Sevoflurane-Induced Learning Deficits and Spine Loss via Nectin-3/CRHR1 Signaling in Neonatal Mice

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Background: General anesthetics neurotoxicity in the developing brain has been investigated in the recent years and raised great concern as a major health issue to the public and doctors. Sevoflurane exposure may induce neurotoxicity expressed as learning and memory impairment in young animals. Recently, nectin-3/CHRH1 signaling was reported as important mediators for memory and learning function and spine number in mice. In the current study, we investigated the role of nectin-3/CHRH1 signaling in the sevoflurane-induced learning deficits and spine loss in neonatal mice.

Methods: Neonatal mice (P7) were treated with 3% sevoflurane for 6h or air. Working memory and spatial learning and memory of mice were evaluated in Y maze and Morris water maze. Hippocampal tissues of the mice were harvested and subjected to western blot to assess nectin-3 expression at 1h before and 1h, 4h, 8h, 1d, 2d, 3d and 2mon after sevoflurane exposure. The spine morphology of hippocampal was determined in the Golgi impregnation.

Results: Sevofluane exposure to neonatal mice had decreased hippocampal nectin-3 level form 1h to 2mon after sevoflurane exposure and attenuated working and spatial memory and spinal number in adulthood, which could be attenuated by nectin-3 overexpression and CRHR1 inactivation. Nectin-3 knockdown caused spatial learning deficits and spine loss and decreased L-afadin protein expression, whereas hippocampal nectin-3 overexpression rescued the learning deficits and spine loss and L-afadin protein level in adulthood.

Conclusion: Our findings suggest that hippocampal nectin-3/CRHR1 signaling is necessary for sevoflurane-induced learning deficits and spine loss and L-afadin was a potential molecular substrate that mediates nectin-3 dependent learning changes.

