Brief Communication

Administration of the phosphodiesterase type 4 inhibitor rolipram into the amygdala at a specific time interval after learning increases recognition memory persistence

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Here we show that administration of the phosphodiesterase type 4 (PDE4) inhibitor rolipram into the basolateral complex of the amygdala (BLA) at a specific time interval after training enhances memory consolidation and induces memory persistence for novel object recognition (NOR) in rats. Intra-BLA infusion of rolipram immediately, 1.5 h, or 6 h after training had no effect on retention tested at 1, 7, and 14 d later. However, rolipram infused 3 h post-training promoted memory persistence for up to at least 14 d. The findings suggest that PDE4 inhibition in the BLA can enhance long-term memory formation when induced specifically 3 h after learning.

The basolateral complex of the amygdala (BLA) is involved in enhancing the consolidation of memories for emotionally arousing events (for review, see McGaugh 2004). Recent evidence suggests that the BLA may also modulate memories for low-arousing tasks, including novel object recognition (NOR), a task based on the natural preference toward novel objects displayed by rats and mice (Roozendaal et al. 2006, 2008; Okuda et al. 2004).

Phosphodiesterase type 4 (PDE4), an enzyme that catalyzes hydrolysis of cAMP, plays a critical role in regulating the activity of protein kinase A (PKA). The cAMP/PKA/cAMP regulatory element-binding protein (CREB) signaling pathway in the BLA is involved in regulating memory for fear-motivated tasks (Schafe and LeDoux 2000; Roozendaal et al. 2002). The specific PDE4 inhibitor rolipram enhances synaptic plasticity and memory formation in rodents, particularly when animals are given a mild training, and also ameliorates memory deficits in models of cognitive impairment (Barad et al. 1998; Bourboulouladez et al. 2003; Rutten et al. 2006; de Lima et al. 2008). The possible effects of amygdalar PDE4 inhibition on NOR memory have not been investigated. In this study, we examined the effects of administration of rolipram into the BLA at different time intervals after training on long-term retention of NOR memory in rats.

Male adult Wistar rats (age 3–4 mo; weight 270–330 g) were obtained from our institutional breeding colony (CREAL-UFRGS). The animals were housed five to a plastic cage with sawdust bedding and were maintained on a 12-h light:12-h dark cycle (lights on at 7 a.m.) with room temperature of 22 ± 1 °C. Food and water were available ad libitum. Behavioral procedures were conducted between 9 a.m. and 7 p.m. All experimental procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH publication number 80-23, revised 1996) and approved by the institutional animal care committee under protocol number 10–0552.

Animals were bilaterally implanted under ketamine (75 mg/kg) and xylazine (25 mg/kg) with a 14.0-mm, 22-gauge guide cannula aimed 1.0 mm above the BLA (Roesler et al. 2003, 2004b). Stereotaxic coordinates were according to the atlas of Paxinos and Watson (2007): A 2.8 mm, L ± 4.8 mm, V 7.5 mm. Rats given at least 5 d to recover before the experimental procedures.

NOR training and testing was conducted in a 40-cm × 50-cm open field surrounded by 50-cm-high walls made of plywood with a frontal glass wall, placed in a dimly illuminated room. The floor was covered with sawdust. The stimulus objects used in training and tests presented distinctive colors and shapes and were made of metal, glass, or plastic. In pilot experiments, all objects were behaviorally irrelevant and equally distinguished for the rats. Between trials, the objects were washed with a 70% ethanol solution. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Sitting on the object was not considered exploration. Training and tests procedures followed the general methods described in previous reports (Roesler et al. 2004a; de Lima et al. 2008; Reolon et al. 2011). Before training, the animals were habituated to the experimental arena by allowing them to freely explore it for 2 min in the empty arena. Twenty-four
hours after habituation, training was carried out by placing the rats in the open field containing two identical objects (objects A1 and A2) and leaving them to freely explore for 5 min. The objects were positioned in two adjacent corners, 9 cm from the walls. The rats were trained to a criterion of 30 sec of total time exploring both objects. Five rats were excluded from the experiment because they did not accumulate 30 sec of object exploration during the 5-min training. On retention tests trials given 1, 7, and 14 d after training, each rat was placed in the open field for 5 min and left to freely explore. One of the objects (A) was exchanged for a novel object (B, C, or D). The new object was placed in the same location of objects as stimuli during the training trial. To reduce potential bias due to the preference of the animal for a specific location or object, all combinations and positions of objects were used in a balanced manner. Object exploration was measured by one experimenter blind to group treatment assignments. To measure retention, a discrimination exploration was measured by one experimenter blind to group assignment. To reduce potential bias due to the preference as stimuli during the training trial. To re-

