Supporting information

Structure-activity studies reveal the molecular basis for $GABA_B$-receptor mediated inhibition of high voltage-activated calcium channels by α-conotoxin Vc1.1.

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Table of Contents

Figures S1 - S5 2
Tables S1 – S2 6

Figures S1 - S5

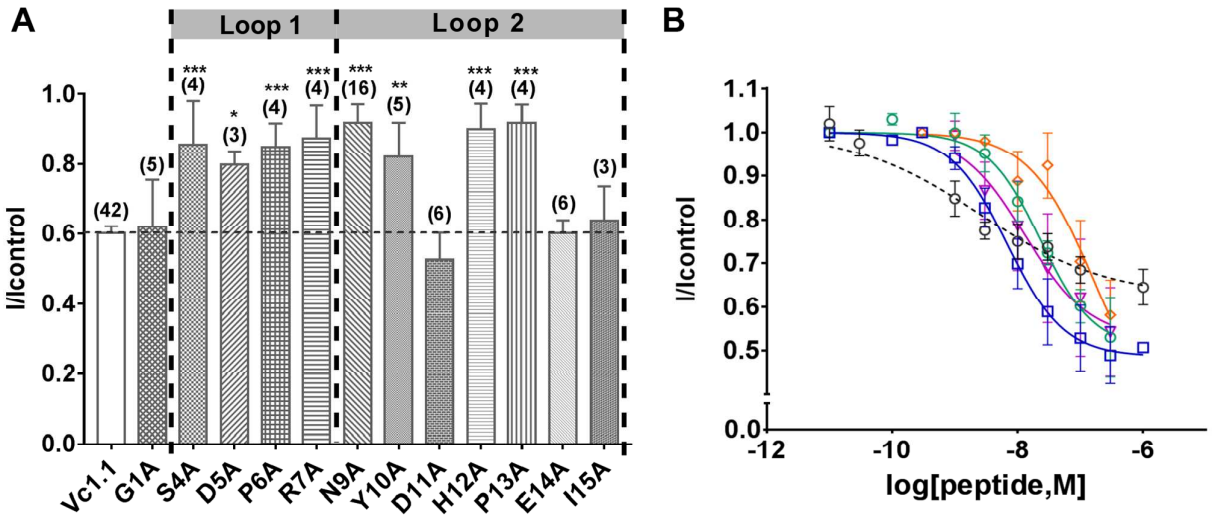

Figure S1. Effect of Vc1.1 point mutation analogues (100 nM) on HVA calcium channels in rat DRG neurons. (A) Bar graph shows inhibition of HVA calcium channel current amplitude by alanine substituted analogues of Vc1.1. Loop regions are highlighted by the dotted lines and grey horizontal bars. The *asterisk* indicates mutants with significantly decreased potency relative to Vc1.1 (unpaired Student's t-test. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$). Similar to mouse DRG data, only G1A, D11A, E14A and I15A, retain activity at $GABA_B$ R-mediated inhibition of HVA calcium channels in DRG neurons. Dotted line indicates the level of I/I_{Ba} inhibition by Vc1.1. The number of experiments, n, is in parentheses. Data points are mean ± SEM. (**B**) Concentration-response relationship for inhibition of HVA calcium channel currents by Vc1.1 and the active alanine substituted analogues in rat DRG neurons. Pooled data indicate the IC_{50} for Vc1.1 (black dashed line, 2.5 nM, n = 8-25), D11A (blue, 6.8 nM, n = 3-6), E14A (green, 23.1 nM, n = 3-6), I15A (orange, 170.7 nM, n = 3-6) and G1A (purple, 13.1 nM, n = 5-6). Data points are mean ± SEM.

