Optimising the fibroblast-to-sensory-neuron differentiation protocol

BY

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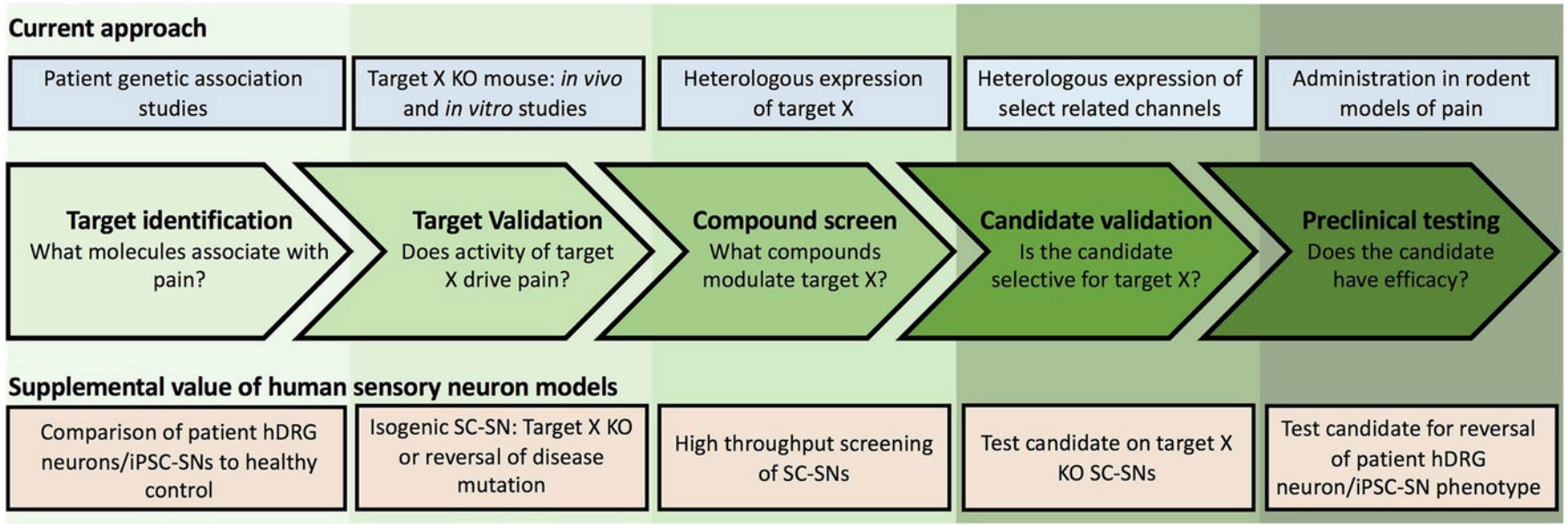
virtual work experience

at

LifeArc

Introduction

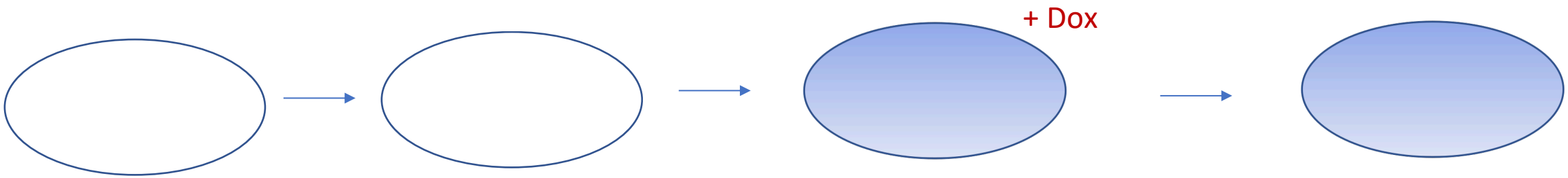
- *In vitro*-derived sensory neurons are gaining momentum in pain research and drug development



Chrysostomidou et al., *Neurobiol Pain*2021

Introduction

- A fibroblast-to-sensory-neuron differentiation protocol (Blanchard et al., *Nat Neursci 2015,* with modifications)



Aims

Optimisethe differentiation protocol conditions with respect to:

- The concentration of lentivirus delivering inducible *NGN2*
- Should the NT3 supplement be added to the neural induction media?

