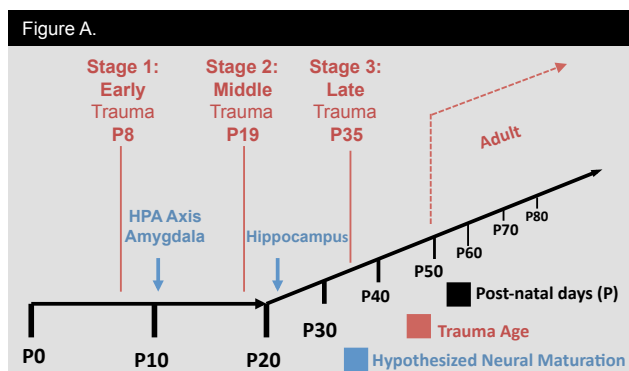


SPECIFIC AIMS

Childhood trauma is a major risk factor for the adult development of Post-traumatic stress disorder (PTSD), yet determining the underlying causes of this relationship has remained extraordinarily challenging. In particular, determining how the functional state of the brain at the time of trauma contributes to PTSD at its biological underpinnings remains an elusive goal. Animal models focused on efforts to examine early life trauma during the first 2 weeks of life have mainly used non-painful stressors such as mother-pup separation procedures. These efforts have uncovered varying results on adult learning, anxiety and neuroendocrine function. A disparity between human physical abuse, which has a pain component and the animal mother-pup separation paradigm, may pose a significant hurdle in determining how the maturational state of the brain at the time of trauma contributes to adult PTSD.

Standard fear conditioning procedures in the developing rodent can be applied to this problem, by delivering a series of painful footshocks at different functional stages of brain maturation. This procedure when presented at different post-natal ages has yielded important insights into the ontogeny of fear learning. Our laboratory has substantial experience in adult fear conditioning, with the above procedure in the mature animal resulting in a life-long memory of the trauma that depends on the basolateral amygdala (BLA) hippocampus (HIP) and prefrontal cortex (PFC). Importantly, if this fear experience is strong enough it results in a sustained vulnerability to over exaggerate fear responses even to mild reminders of the original trauma. This sensitized fear responding along with anxiety are central characteristics of PTSD.

Our conceptual framework is that the neural circuits that regulate fear are driven by two non-mutually exclusive processes: 1) an associative mnemonic component that establishes predictions between distributed contextual-spatial information and painful nociceptive input. 2) a non-associative process that sensitizes future fear responding to painful nociceptive input. Thus, under conditions in which predictions are limited, non-associative processes prevail at the cost of an overt fear memory. We apply this dual process view of fear regulation to the knowledge that infantile amnesia results from the immaturity of the hippocampal systems that process contextual spatial cues and this inability to use these predictive cues in the face of danger may result in the long-term sensitization of fear and neuroendocrine circuits. This sensitization, under conditions of diminished prediction is a possible mechanism by which early traumatic experience results in adult PTSD. It should be noted that depression, which is often co-morbid with anxiety disorder is often theorized to result similarly from unpredictable/uncontrollable stressful events.



Our approach identifies 3 key hypotheses based on the timing of early life trauma (Figure A): 1) **Early (P8) Post-natal trauma** during **Stage 1**: Prior to the functional maturation of the amygdala, fear learning systems cannot be sufficiently activated. 2) **Middle (P19) Post-natal trauma** during **Stage 2**: After the functional maturation of the amygdala, but prior to the maturation of the hippocampal context processing pathways, associative fear responses cannot be established, yet non-associative sensitization of fear and neuroendocrine responses persist. 3) **Late (P35) Post-natal trauma** during **Stage 3**: With the maturation these two systems both appropriate conditional responses can be acquired as well as a sustained adult enhancement of amygdala-based fear learning. It will be shown in

the Preliminary Studies that we have begun to solve this by showing that unlike adult animals that trauma in **early post-natal** rats neither shows a fear memory nor later adult sensitized fear responses. However, foot shock trauma delivered during a **middle post-natal** period, which similarly fails to show a fear memory, yet results in a long-lasting sensitization of adult fear and neuroendocrine systems. We are proposing to identify the functional status of fear neural pathways during early life trauma and how this contributes to development of adult PTSD related symptomology including fear, depression and social and generalized anxiety. We address this, in the following specific aims:

Aim 1: Immediate Impact & Functional Status of neural pathways and neuroendocrine responses to Early-life Trauma.

To test this both retrograde and anterograde neuroanatomical tract tracers will be targeted within the basolateral amygdala (BLA) in combination with immediate early gene (IEG) expression to repeated footshock trauma or upon being returned to the original trauma context. The level and locus of IEG activity in regions that project to and from the BLA will be analyzed in respect to behavioral and neuroendocrine measures of fear and stress, respectively.

Aim 2: Long-Lasting Impact of Early Life Trauma on Adult Neuroendocrine Function, Fear, Anxiety and Depression.

To test this: 1) Adult fear memory of early-life trauma will be assessed as well as the characterization of new fear learning, depression and (social and generalized) anxiety. 2) Neuroendocrine biomarkers of long-lasting stress related changes including basal and evoked levels of corticosterone (stress), neuropeptide Y (stress resilience) and insulin like growth factor-2 (fear memory).