

Posttraumatic administration of a sub-anesthetic dose of ketamine exerts neuroprotection via attenuating inflammation and autophagy

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Overview

- Basics
- Experimental procedures
- Results
- Discussion
- My opinion and criticism

Ketamine

- NMDA receptor antagonist
- induces trance like state
- used for pediatric procedural sedation, neuropathic pain management and peripheral inflammation
- heart function, breathing and airway reflex remain functional
- side effects: psychological reactions, hypertension, muscle tremor
- drug of choice for patients in traumatic shock with risk of hypotension

TBI Traumatic Brain Injury

- traumatically induced structural injury and/or physiological disruption of brain function due to an exterior force
- 2 typical processes of injury: primary and secondary brain injury
- can result in long-term psychiatric changes and sensorimotor and cognitive impairments
- memory loss and long-term cognitive dysfunction

Experimental Overview

- 96 male rats divided into 3 groups
 - group 1: ketamine group
 - group 2: DMSO group
 - group 3: sham-injured group
- 4 time points after TBI: 2h, 1d, 3d, 7d
- 8 rats out of each group were examined at each time point

Experimental Procedure - Surgery

- anesthesia: chloral hydrate in a 10% solution
- TBI to the left portion of the brain
- Symptoms after the hit:
limb twitch, urinary incontinence, nasal bleeding

Experimental Procedure - MWM

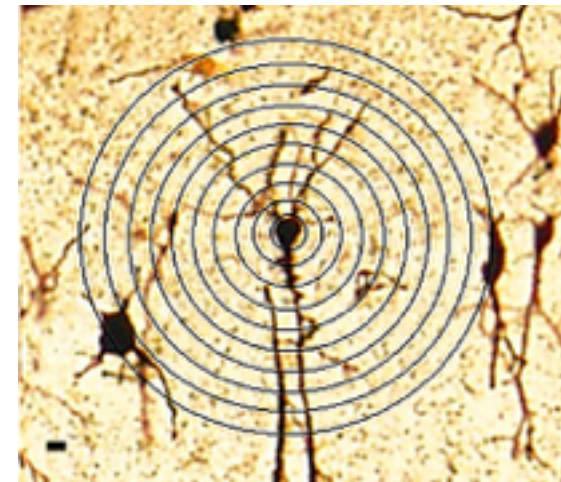
- round dark metallic pool: 160cm diameter, 60cm deep
- escape platform: 12 x 12cm, 30cm from the edge of pool
- search for hidden platform: up to 1 minute
- 5 consecutive days, 4 training trials, then at the 4 time points
- sacrifice for hippocampal tissue samples

Experimental procedure - Hematoxylin eosin staining

- 1 brain tissue sample from each group
- fixed in 10% buffered formalin
- sliced and stained with a
 - 0,5% hematoxylin staining for 2 minutes
 - 1% hydrochloric acid alcohol for 20 seconds
 - 0,5% eosin for 1 minute
- dehydration with 50%, 75%, 90%, 95% and anhydrous ethanol
xylene 3 times for 2 minutes each

Experimental Procedure - Golgi-cox staining and microscopy

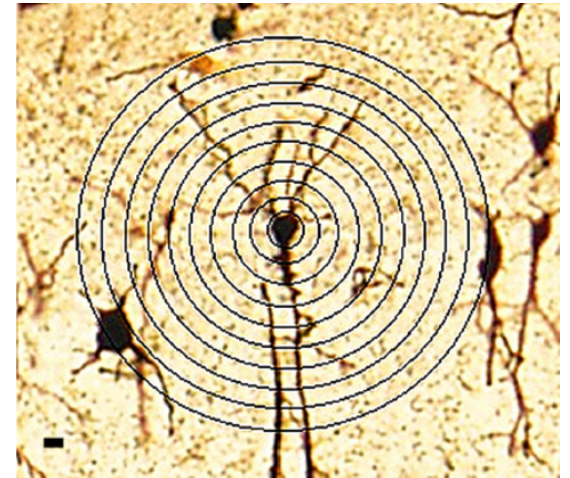
- Dendritic spines:
location of more than 90% of all excitatory synapses
- dendritic arborization and length measured by the amount of ring intersections with dendritic tree
- Over 10 primary dendritic branches were traced
- amount of dendritic spine was computed





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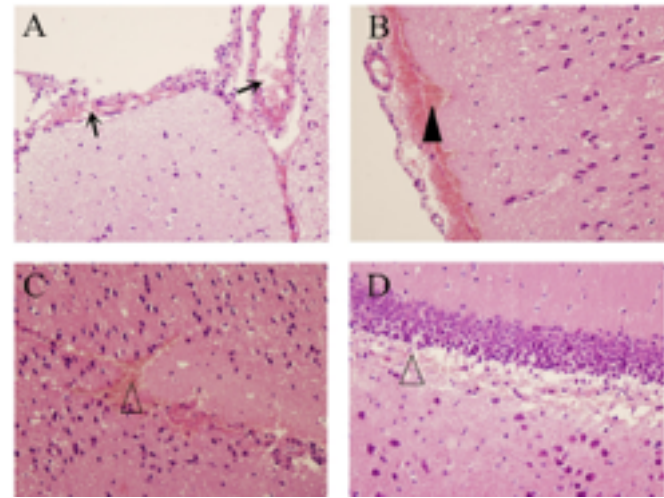
Experimental Procedure - IL-6 TNF assay

- hippocampal tissue was harvested immediately
- levels of IL-6 and TNF-alpha were determined

