N°Anonymat:

The candidate will provide (i) a title to the article, (ii; iii) a short description of the biological context and of the specific problematics of the article and (iv) an analysis of the data (for each panel, describe the aim of the experiment, the general methodology, the results and conclude). Finally the candidate will propose a model (v) and formulate 2 to 4 questions raised by the study (vi).

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(i) TITLE?????

INTRODUCTION

(ii)- Biological context of the article

In *Drosophila*, neural stem cells (NSCs) enter quiescence at the end of embryogenesis and are reactivated during early larval life in response to feeding (**Figure 1A**). Amino acid availability is sensed by the fat body, the functional equivalent of the mammalian liver and adipose tissue. The fat body sends an as-yet-unidentified signal, or signals, to the brain to induce the production and secretion of insulin-like peptides (dIlps) by blood-brain barrier (BBB) glial cells. dIlps act locally to trigger the insulin/insulin-like growth factor receptor pathway in underlying NSCs. Consequently, the NSCs enlarge and re-enter the cell cycle. NSC reactivation occurs synchronously in all neurogenic zones of the CNS, suggesting that BBB glial cells and/or NSCs are linked by an intercellular signaling mechanism. Gap junctions are intercellular channels formed by the juxtaposition of connexin hexamers…

(iii)- Specific problematics of the article

RESULTS

(iv)- Data analysis (Fig. 1 to 3)

