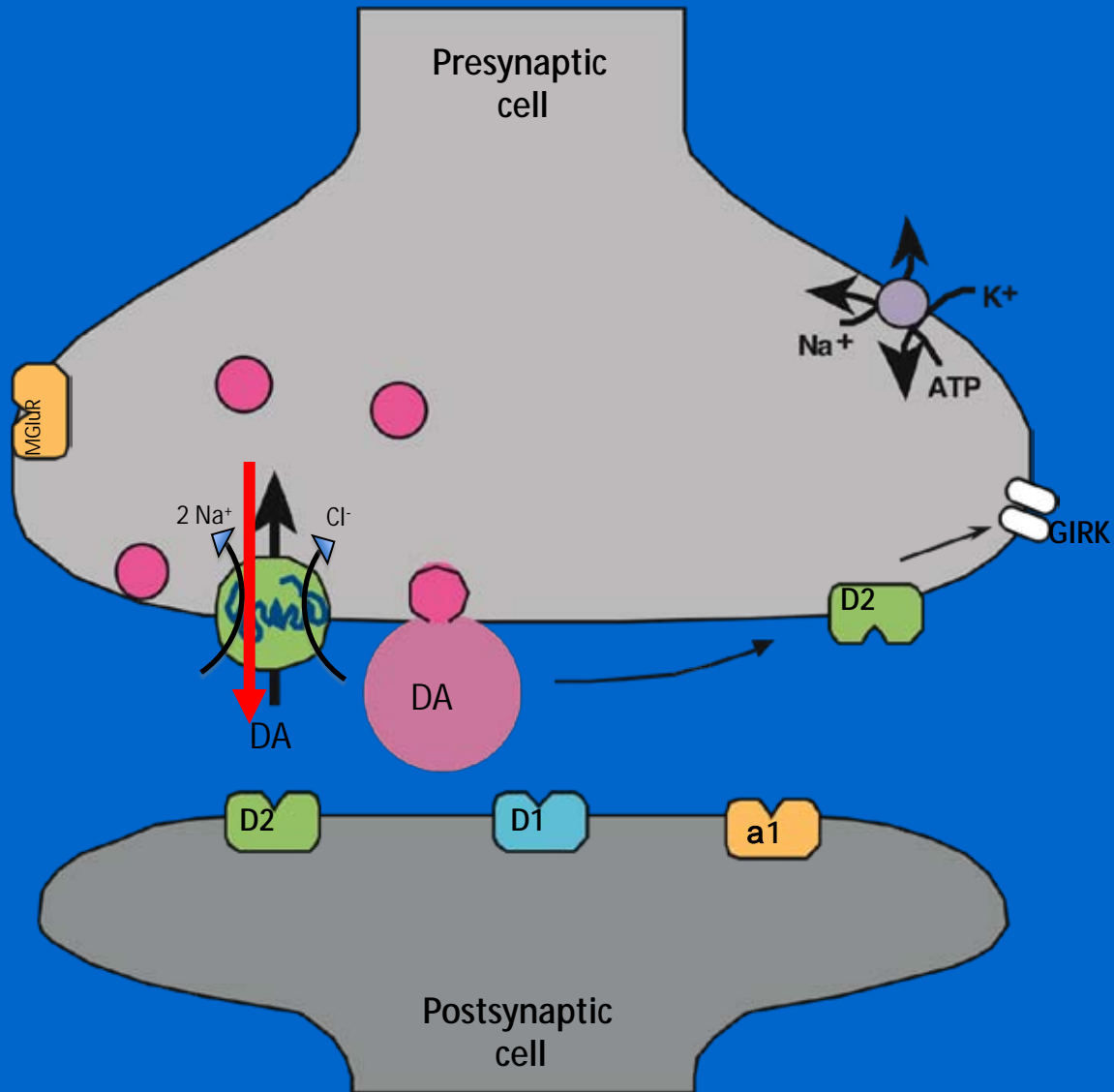




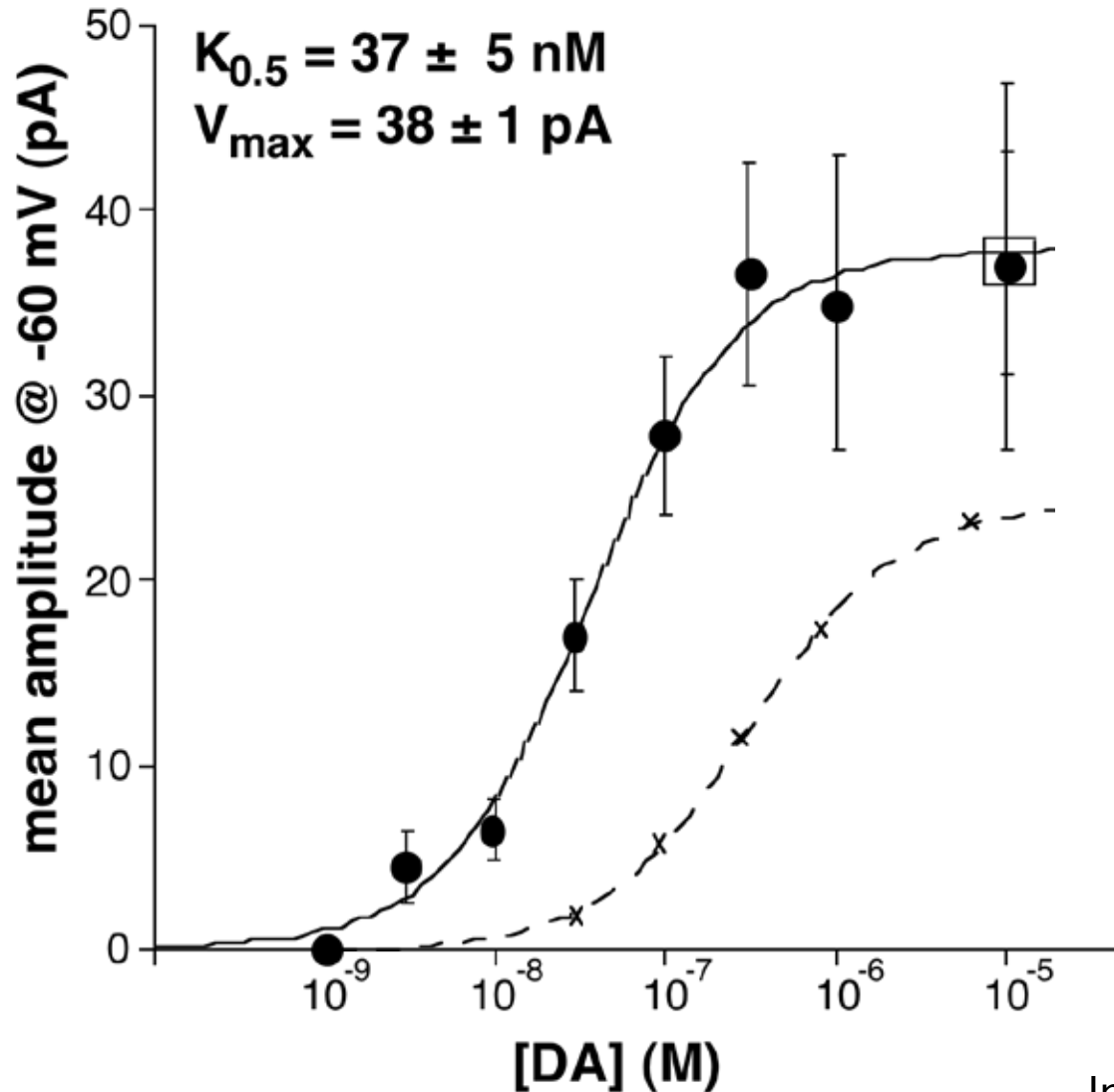
The Dopamine Transporter: More Exciting than Housekeeping