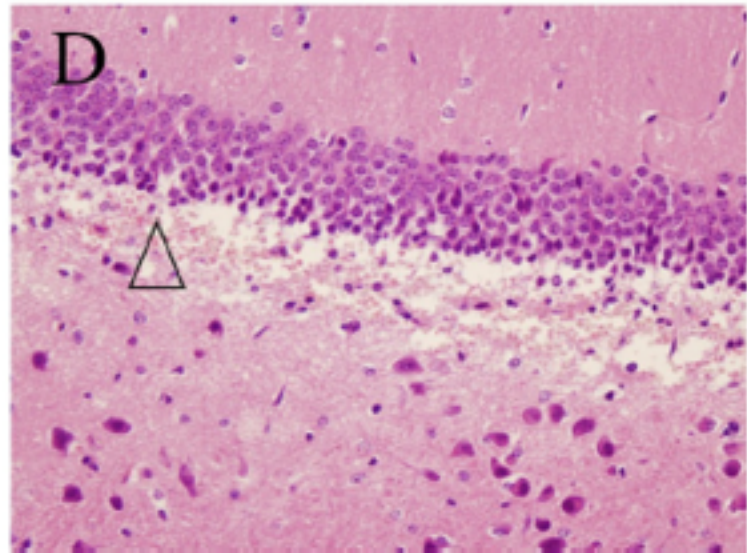
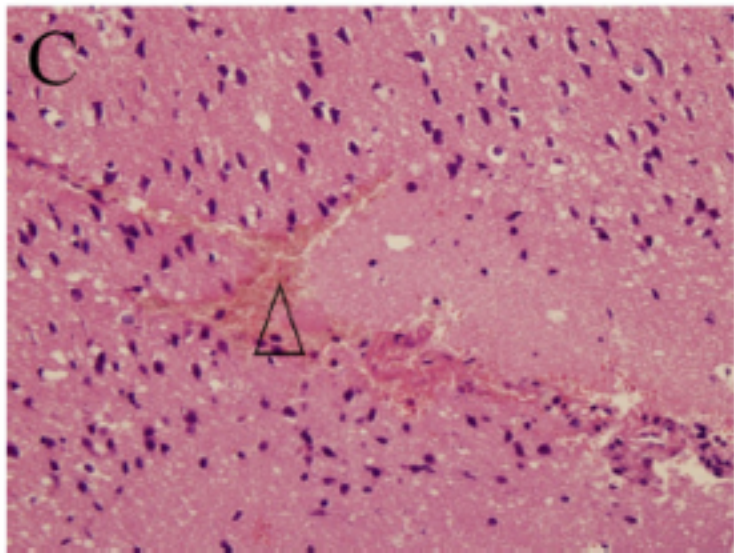
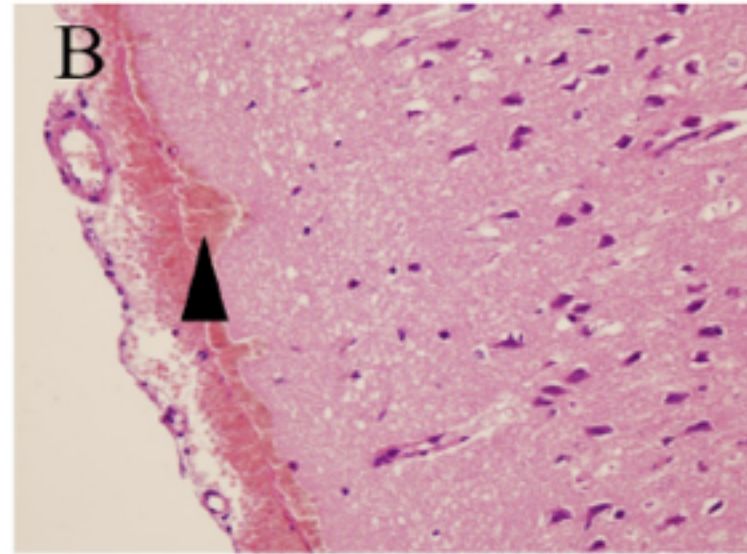
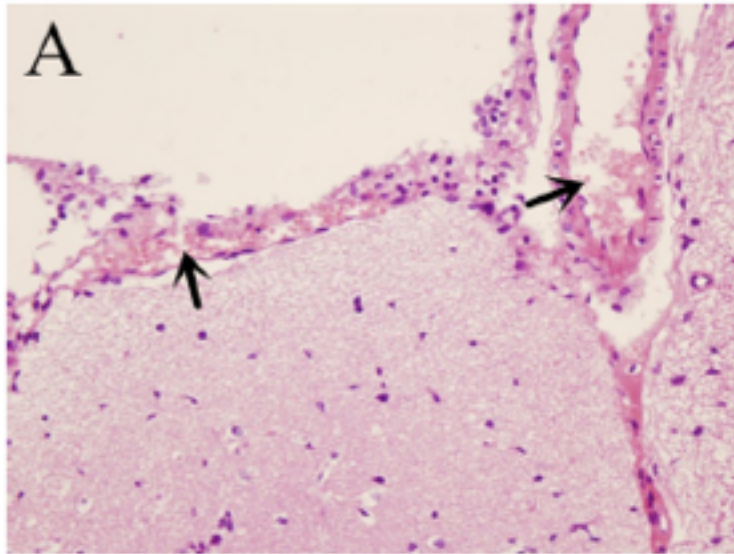
Experimental Procedure - Western blot analysis

- hippocampal tissue was centrifuged and homogenized for 15min
- BCA protein assay kit to measure concentration of protein
- Beclin-1, LC3 and p-mTOR were separated by electrophoresis
- after several washes with TBST buffer: blocking of membranes
- incubated with
rabbit anti-Beclin-1, anti-mTOR, anti-GAPDH, anti-LC3B

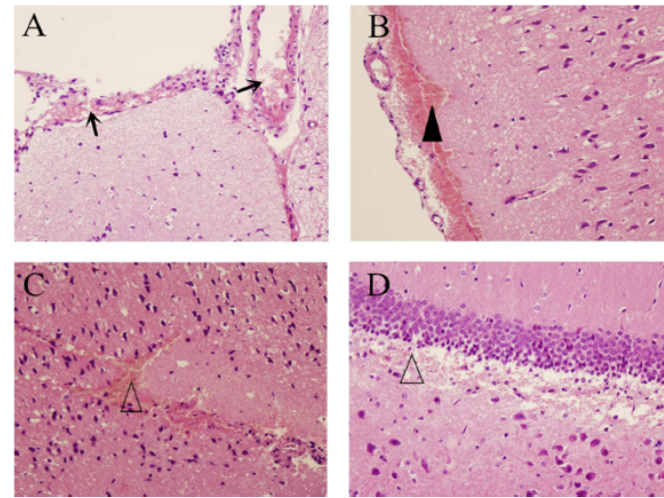
Results - Histology



- brains with a histologically verified TBI were processed with HE-staining
- histological findings:
subarachnoid congestion and focal hemorrhage,
small vessel congestion and small focal hemorrhage