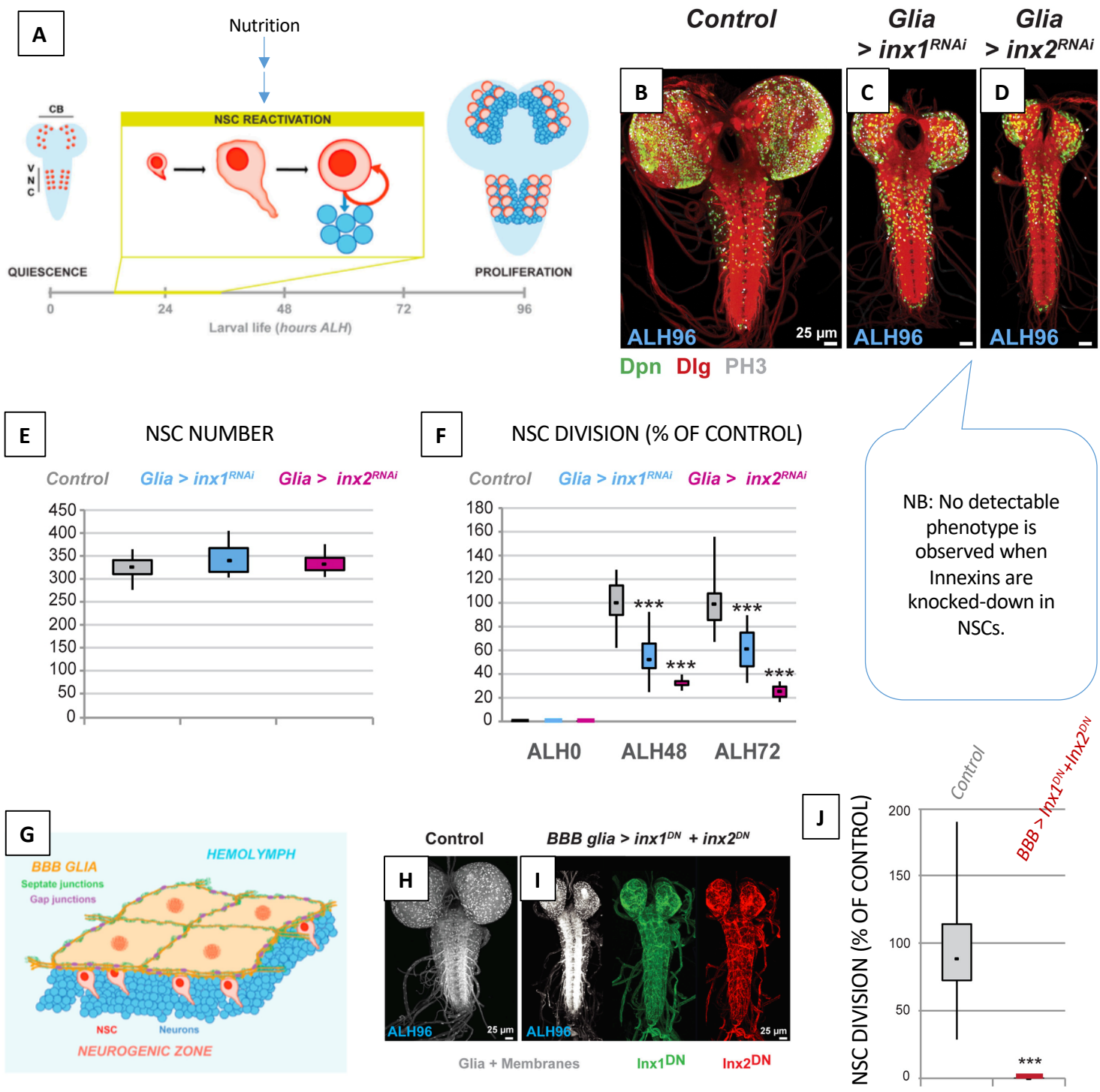


Fig.1: Analysis of *Inx1/2* loss of function in all glial cells or in the blood-brain barrier (BBB) glial cells.

(A) Schematic showing that *Drosophila* quiescent NSCs are reactivated within a 24 hr time window in response to nutrition in the central nervous system (CNS). CB, central brain; VNC, ventral nerve cord; ALH, hours after larval hatching.

(B-F) RNAi-mediated knockdown of Innexin 1 or 2 in glia (*Glia > Inx1^{RNAi}* or *Glia > Inx2^{RNAi}*) . Drosophila Innexins are equivalents of vertebrate connexins, the functional units of Gap junctions. Inx1 and Inx2 are known to form heteromeric complexes. (B-D) *Drosophila* larval CNS immunostained at stage ALH96 with antibodies against Dpn (marker of NSC nuclei in green), Dlg (marker of cell bodies in red) and PH3 (marker of mitosis in grey). (E, F) Quantification of NSC number (E; stage ALH0) and proliferation (F, stages ALH0, ALH48 and ALH72).

(G) Schematic showing the relationship between the BBB and the neurogenic zone that contains NSCs.

(H-J) Targeted overexpression in BBC glial cells of dominant-negative versions of Inx1 (*BBB > Inx1^{DN}*; fused to GFP, green) and Inx2 (*BBB > Inx2^{DN}*; fused to RFP; red). (H-I) *Drosophila* larval CNS immunostained at stage ALH96 with antibodies labelling glial nuclei and cell bodies (grey). (J) Quantification of NSC proliferation.