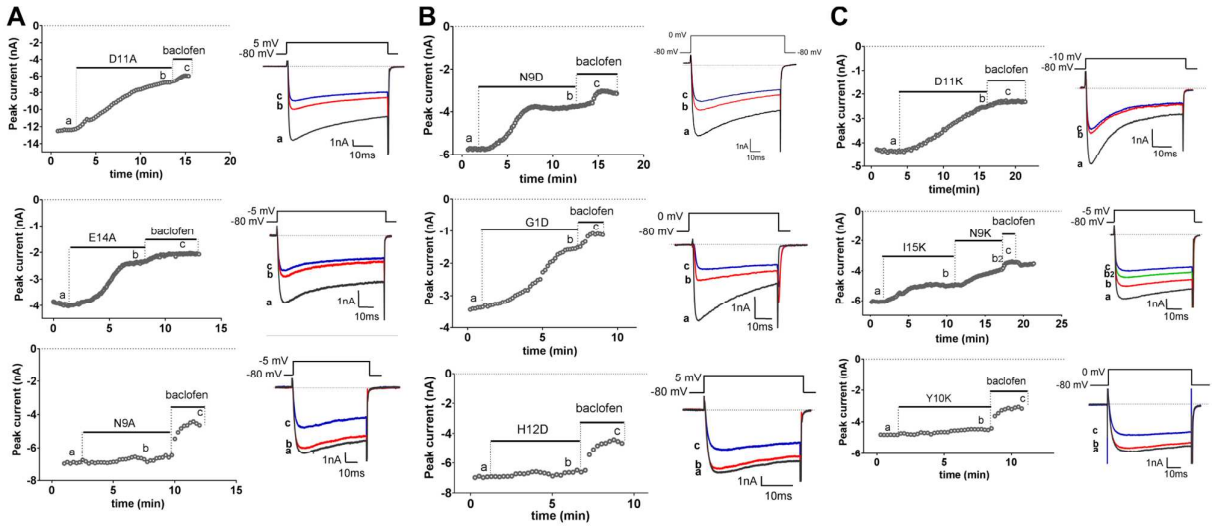

Figure S2. Representative time courses of HVA calcium channel inhibition by single point Vc1.1 mutants (A) Representative time course of peak I_{Ba} inhibition in the presence of 100 nM alanine-substituted analogues of Vc1.1: D11A, E14A and N9A. **(B)** Aspartic acid substituted analogues of Vc1.1: N9D, G1D and H12D. **(C)** Lysine substituted analogues of Vc1.1: D11K, I15K and Y10K. (A-C) the first and second rows show the substituted analogue of Vc1.1 that is equipotent to Vc1.1 and the third row shows the Vc1.1 analogues that are less potent than Vc1.1 on $GABA_BR$-mediated inhibition of HVA calcium channel currents. In all experiments, the $GABA_BR$ agonist baclofen (50 μM) has been applied at the end of experiment. Bar indicate the duration of α-conotoxin or baclofen application. Inward I_{Ba} were evoked by voltage steps from a HP of −80 mV to −5, 0 or +5 mV applied at 0.1 Hz, based on the peak I_{Ba} amplitude (*insets*). Superimposed representative I_{Ba} traces (*insets*) obtained in the absence (black, a) and presence of peptide (red, b), and 50 μM baclofen (blue, c), are shown at the times indicated by lowercase letters. Dotted lines indicate zero-current level.

