

Supplementary Information

***Xenopus borealis* as an alternative source of oocytes for biophysical
and pharmacological studies of neuronal ion channels**

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Supplementary Table S1

Comparison of mean values for fast and slow time constants (τ) of deactivation for $K_V10.1$ channels tested in *X. laevis* and *X. borealis* oocytes. There is no evidence of a difference between the two species for all measured parameters (unpaired t-test, $P > 0.05$).

Ion Channel	Tau – deactivation (ms)		P value	Reference
	<i>X. laevis</i>	<i>X. borealis</i>		
$K_V10.1$				
Slow (–120 mV)	145.9	169.7	0.075	
Slow (–110 mV)	147.6	169.8	0.085	1
Slow (–100 mV)	174.4	193.7	0.257	
Slow (–90 mV)	180.0	198.9	0.187	
Fast (–120 mV)	25.6	27.1	0.725	
Fast (–110 mV)	21.9	25.2	0.406	1
Fast (–100 mV)	22.4	27.3	0.289	
Fast (–90 mV)	20.5	23.9	0.319	

Supplementary Table S2

Properties of MTS-TAMRA labelled α_1N203C and MTSR labelled α_1R271C human GlyRs. Comparison of EC_{50} , slope, maximal current change (ΔI_{max}), and maximal fluorescence change (ΔF_{max}) values tested in *X. laevis* and *X. borealis* oocytes. Data are mean \pm s.e.m. There is no evidence of a difference between the two species for all measured parameters (unpaired t-test, $P > 0.05$). Data are comparable to literature values for α_1N203C ² and α_1R271C ³.

Ion Channel	EC_{50} (μM)		P value
	<i>X. laevis</i>	<i>X. borealis</i>	
GlyR			
$\alpha_1N203C \Delta I$	145 \pm 10	136 \pm 8.7	0.69
$\alpha_1N203C \Delta F$	548 \pm 25	408 \pm 51	0.63
$\alpha_1R271C \Delta I$	1926 \pm 175	1698 \pm 104	0.52
$\alpha_1R271C \Delta F$	872 \pm 58	1006 \pm 98	0.46
	Slope		P value
	<i>X. laevis</i>	<i>X. borealis</i>	
$\alpha_1N203C \Delta I$	1.9 \pm 0.2	1.5 \pm 0.1	0.82
$\alpha_1N203C \Delta F$	1.4 \pm 0.2	1.8 \pm 0.1	0.62
$\alpha_1R271C \Delta I$	1.4 \pm 0.2	2.0 \pm 0.2	0.1
$\alpha_1R271C \Delta F$	1.3 \pm 0.2	1.0 \pm 0.1	0.46
	ΔI_{max} (μA) or ΔF_{max} (%)		P value
	<i>X. laevis</i>	<i>X. borealis</i>	
$\alpha_1N203C \Delta I$	8.4 \pm 0.2	8.0 \pm 0.8	0.63
$\alpha_1N203C \Delta F$	25.9 \pm 4.2	27.8 \pm 4.4	0.77
$\alpha_1R271C \Delta I$	3.4 \pm 1.7	2.7 \pm 0.5	0.69
$\alpha_1R271C \Delta F$	12.2 \pm 2.2	9.0 \pm 2.2	0.34

Supplementary Table S3

Comparison of half-maximal response and slope values for ion channels tested in *X. laevis* and *X. borealis* oocytes and references to studies reporting similar values for these parameters. Steady-state desensitisation: SSD; Activation: act. There is no evidence of a difference between the two species for any of the measured parameters (unpaired t-test, $P > 0.05$).