***p < 0.05. Two-sided Student's t test. Average and SD were calculated from two biological replicates.

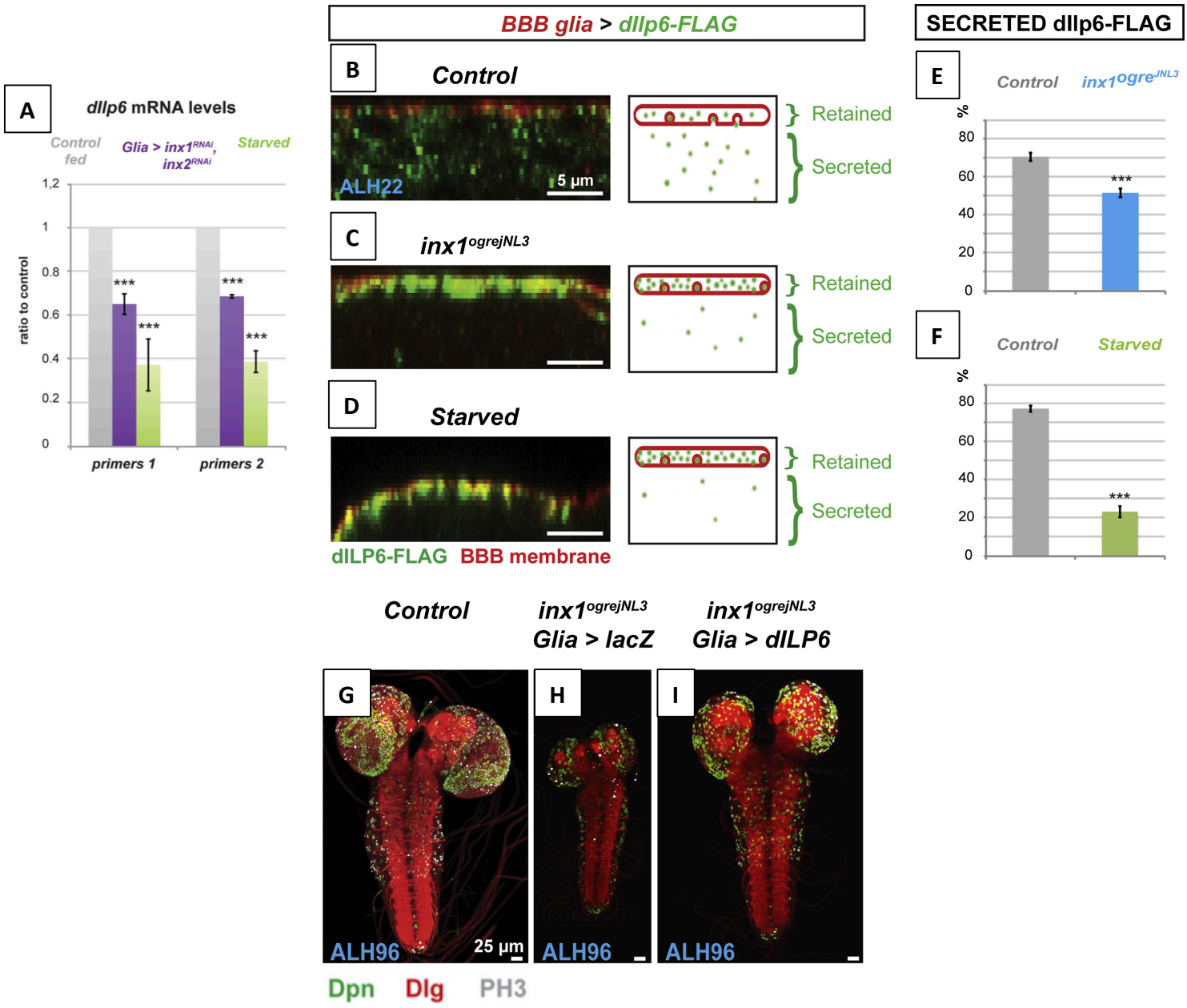
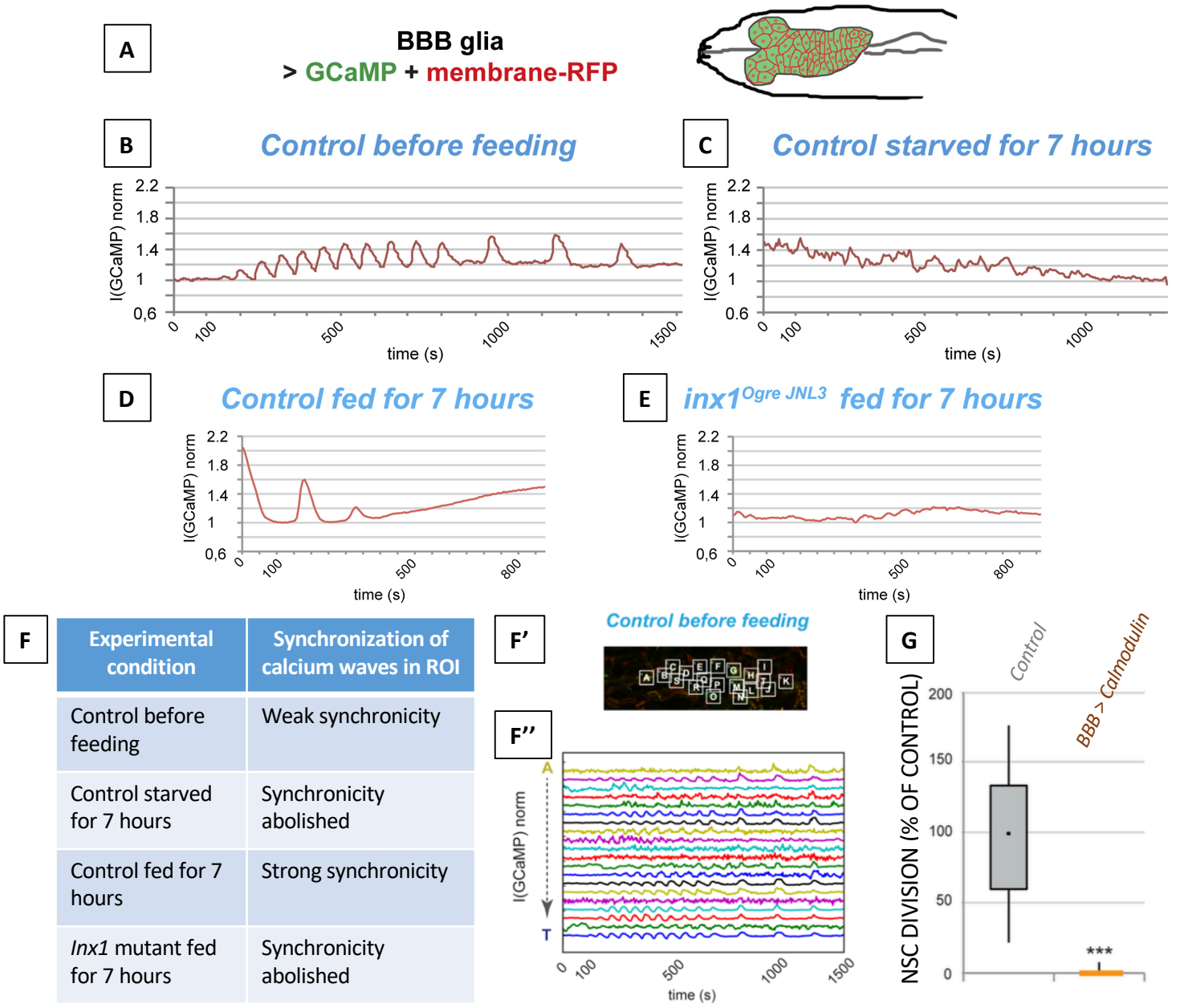


