FASS and FAST-FIN, two new FACS based methods for the purification of brain tissue organelles after subcellular fractionations

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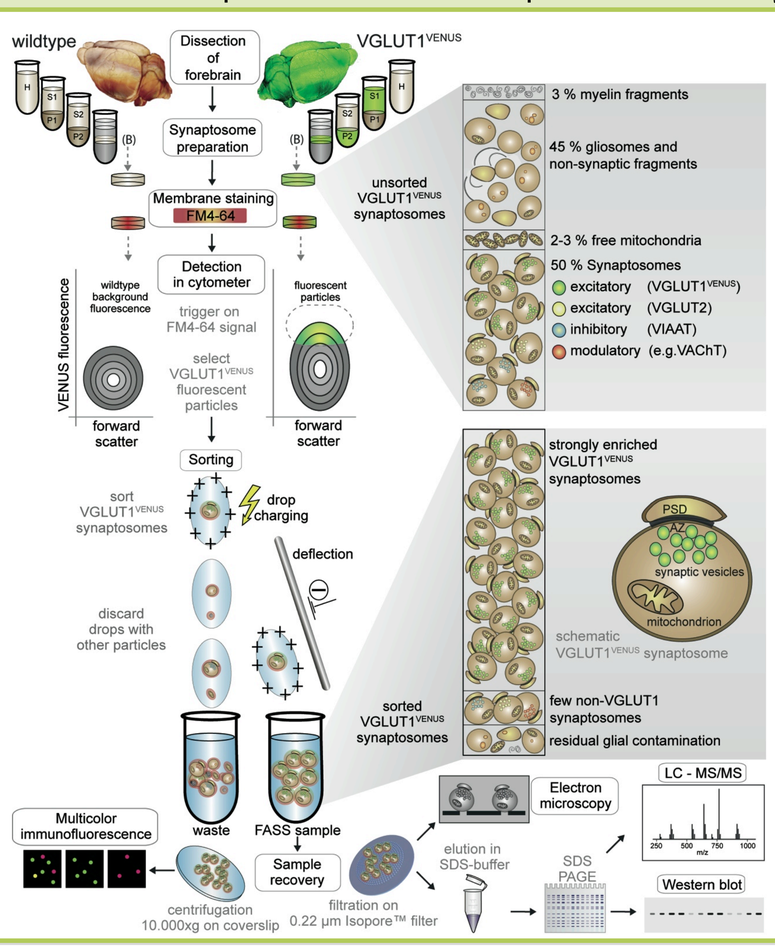
ABSTRACT

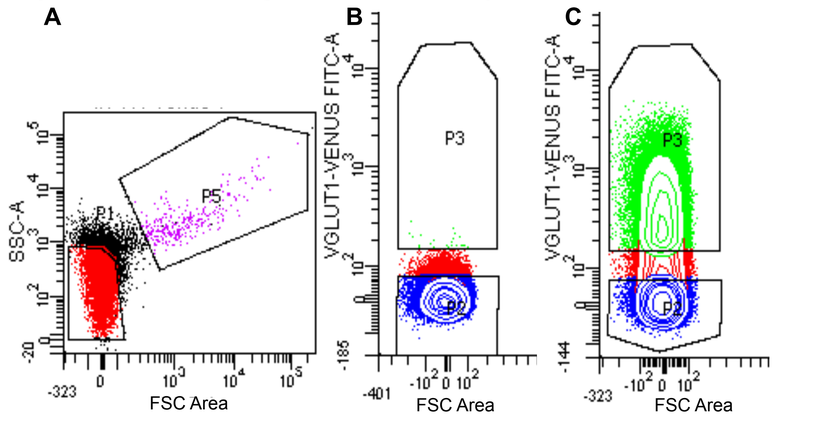
The mammalian brain contains a complex network of neurons that communicate via many different types of synapses. A major difficulty in investigating brain tissue comes from its cellular heterogeneity. Standard fractionation methods can therefore only provide information on a mixture of organelle populations from various cell types. Moreover, at the nucleus level, standard cell sorting methods do not allow the study of the labile post-translational modifications triggered by synaptic activity. To solve these issues, we established two robust sorting methods for the purification of specific synaptic populations **(1)** and identified fixed neuronal nuclei **(2)**.

