# Effect of the voltage sensor dipicrylamine on neuronal action potential propagation.

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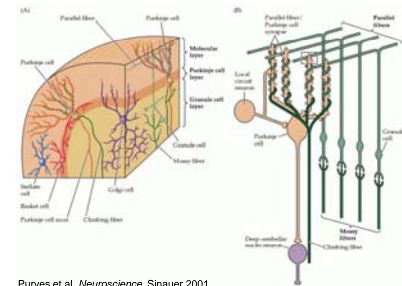
## Introduction.

Microelectrodes can be used to record electrophysiological events. However, this method is limited when it comes to monitoring a large number of cells in a circuit as well as measuring electrical signals in subcellular structures such as axons and dendrites. Fluorescence-based voltage detection based on the quenching of a fluorescence donor by a voltage sensitive fluorescence acceptor is a new approach that aims to ameliorate these limitations. The technique relies on DiO, a fluorescent, lipophilic neuronal tracer dye, and dipicrylamine (DPA), a lipophilic anion whose partitioning in the membrane is voltage sensitive. When DPA is in close proximity to DiO, it can quench the fluorescence emitted from DiO through FRET (Förster resonance energy transfer), and the degree to which it does so is directly related to the membrane potential of the cell. Together, DiO and DPA function as an optical reporter of membrane potential dynamics. The benefits of DPA include rapid kinetics, optimal voltage sensitivity within the physiological range, good aqueous solubility, and low phototoxicity. However, at concentrations above 5 uM, DPA may disturb membrane excitability. To better understand the effects of DPA on action potential propagation, this study compared fiber volley conduction velocity in parallel fibers of the cerebellar molecular layer in slices with varying concentrations of DPA applied. 5um and 10uM DPA were the concentrations evaluated in comparison to the control condition in aCSF. Fiber volleys, which are extracellular field responses to action potentials traveling along the fibers, were recorded in current clamp in order to measure changes in voltage subsequent to DPA application.

## Cerebellar Circuit.

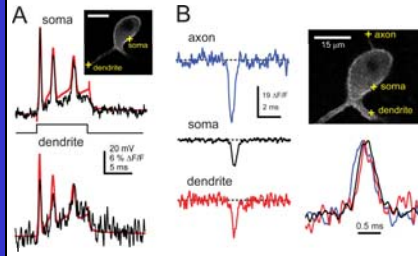


Purves et al, *Neuroscience*, Sinauer 2001.

## Methods.

Transverse cerebellar slices (270-300 mm) were cut from 20 to 28 day old mice. The external aCSF contained (in mM) 119 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1 $NaH_2PO_4$, 26.2 $NaHCO_3$, 25 glucose. Fiber volley stimulus (120-250 uA) and recording electrodes were placed on parallel fibers in the molecular layer 862.5-1150 um apart. After eliciting a fiber volley, DPA (5uM and 10uM) was washed into the bath. Fiber volley recordings were done from 5 minutes up to 50 minutes in 5-minute increments. Each trial consisted of 20 sweeps of increasing stimulus duration from 20-400 microseconds by increments of 20 microseconds.

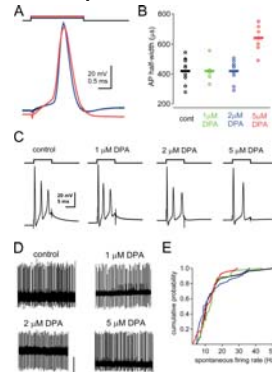
## DiO/DPA FRET pair reports Purkinje neuron firing in brain slices.




Bradley et al. J. Neurosci., 29(29):9197-9209, 2009

In 2uM DPA, the DPA/DiO fret pair images cell complex spike-like bursting in the soma and dendrites of Purkinje neurons as reflected in the averaged (n=16) optical recordings (A). The complex spike evoked by high current injection is intended to mimic a climbing fiber response. Similarly, responses to a single action potential in different subcellular locations can be imaged simultaneously (B).

