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"CHRI"sis at the NICU: The Medley with Midazolam

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“CHRI”sis in the NICU: The Medley with Midazolam

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Summer Undergraduate Research Program

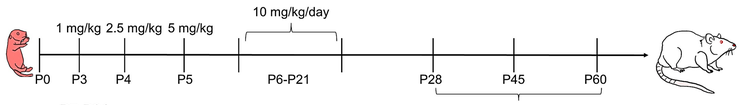
Introduction

Approximately 1.5 million neonates undergo anesthesia for surgical procedures in the United States every year[1]. Notably, **Midazolam,** a commonly used anesthetic agent is used in the Neonatal Intensive Care Unit (NICU) to sedate neonates and facilitate complex procedures such as mechanical ventilation.[2] One knowledge gap pertaining the use of midazolam is its ability to affect cognitive development of infants. In 2014, the International Anesthesia Research Society released a statement saying, *“Surgeries and procedures requiring anesthetic and sedative drugs that could reasonably be delayed should possibly be postponed because of the potential risk to the developing brain of infants, toddlers, and preschool children”.* [3] Although some evidence has shown midazolam exposure could harm an infant’s cognitive development, little is known about the molecular and behavioral underpinnings and if these changes persist into adulthood. In our current study employing a preclinical animal model system, we for the first time present a comprehensive characterization on how early life exposure to midazolam impacts neurodevelopment outcomes at different tiers – phenotypic, molecular and behavioral levels and if these changes persist during early adulthood.

Central Hypothesis

Long term exposure to midazolam at early stages of life can potentially perturb neurodevelopmental outcomes that could further persist into adulthood.

Methods & Design

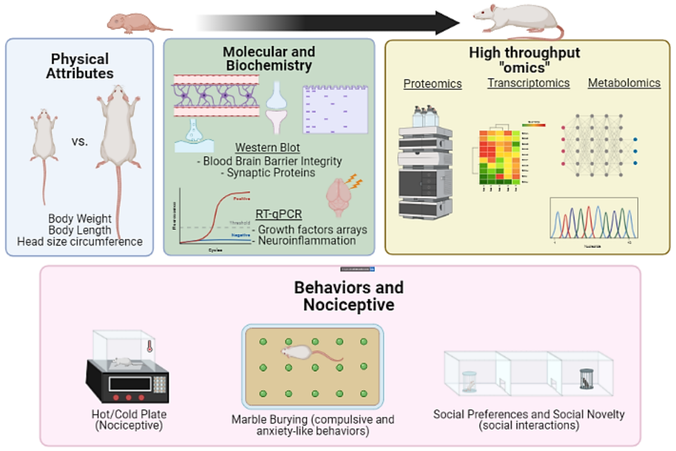


P7-P21:
- Phenotypic measurement at P7, P14, and P21
- Brain collection at P7, P14, and P21
- At P21:
  - Nociception assay (Hot/Cold Plate)
  - Plasma and organ collection

P28-P60:
- Nociception assay (Hot/Cold Plate)
- Marble burying
- Social Novelty and Social Preferences
- At P60: Plasma and organ collection

- P3 ≈ pre-term infant
- P7 ≈ full-term infant
- P14 ≈ critical stage for synaptogenesis
- P21 ≈ early childhood (2-3 yrs)

