

Erasing sensorimotor memories via PKM ζ inhibition

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Abstract

Sensorimotor cortex has a role in procedural learning. Previous studies suggested that this learning is subserved by long-term potentiation (LTP), which is in turn maintained by the persistently active kinase, PKM ζ . Whereas the role of PKM ζ in animal models of declarative knowledge is established, its effect on procedural knowledge is not well understood. Here we show that PKM ζ inhibition, via injection of zeta inhibitory peptide (ZIP) into the rat sensorimotor cortex, disrupts sensorimotor memories for a skilled reaching task even after several weeks of training. The rate of relearning the task after the memory disruption by ZIP was indistinguishable from the rate of initial learning, suggesting no significant savings after the memory loss. These results indicate a shared molecular mechanism of storage for declarative and procedural forms of memory.

Introduction

Memories have been classified into several varieties characterized by different acquisition paradigms, temporal stability, and neural and molecular substrates. Procedural memories, like the skill of riding a bicycle, are thought to be fundamentally different from declarative (explicit) memories because they accumulate slowly through repetition, are expressed automatically in performance, rather than consciously through explicit knowledge, and are subserved by a separate neural system [1,2]. However, both forms of long-term memory have been proposed to be mediated by the strengthening of synaptic connections through long-term potentiation (LTP) [3-5].

In recent years the persistent activity of an atypical and autonomously active form of protein kinase C (PKM ζ) [6] has been shown necessary for the maintenance of LTP and the storage of spatial memories involving the hippocampus, a region required for declarative memory [4]. It was later shown that PKM ζ activity in the hippocampus, gustatory cortex, and basolateral amygdala is necessary for several forms of specific and accurately learned memories, but PKM ζ has not yet been proven necessary for procedural memories [5,7].

We therefore tested whether PKM ζ activity is necessary for the maintenance of a well-established, consolidated, skilled sensorimotor memory (a paradigmatic procedural memory). Specifically, we tested whether the PKM ζ inhibitor, zeta inhibitory peptide

(ZIP), disrupts performance on a rat reach-to-grasp and retrieve task. This task has previously been associated with changes in sensorimotor cortex cutaneous receptive field size, baseline level of synaptic potentiation, and the number and stability of dendritic spines [8-13].

Materials and Methods

Ethics Statement: All work adhered to NIH guidelines and was approved by SUNY Downstate's IACUC (permit #: 02-409-09).

Animals: Twenty two adult female Long-Evans rats (375-450 g) were used. Eleven rats were randomly selected to be intracortically injected with ZIP (10 nmol/ μ l) and 11 with saline. Five ZIP/control pairs were injected in the sensorimotor cortex at posterior: 1.5 mm; lateral: 1.5 mm and 2.5 mm, relative to Bregma, while the remaining six pairs were injected in the motor cortex: anterior 1.5 mm; lateral: 1.5 mm and 2.5 mm, relative to Bregma. Of the animals injected in posterior sensorimotor cortex, ZIP was injected with a 24 hr delay after the last training episode for two rats, and with a 4 hr delay for three rats; the results were indistinguishable and therefore combined.

Task paradigm: Rats were food deprived to ~85% of free-feeding body weight and then trained to reach through a narrow vertical slot (15 mm x 75 mm) to obtain a food pellet (45 mg 'dustless precision' food pellet, Bio-Serve) resting in a metal washer (5mm ID), 15 mm away on a 40 mm high platform. This same platform extended into the training chamber by 15 mm, towards the rat. For rats injected in the posterior sensorimotor cortex, a 2 mm diameter wooden dowel was placed across the pellet's platform (Fig. 1A, insert) to increase the difficulty of the task. A successful reach, the procedural component of the task, was one in which the rat maintained its grasp and brought the pellet to its mouth. The experimenter did not assist with pellet retrieval. A new pellet was not placed onto the reaching platform by the experimenter until the rat had moved 35 cm to the rear of the cage and then back, resetting its stance. In the case of a successful reach a pellet was placed at the back of the cage as an additional reward. Each rat was trained for 30 min a day until reaching a criterion of >80% average success for 4 consecutive days, with <0.05 SEM; this was at least 24 days after the first

training session. The experimenter was not informed of the rats' group assignment either prior to or after the ZIP/control injections.

