Investigating the Role of the Blood Brain Barrier in *Drosophila* sleep during Early Development

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Abstract

Across the animal kingdom, sleep during early life is essential for brain development and cognitive functions. Accumulating evidence across species, including the fruit fly Drosophila, has demonstrated a close relationship between the blood-brain barrier (BBB) and sleep in mature adulthood. However, the role of the BBB in sleep during early life has not been evaluated. Recently, Matthew Kayser's laboratory discovered and characterized a sleep state during the second instar larval stage (L2), establishing Drosophila larvae as a powerful model for studying sleep during early developmental stages. In this model system, I evaluated the role of different cellular components of the BBB on larval sleep using genetic and behavioral approaches. First, as Ca^{2+} levels are essential for BBB physiology in Drosophila larvae, I reduced the expression of various Ca^{2+} signaling components in the perineurial (PG) and sub-perineurial (SPG) cells, which constitute the BBB. The majority of Ca^{2+} signaling components evaluated in the SPG, but not in PG cells, affected larval sleep. Additionally, since intercellular communication between BBB cells is known to be relevant for Ca^{2+} homeostasis across the BBB in Drosophila, I reduced the expression of innexin 2, a key component of intercellular communication. I found that reduced expression of Inx2 in the SPG, but not in the PG, affects total sleep. Finally, as in adult flies, inhibiting endosome recycling in BBB cells increases total sleep time, I assessed whether this cellular mechanism is relevant for larval sleep. Through genetic manipulations of Rab11, a critical protein for endosome recycling, I demonstrated that this mechanism in the BBB is not relevant for larval sleep. My findings support the idea that BBB signaling is relevant for larval sleep, possibly utilizing mechanisms distinct from those involved in BBB-sleep interactions in adult flies.

Background & Methodology

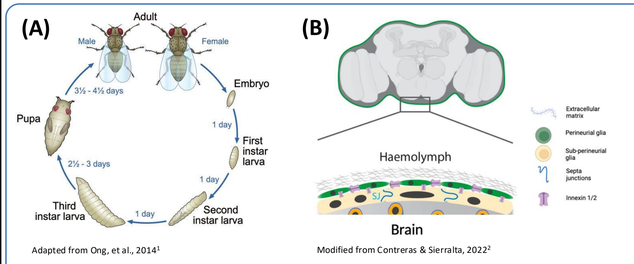

Fig 1. **(A)** Life cycle of *Drosophila* adapted from Ong et al., 2014. **(B)** Schematic representation of the BBB, which is formed by 2 types of glial cells: sub-perineurial glia and perineurial glia (SPG and PG respectively). Intercellular communication between SPG and PG cells is mediated by Innexin 1 and 2 (Inx1 and Inx2 respectively). SPG form septate junctions to block paracellular diffusion. Adapted from Contreras & Sierralta, 2022.

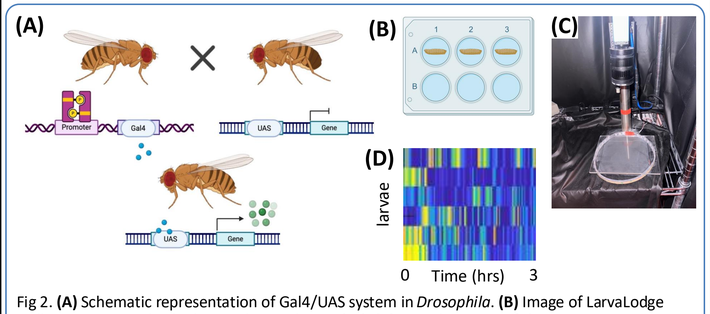

Fig 2. **(A)** Schematic representation of Gal4/UAS system in *Drosophila*. **(B)** Image of LarvaLodge wells containing individual second instar larvae (L2). **(C)** Complete view of the imaging system used to study *Drosophila* larval sleep. **(D)** Rastor plot showing activity (yellow) and quiescence (blue) of 6 larvae monitored from early L2.

