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

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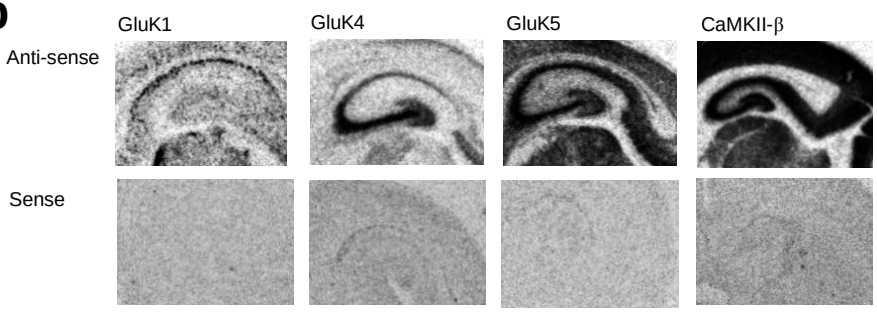
SUPPLEMENTARY DATA

Supplementary Figure 1

a

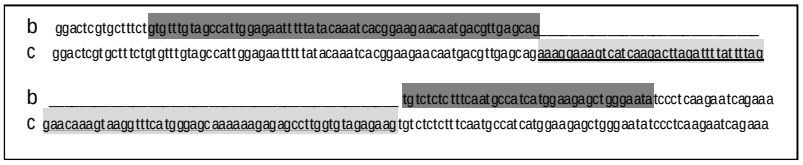
Probe	Accession number	Nucleotides	Length
CaMKII-β	NM_021739.2	893-1393	501 bp
GluK1	NM_001111117.1	1139-1641	503 bp
GluK4	NM_012572.1	296-786	491 bp
GluK5	NM_031508.2	1532-2082	551 bp

b

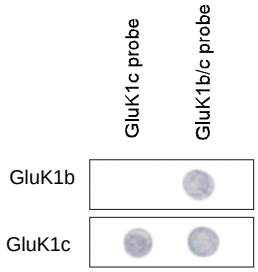


c

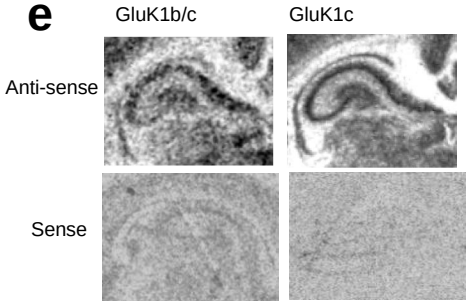
Probe	Accession number	Nucleotides	Length
GluK1b/c	NM_017241.2	2663-2761	99bp
GluK1c	NM_001111117.1	2793-2879	87bp



d



e



a. The probes used for *in situ* hybridization.

b. Autoradiography showing the signal with the various antisense and sense S^{35}-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^{35}-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™-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).