Surgery: After reaching criterion, rats were intracortically injected with ZIP (10 nmol/ μ l) or saline. Rats were anesthetized with Nembutal and placed in a stereotaxic device. Two small craniotomies were made along with small incisions in the dura to allow for four microinjection sites (two in each hemisphere) with coordinates as described above. For each injection site, a 33 gauge (0.0065" ID, 0.00825" OD) stainless steel cannula was initially lowered to 1.5 mm depth to create a fluid repository before drawing back to administer two injections of 1.25 μ l at depths of 1 and 0.5 mm. After the injections, the craniotomies were sealed with bone wax, and the scalp was sutured and treated with antibiotic ointment. Ad libitum food and water were provided over the next 2 days. Measurements for reaching success were begun 4 days after the injections, when previous results have shown ZIP is eliminated [4,7].

Drug diffusion analysis: To estimate ZIP spread from our injection sites additional rats were injected with biotinylated ZIP [4], deeply anesthetized after 2.5 hr, perfused with 4% paraformaldehyde, and decapitated. The brain was then removed, vibratome sectioned at 50 μ m thickness, and stained with an ABC kit (Vector Laboratories).

Statistical analysis: Unless otherwise stated, statistics were conducted using ANOVA for within group effects, and ANOVA followed by Tukey's honestly significant difference (HSD) test for comparisons between groups. Linear regression slope and y intercept comparisons were performed using Matlab's ANCOVA function.

Results

After 3-4 weeks of training to maximal ability on the reaching task, the intracortical injection of ZIP into sensorimotor cortex disrupted the sensorimotor memory (Fig. 1, 2A, Supplementary Fig. 1), causing an $84 \pm 21\%$ decrease in learned ability (measured between naïve ability and performance prior to ZIP injection; ANOVA $p < 0.0001$, $F = 54$). Saline controls showed no change in learned ability post-injection (ANOVA $p = 0.98$, $F = 0.0007$). An inactive scrambled version of ZIP [4,7] had no effect on memory retention ($N = 4$, ANOVA $p = 0.92$, comparing performance pre- and post-injection). When comparing all three treatments, only ZIP vs. saline and ZIP vs. scrambled ZIP

showed significant differences, while saline vs. scrambled ZIP did not (ANOVA followed by Tukey's HSD test; $p < 0.01$, $p < 0.01$, and $p = 0.46$, respectively). The drug did not affect the number of attempted reaches and stance resets (total reaches and resets per day during relearning days 4-11: saline, 75.5 ± 2.3 ; ZIP, 69.3 ± 6.4 ; unpaired 2-tailed t-test $p = 0.43$), and the animals' gait appeared normal on the first day of task administration post-injection. Animals injected with ZIP anteriorly into the hand/wrist motor area exhibited a smaller memory disruption with a $23 \pm 10\%$ decrease in performance (two tailed t-test, $p < 0.05$, $N = 6$); saline controls showed no change (two tailed t-test, $p = 0.78$, $N = 6$). Comparison of treatment effect between groups showed a significant difference (two tailed t-test, $p < 0.05$, $N = 6$).

Fig. 1 shows video captures of representative reaches for the control and ZIP-injected rats. The stereotypical reaching behaviors of pronation, grasp, and supination were accurately carried out by the control rats (Fig. 1A). In contrast, while the ZIP-injected rats were able to pronate their arms in an accurately aimed reach, they were unable to effectively grasp the food pellet (Fig. 1B). Further studies with high speed, multi-angle video, EMG and neural recording will be necessary to further characterize these differences.

Fig. 2A shows the average percentage of successful reaches for both control and ZIP groups during initial learning and relearning after injection into the sensorimotor cortex. After reaching asymptotic levels of success, the animals were injected with either saline or ZIP. After 4 days rest, the animals were tested on the reaching task. Following the initial decline in performance to naive levels, the ZIP-injected rats relearned the task, and there was no significant difference between the initial learning and the relearning curves of the ZIP-injected rats (Fig. 2B; ANCOVA $p = 0.80$, slope; $p = 0.35$, y intercept). This suggests that there were no significant memory savings or damage to the cortex due to the injections, as also indicated from the lack of change in the control animals' performance post-injection.

