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BBSRC bioscience for the future

Role of nitric oxide during nervous system development

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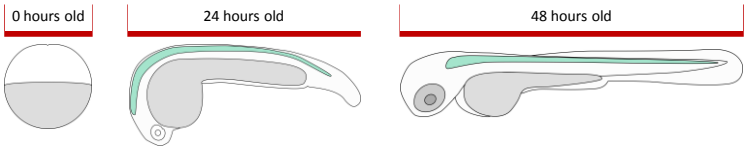
1 INTRODUCTION

The nervous system, which is responsible for a range of activities including locomotion, consists of specialised cells known as neurons. Each neuron has a cell body which extends a long process called the 'axon'. The axon's main role is to enable neurons to make contact and communicate with other cells (such as other neurons and muscle cells) which may be at some considerable distance from them. Between each axon and its coupled cell is a small physical gap called a 'synapse'. Information is passed across this synapse through release of 'neurotransmitters', small molecules secreted from the end of the axon. In this way neurons can communicate with one another and with muscle cells to generate behaviour.

During development, the nervous system must be assembled. An important process is the growth of axons to their target cells and the formation of synaptic connections. Many genetic and environmental signals are believed to influence this process. Amongst these is the gaseous molecule nitric oxide (NO). **Here we asked how NO affects development of synaptic connections between nerve and muscle cells, which are important for controlling animal movement.**

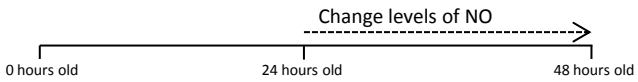
2 WHY ZEBRAFISH?

Zebrafish are a very useful animal for studying the development of axons and synapses: as vertebrates they contain a nervous system which is functionally similar to our own, yet it is much simpler, which makes it easy to study. Zebrafish develop outside of the mother and during early life are optically transparent, which makes it is easy to monitor developmental processes.



3 METHODS: WHAT WE DID

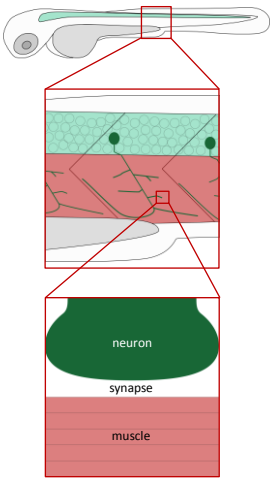
First we manipulated NO levels during development:



When embryos were 48 hours old, we used two techniques which allowed us to address whether changing levels of NO had any **anatomical** or **functional** consequences at the synaptic boundary between axons and muscle tissue.

1. Anatomical effects

Neurons are stained green and muscle is labelled red:

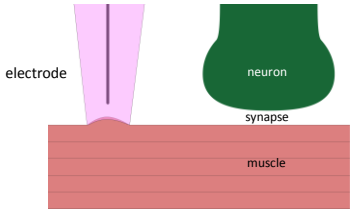


Overlapping of green and red allows us to identify synapses:

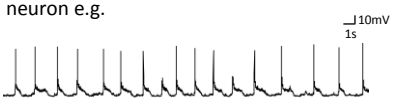
2. Functional effects

When neurotransmitters are released at the synapse, the electrical activity within the coupled cell changes. We can measure this activity and determine whether manipulating NO levels effects the function of the synapse between neurons and muscles.

To do this, a glass pipette containing an electrode is placed onto the cell of interest:

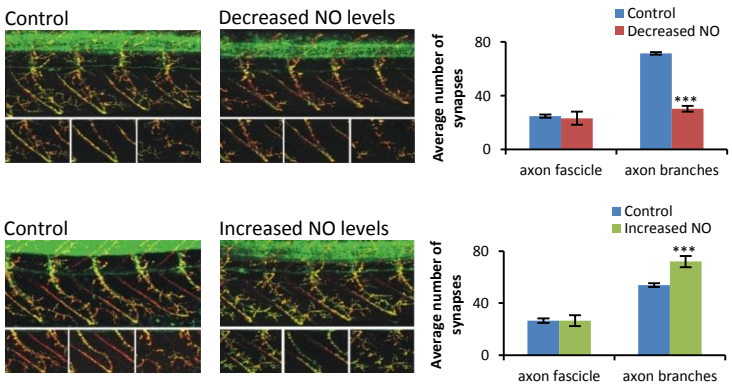


After we rupture the cell surface membrane, we can then observe the input the coupled cell receives from the neuron e.g.



4 RESULTS: ANATOMICAL CONSEQUENCES

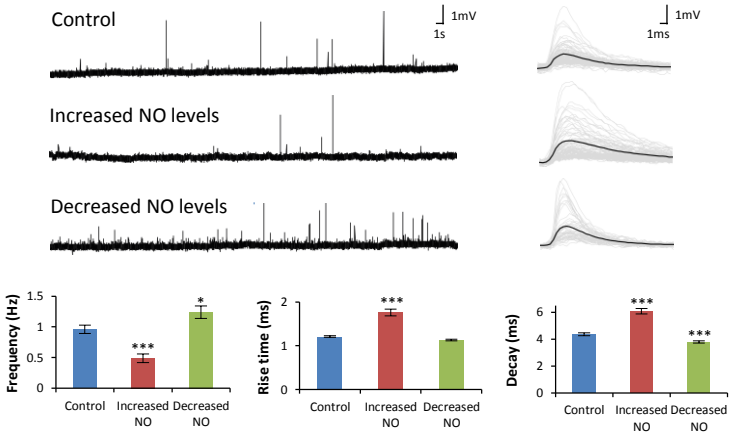
Disruption of NO signalling controls formation of nerve-muscle synapses.



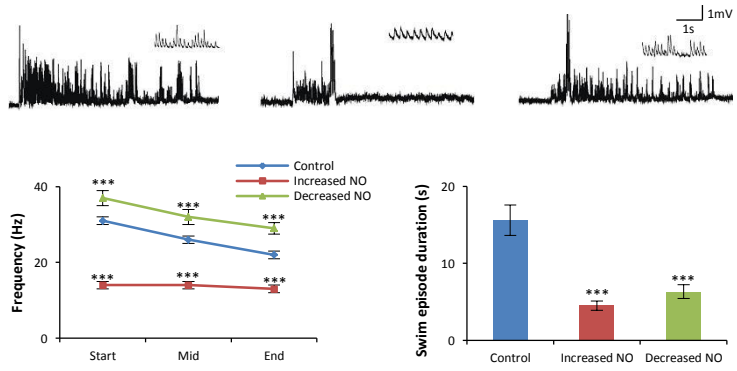
5 RESULTS: FUNCTIONAL CONSEQUENCES

NO affects electrical properties of neurotransmitter signalling at nerve-muscle synapses.

After blocking normal synaptic activity, spontaneous events can be observed when recording from muscle cells. From these events we can determine whether changes in NO levels affects the properties of synapses.



Disruption of NO levels impacts the electrical activity underpinning swimming.



6 SUMMARY

- **NO signalling regulates addition of synapses between neurons and muscle cells.**
  - Increasing NO causes a decrease in synapse number whilst a decrease in NO levels causes an increase in synapses.
- **Subsequent innervation of muscle tissue is affected by changes in NO levels.**
  - Increased NO levels causes a decrease in synaptic activity while conversely, decreasing NO levels causes an increase in synaptic activity.
- **Fictive swimming activity is perturbed by disruption of NO signalling.**