Impaired Spatial Learning is Associated with Disrupted Integrity of the White Matter in Akt3 Knockout Mice

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Received 8 August 2016; revision 1

September 2016; accepted 3 September 2016

doi: 10.1111/cns.12647

The PI3K/Akt signaling pathway is important for various biological processes [1], and impaired PI3K/Akt signaling has been observed in neurodevelopmental and neurodegenerative diseases [2,3]. In mammals, whereas Akt exists in three isoforms including Akt1, Akt2, and Akt3 [1], Akt3 is the dominant isoform to be expressed in the brain [4]. Recently, abundant evidence has shown that mutations on Akt3 (loss-of-function) but not Akt1 or Akt2 are associated with microcephaly, a disease displaying intellectual disability and decreased size of the brain [3]. However, it remains unknown whether Akt isoforms differentially regulate brain development and affect learning ability. In this study, two different lines of Akt knockout (KO) mice, *Akt3*^{-/-} [5] and *Akt1*^{-/-} [6], were used for behavioral and morphological analyses.

To assess spatial learning and memory, *Akt3*^{-/-} mice and age-matched littermate wild-type (WT) animals were examined by a Morris watermaze task. First, a visible cuetask was performed. Comparable performance was observed between *Akt3*^{-/-} mice and WT (data not shown), suggestive of normal basic learning in *Akt3*^{-/-} mice. Second, a hidden platform task of the watermaze [7] was conducted. The mice were trained to learn a submerged platform, and the training protocol consisted of two blocks per day and two trials per block over 5 days. Analysis of variance (ANOVA) revealed a highly significant main genotype effect on the latency to escape (Fig. 1A: $F = 13.5$, $df_{1/18}$, $P < 0.005$) during the training period, suggesting that spatial learning in *Akt3*^{-/-} mice was impaired. The ANOVA also showed a significant block effect ($F = 18.4$, $df_{4.2/75.1}$, $P < 0.001$), suggesting that the training protocol worked well so that all the mice had improved the

performance significantly after the training. Third, spatial reference memory was accessed by a probe test 24 h after the hidden platform training. We found that WT and *Akt3*^{-/-} mice displayed comparable performance on the time spent in the target quadrant (Fig. 1B: $P > 0.2$, two-tailed Student's *t*-test). The target quadrant occupancy for WT and *Akt3*^{-/-} mice was significantly better than the chance performance (25%) (Fig. 1B: $P_s < 0.05$), suggesting that *Akt3*^{-/-} mice had formed solid spatial reference memory after the 5-day training.

Whereas Akt3 is the most abundant Akt isoform to be expressed in mammals, Akt1 is the second [4,5]. To study whether Akt1 is also required for spatial learning, we trained *Akt1*^{-/-} mice in the watermaze using the same protocol for *Akt3*^{-/-} mice. The learning curve was plotted in Fig. 1C. ANOVA did not reveal any significant genotype effect on the latency to escape ($F = 0.3$, $df_{1/19}$, $P > 0.4$), suggesting that learning ability of *Akt1*^{-/-} mice was not impaired. The ANOVA showed a significant block effect ($F = 18.4$, $df_{3.8/73.1}$, $P < 0.001$), indicating effective learning by all the mice. Moreover, WT and *Akt1*^{-/-} animals exhibited equivalent level of the time spent in the target quadrant during the probe test (Fig. 1D), suggesting that spatial reference memory of *Akt1*^{-/-} mice was not affected. In addition, results from a cuetask showed no difference on the performance for *Akt1*^{-/-} and WT animals (data not shown).

To understand molecular mechanism by which Akt3 regulates learning ability, we examined brain morphology of mutant mice. First, using an antibody against MBP (myelin basic protein), we conducted immunohistochemistry (IHC) [8] on MBP and found

Figure 1 Impaired spatial learning in *Akt3*^{-/-} but not *Akt1*^{-/-} mice. **A.** Escape latency for *Akt3*^{-/-} and age-matched littermate WT mice. These animals were trained in the Morris watermaze for 10 blocks over five consecutive days. Escape latency by *Akt3*^{-/-} mice was significantly longer than that by WT animals in finding the hidden platform (WT: $n = 8$; *Akt3*^{-/-}: $n = 12$). There was significant difference between the two genotypic groups (* $P < 0.05$; ** $P < 0.01$; **** $P < 0.001$). **B.** Quadrant occupancy of the watermaze in a probe test, which was conducted 24 h after the last training trial. The animals were allowed to spend 60 seconds searching for the location of the hidden platform. There was no significant difference in the time spent in the target quadrant between *Akt3*^{-/-} and control animals ($P > 0.05$). **C.** Escape latency for *Akt1*^{-/-} and age-matched littermate WT mice. Escape latency by *Akt1*^{-/-} mice did not differ from that by WT animals in finding the hidden platform (WT: $n = 9$; *Akt1*^{-/-}: $n = 12$). **D.** Quadrant occupancy of the watermaze in a probe test. There was no significant difference in the time spent in the target quadrant between *Akt1*^{-/-} and control animals ($P > 0.05$).

