Study of EV microRNAs from microglia involved in the crosstalk to neurons

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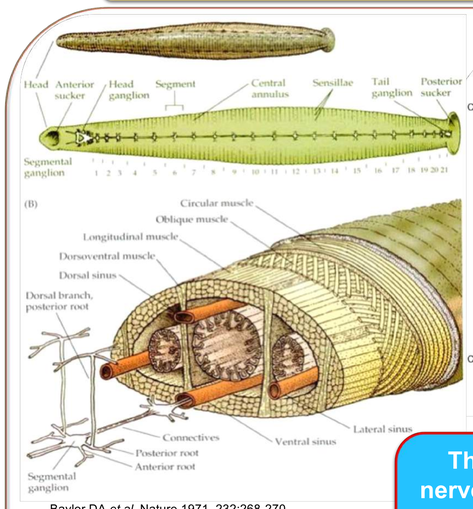
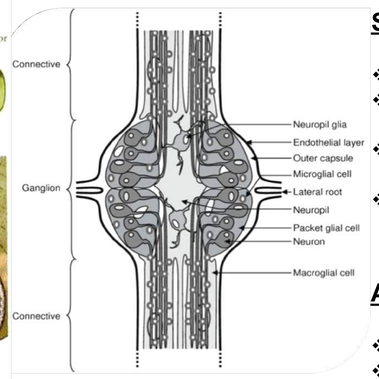
I- Introduction

Unlike vertebrates, the medicinal leech (*Hirudo medicinalis*) is able to functionally repair its central nervous system (CNS) after an injury (Baylor *et al.* Nature 1971). Interestingly, when the CNS is injured, microglial cells migrate to the site of lesion. This accumulation is known to be essential for the usual sprouting of injured axons that leads to a functional nerve repair (Ngu *et al*. J comp neurol 2007). If the accumulation of microglia is blocked, the nerve repair is significantly delayed. Microglial cells are able to release extracellular vesicles (EVs) (Zuppulli *et al*. J Clin Invest 2016) and we show that leech microglial cells release EVs at the lesion site and in ganglion around the neuronal cell bodies (Raffo-Romero *et al.* IJMS 2018). The EVs contain many molecules (proteins, lipids, mRNA and miRNA) (Cocucci & Meldolesi. Trends Cell Biol 2015). We also know that miRNAs are implicated in some neurophysiological processes (brain development, homeostasis…) but also in neuropathologies (Alzheimer, Parkinson…). The present study explores microglia-neurons crosstalk mediated by the miRNAs in EVs for a better understanding of neuroprotection.

II- Model : The leech *Hirudo medicinalis*





Structure

- 21 ganglions linked by connectives
- Each ganglion contains around 400 neurons
- The connectives contain the axonal processes
- Numerous microglial cells are dispersed in the CNS

Advantages

- No blood cell infiltration
- Resident microglia is recruited at the lesion site
- Easy collection of microglia as well as neurons
- Fragments of CNS can be collected and injured *in vitro* for analyses

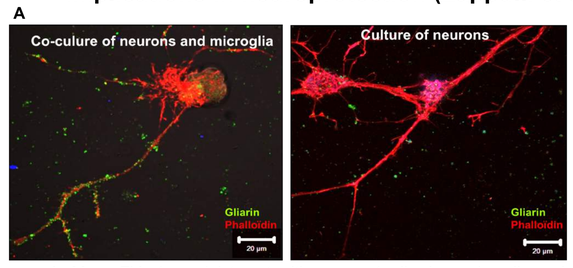
The tubular structure of the nerve chain makes it possible to specifically damage the axons and keeping intact the neuronal cell bodies

Baylor DA *et al.* Nature 1971, 232:268-270
Jansen JK *et al.* Proc Natl Acad Sci U S A 1972, 69:636-639
Muller KJ *et al.* Science 1979, 206:87-89.
Muller KJ *et al.* Nature 1980, 283:89-90.
Salzet M & Macagno E. Annelids in Modern Biology 2009, Chapter 9, pp.156-184.

III- Microglia and EVs

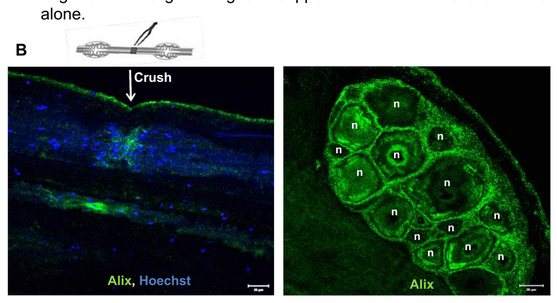
In leech, microglia are accumulated at the lesion site just few minutes after the crush. The EVs are released following the accumulation of microglial cells. In literature, the EVs show many implications in neuroprotection (Zappulli *et al*. J Clin Invest 2016).