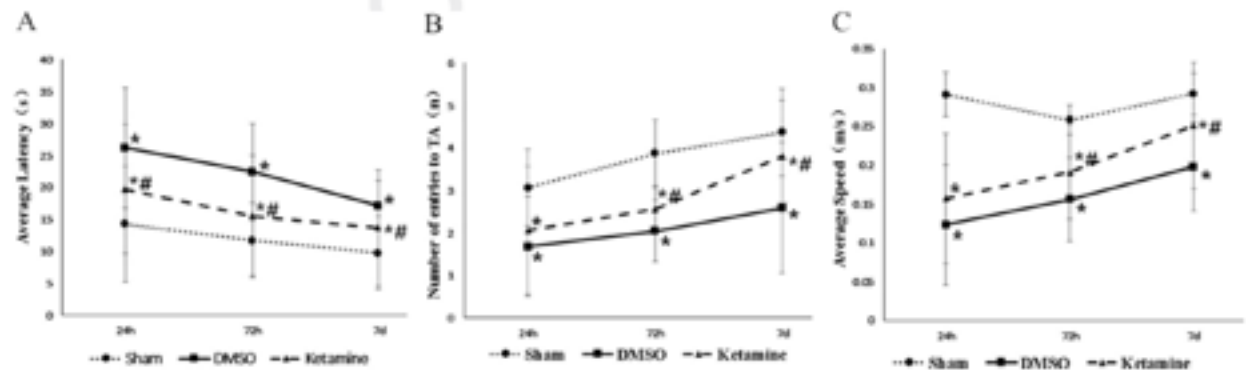
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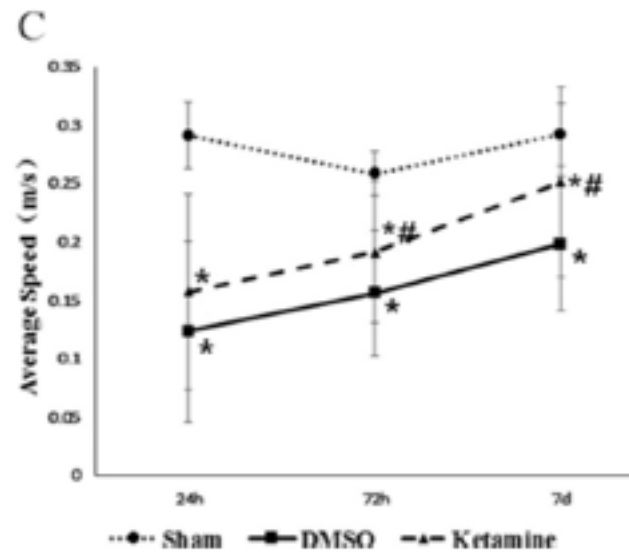
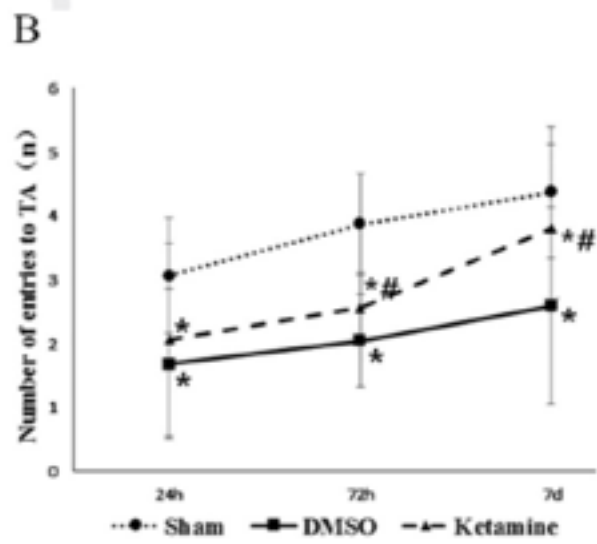
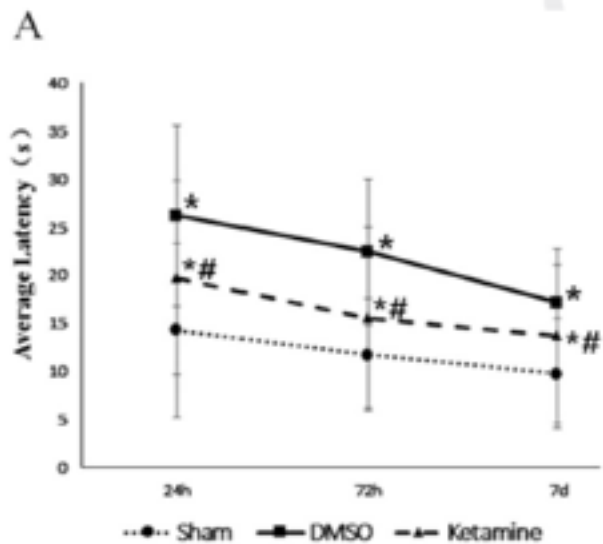


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Results - Memory and spatial learning

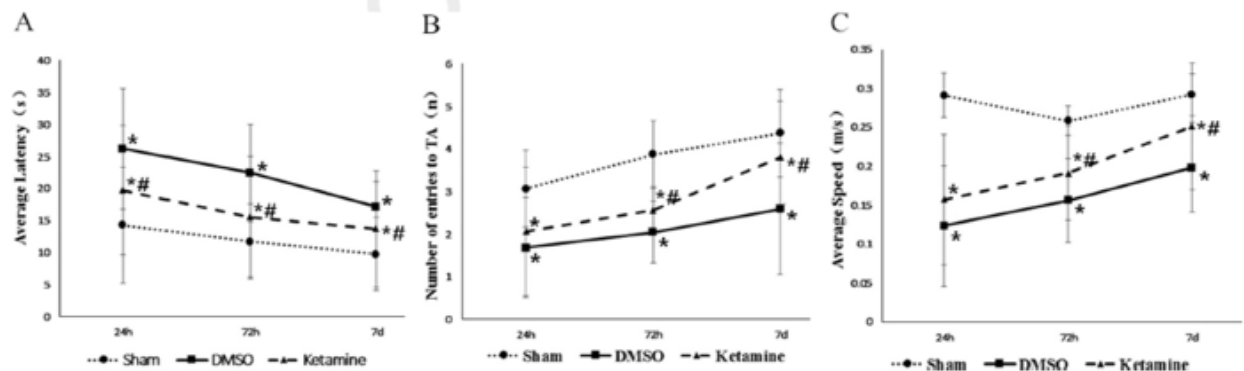
- escape latency in the MWM
- number of entries into the target quadrant TA
- average swimming speed