Fig.2: Analysis of Insulin secretion by the BBB cells following *Inx*1/2 loss of function.
(A) Quantification of *dllp6* transcript levels by q-PCR in fed larvae with RNAi-mediated knockdown in glial cells of both *Inx1* and *Inx2*, compared to fed or starved control larvae. Two pairs of *dllp6* primers were used. *Dllp6* encodes one of the eight insulin-like peptides identified in *drosophila* and its transcription is known to be dramatically up-regulated upon feeding.
(B-F) dIlp6 secretion was assayed by expressing a tagged version in the BBB glia only (*BBB > dllp6-FLAG)* and assessing at stage ALH22 what is found out of the BBB glia, in the neurogenic zone (see Fig. 1G). (B-D) Cross-section of individual BBB glial cells immunostained with an anti-FLAG antibody (green) and against a BBB glial cell marker (red). On the right are corresponding schematic representations. Experimental conditions tested are: (B) control, (C) *Inx1* loss of function mutant (*inx1^{ogrejNL3}*), (D) starved larva. (E-F) Quantifications of dIlp6 secretion.
(G-I) Forced expression of dIlp6 in glial cells of *Inx1* mutants. *Drosophila* larval CNS immunostained at stage ALH96 with antibodies against Dpn (marker of NSC nuclei in green), Dlg (marker of cell bodies in red) and PH3 (marker of mitosis in grey). Experimental conditions tested are: (G) control, (H) *Inx1* loss of function mutant expressing ß-galactosidase in glial cells (*inx1^{ogrejNL3}; Glia > lacZ*), (I) *Inx1* loss of function mutant expressing dIlP6 in glial cells (*inx1^{ogrejNL3}; Glia > dllp6*).
***p < 0.05. Bar graphs represent mean ± SEM.

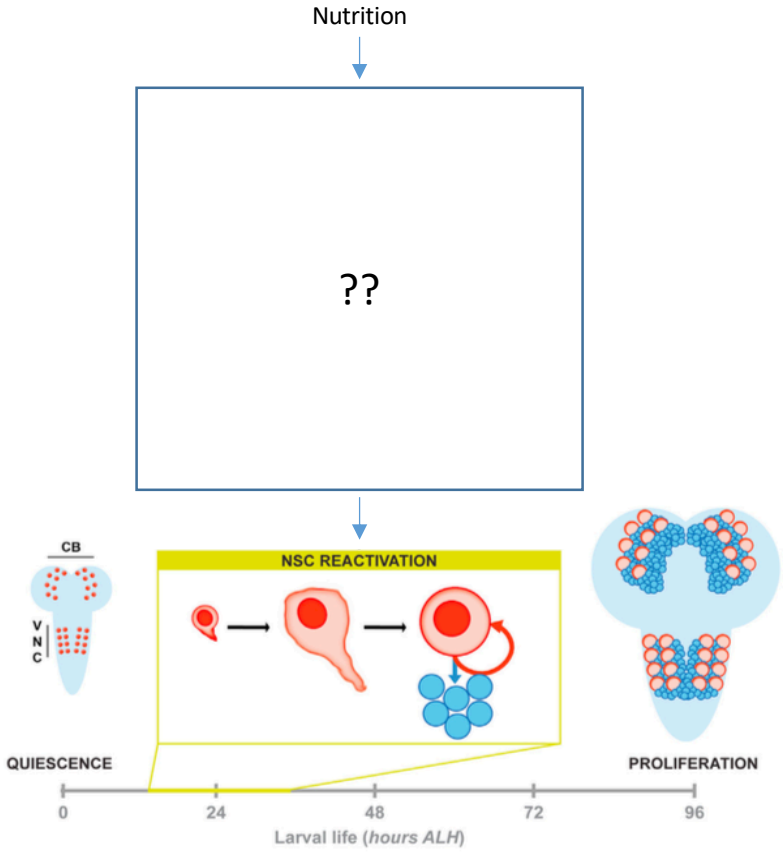


Experimental condition	Synchronization of calcium waves in ROI
Control before feeding	Weak synchronicity
Control starved for 7 hours	Synchronicity abolished
Control fed for 7 hours	Strong synchronicity
Inx1 mutant fed for 7 hours	Synchronicity abolished

Fig.3: Analysis of calcium oscillations in the BBB Glia.
(A) Schematic representation of the experimental procedure used in (B-F). Expression of the fluorescent calcium sensor GCaMP3 (green) and of a membrane marker (red) was driven specifically in the BBB glia to measure calcium oscillations.
(B-E) Calcium oscillations measured in individual CNS from a control larva before feeding (B, stage ALH0), a control larva starved for 7 hours (C, stage ALH7), a control larva fed for 7 hours (D, stage ALH7) and a *Inx* mutant larva (*inx1*^{ogrejNL3}) fed for 7 hours (E, stage ALH7). Shown in graphics is GCaMP3 normalized mean intensity over time for the entire plane.
(F) Summary of correlations analyses. To assess the coordination of calcium oscillations within the BBB glia, several regions of interest (ROI) were selected randomly in individual larvae from each conditions (see example in F') and calcium oscillations were measured in each of them (as shown in F''). The table indicates whether synchronicity was observed or not between the considered regions.
(G) Quantification of NSC proliferation following calmodulin overexpression in BBB glia (*BBB > Calmodulin*). Calmodulin is a calcium-binding protein. ***p < 0.05. Two-sided Student's t test. Average and SD were calculated from two biological replicates.

CONCLUSION AND DISCUSSION

(v) Using the informations of the introduction and all the results obtained, propose a simple model of the successive signals leading to NSC reactivation in the *drosophila* larval CNS.



(vi)- Ask questions