Supporting Information for:

TRPC channels are not required for graded persistent activity in entorhinal cortex neurons

Alexei V. Egorov[1], Dagmar Schumacher[2], Rebekka Medert[2], Lutz Birnbaumer[3], Marc Freichel[2] and Andreas Draguhn[1]

[1]Institute of Physiology and Pathophysiology, Heidelberg University, 69120 Heidelberg, Germany

[2]Institute of Pharmacology, Heidelberg University, 69120 Heidelberg, Germany

[3]Neurobiology Laboratory, National Institute of Environmental Health Sciences, Durham, North Carolina 27709, USA and School of Medical Sciences, Catholic University of Argentina, Institute of Biomedical Research (BIOMED UCA-CONICET), Buenos Aires, Argentina

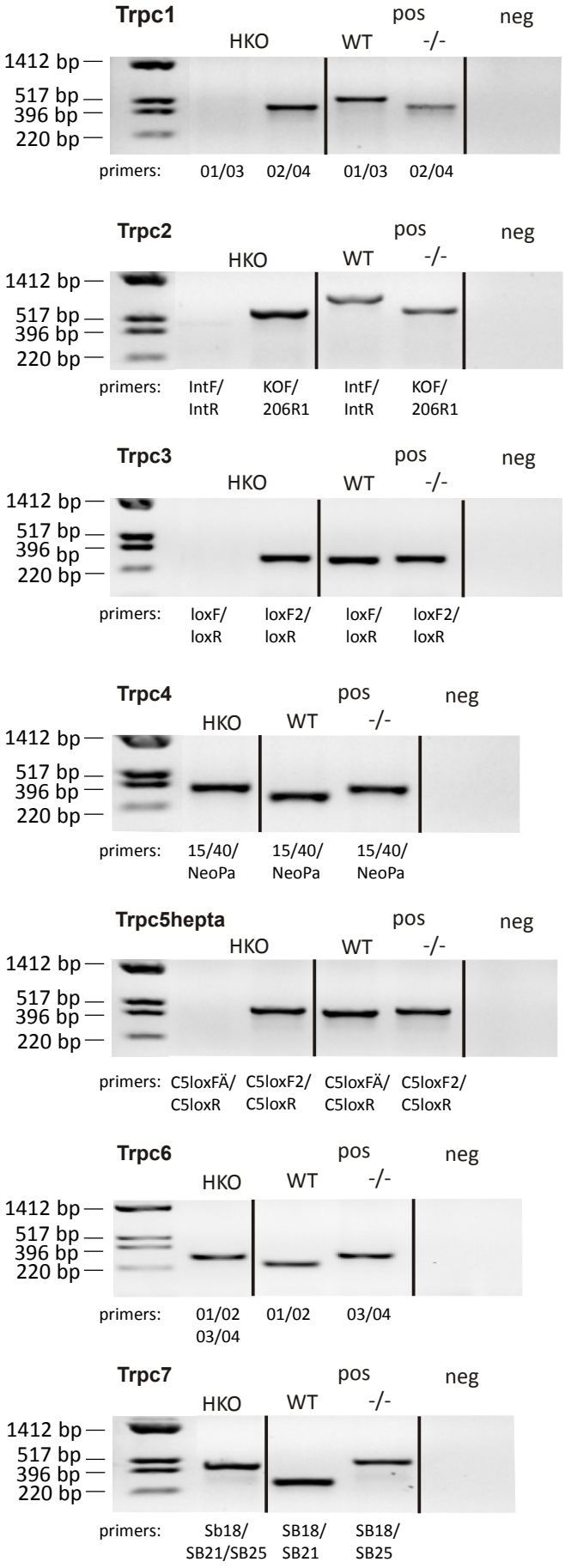

Figure S1. PCR-based genotyping of Trpc1/2/3/4/5/6/7 $^{-/-}$ animals

Ethidium bromide stained DNA fragments obtained by PCR from Trpc1/2/3/4/5/6/7 $^{-/-}$ Hepta KO (HKO) mice following gel electrophoresis of the amplification products of genomic DNA fragments that are diagnostic for each of the seven TRPC wild type and TRPC null alleles, respectively, are shown. Allele specific primer combinations are indicated and their sequence is listed in Table S1. The sizes of marker DNA fragments are indicated. pos, positive control sample; neg, negative control sample without template DNA.

Table S1. Genotyping of TRPC-deficient mice

Genotyping of TRPC wildtype and Null alleles were performed by PCR using the amplification protocol and primers as indicated below.

