



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

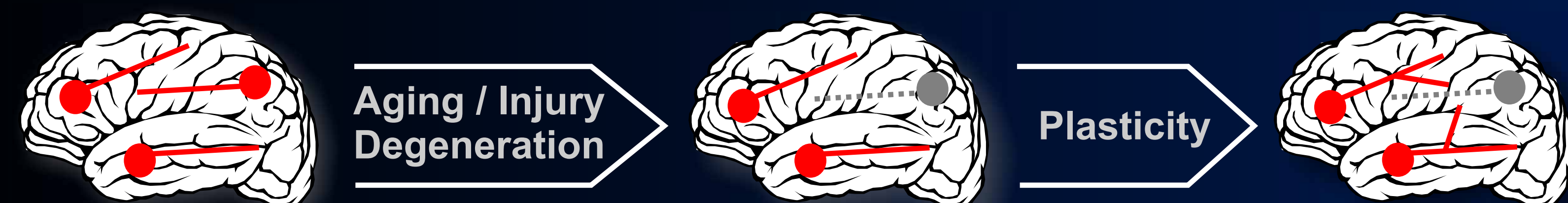
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INTRODUCTION

- 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.

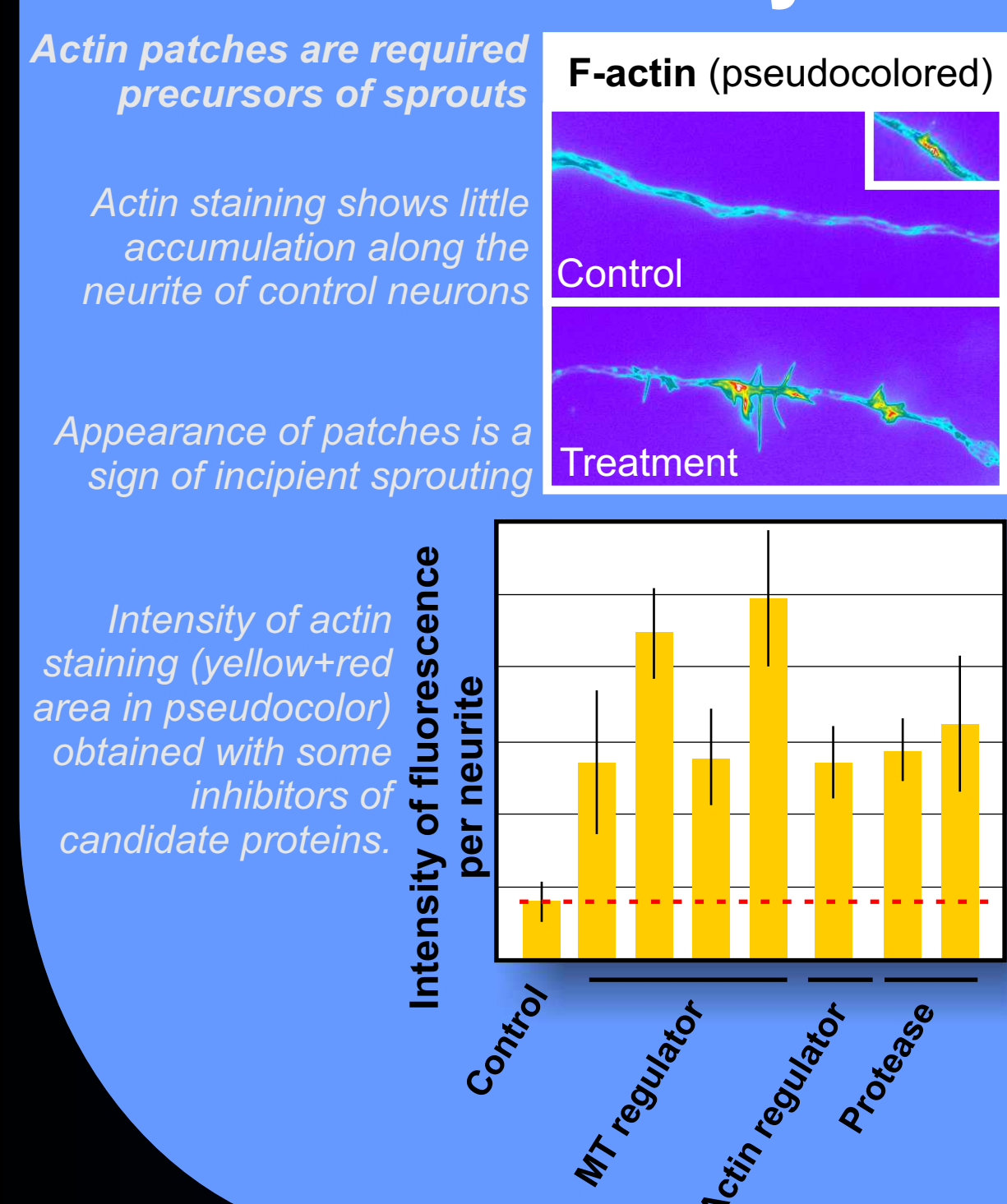


Goal: Identify therapeutically relevant drug targets to stimulate neuronal plasticity.

