Supplemental text

Derivation of Equations 1a,b

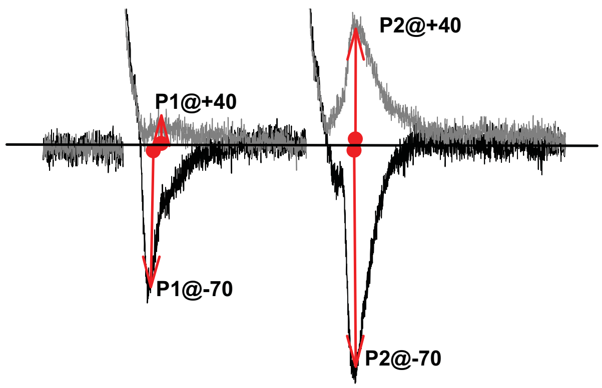
<u>Define (see graph):</u>

P1@$^{+}$40 = Peak current at +40 mV after first/single pulse

P2@$^{+}$40 = Peak current at +40 mV after second pulse

P1@$^{-}$70 = Peak current at -70 after first/single pulse

P2@$^{-}$70 = Peak current at +40 after second pulse

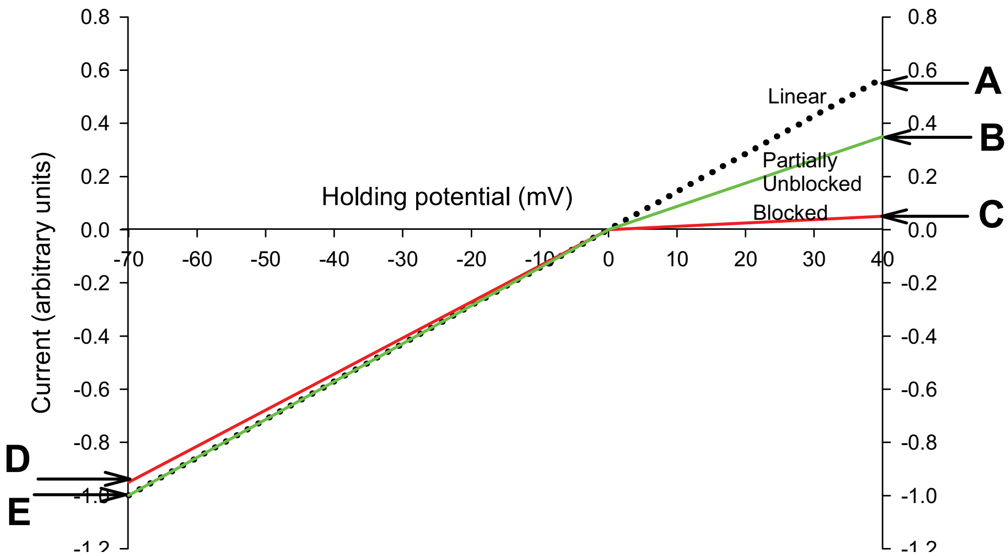


Supplemental figure A. Data reproduced from Figure 2C1 and detailed to show definitions of peaks used to derive equation for dependence of UBI on PRI.

PRI = fraction (0 to 1) of peak current contributed by inward rectifying AMPAR

This is the independent variable.

(1 – PRI) = fraction (0 to 1) of peak current contributed by linear AMPARS



F1 = maximal block of a pure inward rectifier as

First peak current at +40 mV/First peak current at -70 mV

= C / D

Estimated to be ≈ 0.05 based on (70), their Figure 4, from HEK293 cells expressing GluA2-B(Q) (inward rectifying mutant GluA2). Quantitatively this is similar to that seen (Washburn, M.S. and Dingledine, R. **JPET**, 278: 669-678(1996), their Figure 5) in CA3 interneurons. Given our highest estimate of RI was 21.0, if this was reflective of a nearly pure population of inward rectifying AMPARs, this would equate to F1 = 1/RI = 1/21 = 0.048. See sensitivity analysis below (Supplemental Figure B).

F2 = unblock of a pure population of inward rectifiers after a single pre-pulse at + 40 mV as

Second peak current at +40 mV/First peak current at -70 mV

= B / D

B is unknown

F3 = for a pure population of non-rectifying (linear) AMPARs

Peak current at +40 mV/Peak current at -70 mV

For a linear conductance G(V), I = G*V

= (G*40)/ (G*70) = 40/70 = 0.57

= A / E

F3’ = for a completely unblocked pure population of inward rectifying (temporarily linear) AMPARs

Peak current at +40 mV/Peak current at -70 mV

For a linear conductance G(V), I = G*V

= (G*40)/ (G*70) = 40/70 = 0.57

= A / E

Unblock ratio = amount (0 to 1) of unblock after pre-pulse

= F2 / F3’

F4 = unblock of a pure inward rectifier after a single pre-pulse at -70 mV as

Second peak current at -70 mV/First peak current at -70 mV

= E / D

This is assumed to be greater than or equal to unity (1). While there is some unblock at -70, this is assumed to be minimal.

PPR = paired pulse ratio. This is the fractional increase in the second pulse solely due to presynaptic factors. It is assumed that presynaptic function, as it affects different pools of inward rectifying and linear AMPARs, is the same.

PPR@$^{-}$70 = apparent paired pulse ratio at -70 mV.