Experimental design

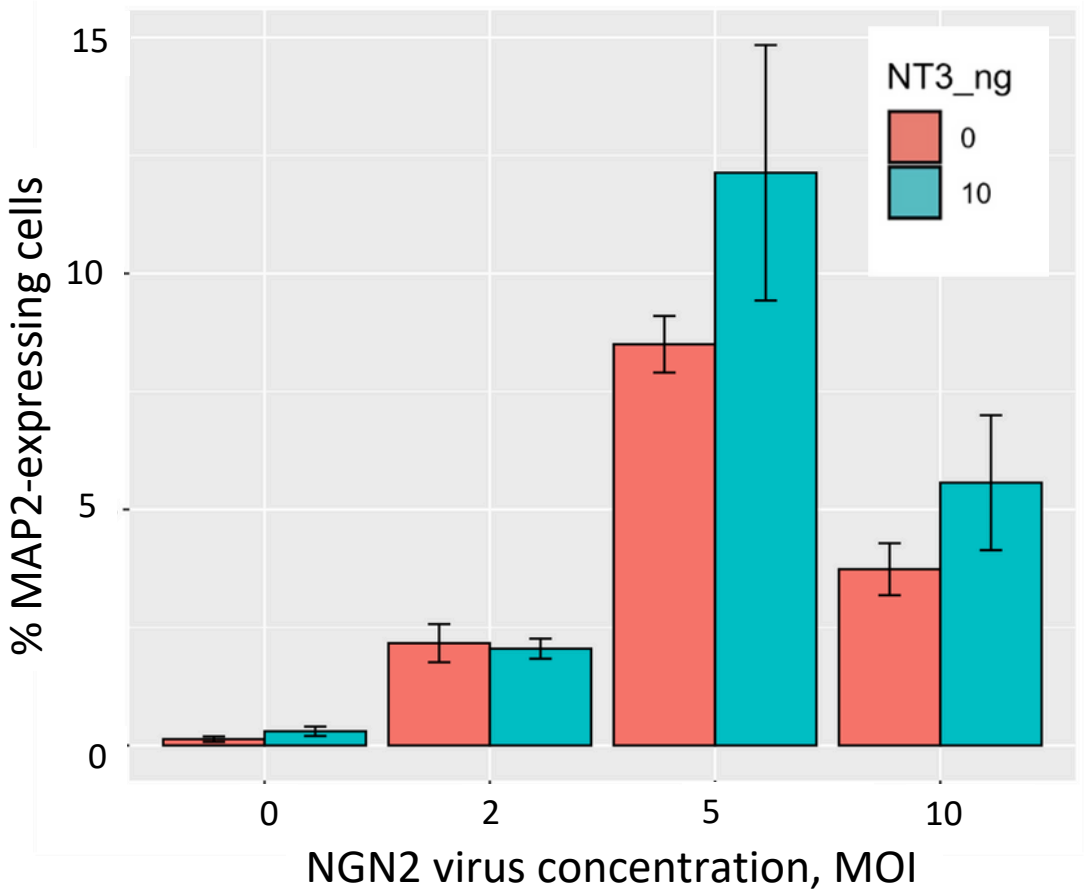
- Treatments tested:
  - 0, 2, 5, 10 MOI of the virus
  - 0, 10 ng/mL NT3

- Testing all combinations of these treatments (4 MOIs x 2 NT3 = 8), each combination in triplicate

- Using 5 MOI of the empty vector virus in the 0 MOI condition

- Assessing the expression of mature neuronal marker MAP2 (by immunostaining with anti-MAP2 Ab) after 14 days

Results



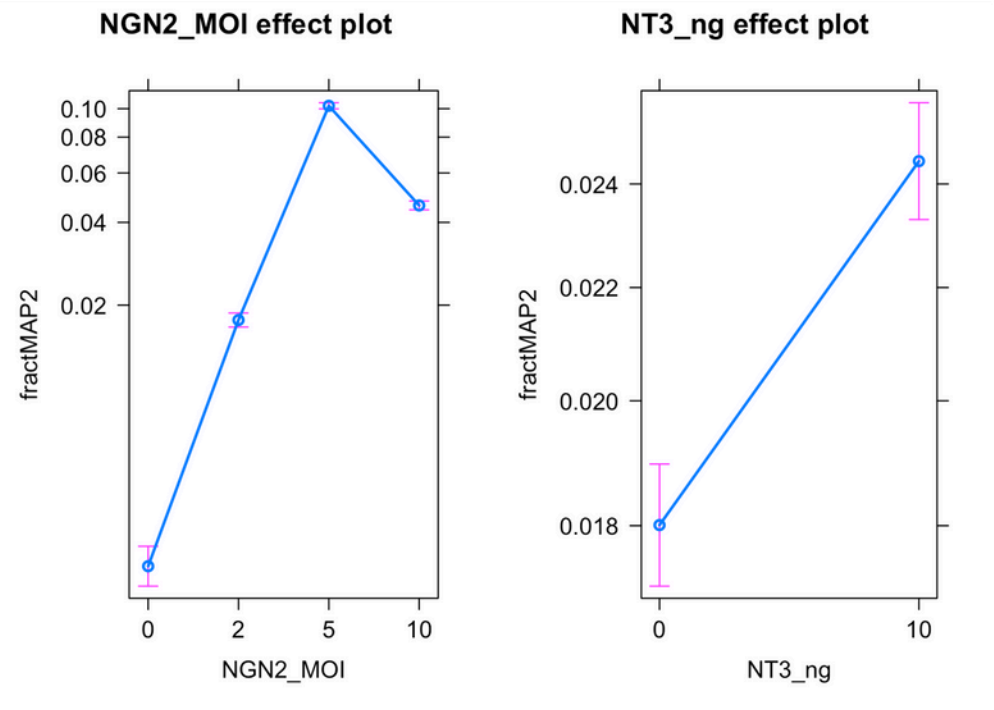
- One outlier replicate (~0% MAP2+ cells) was removed from 2 MOI+NT3, without much effect on the overall results