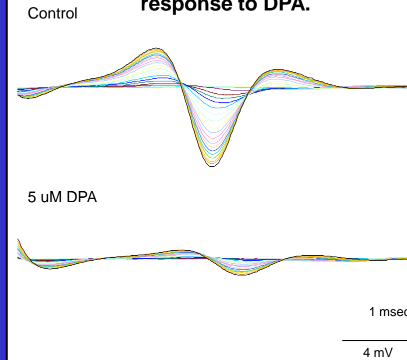
## DPA does not affects excitability of Purkinje neurons in vitro.




Bradley et al. J. Neurosci., 29(29):9197-9209, 2009

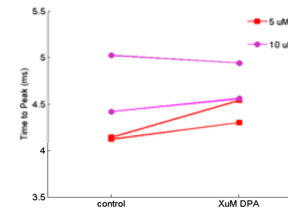
As previously shown by Bradley et al. in whole cell current clamp recordings of Purkinje neuron action potentials following 2 ms step current injection, 5 uM DPA prolongs the AP time course (A, B). Additionally, the diminished complex spike (C) indicates that DPA can modify spike shape. However, DPA does not affect excitability in vitro because DPA does not significantly affect the spontaneous firing rate of Purkinje neurons (D, E).

## Attenuation of fiber volleys in response to DPA.



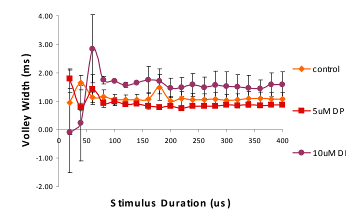

Each trace represents 1 run containing 20 sweeps from 20-400 usecond stimulus duration in 20 usecond increments. As stimulus duration increases, stimulus intensity increases, thereby exciting more fibers and increasing the amplitude of the volley. 5uM DPA delays the time to peak of the volley.

## Time to peak of fiber volley increases in DPA.



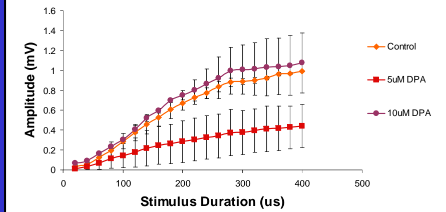

The time to peak measurements were taken at maximum stimulus. Based on the fact that 1 field is 575um in diameter, the average conduction velocity in control trials for 5 uM DPA is 208.83 mm/sec, compared to 194.83 mm/sec in 5uM DPA. These velocities are on the order of those calculated by Vranesic et al. (1994).

## Volley time width averaged across cells.



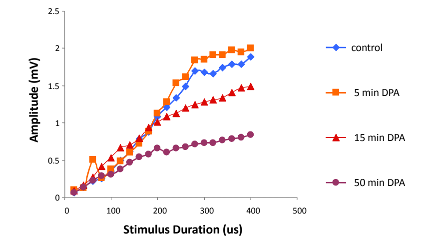

The volley width is the difference between the time of the volley maximum and minimum. In the presence of 10uM DPA, the duration of the fiber volley is prolonged.

## Amplitude averaged across cells.




Averaged values of the volley amplitude (n=4 for control, n=2 for 5uM and 10 uM DPA), normalized to the maximum value in control, indicate that fiber volley amplitude decreases in 5uM DPA. 10uM result obtained in <15 min DPA wash.

## Amplitude change over time in 10uM DPA.




Single measurements of the volley amplitude at different stimulus durations in 10 uM DPA shows that over the time course of DPA application the volley amplitude decreases.

## Conclusions.

DPA tends to decreases conduction velocity in 5uM and 10 uM DPA as indicated by an increase in the time to peak of the fiber volley. Also, the increase in volley width in 10uM DPA suggests that DPA prolongs the duration of fiber volleys. This increase in volley duration as a result of a possible increased capacitance of the fibers, suggests that DPA changes the shape of the action potential. Furthermore, the amplitude of the fiber volleys decrease in 5 uM DPA over time. The time dependence of the response suggests that DPA requires time to permeate the slice. The decrease in amplitude size is likely the result of less fibers firing as DPA enters the tissue.

## References.

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The authors would like to thank Dr. Shlomo S. Dellal for help in preparing this presentation and the URFP for its funding and support.