PPR@$^{+}$40 = apparent paired pulse ratio at + 40 mV

UBI = unblocking index = PPR@$^{+}$40/PPR@$^{-}$70

This is the dependent variable

Derivation of dependence of UBI on PRI:

P1@$^{-}$70 = fraction of current due to inward rectifying AMPARs

+ fraction of current due to linear AMPARs

= PRI * P1@$^{-}$70 + (1 – PRI) * P1@$^{-}$70

= P1@$^{-}$70

P2@$^{-}$70 = (P1@$^{-}$70 * PRI*F4 + P1@$^{-}$70 * (1 – PRI))) * PPR

= P1@$^{-}$70* (PRI*F4 + (1 – PRI)) * PPR

$= P1@^{-}70*(PRI*(F4 – 1) + 1) * PPR$

$PPR@^{-}70 = P2@^{-}70/P1@^{-}70$

$= P1@^{-}70*(PRI*(F4 – 1) + 1) * PPR / P1@^{-}70$

$= (PRI*(F4 – 1) + 1) * PPR$

(We assume that $F4 \geq 1$)

$P1@^{+}40 = P1@^{-}70 * PRI * F1 + P1@^{-}70 * (1 – PRI)*F3$

$= P1@^{-}70 * (PRI * F1 + (1 – PRI)*F3)$

$= P1@^{-}70 * (PRI * (F1 – F3) + F3)$

$P2@^{+}40 = (P1@^{-}70 * PRI * F2 + P1@^{-}70 * (1 – PRI) * F3) * PPR$

$= P1@^{-}70 * PPR * (PRI * F2 + (1 – PRI) * F3)$

$= P1@^{-}70 * PPR * (PRI * (F2 – F3) + F3)$

$PPR@^{+}40 = P2@^{+}40 / P1@^{+}40$

$$= \frac{P1@^{-}70 * PPR * (PRI * (F2 – F3) + F3)}{P1@^{-}70 * (PRI * (F1 – F3) + F3)}$$

$$= \frac{PPR * (PRI * (F2 – F3) + F3)}{(PRI * (F1 – F3) + F3)}$$

$UBI = PPR@^{+}40/PPR@^{-}70$

$$= \frac{\dfrac{PPR * (PRI * (F2 – F3) + F3)}{(PRI * (F1 – F3) + F3)}}{PPR * (PRI*(F4 – 1) + 1)}$$

$$= \frac{PRI(F2 – F3)/(PRI(F1 – F3) + F3)}{PRI(F4 - 1) + 1} \qquad (1a)$$

$RI_{apparent}$ = apparent rectification index (dependent on PRI)

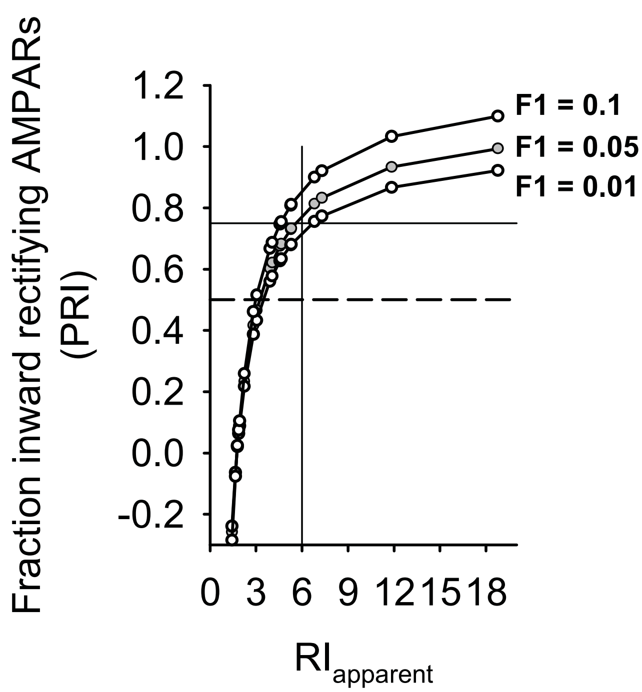
$$= P1@^{-}70 \,/\, P1@^{+}40$$

$$= \frac{P1@^{-}70}{P1@^{-}70 * (PRI * (F1 - F3) + F3)}$$

$$= 1 \,/\, (PRI * (F1 - F3) + F3)$$

Rearranging, solving instead for PRI as the independent variable:

$$PRI = \frac{1 - RI_{apparent} * F3}{RI_{apparent} * (F1 - F3)} \qquad (1b)$$



Supplemental Figure B. Data and fit replotted from Figure 2E. Effect of estimates of F1, the rectification index of a pure population of inward rectifying AMPARs, on PRI. Values underlying greater rectification (0.01) still suggest that > 20% of synaptic AMPAR clusters have > 75% inward rectifying AMPARs. This value (0.01) would suggest that peak synaptic current at -70 mV was 100 times greater than that seen at +40 mV, which has never been reported. Values underlying less rectification (0.1) result in the suggestion that synaptic AMPAR clusters have greater than 100% inward rectifying AMPARs, which is not physically possible but also then overestimate (39%) the contribution of inward rectifying AMPARs. This validates our use of F1 = 0.05 by demonstrating that a robust range still supports our findings.