

A Mixed Periodic Paralysis & Myotonia Mutant, P1158S, Imparts pH-Sensitivity in Skeletal Muscle Voltage-gated Sodium Channels

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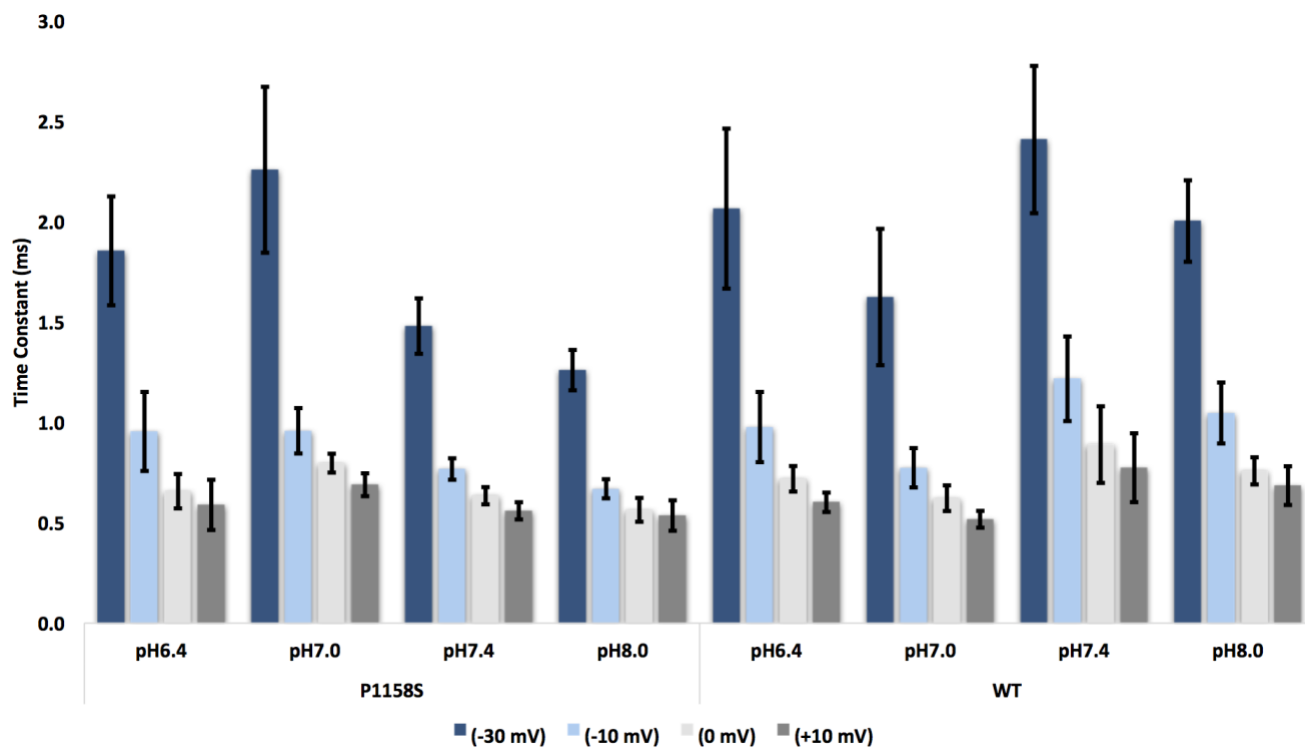
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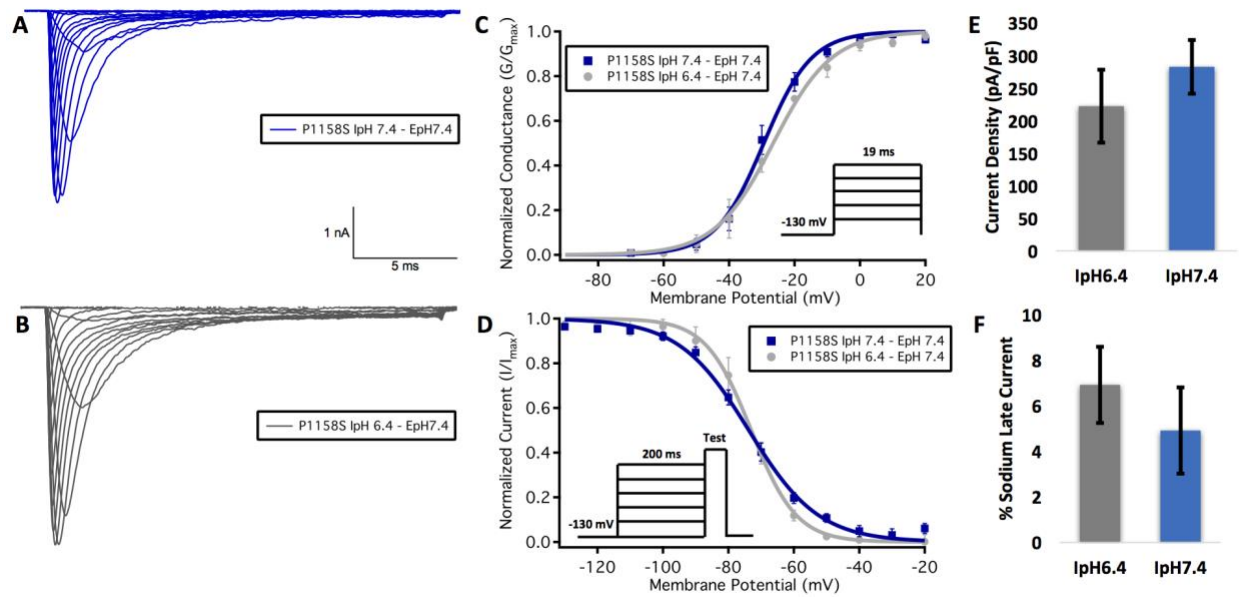
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P1158S Imparts pH-Sensitivity in Nav1.4



