# Pre- and Postsynaptic Actions of Pancuronium in Spinal Cord - Skeletal Muscle -Cocultures

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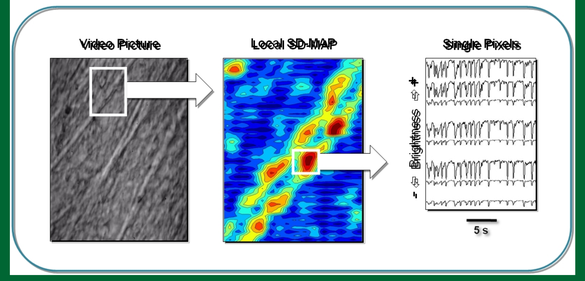
## Introduction

In organotypic spinal cord-skeletal muscle-cocultures of mice, functional neuromuscular junctions are forming during the first week in vitro. Thereafter, muscle contractions, driven by the firing of spinal neurons, can be readily observed.
Because drug access to the neuromuscular junction is not limited by diffusion, these cocultures seem to be a promising tool for the study of compounds modulating neuromuscular transmission directly or indirectly.
However, the significance of in vitro studies critically depends on the question of whether the reported drug effect is observed at a clinically relevant concentration. In order to address this issue, we investigated the action of the non-depolarizing muscle relaxant pancuronium.

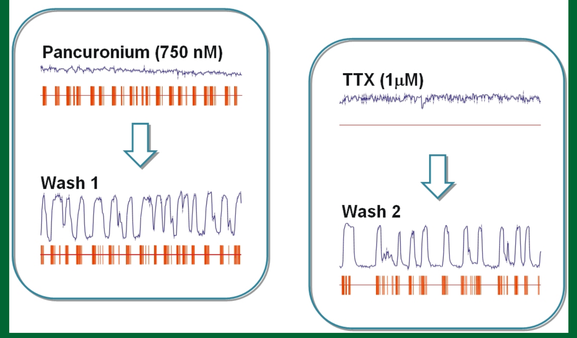
## Monitoring Muscle Contractions




Left: A video picture taken from a spinal cord-skeletal muscle-coculture.
Middle: The colors are coding the variation in brightness of single pixels. Red indicates a high standard deviation. Video pictures were sampled at 30 Hz. Total sampling time was 60 seconds.
Right: The curves show rhythmic changes in brightness (relative units) of single pixels, which were caused by spontaneous displacements of muscle fibers.

Correlation between muscle tension (upper panel, blue trace) and the discharge pattern of a neuron in the ventral horn of the spinal cord (lower panel, red trace).The neuron shows rhythmic activity. Bursts of action potentials are accompanied by muscle contractions.

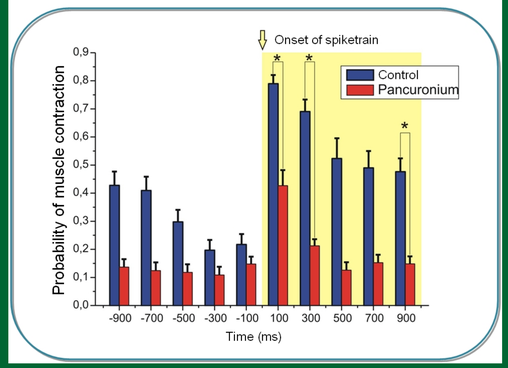
## Actions of Pancuronium and TTX




Left: Effects of pancuronium in a spinal cord-skeletal-muscle-coculture. Pancuronium acts as a competitive antagonist at nicotinic acetylcholine receptors. The drug depressed muscle activity without affecting spontaneous action potential firing of ventral horn neurons. After removing pancuronium (wash 1), muscle activity recovered.
Right: Effects of tetrodotoxin (TTX) in the same culture. TTX depressed both, action potential firing of spinal neurons and muscle activity. These actions were reversed after removing the drug (wash 2).

Muscle activity before and during action potential firing of neurons in the ventral horn of the spinal cord in the presence and absence of pancuronium. Note that under drug-free conditions, each train of action potentials triggers a muscle contraction.

