context, 2 days after training without foot shocks, during 7 min to induce memory reconsolidation.

Extinction session Depending on the experiment performed, subjects were reexposed to the context, 2 days after training without foot shocks, during 30 min to induce memory extinction.

Test session Animals were tested for 4 min in the same context.

Behavioral Measurement

Freezing behavior was used as a memory index, being registered using a stopwatch in real time by an experienced observer that was unaware of the experimental conditions. Freezing was defined as total immobilization except for movements required for respiration [45].

Statistical Analysis

Since data from all experimental groups was proven to be both homoscedastic and normally distributed (Kolmogorov-Smirnov test with Lilliefors correction, $P > 0.05$), results were analyzed with dependent and independent t test, one-way ANOVA, or repeated measures ANOVA followed by a Fisher's (LSD) post hoc test for within and between comparisons. Significance level was set at $P < 0.05$.

Results

Hippocampal LIMK-Mediated Signaling Controls Memory Consolidation for Contextual Fear

Immediately after training to fear conditioning, memory becomes labile and sensitive to modulation, in which it may be strengthened or disrupted. Gradually, memory is transformed into a more stable form. This post-acquisition process of stabilization is called consolidation [46, 47].

Learning induces changes in the size and number of dendritic spines [48, 49]. Since LIMK plays an important role in actin cytoskeleton reorganization, spine morphology, and LTP induction [20, 50, 51], we hypothesized that LIMK inhibition would affect memory consolidation. In order to address this question, the LIMK inhibitor BMS-5 (20 or 200 μM/side) or vehicle was bilaterally infused into the hippocampus of rats immediately after contextual fear conditioning training. Rats were tested for memory consolidation 48 h after fear conditioning (Fig. 1a).

One-way ANOVA revealed significant effects of treatment (Fig. 1b, $F_{2,23} = 5.680$, $P = 0.009$). Post hoc analysis showed that the group treated with 200 μM BMS-5 expressed lower freezing levels compared to the 20 μM and vehicle groups ($P < 0.01$). These results show that the hippocampal LIMK inhibition immediately after training disrupts memory consolidation.

An additional experiment was performed in order to test if a short-term fear memory (STM) could be affected by LIMK inhibition. Animals were infused with 200 μM BMS-5 immediately after training and were tested in the same context 2 h later (Fig. 1c). No significant difference was found between both groups, BMS-5 and vehicle, in the test (Fig. 1d, $P = 0.1565$ in Student's t test), suggesting that actin dynamics briefly after training is not crucial to STM expression.

Inhibition of Hippocampal LIMK Activity Disrupts the Acquisition of Contextual Fear Memories

Previous studies have reported that local administration of cytochalasin D, an actin polymerization inhibitor, impairs long-term fear memory when applied immediately before training [52, 53]. Since 200 μM BMS-5 was able to impair memory consolidation, next, we addressed whether LIMK activity would play a role in the acquisition of contextual fear memories. BMS-5 (200 μM) or vehicle were infused into the dorsal hippocampus of rats 20 min before training (Fig. 2a). Animals infused with BMS-5 expressed lower freezing levels in the test compared to the vehicle group (Fig. 2b, $P = 0.023$ in Student's t test). Our findings indicate that memory acquisition requires LIMK activity, suggesting that contextual fear conditioning involves immediate changes in actin reorganization within the hippocampus. In order to rule out the

