Mechanism and consequence of parallel fiber excitation by $GABA_A$ receptor activation in the cerebellum.

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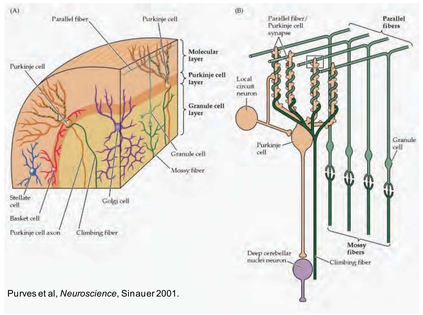
Introduction.

GABA has been found to be excitatory in the cortex and the hippocampus [1]. In the cerebellar molecular layer, previous studies [2] have shown that the $GABA_A$ receptor agonist muscimol causes an increase in spontaneous EPSC frequencies in Purkinje cells and interneurons. We confirm that this excitation is caused by the activation of $GABA_A$Rs located on parallel fibers using molecular layer calcium imaging [3], fiber volley measurements, and recording of antidromic spikes in granule cells [4]. How does this occur? $GABA_A$R activation may be recruiting more fibers to threshold for firing or raising the calcium level in each fiber. Since NKCC1 transporter accumulates chloride, allowing activation of $GABA_A$Rs to be excitatory during development [5], it may be involved in forming a chloride gradient for GABA excitation. Why does this occur? Parallel fiber $GABA_A$R activation may affect orthodromic spiking of granule cells in addition to detection of antidromic spikes. GABA can affect speed of spike initiation rather than conduction velocity. A growing body of evidence suggests the involvement of δ subunit containing $GABA_A$Rs in transmission enhancement [6].

Circuitry of the cerebellum.



Purves et al, *Neuroscience*, Sinauer 2001.

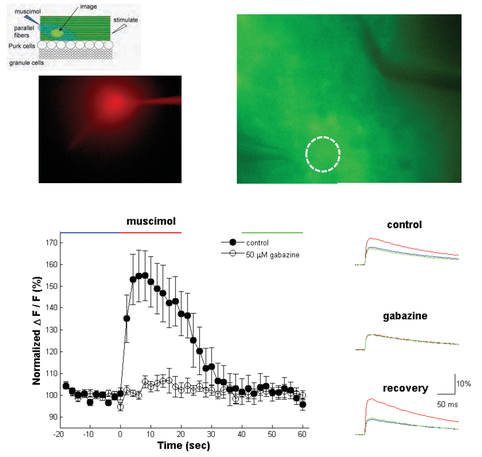
Methods.

Transverse cerebellar slices (275-300 μm) were cut from 20 to 37 day old Sprague-Dawley rats and C57BL6 mice. The aCSF contained (mM) 119 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1 NaH_2PO_4, 26.2 $NaHCO_3$, 25 glucose. Calcium imaging: parallel fibers were labeled locally using a 5-10 μm pipette of 75 μM Oregon Green BAPTA 1 and a 20 μm suction pipette to form a plume. Fiber volleys: stimulus (100 μA) and recording electrodes were placed 0.6-1.3 mm apart. Granule cell recordings: 10 MΩ pipettes contained internal 130 $KMeSO_3$, 4 NaCl, 1 $CaCl_2$, 10 HEPES, 2 ATP, 5 EGTA.

References.

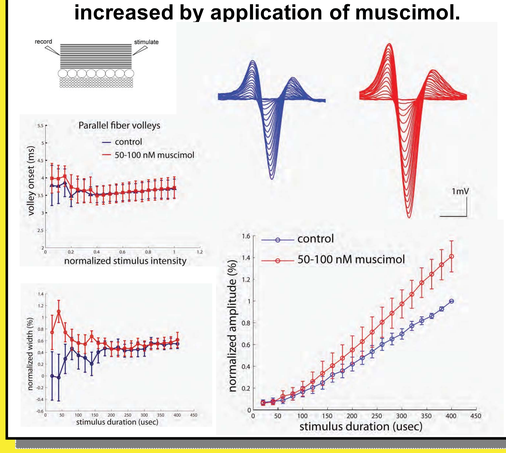
1. Marty and Llano. (2005). Trends in Neuroscience 28(6):284-289.
2. Stell et al. (2007). J Neuroscience 27(34):9022-9031.
3. Regehr and Atluri. (1995). Biophysical Journal 68:2156-2170.
4. Paradiso and Wu. (2009). Nature Neuroscience 12(5):541-543.
5. Dzhala et al. (2005). Nature Medicine 11(11):1205-1213.
6. Ruiz et al. (2010). Nature Neuroscience 13(4):431-440.

Presynaptic calcium transients are enhanced by $GABA_A$R agonist muscimol.



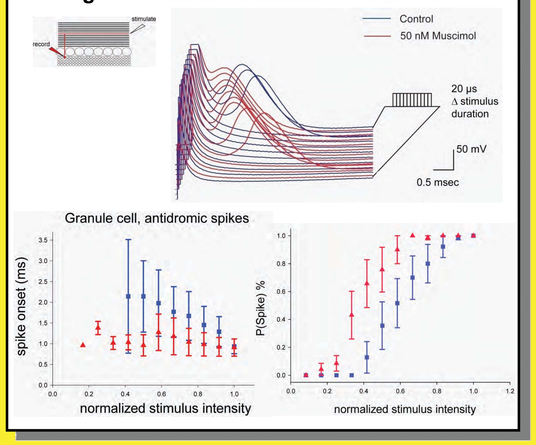
Plume formed by injection and suction pipettes in and above the slice visualized in red using Alexa 568 (top left). Fluorescent image of parallel fibers labeled by Oregon Green BAPTA 1 and puffer pipette next to the recording site with the shadow of the stimulation pipette farther away (top right). 10 μM muscimol applied by pressure pipette to parallel fibers enhances calcium signals evoked by bipolar stimulation (p=0.00032, n=10). This effect is prevented by application of $GABA_A$R antagonist gabazine (50 μM). Averaged example traces from a single spot before (blue), during (red), and after (green) muscimol application (right).