We believe that the FASS and FAST-FIN methods represent the beginning of a new era of investigations on brain tissue in subcellular fractionations in physiology and pathology.

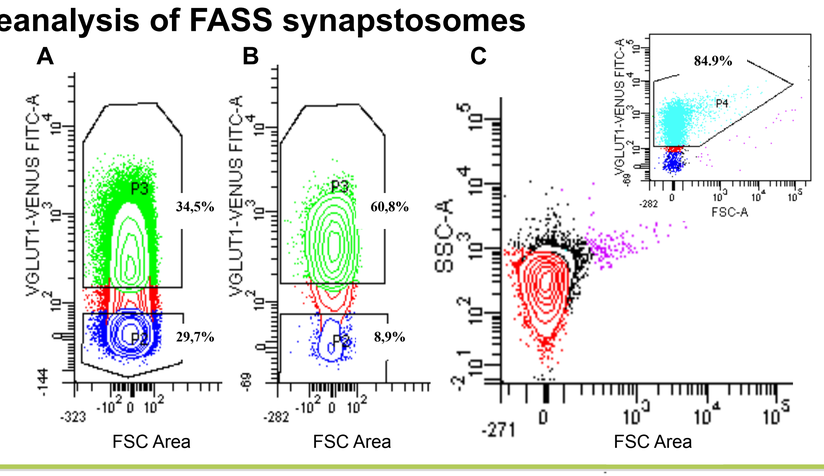
(1) - Quantitative proteomic approaches should allow us to understanding the composition of synaptic protein machinery. However, synapse preparations called « synaptosomes » suffer from a lack of purity and the presence of numerous glial and neuronal contaminants. We here use knock-in mice with fluorescence glutamatergic synapses to perform Fluorescence Activated Synaptosome Sorting (**FASS**) and substantially improve conventional synaptosome enrichment protocols. Wide-type and knock-in mouse forebrain were dissected, homogenized and fractinated following Whittaker synapstome preparation[1]. All particles of the synaptosomal fraction are bulk stained using the lipophilic dye FM4-64 to allow for thresholding of the detection in the sorter as FSC parameters were not sensitive enough. WT mouse sample is used to determine the baseline of autofluorescence in the GFP/VENUS channel. Fluorescent particles brighter than autofluorescence are sorted in the VGLUT1^{venus} samples. The FASS sample is collected by filtration on isopore polycarbonate or centrifugation on coverslips, depending on the subsequent Analysis[2].