Histological analysis of brain sections indicates the spread of ZIP did not extend into subcortical regions (Fig. 3A), but encompasses several areas involved in skilled reaching including M1, M2, and S1 limb regions [14,15] (Fig. 3B).

Discussion

Here we demonstrated that a sensorimotor, procedural memory is dependent on the persistent activity of the autonomously active protein kinase $M\zeta$ in the sensorimotor cortex. Previous results have shown that $PKM\zeta$ is present in neocortex, including motor cortex [16-18]. We found that bilateral injections of the $PKM\zeta$ inhibitor ZIP into the sensorimotor cortex of rats erased the sensorimotor memories learned in a skilled reach-to-grasp task [19]. This is the first demonstration that a procedural memory shares the same underlying molecular mechanism for persistence as declarative memories [4,5,7]. Our results support previous studies that have suggested the importance of LTP in sensorimotor memories in the sensorimotor cortices [3,10] and confirm that the information mediating the reaching task is stored in these brain regions.

There were no apparent postinjection savings in the ZIP-injected rats as indicated by their learning and relearning curves, which were indistinguishable in both slope and y-intercept (Fig. 2B). As our injections did not penetrate subcortically (Fig. 3A), erasing information in the sensorimotor cortex is apparently sufficient to require complete relearning of the task. This suggests that the sensorimotor memory was either maintained by $PKM\zeta$ -dependent activity entirely within the injected cortical regions, or was distributed over several brain areas with maintenance/retrieval entirely dependent upon $PKM\zeta$ in the cortex. The latter would be consistent with previous work that proposes a striato-cortico circuit is responsible for habitual, olfaction-guided reaching movements [20,21].

There are other possible interpretations of our data. For instance, there could have been differences in the level of attention to the task between the ZIP- vs. saline-injected animals. However, if this were the case, we would expect a difference between the number of reaching attempts made between the two groups. No significant difference was observed. In addition, we have presented our results as a percentage of success, thus normalizing the data by the number of attempts. Whether the disruption of sensorimotor memory by ZIP extends to even more basic neural plasticity such as that

which establishes and maintains the sensorimotor homunculus will require future investigation.

In recent years there has been a great deal of interest in the concept of consolidation as it pertains to reaching movements [22-24]. In our experiments, ZIP disrupted memory after the animals had reached asymptotic performance following several weeks of training, but it cannot be excluded that at some point in the future the memory would become independent of the kinase. Indeed, it will be interesting in future studies to determine the influence that the passage of time since memory activation has on PKM ζ dependence for such procedural memories. It is still unclear what holds consolidated long-term memories in their stable form; however, new synaptic growth has been proposed as one possibility. Whether this new synaptic growth depends upon the persistent activity of PKM ζ remains to be determined. Alternatively, synaptogenesis associated with sensorimotor training may play a different role, such as allowing sensorimotor cortex to maintain a balance between excitation and inhibition, or to reestablish the potentiation dynamic range of the neural ensemble [25,26]. In this sense, the learning correlated synaptic growth observed by previous researchers may be a homeostatic response to memories maintained by PKM ζ activity. Synaptic creation and destruction is a dynamic process, and PKM ζ stabilizes synapses [27]. Therefore, what appears to be an increase in synaptogenesis after motor learning may not be due solely to an increase in synaptic formation, but to a shift in the equilibrium between synaptic creation and destruction, thus leading to an overall increase in the number of synapses.

Finally, the results from this study may have direct clinical relevance for the treatment of focal dystonia and chronic pain caused by amputation or peripheral nerve injury, because these are believed to be due to aberrant LTP in sensorimotor areas [28,29]. As we have now shown that ZIP can erase such sensorimotor memories, the application of ZIP to animal models of these disorders should be tested for therapeutic value.