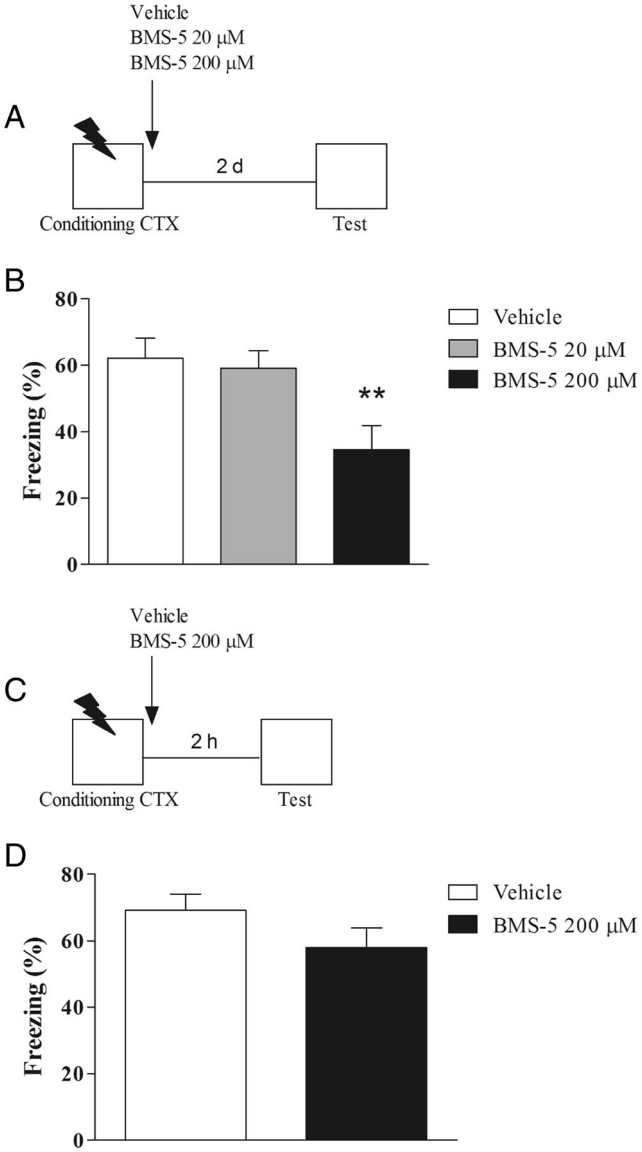

Fig. 1 LIMK activity regulates memory consolidation at higher dose. The graph shows the percentage of freezing time expressed as mean ± SEM. **a** Experimental design: animals were injected with either vehicle (DMSO 1%) or BMS-5 at two different concentrations, 20 and 200 μM, immediately after conditioning CTX and tested 2 days later for memory consolidation. **b** During the test, only animals infused within dorsal hippocampus with BMS-5 at 200 μM expressed lower freezing levels (the *asterisks* indicate $P < 0.01$, Fisher's LSD post hoc test after one-way ANOVA—vehicle, $N = 9$; BMS-5 20 μM, $N = 8$; BMS-5 200 μM, $N = 9$). **c** Experimental design: animals were injected with either vehicle (DMSO 1%) or BMS-5 at 200 μM immediately after conditioning CTX and tested 2 h later for short-term memory formation. **d** No difference on freezing levels was found between the groups ($P = 0.1565$ in Student's t test—vehicle, $N = 13$; BMS-5 200 μM, $N = 14$)