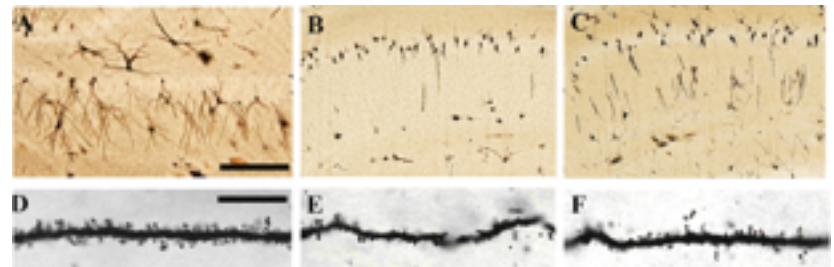
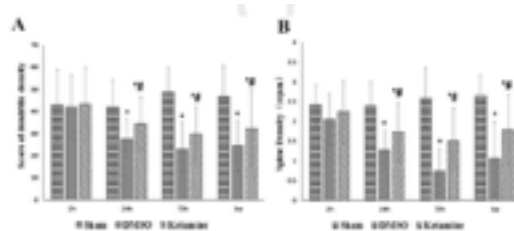
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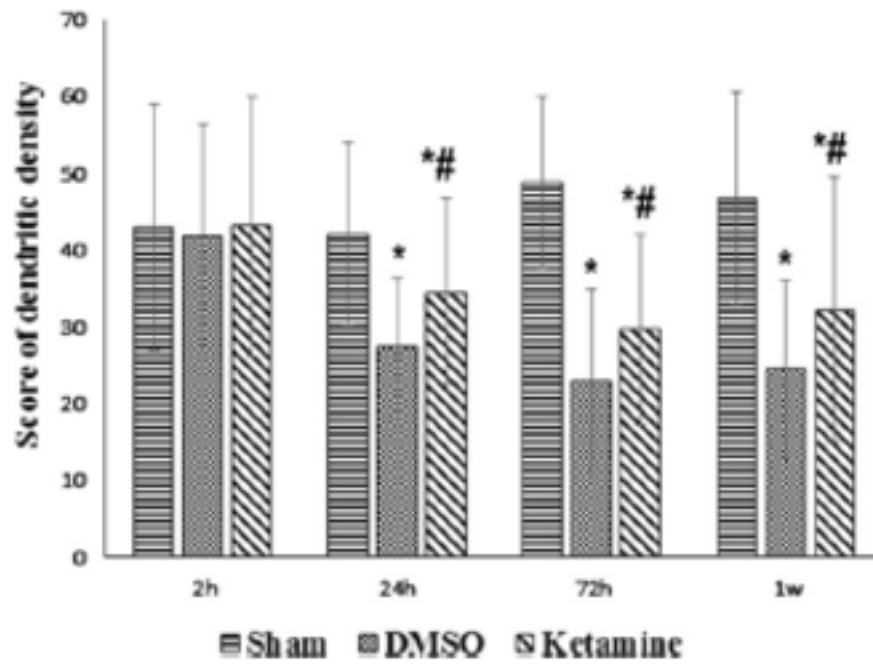
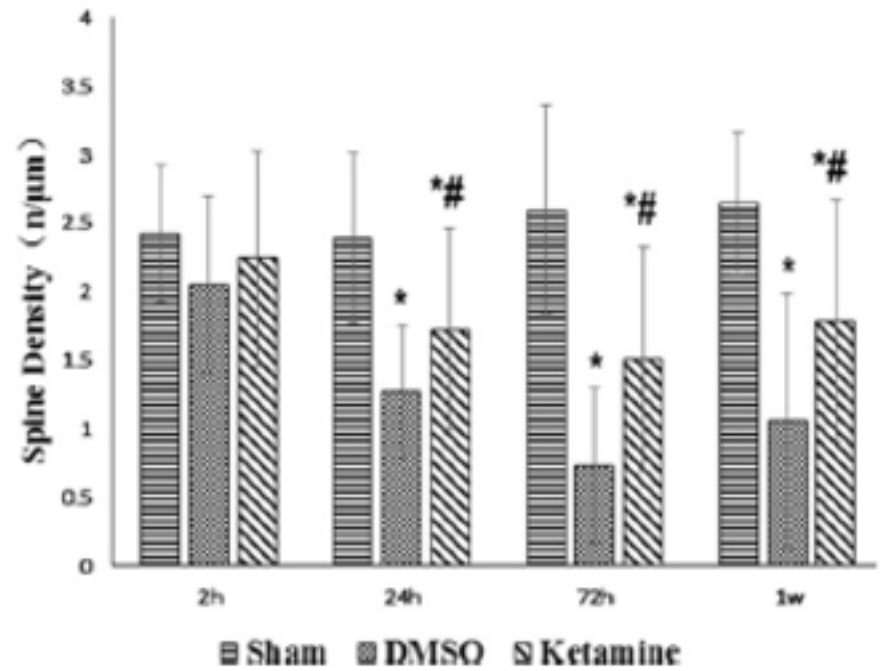
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Results - Changes in dendrites and dendritic spines