Susan Ingram
Washington State University Vancouver

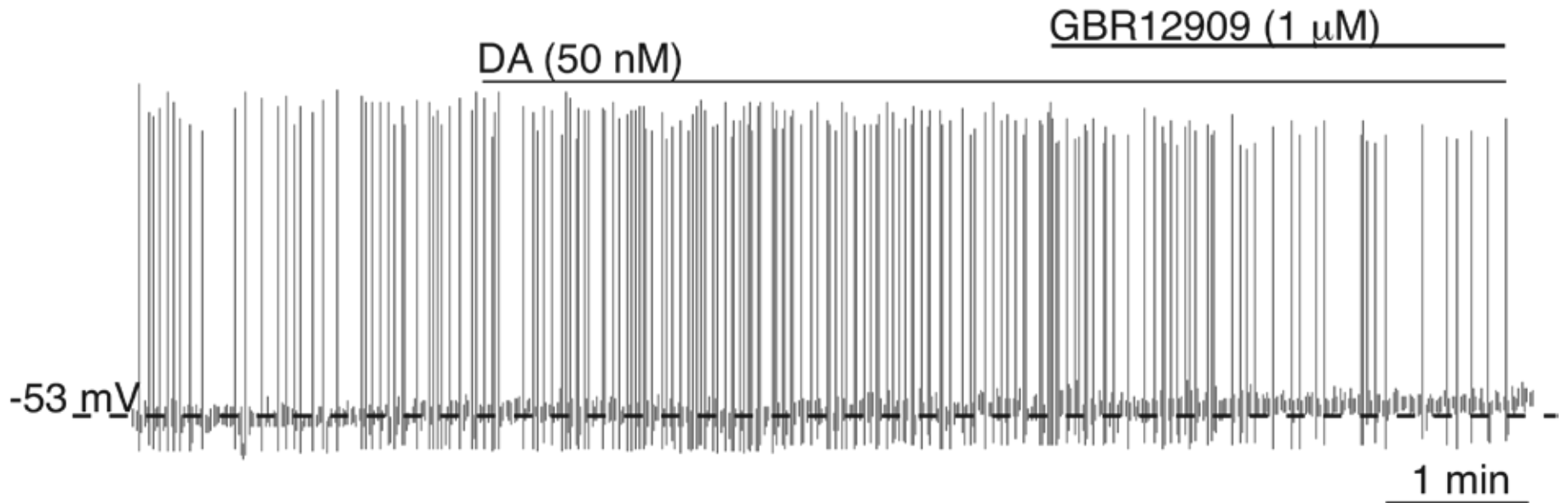
Dopaminergic Synapse



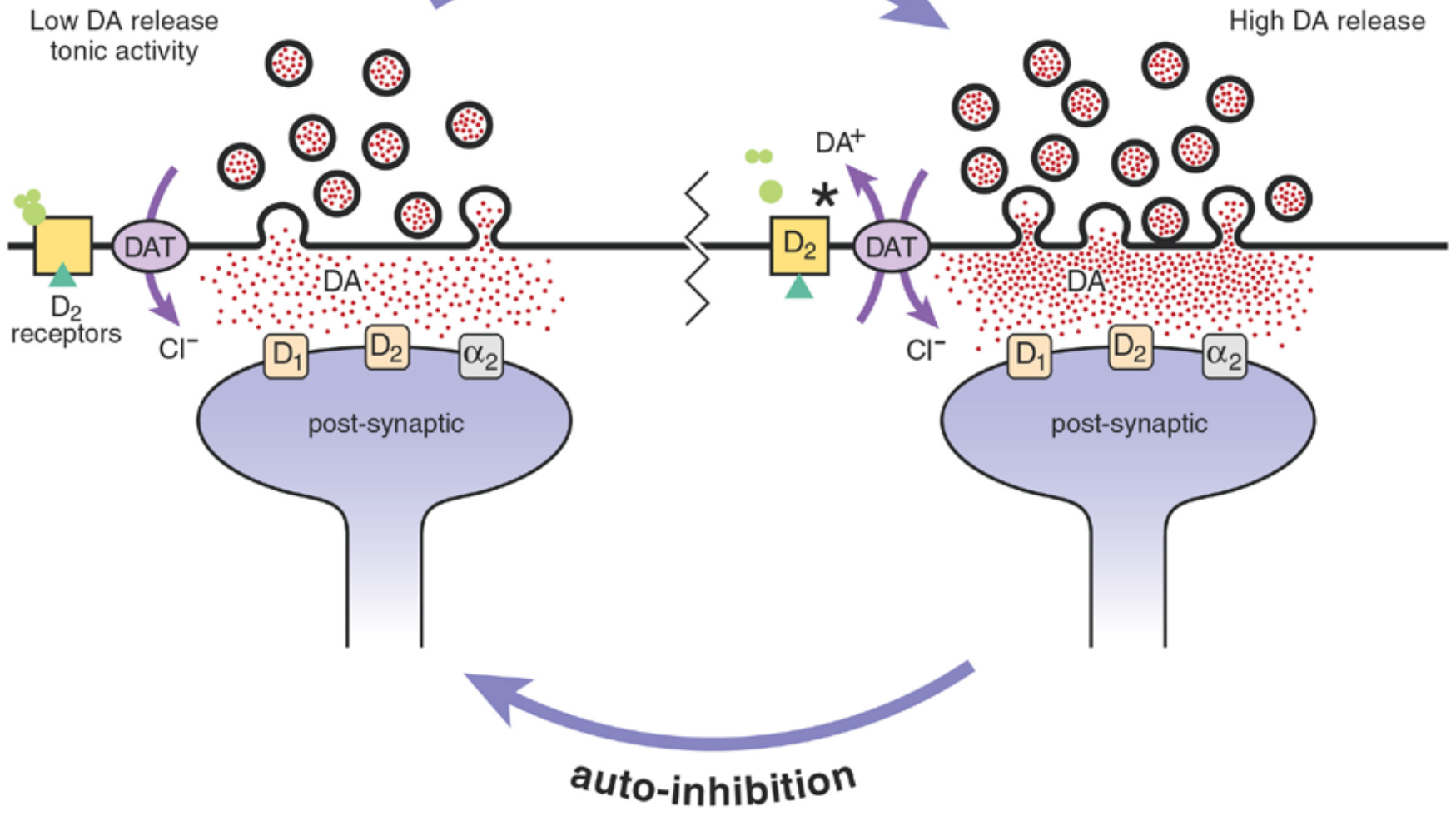
Currents are activated at lower dopamine concentrations than are required for transport