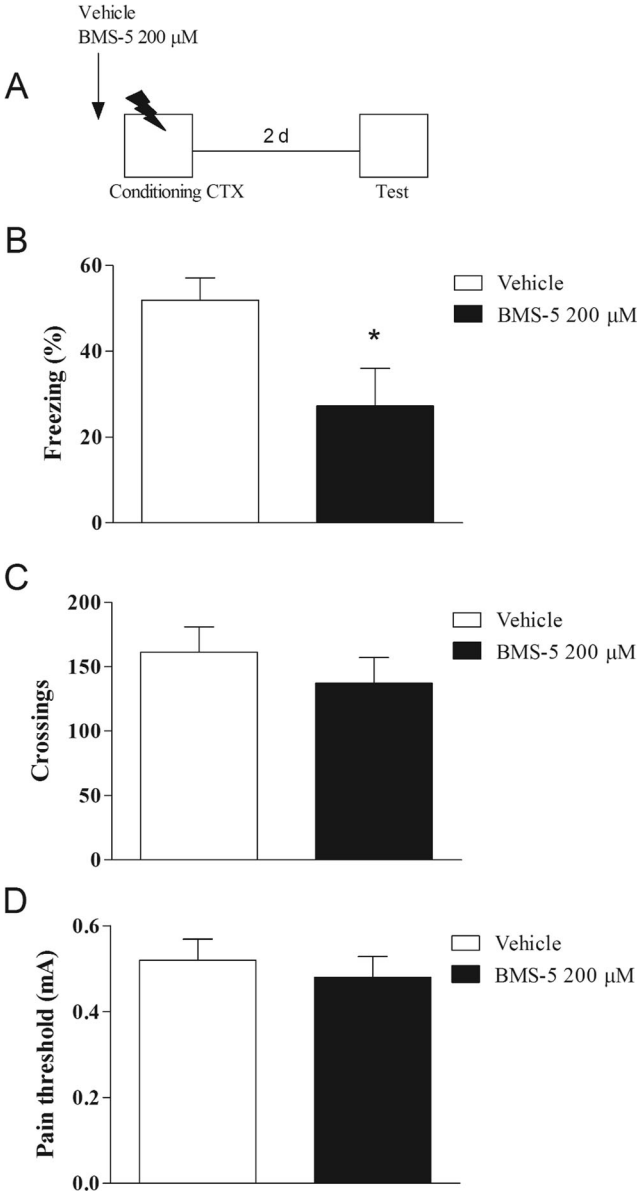

Fig. 2 LIMK inhibition prevented learning. The graph shows the percentage of freezing time expressed as mean ± SEM. **a** Experimental design: animals were injected with either vehicle (DMSO 1%) or BMS-5 200 μM 20 min prior conditioning CTX and tested 2 days later to evaluate memory acquisition. **b** During the test, animals infused within dorsal hippocampus with BMS-5 200 μM showed less freezing levels than the vehicle group (the *asterisk* indicates $P < 0.05$, Student's t test, $N = 10$ for both groups).**c** BMS-5 200 μM injection has no effects in motor activity ($P = 0.4148$ in Student's t test, $N = 5$–6) as well as in (**d**) pain sensitivity ($P = 0.5796$ in Student's t test, $N = 5$)

possibility that the BMS-5 could induce non-mnemonic effects, animals received intra-hippocampal infusion of BMS-5 or vehicle and, 20 min later, were tested either in open-field locomotor activity test or in a pain sensitivity test. No significant difference between the groups were found in the crossings during an open-field task (Fig. 2c, $P = 0.4148$ Student's t test) or pain sensitivity to a rising electric foot shock (Fig. 2d, $P = 0.5796$ in Student's t test).

LIMK Activity Is Necessary for Memory Retrieval

Some evidence suggests that memory retrieval is not a passive process. For instance, a recent study demonstrated that

retrieval of auditory fear memories depends on protein synthesis and NMDAR activity-mediated AMPAR trafficking [54]. Since AMPAR trafficking has been shown to be highly dependent on the structure and turnover rates of actin filaments [12, 14, 55], we hypothesized that LIMK inhibition could affect memory retrieval.

In order to address this question, BMS-5 (200 μM) or vehicle was infused into the dorsal hippocampus of rats 20 min before the test (Fig. 3a). Animals infused with BMS-5 expressed lower freezing levels compared to the vehicle group (Fig. 3b, $P = 0.005$ Student's t test). These results show that pre-test inhibition of LIMK in the hippocampus impairs memory retrieval. It also suggests that this process requires an ongoing balance between actin polymerization and depolymerization (and ultimately spine morphology) in order to retrieve memory.

LIMK Inhibition Disrupts Memory Reconsolidation

Fear memory retrieval in the absence of the unconditioned stimulus (foot shock) could permit two different post-retrieval processes: reconsolidation or extinction. Previous experiments from our lab demonstrated that a reexposure session with a duration similar to the one used here was able to turn memory susceptible to modifications via reconsolidation [41, 56]. However, whether actin dynamics mediated by LIMK are crucial for memory reconsolidation is still an open question. Thus, in order to address this possibility, animals were infused with BMS-5 (200 μM) or vehicle into the hippocampus immediately after the reexposure to the training context (retrieval, 7 min) and tested 24 h later (Fig. 4a). Repeated measures ANOVA revealed significant effects of treatment (vehicle or LIMK; $F_{1,16} = 4.854$, $P = 0.042$) and interaction of treatment × time (reactivation vs. test; $F_{1,16} = 17.461$, $P = <0.001$) but not for time ($F_{1,16} = 0.981$, $P = 0.336$). Fisher's (LSD) post hoc analysis showed that animals treated with intra-hippocampal LIMK inhibitor expressed less freezing compared to vehicle during the test ($P < 0.01$) and