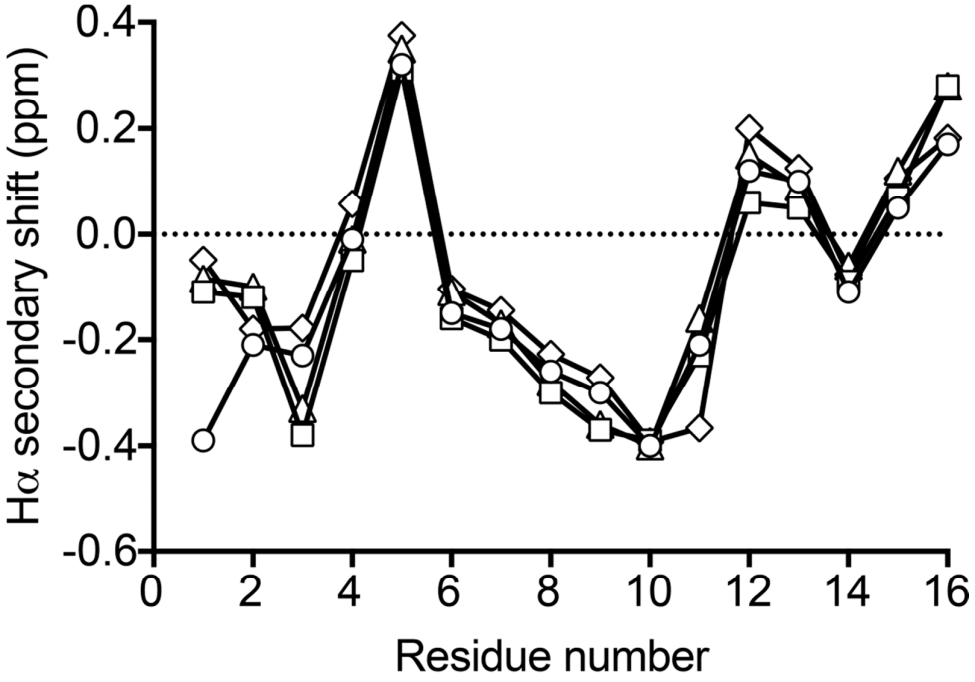

Figure S3. NMR Hα secondary shift comparison of Vc1.1[D11A, E14A] (circles) and cVc1.1[D11A, E14A] (squares) with cVc1.1 (triangles) and Vc1.1 (diamonds) from residues 1 to 16. All peptides exhibit almost identical secondary shift values across the entire sequence. NMR data was collected in H_2O/D_2O (90%/10%), pH 3.5 – 4.5 at 280 K and referenced to DSS at 0 ppm.

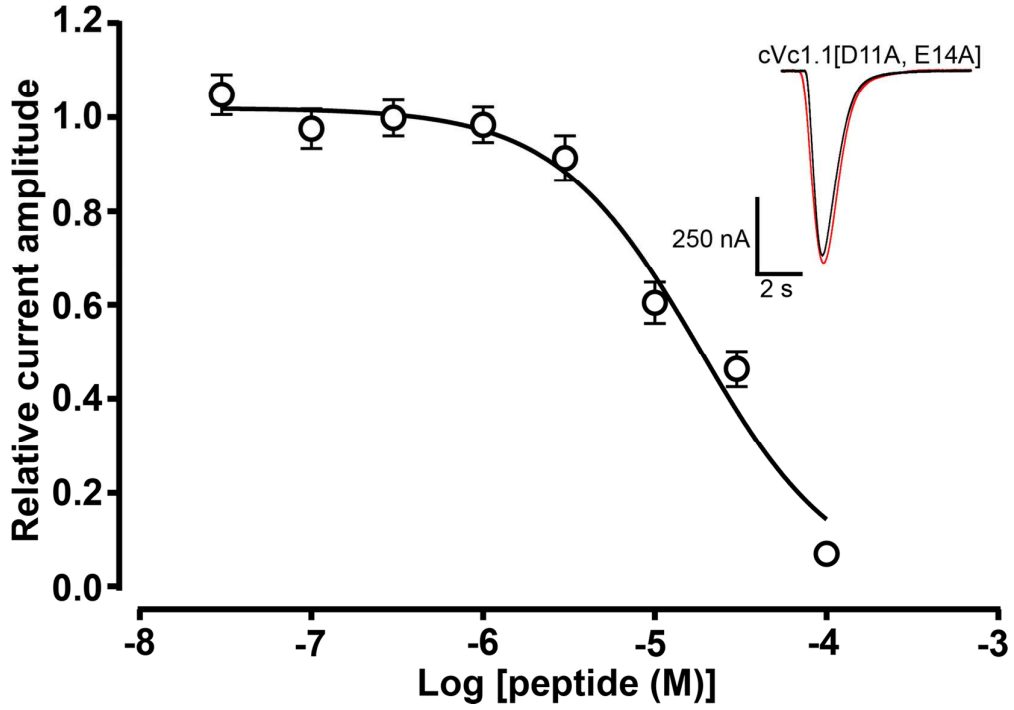

Figure S4. Inhibition of rat(r) α9α10 nAChRs expressed in *Xenopus* oocytes by cVc1.1[D11A,E14A]. Concentration-response relationship for cVc1.1[D11A,E14A] inhibition of ACh-evoked current amplitude giving an IC_{50} of 17.9 ± 2.6 μM. Current amplitudes (mean ± SEM, n = 3-8) were normalized to the response elicited by 10 μM ACh (corresponding to the EC_{50} value at rα9α10 nAChRs). ***Inset.*** Superimposed representative of ACh (10 μM)-evoked currents mediated by rα9α10 nAChRs in the absence (black) and presence (red) of 1 μM cVc1.1[D11A,E14A].

Tables S1 – S2

Table S1. NMR chemical shifts for cVc1.1[D11A, E14A] at 280K (pH 3.5, referenced to 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) at 0 ppm).