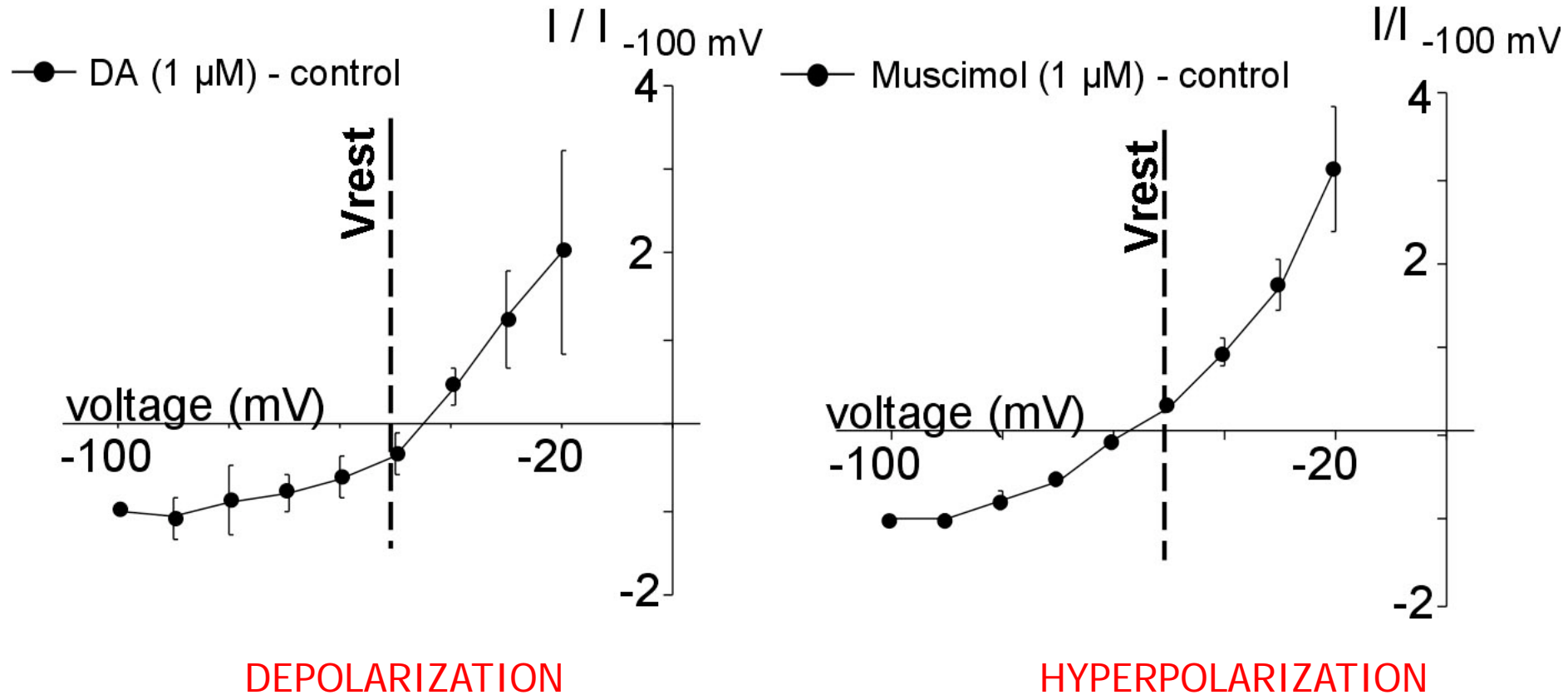
Low DA concentrations increase firing



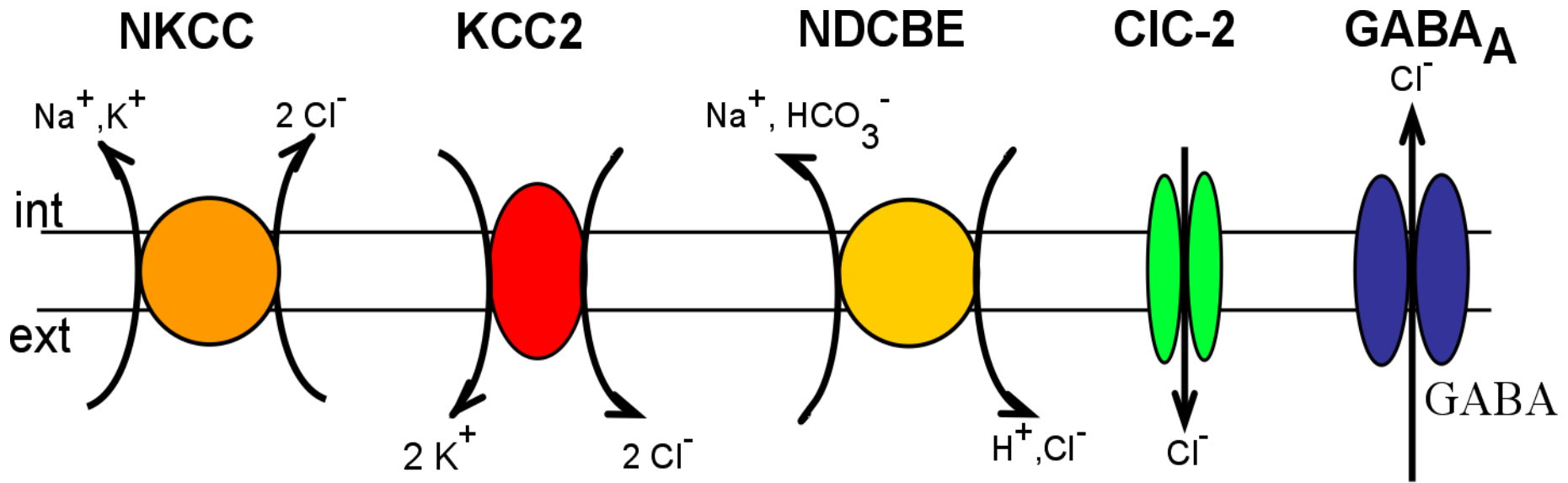
excitation



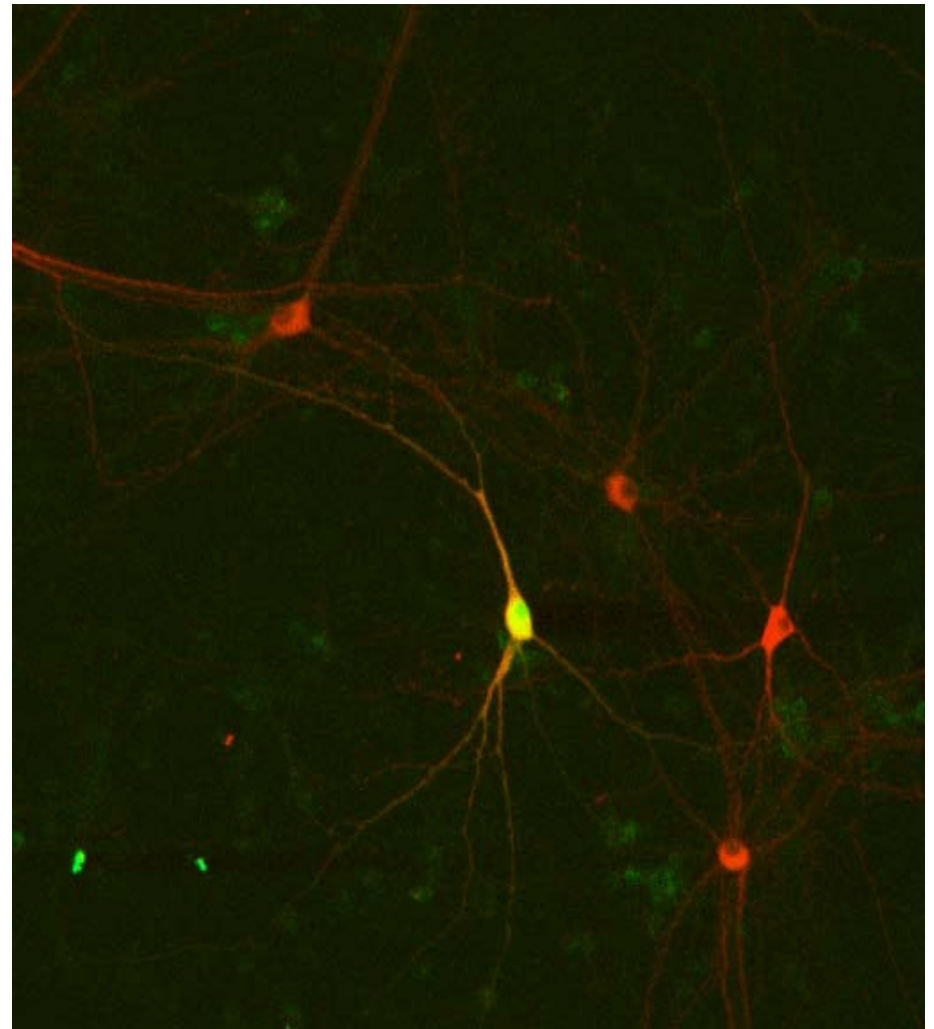
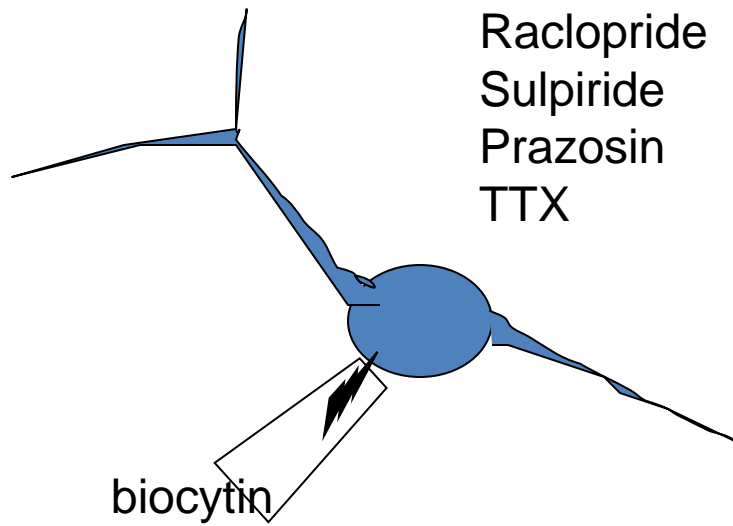
DAT-mediated chloride current is excitatory in cultured midbrain DA neurons