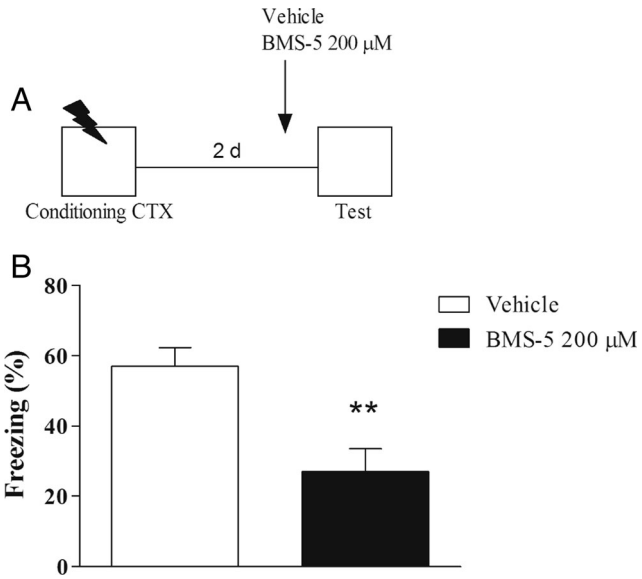

Fig. 3 Memory retrieval is sensible to LIMK inhibition. The graph shows the percentage of freezing time expressed as mean ± SEM. **a** Experimental design: animals were injected with either vehicle (DMSO 1%) or BMS-5 200 μM 20 min prior the test and freezing levels were analyzed subsequently. **b** Animals that received BMS-5 200 μM within the dorsal hippocampus expressed lower freezing levels compared to the vehicle group (the *asterisks* indicate $P < 0.01$, Student's t test—vehicle, $N = 6$; BMS-5 200 μM, $N = 8$)

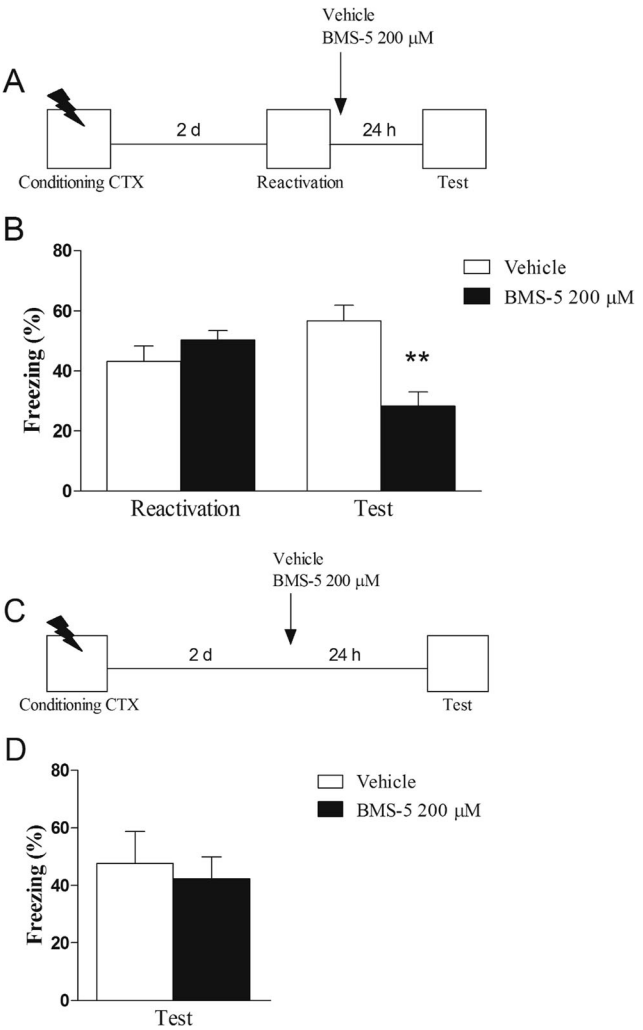

Fig. 4 After retrieval, blockade of LIMK activity impairs memory reconsolidation. The graph shows the percentage of freezing time expressed as mean ± SEM. **a** Experimental design: animals were injected with either vehicle (DMSO 1%) or BMS-5 200 μM immediately after reexposure to the conditioning CTX (reactivation, 7 min) and tested 24 h later. **b** During the test, animals that were infused with BMS-5 200 μM within dorsal hippocampus showed less freezing levels than the vehicle group (the *asterisks* indicate $P < 0.01$, ANOVA for repeated measures with Fisher's LSD post hoc analysis—vehicle, $N = 8$; BMS-5 200 μM, $N = 7$). **c** Experimental design for non-reactivation test: animals were injected either vehicle (DMSO 1%) or BMS-5 200 μM 2 days after training and tested 24 h later. **d** No difference on freezing levels was found between the groups ($P = 0.4800$ in Student's t test, $N = 8$–9)

