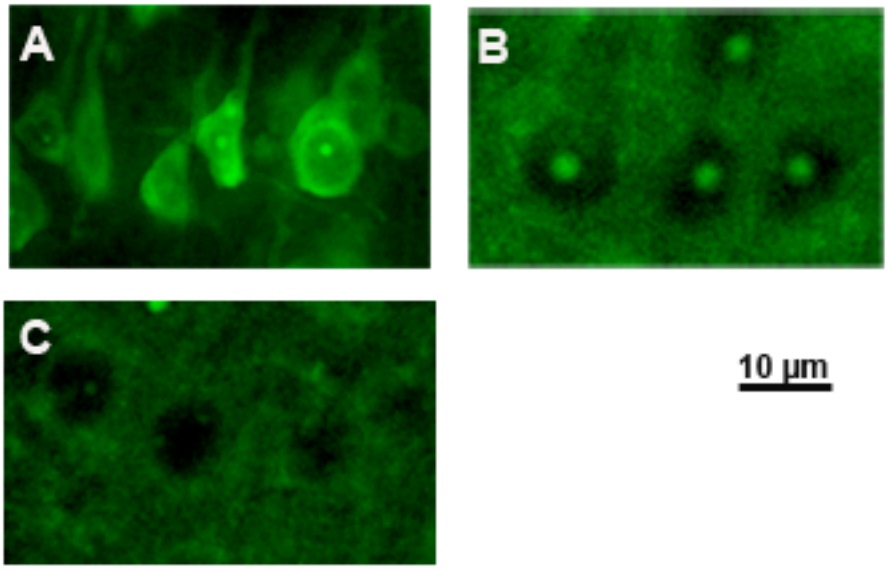
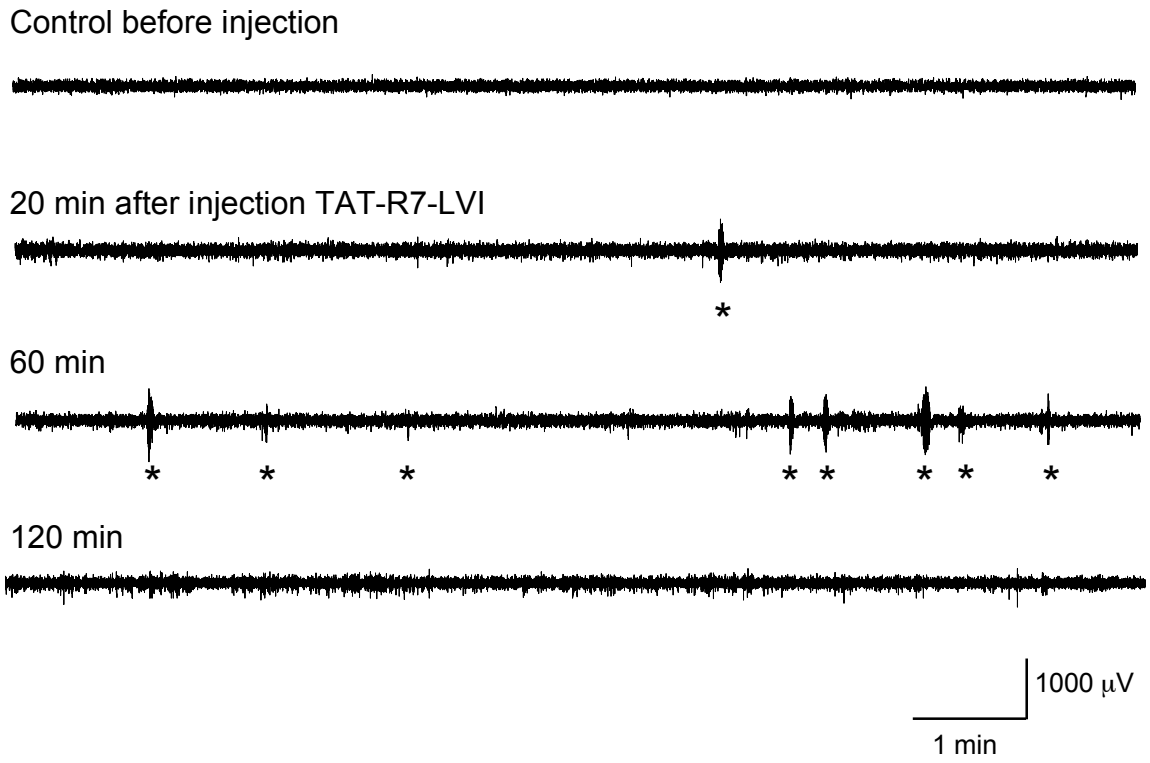
"PICK1 uncoupling from mGluR7a causes absence-like seizure"
Bertaso F, Zhang C, Scheschonka A, de Bock F, Fontanaud P, Marin P, Huganir RL, Betz H, Bockaert J, Fagni L, Lerner-Natoli M