A



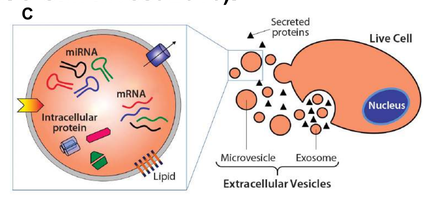
A. Many EVs (green) interact with neuron (red) when microglia and neurons are in co-culture. We suppose those EVs to have a microglial origin since the green signal disappears when neurons are cultured alone.

B



B. Immunopositive structures for Alix are observed at the site of lesion (white arrow) and arround the neuronal cell bodies. Microglia nuclei are shown by Hoechst counterstaining.

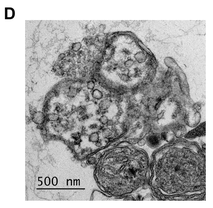
C



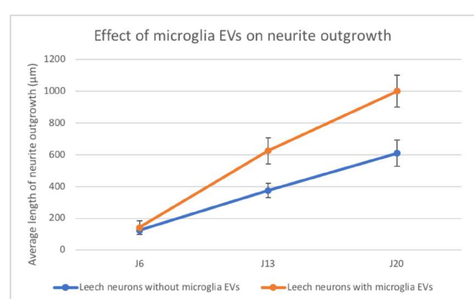
James A. Smith *et al*, (2015)

C. The EVs are subdivided in two main groups, the exosomes (40 – 120 nm) and microvesicles (50 - 1,000 nm). The main difference is the origin : exosome coming from multivesicular bodies (MVBs) and microvesicles coming from cell surface (outward budding) (J.A Smith *et al*, (2015)) D. TEM analyses of microglial cells. Activated microglia produce multivesicular bodies containing exosomes.

D



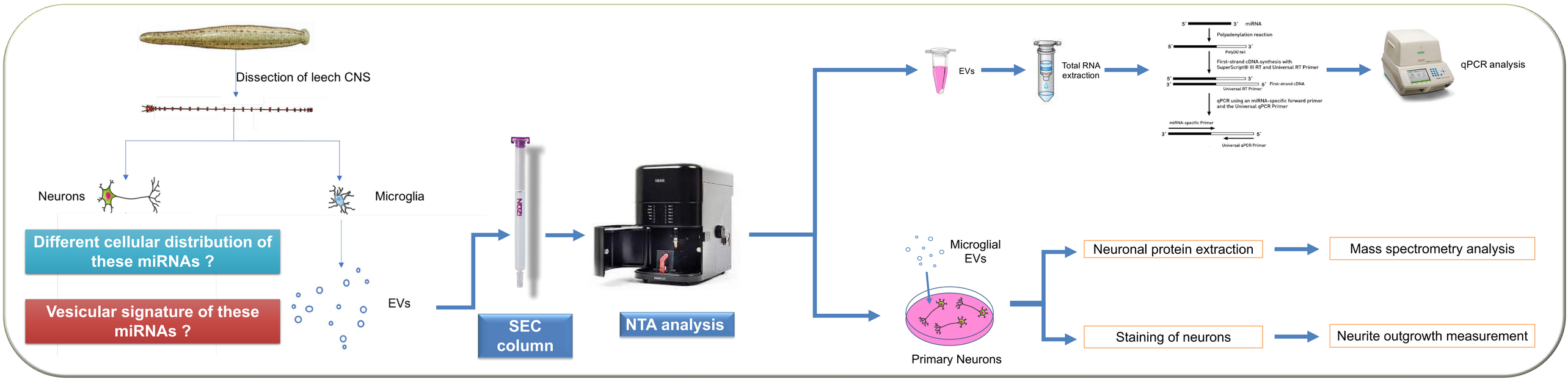
E



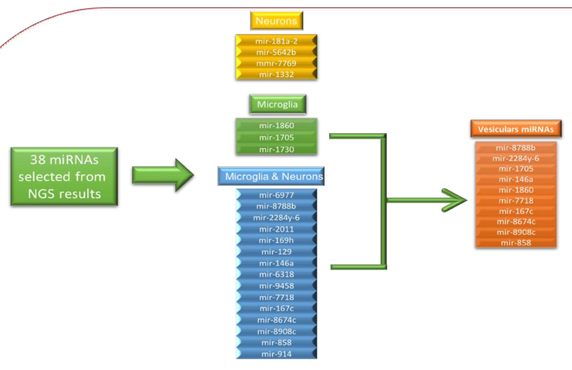
E. Effect of microglia EVs on neurite outgrowth. The leech neurons show an increase of neurite outgrowth in presence of EVs compared to the non-treated condition.