during the test compared to the reactivation session ($P < 0.01$) (Fig. 4b). These results indicate that LIMK inhibition disrupts reconsolidation, suggesting that memory reactivation induces an actin remodeling process that requires LIMK activation in order to maintain LTM.

Additionally, we performed a test without memory reactivation (Fig. 4c) in order to rule out the possibility of unspecific effects of BMS-5 administration and the necessity of actin rearrangement triggered by memory reactivation [57]. We found no differences between the groups (Fig. 4d, $P = 0.4800$ Student's t test). This result suggests that LIMK activity is essential only when reconsolidation induces synaptic plasticity associated to destabilization/reconsolidation process.

Contextual Fear Extinction Is Not Sensitive to LIMK Inhibition in the Hippocampus

Fear extinction depends on the inhibition of previously acquired memories. However, in contrast to reconsolidation, the original fear memory may return over time or can be reinstated by presenting the unconditioned stimulus [58].

In order to evaluate the involvement of LIMK activity during contextual fear extinction, BMS-5 (200 μM) or vehicle was infused into the dorsal hippocampus of rats 20 min before the extinction session (Fig. 5a). Repeated measures ANOVA revealed significant effects of time (0–5 vs. 25–30 min; $F_{1,16} = 6.426$, $P = 0.021$) but not treatment (vehicle vs. BMS-5; $F_{1,16} = 3.512$, $P = <0.079$) and a time × treatment interaction ($F_{1,16} = 1773$, $P = 0.201$). Post hoc analysis showed that animals treated with intra-hippocampal LIMK inhibitor expressed less freezing compared to vehicle during the first 5 min of the extinction session ($P < 0.05$), as verified in our previous experiments with memory retrieval showed previously. The vehicle group was able to extinguish fear memory, since in the last 5 min of the extinction session, animals expressed less freezing compared to the first 5 min ($P < 0.05$; Fig. 5b). No differences were detected within the BMS-5 group during the extinction session.

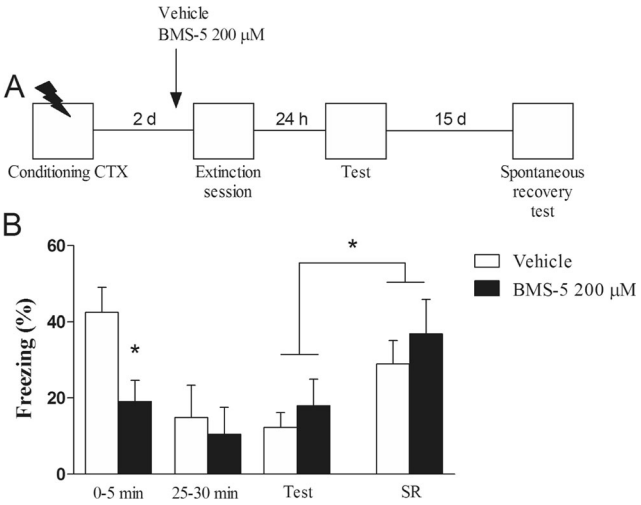

Fig. 5 Contextual fear extinction was not sensitive to LIMK inhibition. The graph shows the percentage of freezing time expressed as mean ± SEM. **a** Experimental design: animals were injected with either vehicle (DMSO 1%) or BMS-5 200 μM 20 min prior the extinction session (30 min) and tested 24 h later. **b** Animals infused with BMS-5 200 μM within dorsal hippocampus expressed less freezing levels than the vehicle group during the first 5 min of extinction session, and all groups expressed spontaneous recovery (SR) (the *asterisk* indicates $P < 0.05$, ANOVA for repeated measures with Fisher's LSD post hoc analysis—vehicle, $N = 10$; BMS-5 200 μM, $N = 8$)

During the test performed 24 h after fear extinction and spontaneous recovery session, repeated measures ANOVA revealed significant effects of time (test vs. spontaneous recovery; $F_{1,16} = 13.949$, $P = 0.001$) but not for treatment (vehicle vs. BMS-5; $F_{1,16} = 0.750$, $P = 0.398$). No significant time × treatment interaction was observed ($F_{1,16} = 0.057$, $P = 0.813$). Post hoc analysis showed that both the vehicle and the BMS-5 groups expressed more freezing during the spontaneous recovery session compared to the test 24 h after extinction ($P < 0.05$), demonstrating that fear responses returned independent of LIMK inhibition (Fig. 5b).

