Analysis of Corpus Callosum and Ventricles in Brain Tissue of MK801/CDPPB Treated C57BL6/J Mice

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Background

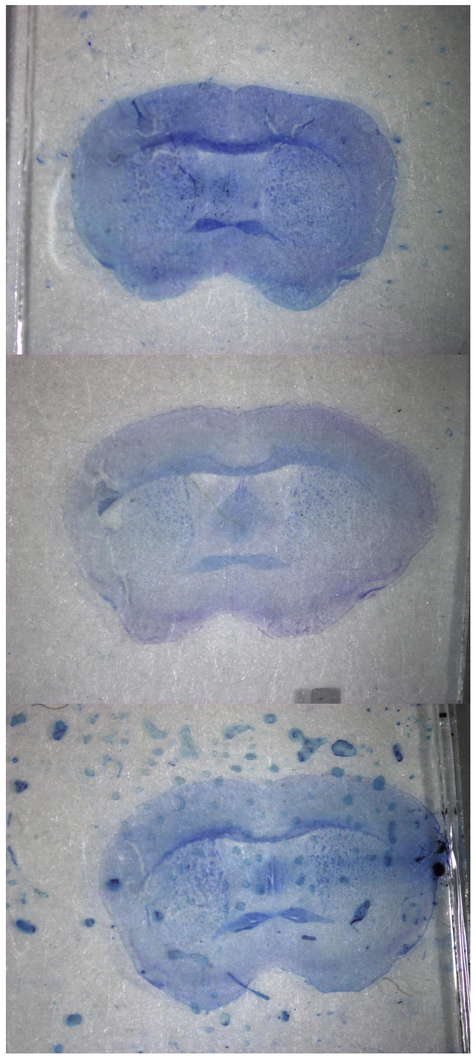
The drug MK-801 has been used to create a rodent model of schizophrenia. MK-801 noncompetitively blocks the NMDA glutamate receptor in rodents and humans, and receptor hypofunction is associated with positive, negative and cognitive symptoms of schizophrenia. NMDA hypofunction also contributes to glutamate excitotoxicity. Subsequent effects such as enlarged cerebral ventricles can be seen in clinical schizophrenia and its animal models. Exposure to MK-801 during early development has also been shown to reduce the size of the corpus callosum in rodent models.

The present study examined whether the positive allosteric modulator of the NMDA receptor, CDPPB, would affect the size of the ventricles and corpus callosum in rodents treated with MK-801. CDPPB is thought to increase NMDA activity, possibly inhibiting some of MK-801's NMDA blockade. Previously, our lab found that CDPPB inhibits some of the psychotomimetic effects in the MK-801 rodent model of schizophrenia. The present study could further elucidate the mechanism of these effects by examining whether treatment with CDPPB can block the ventricle enlargement and corpus callosum shrinkage in rodents exposed to MK-801 during the neonatal period.

Methods

- **Mouse brains from a no-injection control group (n=4), a saline-saline control group (n=2), an MK-801-saline group (n=4), and MK-801-CDPPB group (n=2) were examined. Brains were preserved, flash-frozen, and sectioned using a cryostat.**
- **Relevant areas of brain tissue in the Bregma 0.26mm region were then collected and stained with Cresyl Violet and Luxol Fast Blue to highlight myelinated structures, including the corpus callosum.**
- **After coverslipping, tissue sections were imaged with Infinity Analyze software running a Luminera Infinity 2 camera mounted on a Zeiss Stemi 2000-C microscope.**
- **Morphometric measures were collected from the imaged tissue sections using ImageJ software. First, calibration was performed using a microscope reticle and ImageJ's "Set Scale" tool.**
- **After setting the scale, tissue sections were measured using the "polygon selections" tool to trace relevant neural structures. Traced sections were then measured for area. The total section area, the area of the ventricles, and the area of the corpus callosum were all recorded.**
- **Ventricles to total area ratios and corpus callosum to total area ratios were calculated, and each group's ratios were averaged together. Final averages were compared across each group to produce the results.**

Results



Top: representative section of a mouse brain treated with saline as a neonate and as a juvenile (control condition)

Middle: mouse brain treated with MK-801 as a neonate and saline as a juvenile (toxicity condition)

Bottom: mouse brain treated with MK-801 as a neonate and CDPPB as a juvenile (rescue condition)

The saline and CDPPB treated sections have ventricles of a similar size, while the mouse treated with MK-801 and saline (middle), has visibly larger ventricles.

Limitations

- **Sample size was limited due to the small number of properly sectioned brains.**
- **Many brain sections were lost due to cryostat equipment malfunctions.**
- **The time span between behavior testing and imaging was lengthy, which caused problems with tissue preservation.**
- **Many available brain sections did not contain the ventricle and corpus callosum regions of interest examined in this study.**

Discussion

- **This study seemed to indicate some increase in ventricle size and a decrease in corpus callosum in the brains of mice treated with MK801.**
- **Due to the overall small sample size (n=10), no firm conclusions can currently be drawn about the effect of MK801 or CDPPB on the myelinated structures in the mice.**
- **The staining and measuring procedures proved useful for collecting data for the study and going forward, could make completing this project attainable.**
- **It is important to determine if MK-801 and/or CDPPB effects can produce sex-specific effects.**

Future Directions

- **Generating a new cohort will boost sample size. This will allow for a more comprehensive analysis of susceptible behaviors as well as myelinated brain structures.**
- **The lab cryostat has been replaced, which allows us to freeze the sample brains at a lower temperature, thus producing better quality tissue sections.**
- **Staining and imaging in a timely manner, immediately after cryostat sectioning will reduce tissue section loss.**
- **CDPPB effects on additional brain structures, as well as other NMDA antagonist drugs, would be informative.**
- **Additional research with appropriate animal models could indicate whether CDPPB should be evaluated in clinical trials as a possible treatment for schizophrenia.**

References

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