Supporting Information

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Fig. 51. Excitation of granule cells cultured at the physiological KCl concentration. Granule cells were cultured in medium containing serum for 24 h and then in serum-free medium for 96 h. During the last 30 min of culture, the cells were loaded with Fluo-4 Ca²⁺ indicator. They then were incubated with the fresh medium in a micro CO₂ incubator (Tokai Hit) mounted on a stage of an Olympus BX51Wl upright fluorescence microscope equipped with an EM-CCD camera (Andor). Ca²⁺ transients were measured by recording fluorescent changes at 510 nm by excitation at 488 nm and were analyzed by using MetaMorph software (Molecular Devices). Frequencies of Ca²⁺ transients were calculated by counting Ca²⁺ transients that exceeded 3% in changes of fluorescence intensity (ΔF) relative to that of the baseline fluorescence (*F*). Representative traces (*A*) and frequencies (*B*) of Ca²⁺ transients under the different culture condition (*n* > 100 cells) are indicated. *** *P* < 0.001 vs. control. CPP, 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid; NBQX, 2, 3-dioxo-6-nitro-1, 2, 3, 4-tetrahydrobenzo [f] quinoxaline-7-sulfonamide; TTX, tetrodotoxin.



Fig. 52. Inhibition of 17 maturation genes by TTX. Granule cells were cultured in medium containing serum for 24 h and then in serum-free medium in the presence or absence of TTX (5 μ M) for 96 h, and mRNA levels were quantified by PCR. Experiments were performed in triplicate. β -actin mRNA was quantified as an activity-insensitive control mRNA. Data are expressed as percentages of mRNA levels in TTX-treated cells relative to those in untreated cells (100%) and are shown as mean \pm SEM. **P* < 0.05; ***P* < 0.01; ****P* < 0.001 vs. untreated. n.s., not significant. Ets2, E26 avian leukemia oncogene 2; NR2C, NMDA glutamate receptor subunit 2C; VGIUT1, vesicular glutamate transporter 1; Tiam1, T-cell lymphoma invasion and metastasis 1; Nptx1, neuronal pentraxin 1; Etv1, Ets variant gene 1; Kv1.1, voltage-gated potassium channel, shaker-related subfamily, member 1; Wnt7a, wingless-related MMTV integration site 7A; GABA_AR\alpha6, GABA_A receptor α 6; CaMKK β , calcium/calimodulin-dependent protein kinase kinase β ; TASK1, TWIK-related acid-sensitive potassium channel 1; KCC2, potassium-chloride cotransporter 2; GABA_ARa(1, GABA_A receptor α 1.



Fig. S3. Knockdown of voltage-gated sodium channel type II (*Nav1.2*) mRNA and Ets variant gene 1 (*Etv1*) mRNA by siRNA treatments and blockade of action potential by Nav1.2 siRNA. (*A*) Nav1.2 siRNA or scrambled siRNA (scRNA) (6 μ g each) was electroporated into dissociated granule cells, which then were cultured in medium containing serum for 24 h and in serum-free medium for 96 h. Experiments were performed in triplicate, and mRNA levels were quantified by PCR. Statistical analysis was performed, and data are presented as in *P* for 600 ms at the holding potential of -70 mV. Representative voltage traces following current injection of +12 pA, -12 pA, or no injection (*B*) and frequencies of action potential sta +12 pA (C) are indicated (*n* = 12 for scRNA; *n* = 6 for Nav1.2 siRNA.). (*D*) Etv1 siRNA-1, Etv1 siRNA-2, or scRNA (6 μ g each) was electroporated into dissociated granule cells, and culture was conducted as in *A* (*n* = 3). *Etv1* mRNA by siRNA treatments are shown as mean \pm SEM. **P* < 0.05; ****P* < 0.001 vs. scRNA.