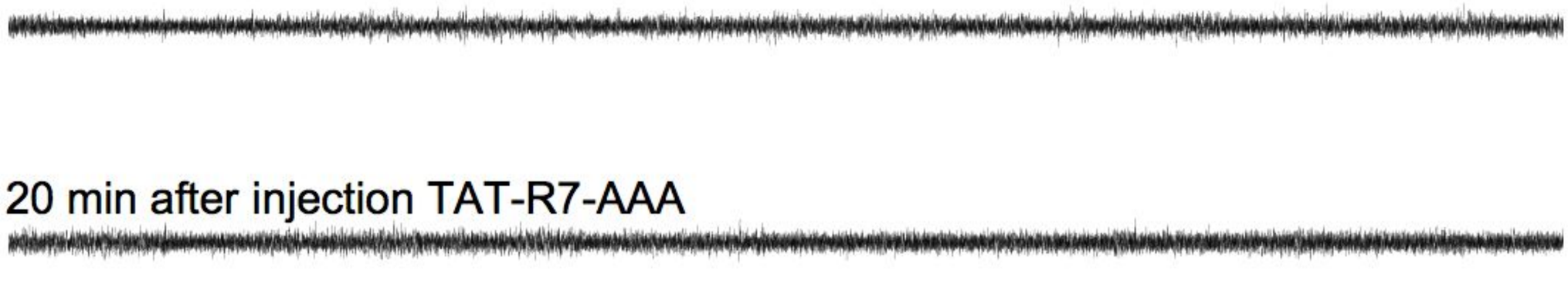
Supplementary Figure 1

Time course of the distribution of TAT-R7-LVI peptide.
FITC-conjugated avidin was used to label biotin-conjugated TAT-R7-LVI peptide in mouse cortex slices obtained 1 hr (A), 2 hr (B) and 3 hr (C) after intravenous injection of the peptide.