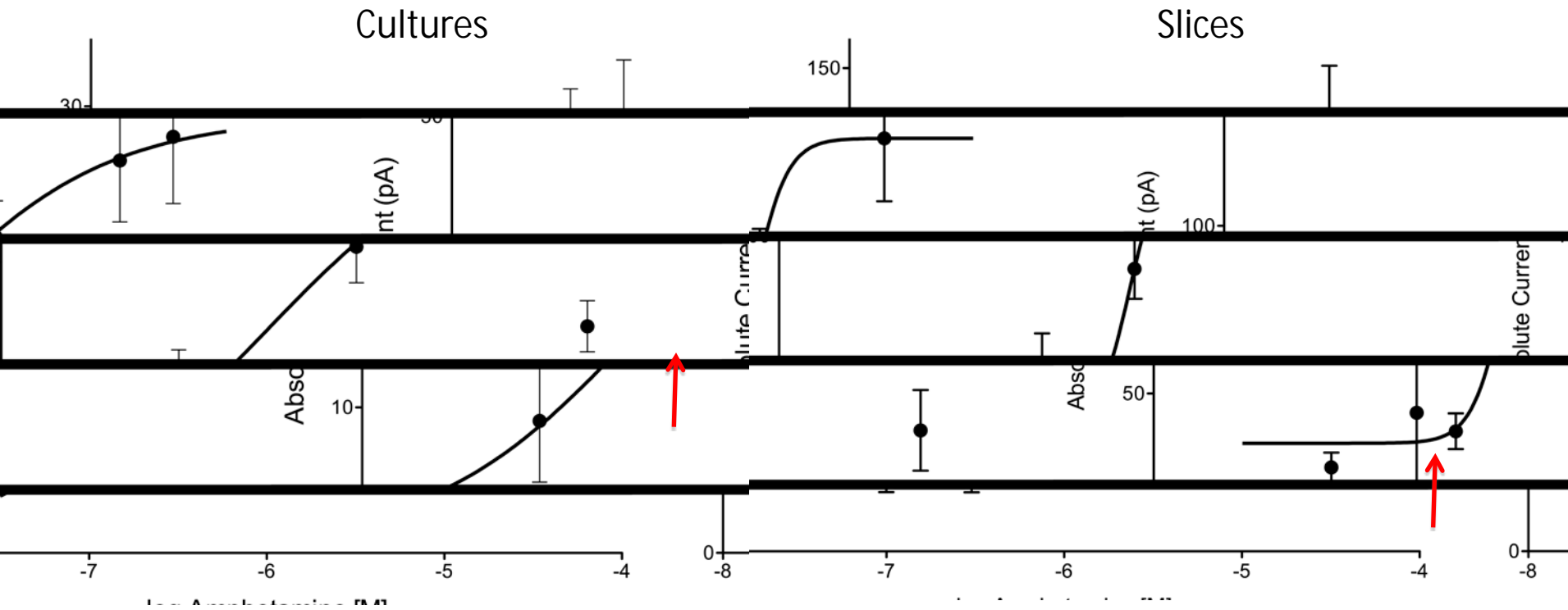
Proteins that regulate intracellular Cl⁻



Whole-cell patch clamp recordings from DA neurons

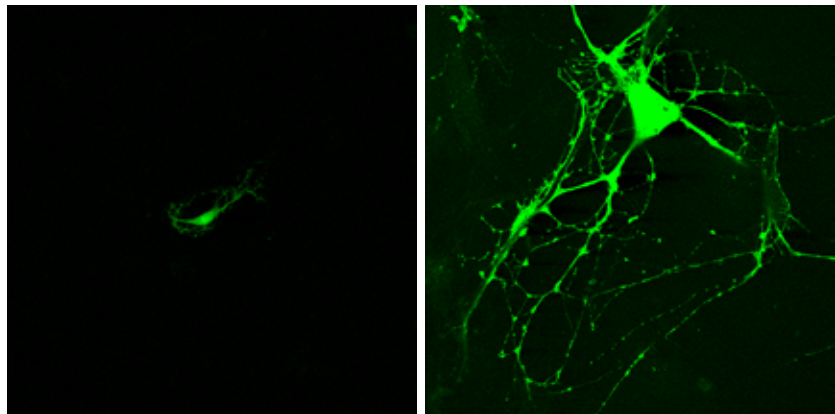
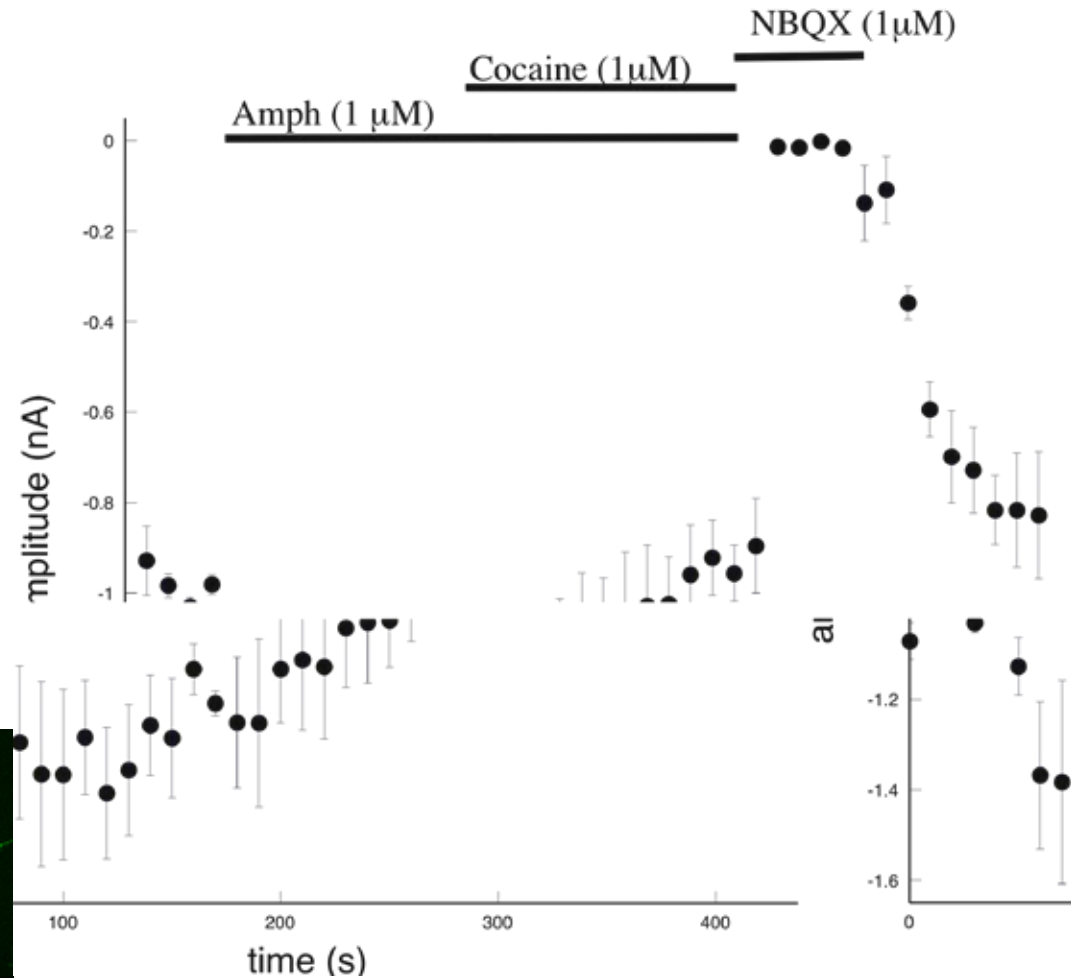
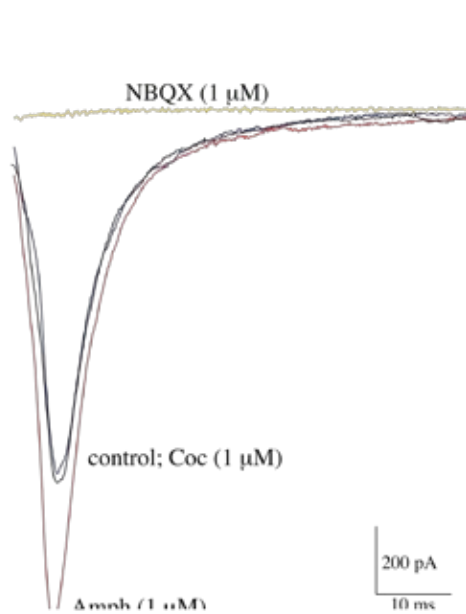


