Transporters

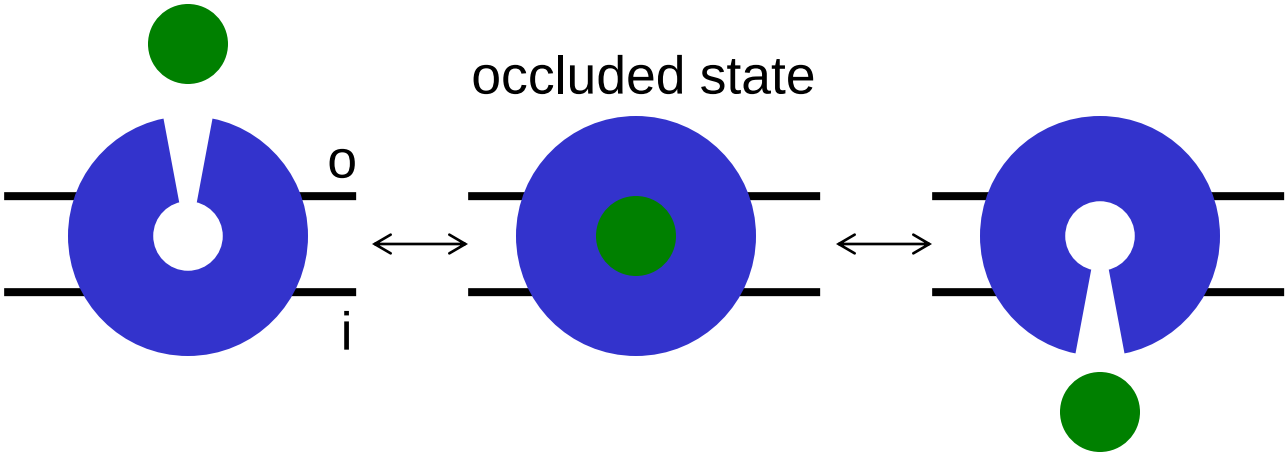
different from channels:

speed

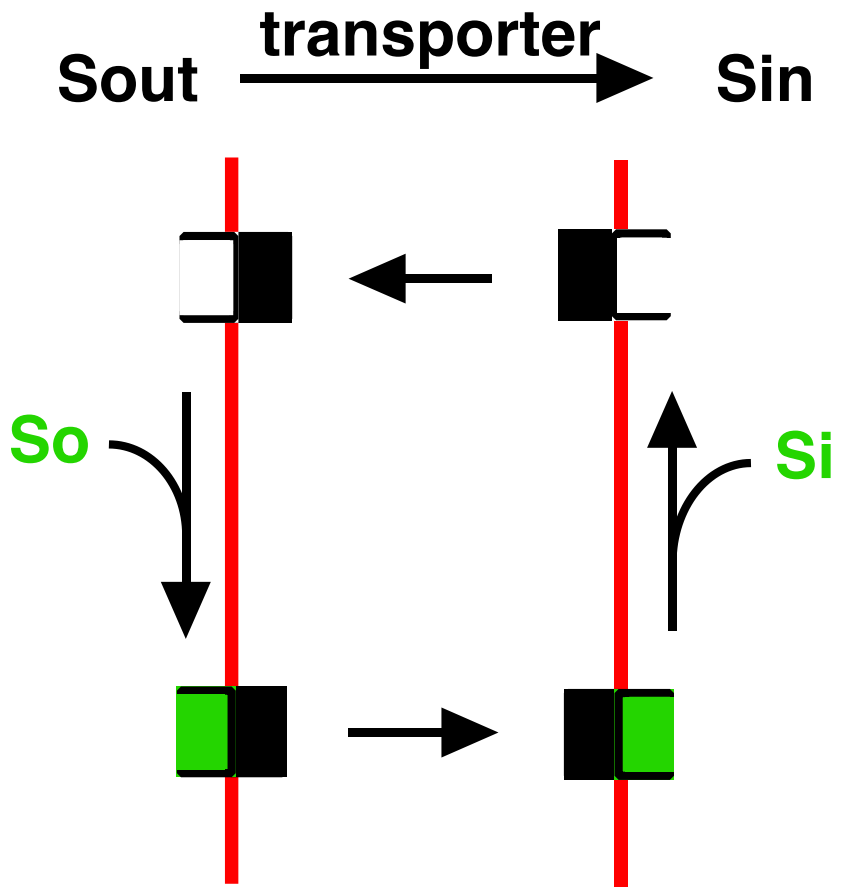
saturation

concentration

--not exactly

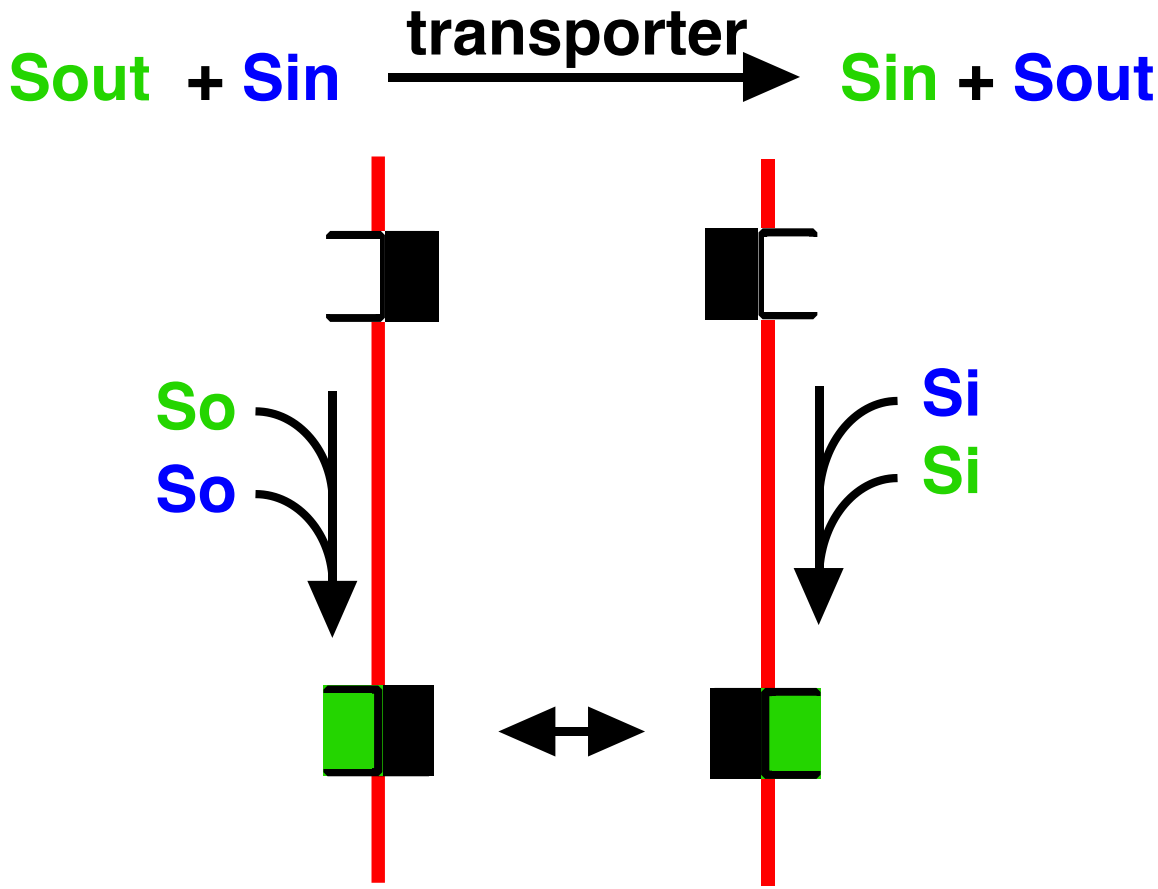


alternating access



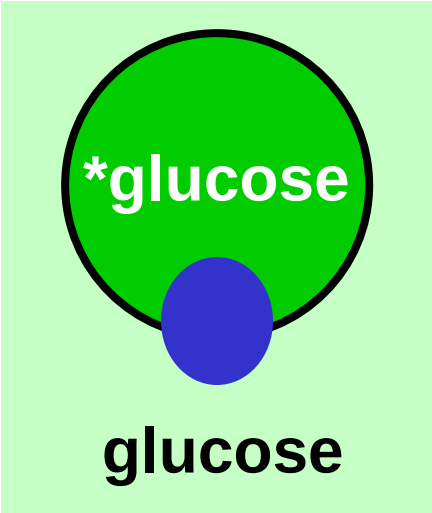
movement of unloaded carrier crucial for net transport

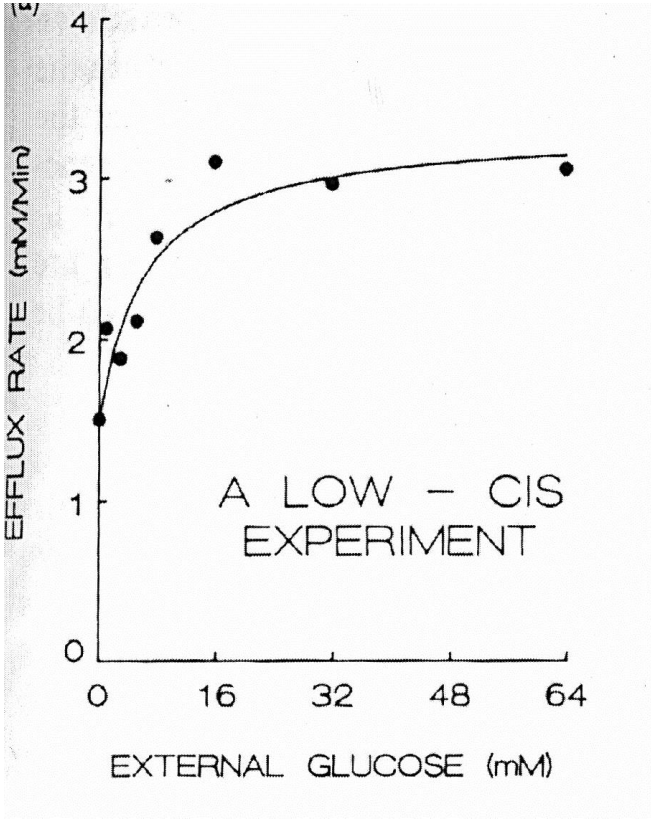
exchange



--no net flux

membranes preloaded with ^{14}C-glucose
diluted into medium with unlabelled glucose

