

Supplementary material

***Drosophila* expressing mutant human *KCNT1* transgenes make an effective tool for targeted drug screening in a whole animal model of KCNT1-epilepsy**

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Supplementary Table 1. P values obtained using One way ANOVA with Dunnett’s multiple comparisons test of the data presented in Figure 6. Blue asterisks denote the statistically significant decrease in seizures compared to vehicle control, whereas red asterisks denote significant increase; ns – not significant. N is the number of independent fly crosses; number in brackets is the total number of flies analysed in each condition. The number of the control groups and the total number of flies for G288S, R398Q and R928C are 4 (54), 18 (314) and 4 (103) respectively.

	G288S					R398Q					R928C				
[μ M]	CBD	Vigabtr	Valpr	Carbam	Quin	CBD	Vigabtr	Valpr	Carbam	Quin	CBD	Vigabtr	Valpr	Carbam	Quin
0.001	<0.0001 **** N=4(60)	0.9604 ns N=4(60)	0.9999 ns N=4(60)	0.9996 ns N=3(62)	0.9979 ns N=5(54)	0.0136 * N=4(73)	0.1252 ns N=5(101)	0.0526 ns N=5(54)	0.6311 ns N=5(77)	0.3140 ns N=4(70)	0.9608 ns N=4(73)	0.9998 ns N=5(70)	0.8910 ns N=5(54)	0.8675 ns N=3(50)	0.9996 ns N=4(67)
0.01	<0.0001 **** N=4(60)	0.9819 ns N=4(60)	0.4985 ns N=4(60)	0.9977 ns N=3(60)	0.9758 ns N=5(50)	<0.0001 **** N=4(50)	>0.9999 ns N=4(50)	0.0613 ns N=5(50)	0.9479 ns N=5(74)	0.1634 ns N=4(66)	0.5964 ns N=4(52)	0.9996 ns N=5(79)	0.0904 ns N=5(50)	0.0550 ns N=4(54)	0.8641 ns N=4(51)
0.1	<0.0001 **** N=4(60)	0.9944 ns N=4(60)	0.2154 ns N=4(60)	0.9924 ns N=4(55)	0.7269 ns N=5(50)	<0.0001 **** N=6(158)	>0.9999 ns N=4(76)	0.1073 ns N=5(50)	0.4074 ns N=5(51)	0.0012 ** N=5(54)	0.1644 ns N=6(94)	0.2033 ns N=4(74)	0.0037 ** N=5(50)	0.0023 ** N=5(55)	0.0125 * N=6(58)
1	<0.0001 **** N=4(60)	0.0124 * N=4(60)	0.1815 ns N=4(60)	0.9997 ns N=3(52)	0.1240 ns N=4(55)	<0.0001 **** N=5(126)	<0.0001 **** N=5(132)	0.0006 *** N=5(68)	0.0569 ns N=6(58)	0.0022 ** N=4(55)	0.0583 ns N=5(91)	0.1963 ** N=5(99)	<0.0001 **** N=5(50)	0.0003 *** N=3(53)	<0.0001 **** N=5(51)
10	<0.0001 **** N=4(60)	0.0240 * N=4(60)	0.9999 ns N=4(60)	0.9996 ns N=3(65)	0.2295 ns N=5(50)	<0.0001 **** N=6(117)	0.0010 ** N=4(106)			0.0001 *** N=5(66)	0.0021 ** N=6(94)	0.0014 *** N=5(80)		<0.0001 **** N=5(67)	<0.0001 **** N=5(51)
50	<0.0001 **** N=4(60)				0.0153 * N=5(50)	<0.0001 **** N=7(96)				<0.0001 **** N=5(59)	0.0096 ** N=7(86)				<0.0001 **** N=5(56)
100							0.0153 * N=5(62)					0.0005 *** N=5(75)			