Residue	H_N	Hα	Additional Hs	^{15}N[a]	^{13}C[b]
Gly[1]	8.691	3.79, 3.93		108.7	Cα 48.9
Cys[2]	8.83	4.59	Hβ 2.73, 3.22	122.1	Cα 60.5; Cβ 44.3
Cys[3]	8.25	4.33	Hβ 2.82, 3.27	113.2	Cα 58.8; Cβ 43.8
Ser[4]	7.85	4.43	Hβs 3.97	114.8	Cα 63.0; Cβ 66.4
Asp[5]	7.92	5.20	Hβ 2.70, 3.18	124.1	Cα 52.4; Cβ 43.8
Pro[6]	-	4.26	Hβ 1.99, 2.37; Hγ 2.06, 2.11; Hδ 3.92, 4.08	-	Cα 68.5; Cβ 35.0; Cγ 30.2; Cδ 53.6
Arg[7]	7.98	4.15	Hβs 1.89; Hγ 1.64, 1.73; Hδs 3.20; Hε 7.40	115.8; Nε 124.3	Cα 61.2; Cβ 32.3; Cγ 29.9; Cδ 45.7;
Cys[8]	8.09	4.41	Hβ 3.26, 4.23	121.4	Cα 62.0; Cβ 43.8
Asn[9]	9.02	4.35	Hβs 2.86	121.7	Cα 59.1; Cβ 41.6
Tyr[10]	7.90	4.16	Hβ 3.01, 3.19	117.5	Cα 62.6; Cβ 40.9
Ala[11]	7.40	4.10	Hβs 1.31	117.9	Cα 55.0; Cβ 21.8
His[12]	7.84	5.06	Hβ 3.05, 3.25	115.9	Cα 56.6; Cβ 31.3
Pro[13]	-	4.48	Hβ 1.96, 2.27; Hγ 1.95; Hδ 3.38, 3.47	-	Cα 67.5; Cβ 34.0; Cγ 30.0; Cδ 52.9
Ala[14]	8.41	4.24	Hβs 1.44	120.6	Cα 64.6; Cβ 21.2
Ile[15]	7.47	4.24	Hβ 1.99; Hγ 1.20, 1.62, 0.87; Hδ 0.80	114.1	Cα 56.8; Cβ 41.5; Cγ 15.5, 30.2; Cδ 15.6
Cys[16]	8.021	4.99	Hβ 2.74. 3.30	118.4	Cα 56.9; Cβ 40.9
Gly[17]	8.36	3.86, 4.07	-	110.6	Cα 48.7
Gly[18]	8.45	3.85, 4.00	-	110.0	Cα [c]
Ala[19]	8.21	4.37	Hβs 1.40	124.1	Cα 54.8; Cβ

					21.9
Ala[20]	8.19	4.29	Hβs 1.39	122.8	Cα 55.1; Cβ 21.9
Gly[21]	8.14	3.86, 4.01	-	107.8	Cα 47.5
Gly[22]	8.31	3.93, 4.11	-	-[c]	Cα 47.2

[a]From ^{15}N-HSQC spectrum. [b]From ^{13}C-HSQC spectrum. [c]Not assigned

Table S2. Structure statistics for cVc1.1[D11A, E14A]

Experimental restraints	
Distance restraints	
Total NOE	78
Intraresidue	28
Sequential ($\|i - j\| = 1$)	37
Medium range ($\|i - j\| < 4$)	12
Long range ($\|i - j\| > 5$)	1
Hydrogen-bond restraints	0
Total Dihedral-angle restraints	39
φ	17
ψ	16
χ_1	6
Total number of restraints per residue	5.3
Target function (Å)	0.36 ± 0.016
Root-mean-square deviation to mean coordinate structure (Å)	
Backbone atoms (residues 2 – 16)	0.21 ± 0.11
All heavy atoms (residues 2 – 16)	0.61 ± 0.13
Stereochemical quality[b]	
Residues in most favoured Ramachandran region (%)	91.0 ± 5.3
Residues in allowed Ramachandran region (%)	9
Ramachandran outliers (%)	0
Unfavourable side chain rotamers (%)	21.6 ± 7.7
Cβ deviations >0.25 Å	0
Clash score, all atoms	0
Overall MolProbity score	1.943 ± 0.287

[a]Root-mean-square deviation values were calculated over the entire structure

[b]Stereochemical quality was assessed via MolProbity {Chen, 2010 #1826}. Clash score is the number of steric overlaps >0.4 Å per 10^3 atoms