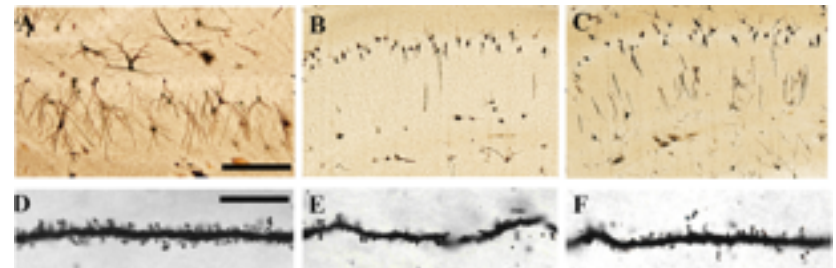
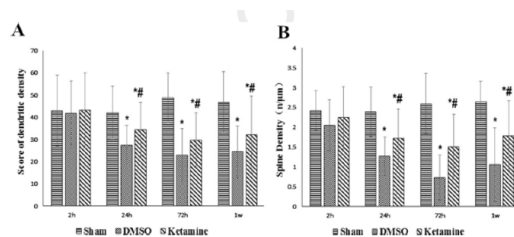
- The branching and dendritic length of the neurons in the DMSO group was considerably less than that in the sham group at 1d, 3d and 7d. No considerable difference at 2h.
- In the ketamine group the deficits in the dendrites were enhanced when compared with that observed in the sham group, yet they were less than that in the DMSO group.
- the change in the dendritic spines in the 3 groups showed a similar trend



A**B**

Results - Changes in dendrites and dendritic spines

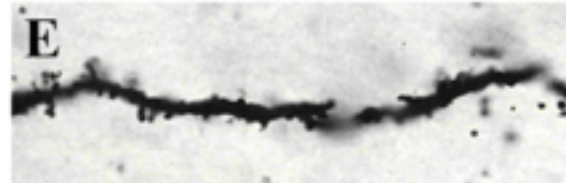
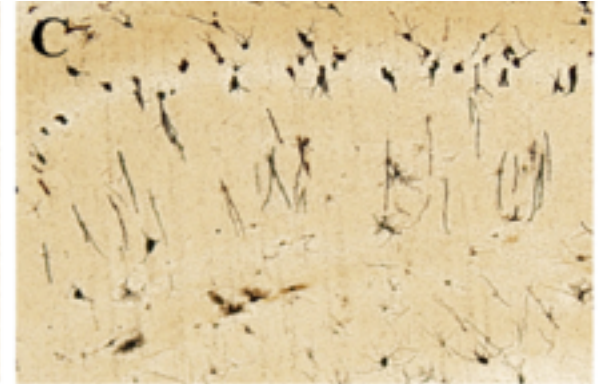
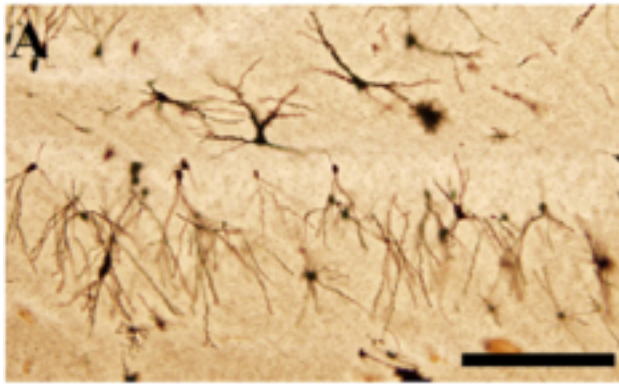
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Sham group

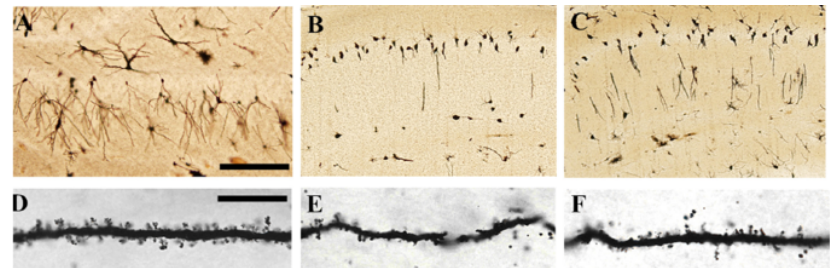
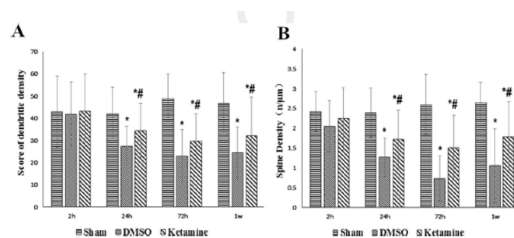
DMSO group

