Restoration of spatial working memory by genetic rescue of GluR-A–deficient mice

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Gene-targeted mice lacking the AMPA receptor subunit GluR-A (also called GluR1 encoded by the gene *Gria1*,) have deficits in hippocampal CA3–CA1 long-term potentiation (LTP) and have profoundly impaired hippocampus-dependent spatial working memory (SWM) tasks, although their spatial reference memory remains normal. Here we show that forebrain-localized expression of GFP-tagged GluR-A subunits in GluR-A–deficient mice rescues SWM, paralleling its rescue of CA3–CA1 LTP. This provides powerful new evidence linking hippocampal GluR-A–dependent synaptic plasticity to rapid, flexible memory processing.

Induction of hippocampal LTP requires NMDA receptor activation. Its expression depends in part on recruiting to activated synapses AMPA receptors that contain GluR-A subunits[1,2]. Gene-targeted mice lacking GluR-A AMPA receptor subunits do not show tetanus-induced, rapid-onset hippocampal CA3–CA1 LTP[3] but can show a form of LTP after theta-burst stimulation[4]. In parallel, GluR-A–deficient mice are impaired in hippocampus-dependent SWM tasks but show normal hippocampus-dependent spatial reference memory (SRM) performance[5,6]. Together, these results suggest that GluR-A–independent neuronal mechanisms within the hippocampus can support gradual acquisition of a fixed-location, hidden-platform water maze task[5] or discrimination of initially baited arms from never-baited arms on a radial maze[6]. Nonetheless, GluR-A–dependent hippocampal synaptic plasticity seems necessary for rapid, flexible, trial-specific memory[7–9] such as keeping track of which initially baited arms have been chosen within a given trial.

We tested this by transferring a GFP-tagged GluR-A expression system into GluR-A–deficient mice. This produces a mosaic pattern of subunit expression across the CA1 cell population such that LTP can be induced in CA1 neurons expressing GFP–GluR-A, but not in GluR-A–deficient cells[10]. This results in partial recovery of tetanus-induced field LTP. We assessed whether this could restore SWM performance.

Constitutively rescued GluR-A–deficient mice expressing GFP–GluR-A ($n = 10$) were compared with GluR-A–deficient knockout mice ($n = 10$) and wild-type controls ($n = 10$) on a radial maze task. The same three out of six arms were always baited, but milk rewards were not replaced within trials[6], allowing assessment of SWM and SRM. The animals collected rewards from the ends of the maze arms guided by distal extramaze cues. As rewards were not replaced, the animal had to adopt a 'win-shift' strategy and remember which arms it had already visited on that trial. This provided a test of SWM. By only baiting three