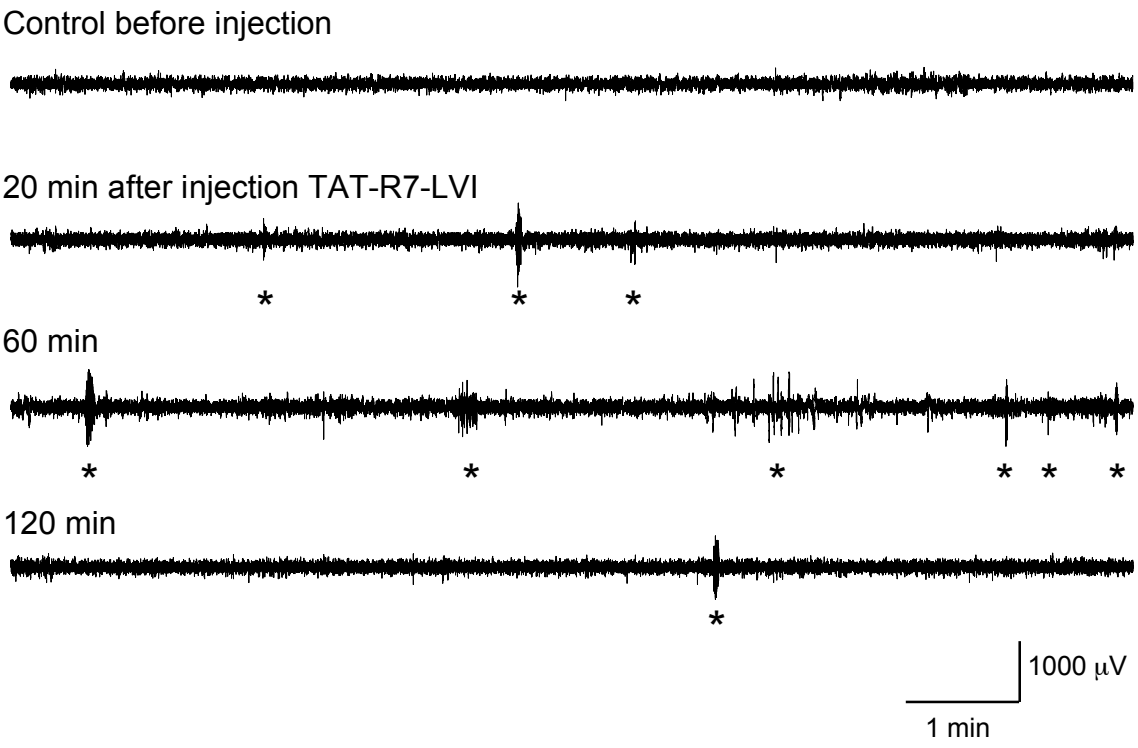
Supplementary Figure 2

Effect of the TAT-R7-LVI peptide on rat cortical EEG activity.
EEG recordings obtained from a rat 15 min before (control) and at different times after intravenous injection of 1 μmol TAT-R7-LVI peptide. Asterisks indicate absence-like discharges.