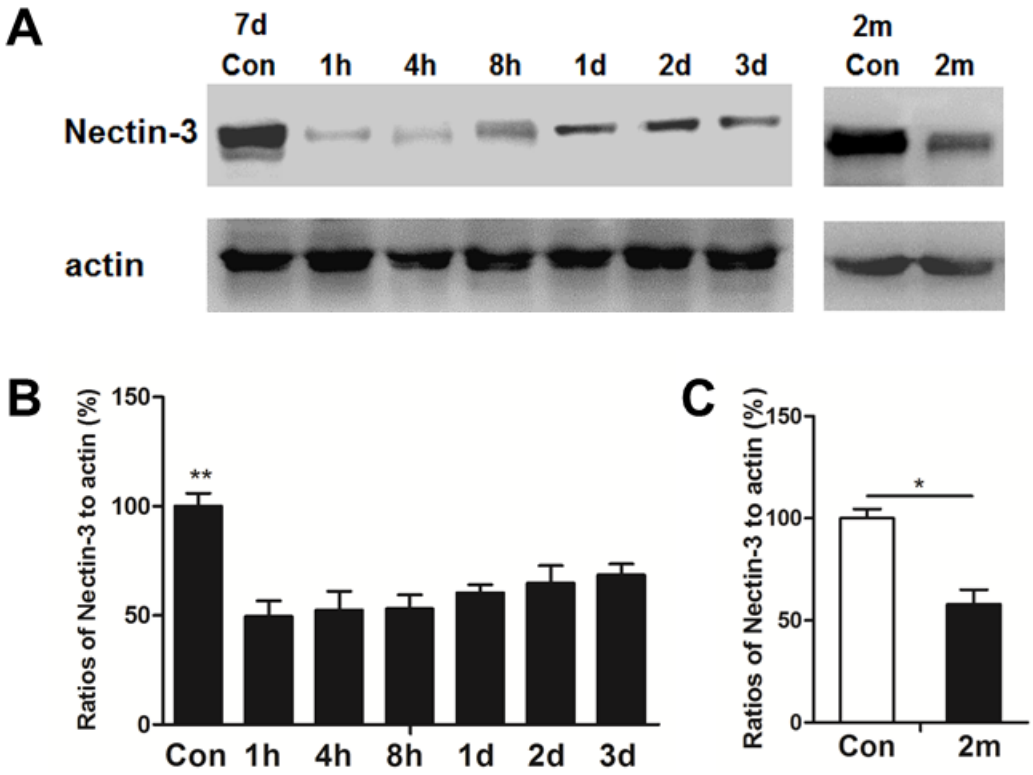
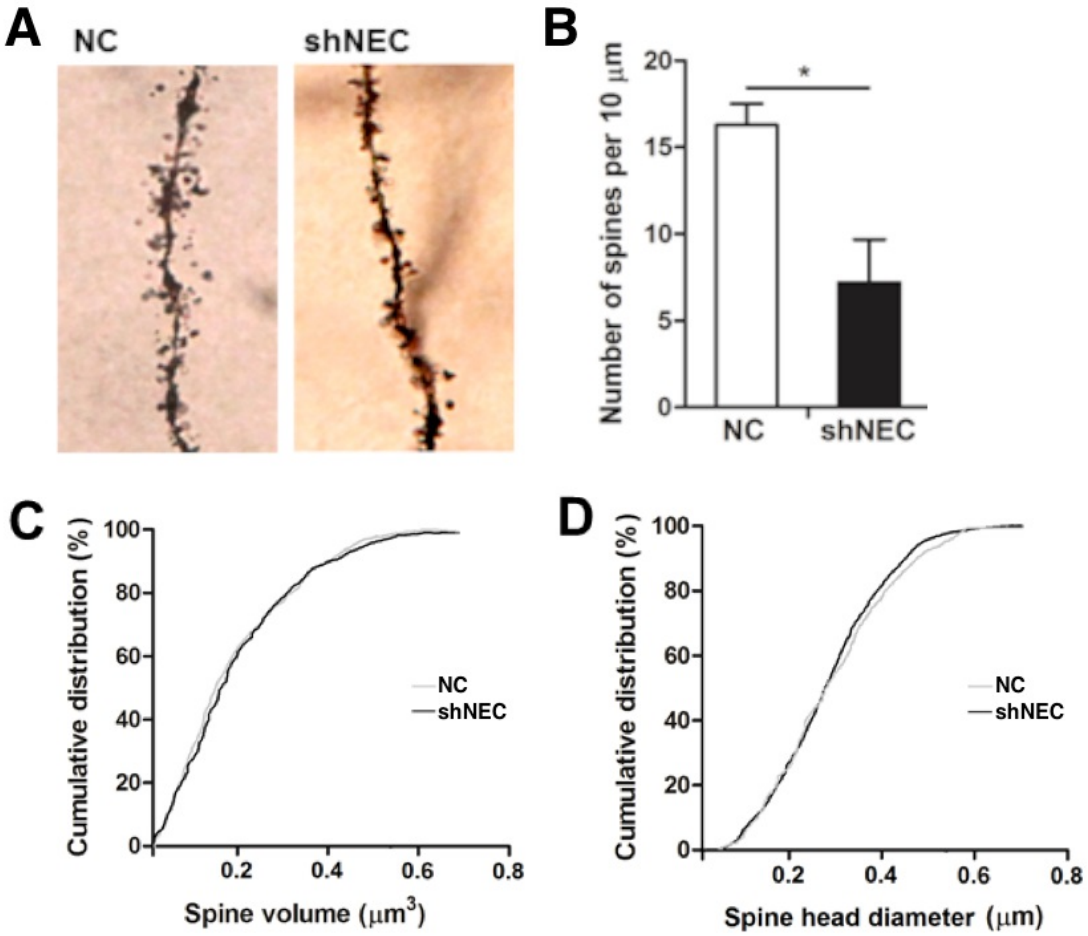


Figure1. Sevofluane exposure to neonatal mice had decreased hippocampal nectin-3 protein level form 1h to 2mon after sevoflurane exposure. Data represent mean ± sem. Compared with Control group (Con), $^{*}P < 0.01$, $^{**}P < 0.001$, ANOVA, post-hoc Turkey.



Figrue2. Nectin-3 knockdown reduced dendritic spine density in CA3 pyramidal neurons. (A) Representative Golgi dying figures of negative control group (NC) and nectin-3 knockdown group (shNEC) in CA3. (B) Suppression of nectin-3 decreased spine density in CA3 pyramidal neurons ($^{**}P < 0.01$, unpaired t test). (C) Nectin-3 knockdown did not affect spine volume (P = 0.536, Welch's t test) or spine head diameter (P = 0.498, Welch's t test). Mice were 1 mon old when they injected with virus and were killed after 4 weeks of recovery. For each mouse, 8-16 dendrites were analyzed.