Cyling programm:

94°C	1:30	
94°C **65**°C 72°C	0:30 0:30 -0,5°C decrease per cycle 0:30	**10 cycles**
94°C **60**°C 72°C	0:30 0:30 0:30	**26 cycles**
72°C 4°C	5:00 ¥	

TRPC1:	Primer 01:	GAGACTGTTGTCACAAGATGC
	Primer 02:	TCAGTTAATGTCCCATTCCGGC
	Primer 03:	ACTTTGAGGGCAAAGGTTGCC
	Primer 04:	AGAGGCCACTTGTGTAGCGC
TRPC2:	IntF:	ATGACGGGTTTATGGCTCAG
	IntR:	GATCCCCTGGAATTGGAGTT
	KOF:	CTGTAGCCATCTTCAGACACACC
	206R1:	ACGAGACTAGTGAGACGTGCTAC
TRPC3:	loxF:	GCTATGATTAATAGCTCATACCAAGAGATC
	loxF2:	GAATCCACCTGCTTACAACCATGTG
	loxR:	GGTGGAGGTAACACACAGCTAAGCC
TRPC4:	15:	ACAGTGCTCTGAACCCACGG
	40:	CTCGCACCGGATGCCTTTGC
	NeoPa:	GCCTGCTCTTTACTGAAGGCTCT
TRPC5hepta:	C5loxFÄ:	GGCGCAGAAAGAGTTTATGGGGA
	C5loxR:	GGATGTTGGCTCTGTGAAACAATGACTC
	C5loxF2:	AGCTAACAGTATCCCTAAGTGATCC
TRPC6:	Primer 01:	ACGAGACTAGTGAGACGTGCTACTTCC
	Primer 02:	GGGTTTAATGTCTGTATCACTAAAGCCTCC
	Primer 03:	CAGATCATCTCTGAAGGTCTTTATGC
	Primer 04:	TGTGAATGCTTCATTCTGTTTTGCGCC
TRPC7:	SB18:	CGTAGCAATAGAAACTTAACTTAG
	SB21:	CCACTAAACCCAAGCACATGG
	SB25:	GACACACCTGCCAGGCAC

Table S2. Intrinsic properties of mEC LV neurons of TRPC1/4/5 knockout mice, and hepta-TRPC knockout versus WT control mice

mEC LV	TRPC1/4/5 KO (C57Bl6/N background)			hepta-TRPC KO		*vs.*	WT control	
		n			***n***	***p***		***n***
Resting mp (mV)	-65.3 ± 1.2	8		-73.4 ± 5.4	11	0.002*	-64.9 ± 2.2	6
Input resistance (MΩ)	66 ± 27	8		83 (59; 89)	11	0.024*	103 ± 24	6
AP threshold (mV)	-48.0 ± 2.4	8		-47.7 ± 2.4	12	0.757	-48.1 ± 1.1	6
AP amplitude (mV)	64.9 ± 3.7	8		62.9 ± 6.7	12	0.803	62.1 ± 5.4	6
AP half-width (ms)	0.67 ± 0.11	8		0.87 ± 0.1	12	0.118	0.80 ± 0.1	6
AP rise slope (mV/ms)	147.5 (142; 152)	8		141.1 ± 17.9	12	0.916	140.2 ± 15.3	6
AP fall slope (mV/ms)	-90.3 ± 17.5	8		-62.0 ± 11.2	12	0.190	-69.3 ± 9.6	6
AHP following single AP								
fAHP amplitude (mV)	10.6 ± 3.4	7		9.4 ± 2.2	8	0.301	8.4 ± 1.1	6
fAHP latency (ms)	1.9 ± 0.3	7		2.4 ± 0.4	8	0.588	2.3 ± 0.2	6
mAHP amplitude (mV)	14.5 (13.7; 15.2)	8		16.0 ± 2.5	12	0.218	14.6 ± 1.6	6
mAHP latency (ms)	14.6 ± 7.6	8		24.1 ± 9.7	12	0.754	25.5 ± 5.8	6
mAHP following train of APs								
mAHP amplitude (mV) Δ to AP threshold	17.9 ± 4.8	8		19.7 ± 2.5	7	0.004*	15.5 ± 1.6	6
mAHP amplitude (mV) Δ to recording mp	4.6 ± 2.6	8		7.0 ± 2.8	7	0.019*	3.7 ± 1.1	6
Recording mp (mV)	-59.8 ± 3.1	8		-60.6 ± 2.5	7	0.628	-59.9 ± 1.9	6

All values were obtained in the presence of CCh and during blockade of AMPA-, NMDA- and GABA(A)-mediated neurotransmission. Averaged data are given as mean ± SD or as median (P_{25}; P_{75}). P values for hepta-TRPC KO vs. WT control mice (Unpaired two-tailed t-test; Mann-Whitney U-test for input resistance, * $p < 0.05$). AP: action potential, fAHP: fast after-hyperpolarization, mAHP: medium afterhyperpolarization, mp: membrane potential.