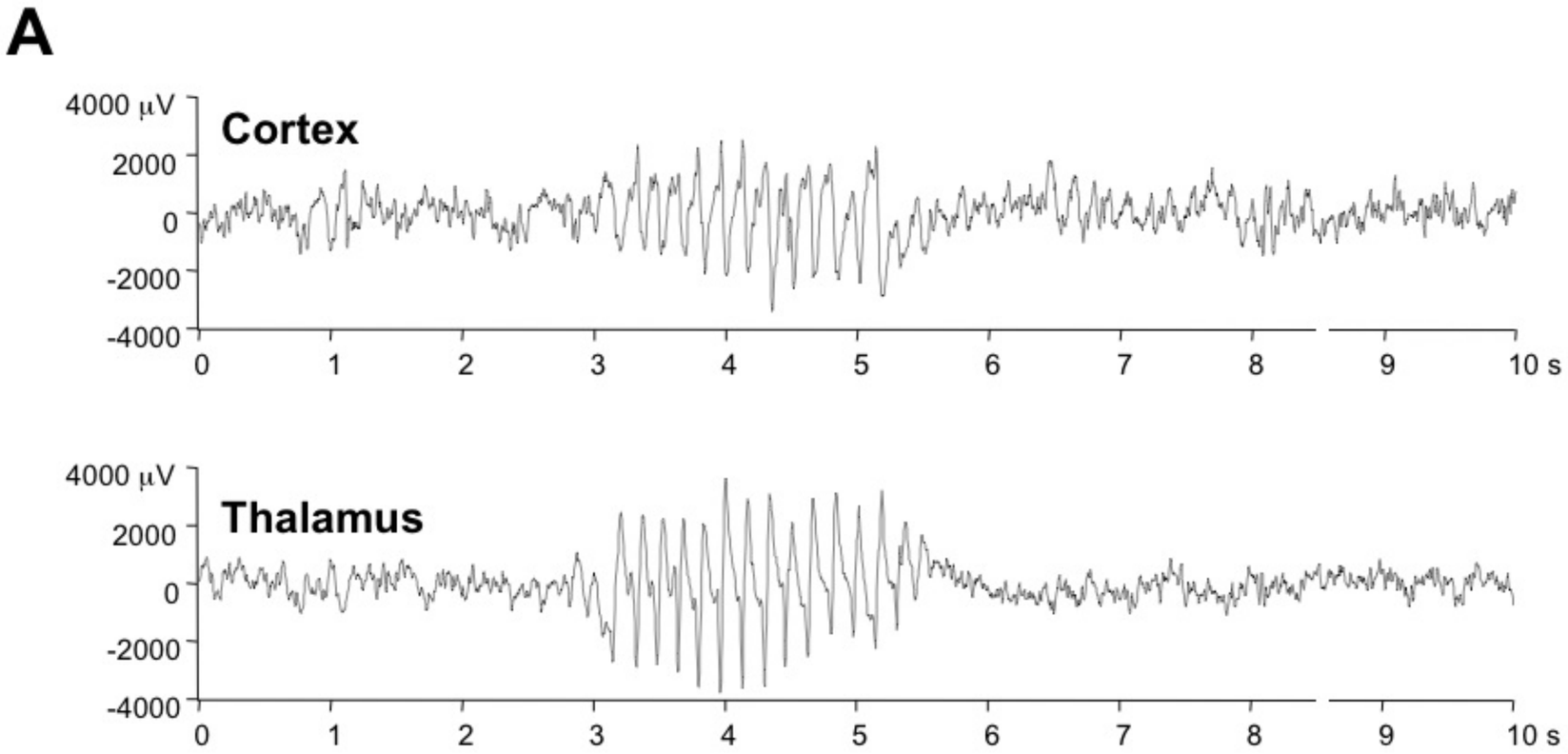
Supplementary Figure 3

Effect of the TAT-R7-AAA peptide on rat cortical EEG activity.
EEG recordings obtained from a rat 15 min before (control) and at different times after intravenous injection of 1 µmol TAT-R7-AAA peptide.



Supplementary Figure 4

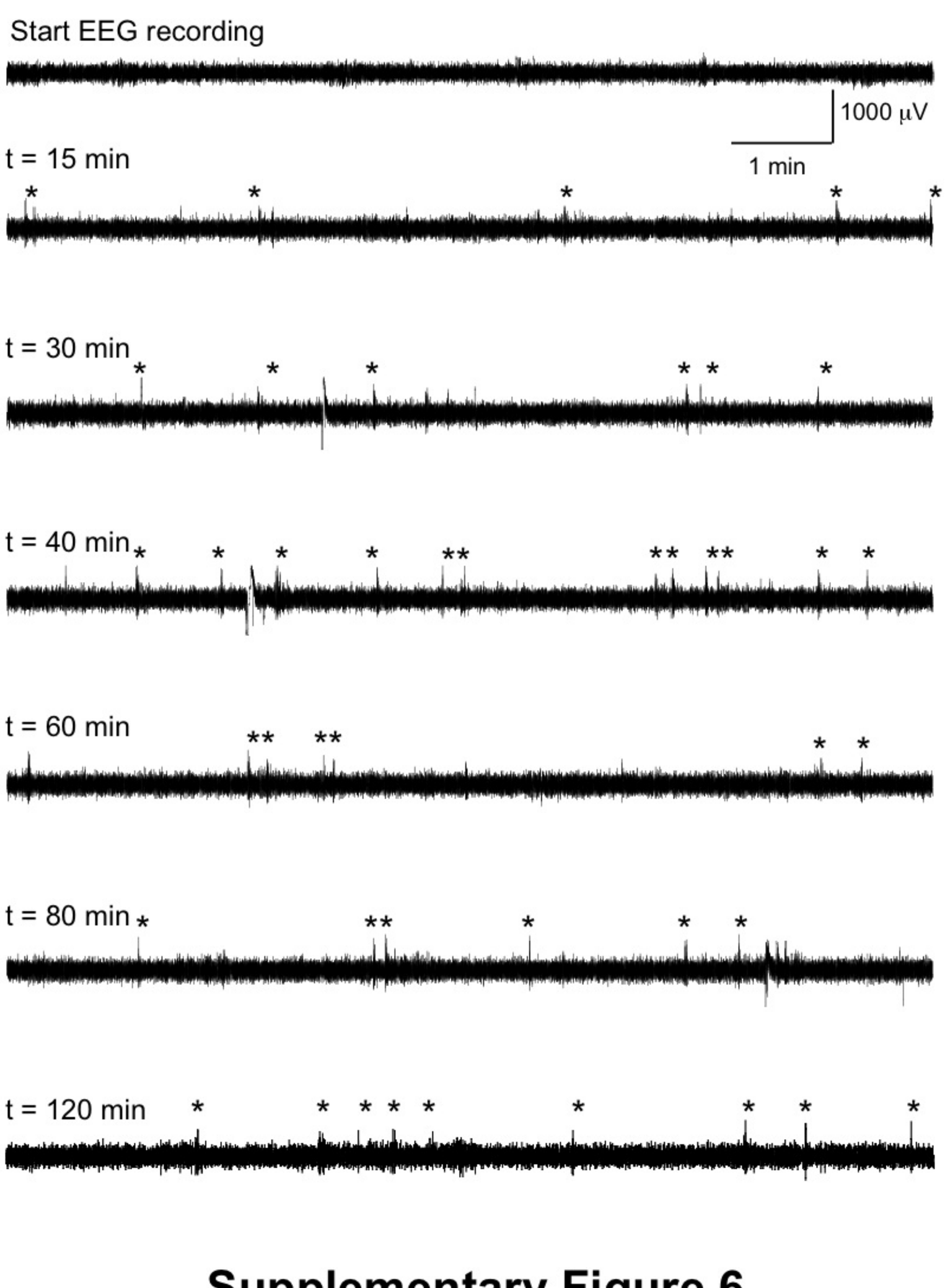
Effect of the TAT-R7-LVI peptide on mouse cortical EEG activity.
EEG recordings obtained from a wild-type mouse 15 min before (control) and at different times after intravenous injection of 400 nmol TAT-R7-LVI peptide. Asterisks indicate absence-like discharges.