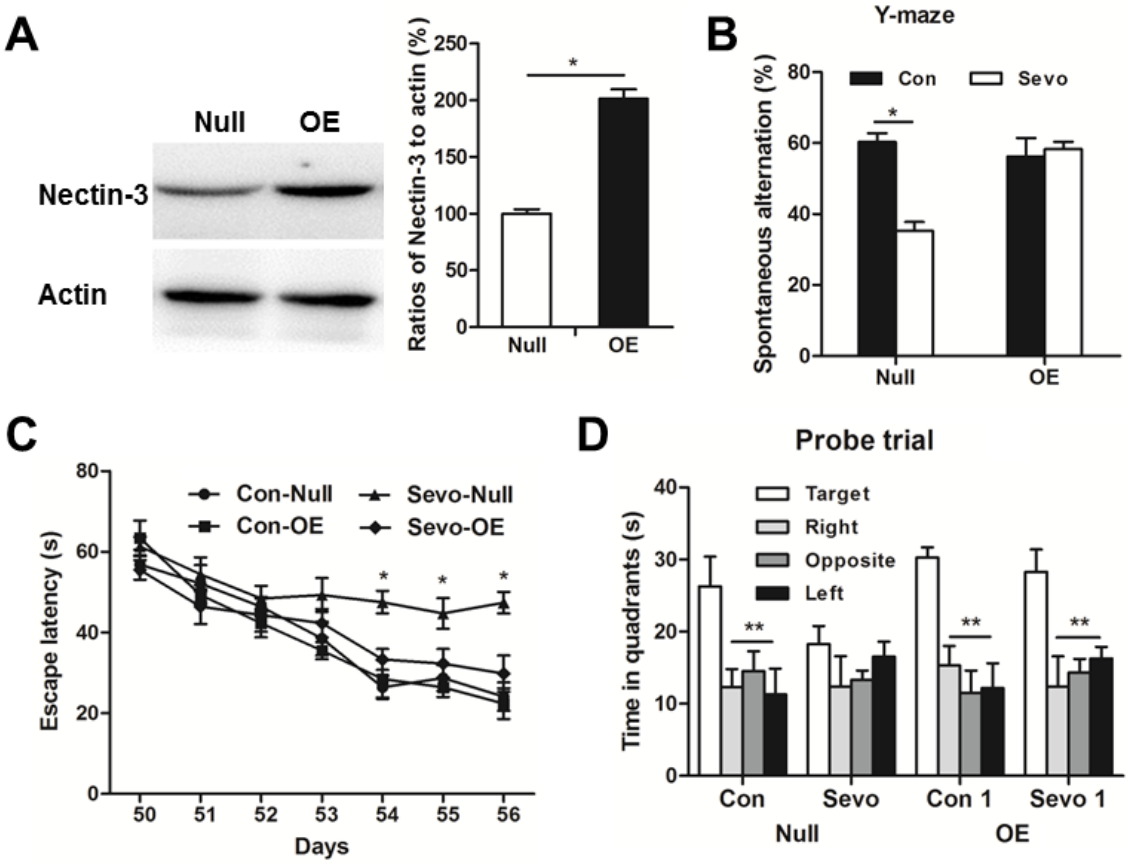


Figure3. Hippocampal nectin-3 overexpression reversed sevoflurane-induced learning deficits. (A) Hippocampal nectin-3 protein level was determined by western blot after nectin-3 overexpression. Nectin-3 protein level was increased by virus overexpression (OE). Mice were 1 mon old when they injected with virus intra-hippocampally and were killed after 4 weeks of recovery. For each mouse, 8-16 dendrites were analyzed. (B) In the Y-maze test, sevoflurane exposure to neonatal mice impaired working memory ($^{*}P < 0.01$, ANOVA) and nectin-3 overexpression restored working memory ($P = 0.671$, ANOVA). (C) In the Morris water maze test, sevoflurane exposure (Sevo-Null) group showed significant increased acquisition time (All $^{*}P < 0.01$, two-way ANOVA) from P54 to P56 and nectin-3 exposure decreased acquisition time (All $P > 0.0942$, two-way ANOVA). (D) In the probe trial, nectin-3 overexpression (OE) increased the ratio of time spent exploring the target quadrant over non-target quadrants ($^{**}P < 0.001$, paired t test). Data represent mean ± sem.