## Peri-Spiketrain Muscle Tension: Grand Averages

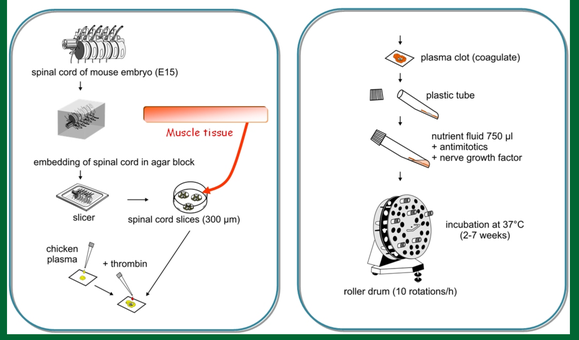



Probability of muscle contraction before and after onset of action potential activity in the ventral horn of the spinal cord. Under drug-free conditions, muscle activity is lowest before and highest immediately after spinal neurons start firing. Pancuronium reduces muscle activity before and after the onset of neuronal activity in the spinal cord. This effect is statistically significant at 100, 300 and 900 ms (paired t-test, $p<0.05$). Bin width is 200 ms. The data represent grand averages.

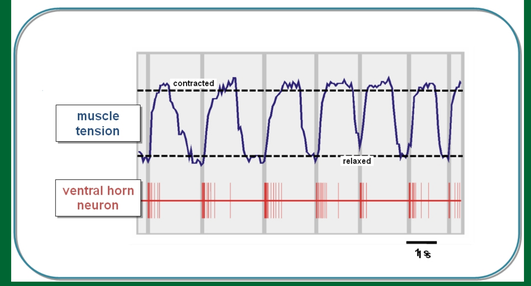
## Results

| | |
|---|---|
| 1 | In spinal cord-skeletal muscle-cocultures, spontaneously occurring bursts of action potentials, recorded from neurons in the ventral horn of the spinal cord, are preceeding muscle contractions by 1-100 ms. |
| 2 | At a clinically relevant concentration of 750 nM, Pancuronium attenuates muscle contractions without modifying action potential firing of spinal neurons. |
| 3 | The efficacy of pancuronium to block neuromuscular transmission is time-variant, reaching a minimum when ventral horn neurons are firing at their maximal rates. |

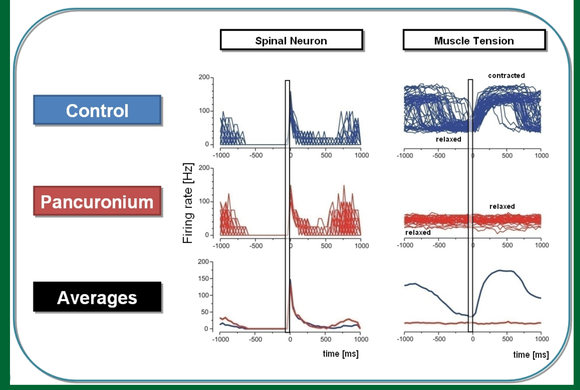
## Methods: Co-Culturing Spinal Cord and Muscle Cells



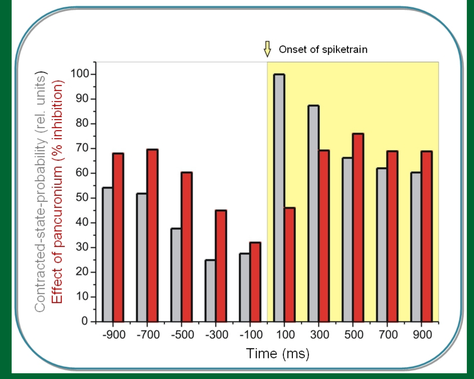

## Spinal Neurons Trigger Muscle Contractions




## Burst-Triggered Muscle Activity




## Efficacy of Pancuronium in Causing Muscle Relaxation




Efficacy of pancuronium in decreasing muscle activity is time-variant, reaching a minimum just before spinal neurons get active. During this period, spontaneous muscle activity is minimal. Note that the efficacy of pancuronium slowly increases after the onset of neuronal activity in the ventral horn of the spinal cord.