Ketamine group

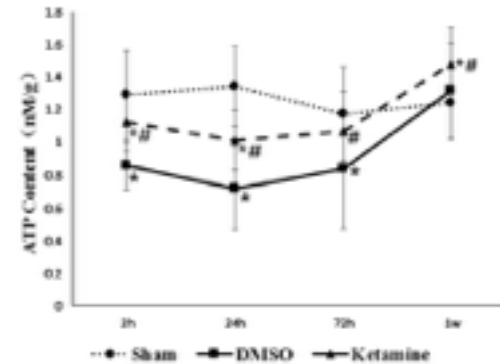


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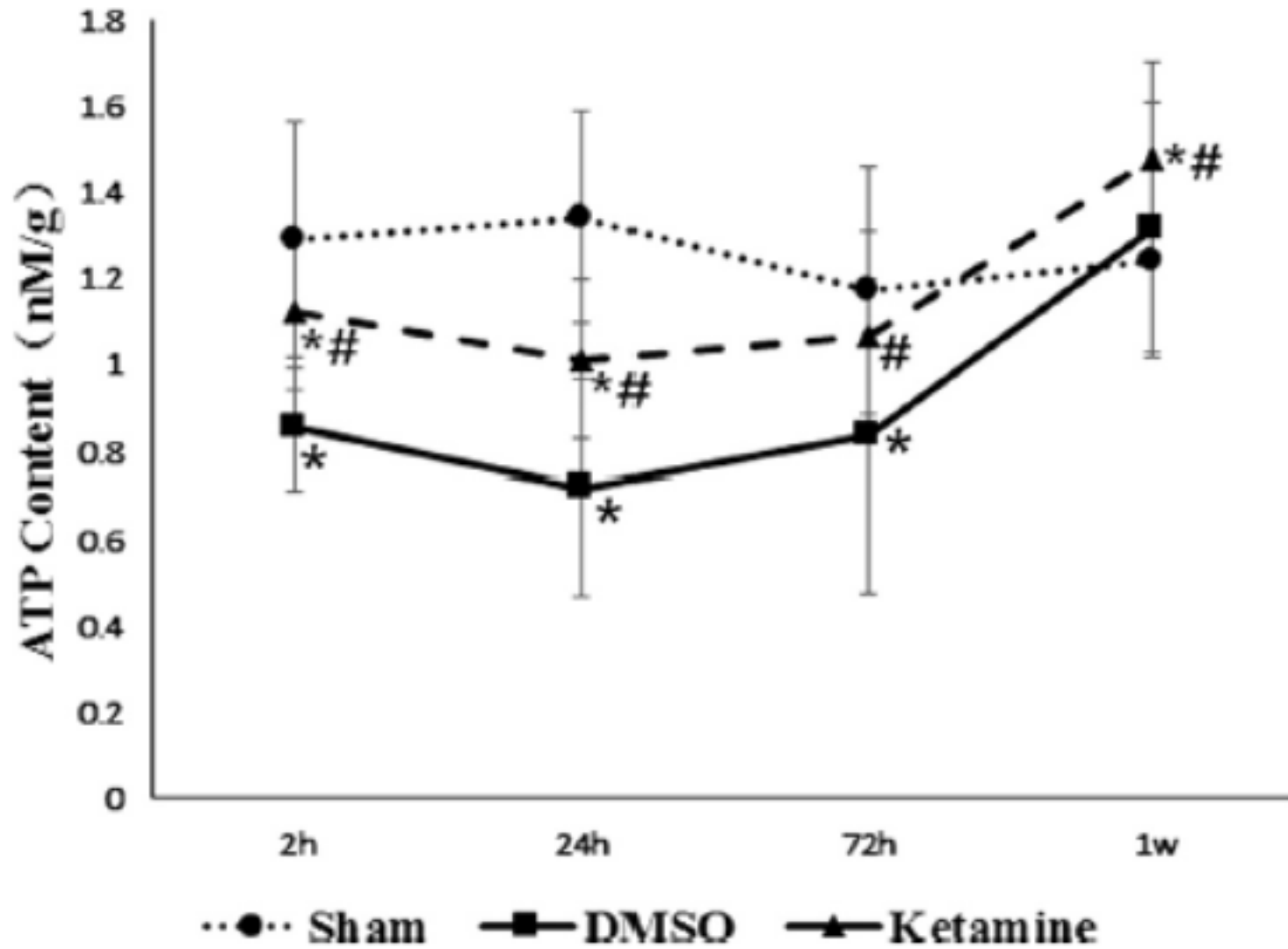
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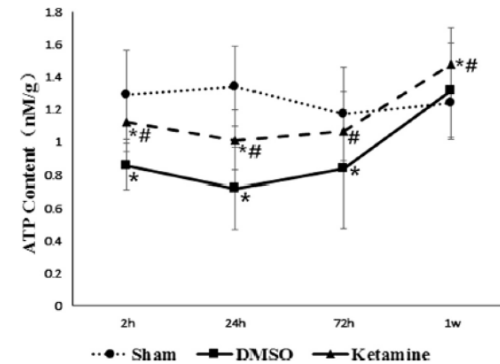
Results - Effect on ATP



- mitochondrial dysfunction as a common characteristic after TBI
- changes in ATP concentration in the DMSO and Ketamine group
initial considerable reduction 1d ($P < 0,001$)
followed by a spontaneous recovery until the 7th day
- Ketamine group: decreased reduction and increased recovery
7th day: compared to DMSO $p = 0,011$; to sham $p < 0,001$



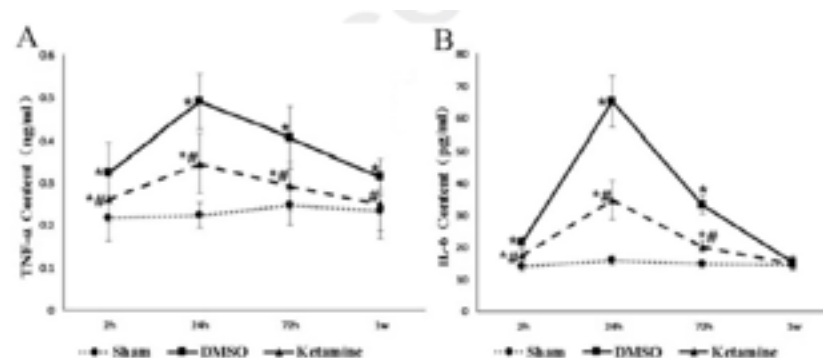
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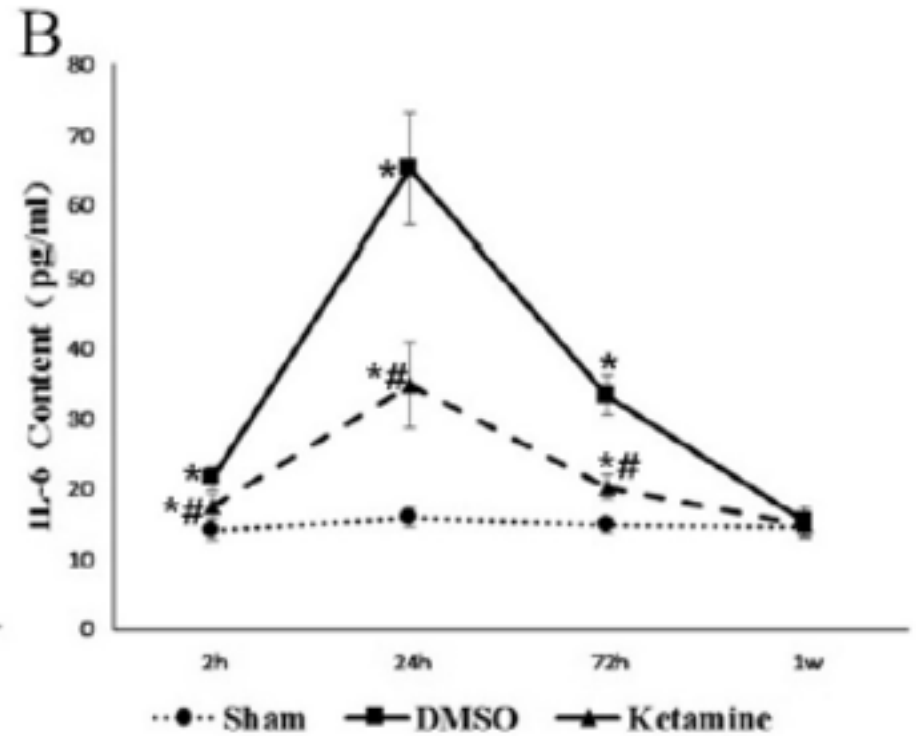
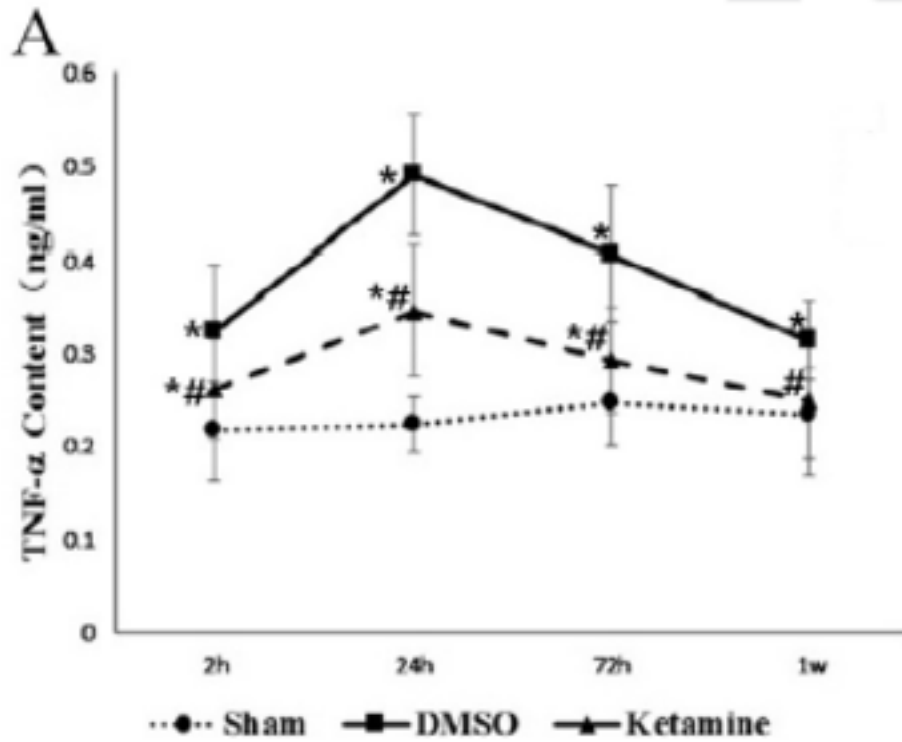