Table 1. Total time exploring both objects during NOR training and retention test trials in rats given infusions of vehicle or rolipram into the BLA

| Group                  | n | Training | 1-d test | 7-d test | 14-d test |
|------------------------|---|----------|----------|----------|-----------|
| Immediately post-training infusions |   |          |          |          |           |
| Vehicle                | 8 | 42.19 ± 3.63 | 45.66 ± 4.65 | 41.49 ± 3.19 | 28.60 ± 2.34 |
| Rolipram               | 9 | 38.21 ± 2.72 | 46.76 ± 3.94 | 31.36 ± 3.20 | 27.75 ± 1.74 |
| 1.5 h post-training infusions |   |          |          |          |           |
| Vehicle                | 10| 40.96 ± 2.35 | 37.56 ± 2.71 | 36.86 ± 2.14 | 41.29 ± 4.57 |
| Rolipram               | 10| 41.31 ± 2.26 | 35.94 ± 1.45 | 29.84 ± 1.95 | 33.07 ± 2.36 |
| 3-h post-training infusions |   |          |          |          |           |
| Vehicle                | 11| 41.40 ± 1.87 | 38.44 ± 3.18 | 45.04 ± 3.04 | 32.22 ± 3.00 |
| Rolipram               | 12| 40.05 ± 1.58 | 49.53 ± 3.84 | 49.69 ± 3.85 | 38.94 ± 4.31 |
| 6-h post-training infusions |   |          |          |          |           |
| Vehicle                | 9 | 50.39 ± 3.22 | 45.42 ± 4.13 | 41.28 ± 6.45 | 40.30 ± 4.02 |
| Rolipram               | 9 | 51.84 ± 2.66 | 49.02 ± 5.87 | 42.85 ± 4.25 | 41.18 ± 4.20 |

(*) P < 0.05 compared to the previous trial within the same group.
Amygdalar PDE4 and recognition memory persistence

Recognition memory in rats infused with rolipram into the BLA at different time intervals after training. Rats were given NOR training and infused with vehicle or rolipram (7.5 µg/side) immediately (A), 1.5 h (B), 3 h (C), or 6 h (D) after training. Retention test trials were carried out 1, 7, and 14 d later. Data are expressed as mean ± SEM discrimination indexes; n = 8–12 animals per group. (*) P < 0.05; (**) P < 0.01 compared to control rats in the same trial; (#) P < 0.05; (##) P < 0.01 compared to the training trial within the same group.

The main finding of the present study was that rolipram given into the BLA 3 h after training, but not at other post-training intervals, induced memory enhancement that persisted for at least 14 d. The results indicate for the first time that inhibition of PDE4 in the BLA at a specific later time point after learning can induce a persistent enhancement of NOR memory.

Previous evidence has indicated a role for cAMP/PKA in the BLA in memory formation. Thus, intra-BLA infusion of a PKA inhibitor impairs fear conditioning (Schaef and LeDoux 2000) and blocks glucocorticoid-induced enhancement of fear memory (Roozendaal et al. 2002). Conversely, administration of a PKA activator into the BLA enhances memory for rewarding conditioning (Jentsch et al. 2002). A late phase (around 3 h after induction) of long-term potentiation (LTP) in the amygdala requires PKA as well as protein synthesis and mitogen-activated protein kinase (MAPK) and is regulated by agonists of β-noradrenergic receptors (which act upstream of cAMP/PKA signaling) (Huang et al. 2000). Recent evidence suggests that PDE4 in the BLA interacts with β-arrestin to negatively influence PKA activity and formation of memory for fear conditioning (Li et al. 2009). In studies using the NOR task, post-training pharmacological stimulation of noradrenergic receptors in the BLA enhanced 1-d retention of NOR (Roozendaal et al. 2008), and systemic injection of adrenaline immediately after training produced persistence of NOR memory at least up to 4 d (Dornelles et al. 2007). However, previous studies have not directly examined the role of PDE4 or other intracellular components of the cAMP/PKA/CREB pathway in the BLA in NOR memory. The finding that rolipram had an effect when given specifically 3 h after training is consistent with previous evidence that rolipram injected systemically at 3 h, but not at 1 or 6 h, after training enhanced the consolidation of NOR memory (Rutten et al. 2007). Our results suggest that stimulation of cAMP/PKA signaling in the BLA can significantly enhance memory formation at a time point in which PKA activity is required to promote enduring LTP in the amygdala and support the view that the BLA regulates the consolidation of memories for tasks involving low-arousing stimuli.