Consistent with our results upon retrieval, hippocampal LIMK inhibition was able to impair memory expression during the extinction session; however, the absence of behavioral fear response within the extinction session did not prevent the acquisition of fear extinction. These findings suggest that actin dynamics mediated by hippocampal LIMK during memory retrieval can take place in parallel to additionally plastic events that occur during fear extinction.

Discussion

Our findings provide evidence for a central role of LIMK activity in different memory processes in the hippocampus. Using a potent LIMK inhibitor (BMS-5), here, we demonstrate that LIMK activity in the hippocampus is necessary for consolidation, acquisition, retrieval, and reconsolidation of contextual fear conditioning in rats. Surprisingly, only fear extinction was resistant to LIMK manipulation, allowing the natural course of fear extinction acquisition and subsequent spontaneous recovery.

Actin remodeling plays a pivotal role in the structural plasticity involved in LTP induction and memory formation [59–61]. LIMK is the convergent node of two key upstream pathways controlling spine morphogenesis—the Rac1/PAK and RhoA/ROCK cascades. We chose to inhibit LIMK because it controls cofilin, a crucial regulator of actin dynamics [36, 37]. The phosphorylated form of cofilin prevents the cleavage and de-polymerization of actin filaments [55]. It has been showed that LIMK-1 knockout mice exhibit changes in LTP, fear conditioning, and spatial learning [20, 40]. Our results are consistent with this evidence, since BMS-5 was

able to impair memory acquisition (Fig. 2b) and consolidation (Fig. 1b) of contextual fear conditioning. Studies in cultured hippocampal neurons using latrunculin A, a compound able to disrupt actin filaments, showed that actin de-polymerization elicits NMDA and AMPA receptor internalization that requires stable F-actin [62]. It is well known that NMDA receptors lead to the activation of intra-cellular cascades such as calcium-dependent enzymes and AMPAR trafficking [63, 64]. NMDAR also triggers the structural and functional changes in dendritic spines that are characteristic of LTP induction [18]. Therefore, we suggest that LIMK inhibition may disrupt the early stages of synaptic potentiation during memory LTM acquisition and consolidation, while short-term memory is unaffected (Fig. 1d). Accordantly, several studies have demonstrated that actin polymerization processes are not needed for generate LTP, but it is essential for LTP endurance [65, 66; see review in 47].

Retrieval of cue fear conditioning leads to the rapid insertion of CP-AMPAR and removal of CI-AMPAR via endocytosis of GluA2 into lateral amygdala synapses [67]. Because GluA2 controls spine size and density in hippocampal neurons [68], these results led us to hypothesize that cytoskeletal reorganization could be a critical step during the retrieval of contextual fear memories. In fact, it has been recently shown that ongoing protein synthesis is required to retrieve fear memories by controlling AMPAR expressions in the PSD [54]. Here, we demonstrated that LIMK inhibition was able to disrupt fear memory retrieval, suggesting that retrieval is an active process relying on actin remodeling mechanisms. Interestingly, MAPK activity related to memory retrieval is also known to phosphorylate cortactin, a structural protein associated with actin, which promotes changes within spines via F-actin and actin-related protein (Arp) 2/3 complexes [69]. Taken together with the present study, these results suggest that memory retrieval is a dynamic process mediated by constant actin recasting that in turn depends on AMPAR trafficking.