Supplementary Fig. 1- Open-state fast inactivation time constants across all conditions at: -30, -10, 0, +10 mV.

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Supplementary Fig. 2- Effects of changing intracellular pH to 6.4. **(A-B)** Representative current traces of P1158S with both intra- and extracellular pH at 7.4, and intracellular pH6.4 and extracellular pH7.4, respectively. **(C)** Shows the voltage-dependence of activation for P1158S at intracellular pH7.4 (blue squares) and intracellular pH6.4 (grey circles). **(D)** Shows the voltage-dependence of steady-state fast inactivation for P1158S at intracellular pH7.4 (blue squares) and intracellular pH6.4 (grey circles). *Insets* show voltage protocols. **(E)** Shows average current density for P1158S channels at intracellular pH7.4 and 6.4. **(F)** Shows late sodium current as a percentage of peak sodium current for WT and P1158S channels.

Supplementary Table 1- Conductance comparison across pH6.4 to 8.0 between WT and P1158S

Channel Type-pH	Mean $V_{1/2} \pm SE$ (mV)	Mean $z \pm SE$ (slope)	n
P1158S-pH6.4	-19.48 ± 2.22^A	2.76 ± 0.32	9
P1158S-pH7.0	-22.11 ± 2.52^A	2.68 ± 0.36	7
P1158S-pH7.4	-29.95 ± 2.35^B	4.71 ± 0.34	8
P1158S-pH8.0	-34.77 ± 2.35^B	3.02 ± 0.34	8
WT-pH6.4	-25.86 ± 2.72	3.15 ± 0.39	6
WT-pH7.0	-24.87 ± 2.52	3.56 ± 0.36	7
WT-pH7.4	-24.84 ± 2.52	3.14 ± 0.36	7
WT-pH8.0	-21.92 ± 2.98	3.16 ± 0.43	5

Letter codes indicate statistical significance. Numbers with different letter codes are significantly different.

Supplementary Table 2- Peak conductance and current densities

Channel Type-pH	Mean density $\pm SE$ (pA/pF)	n	Mean Conductance $\pm SE$ (nS/pF)	n
P1158S-pH6.4	45.7 ± 19.5^A	9	1.06 ± 0.45^A	9
P1158S-pH7.0	70.4 ± 20.7^A	8	1.68 ± 0.48^A	8
P1158S-pH7.4	$192.0 \pm 20.7^{B*}$	8	$3.40 \pm 0.48^{B*}$	8
P1158S-pH8.0	167.6 ± 22.1^B	7	3.86 ± 0.51^B	7
WT-pH6.4	77.2 ± 26.1	5	1.69 ± 0.57	5
WT-pH7.0	88.0 ± 26.1	5	1.90 ± 0.57	5
WT-pH7.4	$110.2 \pm 18.5^*$	10	$2.22 \pm 0.40^*$	10
WT-pH8.0	94.0 ± 23.9	6	1.78 ± 0.52	6

Letter codes and asterisk indicate statistical significance. Numbers with different letter codes and/or matching asterisks are significantly different.