Although many aspects of the molecular basis of memory formation have been uncovered, much less is known about the mechanisms underlying long-term memory persistence, or maintenance, one of the main attributes of long-term memories (Dudai 2002; Bekinschtein et al. 2007). Evidence indicates that persistence of memory for a fear-motivated task can be selectively produced by administration, at a late interval (12 h) after training, of brain-derived neurotrophic factor (BDNF) into the hippocampus and disrupted by hippocampal protein synthesis inhibition at the same time point (Bekinschtein et al. 2007, 2008). Since treatment with rolipram can increase BDNF levels in the rat brain (Nibuya et al. 1996; DeMarch et al. 2008), and the late phase of rolipram-induced LTP is dependent on BDNF (Navakkode and Korte 2012), an increase in amygdalar BDNF levels emerges as a candidate mechanism involved in mediating memory persistence induced by intra-BLA rolipram. Importantly, increases in amygdalar BDNF levels might be involved in mediating long-term memory persistence (Ou et al. 2010). Moreover, since pharmacological stimulation of the BLA results in increased expression of genes related to memory formation in other brain regions such as the hippocampus (McIntyre et al. 2005), it is also possible...
that intra-BLA rolipram leads to enhanced BDNF levels in other areas, which might be involved in mediating the enhanced NOR memory observed in the present study. Further experiments should address these possibilities.

In conclusion, the present findings provide the first evidence suggesting that PDE4 inhibition in the BLA after learning can enhance memory formation and produce long-term memory persistence. Experiments aimed at further characterizing the relationship between amygdalar cAMP/PKA/CREB signaling and memory persistence, as well as detailing the molecular mechanisms underlying the effects of PDE4 inhibition on long-term memory maintenance, are warranted.

Acknowledgments

This research was supported by the National Council for Scientific and Technological Development (CNPq) grant 303703/2009-1 to R.R., National Institute for Translational Medicine (INCT-TM), Coordination for the Improvement of Higher Education Personnel (CAPES; fellowships to M.B., P.F.C.J., and T.R.P.), and the HCPA Institutional Research Fund (FIPE/HCPA).

References

Barad M, Bourboulouzade R, Winder DG, Golan H, Kandel E. 1998. Rolipram, a type IV-specific phosphodiesterase inhibitor, facilitates the establishment of long-lasting long-term potentiation and improves memory. Proc Natl Acad Sci 95: 15020–15025.

Bekinschtein P, Cammarota M, Igaz LM, Baradi M, Bourtchouladze R, Winder DG, Golan H, Kandel E. 1998. The basolateral amygdala is critical for fear memory. Proc Natl Acad Sci 95: 267–271.

Bekinschtein P, Cammarota M, Igaz LM, Baradi M, Bourtchouladze R, Winder DG, Golan H, Kandel E. 1998. The basolateral amygdala is critical for fear memory. Proc Natl Acad Sci 95: 267–271.

Bourboulouzade R, Lidge R, Catapano R, Stanley J, Gossweiler S, Bekinschtein P, Cammarota M, Igaz LM, Bevilaqua LR, Izquierdo I, Schröder N. 2008. Amelioration of methylene blue in the BLA for rats included in the statistical analysis.

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Figure 2. Infusion placements into the BLA. Schematic diagrams of infusion placements into the BLA. Schematic diagrams of infusion placements into the BLA.
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Learn. Mem. 2012 19: 495-498
Access the most recent version at doi:10.1101/lm.026997.112

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