significantly reduced MBP immunoreactivity in the neocortex and the hippocampus of *Akt3*^{-/-} (Fig. 2A) but not *Akt1*^{-/-} mice (Fig. 2B). Moreover, the complexity of MBP+ fiber-like structure was diminished in *Akt3*^{-/-} (Fig. 2A) but not *Akt1*^{-/-} mice (Fig. 2B). Second, IHC on MAG (myelin-associated glycoprotein) revealed significantly reduced MAG immunoreactivity in *Akt3*^{-/-} (Fig. 2C) but not *Akt1*^{-/-} mice (Fig. 2D). Consistent with the IHC data, Western results on MAG, MBP, and PLP revealed decreased protein levels for them (data not shown). Overall, the above findings suggest that *Akt3*^{-/-} but not *Akt1*^{-/-} mice displayed disrupted white matter integrity. Third, MAP2 (microtubule-associated protein 2) IHC showed unaltered MAP2 immunoreactivity in brains of *Akt3*^{-/-} (Fig. 2E) or *Akt1*^{-/-} mice (Fig. 2F). Lastly, we found no significant change on SVP38 (synaptophysin) immunoreactivity in *Akt3*^{-/-} or *Akt1*^{-/-} mice (data not shown), suggesting that dendritic or synaptic morphology was unaltered in *Akt3*^{-/-} or *Akt1*^{-/-} mice.

Akt3 and Akt1 are two major Akt isoforms which are expressed abundantly in the brain. Recent evidence has demonstrated that loss-of-Akt3-function is associated with microcephaly [3]. Our behavioral data on *Akt3*^{-/-} and *Akt1*^{-/-} mice have demonstrated that Akt3 but not Akt1 is required for spatial learning (Fig. 1). Therefore, the above findings indicate that there is a differential role for Akt isoforms on learning ability. Our histological analyses on synapse, dendrite, and myelin markers have suggested that Akt3 but not Akt1 is important for the white matter integrity, and that Akt single isoform does not play a pivotal role on neuronal survival. As abundant evidence has shown that disrupted integrity of the white matter significantly impairs learning [9,10], it is reasonable to conclude that Akt3 may affect learning ability via the regulation of the integrity of the white matter. Moreover, understanding molecular mechanism underlying

Figure 2 Disrupted white matter integrity in *Akt3*^{-/-} but not *Akt1*^{-/-} mice. **A.** MBP IHC in *Akt3*^{-/-} mice. There was significant difference on the immunoreactivity of MBP in the cortex (a,b) and hippocampus (c,d) between WT and *Akt3*^{-/-} mice. **B.** MBP IHC in *Akt1*^{-/-} mice. There was no difference on the immunoreactivity of MBP between WT and *Akt1*^{-/-} mice. **C.** MAG IHC in *Akt3*^{-/-} mice. There was significant difference on the immunoreactivity of MAG in the cortex (a,b) and hippocampus (c,d) between WT and *Akt3*^{-/-} mice. **D.** MAG IHC in *Akt1*^{-/-} mice. There was no difference on the immunoreactivity of MAG between WT and *Akt1*^{-/-} mice. **E.** MAP2 IHC in *Akt3*^{-/-} mice. There was no difference on the immunoreactivity of MAP2 between WT and *Akt3*^{-/-} mice. **F.** MAP2 IHC in *Akt1*^{-/-} mice. There was no difference on the immunoreactivity of MAP2 between WT and *Akt1*^{-/-} mice. In **A–F**, scale bar = 50 μ m.

Akt3-dependent learning may help find novel therapeutic targets for the prevention or the treatment of microcephaly.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (31271123 and 81471157) and the National Basic Research Program of Ministry of Science

and Technology of China (2014CB942804 and 2014CB943303). We thank Prof. Zhongzhou Yang at the MARC of Nanjing University for providing *Akt1*^{+/-} and *Akt3*^{+/-} mice for breeding.

Conflict of Interest

The authors declare no conflict of interest.

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