Supplementary Figure 5

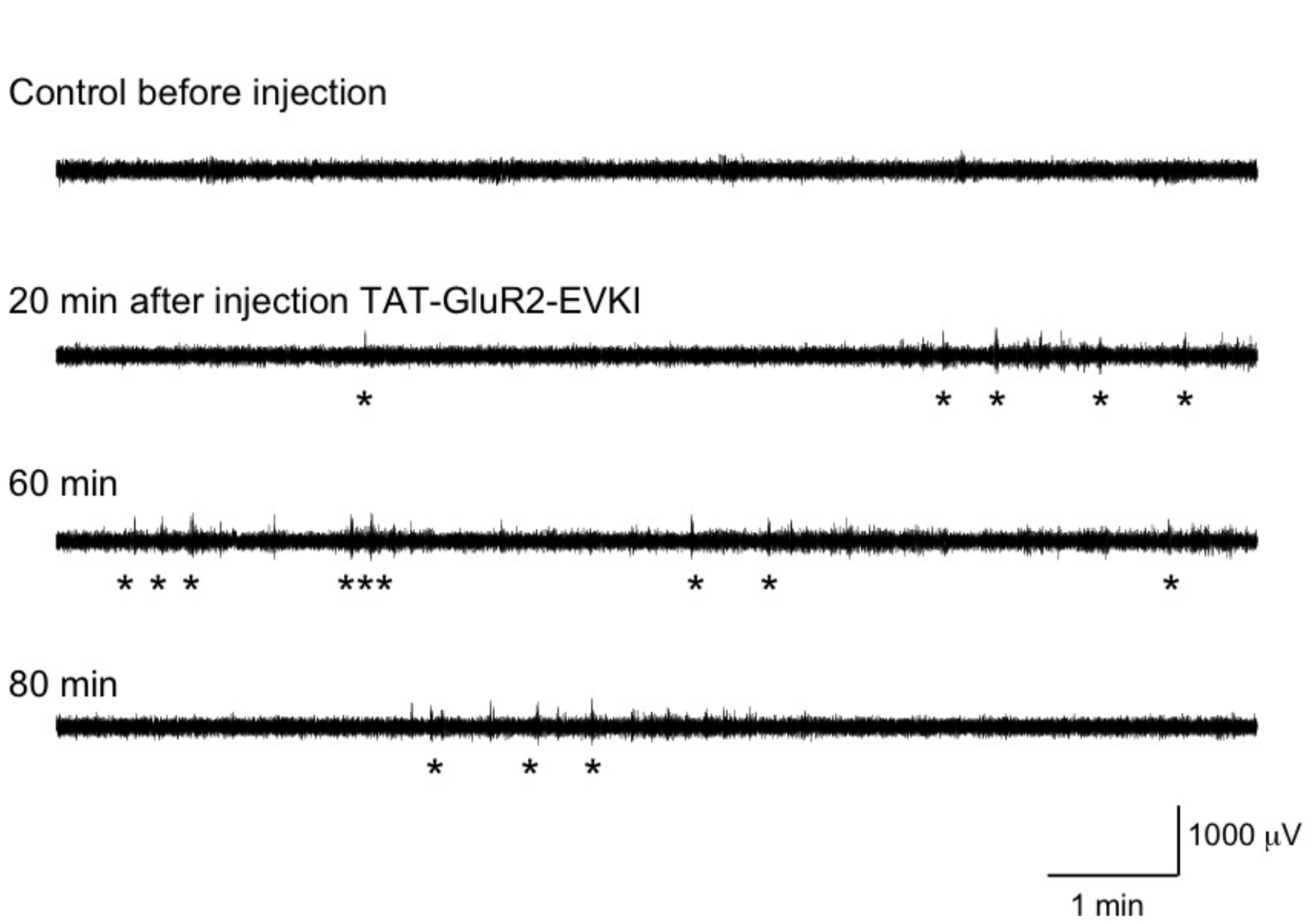
Concomitant EEG discharges in cortex and thalamus in response to TAT-R7-LVI peptide.

