CREB down-regulation in the laterodorsal thalamic nucleus deteriorates memory consolidation in rats

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The laterodorsal thalamic nucleus (LD) is believed to play roles in learning and memory, especially spatial tasks. However, the molecular mechanism that underlies the cognitive process in the LD remains unclear and needs to be investigated. So far, there is plenty of evidence indicating that plasticity has been in some of the cortico- and subcortical regions closely related to the LD, particularly stimulated by external learning tasks. Therefore, the present study aimed to test the hypothesis that similar effect exists in the LD. The transcription factor, cAMP-response element binding protein (CREB), works essentially in brain plasticity by tightly regulating the transcriptional level of memory-related target genes, and the increase of activated CREB (phosphorylated CREB, p-CREB) could facilitate memory consolidation. In this study, the siRNA against CREB was synthesized to down-regulate the CREB mRNA in the LD. After Morris water maze behavioral training, CREB siRNA rats exhibited a memory deficiency, significantly diverging from the control groups. In subsequent detection, the expression of p-CREB of these memory impairment rats attenuated. These results support the hypothesis that CREB-mediated plasticity contributes to memory facilitation and consolidation in the LD.

SiRNA against CREB suppresses CREB mRNA expression in the LD of rats

CREB was down-regulated by stereotaxically injecting siRNA into the LD. The control rats were injected with scrambled siRNA (siScr) or liposome vehicles (LIPO). The injection site is shown in the stereotaxic coordinate diagram (Paxinos 2013) in Figure 1A. The animals were sacrificed 48, 72, and 96 h, respectively, after the injection manipulation, and reverse transcription polymerase chain reaction (RT-PCR) was performed to detect the CREB mRNA of these three timing groups. Figure 1B,C shows the RT-PCR result. Statistical analysis of the three groups was tested using the one-way ANOVA test. There was an overall significant difference among the three groups, indicating that CREB mRNA expression in the LD was reduced by siRNA injection.
the LD implicates in spatial memory

SiCREB rats exhibit memory impairment in Morris water maze, while acquisition stays intact

Next, Morris water maze behavioral testing was conducted in injected (siCREB, siScr, LIPO) rats and wild-type rats. There are four different shaped distal clues placed around the tank to help rats navigate their path toward the platform (Position: N, S, E, and W). Besides, for avoiding the situation that rats learn a specific order in their movement to find the platform, we used different start positions that are approximate in length from the platform in each group. Lane 1: siCREB, lane 2: siScr, lane 3: LIPO. Error bars represent SD and significant differences are indicated by (*) $P<0.05$, (**) $P<0.01$. N=9.

(F=1.947, $P=0.163$, one-way ANOVA, Fig. 2E), and we will discuss this situation below.

The expression of p-CREB in rats with memory impairment is significantly decreased

In many research studies that concentrated on other memory-related brain areas, like hippocampal formation or ATN, the level of phosphorylated CREB (p-CREB) would elevate after memory acquisition, on the other hand, its decrease would impair spatial memory (Li et al. 2017; Yu et al. 2017). To examine this alteration whether it also exists in the LD, western blot and immunofluorescence were performed to measure p-CREB levels after behavioral testing. In addition, we set another untrained naive group, which all rats within it were put into tank to swim (the time of their swim was determined by the average of escape latency of the trained groups), but without the platform. A one-way ANOVA showed that siScr, LIPO and wild-type groups made considerably more times crossing the platform and had less escape latency than siCREB group (EL: siCREB vs. siScr, $P=0.025$; siCREB vs. LIPO, $P<0.001$; siCREB vs. wild-type, $P=0.001$). Crossings: siCREB vs. wild-type, $P=0.003$; siCREB vs. wild-type, $P=0.001$, Fig. 2C,D). Interestingly, differing from results above, analysis indicated the difference about platform quadrant occupation time was statistically nonsignificant.

In Figure 3, the expression of p-CREB in rats was determined by western blot. The level of phosphorylated CREB (p-CREB) would elevate after memory acquisition, on the other hand, its decrease would impair spatial memory. (F=17.666, $P<0.001$, Fig. 3C,D). Post hoc test (Bonferroni) confirmed a significant decrease in siCREB group compared with control groups (siScr, $P=0.001$; LIPO, $P<0.001$; wt, $P<0.001$). Crossings: siCREB vs. wild-type, $P=0.001$; siCREB vs. wild-type, $P=0.001$, Fig. 2C,D). Interestingly, differing from results above, analysis indicated the difference about platform quadrant occupation time was statistically nonsignificant.
positive cells among four groups ($F = 35.434, \ P < 0.001$). Positive cells were strongly expressed in the control groups, and almost absent from the siCREB and NG groups (siScr vs. siCREB, $P < 0.001$; LIPO vs. siCREB, $P = 0.001$; siScr vs. NG, $P < 0.001$; LIPO vs. NG, $P < 0.001$; WT vs. NG, $P = 0.006$. Post hoc comparison). The expression among control groups were not statistically significant (siScr vs. LIPO, $P = 1.000$; siScr vs. WT, $P = 0.372$; LIPO vs. WT, $P = 0.288$). The results of immunofluorescence also support those of the western blot.