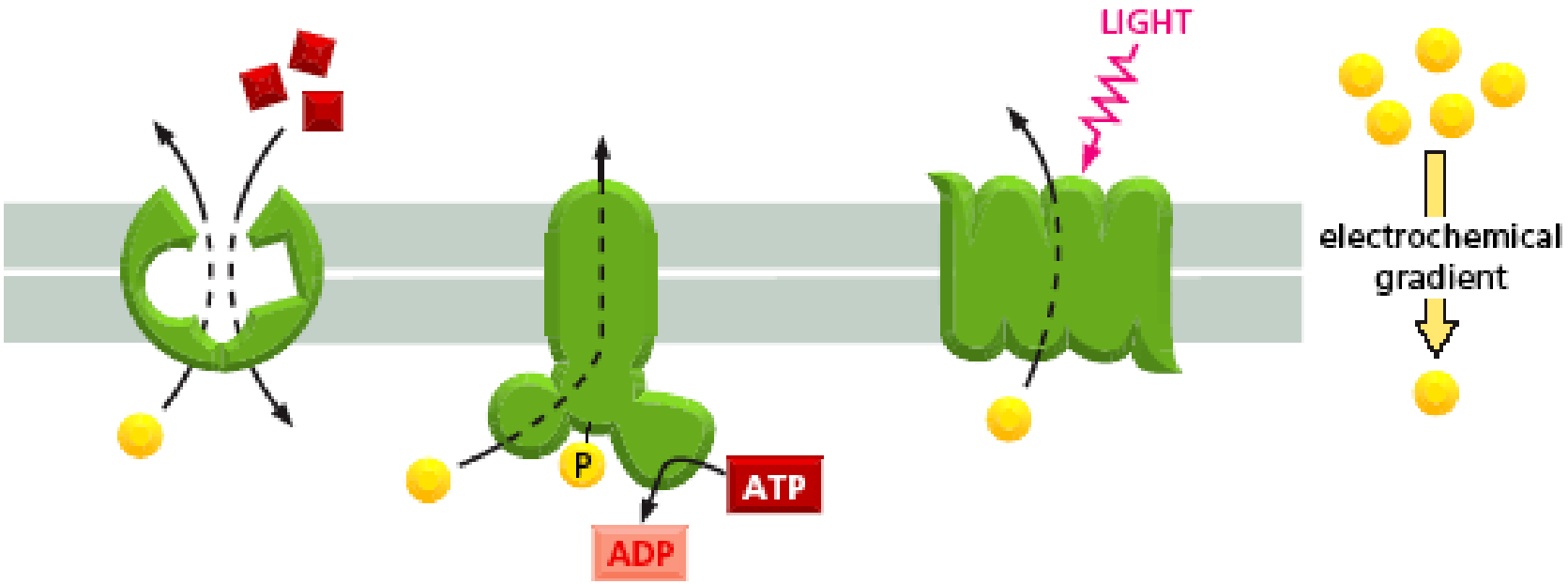




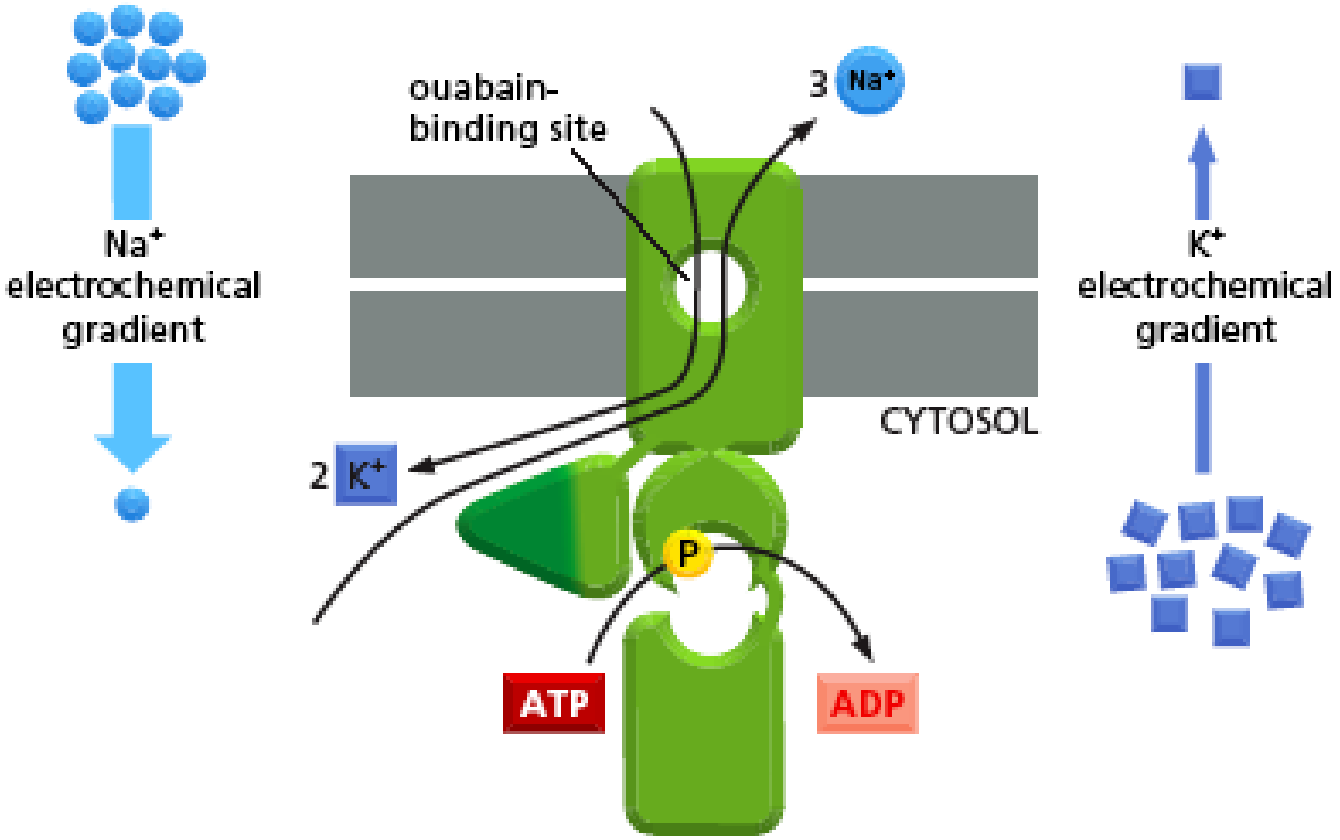
only loaded carrier moves
—not unloaded
translocation steps slow
--channel cannot do this

heteroexchange
obligate exchangers cannot do net flux
amphetamines may release monoamines this way (exchange-diffusion)

different classes

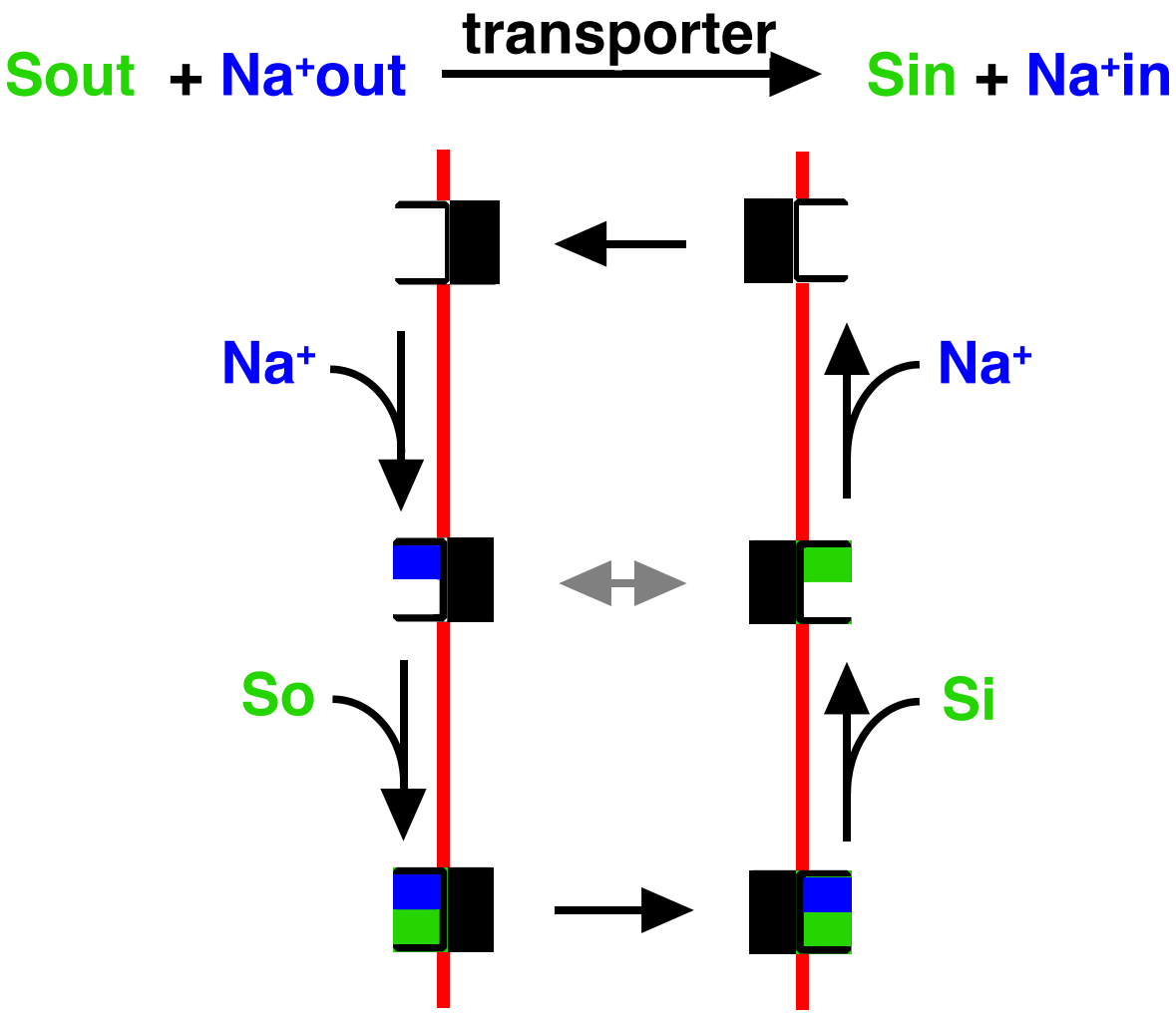


P-type ATPase: Na^+/K^+-ATPase



P-type involve phosphorylated intermediate
how can phosphorylation outside membrane
trigger movement of ions across membrane?

ionic coupling



coupling rules: Na^+ can only move with S--why?
AND gate, ~coincidence detector

equilibrium

$$S_o + Na^+_o \longleftrightarrow S_i + Na^+_i$$

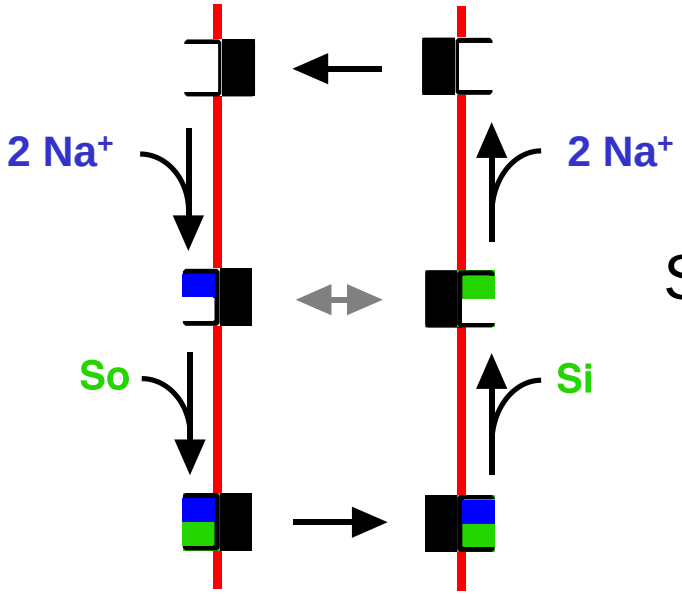
stoichiometry of 1 Na^+ : 1 S and ~12-fold Na^+ gradient will generate ? gradient of S

at equilibrium, equal rates in and out of cell

$$[Na^+]_o \times [S]_o = [Na^+]_i \times [S]_i$$

$$[Na^+]_o / [Na^+]_i = [S]_i / [S]_o$$

what if it is an exchanger?