Ion Channel	Half-maximal response		<i>P</i> value	Slope		<i>P</i> value	Reference
	<i>X. laevis</i>	<i>X. borealis</i>		<i>X. laevis</i>	<i>X. borealis</i>		
Voltage-gated potassium channels							
K _V 10.1	19.7 mV	20.2 mV	0.86	20.8	20.6	0.90	1
K _V 11.1	-24.3 mV	-23.7 mV	0.18	6.98	7.53	0.16	4
Voltage-gated sodium channels							
Na _V 1.2	-22.5 mV	-22.3 mV	0.61	4.74	4.60	0.84	5
Na _V 1.5	-33.5 mV	-33.8 mV	0.49	4.81	4.22	0.08	6
Na _V 1.7	-14.2 mV	-14.8 mV	0.09	5.24	4.88	0.27	7
Acid-sensing ion channels							
ASIC1a pH _{act}	pH 6.11	pH 6.06	0.27	1.95	1.86	0.83	8
ASIC1a pH _{SSD}	pH 7.19	pH 7.19	0.66	10.39	9.97	0.69	8
ASIC1b pH _{act}	pH 5.95	pH 5.97	0.11	3.98	3.36	0.12	9
ASIC1b pH _{SSD}	pH 6.78	pH 6.78	0.72	4.17	4.09	0.91	10
ASIC2a pH _{act}	Ambiguous fits with sigmoidal non-linear regression model						
ASIC2a pH _{SSD}	pH 5.99	pH 5.98	0.66	2.27	2.37	0.78	11
ASIC3 pH _{act}	pH 6.16	pH 6.12	0.13	1.46	1.60	0.40	9
ASIC3 pH _{SSD}	pH 7.04	pH 7.02	0.19	8.25	9.04	0.70	12
Pc1a:ASIC1a	1.00 nM	1.03 nM	0.63	1.44	1.60	0.34	13
APETx2:ASIC3	64.7 nM	51.1 nM	0.05	0.84	0.93	0.37	14
GABA _A receptors							
$\alpha_1\beta_2\gamma_{2L}$	25.1 μ M	17.9 μ M	0.27	0.56	0.60	0.71	15
$\alpha_5\beta_2\gamma_{2L}$	5.18 μ M	4.71 μ M	0.84	0.49	0.74	0.16	16
$\alpha_5\beta_3\gamma_{2L}$	3.10 μ M	3.92 μ M	0.35	0.75	0.93	0.27	17

Supplementary Figure S1

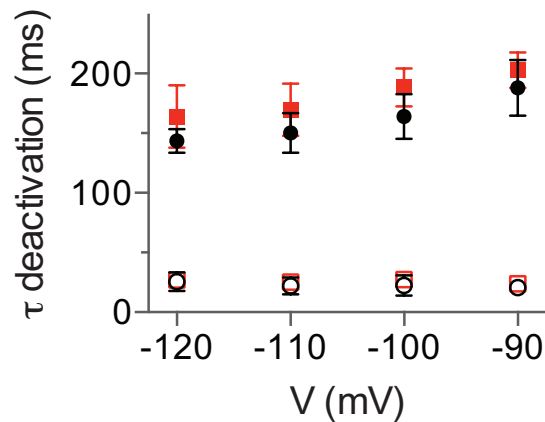


Figure. S1. Deactivation kinetics of Kv10.1 channels expressed in *X. laevis* (●) and *X. borealis* (■) oocytes. Fast (open symbols) and slow (closed symbols) time constants (τ) of deactivation (analysed from the tail current at different voltages following a maximally activating pre-pulse to +80 mV) ($n = 12-15$). Error bars indicate 95% confidence intervals.

Supplementary References

- 1 Simons, C. *et al.* Mutations in the voltage-gated potassium channel gene *KCNH1* cause Temple-Baraitser syndrome and epilepsy. *Nat. Genet.* **47**, 73-77, doi:10.1038/ng.3153 (2015).
- 2 Pless, S. A. & Lynch, J. W. Ligand-specific conformational changes in the $\alpha 1$ glycine receptor ligand-binding domain. *J. Biol. Chem.* **284**, 15847-15856, doi:10.1074/jbc.M809343200 (2009).
- 3 Pless, S. A., Dibas, M. I., Lester, H. A. & Lynch, J. W. Conformational variability of the glycine receptor M2 domain in response to activation by different agonists. *J. Biol. Chem.* **282**, 36057-36067, doi:10.1074/jbc.M706468200 (2007).
- 4 Twiner, M. J. *et al.* Marine algal toxin azaspiracid is an open-state blocker of hERG potassium channels. *Chem. Res. Toxicol.* **25**, 1975-1984, doi:10.1021/tx300283t (2012).
- 5 Nguyen, H. M. & Goldin, A. L. Sodium channel carboxyl-terminal residue regulates fast inactivation. *J. Biol. Chem.* **285**, 9077-9089, doi:10.1074/jbc.M109.054940 (2010).
- 6 Jones, D. K., Peters, C. H., Tolhurst, S. A., Claydon, T. W. & Ruben, P. C. Extracellular proton modulation of the cardiac voltage-gated sodium channel, Nav1.5. *Biophys. J.* **101**, 2147-2156, doi:10.1016/j.bpj.2011.08.056 (2011).

