RESEARCH SUBJECT AND OUTLINE: neuronal wiring seen by the cytoskeleton

How the brain and neural activities are formed and altered during development, learning, senescence, disease and regeneration are intertwined questions with underpinning structural and molecular explanations. Very little is known about the role of microtubule (MT) polymerization during neurite outgrowth, guidance, synaptogenesis and nerve regeneration, despite the essential role of MTs for axonal/dendritic transport and generation of neuronal morphology/polarity. By pharmacological means, it has by real-time microscopy been demonstrated convincingly that MT dynamics apparently are *not* critical for neurite extension but intriguingly are required for growth cone turning at a substrate boundary[a,b]. However, the underlying molecular mechanism(s) remain unknown[c-e]. Using a combined *in vitro* and *in vivo* approach, this study aims, amongst other, to develop further a zebrafish spinal neuron culture system (Fig.1), and initially use it to address how MTs regulate neurite outgrowth, guidance and synaptogenesis[c,f-h]. The topic is part of the general efforts to unravel the mechanics of CNS neuronal wiring and cell migration, that also encompass upstream receptor dynamics, of which neurons offer a delicate example to be contrasted and compared with findings from epithelial systems[c-f,i,j].

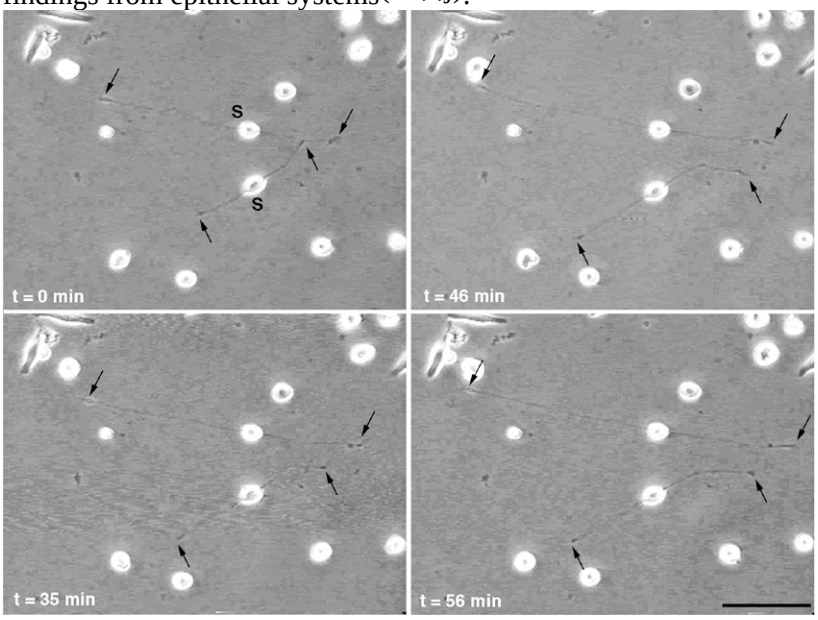

Figure 1: Time course of the growth of two representative bipolar zebrafish neurons on laminin coated glass. S is soma; arrows point to the growth cones. Time in left corners is in minutes. Bar: 70 µm.[f]

SIGNIFICANCE AND OUTLOOK

In vitro zebrafish approaches are yet rare, but considering the full genome sequence, its transparency, and the many mutant and transgenic lines available, the combined *in vivo-in vitro* zebrafish approach has a quantum leap to offer the research on mechanistic studies of neuronal wiring in vertebrates[c,f-h]. Indeed, a combination of *in* vivo and in *vitro* approaches is required to reach a mechanistic description of the wiring question. Other systems, such as Xenopus and rat, may find essential applications where zebrafish shows limitations.

This zebrafish approach[f,g] and hypothesis[c,m], potentially offers to bridge phenomenological clinical discoveries in the areas of neurological disorders, like Schizophrenia[k] and Parkinson[l], with their underpinning molecular mechanisms, as well as providing fundamental molecular mechanistic insights of key importance in our nascent understanding of nerve regeneration[h].

Postscriptum: though the experiments suggested in this proposal, dating back to year 2000-2001, to date remain of relevance addressing, the central aspect of the proposal is ***to combine*** zebrafish *in vitro* and *in vivo* approaches for the study of neuronal wiring of an organism. The latter determined more specifically as a function of the particular research environment where your particular experiments are to be performed. In the proposal, the spinal cord is used, because it is relatively easy to isolate neurons from the spinal cord for mechanistic *in vitro* studies. Yet, there would be no hindrance applying this same approach for the study of brain circuit formation in the zebrafish.

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