$$S_o + 2Na^+_o \longleftrightarrow S_i + 2Na^+_i$$

if coupling involves 2 Na^+ : 1 S, then

$$[Na^+]_o^2 \times [S]_o = [Na^+]_i^2 \times [S]_i$$

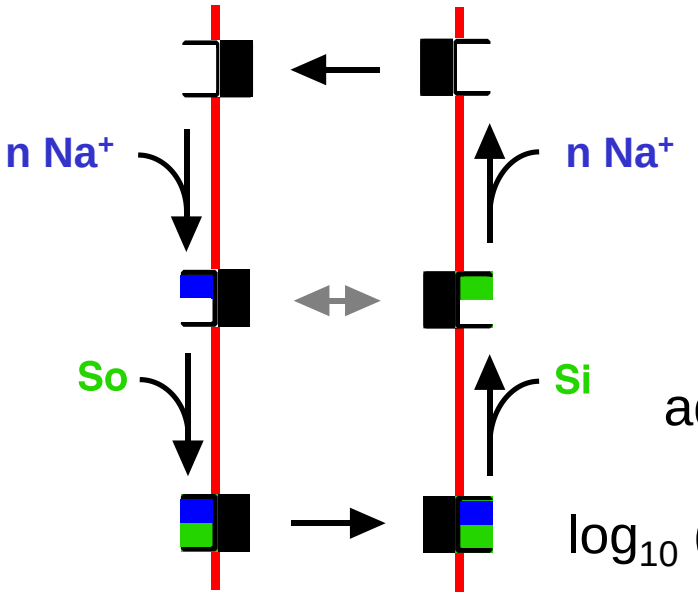
$$([Na^+]_o / [Na^+]_l)^2 = [S]_l / [S]_o \quad \text{or}$$

$$\log_{10} (S_{in}/S_{out}) = 2 \log_{10} (Na^+_{out}/Na^+_{in})$$

why not just make the stoichiometry very high?

what if net flux involves charge movement?

electrogenic transport (transport that moves net charge)



negative resting membrane potential
augments chemical gradient for Na^+ by

$$-z_T\Delta\Psi / 60\ mV$$

where z_T = net charge moved and $\Delta\Psi$ is Vm

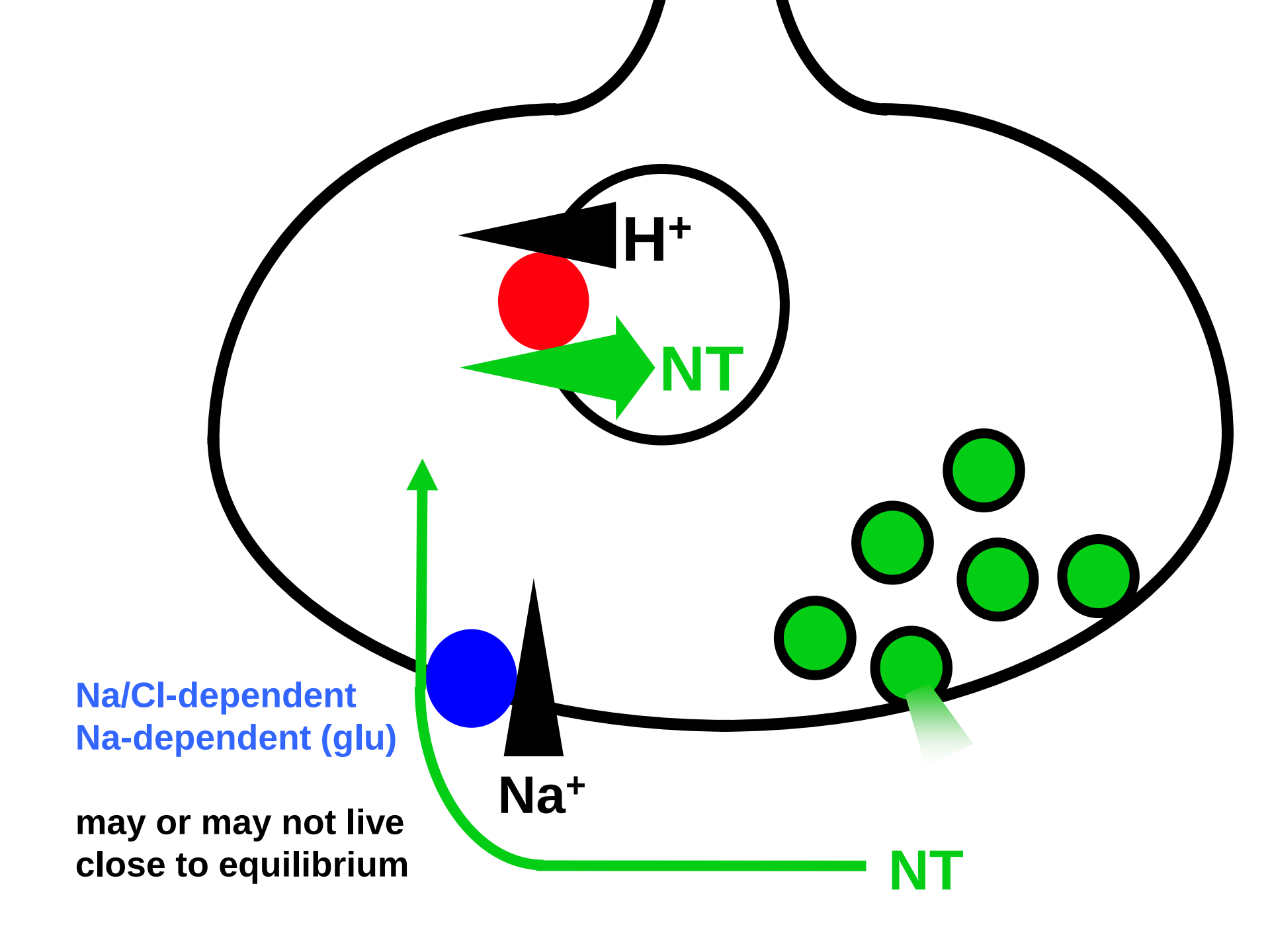
added to the concentration gradient,

$$\log_{10}(S_{in}/S_{out}) = n \log_{10}(Na^+_{out}/Na^+_{in}) - z_T\Delta\Psi / 60\ mV$$

where n = # Na^+ ions cotransported

--power of membrane potential
equation changes for different ionic coupling

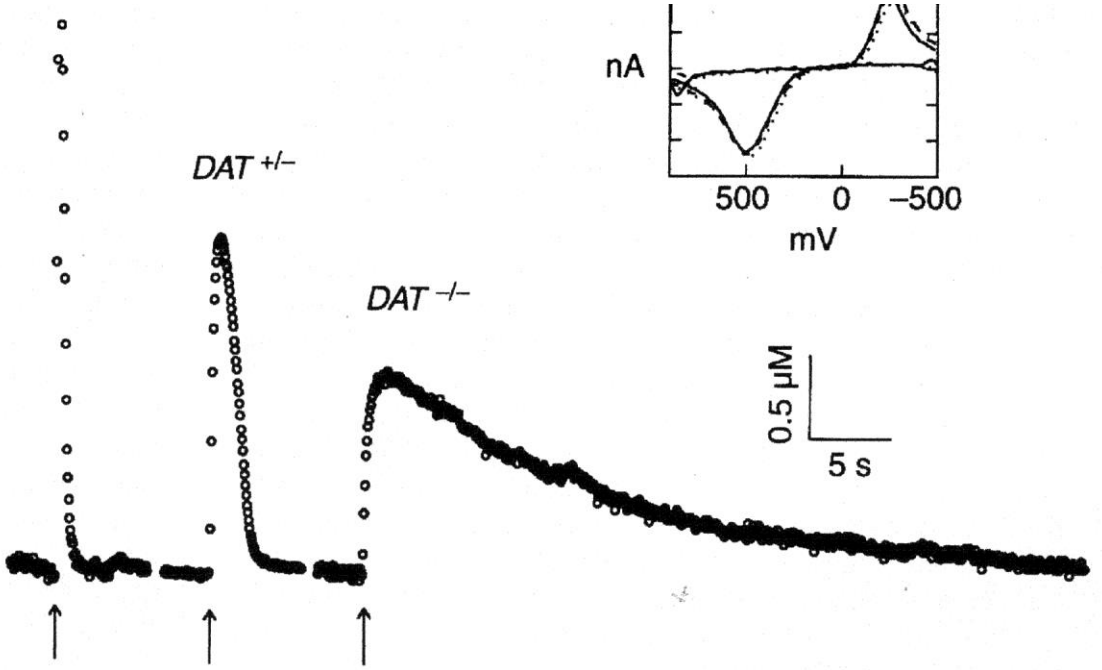
ionic coupling determines direction of flux
magnitude of gradient (can exceed 10^6:1)
regulation by membrane potential



may or may not live close to equilibrium

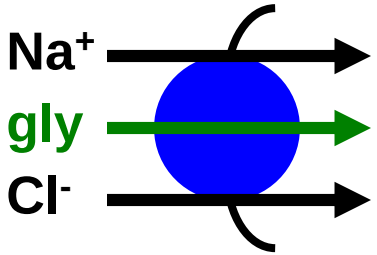
reuptake: Na/Cl-dependent transport

striatal slice voltammetry



DAT KO:
impaired **rate** of dopamine *clearance* in striatum
also, KO has 95% decrease in dopamine stores!
--crucial role in *recycling*

glycine transport

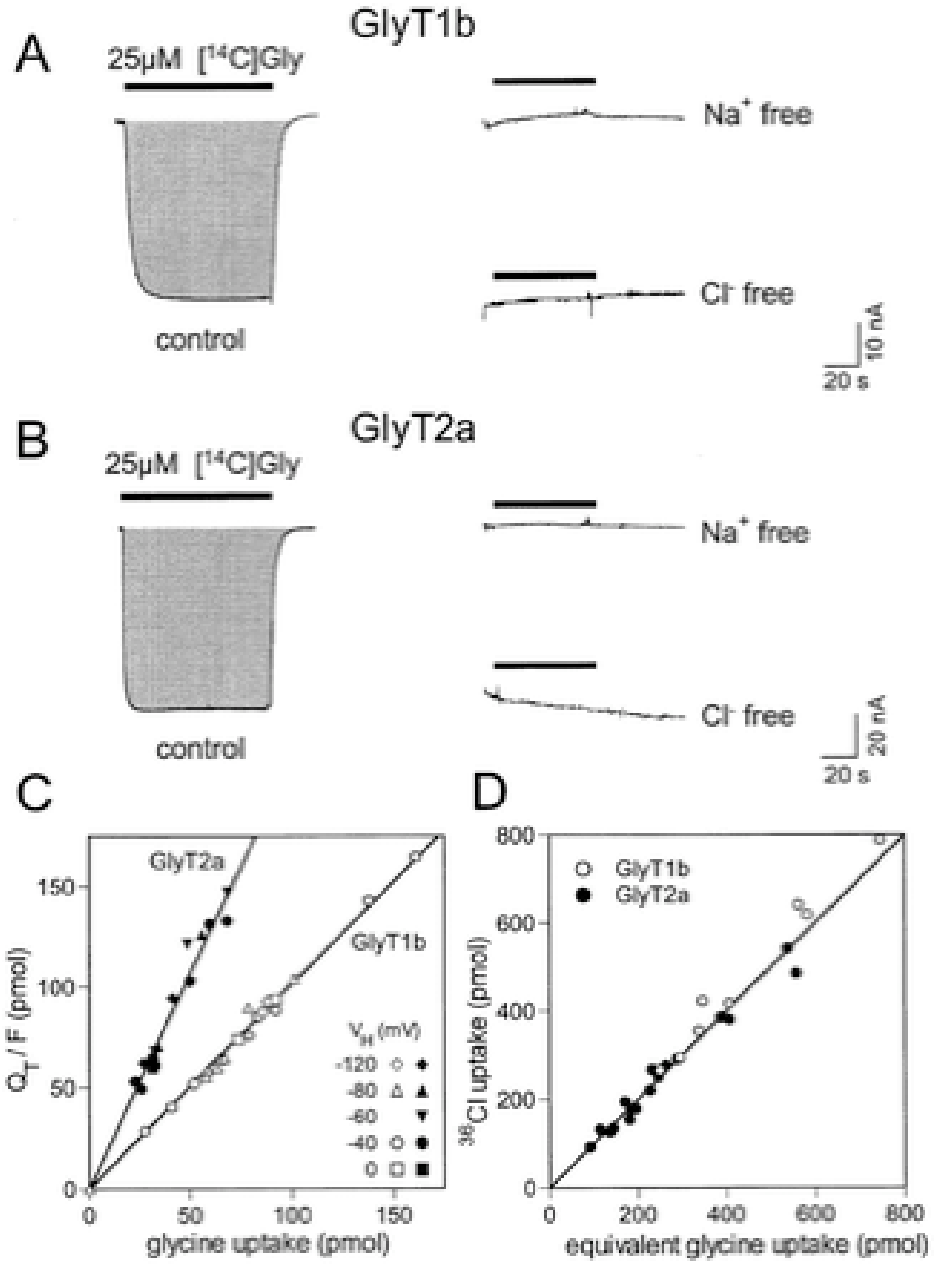


electrogenic transport produces currents: **rate**
--depend on Na^+, Cl^-

defined by gly addition
--strictly rectifying

can measure charge:flux using labeled glycine, Cl
suggests fixed stoichiometry

(Roux and Supplisson, 2000)



