## Expression of GluK1c underlies the developmental switch in presynaptic kainate receptor function

Aino Vesikansa<sup>1,2</sup>, Prasanna Sakha<sup>1,2</sup>, Juha Kuja-Panula<sup>1</sup>, Svetlana Molchanova<sup>1,2</sup>, Claudio Rivera<sup>1,3</sup>, Henri J. Huttunen<sup>1</sup>, Heikki Rauvala<sup>1</sup>, Tomi Taira<sup>1,4</sup>, and Sari E. Lauri<sup>1,2,\*</sup>

- 1. Neuroscience Center, University of Helsinki, Finland
- 2. Department of Biosciences, Physiology, University of Helsinki, Finland
- Université de la Méditerranée, UMR S901 Aix-Marseille 2, INMED (Institut de Neurobiologie de la Méditerranée), Marseille 13009, France
- 4. Department of Veterinary Biosciences, University of Helsinki, Finland

## SUPPLEMENTARY DATA

## Supplementary Figure 1

а	Probe	Accession number	Nucleotides	Length	
	CaMKII-β	NM_021739.2	893-1393	501 bp	
	GluK1	NM_001111117.1	1139-1641	503 bp	
	GluK4	NM_012572.1	296-786	491 bp	
	GluK5	NM_031508.2	1532-2082	551 bp	
la la					
b	GluK1	GluK4	GluK5		CaMKII-β
Anti-sense	Constanting of the second				
	C port		N. 120	203	GN S
	5		1	100	
Sense					
		a forma			
		-	N.L.	S. Mary	
	State Contractor			State M	
_					
C	Probe	Accession number	Nucleotides	Length	
	GluK1b/c	NM_017241.2	2663-2761	99bp	
	GluK1c	NM_001111117.1	2793-2879	87bp	
	b ggactcgtgctttctgtgtttgtagccattggagaatttttalacaaatcacggaagaacaatgacgttgagcag				
	C ggactcgtg	jett tetgt gttt gta ge catt gg ag aa tt tt ta	t aca aa toa oggaa gaa caa	tgacgttgagcag <u>aa</u> a	ag ga aagt cat caaga cttaga ttt tat tttag
	b				aaga got ggga ata tooct caa ga a toag aa a
	C gaacaaagta	aaggtttcatgggagcaaaaaagagagcctt	ig gtg ta gaga ag tgt etetett	t tcaa tg ccat catg ga	aa gaget ggg aa ta teeete a gaate ag aaa
d		e	<b>e</b> Glu	K1b/c	GluK1c
•	obe	prok			
	GluK1c probe	GluK1b/c probe	Anti-sense	Hren ?	
	uK1	uK 1		1919 994	CP and
	Ū	<u></u>	1		
GluK1b					
		~	Sense	and the second	
GluK1c			Sense	Contraction of	A Read and the second
			100 A 100	and the second sec	

- **a.** The probes used for *in situ* hybridization.
- **b.** Autoradiography showing the signal with the various antisense and sense S<sup>35</sup>-labeled probes at P3 hippocampal sections. No unspecific staining was detected with the sense- probes.
- **c.** The sequences of short oligonucleotide probes designed to differentiate between GluK1b- and c splice variants.
- **d.** Cross-reactivity of the short GluK1-probes used in *in situ* hybridization. Dot blot of digoxigenin-labeled GluK1b/c and GluK1c antisense probes against GluK1b and GluK1c mRNA.
- e. Autoradiography with the GluK1b/c and GluK1c antisense and sense S<sup>35</sup>-labeled probes at P3 hippocampal sections. No unspecific staining was detected with the sense- probes.

## Supplementary methods:

For dot blotting, Flag-GluK1-2b/pcDNA3 and Flag-GluK1-2c/pcDNA3 were linearized with ApaI and transcribed *in vitro* using T7 RNA polymerase (mMessage mMachine-kit, Ambion). 5 ng of produced mRNA was pipetted into Hybond<sup>TM</sup>-N+ membrane (Amersham Biosciences) and crosslinked with UV. Hybridizations of DIG-labeled GluK-1b and GluK1-2c antisense probes were done overnight in DIG easy hybridization buffer (Roche Applied Science). After low (0.1xSSC, 0.1% SDS) and high (2xSSC, 0.1% SDS) stringency washes, membrane was incubated with anti-DIG-AP-antibody and detected with NBT/BCIP (Roche Applied Science).