Amphetamine activates a DAT-mediated current at low concentrations

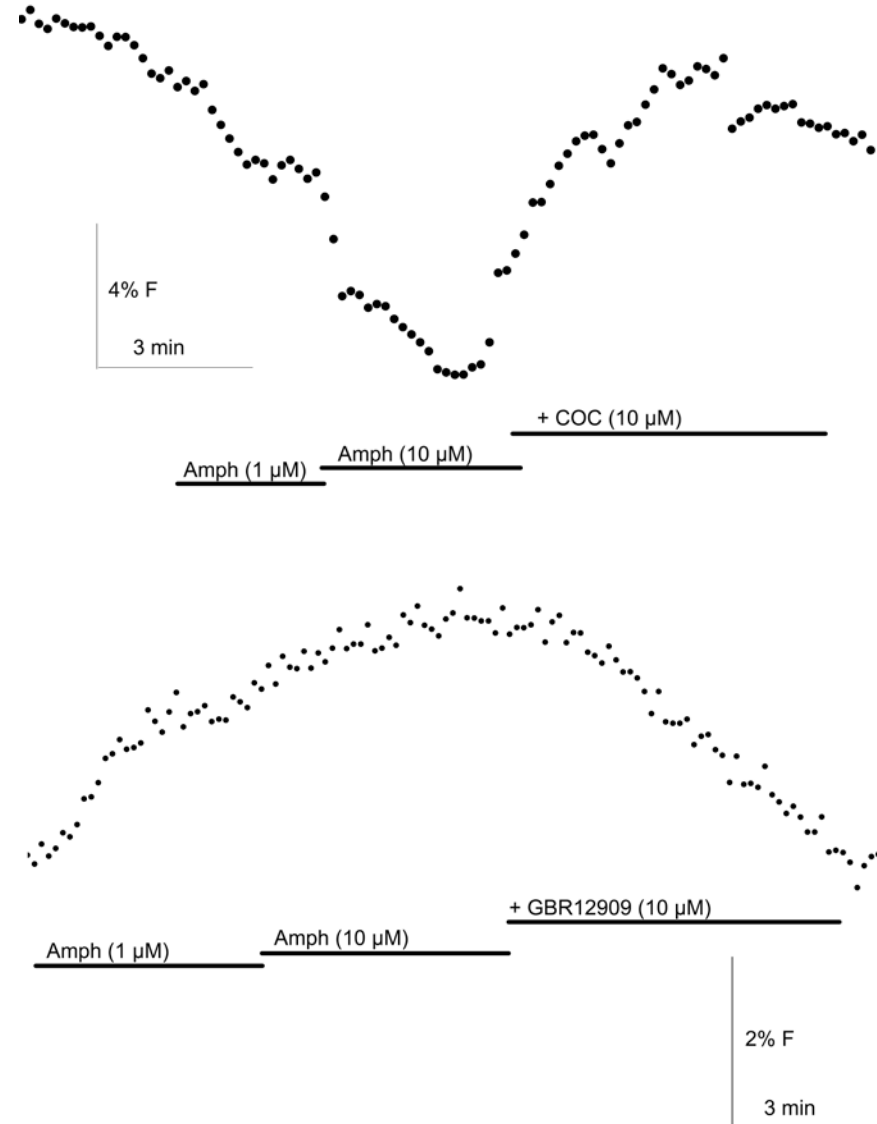
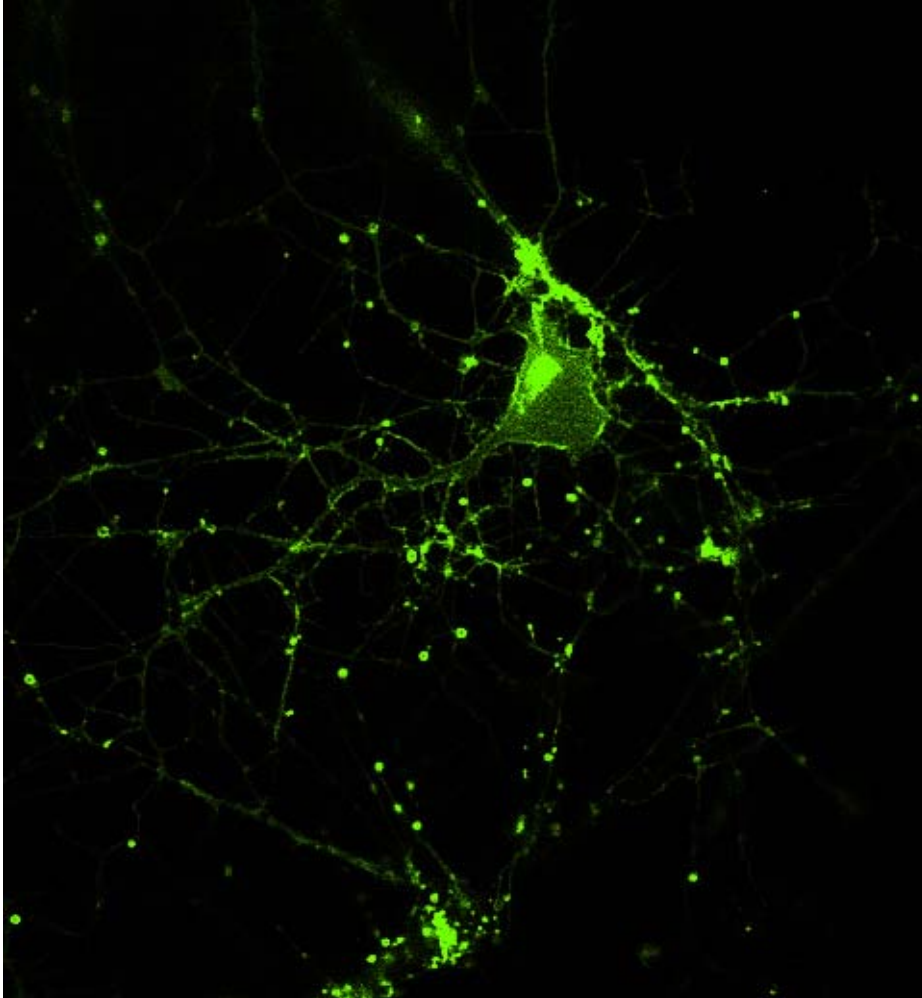


Watts, Fyfe and Ingram, unpublished data.

