Neuronal plasticity: Cell-based strategy for target identification and validation

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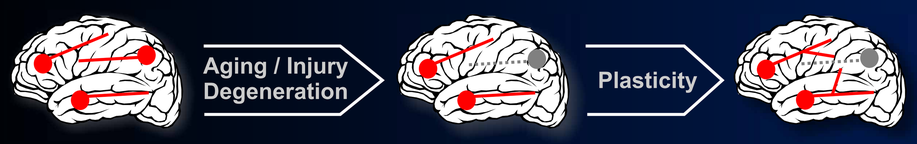
INTRODUCTION

- **Growing neurons are relatively plastic during development, which allows their neurites to sprout and generate new connections.**
- **Plasticity levels drop rapidly as neurons mature and become integrated into neuronal networks.**
- **As a consequence, the central nervous system ability to reorganize itself in response to injury or disease is insufficient.**
- **One of the main limitations for the design of therapeutic strategies to enhance neurite sprouting following neurological diseases is our poor understanding of the mechanisms underlying neurite structural plasticity.**



Goal: Identify therapeutically relevant drug targets to stimulate neuronal plasticity.

1 Candidate targets taken from the literature

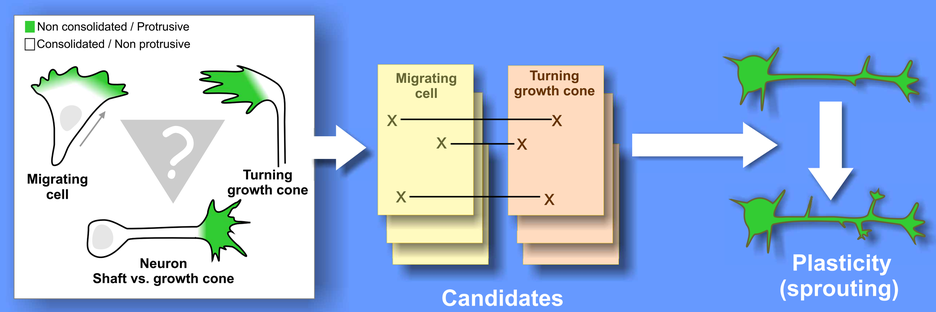
Candidate targets for neuronal plasticity

Rationale and strategy:

- Similar cellular systems are regulated by similar signaling pathways.
- Candidate targets are identified from the literature based on predetermined criteria (e.g. involvement in cell migration and growth cone collapse)

Candidate approach allows to identify new functions of known proteins, and therefore:

- Fast discovery of suitable targets,
- Identify new uses for known drugs,
- Minimize development risk (known drugs),
- Maximize speed to market,
- Fast track and orphan drug status (for many CNS indications).



Cell-based assay using hippocampal neurons

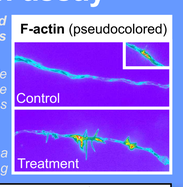
2 Cell-based assays for: a) desired and b) undesired effects

Actin assay

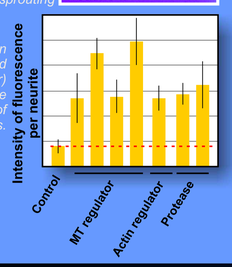
Actin patches are required precursors of sprouts

Actin staining shows little accumulation along the neurite of control neurons

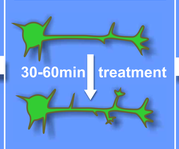
Appearance of patches is a sign of incipient sprouting



Intensity of actin staining (yellow+red area in pseudocolor) obtained with some inhibitors of candidate proteins.



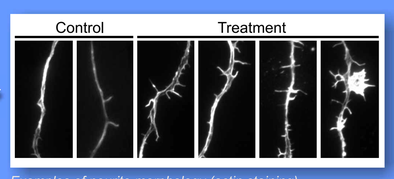
Experimental set up



One day in vitro neurons:
- *Fast (2 days) experiment*
- *Actin staining in 20 mins*
- *still very few branches*
- *great potential to branch*

Good cell-assay for inhibitors of potential targets.

Sprouting assay

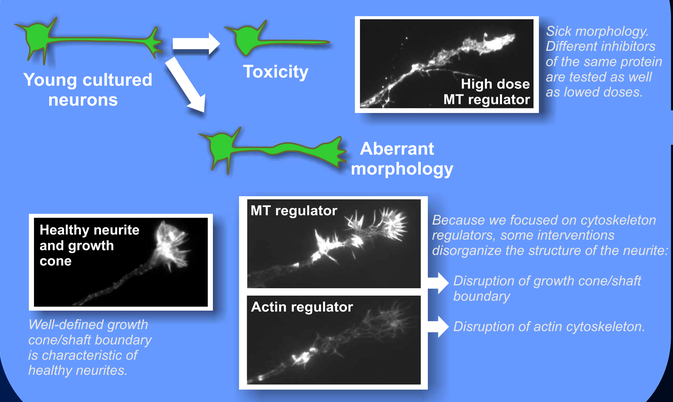


Examples of neurite morphology (actin staining).

