

Dr R. Anne McKinney Project Outline for 2 years

The protein sascin has been found to be mutated in the early onset neurodegenerative disease autosomal recessive spastic ataxia of Charlevoix-Saquenay (ARSACS). The disease is a neurodegenerative disorder characterized by early-onset spastic ataxia, dysarthria, nystagmus, distal muscle wasting, finger and foot deformities, and retinal hypermyelination. The principal neuropathology comprises atrophy of the upper vermis and the loss of Purkinje cells in cerebellum. The role of sascin in the ARSACS has yet to be fully determined. We will use a novel method, the organotypic slice culture technique, to help further understand the role of sascin in ARSACS.

Background on organotypic slice culture

The rollerdrum organotypic slice culture system was invented by Prof Beat Gähwiler in Switzerland in 1980. Prof Gähwiler has optimized many different brain areas for the organotypic slice culture method including hippocampus, striatum, and cerebellum. Organotypic slice cultures are established from newborn or 10 to 15-day-old mice. After 3-4 weeks in culture, the slices flatten to form a cell monolayer allowing easy visualization of fluorescently labeled neurons. The major benefit of the organotypic slice culture is that it maintains a native cytoarchitecture of the brain region placed in vitro, unlike that in dissociated cultures and has an intact network unlike in acute slices. Nonetheless, it has similar physiological properties to a similar aged acute slice. It is easily accessible for intervention to obtain a better understanding of fundamental mechanisms. Furthermore, as the preparations can survive for many months, it allows for long term studies and chronic pharmacological interventions.

Cultured cerebellar slices afford many of the advantages of dissociated cultures of neurons and thin acute slices. The main types of cerebellar neurons have been identified with immunostaining techniques, while their electrophysiological properties have been easily characterized with the patch-clamp recording technique. When slices are taken from newborn mice and cultured for 3 weeks, aspects of the cerebellar development are displayed. A functional neuronal network is established despite the absence of mossy and climbing fibers, which are the two excitatory afferent projections to the cerebellum. In contrast when slices are made from 10-15-day-old mice, which are at a developmental stage when cerebellum organization is almost established, the structure and neuronal pathways are intact after 3-4 weeks in culture. These unique characteristics make organotypic slice cultures of mouse cerebellar cortex a valuable model for analyzing the consequences of gene mutations that profoundly alter neuronal function and compromise postnatal survival

I carried out my postdoctoral studies in the Brain Research Institute under the direction of Prof Beat Gähwiler where I learned how to make the organotypic slice cultures. I have successfully established this method at McGill and we use these cultures routinely in our investigations.

Method

Either hippocampi or cerebellums are removed from mice at P0 to P15. Slices are made at 400 μ m thick sections which are placed on top of a glass coverslip where the tissue adheres by chicken plasma and thrombin. The cultures are prepared and maintained under aseptic conditions and no antibiotics are added, as they can affect inhibitory synaptic activity. The cultures are placed in a special tube which has a flat side and a bulbous side. The tubes are rotated in a wheel at a certain speed. During the rotation process the cultures are part of the time in media and part of the time out of the media allowing an oxygen nutrient interchange. Media or drugs of interest can be added to the media. After 3 weeks the cultures have flattened to 2 or 3 cells thick but maintain the cytoarchitecture.

Aim 1 To establish the impact of RNAi for SACS on the synaptic structure and synaptic function of cells

We will use organotypic slice cultures from hippocampus to optimize the injection parameter to introduce constructs into identified neurons. We will use Sutter manipulators attached to a fixed stage microscope to specifically inject the Sascin construct tagged with GFP into an identified specific cell type. To precisely control the amount of construct injected, we will use a picospritzer system. As the construct has a fluorescent tag, we will be able to determine the localization of the injected construct within the slice.

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In addition, we will inject constructs which have a red fluorescent dye attached (mCherry) into organotypic slice cultures made from transgenic mice which have a subgroup of cells labeled in green. Some cells are green due to GFP tagged to the membrane marker MARCKS, under the Thy1 promoter. This results in just a few cells being labeled in each culture allowing for better visualization of fine synaptic structures and so we can compare both the morphology and functional activity of both control cells (green) and cells where sascin has been silenced (red) which are in the same slice and are exposed to the same neuronal activity. We will perform basic electrophysiological experiments to measure synaptic transmission and we will assess any changes in the morphology of the cells using confocal microscopy and morphometric analysis.

Twenty-five distinct lines have been generated of MARCKS GFP mice, each with subtly different patterns of expression by our collaborator, Dr Pico Caroni FMI Basel, Switzerland. To date we have chosen lines which have low but consistent levels of membrane GFP positive cells within either area CA1 or CA3 of the hippocampus.

Aim 2 To establish cerebellar slice cultures expressing membrane GFP in a subset of Purkinje cells and establish mice which have a knockout for sascin which express membrane GFP in a subset of Purkinje cells.

Method

Two of the 25 lines have a low but consistent level of membrane GFP positive Purkinje cells in the cerebellum. We will transfer 2 males of this line to McGill and once they have been released from quarantine, we will establish and maintain the breeding colony. We will make cerebellar slice during late or early development and observe the affect of silencing sascin on synapse viability, development, maintenance and synaptic transmission by combining dynamic imaging using 4D confocal microscopy and electrophysiological techniques.

We will also cross the knockout animals, which are currently being made by NorCOMM, with membrane GFP transgenic mice to hopefully establish a transgenic mouse line which are knockout for sascin and have a low but consistent labeling of Purkinje cells expressing membrane GFP allowing the visualization of the Purkinje cells. We will perform dynamic 4D confocal imaging of the Purkinje cells during development and assess viability of the cells, formation of synapses and any functional changes.

Benefits

The findings from this work will add to our understanding of the role of sascin in the development of ARSACS and hopefully give us some insight to potential timing for delivery of therapeutic targets.

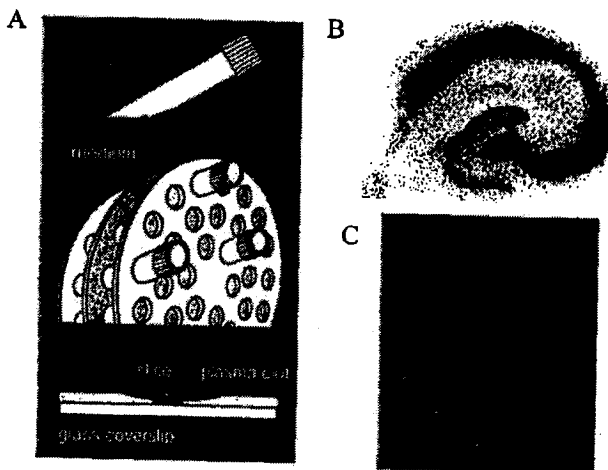


Figure Organotypic Slice culture

A. Illustration of how slice cultures are made, B. example of Nissil stained organotypic hippocampal slice 3 weeks in vitro. Note the cytoarchitecture of the hippocampus is maintained, C. 3 week old hippocampal slice culture made from transgenic mouse expressing membrane GFP (green) in a subset of neurons and dye injection in individual CA3 neurons (red).