for electrogenic glycine transport,

$$\log_{10} (gly_{in}/gly_{out}) = m \log_{10} (Na^+_{out}/Na^+_{in}) + n \log_{10} (Cl^-_{out}/Cl^-_{in}) - z_T\Delta\Psi / 60\ mV$$

$$z_T\Delta\Psi / 60\ mV = \log_{10} \frac{Na^+_o{}^m \times Cl^-_o{}^n \times gly_o}{Na^+_i{}^m \times Cl^-_i{}^n \times gly_i}$$

$$\Delta\Psi = \frac{60\ mV}{z_T} \log_{10} \frac{Na^+_o{}^m \times Cl^-_o{}^n \times gly_o}{Na^+_i{}^m \times Cl^-_i{}^n \times gly_i} = E_{rev}$$

--like Nernst equation:

$$E_{Na} = 60\ mV \log_{10} Na^+_o/Na^+_i$$

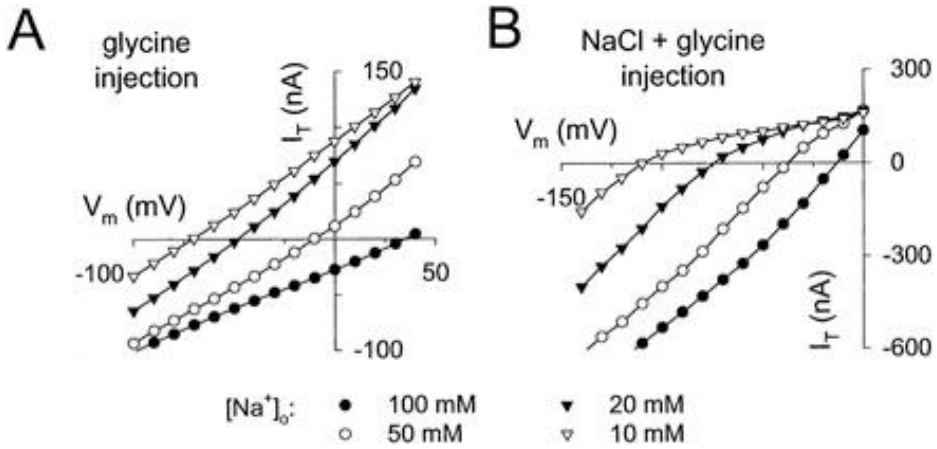
what are the differences?

$$Erev = \frac{60\ mV}{(m_{Na} - n_{Cl})} \log \frac{Na^+_o{}^m \times Cl^-_o{}^n \times gly_o}{Na^+_i{}^m \times Cl^-_i{}^n \times gly_i}$$

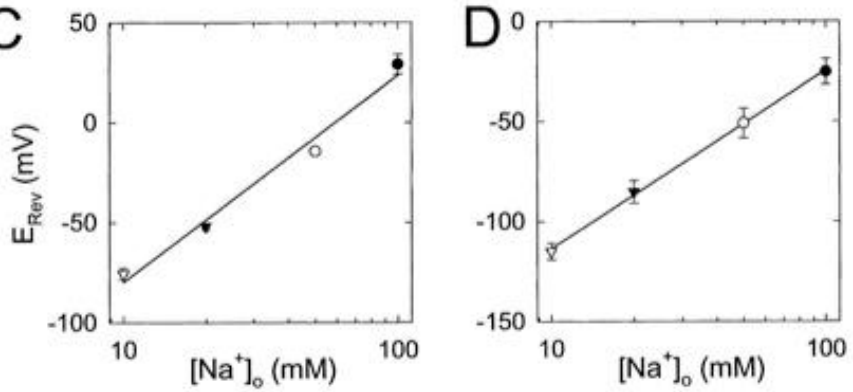
assume n = 1

can use Erev at different ionic gradients to determine n, p
BUT S-induced currents rectify--need them to reverse

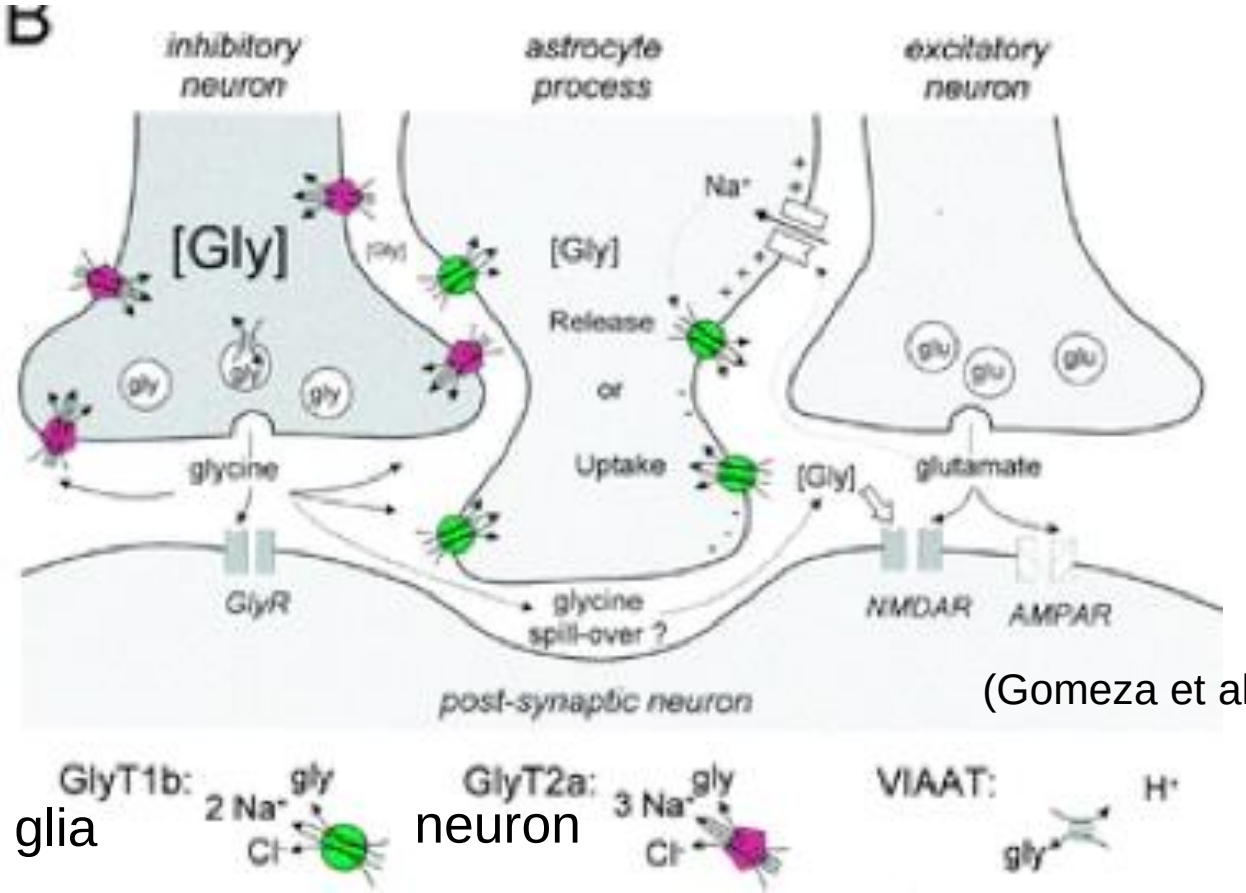
GlyT1 **GlyT2**



m = ?



m = ?



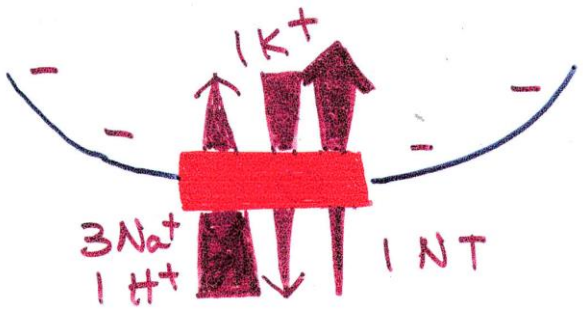
(Gomeza et al, 2003)

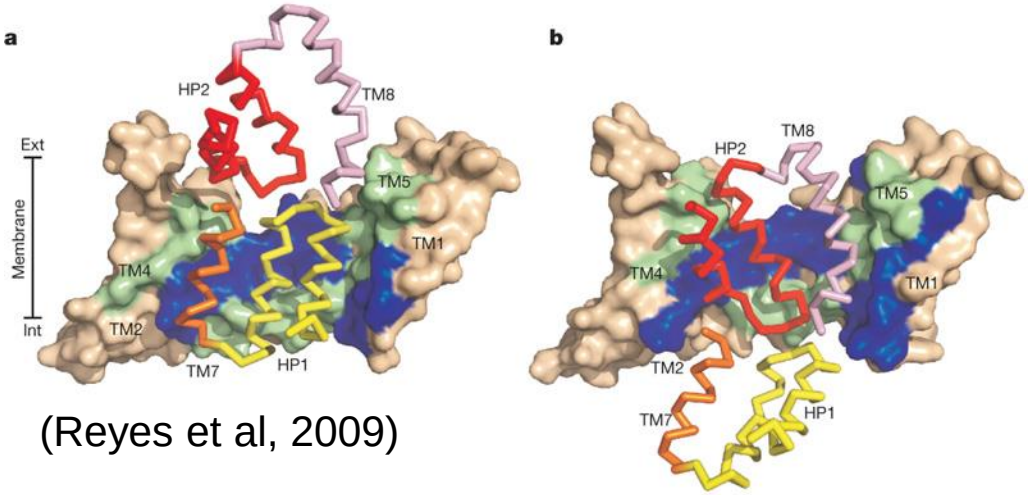
GlyT1 (2 Na^+) allows higher gly_o than GlyT2 (3 Na^+)
--to activate NMDA-type glutamate receptors?
GlyT1 KO: excess glycine (excess inhibition)--main role clearance
GlyT2 KO: resembles GlyR KO (startle)--main role packaging
differences in ionic coupling can also confer transfer between cells

can these transporters release neurotransmitter? how?