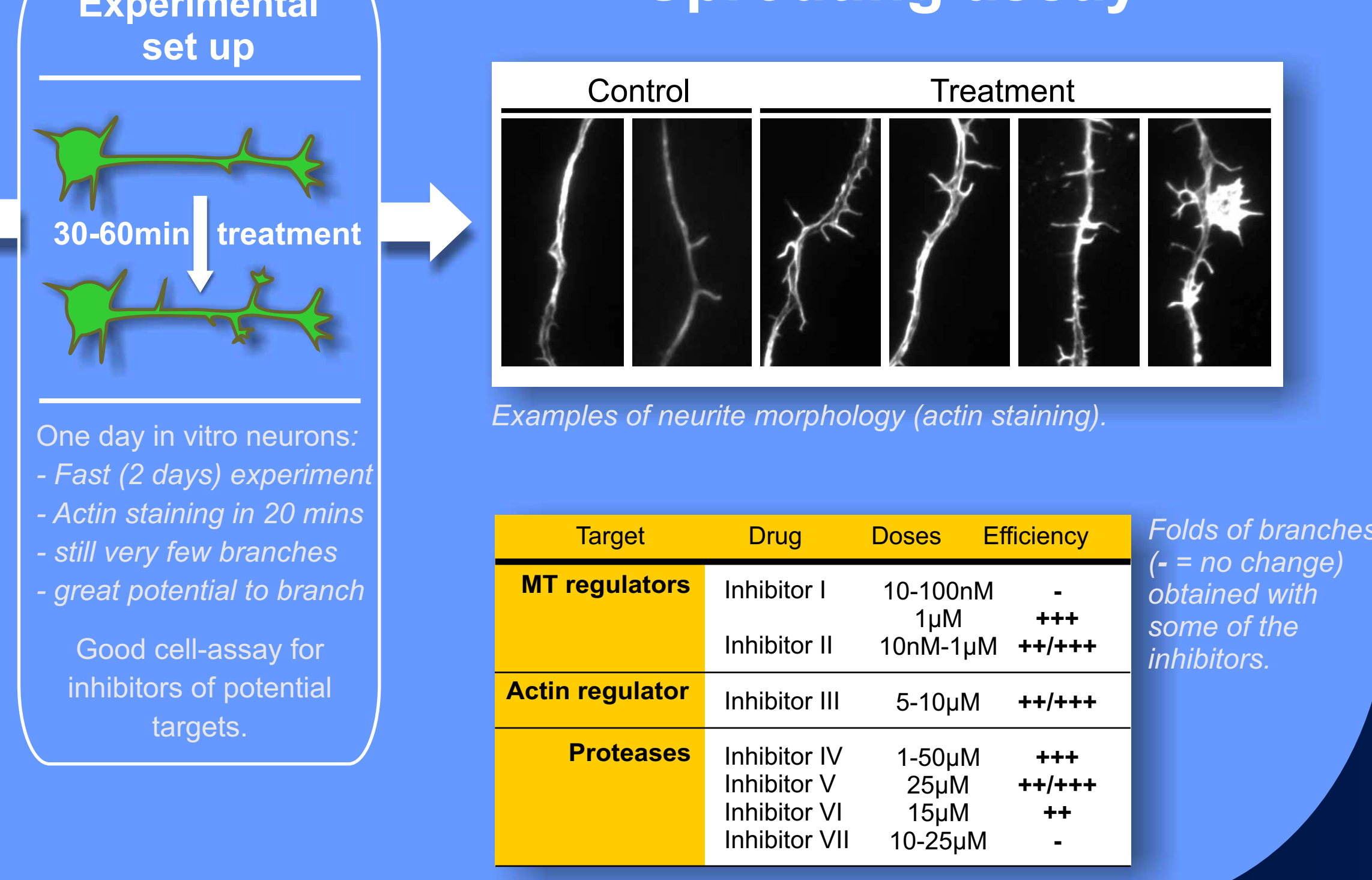
Cell-based assay using hippocampal neurons