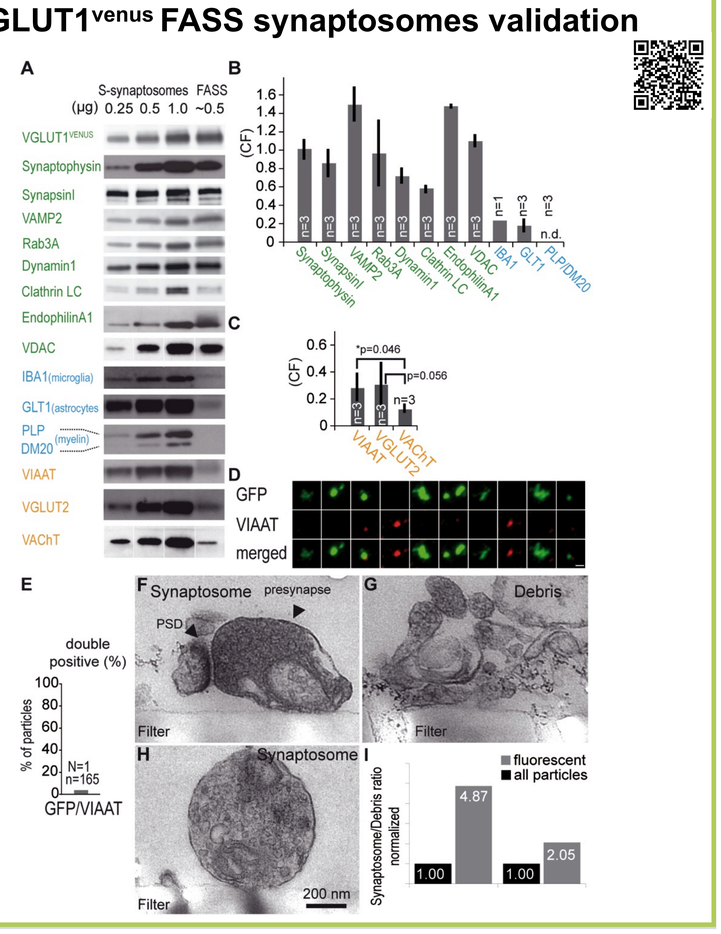
Analysis and Gating of Sucrose Synaptosomes



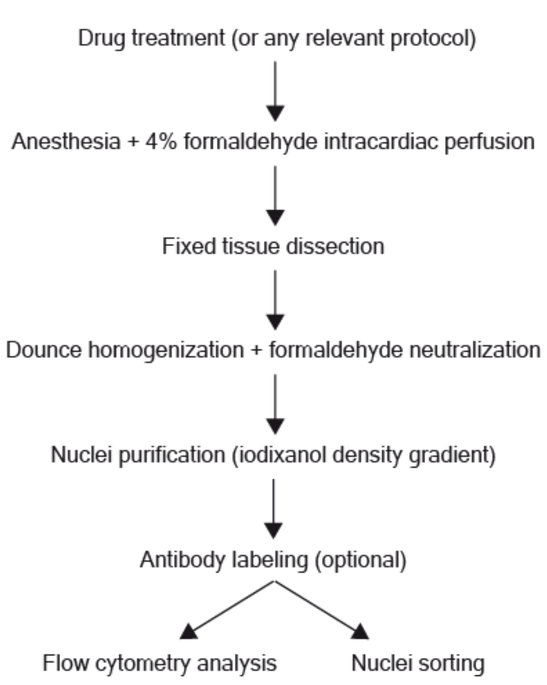
Reanalysis of FASS synapstosomes



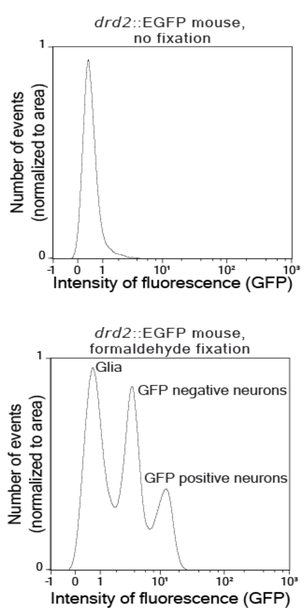
VGLUT1^{venus} FASS synaptosomes validation