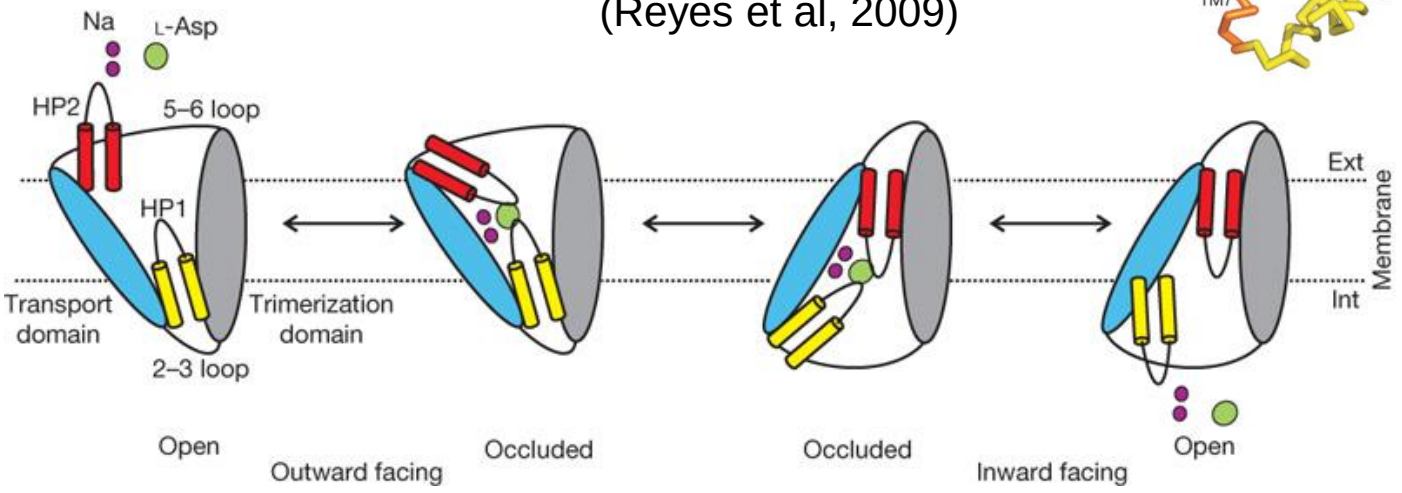
excitatory amino acid transporters (EAATs)

rigid body motion



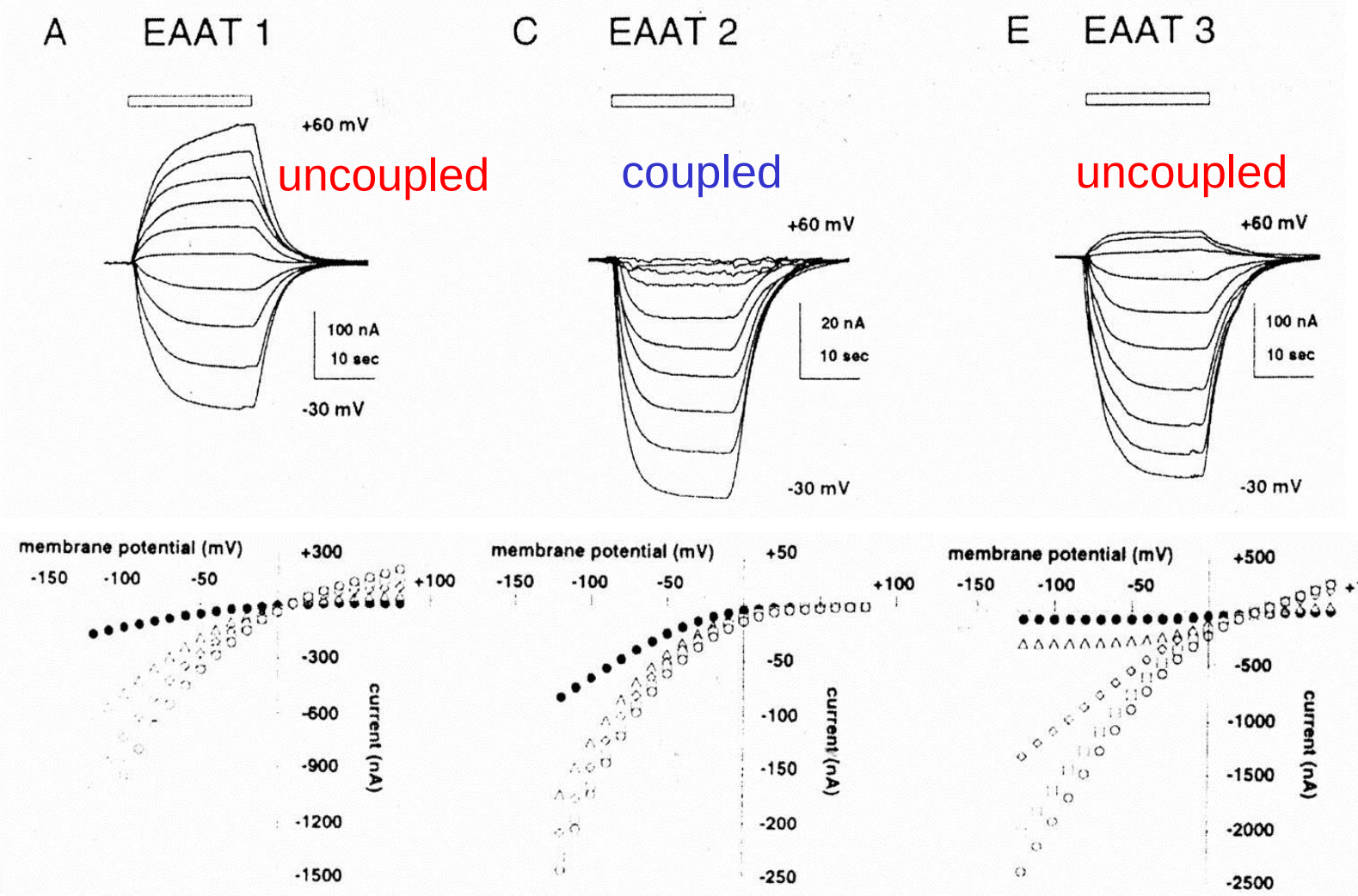


(Reyes et al, 2009)



little effect on kinetics of EPSC (buffer—translocation too slow)
controls activation of perisynaptic receptors, spillover
longer-term effects (seizures, degeneration): equilibrium

$3\ Na^+ : 1\ H^+ : 1\ glu^- \longleftrightarrow 1\ K^+$



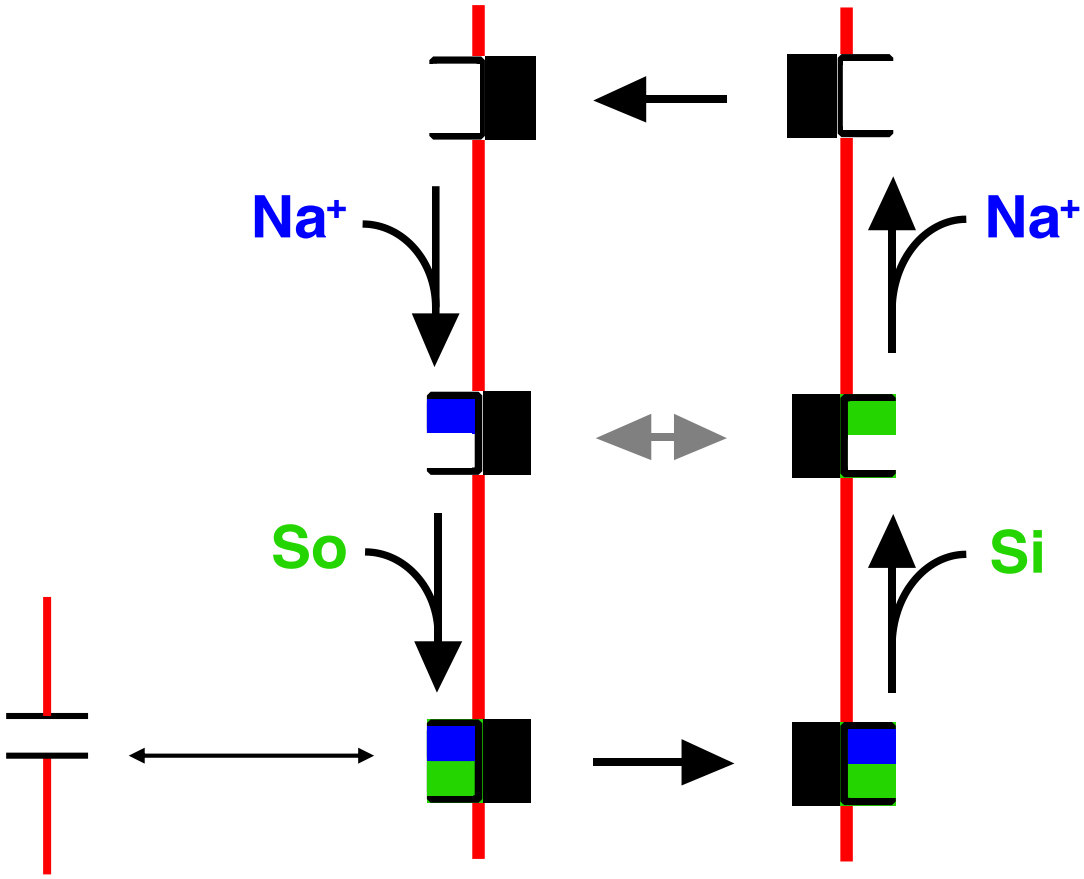
(Wadiche et al, 1995)

glutamate-induced currents can reverse

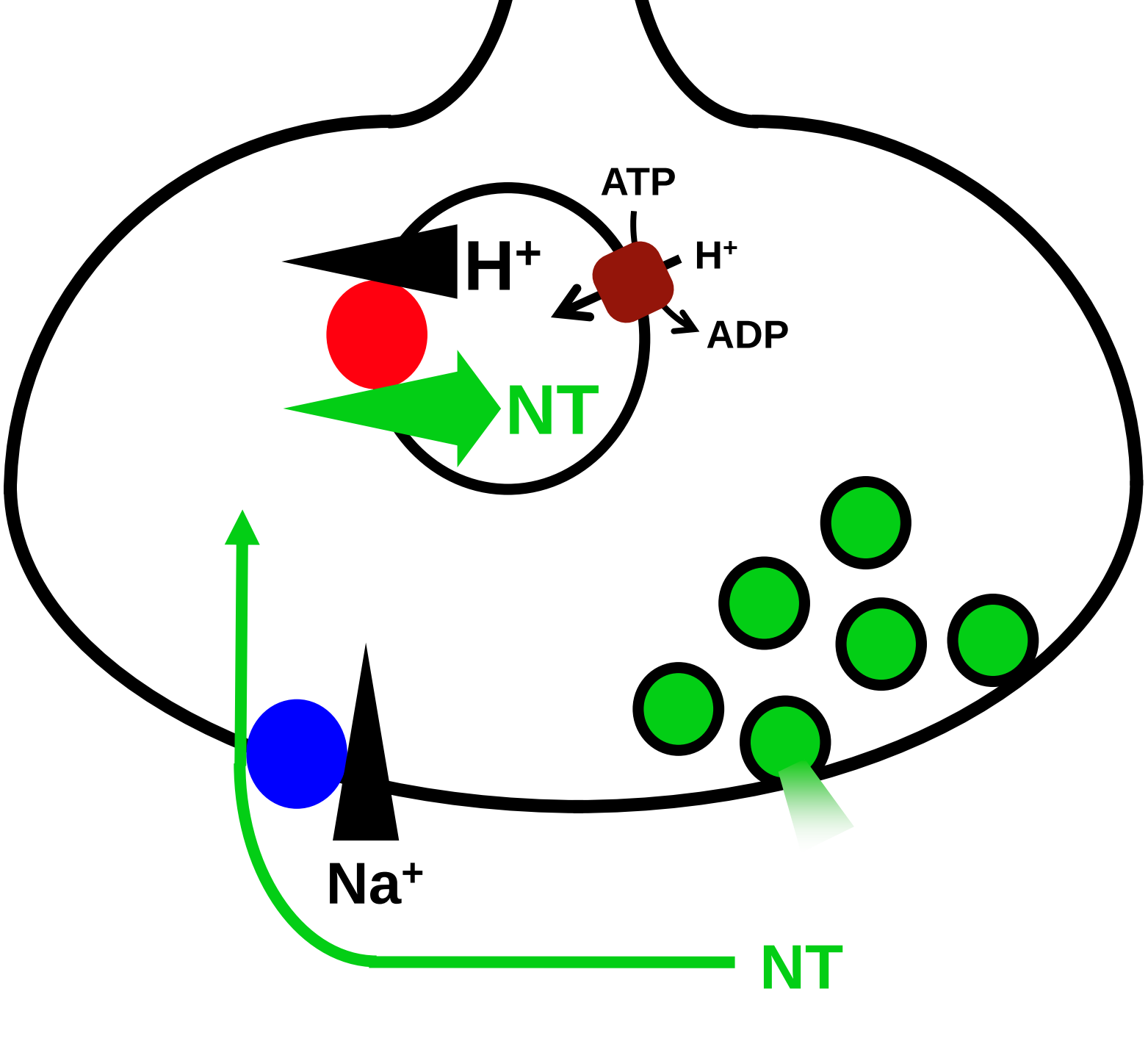
--glutamate-gated chloride channel (receptor)