Simultaneous cortical and thalamic EEG recordings obtained from a rat injected with TAT-R7-LVI peptide. (A) Short time scale representation of single absence-like concomitant EEG discharges in cortex and thalamus. (B) Longer time scale traces of consecutive absence-like EEG discharges in both cortex and thalamus, showing correspondence of the events.



Supplementary Figure 6

Cortical EEG activity of an mGluR7$^{AAA/AAA}$ KI mouse.
Typical EEG traces obtained at the indicated time during the experimental session. Asterisks indicate absence-like discharges.



Supplementary Figure 7

Effect of the TAT-GluR2-EVKI peptide on mouse cortical EEG activity. EEG recordings obtained from a wild-type mouse 15 min before (control) and at different times after intravenous injection of 400 nmol TAT-GluR2-EVKI peptide. Asterisks indicate absence-like discharges.

SUPPLEMENTARY METHODS

Whole-cell patch-clamp recording of Ba^{2+} currents.

Cerebellar granule cells expressing the transfection marker EGFP and mGluR7a cDNAs were recorded at room temperature (20—24°C). The neurons were continuously perfused with an extracellular solution containing 20 mM $BaCl_2$, 10 mM HEPES, 9 mM tetraethylammonium acetate, 130 mM Na-acetate, 15 mM glucose, and 0.3 M tetrodotoxin, adjusted to pH 7.4 with NaOH and to 330 mosm with Na-acetate. Recording pipettes pulled from borosilicate glass had a resistance of 3—5 MOhms when filled with a solution containing 100 mM Cs-acetate, 20 mM CsCl, 2 mM $MgCl_2$, 10 mM HEPES, 20 mM EGTA, 1 mM cAMP, 2 mM Na_2-ATP, 15 mM glucose, adjusted to pH 7.2 with CsOH and to 300 mosm with Cs-acetate. Barium currents were recorded using an Axopatch-200 amplifier (Axon Instruments, CA). Cell capacitance and series resistance compensation were applied electronically. Data were low-pass filtered at 1 kHz, digitized at 5 kHz and analyzed using pCLAMP 8 (Axon Instruments, CA) and Origin 5.0 (Microcal, Northampton, MA). Records were leak subtracted using the pCLAMP P/4 protocol. Barium currents were evoked by 500 ms pulses at 0 mV from a holding potential of –60 mV.

Western blotting.