Supplementary Figure 1

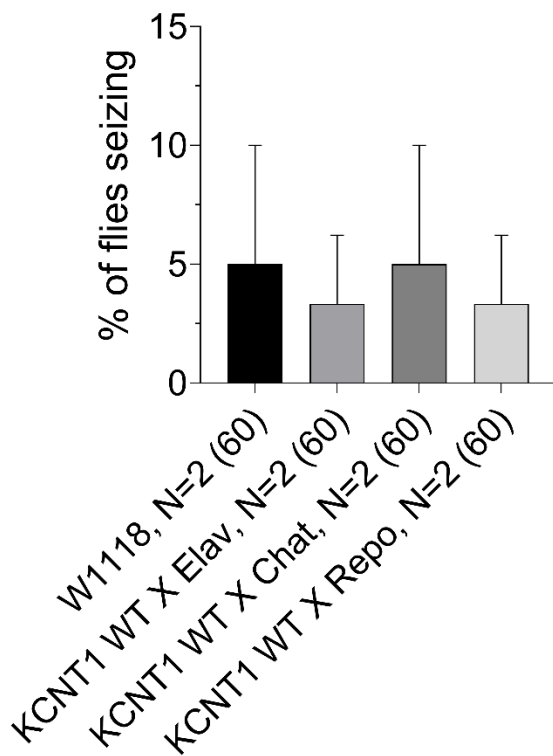


Figure S1. Expressing human *KCNT1* WT in different neurons of *Drosophila* gives no seizures in a bang sensitive behavioural assay. *Drosophila* with the vector control and WT human *KCNT1* expressed pan-neuronal (*elav^{C155}-GAL4*), in excitatory neurons (*CHAT-GAL4*) and in glia (*Repo-GAL4*) were analysed in the bang sensitive behavioural seizure assay. Percentage of *Drosophila* showing a seizure phenotype are shown for each line. N, is the number of independent fly crosses, with total number of flies in all trials shown in the brackets. Brown-Forsythe and Welsh's one-way ANOVA followed by Dunnett's T3 multiple comparisons test showed no significant difference between the experimental groups.

Supplementary Figure 2

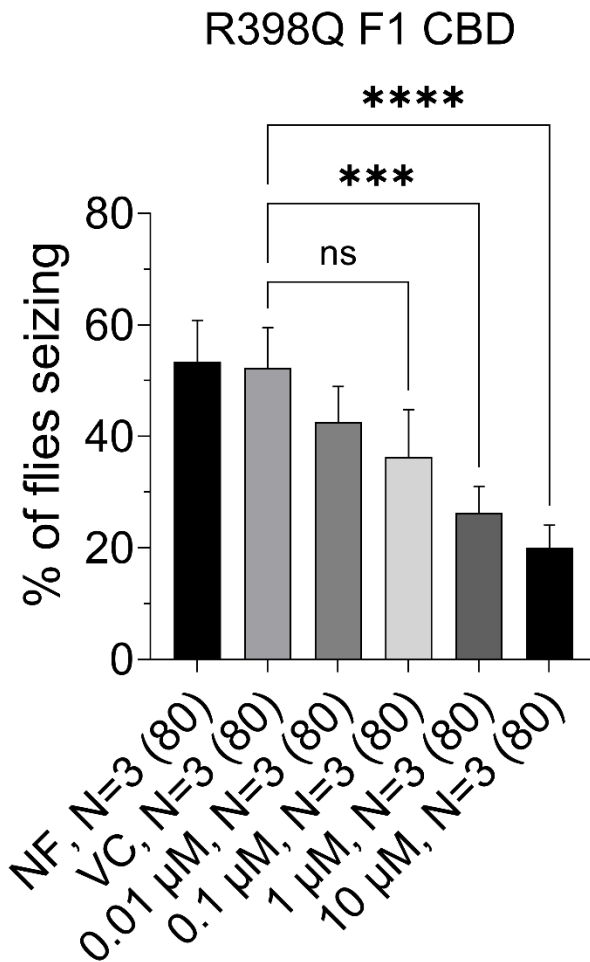


Figure S2. Feeding CBD to adult flies expressing human *KCNT1* mutant (R398Q) in GABAergic neurons of *Drosophila* rescues seizures in the bang sensitive behavioural assay. *Drosophila* expressing mutant human *KCNT1* with the R398Q mutation in GABAergic neurons were raised from embryos on normal food (NF) and the offspring were transferred on a food containing a range of concentrations of CBD (0.01-10 μ M) and then analysed in the bang sensitive behavioural seizure assay. The percentage of *Drosophila* showing a seizure phenotype are shown for each dose of drug. N, is the number of independent fly crosses, with total number of flies in all trials shown in the brackets. All data points were compared to the vehicle control (VC) using Brown-Forsythe and Welch's one-way ANOVA followed by Dunnett's multiple comparisons test. Compared to vehicle control, CBD significantly reduced seizures in R398Q mutants at 1 and 10 μ M concentrations. **** P<0.0001, *** P=0.0006, and ns - no significant difference (P=0.0856 for 0.1 μ M).