CREB has been widely studied as a transcription factor involved in the formation and consolidation of memory, especially long-term memory. Bilateral down-regulation of CREB mRNA in the LD lead to an underperformance in behavioral testing for spatial memory retention, subsequently, the level of activated CREB in memory impairment group was also much lower than that in control groups. Thus we identified a direct association between spatial memory and p-CREB level in the LD, indicating the plasticity in the LD also contributes to spatial memory consolidation.

In behavioral testing, the probe trial, we came across interesting results. The insignificance between treated group and control groups appearing in the behavioral statistics about platform quadrant occupation time was an issue worth attention, as it was not consistent with our other results. To exclude an accident, we increased the number of cases and finally found it remained the same. We found that siCREB group data always had a greater discretization, which is represented in Figure 2E by standard deviation, than that of control groups, even before the increasing of cases. That means occupation time varied widely from rat to rat within siCREB group. Visual inspection of movements of siCREB rats during the probe trial also allied the digits. SiCREB rats whose occupation time was low did not show any specificity for the platform or corresponding quadrant, and their trajectories were "randomly" distributed among four quadrants. Contrastively, high-occupation rats spent more time as well as traveled much longer paths in the platform quadrant. But, crucially, high-occupation rats did not show more accurate sense about the specific location of the platform relative to low-occupation rats, as if they solely knew the general area where the platform is. This could explain why differences were so apparent at the number of

![Figure 2.](image)

**Figure 2.** (A) The tracks of rats during place navigation and probe trial. From day 1 to day 3, all groups experienced a gradual learning process. In day 4 of probe trial, siCREB group obviously differed from siScr, LIPO, and wild-type rats, showing less platform crossings and more indistinct tendency toward the platform. (B) Analysis of escape latency in place navigation. All groups of rats exhibited spatial learning. (C–E) Comparison of escape latency in probe trial, of the number of platform crossings and of the time spent in the platform quadrant. Error bars represent SD and significant differences are indicated by (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$. N=10 rats in each group.

| siCREB | siScr | LIPO | WT | Standards control |
|--------|-------|------|----|-------------------|
| Escape latency | 35.1778 ± 12.22812 | 21.3556 ± 9.84341 | 14.4667 ± 6.55763 | 16.5400 ± 8.58347 | ≤30 |
| Occupation time | 23.4800 ± 14.59579 | 35.0200 ± 3.82714 | 34.3600 ± 5.57387 | 33.7600 ± 7.00914 | ≥30 |
| Crossing times | 1.8000 ± 0.83666 | 3.8000 ± 0.83666 | 4.0000 ± 0.70711 | 4.2000 ± 0.83666 | ≥2 |

Table 1. Escape latency, platform quadrant occupation time, and original platform crossing times are main reference indexes in Morris water maze. Groups that meet the requirements of standard control could be regarded as the ones with intact memory functions.
platform crossings and escape latency (of probe trial). In addition, there is other research supporting this view—for rats that have intact memory function, they may underperform in quadrant preference, when not coming along with higher quality of distal cues (Rogers et al. 2017). In our study, the quality of distal cues is quite moderate, four difform cards which are flat and plain, while the high quality means an array of salient cues. So it also could bring on a narrower gap between siCREB and control group in quadrant preference.

Some animal experiments have revealed the characteristics of LD and the memory system that it may reside in. In some paired-associate learning or spatial processing tasks, when lesions concentrated solely on the LD or other adjacent nuclei, rats tended not to show conspicuous deficits in memory function. Once lesion range extended to multiple nuclei, the damage effect would be much more severe, and would not be able to recover with training sessions increased (Dillingham et al. 2015; Leong et al. 2016). In combination with the result of the present study, the interference of CREB signaling pathway in the LD did not severely restrict the spatial acquisition, we tend to think each nucleus does not work individually on acquisition of an ability to learn or memorize. In brief, our work provides evidence for the presence of memory-related signaling pathway in the LD, which may further explore the role of the LD in the synergy among various nuclei of the thalamus during learning and memory.

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Received March 17, 2019; accepted in revised form April 19, 2019.

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