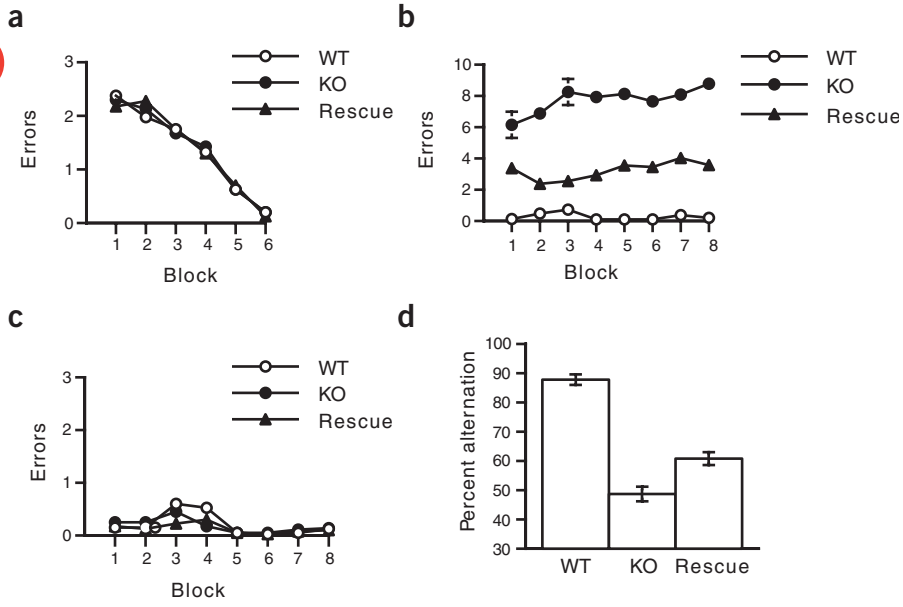

Figure 1 GFP-tagged GluR-A expression rescues spatial working memory performance in GluR-A–deficient mice. (**a**) Acquisition phase: reference memory (RM) errors (all s.e.m. < 0.18) for wild-type (WT; open circles), GluR-A–deficient knockout (KO; filled circles) and GluR-A–deficient mice expressing GFP–GluR-A (Rescue mice; filled triangles) during 6 blocks of training on a 3 out of 6 RM radial maze task. (**b**) Test phase: working memory (WM) errors (± s.e.m., otherwise all s.e.m. < 0.49) during 8 blocks of testing on a 3 out of 6 RM and WM radial maze task. (**c**) Test phase: reference memory errors (all s.e.m. < 0.12) during 8 blocks of testing on a 3 out of 6 RM and WM radial maze task. (**d**) Rewarded alternation: mean percentage of correct responses (± s.e.m.) for wild-type (WT), GluR-A–deficient knockout (KO) mice and GluR-A–deficient mice expressing GFP–GluR-A (Rescue) for 60 trials of spatial non-matching to place testing on the T-maze. All experiments were conducted under the auspices of the UK Home Office Project and personal licenses held by the authors (UK) and the Regierungspräsidium Karlsruhe.

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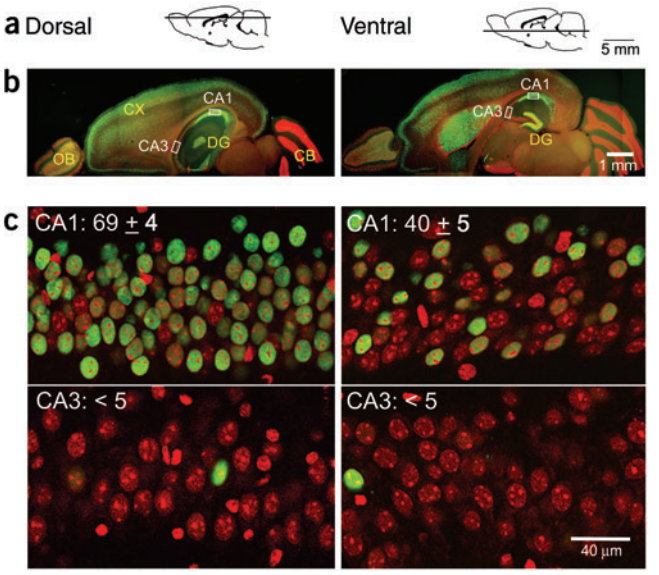

Figure 2 Immunohistochemical comparison of β-gal expression in dorsal and ventral hippocampal CA1 and CA3 of GluR-A–deficient mice expressing GFP–GluR-A. The expression of GFP–GluR-A and β-gal is coregulated. The nucleus-localized β-gal was used as reporter for the presence of GFP–GluR-A in hippocampal pyramidal neurons[10]. (**a**) Schematic sagittal views show the position of horizontal cuts for dorsal (left) and ventral (right) hippocampus (dorsal: bregma –2.8 through –3.3 mm; ventral: –4.0 through –5.0 mm). (**b**) Confocal images of dorsal (left) and ventral (right) horizontal slices from a GluR-A–deficient mouse expressing GFP–GluR-A stained with primary anti–β-gal (rabbit, 1:8,000; ICN) and secondary FITC-labeled antibody (goat, 1:200; Jackson ImmunoResearch; green) and counterstained with propidium iodide (1 μM, Sigma) to label nuclei from all cells (red). CB, cerebellum; OB, olfactory bulb; DG, dentate gyrus; Cx, cortex. Boxed regions represent a single panel in the hippocampal pyramidal cell layer where pyramidal cells were counted. Six or seven such panels were evaluated per slice. (**c**) Dorsal CA1 and CA3, and ventral CA1 and CA3 regions, in higher magnifications. Images with the sharpest and most compact pyramidal cell layering were selected. For quantitative analysis, brains of five GluR-A–deficient mice expressing GFP–GluR-A (age P44–P47) were perfused with paraformaldehyde, sliced horizontally and histochemically stained (see above). CA1 and CA3 images were recorded within a single optical plane using confocal microscopy for five slices per animal at various depths. In total, about 9,000 cells expressing the αCaMKII-driven nucleus-localized β-gal were counted. The number of β-gal–positive cells was slightly variable between slices of different animals (**Supplementary Fig. 1** online). The average percentage of CA1 and CA3 pyramidal cells rescued with GFP–GluR-A and expressing nucleus-localized β-gal is given on the top of panels in **c**.

