# The Effect of Sevoflurane on Dentritic Spine and Spacial Memory is Meditated by α7nAChR-NMDAR in Neonatal Rats

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**Background**: Inhaled anesthesia is a principal method for infants. Howerver, volatile anesthetics may have detrimental effects on the structure and function of the developing brain. As to infants, Sevoflurane is the most common used volatile anesthetic. Although researches show that sevoflurane exposed to neonatal rats can cause long-term memory impairment,while to infants, cause a series of behavioral changes at school age, the mechanism is unclear. Several studies show that α7 nicotinic acetylcholine receptor (α7nAChR) is one of the targets of volatile anesthetics, and N-Methyl-D-Aspartate (NMDAR) plays an important role in LTP. Moreover, α7nAChR can form cohesin complex with NMDAR, regulaing the expression and excitability of NMDAR through direct protein and protein interaction. Therefore, our study aims to investigate the role of α7nAChR in the changes of hippocampus spine morphology and spacial working memory deficits as well as the downregulation of NMDAR induced by sevoflurane exposed to neonatal rats.

**Methods**: Niney-six healthy male Sprague-Dawley rats, 7 d，10~15 g，were randomly divided into 4 groups(n = 24): Control group (group C), in which rats inhaled oxygen of 30% for 6 h ; 3% sevoflurane group (group S), in which rats inhaled 3% sevoflurane for 6 h ; 3% sevoflurane+α7nAChR agonist PNU-282987 group (group PS), in which PNU-282987 (5 mg/kg) was administered intraperitoneally before rats were exposed 6 h to 3% sevoflurane; and α7nAChR antagonist MLA group (group M), in which MLA (3 mg/kg) was administered intraperitoneally before rats inhaled oxygen of 30% for 6 h. Rats in each group (n=16) were guillotined immediately and removed the hippocampus after inhaled oxygen or sevoflurane. The α7nAChR as well as the surface and total NMDAR containing NR1、NR2A and NR2B expression levels in rat hippocampus were determined by western blot. Immunofluorescence was applied to observe the distribution of NMDAR subunits in neurons of CA1 area on the hippocampus slice. Y maze were performed to detect spacial working memory when rats in 4 groups（n = 8） were raised to 2 m. After that, each animal was sacrificed to measure the spine density and spine length of neurons in CA1 area by the method of Golgi-Cox staining.

**Results:** In Y maze test, compared with group C, the ability of recognition memory of rats to novel environment in group S and group M was decreased ($P < 0.05$), while compared with group S, the ability was enhanced in group PS ($P<0.05$).

Golgi-Cox staining shows that compared with group C, the spine density and spine length of hippocampal neurons reduced in group S and group M($P < 0.05$), and compared with group S, they all increased in group PS($P < 0.05$). In Western blot results, compared with group C , the expression of α7nAChR and surface NMDAR containing NR1、NR2A and NR2B subunits were decreased in group S ($P < 0.05$), and the surface expression of NR2B was decreased in group M($P < 0.05$); Compared with group S, the surface NR2B was increased in group PS ($P < 0.05$). In addition, results of immunofluorescence reveal that trafficking of NR1、NR2A and NR2B to the membrane all decreased in group S compared with group C. However, only NR2B increased on the membrane in group PS compared with group S.

**Conclusion:** These data indicate that the expression and trafficking of surface NR2B-containing NMDA receptors are regulated by α7nAChR in neonatal rat hippocampus, which may be involved in sevoflurane-induced changes of hippocampal dentritic spine morphology and spacial working memory deficits.

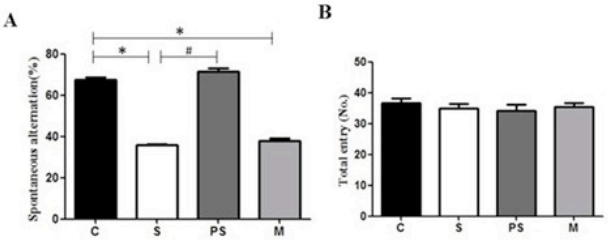


Figure 1.Effect of PNU-282987 and MLA on sevoflurane induced memory deficits in the Y-maze test. *#P<0.05.

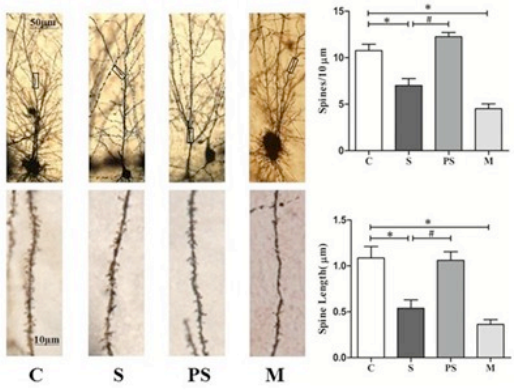


Figure 2. Sevoflurane and MLA induce spine loss and shortness of existing spines, while PNU-282987 reverses the effect of sevoflurane. *#P<0.05.

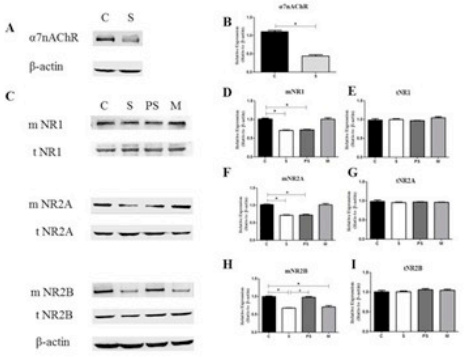


Figure 3.Western blot of of α7nAChR as well as surface and total expression of NMDAR containing NR1、NR2A and NR2B subunits in four groups. *#P<0.05.

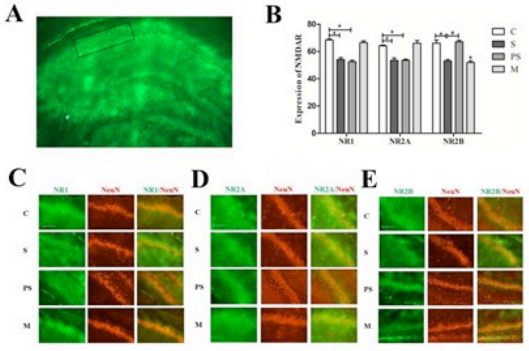


Figure 4.Distribution of NMDAR subunits in CA1 area neurons of hippcampus. (A). Photomicrograph represents an integral hippocampus. (C)、(D) and (E).Representative photomicrographs reveal that sevoflurane and MLA lead NMDAR containing NR1、NR2A and NR2B subunits trafficking to Cytoplasm. However, PNU-282987 only reverses the effect of sevoflurane on NMDAR containing NR2B subunit. *#P<0.05.