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Results - IL-6 and TNF alpha production

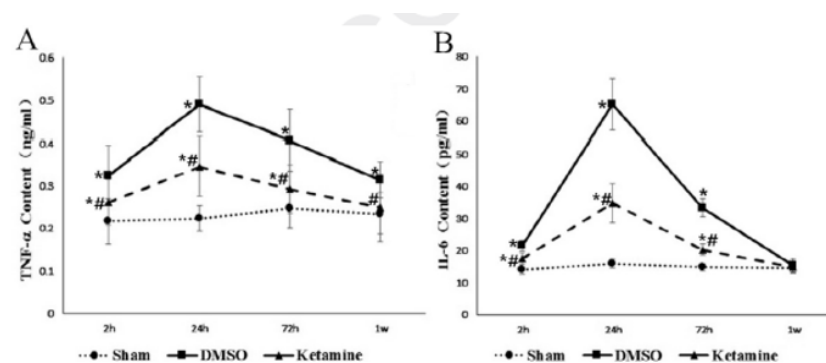
- enhanced concentrations of IL-6 and TNF alpha were observed in the tissue ($P < 0,001$)
- Ketamine reduced the expression of IL-6 and TNF alpha ($P < 0,05$) at 2h, 1d and 3d post TBI
no difference at 7d post TBI
- the degradation of TNF alpha was slower in the DMSO group