- P28 ≈ preadolescent
- P45 ≈ adolescent
- P60 ≈ early adulthood



Results

Phenotypic

1

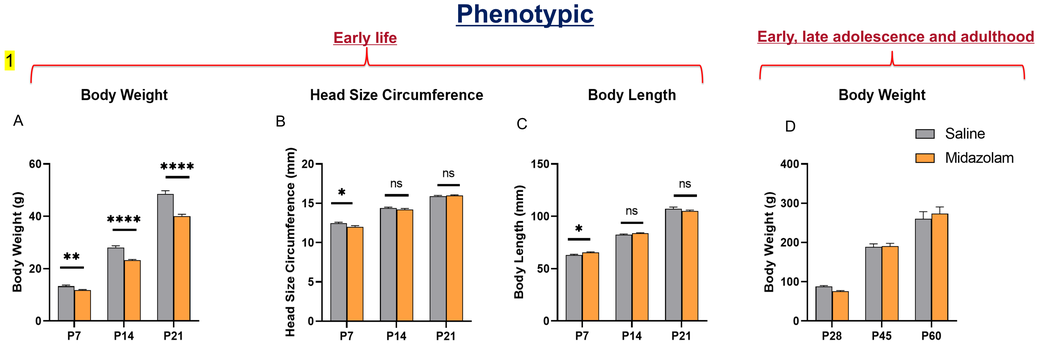


Figure 1. Phenotypic measurements at various time points: At P7 (7 days after birth), there was a significant difference in the body weight, head size circumference, and body length between the saline and midazolam groups. At P14 and P21, there was only a significant difference in body weight between the saline and midazolam groups. These results suggest that physical growth is stunted at childhood but not adulthood. *p <0.05, ** p< 0.01; ****p< 0.0001 as determined by Two-Way ANOVA followed by a post-hoc Sidak’s test. Each bar represents the mean + SEM.

Molecular

2

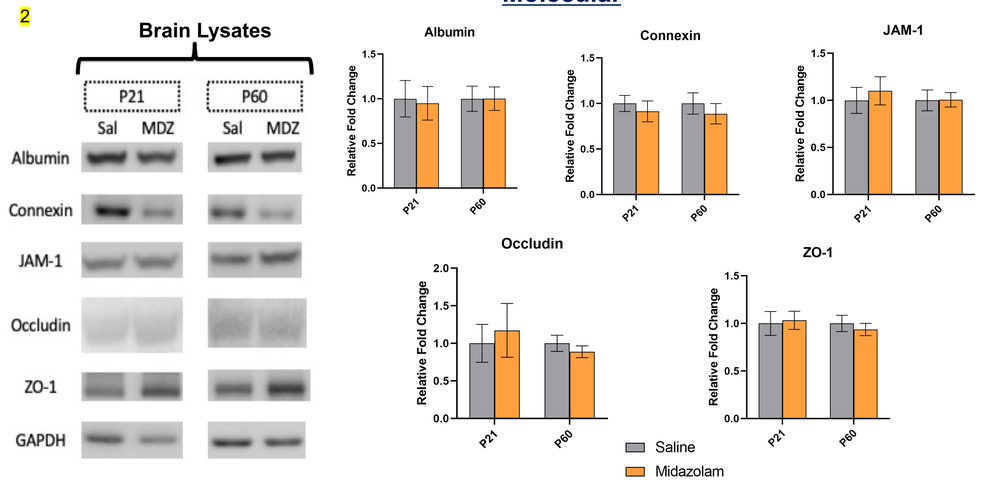


Figure 2. Expression of Blood brain barrier proteins: At P21, the level of albumin, and connexin is slightly downregulated in midazolam-exposed brains, while JAM-1, Occludin, and ZO-1 levels are slightly upregulated. At P60, there is no difference in level of expression of albumin and JAM-1 between saline and midazolam group; however, the level of Connexin, Occludin, and ZO-1 are slightly decreased in midazolam-exposed brains. This suggests that Midazolam potentially alternate the regulation in key blood brain barrier proteins. Two-Way ANOVA followed by a post-hoc Sidak’s test revealed no significant differences (n=12/group). Each bar represents the mean + SEM.