Actin assay

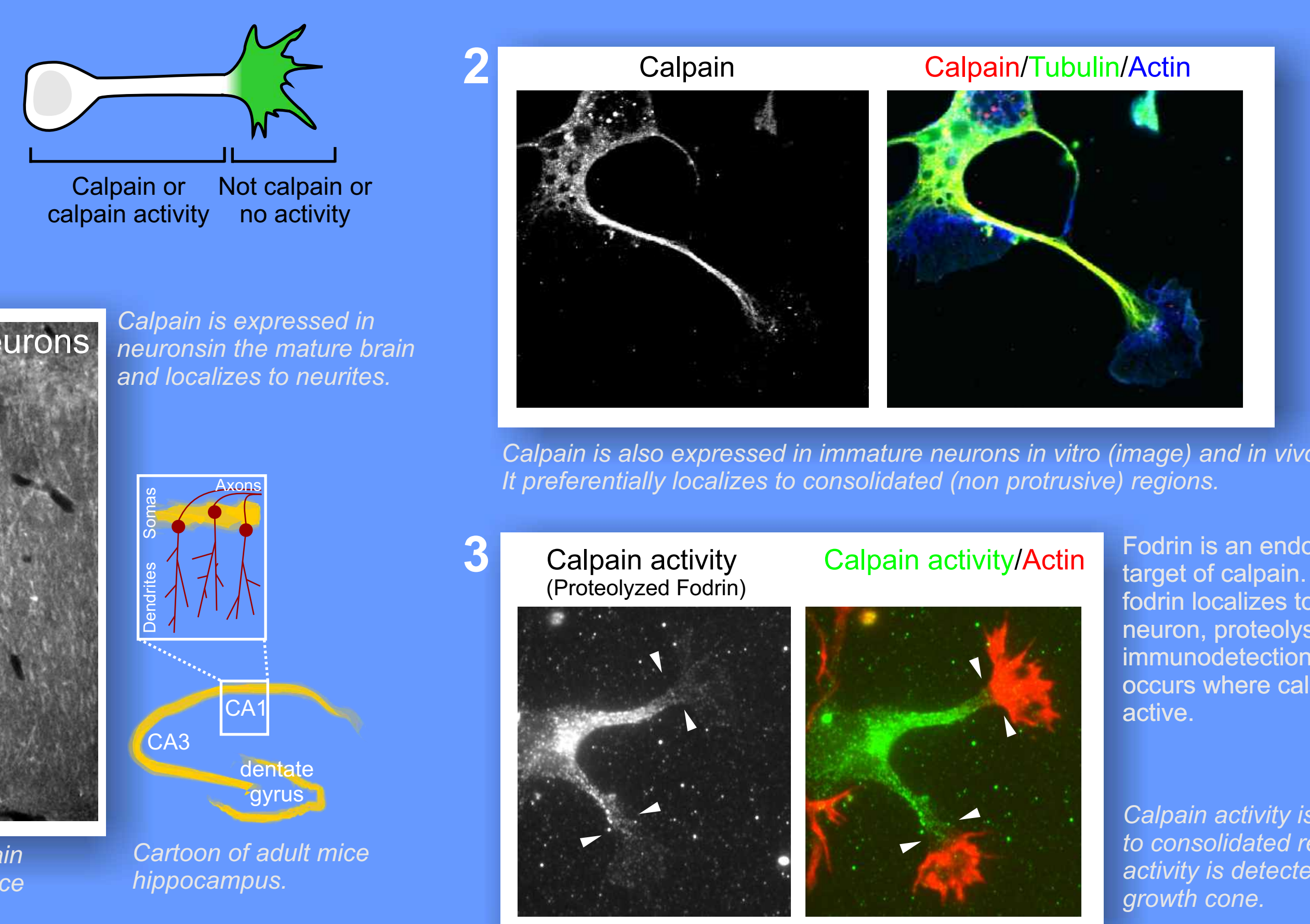


Sprouting assay



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.



1 Candidate targets taken from the literature

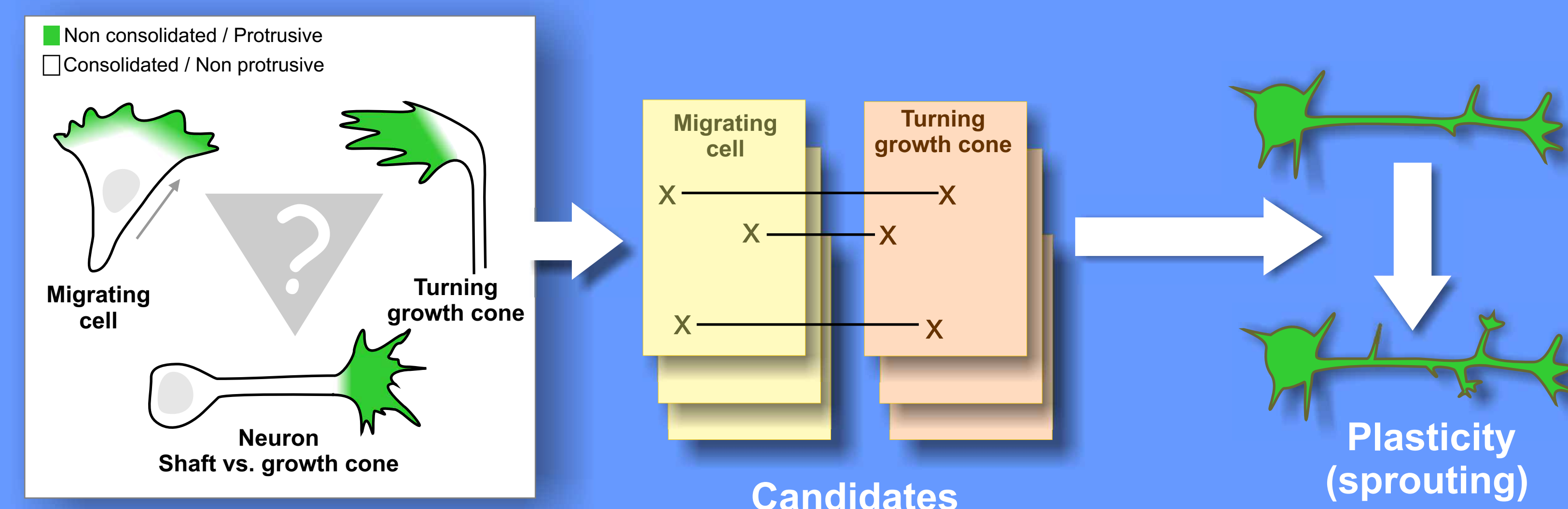
Candidate targets for neuronal plasticity

