# Kir4.1 K+ channels are regulated by external cations

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The inwardly rectifying potassium channel (Kir), Kir4.1 mediates spatial $K^+$-buffering in the CNS. In this process the channel is potentially exposed to a large range of extracellular $K^+$ concentrations ($[K^+]_o$). We found that Kir4.1 is regulated by $K^+_o$. Increased $[K^+]_o$ leads to a slow (mins) increase in the whole- currents of Xenopus oocytes expressing Kir4.1. Conversely, removing $K^+$ from the bath solution results in a slow decrease of the currents. This regulation is not coupled to the $pH_i$-sensitive gate of the channel, nor does it require the presence of K67, a residue necessary for $K^+_o$-dependent regulation of Kir1.1. The voltage-dependent blockers $Cs^+$ and $Ba^{2+}$ substitute for $K^+$ and prevent deactivation of the channel in the absence of $K^+_o$. $Cs^+$ blocks and regulates the channel with similar affinity, consistent with the regulatory site being in the selectivity-filter of the channel. Although both $Rb^+$ and $NH_4^+$ permeate Kir4.1, only $Rb^+$ is able to regulate the channel. We conclude that Kir4.1 is regulated by ions interacting with specific sites in the selectivity filter. Using a kinetic model of the permeation process we show the plausibility of the channel's sensing the extracellular ionic environment through changes in the selectivity occupancy pattern, and that it is feasible for an ion with the selectivity properties of $NH_4^+$ to permeate the channel without inducing these changes.

**Supplementary Material**
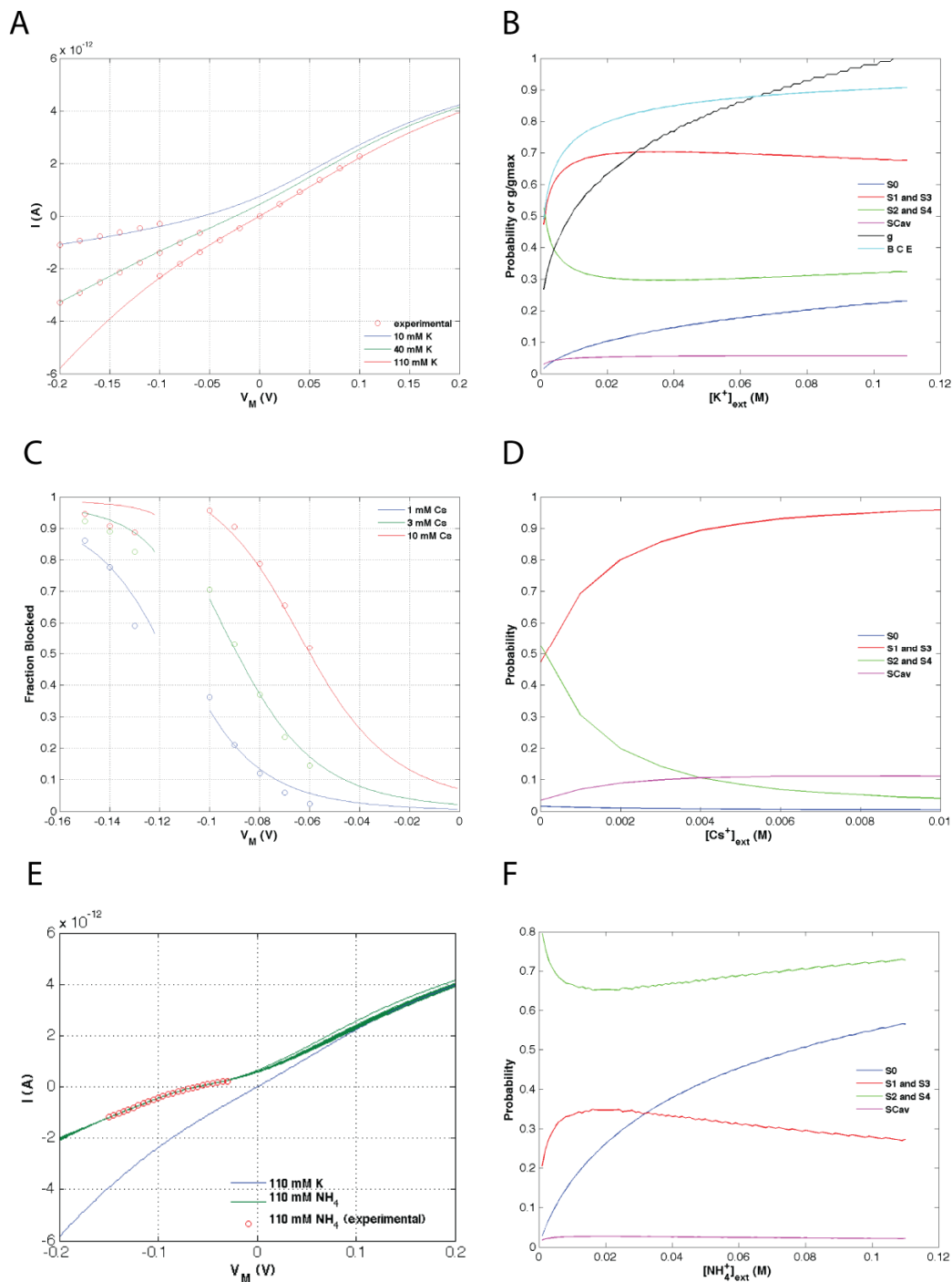
**Figure S1. Results from kinetic model.** $[K^+]_i$ was 110 mM for calculations. **(A)** i-V relationships for different $[K^+]_o$, calculated with the parameter values shown in Table S1, superimposed on experimental values. **(B)** The change in occupancy of different sites as a function of $[K^+]_{ext}$. Green line shows conductance calculated 50 mV negative of the $E_{rev}$. **(C)** Predicted fractional decreases in conductance as a function of voltage with 1 mM $K^+$ and 0, 1, 3, or 10 mM $Cs^+$ in the external solution using the average values from $Cs^+$-fit 1, superimposed on experimental values **(D)** Changes in occupancy of different sites as a function of $[Cs^+]_{ext}$ at the resting potential. **(E)** i-V relationship with 110 mM $NH_4^+$ in the bath superimposed on experimental values. The blue line indicates currents in 110 mM $K^+$.