IV- Materials and Methods

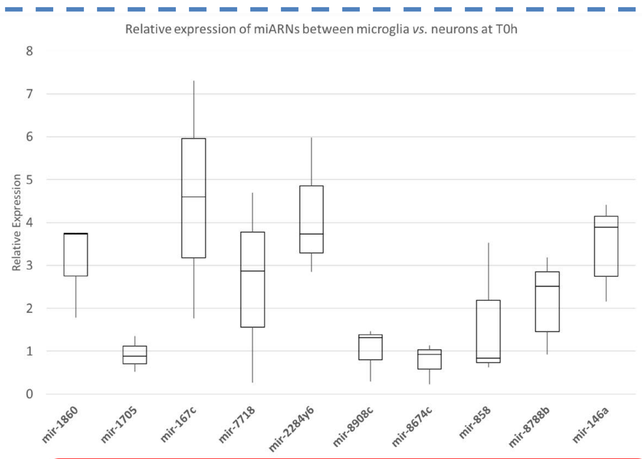
The CNS are dissected and the cells are dissociated. Microglia are cultured for 15 min (early activated state (or T0h)) to permit EVs release by microglia. Then, several differential centrifugations and size exclusion chromatography (SEC) of conditioned medium permitted the collection of extracellular vesicles, used for next experiments.



VI- Results & Prospects



The results of NGS show a large number of miRNAs. For subsequent studies, 38 miRNAs are selected. Some miRNAs are specific to microglia or neuron and some miRNAs are common to both populations. 10 miRNAs were detected in microglial EVs. In order to understand their use in EV-mediated crosstalk, miRNAs from microglia EVs will be analyzed in early activated state (T0h).



qPCR study of 10 miRNAs found in vesicles. Results shows that four miRNAs (mir-1860, mir-167c, mir-2284y6 and mir-146a) are most represented in microglia compare to neurons at T0h.

mRNA targets for miRNAs of interest

mir-1860	mir-167c
Neuropilin 1	Enabled homolog (ENAH)
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SMAD family member 2	Protocadherin 19
Smad nuclear interacting protein 1	Transforming growth factor, beta receptor I
Neural cell adhesion molecule 1	Neural cell adhesion molecule 1
Semaphorin 6A	DCP1 decapping enzyme homolog
Amyloid beta (A4) precursor protein-binding	Neurogenic differentiation 1
mir-2284-y6	**mir-146a**
Semaphorin 3A	Matrix metallopeptidase 16
Cadherin 3	SMAD family member 4
Plexin A2	Neurofascin homolog
Neuroligin 1	Glial cell derived neurotrophic factor
Myelin transcription factor 1-like	
Parvin, alpha	

Bioinformatic study of the putative mRNA targets for miRNAs shows that many targets have an interest in a neuroinflammatory context and nerve repair.

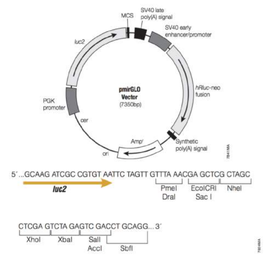
Short-term prospects

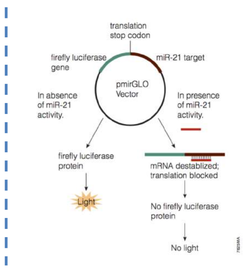
❖ **Impact of leech microglia EVs (modified or not) on neurite outgrowth and neuronal protein on other animal models : rat & mice**

❖ **Comparison of the effects of microglial EVs from mir-146a K-O or WT mice on primary neuronal culture**

Identification of mir-146a effects on neurite outgrowth and neuronal metabolism

❖ **Validation of interaction between mRNA and miRNA with luciferase assay**





Validation of new mRNA targets of mir-146a

Identify miRNAs from microglial EVs to elucidate the neuroprotective influence of EV-mediated microglia on damaged neurons