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:

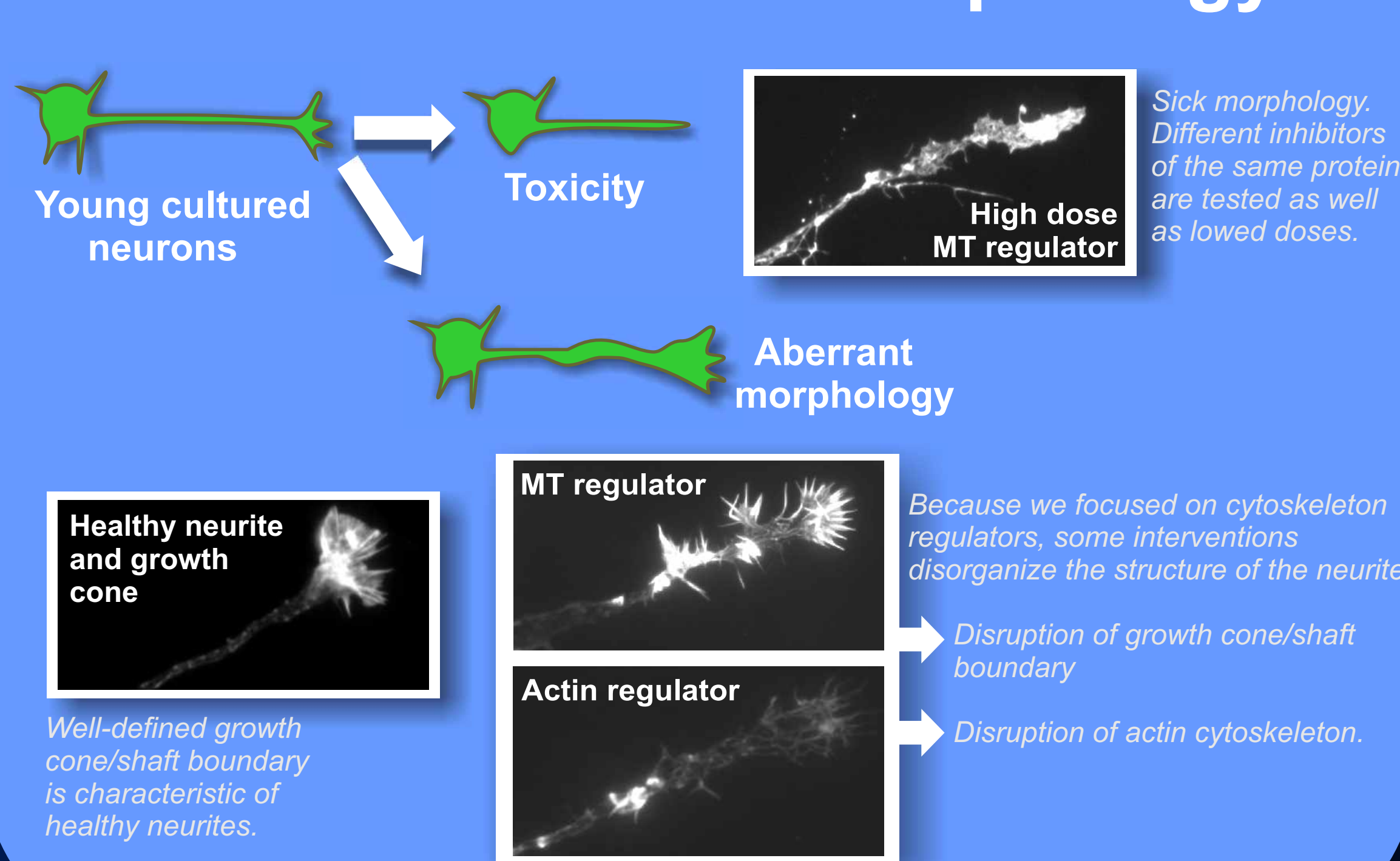
- Fast discovery of suitable targets,
- Identify new uses for known drugs,
- Minimize development risk (known drugs),
- Maximize speed to market,
- Fast track and orphan drug status (for many CNS indications).