WHY?

some transporters also behave like channels
EAATs can behave as glu-gated chloride channels



transport cycle can gate an ion channel
?evolutionary intermediate

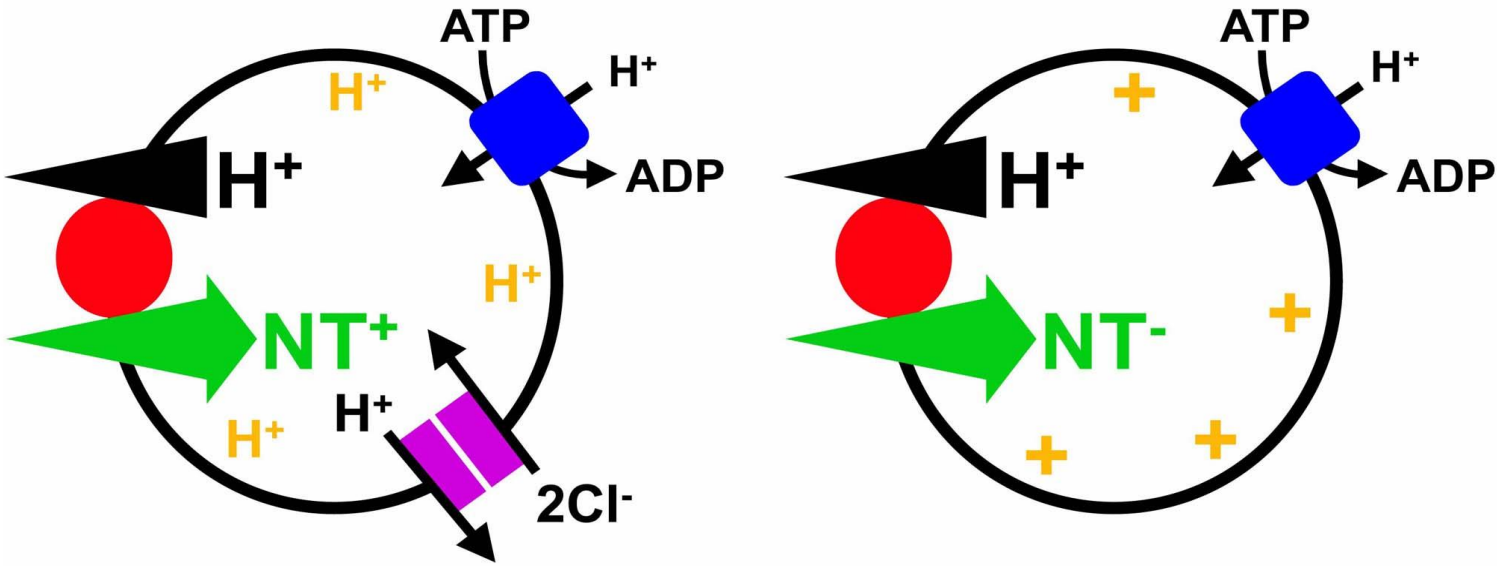

ATP
H+
H+
ADP
NT
Na+
NT

vesicular neurotransmitter transporters

monoamines
acetylcholine

GABA

glutamate



ΔpH > Δψ

Δψ > ΔpH

neurotransmitter per vesicle at **equilibrium**

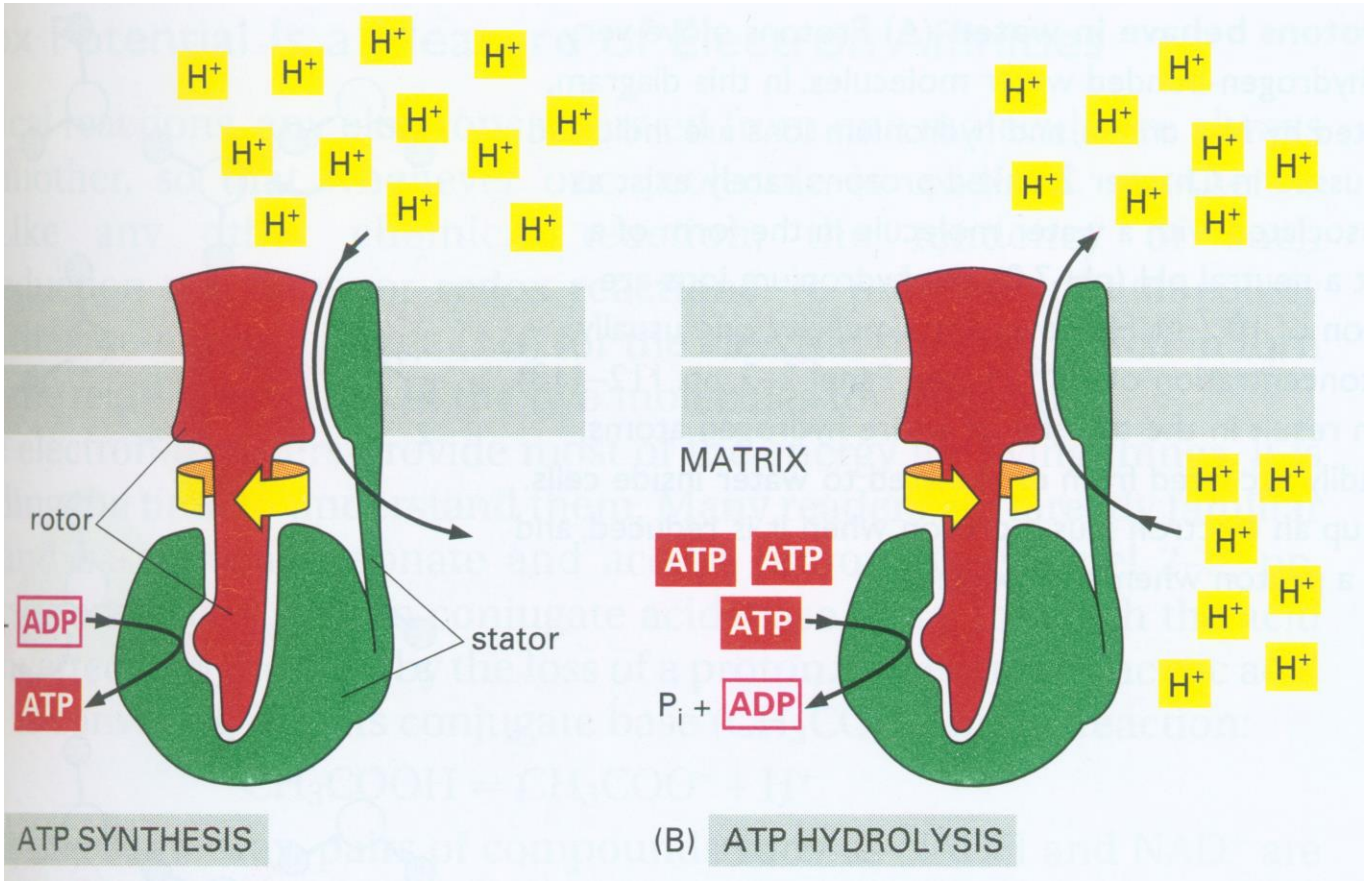
determines location, affinity of receptors activated

depends on H^+ electrochemical gradient: **H^+ ideal**

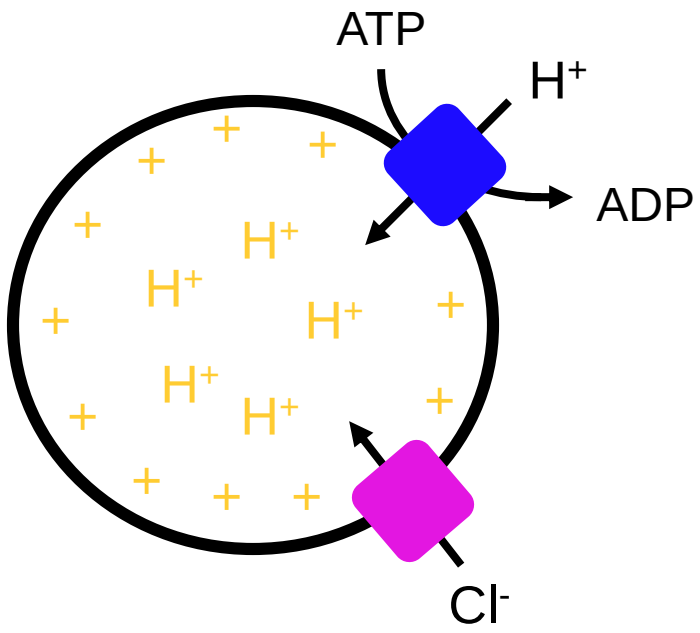
different NT depend on different components (ΔpH and **Δψ**)