- 7 Zhang, M. M. *et al.* Co-expression of Nav β subunits alters the kinetics of inhibition of voltage-gated sodium channels by pore-blocking μ -conotoxins. *Br. J. Pharmacol.* **168**, 1597-1610, doi:10.1111/bph.12051 (2013).
- 8 Schroeder, C. I. *et al.* Chemical synthesis, 3D structure, and ASIC binding site of the toxin mambalgin-2. *Angew. Chem. Int. Ed. Engl.* **53**, 1017-1020, doi:10.1002/anie.201308898 (2014).
- 9 Hesselager, M., Timmermann, D. B. & Ahring, P. K. pH Dependency and desensitization kinetics of heterologously expressed combinations of acid-sensing ion channel subunits. *J. Biol. Chem.* **279**, 11006-11015, doi:10.1074/jbc.M313507200 (2004).
- 10 Chen, X., Kalbacher, H. & Gründer, S. Interaction of acid-sensing ion channel (ASIC) 1 with the tarantula toxin psalmotoxin 1 is state dependent. *J. Gen. Physiol.* **127**, 267-276, doi:10.1085/jgp.200509409 (2006).
- 11 Salinas, M. *et al.* Binding site and inhibitory mechanism of the mambalgin-2 pain-relieving peptide on acid-sensing ion channel 1a. *J. Biol. Chem.* **289**, 13363-13373, doi:10.1074/jbc.M114.561076 (2014).
- 12 Yagi, J., Wenk, H. N., Naves, L. A. & McCleskey, E. W. Sustained currents through ASIC3 ion channels at the modest pH changes that occur during myocardial ischemia. *Circ. Res.* **99**, 501-509, doi:10.1161/01.RES.0000238388.79295.4c (2006).
- 13 Escoubas, P. *et al.* Isolation of a tarantula toxin specific for a class of proton-gated Na⁺ channels. *J. Biol. Chem.* **275**, 25116-25121, doi:10.1074/jbc.M003643200 (2000).
- 14 Diochot, S. *et al.* A new sea anemone peptide, APETx2, inhibits ASIC3, a major acid-sensitive channel in sensory neurons. *EMBO J.* **23**, 1516-1525, doi:10.1038/sj.emboj.7600177 (2004).
- 15 Hall, B. J., Chebib, M., Hanrahan, J. R. & Johnston, G. A. 6-Methylflavanone, a more efficacious positive allosteric modulator of gamma-aminobutyric acid (GABA) action at human recombinant $\alpha_2\beta_2\gamma_{2L}$ than at $\alpha_1\beta_2\gamma_{2L}$ and $\alpha_1\beta_2$ GABA_A receptors expressed in *Xenopus* oocytes. *Eur. J. Pharmacol.* **512**, 97-104, doi:10.1016/j.ejphar.2005.02.034 (2005).
- 16 Karim, N. *et al.* Potency of GABA at human recombinant GABA_A receptors expressed in *Xenopus* oocytes: a mini review. *Amino acids* **44**, 1139-1149, doi:10.1007/s00726-012-1456-y (2013).
- 17 Ramerstorfer, J., Furtmüller, R., Vogel, E., Huck, S. & Sieghart, W. The point mutation γ 2F77I changes the potency and efficacy of benzodiazepine site ligands in different GABA_A receptor subtypes. *Eur. J. Pharmacol.* **636**, 18-27, doi:10.1016/j.ejphar.2010.03.015 (2010).