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

Health and neurite morphology



Case study: Calpain

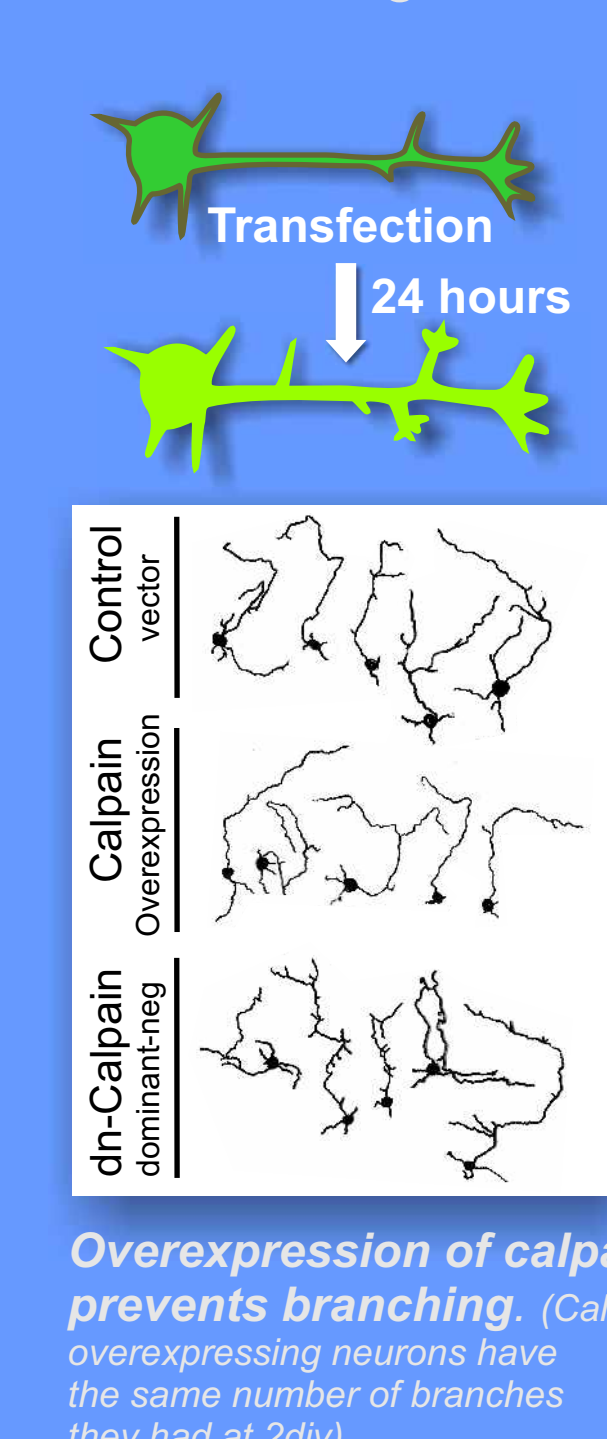
- Several calpain inhibition promote neurite sprouting.
- Endogenous calpain activity doesn't seem to be required for normal neurite morphology/functioning.
- Calpain inhibitors are already being tested for CNS indications as neuroprotectors.
- Because of calpain role in neuronal death, very specific 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)