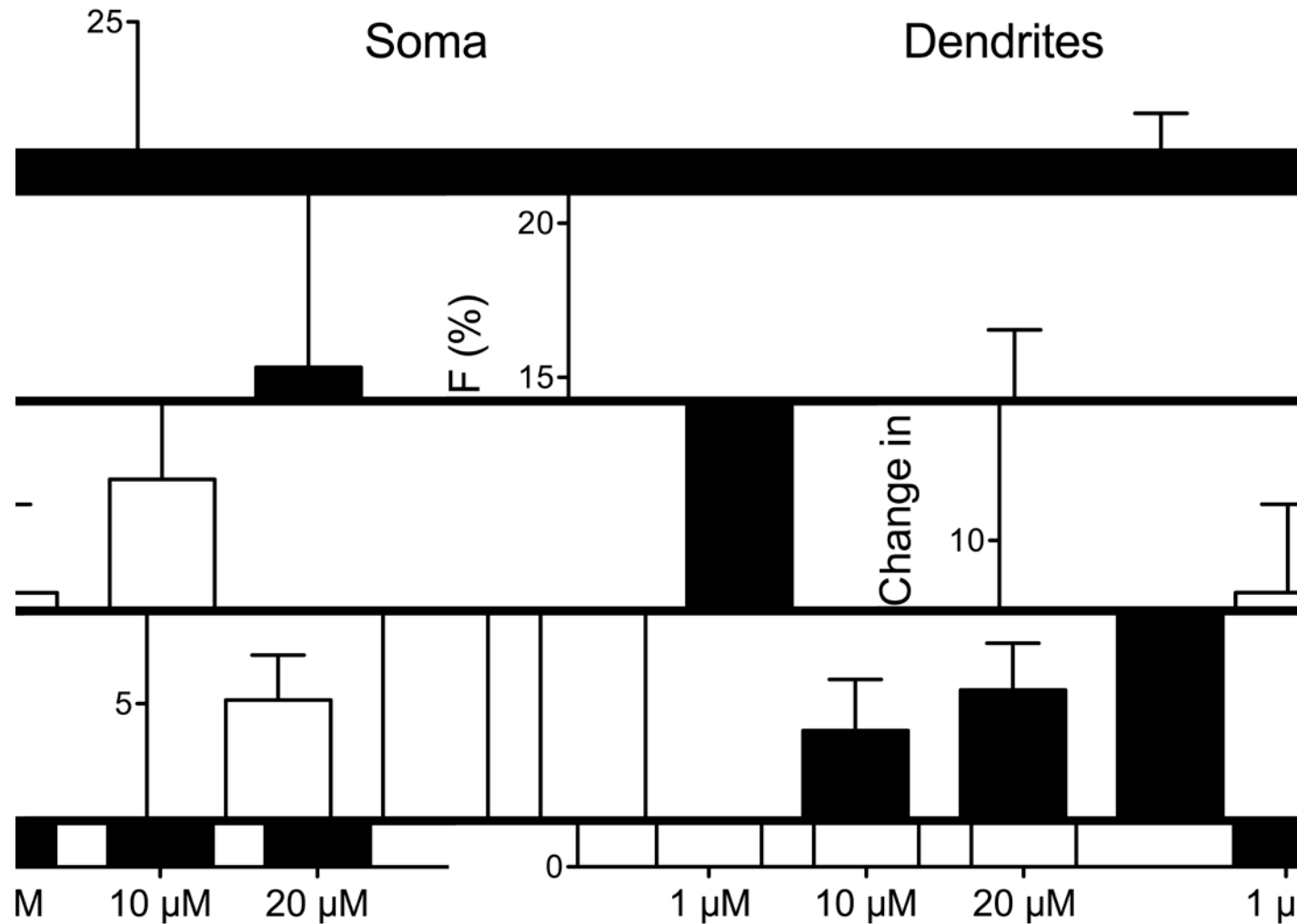
DA neurons make glutamatergic autapses in culture and amphetamine increases AMPA currents



mbYFPQS localizes to the membrane of cultured midbrain neurons



Amphetamine stimulates a dose-dependent change in mbYFPQS fluorescence in both soma and dendrites of DA neurons

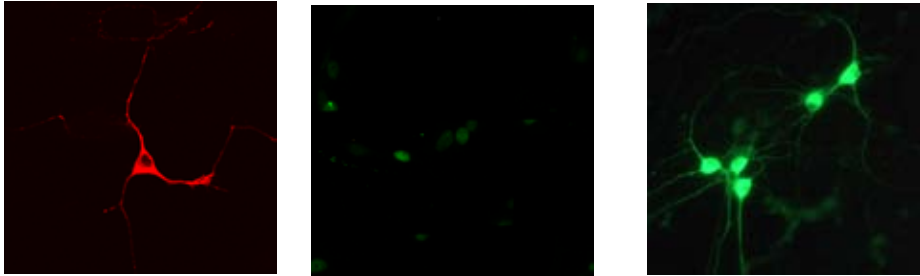


KCC2 Expression is different in cultures and slices

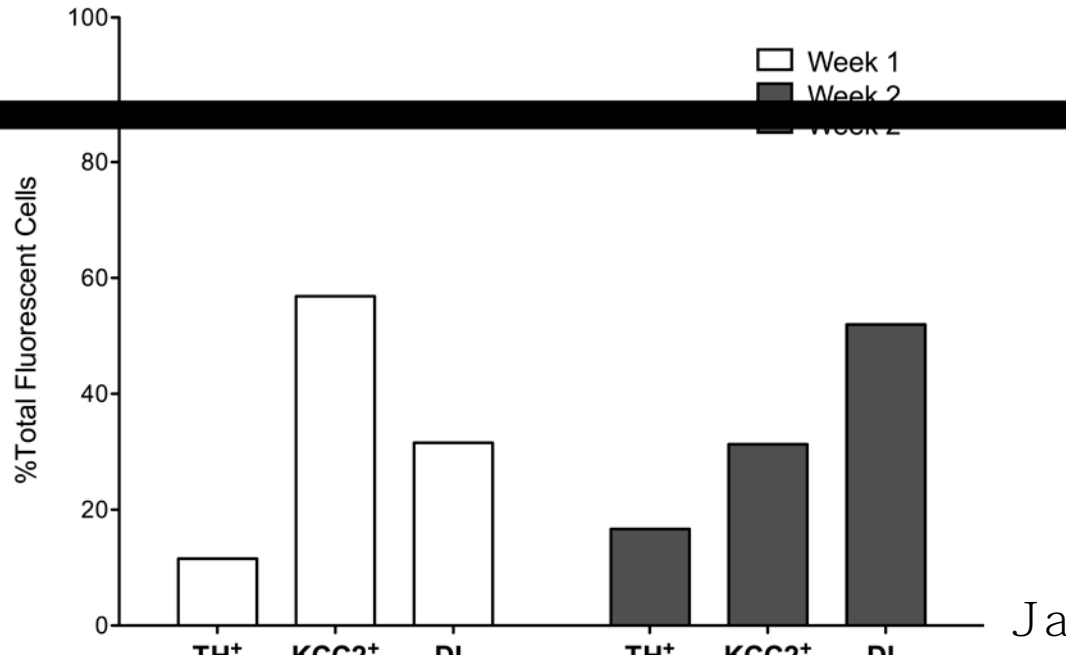
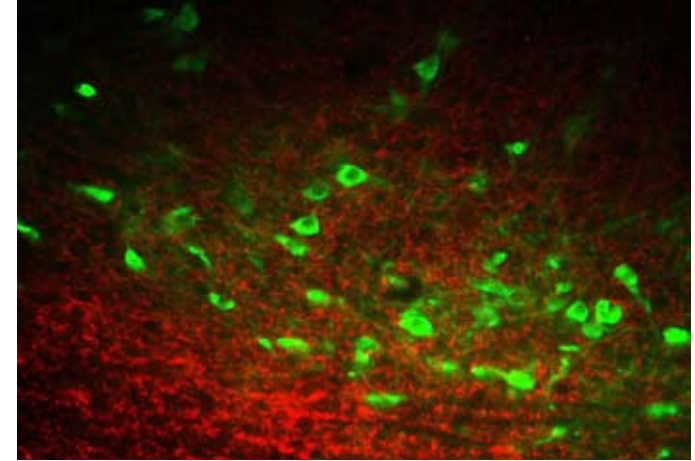
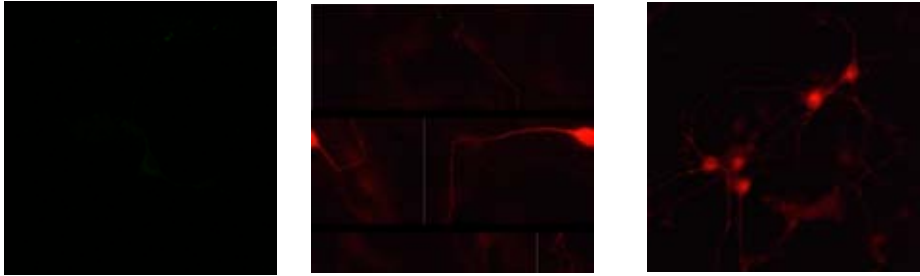
cultures