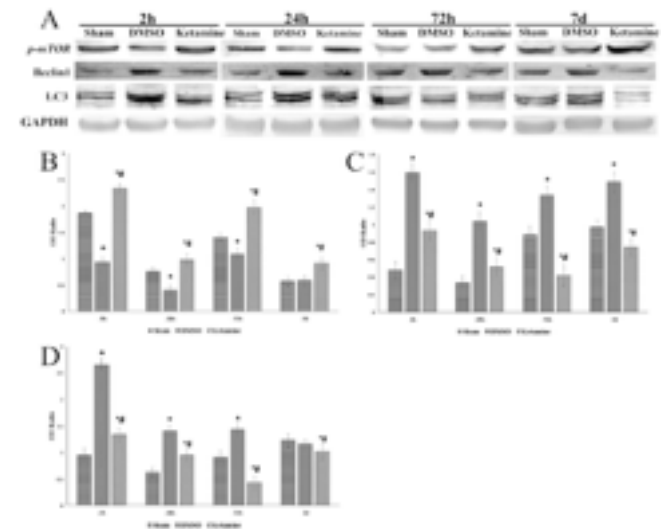
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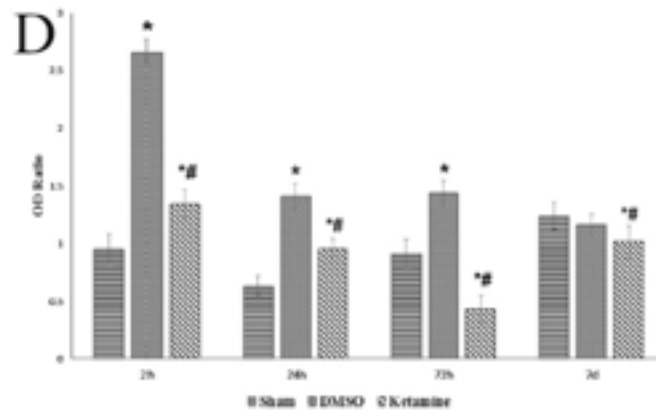
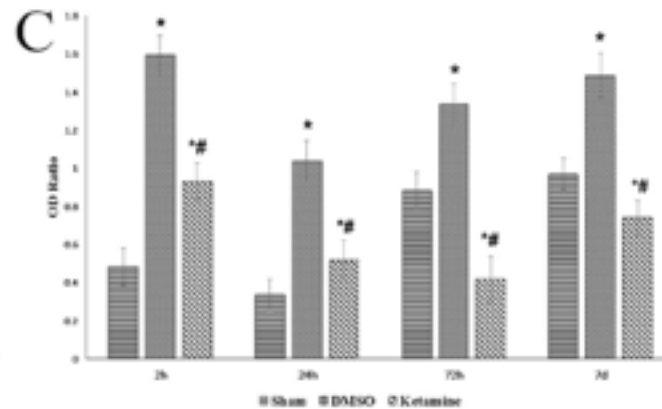
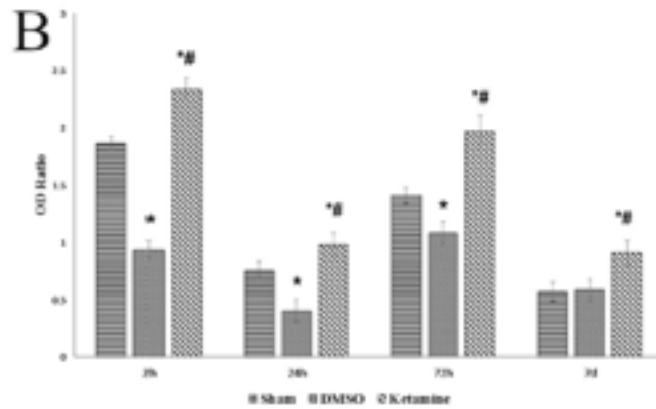
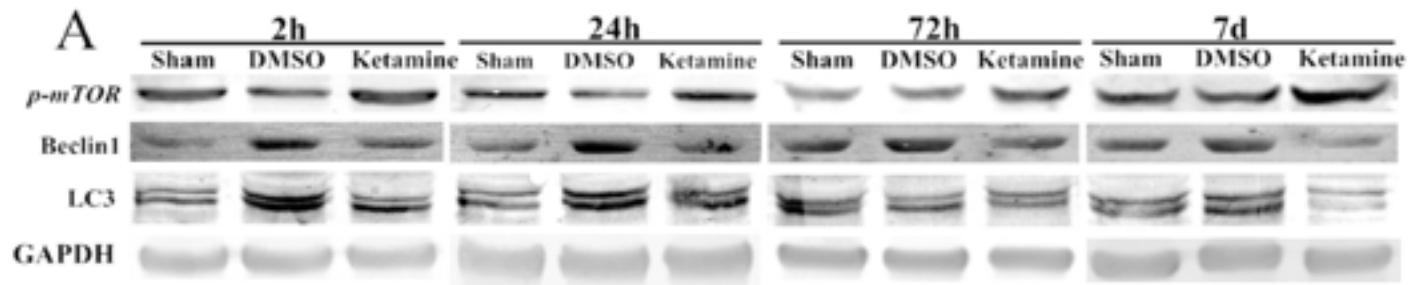
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Results - Autophagy

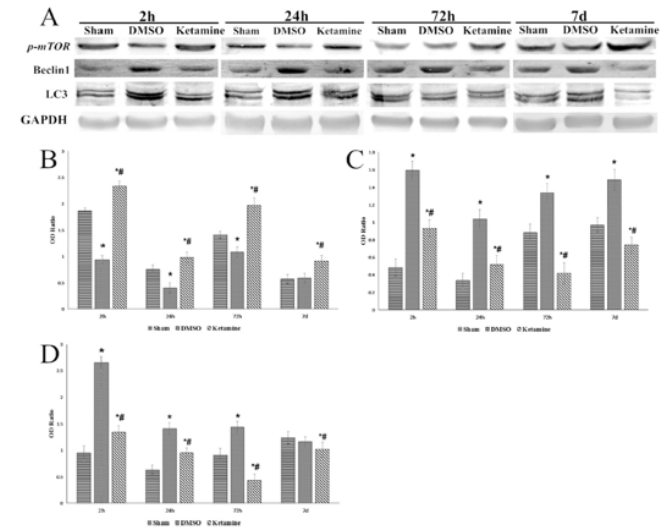
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Discussion 1

- higher and more frequent Ketamine doses may lead to a reduction in the number of glutamate synapses caused by a long term increased neurotoxicity
- lower and smaller frequent Ketamine doses have significant neuroprotective effects, increasing the synaptic spine density and efficiency and improving synaptic plasticity
- Ketamine can result in cognitive impairment, particularly in the domains of semantic and episodic memory
- low doses of Ketamine had a neuroprotective effect, weakening memory impairment and strengthening spatial learning

Discussion 2

- energy stores were maintained when Ketamine was added to cultures immediately before an anoxic exposure
- Ketamine results in a considerable attenuation of the TBI induced increases in IL-6 and TNF alpha, thereby improving survival
- reduction in the expression of the negative regulator of autophagy mTOR was observed, causing an increase in LC3 and Beclin-1 expression

My opinion and criticism

- very well written with a good structure
- the neuroprotective potential of Ketamine was based on only one sub-anesthetic dose used. Therefore the effects of an anesthetic dose of Ketamine on TBI are not clear!
- Ketamine often prevents hypotension in the brain, why not measure the blood pressure at given time points?
- Ketamine is said to increase the ICP, why not measure the ICP at given time points?

Adams SD, Radhakrishnan RS, Helmer KS, Mercer DW (2008) Effects of anesthesia on lipopolysaccharide-induced changes in serum cytokines. *J Trauma* 65:170–174.

Amini M, Ma CL, Farazifard R, Zhu GQ, Zhang Y, Vanderluit J, Zoltewicz JS, Hage F, Savitt JM, Lagace DC, Slack RS, Beique JC, Baudry M, Greer PA, Bergeron R, Park DS (2013) Conditional disruption of calpain in the CNS alters dendrite morphology, impairs LTP, and promotes neuronal survival following injury. *J Neurosci* 33:5773–5784.

Belanger HG, Vanderploeg RD (2005) The neuropsychological impact of sports-related concussion: a meta-analysis. *J Int Neuropsychol Soc* 11:345–357.

Bhutta AT, Schmitz ML, Swearingen C, James LP, Wardbegnoche WL, Lindquist DM, Glasier CM, Tuzcu V, Prodhan P, Dyamenahalli U, Imamura M, Jaquiss RDB, Anand KJS (2012)