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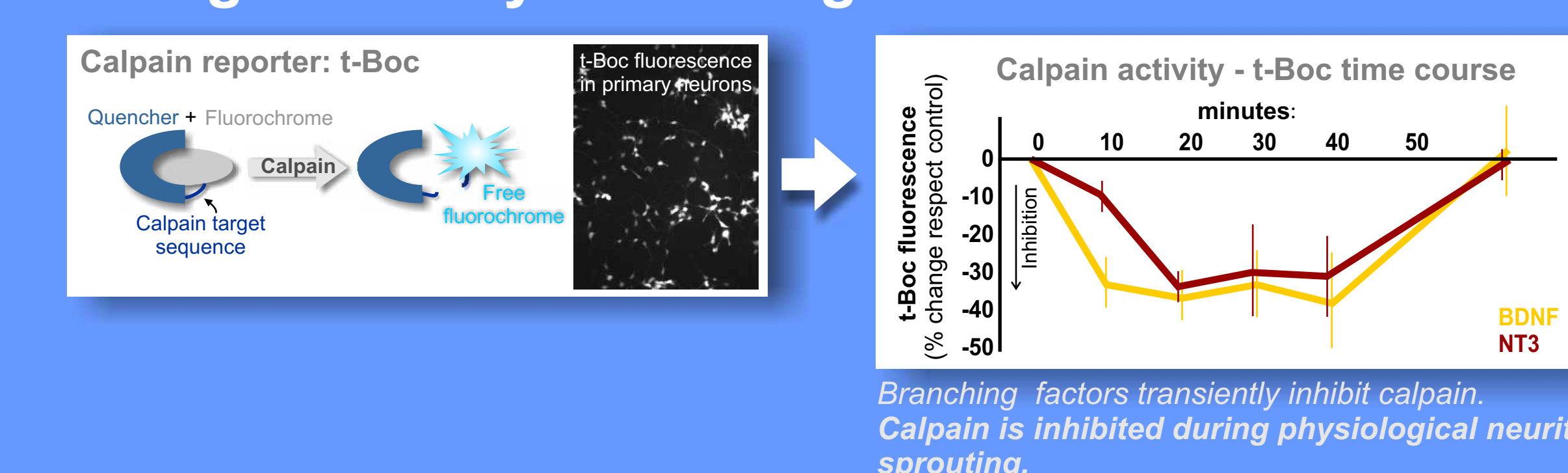
Validation steps: a) Protein expression b) Protein activity

