ASSESSING FEAR AND ANXIETY BEHAVIORS FOLLOWING INDUCTION OF NEONATAL HYPOXIC ISCHEMIC ENCEPHALOPATHY

Hackett, MB.[1]; Ozaydin B.[1,2]; Deveci, N.[1]; Yapici, S[1]; Aycan, N.[1]; Keles N. K.[1]; Ferrazzano P.[1,3]; Levine J.[4]; Cengiz P.[1,3]

[1]Waisman Center, University of Wisconsin, Madison, WI, USA; [2]Department of Neurological Surgery University of Wisconsin, Madison, WI, USA; [3]Department of Pediatrics, Division of Critical Care Medicine, University of Wisconsin, Madison, WI, USA; [4]Department of Neuroscience, University of Wisconsin, Madison, WI, USA

UW Health American Family Children's Hospital

Department of Pediatrics UNIVERSITY OF WISCONSIN SCHOOL OF MEDICINE AND PUBLIC HEALTH

BACKGROUND

Neonatal hypoxia ischemia (HI) related encephalopathy is one of the major causes of learning disabilities and memory deficits in children. The learning disabilities and memory deficits may be related to increased fear and anxiety behavior following neonatal HI. Our recent findings reveal that tyrosine kinase B receptor (TrkB) agonist, 7,8-dihydroxyflavone (7,8-DHF), exerts a neuroprotective effect in the hippocampi of female but not male neonate mice due to TrkB phosphorylation post-HI. We hypothesized that fear response and anxiety behavior is ameliorated with tyrosine kinase B agonist 7,8-DHF only in females following neonatal HI.

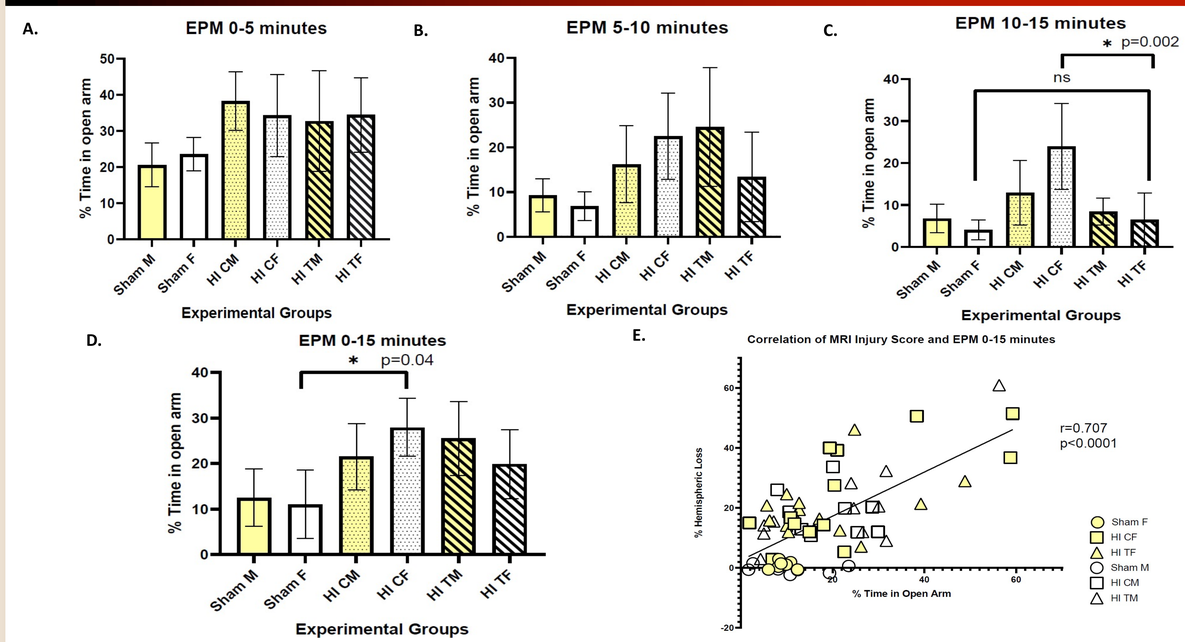
METHODS

HI was induced in postnatal (P) day 9 C57BL/6J mice by using Vannucci's HI model. Mice were treated with 7,8 DHF (5mg/kg, i.p.) or vehicle control starting at 10 min post-HI for 7 days. At P 90+, mice underwent elevated plus maze (EPM) testing for 15 minutes. Fear and anxiety behavior was assessed at 0-15 min, 0-5 min, 5-10 min and 10-15 min intervals. Video recordings of the EPM were analyzed using AnyMaze software. Percent time spent in open arm is reported in 95% CI [LL, UL]. ANOVA was used to analyze the EPM data with significance set at $p \leq 0.05$.

Following the EPM test, MRI was performed under anesthesia using 4.7-tesla small animal MRI. Masks of ipsilateral and contralateral hemispheres were created for each slice using ImageJ. Mean hemispheric areas were calculated from 8-11 slices/brain. CSF containing spaces were excluded from hemispheric area measurements. The data is presented as the difference in CL versus IL area for each brain as (CL−IL)/CL×100 and reported as percent hemispheric area loss. Percent time in open arm (EPM) and IL hemispheric area loss is fitted with linear regression and correlation statistics.

- There was a sexually differentiated effect of treatment with TrkB agonist in fear response, returning HI treated female mice to sham levels of open arm exploration. TrkB mediated long-term neuroprotection in females following hypoxic ischemic brain injury maybe through preserving the neural networking through the limbic system, hippocampus and amygdala.
- Hemispheric area loss due to HI is significantly positively correlated with abnormal fear response as measured by time spent in the open arm of the EPM apparatus. This suggests that the loss of fear response is directly related to the severity of injury.

RESULTS



CONCLUSIONS

- TrkB agonist therapy recovers fear response to sham levels only in female mice. However, this effect is only observed during the final 5 minutes of testing when all the mice included in the analysis. We propose that the mice spend the first 10 min habituating to the open and closed arms. The mice explore the environment prior to interpreting the danger of its surroundings.
- Percent time spent in the open arm of the apparatus has a significant positive correlation with percent hemispheric loss measured by MRI. This correlation indicates that as severity of hypoxic ischemic injury increases, normal fear response decreases in the animal.
- Further stratification of the severity of injury along with additional fear and anxiety testing is needed to explore this phenomenon.

ADDITIONAL KEY INFORMATION

Figure 1: No significant differences between treatment groups were recorded at the 0–5-minute segment **(A.)** and the 5–10-minute segment **(B.)** of testing. During the final 5 minutes of testing **(C.)** significant differences in time spent in the open arm were recorded between HI CF and HI TF mean diff 17.4, CI [4.7, 30.1] p=0.0002 while HI TF and Sham F explored at nearly identical levels mean diff -2.440, CI [-16.1, 11.2] p=0.9. Over the course of the entire test **(D.)**, significant differences in exploration were only recorded between Sham F and HI CF mean diff -15.69 CI [-31.1 -0.3] p=0.04. Hemispheric loss was significantly correlated with percent time spent in the open arm of the EPM apparatus r=0.707, p<0.001 **(E.) n=8-14**

Acknowledgements:

- Department of Pediatrics R and D Grant
- NIH/NINDS K08 NS088563
- NIH/NINDS R01 NS111021
- NIH Waisman Core Grant U54HD090256

References:

1. Cikla et al., eNeuro. 2016.
2. Vannucci, R. C. Ann N Y Acad Sci. 1997.