Parallel fiber volley amplitudes are increased by application of muscimol.



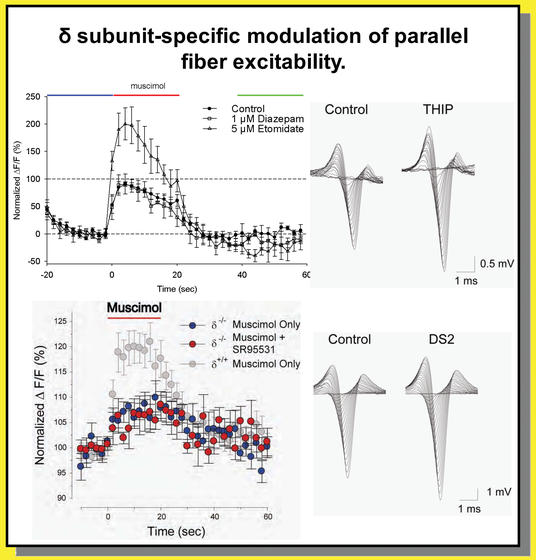
Fiber volleys in response to 100 μA stimulation lasting 20 μs to 400 μs in 20 μs increments before (blue) and after (red) 100 nM muscimol application (top right). Muscimol-induced amplitude (p=0.0131, n=9) (lower right). Muscimol does not change peak-to-peak width or time to peak of volley, indicating no change in conduction velocity (left).

Probability of evoking antidromic spikes in granule cells increases in muscimol.



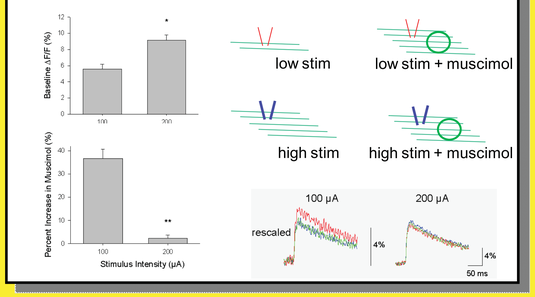
Antidromic spikes are evoked in molecular layer 50-800 μm away from granule cell recording. Spikes induced before (blue) and after (red) 50 nM muscimol application in response to stimulus of durations between 40 and 260 μs (top right). Probability of spikes increased in muscimol (n=7) (lower right). Muscimol reduces time to spike, indicating increases in speed of spike initiation (lower left).

δ subunit-specific modulation of parallel fiber excitability.



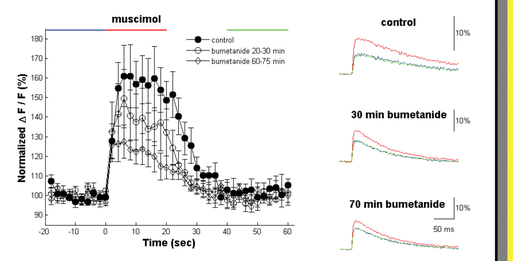
Composition of $GABA_A$R subunits in parallel fibers studied using calcium imaging (left) and fiber volleys (right). 5 μM etomidate enhances musicmol-induced potentiation of stimulus-evoked calcium transients (p=0.022, n=4) but 1 μM diazepam does not (p=0.46, n=4), suggesting that parallel fiber $GABA_A$Rs are not γ subunit containing (top left). Muscimol effect is reduced in $\delta^{-/-}$ mice ($\delta^{-/-}$: n=6 muscimol, n=4 muscimol + gabazine, $\delta^{+/+}$: muscimol n=13) (lower left). Fiber volleys are enhanced by δ subunit specific concentrations of THIP (1 μM) and DS2 (10 μM).

Muscimol brings parallel fibers nearer the threshold for firing.



Muscimol effect on stimulus-evoked calcium transients is diminished at high stimulation intensity (p=0.05, n=3) (left). Illustration showing recruitment of fibers at higher stimulation and expected effect of muscimol--either recruit more fibers or raise calcium level of each fiber (top right). Calcium transient before (blue), during (red), and after (green) muscimol application scaled to control (lower right).

NKCC1 transporter blocker bumetanide reduces the muscimol-induced potentiation of stimulus-evoked calcium transients.



10 to 20 μM bath-applied bumetanide reduces muscimol induced potentiation of stimulus-evoked calcium signals (n=5, 30 minutes p=0.031, 60 minutes p=0.027). Averaged example traces from a single spot before (blue), during (red), and after (green) muscimol application, scaled to the pre-muscimol calcium transient baseline (right).

Conclusions.

Parallel fibers are excited by $GABA_A$R agonists by bringing more fibers closer to threshold for firing an action potential.

$GABA_A$R activation appears to increase the speed of spike initiation but not conduction velocity in parallel fibers.

$GABA_A$R activation in the molecular layer improves the detection of spikes at the granule cell.

Excitatory $GABA_A$Rs do not appear to contain a γ subunit, but does depend on presence of δ subunits.

Excitatory $GABA_A$R effect is seen with endogenous GABA.

Excitatory effect of $GABA_A$Rs appears to arise from an altered Cl^- gradient due to the NKCC1 chloride transporter.