Validation II - Participation in neurite sprouting

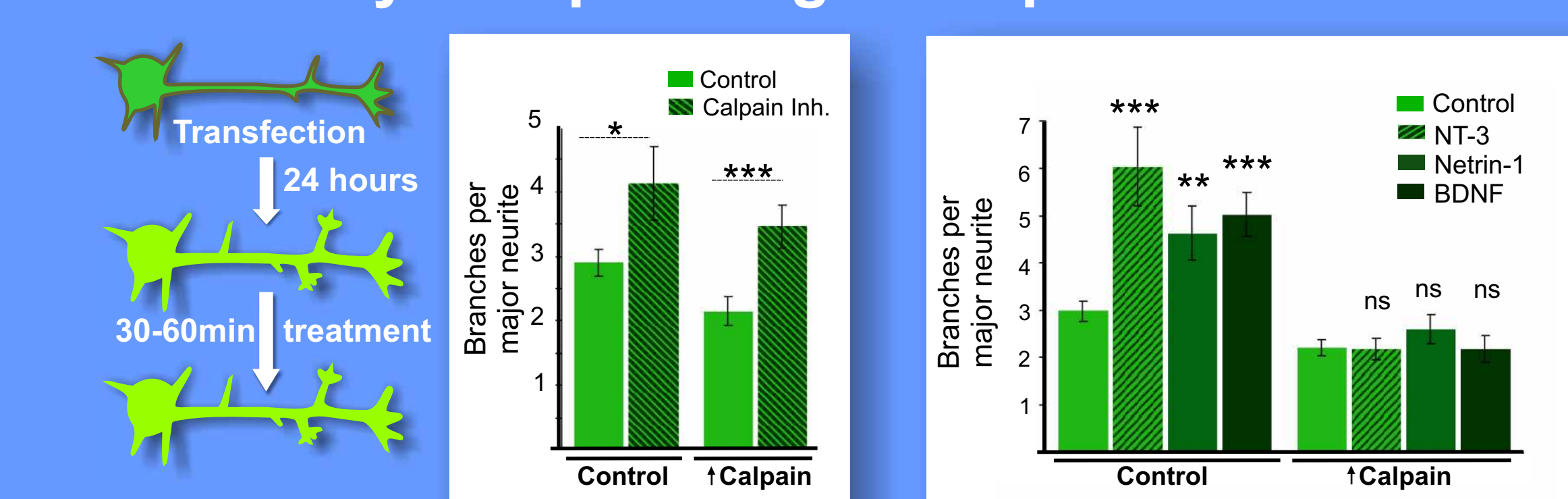
1. Sufficiency Genetic regulation



2. Regulation by branching factors

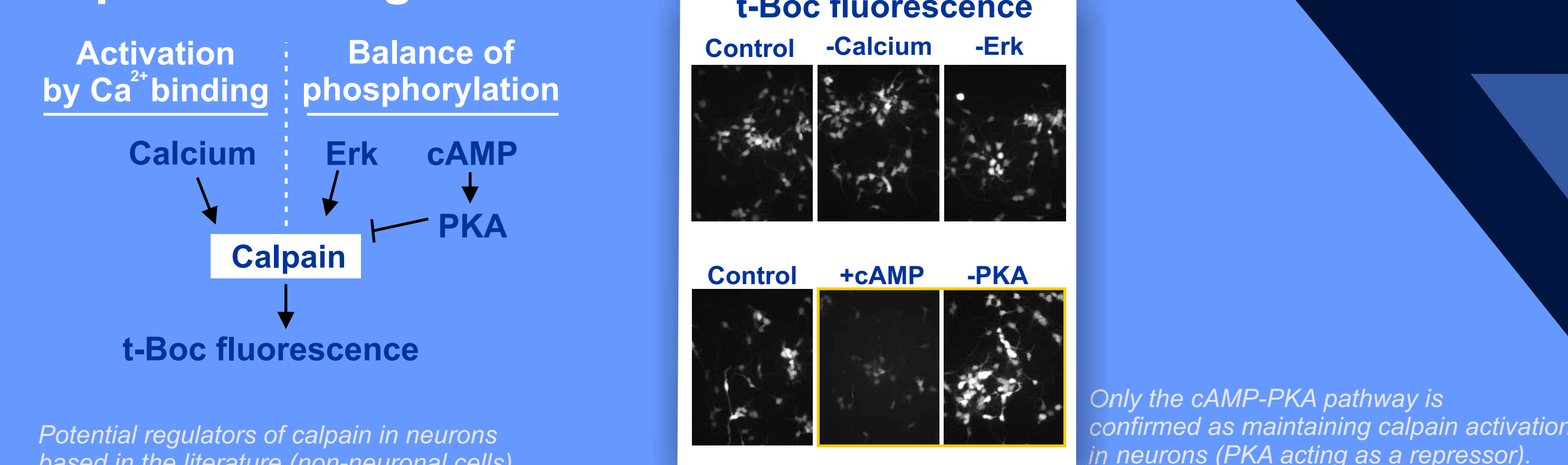


3. Necessity for sprouting in response to br. factors

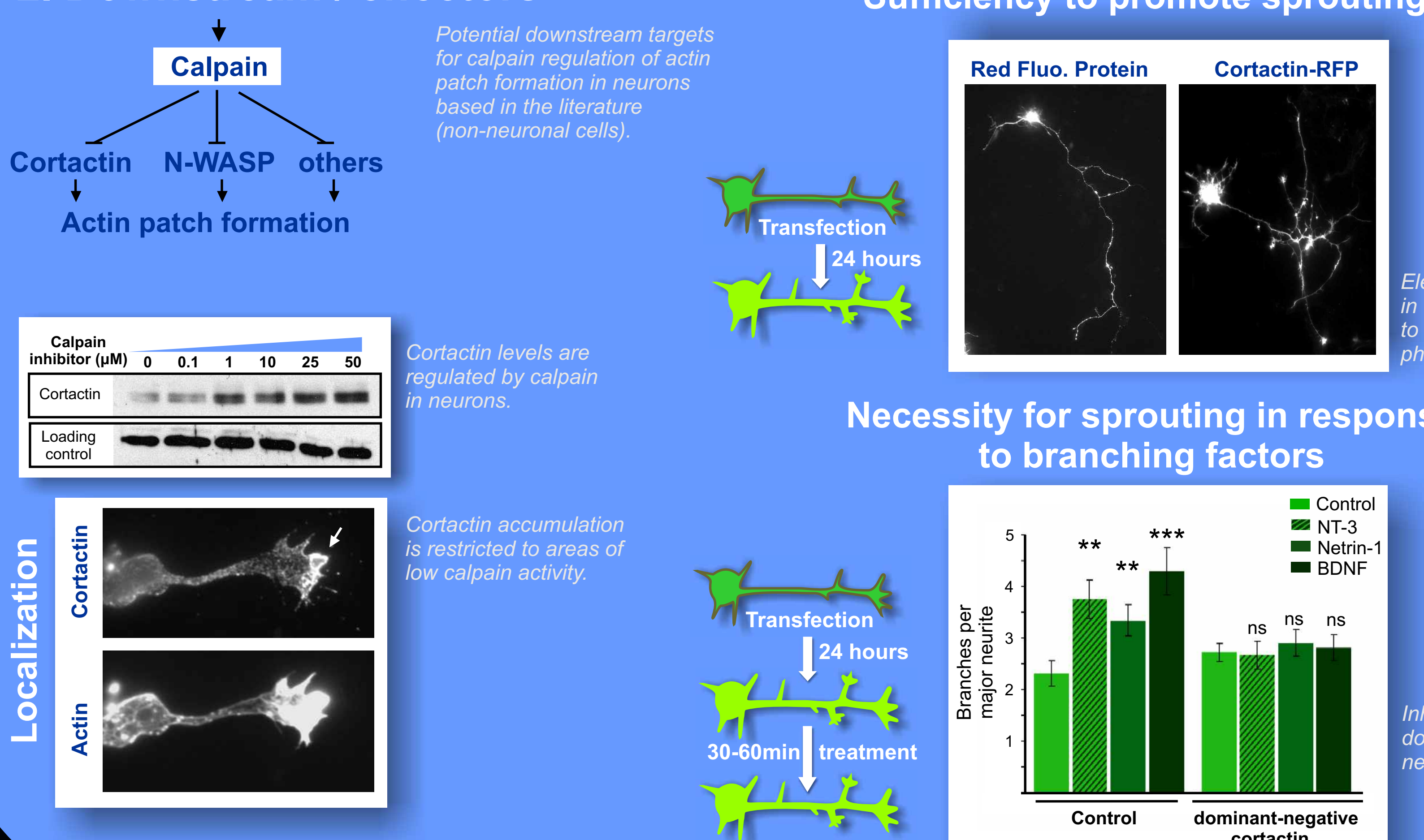


Pathway mapping to identify additional targets.

