Figure 4 The GluN2 S/L site contributes to NMDAR subtype specificity of relative Ca^{2+} permeability. (**a,b**) Representative I-V curves in 143 mM extracellular Cs^+ (**a**) and 1.8 mM extracellular Ca^{2+} (**b**). (**c,d**) Average $V_{rev} \pm$ s.e.m. of wild-type and mutant NMDARs in 143 mM extracellular Cs^+ (**c**) and 1.8 mM extracellular Ca^{2+} (**d**). No significant differences were detected among wild-type and mutant receptor V_{rev} in 143 mM extracellular Cs^+ (one-way ANOVA, $P = 0.45$). Significantly different V_{rev} values (one-way ANOVA followed by Tukey *post hoc* comparison, $P < 0.05$) between wild-type and mutant receptors are marked with an asterisk. (**e**) P_{Ca}/P_{Cs} values of wild-type and mutant NMDARs.

GluN1/2A(S632L) receptors was 34.8 ± 1.4 pS, which was significantly different from the GluN1/2A receptor main state conductance (*t* test for heterogeneity of slopes, $P < 0.0001$), but not significantly different ($P = 0.25$) from that of GluN1/2D receptors (37.4 ± 1.3 pS, measured previously[15] and consistent with other reported values[21]). The GluN1/2D(L657S) receptor main state conductance was 54.9 ± 1.5 pS, which was significantly different from the GluN1/2D receptor main state conductance[15] ($P < 0.0001$), but not significantly different from that of GluN1/2A receptors ($P = 0.23$).

Another single-channel property that distinguishes NMDAR subtypes is the prominent subconductance state of ~20 pS that is exhibited by GluN1/2C and GluN1/2D receptors[15,21]; in contrast, GluN1/2A or GluN1/2B receptors exhibit a larger subconductance state (~40 pS)[20] that is less commonly occupied. GluN1/2A(S632L) receptors exhibited a subconductance state with a conductance (17.0 ± 0.7 pS) that was not significantly different from the subconductance state of GluN1/2D receptors (20.2 ± 1.3 pS, $P = 0.052$; **Fig. 5c**). In contrast, the GluN1/2D(L657S) receptor subconductance state was 49.6 ± 5.6 pS, which was significantly higher than that of GluN1/2D receptors ($P < 0.0001$). We could not consistently resolve a subconductance state in GluN1/2A receptors, although a subconductance state of ~40 pS was occupied infrequently in some patches. Our ability to consistently resolve the subconductance state of GluN1/2D and GluN1/2D(L657S) receptors, but not of GluN1/2A receptors, suggests that subconductance state occupancy by GluN1/2D receptors was not markedly reduced by the mutation. Thus, mutation of the GluN2 S/L site in GluN2D receptors has a powerful effect on the

conductance of the subconductance state, but appears to have a weaker effect on subconductance state occupancy.

To determine whether single-channel kinetics are influenced by the GluN2 S/L site, we constructed open period and shut time histograms (**Supplementary Fig. 2** and **Supplementary Table 2**) and statistically compared the values of weighted mean open periods. The weighted mean open periods of GluN1/2A and GluN1/2A(S632L) receptors were not significantly different (one-way ANOVA followed by Tukey *post hoc* comparison, $P = 0.70$), nor were the weighted mean open periods of GluN1/2D and GluN1/2D(L657S) receptors ($P = 0.84$; see **Supplementary Table 2**). Thus, consistent with previous studies showing that NMDAR subtype-dependence of channel gating depends on the N-terminal domain[8,9], the GluN2 S/L site does not appear to have a substantial effect on channel kinetics.

We conclude that the naturally occurring residue replacement in GluN2 subunits that we mimicked by creating GluN2A(S632L), GluN2C(L643S) and GluN2D(L657S) subunits underlies fundamental NMDAR subtype–dependent variations in multiple channel characteristics: Mg^{2+} block, relative Ca^{2+} permeability, and single-channel conductance of both main and subconductance states. However, the way in which the GluN2 S/L site affects pore properties is unclear on the basis of the above data.

Mechanism of GluN2 S/L site influence on Mg^{2+} block

To search for the mechanisms by which the GluN2 S/L site transmits its effects to the pore, we created additional GluN2A subunits with mutations at the GluN2 S/L site and tested whether the Mg^{2+} IC_{50}
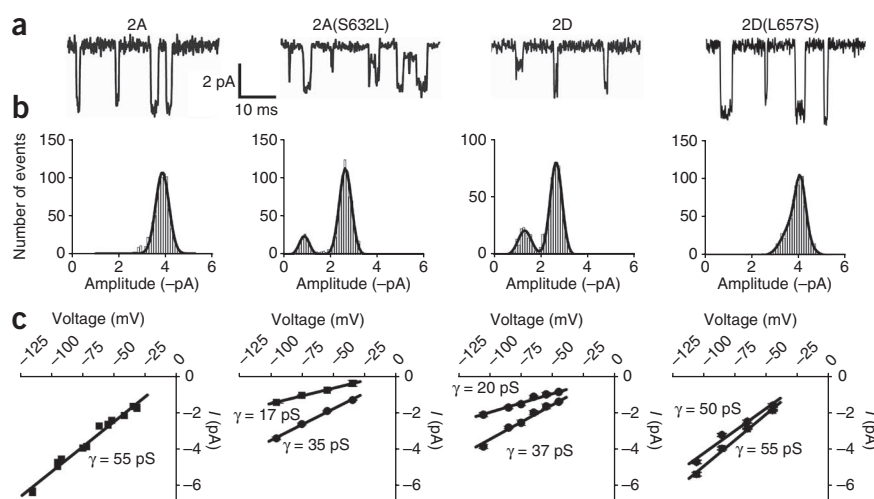
Figure 5 The GluN2 S/L site controls the NMDAR subtype specificity of single-channel conductance. (**a,b**) Representative single-channel current traces (**a**) and amplitude histograms (**b**) of GluN1/2A (left), GluN1/2A(S632L) (center left), GluN1/2D (center right) and GluN1/2D(L657S) (right) receptors recorded in the outside-out patch configuration at −75 mV. (**c**) Current versus voltage plots of single-channel currents and linear regression fits. The slope of the linear fit to each plot (single-channel conductance) is shown next to each fit. Left, GluN1/2A receptors ($n = 17$ single-channel current recordings); center left, GluN1/2A(S632L) receptors ($n = 17$); center right, GluN1/2D receptors ($n = 24$; GluN1/2D data from ref. 15); right, GluN1/2D(L657S) receptors ($n = 16$).