Target	Drug	Doses	Efficiency
MT regulators	Inhibitor I	10-100nM	-
		1µM	+++
	Inhibitor II	10nM-1µM	++/+++
Actin regulator	Inhibitor III	5-10µM	++/+++
Proteases	Inhibitor IV	1-50µM	+++
	Inhibitor V	25µM	++/+++
	Inhibitor VI	15µM	++
	Inhibitor VII	10-25µM	-

Folds of branches (- = no change) obtained with some of the inhibitors.

Health and neurite morphology



Sick morphology. Different inhibitors of the same protein are tested as well as lowed doses.

Well-defined growth cone/shaft boundary is characteristic of healthy neurites.

Because we focused on cytoskeleton regulators, some interventions disorganize the structure of the neurite:

Disruption of growth cone/shaft boundary

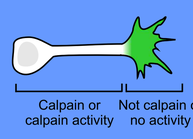
Disruption of actin cytoskeleton.

Case study: *Calpain*

- Several calpain inhibition promote neurite sprouting.
- Endogenous calpain activity doesn't seem to be required for normal neurite morphology/functioning.
- Calpain inhibitors are already being tested for CNS indications as neuroprotants.
- Because of calpain role in neuronal death, very specific inhibitors that cross the blood-brain-barrier are available.
- Data from spinal cord injury research shows delayed recovery in rats treated with calpain inhibitors (potential proof of concept of sprouting)

Validation I - Expression and activity

Since calpain is expected to act as a repressor of neurite sprouting, it must be expressed or active along the shaft, but not at growth cones.



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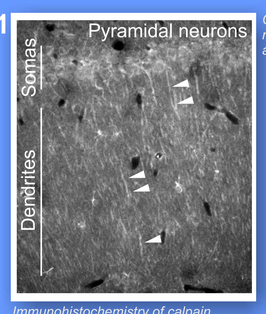
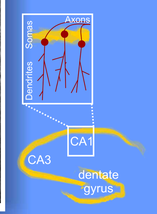


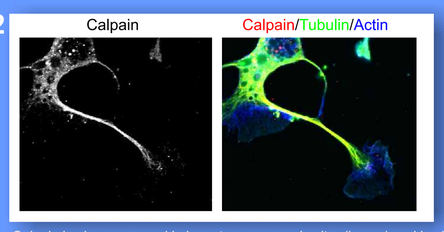
Immunohistochemistry of calpain in the hippocampus of adult mice (CA1 field).

Calpain is expressed in neuronsin the mature brain and localizes to neurites.



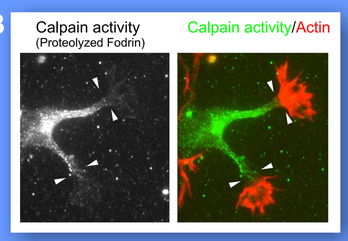
Cartoon of adult mice hippocampus.

2



Calpain is also expressed in immature neurons in vitro (image) and in vivo. It preferentially localizes to consolidated (non protrusive) regions.

3



Fodrin is an endogenous target of calpain. While fodrin localizes to the entire neuron, proteolysis (and immunodetection) only occurs where calpain is active.

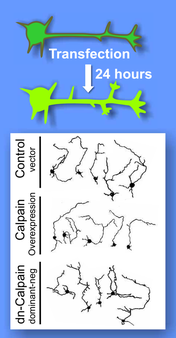
Calpain activity is restricted to consolidated regions. No activity is detected at the growth cone.

3 Validation steps: a) Protein expression b) Protein activity c) Regulation by branching factors

Validation II - Participation in neurite sprouting

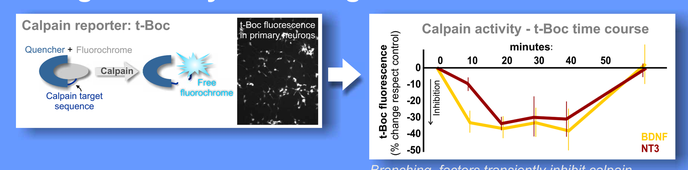
1. Sufficiency

Genetic regulation



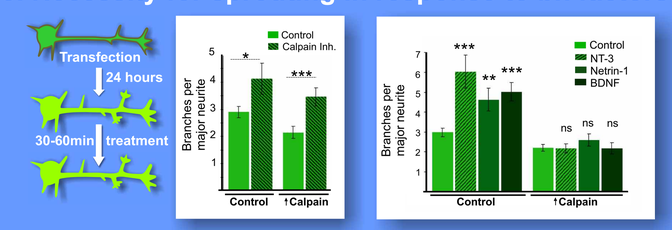
Overexpression of calpain prevents branching. *(Calpain overexpressing neurons have the same number of branches they had at 2div)*