Footnotes

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Fig. 1. Representative video clips of rats performing reaching behavior after A) saline and B) ZIP injection into sensorimotor cortex. Both animals first sniff, then pronate their arm in preparation for grasp; the ZIP-injected rat had difficulty in the grasping phase (B). The rat’s body and limbs are outlined to aid viewing relevant features. Insert above left

shows illustration of the reaching task, including reach and subsequent stance reset (dashed arrows).

Fig. 2. A) ZIP, but not saline injected into the posterior sensorimotor cortex disrupts the retention of sensorimotor memory. Successful reaches/total reaches ($R_{\text{success}}/R_{\text{total}}$) for the initial learning period of 8 days, the 4 days preinjection (days -3 to 0), and, after a delay, the 8 days postinjection (days 4 to 11, with 0 the day of injection). Means \pm SEM; 5 animals per group. B) Linear regressions of learning and relearning curves (pre-ZIP, grey solid line; post-ZIP, black solid line). The 95% confidence interval of the pre-ZIP regression (dashed curves) envelopes the post-ZIP regression line.

Fig. 3. A) Histology showing the extent of diffusion of biotinylated ZIP. Average maximal diffusion of 4 injections is shown by dashed line. There is no diffusion of the biotinylated ZIP to the hippocampus or subcortical structures. Counterstain is cresyl violet. B) Illustration of the diffusion within a single hemisphere from sensorimotor injections sites (dashed circles), mapped onto motor (grey) and sensory (blue) areas: M1, primary motor; M2, secondary motor; and primary sensory (S1) regions, including FL, forelimb; HL, hindlimb; T, trunk; based on (Paxinos and Watson, 1998; Wang and Kurata, 1998).

Supplementary Fig. 1. The individual performances of the rats presented in Figure 2. All rats show a deficit in performance after ZIP (blue); none of the rats show a deficit after saline (orange). Solid lines indicate rats trained 4-6 hr prior to injection; dashed lines indicate rats not trained for 24 hr prior to injection. Thick lines indicate mean performances.

References

1. Cohen NJ, Squire LR (1980) Preserved learning and retention of pattern-analyzing skill in amnesia: dissociation of knowing how and knowing that. *Science* 210:207-210.
2. Squire LR (2004) Memory systems of the brain: a brief history and current perspective. *Neurobiol Learn Mem* 82:171-177.

3. Rioult-Pedotti MS, Friedman D, Hess G, Donoghue JP (1998) Strengthening of horizontal cortical connections following skill learning. *Nat Neurosci* 1:230-234.
4. Pastalkova E, Serrano P, Pinkhasova D, Wallace E, Fenton AA, Sacktor TC (2006) Storage of spatial information by the maintenance mechanism of LTP. *Science* 313:1141-1144.
5. Serrano P, Friedman EL, Kenney J, Taubenfeld SM, Zimmerman JM, Hanna J, Alberini C, Kelley AE, Maren S, Rudy JW, Yin JC, Sacktor TC, Fenton AA (2008) PKMzeta maintains spatial, instrumental, and classically conditioned long-term memories. *PLoS Biol* 6:2698-2706.
6. Sacktor TC, Osten P, Valsamis H, Jiang X, Naik MU, Sublette E (1993) Persistent activation of the zeta isoform of protein kinase C in the maintenance of long-term potentiation. *Proc Natl Acad Sci U S A* 90:8342-8346.
7. Shema R, Sacktor TC, Dudai Y (2007) Rapid erasure of long-term memory associations in the cortex by an inhibitor of PKM zeta. *Science* 317:951-953.
8. Kleim JA, Lussnig E, Schwarz ER, Comery TA, Greenough WT (1996) Synaptogenesis and Fos expression in the motor cortex of the adult rat after motor skill learning. *J Neurosci* 16:4529-4535.
9. Kleim JA, Barbay S, Nudo RJ (1998) Functional reorganization of the rat motor cortex following motor skill learning. *J Neurophysiol* 80:3321-3325.
10. Rioult-Pedotti MS, Friedman D, Donoghue JP (2000) Learning-induced LTP in neocortex. *Science* 290:533-536.
11. Remple MS, Bruneau RM, VandenBerg PM, Goertzen C, Kleim JA (2001) Sensitivity of cortical movement representations to motor experience: evidence that skill learning but not strength training induces cortical reorganization. *Behav Brain Res* 123:133-141.
12. Kleim JA, Barbay S, Cooper NR, Hogg TM, Reidel CN, Remple MS, Nudo RJ (2002) Motor learning-dependent synaptogenesis is localized to functionally reorganized motor cortex. *Neurobiol Learn Mem* 77:63-77.
13. Kleim JA, Bruneau R, Calder K, Pocock D, VandenBerg PM, MacDonald E, Monfils MH, Sutherland RJ, Nader K (2003) Functional organization of adult motor cortex is dependent upon continued protein synthesis. *Neuron* 40:167-176.
14. Paxinos G, Watson C (1998) *The Rat Brain in Stereotaxic Coordinates*, 4th Edition. San Diego: Academic Press.
15. Wang Y, Kurata K (1998) Quantitative analyses of thalamic and cortical origins of neurons projecting to the rostral and caudal forelimb motor areas in the cerebral cortex of rats. *Brain Res* 781:135-147.
16. Naik MU, Benedikz E, Hernandez I, Libien J, Hrabe J, Valsamis M, Dow-Edwards D, Osman M, Sacktor TC (2000) Distribution of protein kinase Mzeta and the complete protein kinase C isoform family in rat brain. *J Comp Neurol* 426:243-258.
17. Oster H, Eichele G, Leitges M (2004) Differential expression of atypical PKCs in the adult mouse brain. *Brain Res Mol Brain Res* 127:79-88.
18. Crary JF, Shao CY, Mirra SS, Hernandez AI, Sacktor TC (2006) Atypical protein kinase C in neurodegenerative disease I: PKMzeta aggregates with limbic neurofibrillary tangles and AMPA receptors in Alzheimer disease. *J Neuropathol Exp Neurol* 65:319-326.
19. Peterson GM (1932-1937) Mechanisms of handedness in the rat. *Comparative Psychology Monographs* 9.
20. Whishaw IQ, O'connor WT, Dunnett SB (1986) The contributions of motor cortex, nigrostriatal dopamine and caudate-putamen to skilled forelimb use in the rat. *Brain*, Vol. 109, No. 5, 805-843
21. Whishaw IQ, Tomie J (1989) Olfaction directs skilled forelimb reaching in the rat. *Behavioural Brain Research* Volume 32, Issue 1, 1 February 1989, Pages 11-21

22. Brashers-Krug T, Shadmehr R, Bizzi E (1996) Consolidation in human motor memory. *Nature* 382:252-255.
23. Caithness G, Osu R, Bays P, Chase H, Klassen J, Kawato M, Wolpert DM, Flanagan JR (2004) Failure to consolidate the consolidation theory of learning for sensorimotor adaptation tasks. *J Neurosci* 24:8662-8671.
24. Criscimagna-Hemminger SE, Shadmehr R (2008) Consolidation patterns of human motor memory. *J Neurosci* 28:9610-9618.
25. Monfils MH, VandenBerg PM, Kleim JA, Teskey GC (2004) Long-term potentiation induces expanded movement representations and dendritic hypertrophy in layer V of rat sensorimotor neocortex. *Cerebral Cortex* 14:586-593.
26. Rioult-Pedotti MS, Donoghue JP, Dunaevsky A (2007) Plasticity of the synaptic modification range. *J Neurophysiol* 98:3688-3695.
27. Shao CY, Sondhi PS, Van De Nes AI, Hernandez T, Sacktor TC (2009) PKM ζ regulates PSD-95 in chemically induced LTP and LTD in cultured hippocampal neurons: Mechanisms for memory maintenance and erasing. In: society for neuroscience annual meeting. Chicago, IL.
28. Byl NN, Merzenich MM, Cheung S, Bedenbaugh P, Nagarajan SS, Jenkins WM (1997) A primate model for studying focal dystonia and repetitive strain injury: effects on the primary somatosensory cortex. *Phys Ther* 77:269-284.
29. Lenz FA, Byl NN (1999) Reorganization in the cutaneous core of the human thalamic principal somatic sensory nucleus (Ventral caudal) in patients with dystonia. *J Neurophysiol* 82:3204-3212.