slices

TH

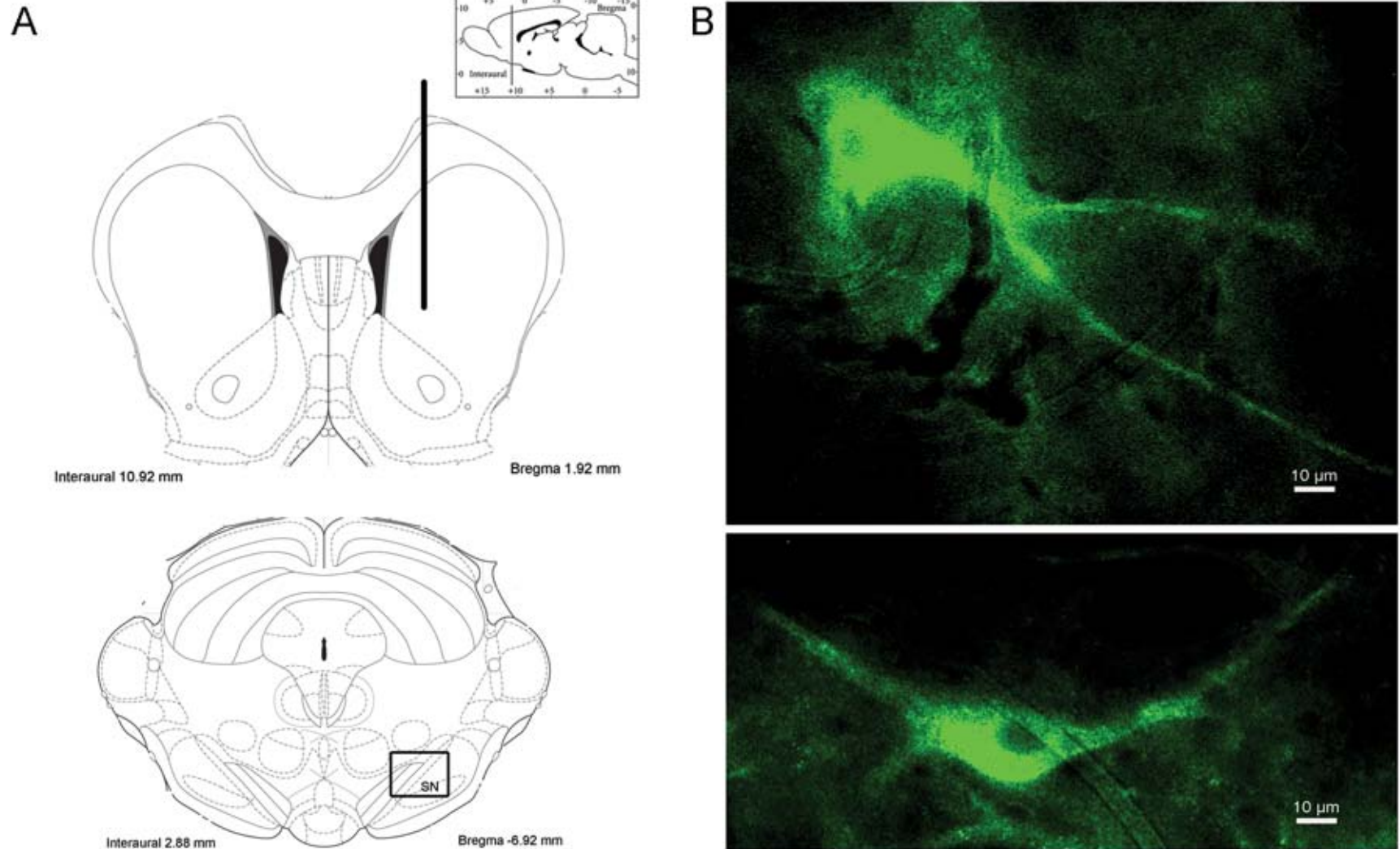


KCC2



Jamieson and Ingram, unpublished data.

Baculoviral Transfections



Watts and Ingram, unpublished data.

Summary

- DAT-mediated chloride current may alter excitability of DA neurons and integration of synaptic activity.
- The current may be activated selectively (relative to transport) by low DA and amphetamine concentrations suggesting a role in increasing release of DA.
- The amphetamine-mediated current is dose-dependent in both cultures and slices of midbrain neurons but is inhibited at high amphetamine concentrations (20 μM).
- The mbYFPQS is a sensitive tool to measure intracellular chloride concentrations ($K_{50} = 30 \text{ mM}$) and is useful for monitoring changes in intracellular chloride concentrations in dendrites.
- Amphetamine can increase influx and efflux of chloride in separate cells in culture suggesting that there are different E_{Cl^-} in DA neurons. These results are supported by the presence of KCC2 in a subpopulation of DA neurons in culture. Therefore the physiological relevance of DAT-mediated currents will have to focus on DA neurons in substantia nigra slices.



Acknowledgements:

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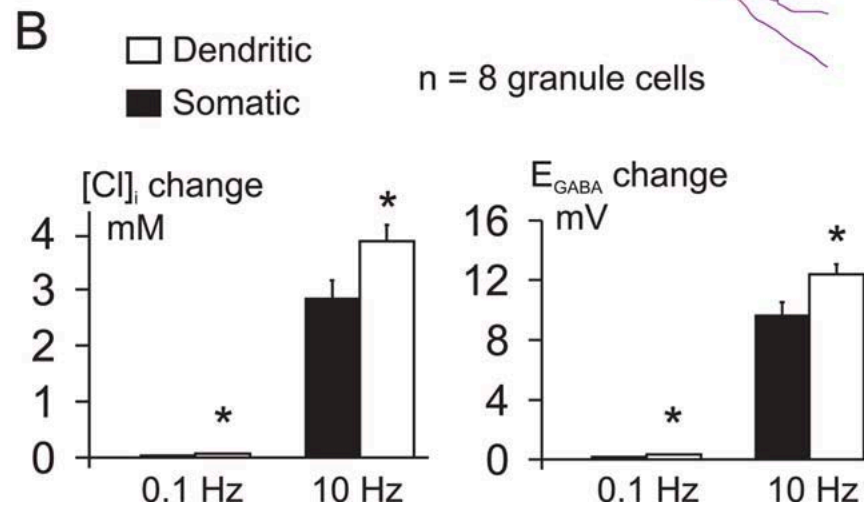
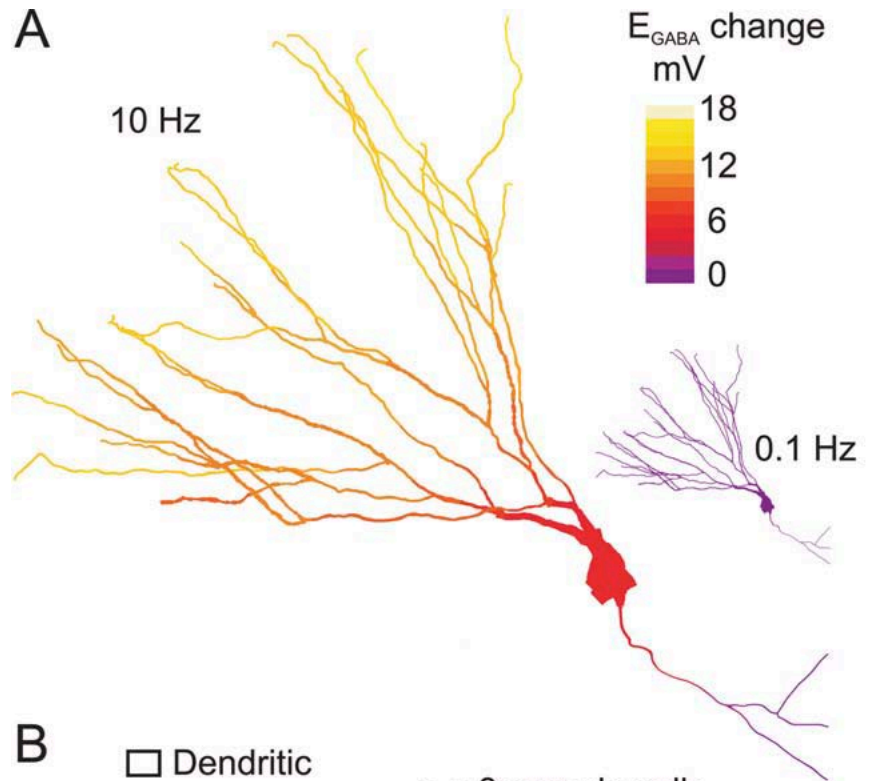
Susan Amara