2. Regulation by branching factors



Branching factors transiently inhibit calpain. ***Calpain is inhibited during physiological neurite sprouting.***

3. Necessity for sprouting in response to br. factors

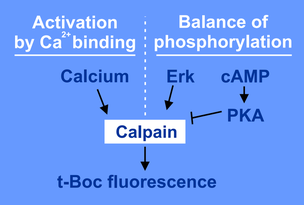


High levels of calpain can be blocked with pharmacological inhibitors. This inhibition induces neurite sprouting.

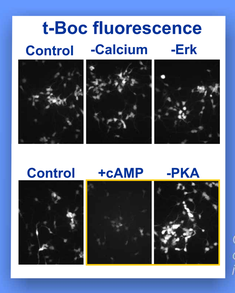
High levels of calpain cannot be blocked by physiological inhibitors (branching factors). ***Calpain inhibition is required for neurite sprouting.***

Pathway mapping to identify additional targets.

1. Upstream / regulators

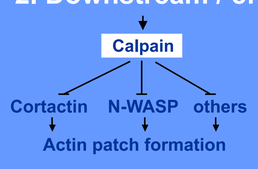


Potential regulators of calpain in neurons based in the literature (non-neuronal cells)

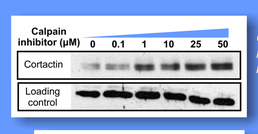


Only the cAMP-PKA pathway is confirmed as maintaining calpain activation in neurons (PKA acting as a repressor).

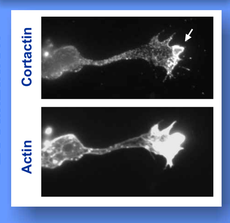
2. Downstream / effectors



Potential downstream targets for calpain regulation of actin patch formation in neurons based in the literature (non-neuronal cells).

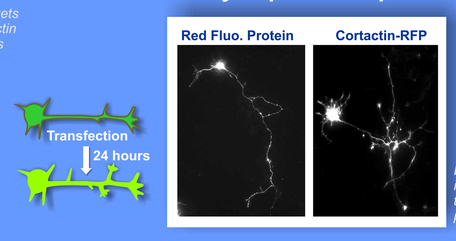


Cortactin levels are regulated by calpain in neurons.



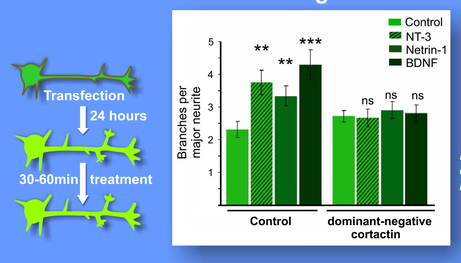
Cortactin accumulation is restricted to areas of low calpain activity.

Sufficiency to promote sprouting



Elevation of cortactin levels in the entire neuron is sufficient to promote an hyperbranched phenotype.

Necessity for sprouting in response to branching factors



Inhibition of cortactin activity using a dominant-negative construct prevents neurite branching.

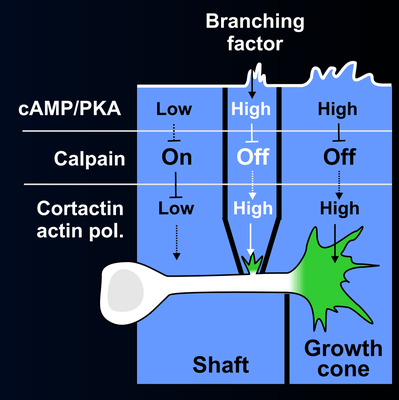
CONCLUSIONS

Approach:

- **Signaling convergency can be used as a starting point when previous knowledge on the signaling pathways involved is missing.**
- **Focusing on known pathways minimizes the time to discovery of suitable targets because of the availability of reagents and (potentially) animal data.**
- **Focusing on known proteins also allows faster mapping of the pathway upstream and downstream of the *positive* candidates (pathway validation vs. discovery), allowing the identification of additional, or better, targets.**

Experimental results:

- **Neurite sprouting is a process of de-repression, as opposed to a "positive event".**
- **Calpain maintains neurite consolidation by constitutively repressing protrusive activity.**
- **The discovery of calpain function in neurons provides a link between cAMP levels and neuronal plasticity.**
- **Calpain and cortactin are two novel targets to stimulate neuronal morphological plasticity.**



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