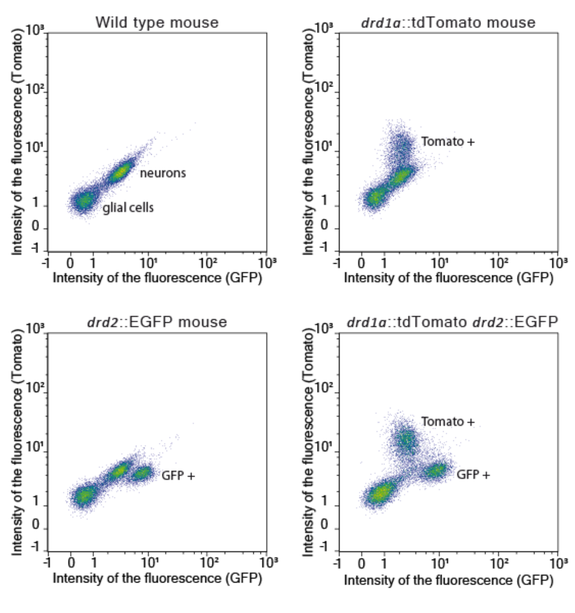
(2) -Long lasting alterations that underlie learning and memory are triggered by synaptic activity. Signalling from the cytoplasm to the nucleus and the resulting changes in transcription and epigenetic modifications are particulary relevant in this context. Using mouse striatum we have developed a rapid and efficient method to isolate cell type-specific nuclei from fixed adult brain, Fluorescence-Activated Sorting of FIxed Nuclei (**FAST-FIN**). Animals are quickly perfused with a formaldehyde fixative that stops enzymatic reactions and maintains the tissue in the state it was at the time of death. Tissue is subsequently dissociated with a Dounce homogenizer and nuclei prepared by centrifugation in an iodixanol density gradient. The purified fixed nuclei can then be immunostained with specific antibodies and either analyzed or sorted by flow cytometry.



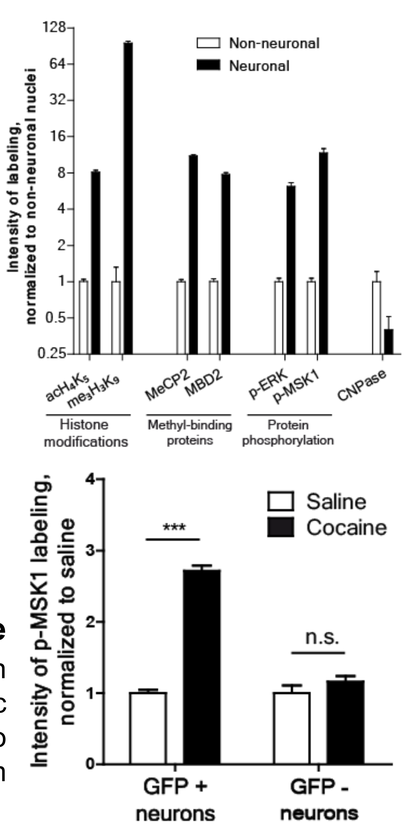
Outline of the procedure.
It can be performed within one or two days.



Fixation prevents soluble proteins such as GFP to leak out of the nucleus.



Glial and neuronal nuclei can be distinguished without any labeling. In this example, using appropriate transgenic mice, we were able to distinguish the two main types of neurons of the striatum simultaneously.



Nuclear proteins can be labeled using antibodies.
In this example, several signaling markers were found more abundant in neuronal nuclei than non-neuronal nuclei.

Dynamic changes in a subpopulation of neurons can be detected.
Mitogen- and stress-activated protein kinase 1 (MSK1) is known to be phosphorylated in D1R expressing neurons only, after cocaine treatment. Here we used D1-GFP transgenic mice (treated with cocaine or saline) and pMSK1 antibody.

The FAST-FIN methods allows the analysis and sorting of fixed nuclei based on multiple criteria (size, fluorescent proteins, antibody labeling). We even sorted nuclei as rare as 0.6% of the total population (paravalbumin interneurons) with a 97% purity. Sorted nuclei are then available for further biochemistry analysis.
As the tissue is being quickly fixed with formaldehyde, labile or transient post-translational modifications can be studied.
This protocol should be applicable to other tissues and species.

We believe that the FASS and FAST-FIN methods represent the beginning of a new era of investigations on brain tissue in subcellular fractionations in physiology and pathology.

[1] Whittaker VP (1993) *J. Neurocytol.* **22:** 735–742
[2] Biesemann C, et al., (2014) *EMBO J* **33:** 157–170

Funding Sources: