INSOMNOTCH
Publishable summary

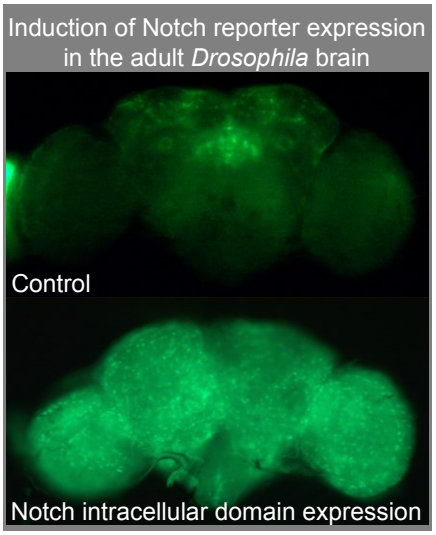
Despite decades of research, the cellular and molecular processes by which sleep maintains normal brain function as well as the mechanisms regulating sleep homeostasis remain elusive. The goal of this project is to use the drosophila model to dissect the function of a neuron-glia signaling pathway that regulates sleep homeostasis and learning. This signaling pathway constitutes a novel function for the trans-membrane receptor Notch and its ligand Delta.

Summary of the Project objectives

-Defining which intracellular components of the *Notch* pathway regulate sleep and learning
-Identifying brain systems regulated by *Notch*.
-Interactions between *Notch* signaling and neurodegenerative processes**.**

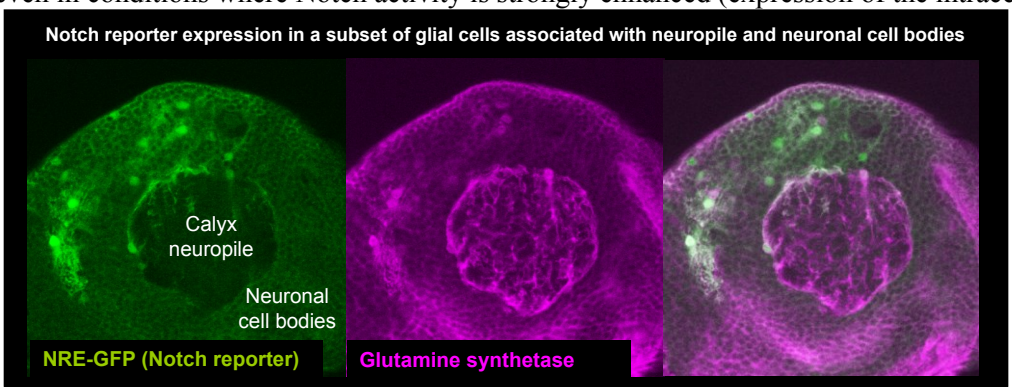
Work performed since the beginning of the project and main results:

Involvement of the Notch canonical pathway.
In the canonical Notch signalling pathway, the receptor is cleaved following binding with the ligand, and the intracellular domain of Notch migrates to the nucleus, where it associates with the transcription factor Suppressor of Hairless (Su(H)) to activate the expression of target genes such as members of the Enhancer of Split (E(spl)) complex. Activation of the Notch canonical pathway can be monitored using a Notch-Reporter-Element (NRE-GFP) reporter transgene containing the Su(H) binding sites combined with a basic promoter and the coding sequence of GFP. We have shown that this reporter is exclusively expressed in a subset of glial cells, indicating that canonical Notch signalling is activated in glial cells. To determine whether the canonical pathway is involved in sleep regulation and learning, we expressed the intracellular domain of Notch (NICD) to constitutively activate Notch signalling in glia. Notch activation in glia prevented learning impairments and sleep rebound following sleep deprivation. As expected, the intracellular domain strongly activated the NRE-GFP reporter expression in glia (see figure in pdf version of the report). Overexpression of Delta, a ligand for Notch, in a neuronal structure involved in learning (the mushroom bodies) also blocked the sleep rebound and prevented learning impairments following sleep deprivation. This work has been published in *Current Biology* (1) in may 2011. It emphasizes the role of Neuro-glial interactions in sleep regulation and function. To further confirm the involvement of the Notch canonical pathway in glia, we examined the expression of two independent Notch reporter constructs. Both of them confirmed Notch activation in glia. Interestingly, we have observed that *Notch* alleles affecting glycosylation result in a down-regulation or an up-regulation of Notch reporter expression. These observations suggested that *fringe*-mediated glycosylation could regulate the level and possibly the pattern of Notch activation within the brain. Indeed, reducing fringe expression in glia using a UAS-RNAi construct resulted in a lower expression of the Notch reporter. Finally, we have shown that reducing the expression of Notch or of Su(H) produced learning impairments and a reduced sleep rebound. Thus, the Notch canonical pathway in glia affects learning and sleep homeostasis. Interestingly, the learning impairments in Notch mutants could be rescued by giving flies L-DOPA, a precursor of dopamine, suggesting they result from impaired dopamine signalling.



Physiological targets of Notch.
Several potential physiological targets of Notch have been tested. We observed that glial cells in which Notch is activated also express glutamine synthetase and the glutamate transporter dEaat1suggesting a role in glutamate regulation (Figure in pdf version). In Notch mutants we observed a reduction of dEaat1 expression at the transcriptional level, however this reduction appeared to be compensated by post-transcriptional mechanisms. We have also tested Fatty Acid Binding Protein (dFABP) and Epidermal Growth Factor (EGF) receptor. However our results do not support the idea that EGFR or EGFR play a major in this context. Finally, we have evaluated the availability of two bioamine neurotransmitters in Notch mutant conditions: dopamine and octopamine (the insect

equivalent of noradrenaline) that play an important role in sleep/wake regulation as well as learning. Using HPLC dosage, we have found that normal levels of dopamine and octopamine were maintained even in conditions where Notch activity is strongly enhanced (expression of the intracellular domain).



Notch activity in a fly model of Parkinson disease. Using the NRE-GFP construct we monitored Notch activity in a *Drosophila* model of Parkinson disease. The activity of Notch remained stable throughout the life of these animals.

Gene profiling in Notch mutant conditions. To identify molecular targets modulated by Notch activity in glia, we used whole genome profiling in five conditions: strong or intermediate Notch activation, control condition, and strong or intermediate inhibition of Notch expression. 1176 genes are differentially expressed across these conditions and 707 are correlated positively or negatively to Notch activity. 64 of these genes were present in other transcriptomics studies looking at genes regulated by sleep and waking. The functional categories that were the most represented are linked to lysosome function, peroxisome function, nucleotide sugar metabolism and glycolysis. 15 of these genes have been tested by inhibiting their expression either in neurons or in glial cells. We are now following up on two of these genes that strongly reduce sleep amounts, when their expression is inhibited in glia. One of the genes is regulating lipid metabolism and the other one is involved in glial coupling.

Results summary and perspectives

This study has evaluated the consequence of Notch activation and inhibition on sleep/wake, learning and brain systems modulating sleep/wake. We described the pattern of Notch activation in the brain and identified potential glial molecular targets that are modulated by Notch and regulating sleep. More specifically, we have shown that:

-Activating canonical Notch signalling by expressing the intracellular domain of Notch reduces learning impairments and the sleep homeostatic response following sleep deprivation.
-Inhibiting canonical Notch signalling results in learning impairments and defective sleep homeostasis.
-Modulating EGF receptor function has no detectable effect on canonical Notch signalling in glia.
-Notch down-regulate transcriptionally the glial glutamate transporter dEaat1.
-Over 700 genes are transcriptionally induced or repressed by Notch activity in glia
-Two Notch-regulated genes, not previously associated with sleep, play an essential role in glia to regulate sleep/wake.

Gene profiling studies in mice have shown that all Notch homologs are predominantly, if not exclusively, expressed in astrocytes, while Delta-like and other Notch ligands are predominantly expressed in neurons. Thus Notch signalling is likely to be mediating an evolutionary conserved neuroglial signalling pathway. Information obtained with the drosophila model is likely to have relevance to mammalian systems and our understanding of Notch signalling in normal brain physiology and disease. Our current data point to a crucial role in the regulation of glial metabolism in this context, which has been so far little explored.

1 - **Seugnet L.,** Suzuki Y., Merlin G., Gottschalk L., Duntley S.P., Shaw P.J (**May 2011**) *Notch* signaling modulates sleep homeostasis and learning after sleep deprivation in *Drosophila*. ***Current Biology*** **(impact factor = 11.435)**. 21, 835-40