F0/F1 ATP synthase

vacuolar H^+-ATPase



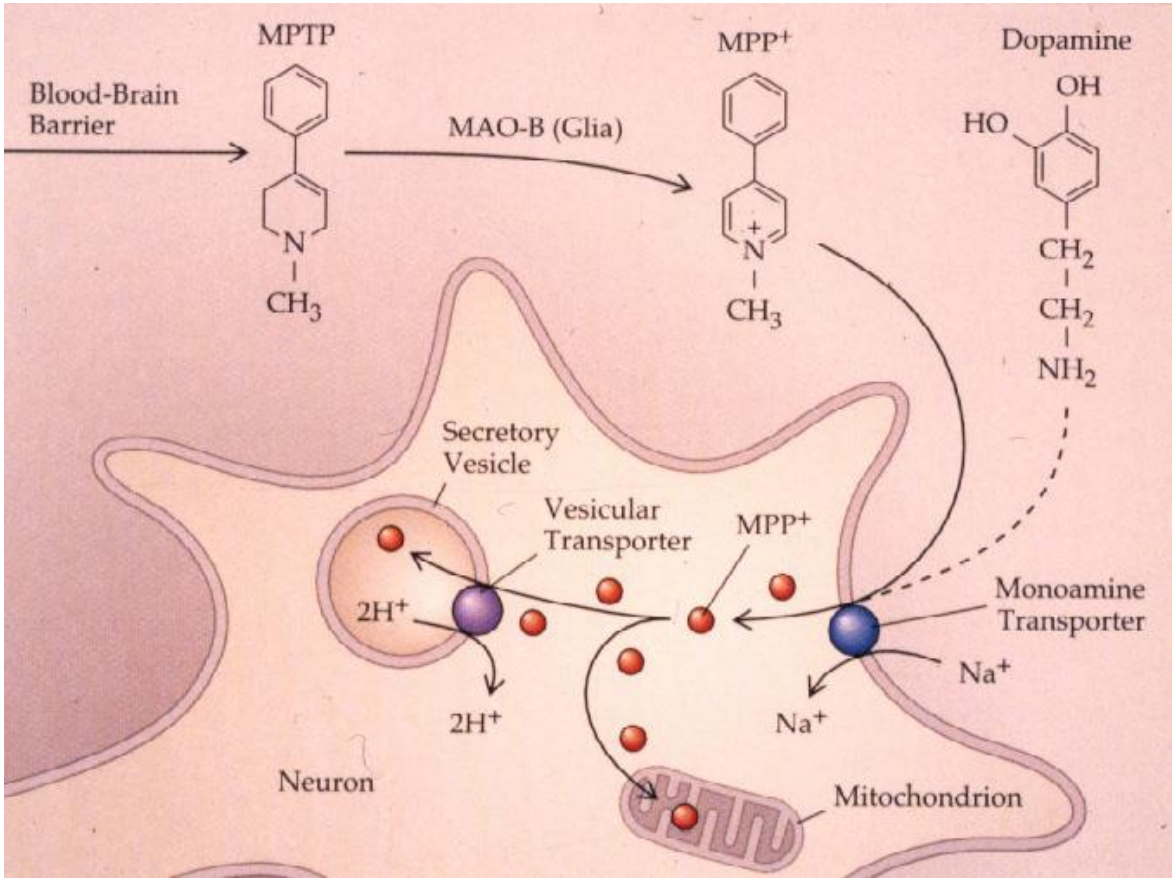
$$\Delta\mu_{H+} = \Delta pH + \Delta\psi$$



Cl^- entry dissipates $\Delta\psi$

cation efflux another way

how to create $\Delta\psi$?



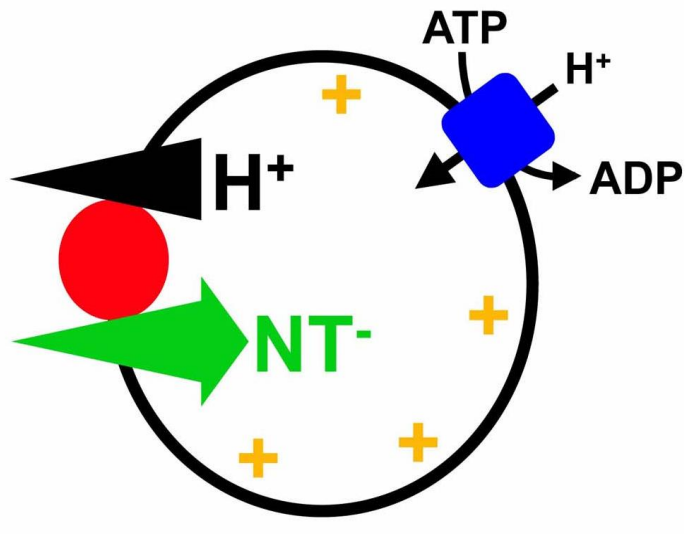
(Liu et al., 1993)

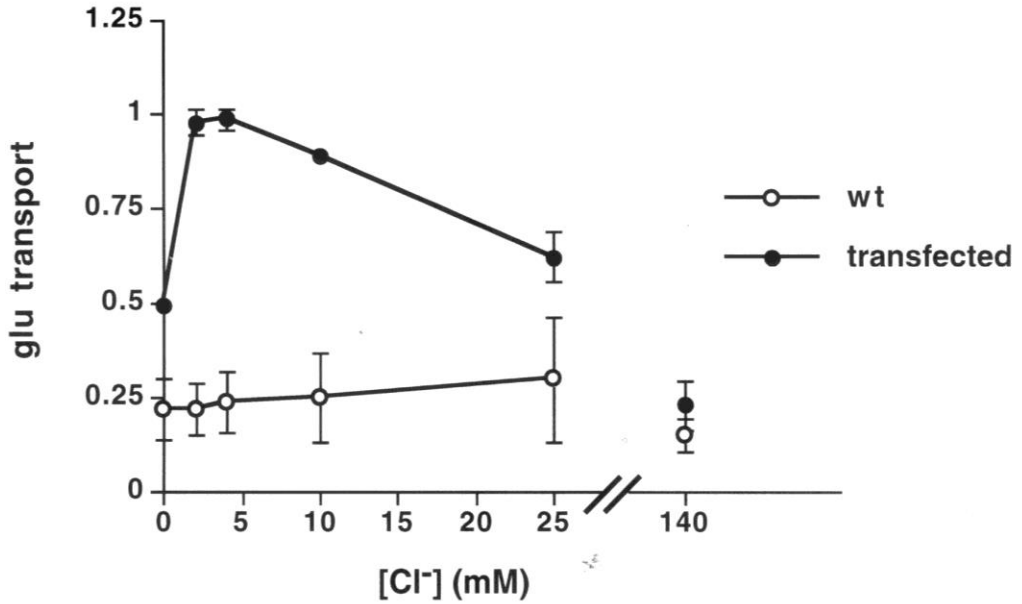
VMAT protects against MPP^+ toxicity

?role in Parkinson's?

Km ~1 µM (high apparent affinity)

vesicular glutamate transport



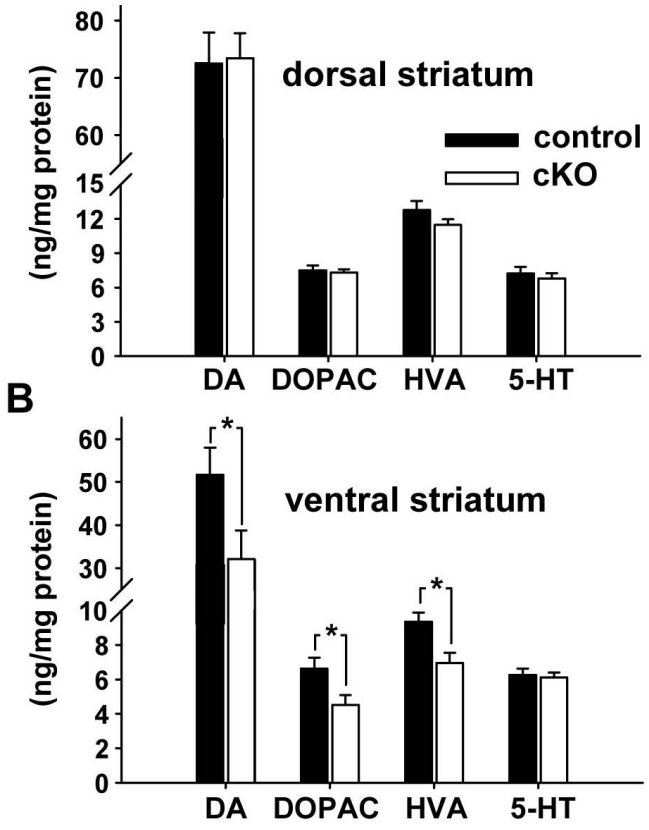


(Bellocchio et al., 2000)

originally identified as Na/Pi cotransporter
depends primarily on $\Delta\psi$
low apparent affinity (Km 1-3 mM)
allosteric activation by chloride (2-10 mM)
defines glutamate neurons

glutamate corelease with dopamine

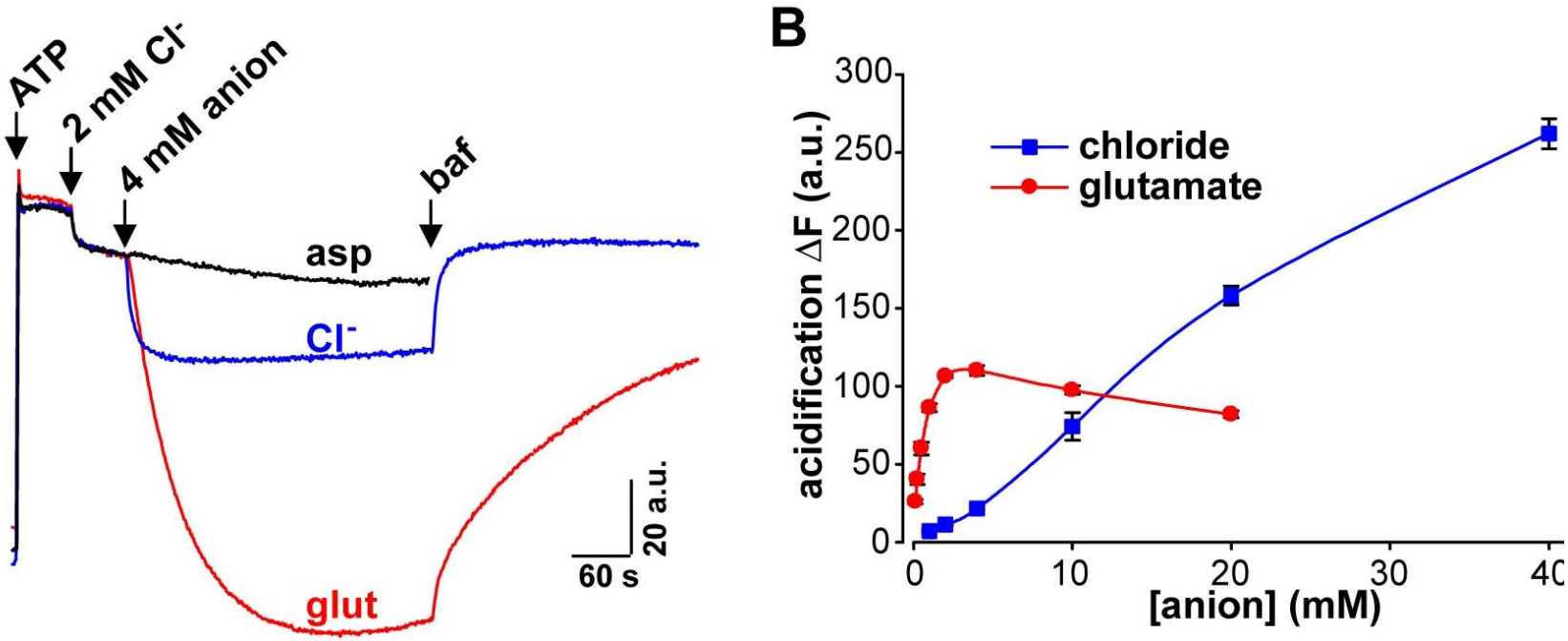
dopamine neurons form glutamatergic autapses *in vitro*
express high levels of VGLUT2 *in vitro*
VTA dopamine neurons express VGLUT2 *in vivo*
especially early in life
DAT-cre:VGLUT2--



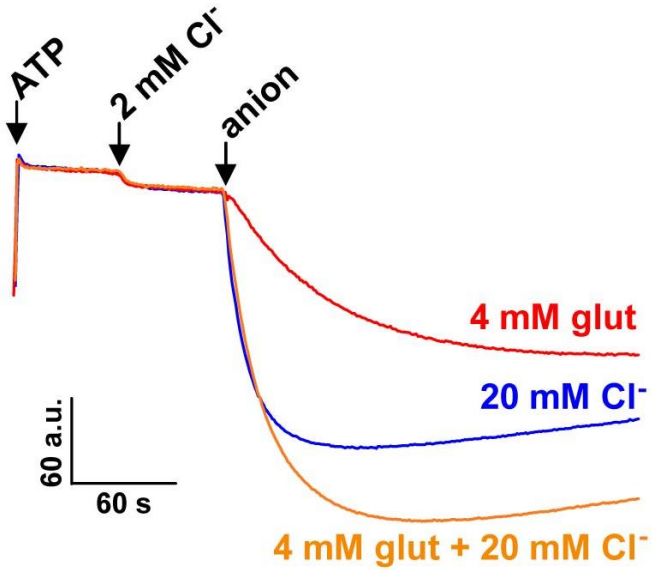
dopamine stores reduced ~35%
selective for ventral striatum
--consistent with localization
of VGLUT2 to VTA