- 5 MOI of NGN2 virus + NT3 seems to be the most effective combination for generating MAP2-expressing cells

Results

- Establish the statistical significance of differences between conditions using **logistic regression**

- Chose logistic regression, because:
  - The outcome is a proportion (over 10,000 scanned cells)
  - Have combinations of treatments and want to establish the effect of each one



Logit(Fraction MAP2+) = k1*NGN2_MOI + k2*NT3_ng

- Using treatment doses as factors to allow for discontinuous trends

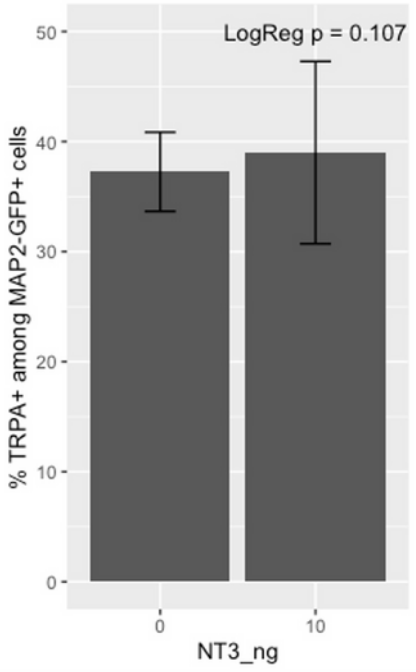
- 0 MOI works significantly worse than all other virus concentrations ($p<2.2e-16$)

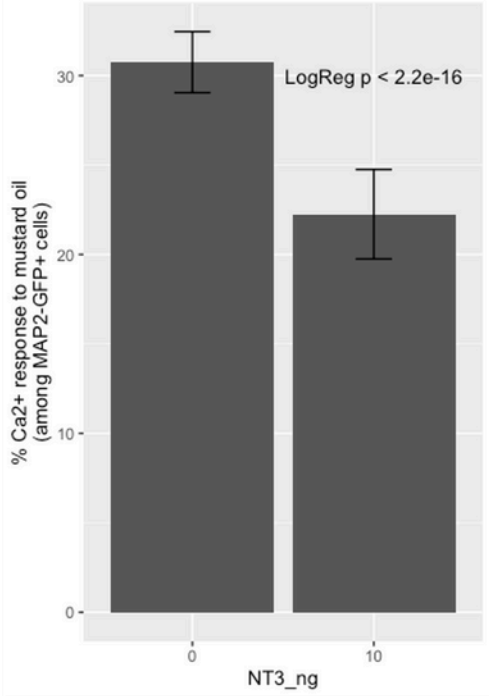
- 5 MOI works significantly better than all other virus concentrations ($p<2.2e-16$; tested by setting it as the control dose)

- NT3 has a smaller effect, but is also highly significant ($p<2.2e-16$)

Follow-up analysis (performed by Ela)

- MAP2 is not a specific marker of sensory/nociceptor neurons
- Does NT3 help generate nociceptors?
- **Ela's analyses using MAP2-GFP fibroblasts**
  - TRPA is a marker of nociceptors
    - No significant difference in % TRPA+ neurons with and w/o NT3
  - Ca2+ response to mustard oil is a **functional** feature of nociceptors. Assessed by FURA-2AM Ca2+ reporter
    - A **lower** proportion of mustard oil responders **with** NT3





Conclusions

- 5 MOI of NGN2 virus is the best concentration for generating mature neurons from fibroblasts

- NT3 increases the yield of MAP2+ cells, but negatively affects %Ca2+ responders to mustard oil

- I suggest using NGN2 virus at 5 MOI and no NT3

- The overall efficiency of generating nociceptor neurons in these conditions is ~2.4%
  (~8% MAP2-expressing cells, of which ~30% respond to mustard oil).

Next steps

- MAP2 may not be the most reliable marker of differentiation efficiency
  - Consider testing Ca2+ response to mustard oil routinely

- The overall efficiency of our protocol remains quite low

  - Consider further protocol optimisation

    - Consider selecting for cells expressing neuronal markers
      - Antibiotic resistance
      - Fluorescent reporters

THANK