| Fit | $G_0$ | $S_0$ | $G_1$ | $S_1$ | $G_2$ | $S_2$ | $G_3$ | $S_3$ | $G_4$ | $S_4$ | $G_{cav}$ | $S_c$ | $G_{cyto}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| K | 0.4±0.0 | -3.1±0.0 | -2.7±0.1 | -2.7 ±0.1 | -0.2±0.3 | -1.6±0.4 | -1.2±0.4 | -1.2±0.4 | 0.8±0.2 | -0.3±0.2 | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 |
| Cs-1 | 1.1±0.8 | -1.2±0.9 | 0.4±1.0 | -1.2±0.8 | 3.2±1.3 | 1.7±1.3 | 4.3±1.1 | -1.2±1.3 | 2.8±1.5 | 0.3±1.7 | 2.5±2.7 | -0.9±4.2 | 4.6±2.7 |
| Cs-2 | 0.9±0.8 | -2.4±0.9 | 0.7±0.5 | 0.1±0.4 | 3.0±1.5 | -0.5±2.1 | 4.9±1.2 | 2.6±2.0 | 3.4±2.0 | -1.8±2.4 | 0.7±2.0 | -1.3±3.1 | 6.3±3.5 |
| $NH_4^+$ | 0.8±0.5 | -3.3±0.2 | 0.7±0.5 | 0.7±0.6 | 3.0±0.6 | 2.1±0.2 | 2.7±0.6 | 2.1±0.8 | 4.1±1.0 | 1.4±0.8 | 1.9±0.9 | -0.1±1.0 | 1.6±0.9 |

**Table S1.** All values are given as units of R·T (1 R·T~ 0.6 kcal/mol at 300 K). The numbers represent means ± SD for 7 fits of the data for $K^+$, 45 for $Cs^+$-fit-1, 19 for $Cs^+$-fit-2, and 95 for $NH_4^+$.