Reducing the expression of itpr, but not ryr, in the PG reduces total sleep

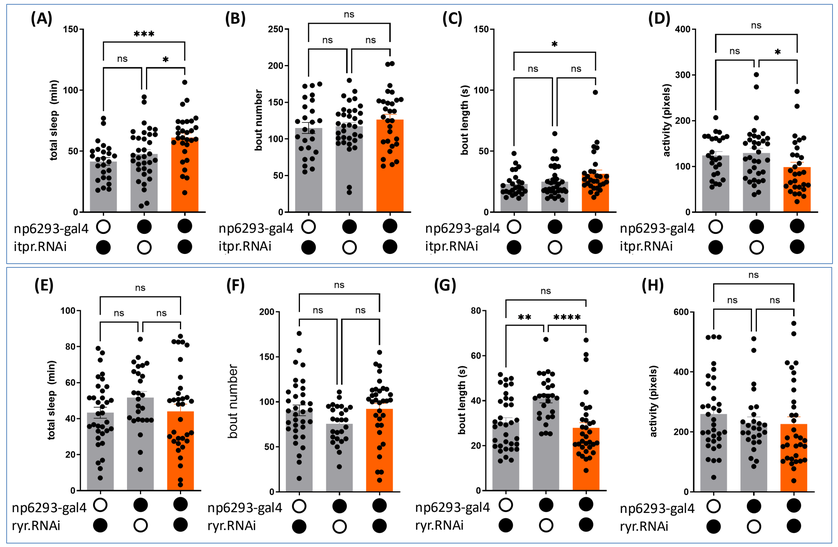

Fig 3. In the PG cells (using np6293-Gal4), knockdown of the Inositol triphosphate receptor (IP3R) **(A-D)** increases total sleep time. Bout length and locomotor activity show a significant difference compared to one control; Ryanodine Receptor (RyR) **(E-H)** does not affect sleep. Data were analyzed using Kruskal-Wallis and one-way ANOVA tests. ****p < 0.0001, ***p < 0.001, **p <0.005, *p < 0.05.

Reducing the expression of inx2 in the SPG, but not the PG, affects total sleep

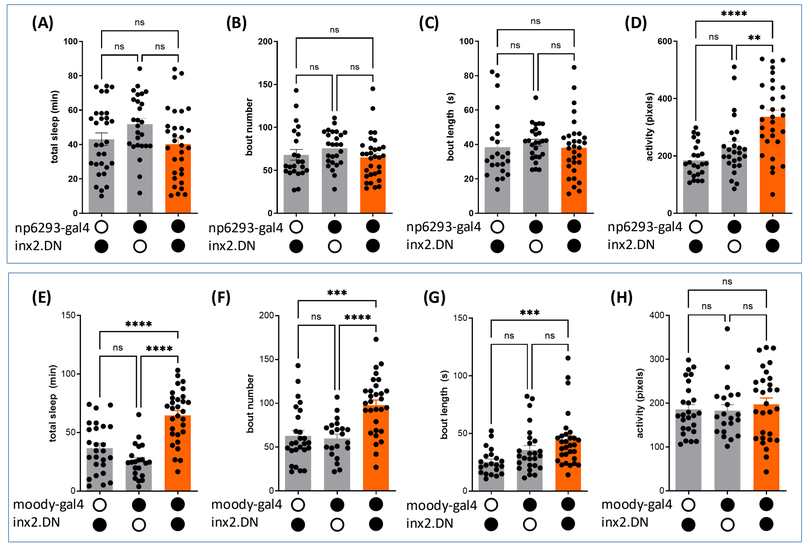

Fig 4. Expression of the dominant negative form of Inx2 in the SPG (using moody-Gal4) **(A-D)** but not the PG **(E-H)** cells affect total sleep. Data were analyzed using one-way ANOVA and Kruskal-Wallis tests. Asterisks (*) denote significance, with increasing asterisks signifying a smaller p-value. ****p < 0.0001, ***p ≤ 0.001, **p < 0.005.