Supplementary Table 3- Steady-state fast inactivation comparison across pH6.4 to 8.0 between WT and P1158S

Channel Type-pH	Mean $V_{1/2} \pm SE$ (mV)	Mean $z \pm SE$ (slope)	n
P1158S-pH6.4	-70.18 ± 3.16^A	-2.96 ± 0.25	6
P1158S-pH7.0	-70.95 ± 2.74^A	-2.53 ± 0.21	8
P1158S-pH7.4	-74.52 ± 2.58^B	-2.63 ± 0.20	9
P1158S-pH8.0	-84.54 ± 3.16^B	-2.41 ± 0.25	6
WT-pH6.4	-68.41 ± 3.16	-3.74 ± 0.25	6
WT-pH7.0	-67.65 ± 2.74	-3.32 ± 0.21	8
WT-pH7.4	-67.77 ± 2.58	-2.49 ± 0.20	9
WT-pH8.0	-67.44 ± 2.74	-3.27 ± 0.21	8

Letter codes indicate statistical significance. Numbers with different letter codes are significantly different.

Supplementary Table 4- Open-state fast inactivation time constants across four voltages

Channel Type-pH	-30 mV $\tau \pm SE$ (ms)	-10 mV $\tau \pm SE$ (ms)	0 mV $\tau \pm SE$ (ms)	+10 mV $\tau \pm SE$ (ms)	n
P1158S-pH6.4	1.85 ± 0.27	0.95 ± 0.20	0.66 ± 0.09	0.59 ± 0.13	7
P1158S-pH7.0	2.26 ± 0.41	0.96 ± 0.11	0.80 ± 0.05	0.69 ± 0.06	4
P1158S-pH7.4	1.48 ± 0.14	0.78 ± 0.05	0.63 ± 0.04	0.56 ± 0.04	13
P1158S-pH8.0	1.26 ± 0.10	0.67 ± 0.05	0.56 ± 0.06	0.54 ± 0.08	7
WT-pH6.4	2.06 ± 0.40	0.98 ± 0.17	0.72 ± 0.06	0.60 ± 0.05	6
WT-pH7.0	1.62 ± 0.34	0.77 ± 0.10	0.62 ± 0.06	0.52 ± 0.04	6
WT-pH7.4	2.41 ± 0.37	1.22 ± 0.21	0.89 ± 0.20	0.77 ± 0.17	8
WT-pH8.0	2.00 ± 0.20	1.05 ± 0.15	0.76 ± 0.07	0.68 ± 0.10	7

Supplementary Table 5- Late sodium current percentage across pH6.4 to 8.0

Channel Type-pH	Mean %INaL $\pm SE$ (%INaL)	n
P1158S-pH6.4	7.67 ± 1.42^A	9
P1158S-pH7.0	8.96 ± 1.81^A	4
P1158S-pH7.4	4.13 ± 1.21^A	6
P1158S-pH8.0	5.18 ± 1.46^A	6
WT-pH6.4	2.66 ± 1.03^B	4
WT-pH7.0	3.33 ± 1.03^B	5
WT-pH7.4	4.34 ± 0.67^B	5
WT-pH8.0	2.57 ± 0.95^B	4

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Supplementary Table 6- Use-dependent inactivation comparison between WT and P1158S

Channel Type-pH	Mean $\tau \pm SE$ (s)	n
P1158S-pH6.4	0.132 ± 0.057^A	4
P1158S-pH7.0	0.725 ± 0.238	8
P1158S-pH7.4	1.643 ± 0.309	3
P1158S-pH8.0	1.753 ± 0.539^B	5
WT-pH6.4	0.416 ± 0.128	3
WT-pH7.0	0.849 ± 0.090	6
WT-pH7.4	0.639 ± 0.290	4
WT-pH8.0	0.711 ± 0.290	4

Letter codes indicate statistical significance. Numbers with different letter codes are significantly different.

Supplementary Table 7- Intracellular acidosis biophysical parameters

Parameter	P1158S IpH7.4	n	P1158S IpH6.4	n
Conductance $V_{1/2} \pm SE$ (mV)	-29.95 ± 2.41	8	-31.67 ± 2.58	7
Conductance $z \pm SE$ (slope)	4.71 ± 0.56	8	3.34 ± 0.60	7
SSFI $V_{1/2} \pm SE$ (mV)	-74.52 ± 2.43	9	-77.78 ± 2.75	7
SSFI $z \pm SE$ (slope)	-2.63 ± 0.24	9	-3.10 ± 0.27	7
Current density $\pm SE$ (pA/pF)	282.7 ± 45.3	10	222.3 ± 50.7	8
%INaL $\pm SE$ (%INaL)	4.91 ± 1.72	6	6.92 ± 2.43	3