Spencer Watts

Supported by NIDA (DA024041)

Activity-Dependent Intracellular Chloride Accumulation and Diffusion Controls GABA_A Receptor-Mediated Synaptic Transmission

Peter Jedlicka,^{1,2*} Thomas Deller,¹ Boris S. Gutkin,^{3,4,5} and Kurt H. Backus²

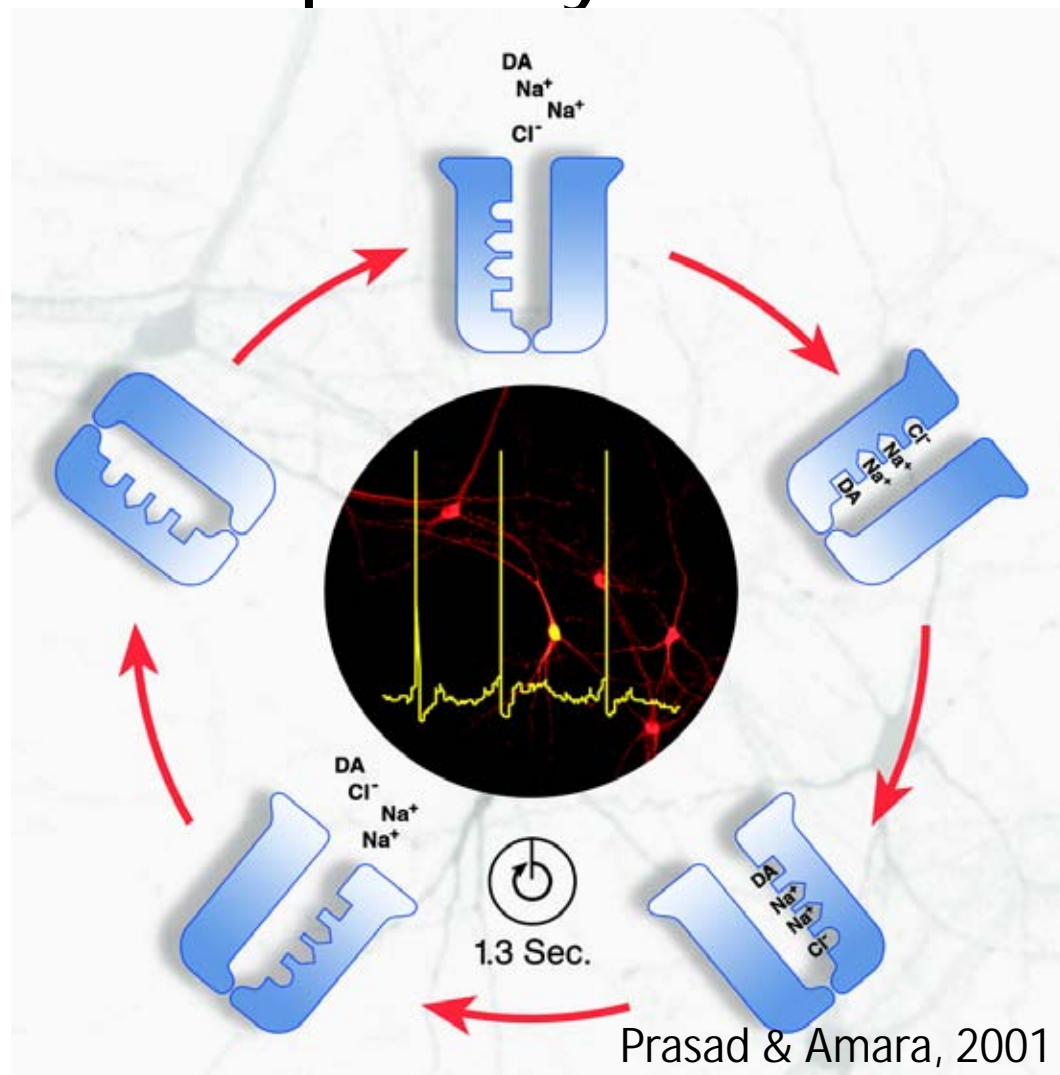


Dopamine Neurons Mediate a Fast Excitatory Signal via Their Glutamatergic Synapses

Nao Chuhma, Hui Zhang, Justine Masson, Xiaoxi Zhuang, David Sulzer, Rene´ Hen, and Stephen Rayport

The Journal of Neuroscience, January 28, 2004 • 24(4):972–981

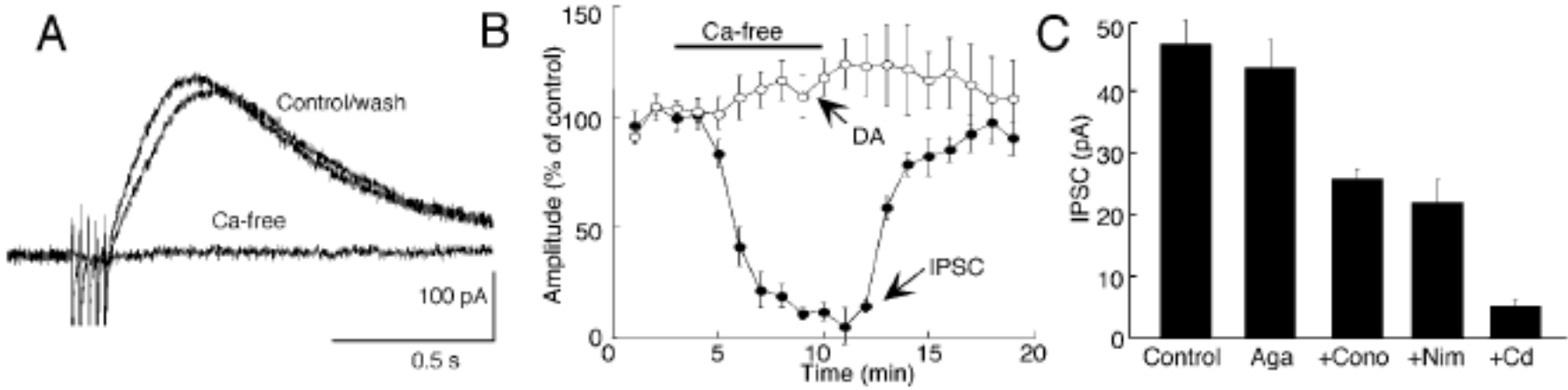
DA transport by DAT is slow



- In midbrain neurons it takes 1.3 sec for one DAT to move one DA molecule.
- Uptake by DAT is insensitive to physiological changes in membrane potential.

Vesicular Dopamine Release Elicits an Inhibitory Postsynaptic Current in Midbrain Dopamine Neurons

Michael J. Beckstead, David K. Grandy, Kevin Wickman and John T. Williams



Neuron, Vol. 42, 939–946, June 24, 2004, Copyright © 2004 by Cell Press

Cl⁻ regulation in neurons