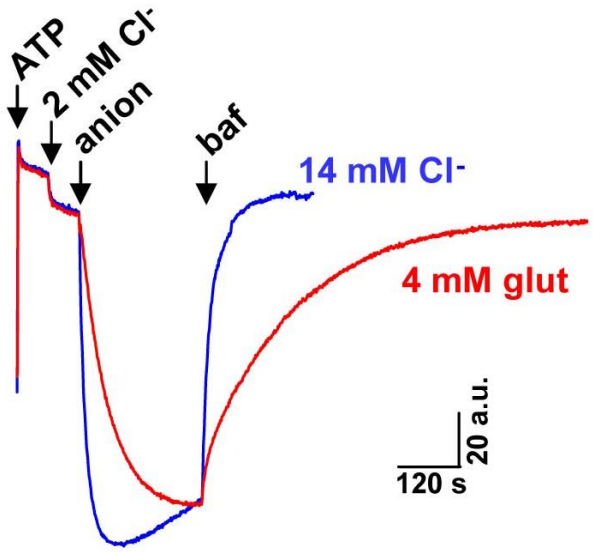
(Hnasko et al., 2010)

acidification: acridine orange



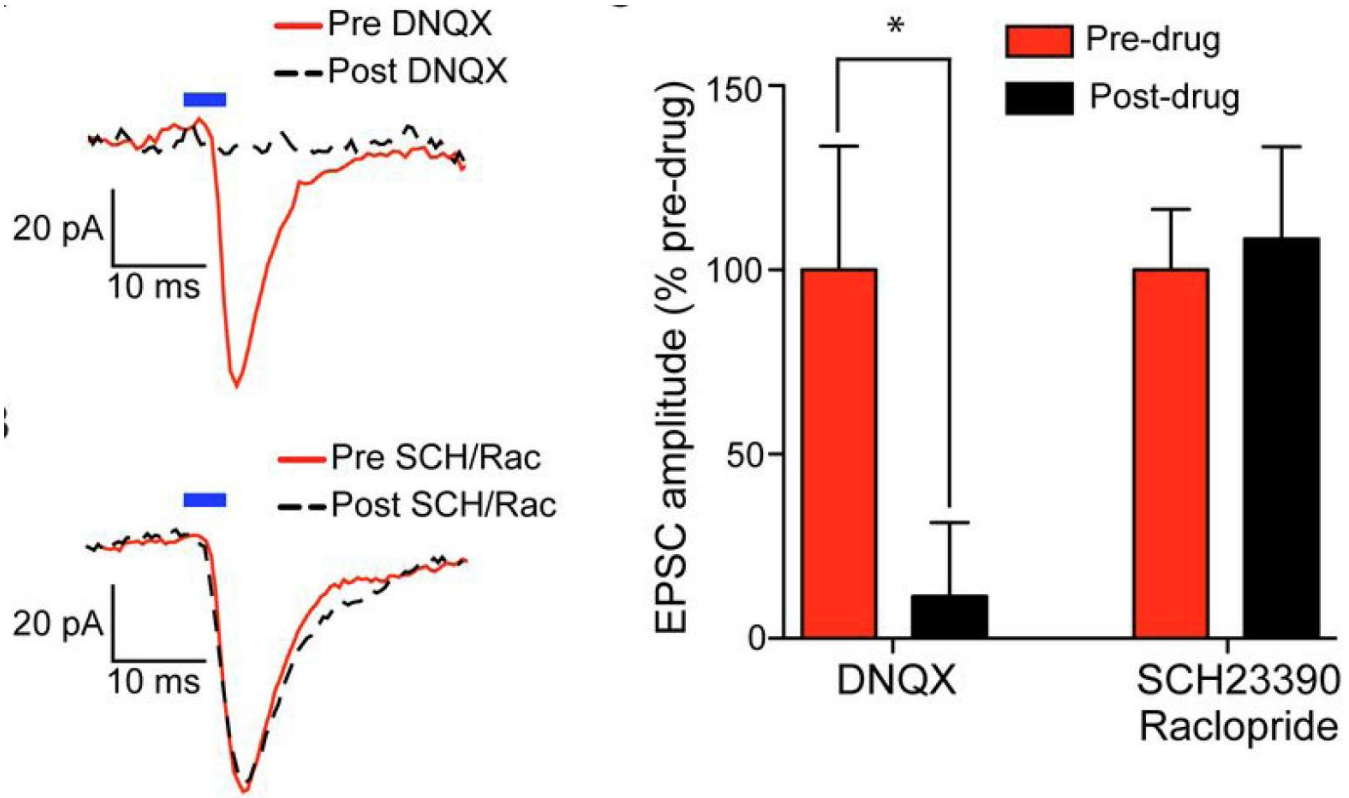
--glutamate also acidifies synaptic vesicles
corelease with other neurotransmitters widespread





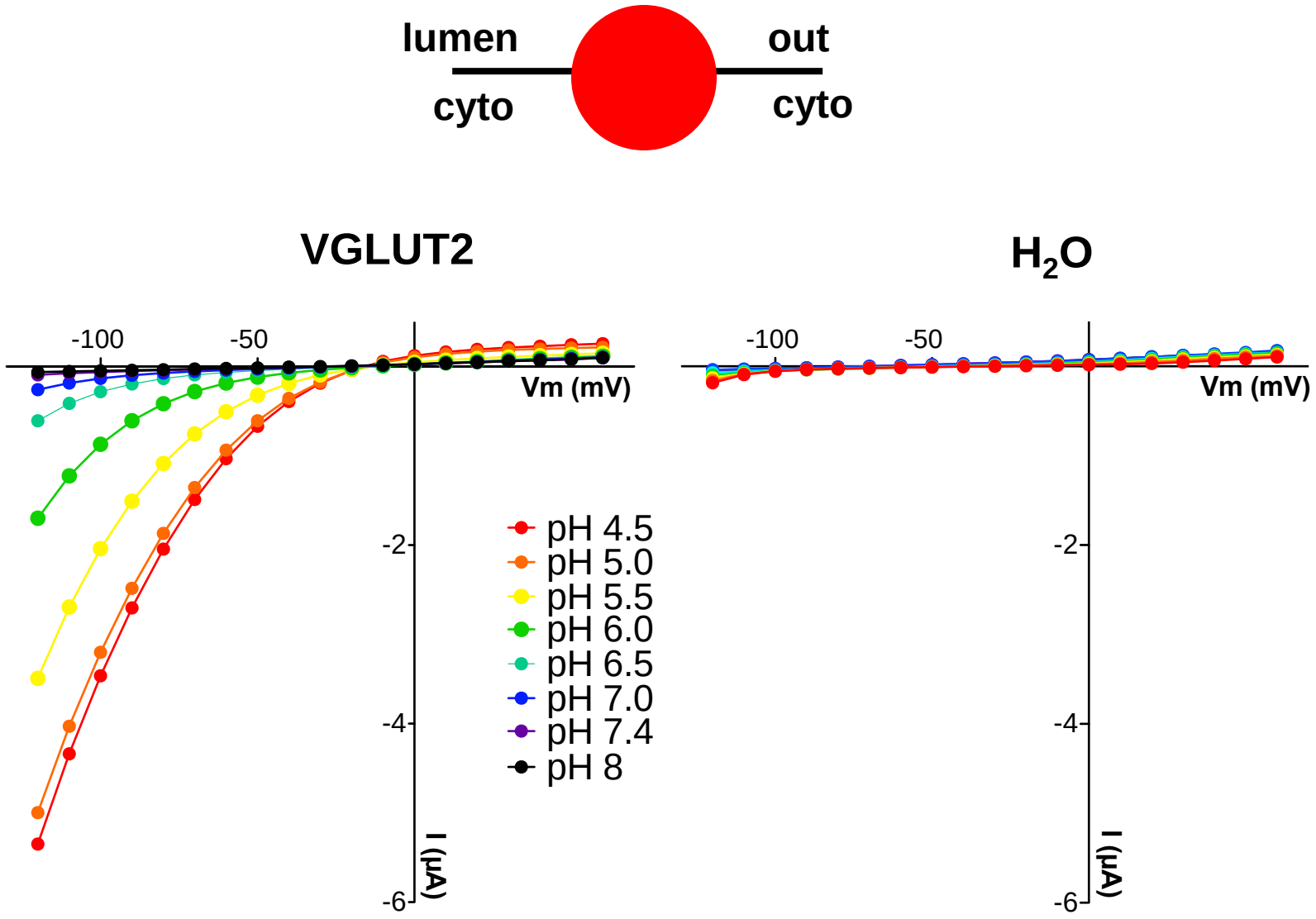
glutamate and Cl^- have additive effects on ΔpH
vesicles acidified with glu retain ΔpH longer
accounts for dopamine storage promoted by glu

optogenetics: cChR2 in DAT-cre mice

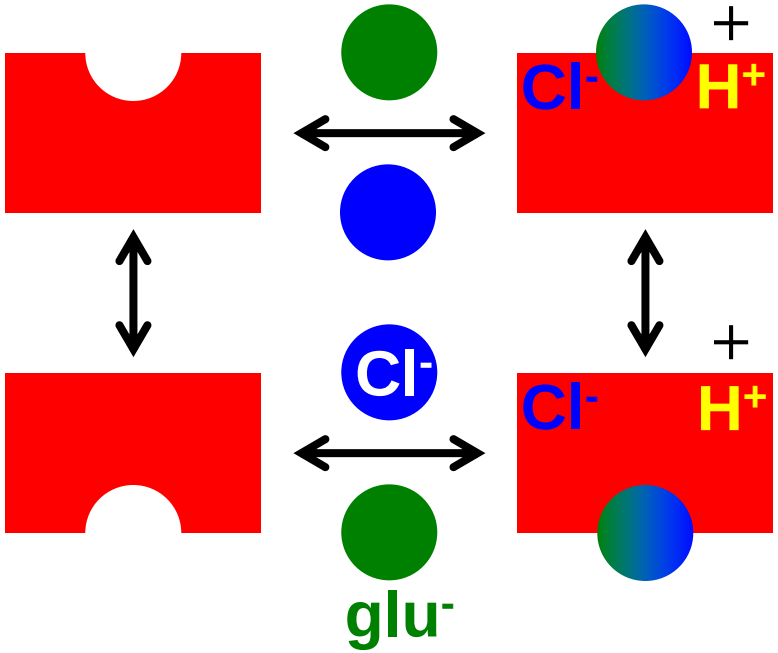


glutamate released by dopamine neurons also acts as an independent signal

?same or different synaptic vesicles?



low pH_o activates an inwardly rectifying Cl^- current



glutamate and Cl^- permeate through similar pathway
both driven by $\Delta\psi$: seems counterproductive
both allosterically activated by lumenal Cl^- and H^+:
role of allosteric activation?

VGLUT function

H^+ pump-dependent activation
Cl^-, glu dissipate Δψ, increase ΔpH
--make it impossible to disentangle
roles of driving force and allosteric activation
predict huge effects on equilibrium and rate of SV filling
voltage clamp would solve this problem
--but how to record from a vesicle transporter?

1) misexpress transporter at plasma membrane
2) record directly from endosomes
--chloride and glutamate conductances
--allosteric activation by H^+ as well as Cl^- (both sides)

Reading: The Synapse, pp. 147-170

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