3

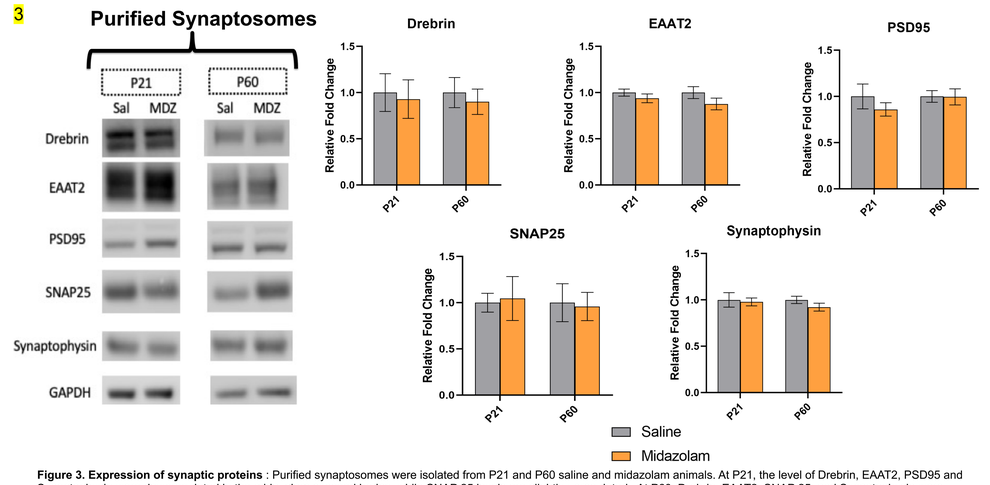


Figure 3. Expression of synaptic proteins : Purified synaptosomes were isolated from P21 and P60 saline and midazolam animals. At P21, the level of Drebrin, EAAT2, PSD95 and Synaptophysin were downregulated in the midazolam exposed brains, while SNAP 25 levels are slightly upregulated. At P60, Drebrin, EAAT2, SNAP 25, and Synaptophysin were downregulated in the midazolam exposed brains; however, there was no difference in the level of expression of PSD95 between the saline and midazolam groups. This suggests that midazolam can potentially alternate the regulation in key synaptic proteins. Two-Way ANOVA followed by a post-hoc Sidak’s test revealed no significant differences (n=12/group). Each bar represents the mean + SEM.

Behavioral

4

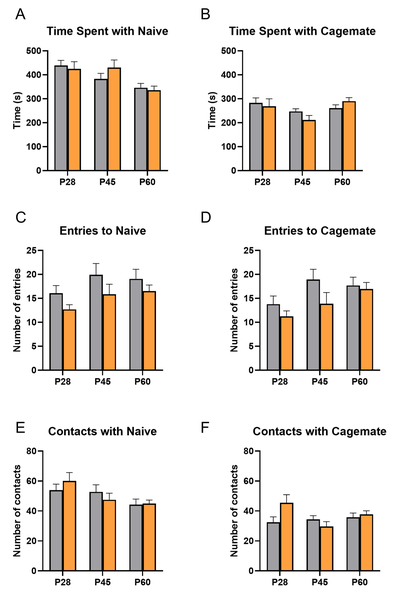


Figure 4. Behavioral tests (Social novelty): Throughout the course of 15 minutes, the number of seconds, contacts, and chamber entries that the rat allocated between the naïve and cage mate rat were recorded. Midazolam-exposed rats had fewer entries to both naïve and cagemate chamber, in comparison to the controls. For contacts, the P28 and P60 Midazolam rats at more contacts for both the naïve and cage mate rat. However, at P45, the Midazolam group had less contacts for both naïve and cage mate as compared to the Saline group. For time spent, the Midazolam group only had a longer duration as compared to the Saline group in P45 for the naïve rat and at P60 for the cage mate rat. Two-Way ANOVA followed by a post-hoc Sidak’s test revealed no significant differences (n=14-18/group). Each bar represents the mean + SEM.

Conclusion and Future Directions

- From these results on the phenotypic, molecular, and behavioral level, it can be established that Midazolam could potentially stunt neurodevelopment during early stages of life/
- Midazolam-exposed rats display significant phenotypic alterations in their earlier stages of life.
- Midazolam exposure has subtle effects on expression of blood brain barrier and synaptic protein levels.
- On the behavioral level, the preference of the midazolam-exposed rats to its cage mate (higher number of contacts) and unwillingness to explore (lower number of entries) can implicate a social deficit.
- Do subtle changes in BBB and synaptic proteins impact synaptic currents (cf. spine density, neurotransmitter release dynamics etc.)?
- Are levels of growth factors impaired in the midazolam exposed animals?
- Increasing the sample size to determine potential sex-specific differences

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