Table S1. Microarray analysis of TTX-suppressive genes in cultured granule cells

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National Center for Biotechnology Information gene ID#	Gene name	Function	Hybridization signals		
			Control (– TTX)	+ TTX	Fold reduction
57816	Tescalcin	Intracellular signaling	984.1	141.4	7.0
23872	Ets2	Transcription factor	735.4	133.8	5.5
14813	NR2C	Receptor	76.4	14.3	5.3
72961	VGluT1	Transporter	1016.1	215.4	4.7
21844	Tiam1	Intracellular signaling	482.0	102.5	4.7
18164	Nptx1	Extracellular signaling	1946.7	472.8	4.1
229759	Olfactomedin3	Extracellular signaling	1439.8	362.4	4.0
14009	Etv1	Transcription factor	2886.3	777.6	3.7
20564	Slit3	Extracellular signaling	20.5	6.1	3.3
16485	Kv1.1	Ion channel	1488.5	529.8	2.8
22421	Wnt7a	Extracellular signaling	855.4	373.3	2.3
12308	Calretinin	Intracellular signaling	1097.8	493.1	2.2
14403	$GABA_AR\delta$	Receptor	732.9	348.6	2.1
69601	Dab2ip	Intracellular signaling	732.3	372.1	2.0
14399	$GABA_AR\alpha 6$	Receptor	5850.2	3006.7	1.9
O 207565	CaMKK β	Kinase	1179.1	720.9	1.6
64297	Gprc5b	Receptor	170.0	108.9	1.6
16527	TASK1	Ion channel	253.8	172.4	1.5
105445	Dock9	Intracellular signaling	633.3	442.6	1.4
226251	Ablim1	Intracellular signaling	392.3	290.4	1.4
57138	KCC2	Transporter	2778.3	2129.8	1.3
69993	Chimerin2	Intracellular signaling	3483.3	2687.9	1.3
14394	$GABA_AR\alpha 1$	Receptor	3007.1	2607.0	1.2
20745	Testican	Extracellular signaling	694.8	780.7	-0.9
	National Center for Biotechnology Information gene ID# 57816 23872 14813 72961 21844 18164 229759 14009 20564 16485 22421 12308 14403 69601 14399 207565 64297 16527 105445 226251 57138 69993 14394 20745	National Center for Biotechnology Information gene ID#Gene name57816Tescalcin23872 $Ets2$ 14813 $NR2C$ 72961 $VGluT1$ 21844 $Tiam1$ 18164 $Nptx1$ 229759Olfactomedin314009 $Etv1$ 20564 $Slit3$ 16485 $Kv1.1$ 22421 $Wnt7a$ 12308Calretinin14403 $GABA_AR\delta$ 69601 $Dab2ip$ 14399 $GABA_ARa6$ 207565 $CaMKK\beta$ 64297 $Gprc5b$ 16527 $TASK1$ 105445 $Dock9$ 226251 $Ablim1$ 57138 $KCC2$ 69993Chimerin214394 $GABA_ARa1$ 20745Testican	National Center for Biotechnology Information gene ID#Gene nameFunction57816TescalcinIntracellular signaling 23872 $Ets2$ Transcription factor14813NR2CReceptor72961VGluT1Transporter21844Tiam1Intracellular signaling18164Nptx1Extracellular signaling229759Olfactomedin3Extracellular signaling14809Etv1Transcription factor20564Slit3Extracellular signaling16485Kv1.1Ion channel22421Wht7aExtracellular signaling12308CalretininIntracellular signaling14403GABA _A R\deltaReceptor69601Dab2ipIntracellular signaling14399GABA_ARa6Receptor64297Gpcc5bReceptor16527TASK1Ion channel105445Dock9Intracellular signaling226251Ablim1Intracellular signaling57138KCC2Transporter6993Chimerin2Intracellular signaling14394GABA _A Ra1Receptor20745TesticanExtracellular signaling	National Center for BiotechnologyHybridization sInformation gene ID#Gene nameFunction57816TescalcinIntracellular signaling984.123872Ets2Transcription factor735.414813NR2CReceptor76.472961VGluT1Transporter1016.121844Tiam1Intracellular signaling482.018164Nptx1Extracellular signaling1439.814009Etv1Transporter2886.3229759Olfactomedin3Extracellular signaling20.516485Kv1.1Ion channel1488.522421Wnt7aExtracellular signaling1097.812308CalretininIntracellular signaling1097.814403GABA _A R δ Receptor732.969601Dab2ipIntracellular signaling732.314399GABA _A R δ Receptor170.016527TASK1Ion channel253.8105445Dock9Intracellular signaling633.3226251Ablim1Intracellular signaling392.357138KCC2Transporter2778.369993Chimerin2Intracellular signaling3483.314394GABA _A R a 1Receptor3007.120745TesticanExtracellular signaling694.8	National Center for Biotechnology Hybridization signals Information gene ID# Gene name Function Control (- TTX) $+$ TTX 57816 Tescalcin Intracellular signaling 984.1 141.4 23872 Ets2 Transcription factor 735.4 133.8 14813 NR2C Receptor 76.4 14.3 72961 VGluT1 Transcription factor 785.4 133.8 18164 Nptx1 Extracellular signaling 482.0 102.5 18164 Nptx1 Extracellular signaling 1439.8 362.4 14009 Etv1 Transcription factor 2886.3 777.6 20564 S/it3 Extracellular signaling 1488.5 529.8 22421 Wnt7a Extracellular signaling 1097.8 493.1 14403 GABA_ARis Receptor 732.9 348.6 69601 Dab2ip Intracellular signaling 1097.8 493.1 14403 GABA_ARis Receptor 5850.2 30

Microarray analysis was performed twice with RNA samples prepared from different cultures, and the data were averaged. Three genes identified as TTXsuppressive genes in this study are marked by black circles. The other 21 genes were reported previously as developmentally up-regulated genes (1), and all but the *testican* gene were found to be TTX suppressive. The 17 genes indicated by black or white circles were selected for detailed analysis. Slit3, slit homolog 3; GABA_ARδ, GABA_A receptor δ; Dab2ip, Dab2 interacting protein; Gprc5b, G protein-coupled receptor, family C, group 5, member B; Dock9, dedicator of cytokinesis 9; Ablim1, actin-binding LIM protein 1.

1. Sato M, Suzuki K, Yamazaki H, Nakanishi S (2005) A pivotal role of calcineurin signaling in development and maturation of postnatal cerebellar granule cells. Proc Natl Acad Sci USA 102:5874–5879.