Proteins from either peptide affinity chromatography or co-immunoprecipitation experiments were resolved on 12.5% gels and electrophoretically transferred onto nitrocellulose membranes (Hybond-C, GE Healthcare). Membranes were incubated in blocking buffer (Tris-HCl, 50 mM, pH 7.5; NaCl, 200 mM; Tween 20, 0.1 % and 5% dried skimmed milk) overnight with goat anti-PICK1 antibody (1:500 in blocking buffer, Upstate) or rabbit anti-mGluR7 antibody (1:500 in blocking buffer, generous gift of Dr R. Shigemoto). They were then washed three times with blocking buffer and incubated with horseradish peroxidase-conjugated rabbit anti-goat antibodies (1:3000 in blocking buffer, Calbiochem) or goat anti-rabbit antibodies (1:4000 in blocking buffer, Amersham) for 1 h at room temperature. Immunoreactivity was detected with an enhanced chemiluminescence method (ECLTM detection reagent, GE Healthcare). *In vivo* co-immunoprecipitation experiments were quantified using the open-access Image J software (NIH http://rsb.info.nih.gov/ij/download.html).

Mutant mice.

The *mGluR7a*$^{AAA/AAA}$ knock-in mouse was generated as follows. Briefly, mouse genomic DNA

isolated from a BAC clone was used for the construction of a targeting vector, which contained exon 10 of the mGluR7 gene. The bases encoding the three C-terminal amino acids of mGluR7a (-LVI) were substituted by site-directed mutagenesis to generate alanine codons (resulting tail sequence -AAA). The targeting vector was introduced into mouse 129/OLA embryonic stem cells, and properly targeted clones were used for blastocyst injection to generate chimeric mice. Chimeras were backcrossed to C57/BL6 mice; the resulting heterozygous offspring was bred further to generate wild-type, heterozygous and homozygous mGluR7a knock-in mice. The presence of the mutation was confirmed by DNA sequencing.

Histological detection of TAT-conjugates and immunohistochemistry

Ten min, 30 min, 1 hour, 2 hours and 3 hours after intracerebral or intravenous injection of the indicated peptides, rats and mice were sacrificed by decapitation. Brains were removed and fixed in paraformaldehyde 4% in 0.1 M phosphate buffer for 24 h and cut in 30 μm coronal sections with a vibratome. Detection of biotinylated peptides was then performed with either peroxidase-conjugated avidin or fluorescent avidin. Sections from animals treated with non-biotinylated peptides were used as controls for endogenous biotin. For peroxidase detection, sections were abundantly washed in PBS containing H_2O_2, in order to quench endogenous peroxidase, and exposed to 0.25% Triton X-100. They were then incubated for 30 min with an avidin–peroxidase complex (Vector, Burlingame, CA), revealed with 3-, 3-diaminobenzidine as a chromogen, mounted on slides and counterstained with haematoxylin.

Double immunostaining was performed to identify the TAT peptide-positive cells. Briefly, after a preincubation in PBS containing 10% horse serum and 0.25% Triton X-100, floating sections were incubated overnight at 4°C with a mouse monoclonal anti-NeuN antibody (1:500, Chemicon, Temecula CA). After several washes, sections were incubated for 3 hours in PBS containing FITC-conjugated avidin (1:200, Vector Labs, ABCYS, France) and donkey Cy3- conjugated anti-mouse IgGs (Jackson Immunoresearch, West Grove, PA). The sections were then abundantly washed and mounted on slides for observation on a Leitz DMRB microscope (Leica) equipped for epifluorescence and with a 1392—1040 resolution cooled color camera CCD (Cool Snap, Princeton Instrument). Data were stored on a computer using Cool Snap® software and transferred to Adobe Photoshop® for image processing.

Expression of c-fos was studied in mice and rats sacrificed 1 hour after injection of TAT-conjugated peptides. The mGluR7 immunostaining was performed on the same animals as for detection of the TAT peptides. The same protocol was used for c-fos and mGluR7. Briefly, after decapitation, fixation, section processing, peroxidase quenching and permeabilization as described above, floating sections were incubated with primary rabbit polyclonal antibodies: anti-mGluR7 (1:1000) or anti-c-fos 1:2000 (Santa Cruz Biotechnology,

Santa-Cruz, CA) in PBS + 10% goat serum. Sections were then washed several times and incubated with a goat anti-rabbit Fab'2 fragment of IgG labelled with horseradish peroxidase (1:1000, P.A.R.I.S., France), revealed by diaminobenzidine and counterstained with haematoxylin. They were then mounted on slides for microscopic observation and data acquisition of the fluorescence and immunoperoxidase staining.