Endosome Recycling in the BBB does not affect larval sleep

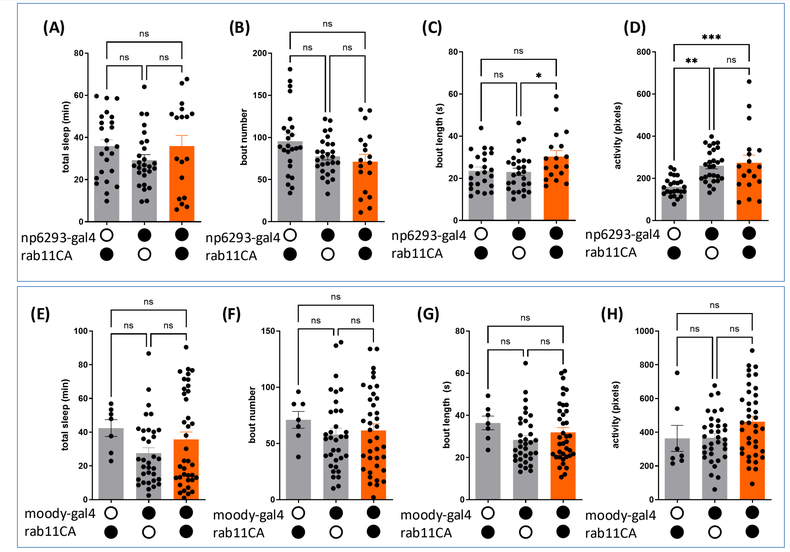

Fig 5. Expression of constitute active form of Rab11 in the SPG **(A-D)** and the PG **(E-H)** does not affect larval sleep. Data were analyzed using one-way ANOVA tests. Asterisks (*) denote significance, with increasing asterisks signifying a smaller p-value. ***p ≤ 0.001, **p < 0.005, *p < 0.05.

Summary & Future Directions

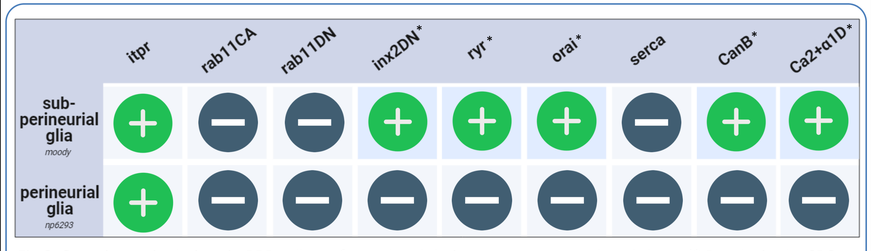

Fig 6. Genetic manipulations in BBB cells performed and their consequences on larval sleep. (* Results in the SPG are from previous manipulations done by a post-doc in the lab.)

Here, we have shown that calcium signaling in SPG cells, as well as intercellular communication between cells in the BBB, regulates sleep. In the future, we hope to assess whether these mechanisms in the BBB can affect the functioning of sleep-relevant neural circuits during early development in *Drosophila* by:

- determining how Ca2+ level changes in SPGs effects BBB function
- testing the idea that BBB manipulations influence sleep via altered excitability of sleep-relevant neurons
- examining how sleep deprivation impacts BBB permeability

References

1. Ong, Cynthia, et al. "Drosophila Melanogaster as a Model Organism to Study Nanotoxicity." *Nanotoxicology*, 22 July 2014, https://doi.org/10.3109/17435390.2014.940405.
2. Contreras, Esteban G, and Jimena Sierralta. "The Fly Blood-Brain Barrier Fights against Nutritional Stress." *Neuroscience Insights*, 19 Aug. 2022, https://doi.org/10.1177/26331055221120252.