Reactivation of an established memory might result in a new labile state that needs to be reconsolidated in order to persist and requires de novo protein synthesis [54]. Some studies suggest that stable dendritic spines provide a physical basis for memory persistence [70, 71]. We found that LIMK inhibition immediately after memory reactivation impairs memory reconsolidation. These findings indicate that reactivation may convert the dendritic spine to a labile state that requires a rearrangement mediated by actin dynamics in order to persist. If such a process is disrupted, memory is lost. In fact, Rao-Ruiz and colleagues (2011) have shown that memory reactivation induces AMPAR endocytosis followed by upregulation of AMPAR exocytosis a few hours later. This AMPAR endocytosis/exocytosis cycle allows memories to be updated [72]. We suggest that this AMPAR trafficking, mediated by memory reactivation, may be accompanied by spine modification via LIMK. Our results are consistent with previous reports showing that inhibition of Rac1 (which reduces spine number and density) disrupts reconsolidation of cue and contextual fear conditioning [30] and cocaine-induced conditioned place preference [73]. Importantly, Rac1-/cofilin-induced reorganization of actin can be prevented by LIMK inhibition [36, 37].

Memory reconsolidation and extinction have been recognized as two faces of the same process [74]. Using a two-photon microscopy and YFP-expressing transgenic mice, Lai et al. (2012) [75] demonstrated that, during cue fear conditioning, foot shock increased the rate of spine elimination in layer V pyramidal neurons in the dorsal medial region of the frontal association cortex. On the other hand, extinction increased the rate of spine formation. Spine elimination and formation processes occurred on the same dendritic branches, but not in the same dendritic spines, suggesting that fear conditioning and extinction have parallel dendritic spine dynamics. Our result showed that, even when treated with the LIMK inhibitor BMS-5, animals were able to acquire and consolidate fear extinction; however, those animals treated with the inhibitor presented significantly lower fear expression in the first minutes of the extinction session. Additionally, LIMK inhibition had no effect on subsequent spontaneous recovery. How can fear extinction memories be established without the proper fear expression? Delorenzi et al. [76] proposed that memory expression could occur independent of the molecular events triggered by memory reactivation. Accordingly, several studies have shown that memory reconsolidation can be disrupted by amnestic treatments independent of adequate memory retrieval [77–79]. We suggest that LIMK inhibition before fear extinction impairs specific mechanisms associated with memory retrieval without interfering with memory extinction, at least in the hippocampus. Indeed, this result differs from a previous report showing that cytochalasin D administration after repetitive and brief context exposure disrupts fear extinction [80]. Other studies have also shown the involvement of actin dynamics for memory extinction. For instance, it has been described the Rac1-Cdk5-PAK1 signaling pathway inhibition impairs memory extinction [81]. A recent work has shown that the inhibition of hippocampal Rac1 activity impaired extinction of contextual fear memory, suggesting a role for Rac1 in fear memory persistence [29]. In the extinction of conditioned taste aversive (CTA) memory, it was observed that there is a temporally enhancement of ADF/cofilin activity in rat infralimbic cortex [13]. Thus, we do not rule out the possibility that the LIMK-cofilin pathway plays a role in the consolidation of fear extinction and future experiments should be conducted to investigate actin dynamics along with different times of context exposure. Moreover, it is possible that other brain structures such as amygdala and medial prefrontal cortex (mPFC) could be the principal locus of LIMK activity in order to acquire or consolidate contextual fear extinction.

Conclusions

The results presented here reveal a broad spectrum of LIMK activity in regulating actin dynamics during the processing of contextual fear memories. Importantly, our pharmacological experiments complement previous genetic approaches while avoiding the compensatory mechanisms that could emerge in knockout mice. Moreover, this pharmacological approach allows the specific evaluation of the role of LIMK in different memory phases in the hippocampus. To our knowledge, this study is the first to demonstrate that LIMK activity is required for memory acquisition and consolidation and that actin dynamics mediated by LIMK are necessary for fear retrieval and memory reconsolidation. However, if the reactivation session is extended in order to promote extinction, LIMK inhibition does not affect the subsequent fear expression, suggesting that cytoskeletal reorganization during retrieval/reconsolidation and extinction are independent mechanisms. Altogether, these results demonstrate that contextual fear memories are regulated by hippocampal LIMK activity and suggest that pharmacological modulation of LIMK may represent a promising therapy for fear-related disorders.

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Compliance with Ethical Standards All experiments were performed in accordance to the national animal care legislation and guidelines (Brazilian Law 11.794/2008) and approved by the Ethics Committee of the Federal University of Rio Grande do Sul.

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