1. Upstream / regulators



2. Downstream / effectors



c) Regulation by branching factors

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Pathway mapping

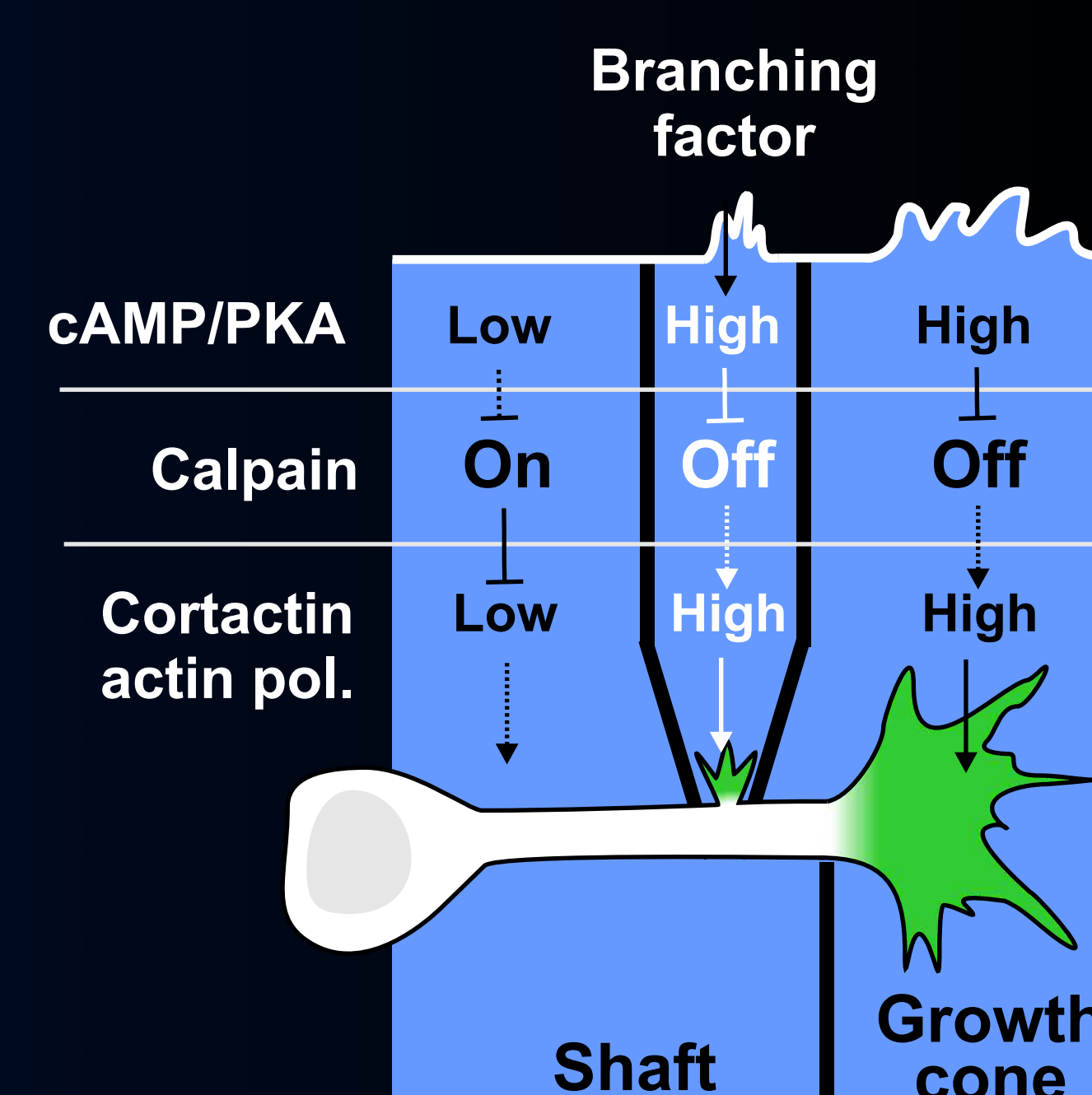
CONCLUSIONS

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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