Effects of a caspase inhibitor on naturally occurring cell death in the mouse brain

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Introduction

Apoptosis , or programmed cell death, is an essential process in the development of the nervous system. More than half of the neurons initially produced are eliminated by cell death, predominantly in the first week of life in mice.

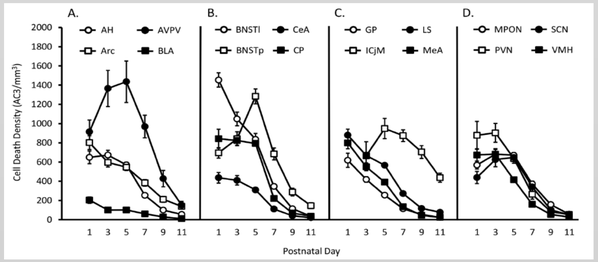
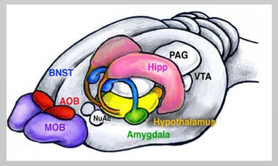
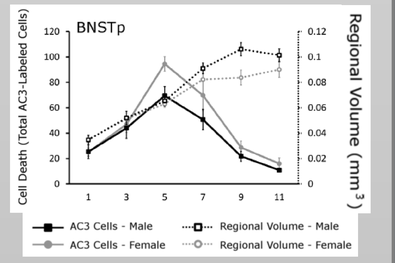


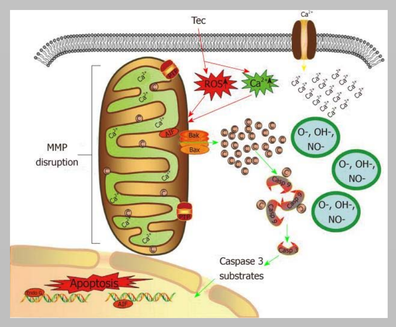
Fig. 1 Cell death in different brain regions over time

There are sex differences associated with this cell death, particularly in the bed nucleus of the stria terminalis (BNST).





Apoptosis is mediated through a cascade of signals that culminates in the activation of caspases.



Introduction

Q-VD-OPh is a recently developed caspase inhibitor shown to prevent cell death after ischemia (induced stroke) and other brain injuries in both neonates and adults.

Experimental Question

- We asked whether Q-VD-OPh could effectively inhibit caspase activation during programmed cell death.
- If so, are sex differences eliminated in animals administered Q-VD-OPh?

Prediction

- If Q-VD-OPh inhibits developmental cell death, the number of caspase-3 activated cells in the BNST will be lower in mice injected with Q-VD-OPh than in control mice. Conversely, the volume of the BNST will be greater in mice injected with drug than in vehicle.
- Cell death in the BNST will be greater in females than males
- Overall volume of the BNST in males will be greater than females.

Methods

64 newborn mice total.

32 injected with 40µg of Q-VD-OPh in 10µL of DMSO, 32 injected with 100% DMSO on postnatal days 3 and 4.

16 from each group (8 males; 8 females) were sacrificed at postnatal day 5, which is the peak of cell death in the BNST, and the remaining 16 in each group were sacrificed at postnatal day 12, which is after cell death.

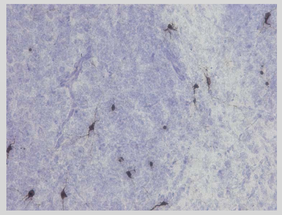
Brains were collected then frozen-sectioned.

Postnatal day 5 brains were labeled for activated caspase-3 (AC3) cells by immunohistochemistry (IHC). The number of AC3 cells in the BNST were then counted.

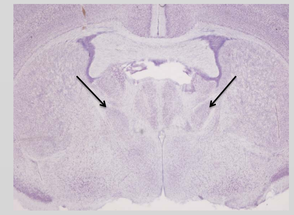
Postnatal day 12 brains were stained with thionin to label all cells and to determine the volume of the BNST.

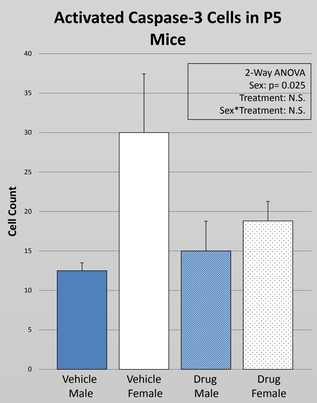
Results

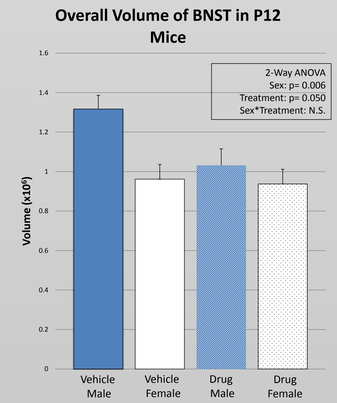
IHC for activated caspase-3



Thionin staining (BNST indicated)







Conclusions

1. We confirmed sex differences in the number of activated caspase-3 cells in the BNST as well as the volume of the BNST in both Q-VD-OPh and vehicle injected animals.
2. Multiple studies have shown that Q-VD-OPh prevents *injury-induced* cell death. Our results suggest that the molecular mechanism of naturally occurring developmental may differ from injury-induced cell death.
3. Lack of an effect of Q-VD-OPh on developmental cell death may indicate a caspase-independent backup pathway of cell death, or that our dose was not optimum

References

Todd H. Ahern et al., 2013. Cell Death Atlas of the Postnatal Mouse Ventral Forebrain and Hypothalamus: Effects of Age and Sex. J Comp Neurol. 521(11):2551-69.

Acknowledgements

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