of the six arms, but always baiting the same three arms, we were able to assess SRM simultaneously.

Mice were first trained solely on the reference memory (RM) component of the task. Doors prevented the mice from reentering an arm that they had already visited on that trial, precluding working memory (WM) errors. RM errors were scored when a mouse entered an arm that had never been baited. All mice learned the RM task at the same rate (main effect of block, $F_{5,135} = 125.0$, $P < 0.0001$; main effect of group and groups by blocks interaction, both $F < 1$; **Fig. 1a**), confirming that GluR-A–deficient mice show normal SRM acquisition[3,5,6].

The WM component was then introduced. All doors were open for every choice, so it was now possible to re-enter previously visited arms. WM errors were scored if a mouse revisited an initially baited arm within a trial. Wild-type mice made virtually no WM errors. In contrast, GluR-A–deficient mice made numerous WM errors (main effect $F_{2,27} = 1{,}588.2$; $P < 0.0001$; Duncan's $P < 0.01$; **Fig. 1b**). The GluR-A–deficient mice rescued by GFP–GluR-A performed at a level intermediate between the wild-type and knockout animals (Duncan's $P < 0.01$ for both comparisons). RM errors remained very low in all three groups (**Fig. 1c**), although wild-type mice showed a slightly increased RM error rate during blocks 3 and 4 (groups × blocks interaction, $F_{14,189} = 2.0$; $P < 0.05$; simple main effects $P < 0.01$). The intact RM performance indicates that GluR-A deletion induces a specific WM deficit rather than a non-mnemonic impairment of general maze behavior.

Additional SWM testing on a discrete trial, rewarded alternation task (spatial non-matching-to-position on an elevated T-maze[11,12]) showed excellent alternation in wild-type but chance performance in GluR-A–deficient mice (main effect of group, $F_{2,27} = 83.8$; $P < 0.0001$; Duncan's, $P < 0.01$; **Fig. 1d**). The GluR-A–deficient mice rescued by GFP–GluR-A again showed intermediate performance (Duncan's $P < 0.01$ versus both wild type and knockout).

Expression of the bidirectionally transgenic-encoded GFP–GluR-A subunit and nucleus-localized β-galactosidase (β-gal) was controlled by a regionally specific CaMKII promoter[10], limiting the GluR-A rescue to forebrain areas such as the hippocampus (**Fig. 2**). Expression was especially high in dorsal CA1 (shown for β-gal), which has been specifically implicated in spatial memory[13,14]. In contrast, constitutive GluR-A–deletion affects the entire brain[3], not only impairing spatial working memory but also inducing a range of behavioral phenotypes including hyperactivity, a subtle motor coordination deficit, changes in aspects of emotionality, and disruption of some species-typical behaviors[15]. It is likely, therefore, that some of these behavioral phenotypes result from deletion of GluR-A subunits from brain areas outside the hippocampus. Those should be unaffected by more region-specific rescue. We therefore also tested emotionality (hyponeophagia), motor behavior (motor activity and coordination) and species-typical behaviors (nesting)[15]. On each test, GluR-A–deficient mice that had and had not been rescued by GFP–GluR-A were equally impaired relative to wild-type mice (**Supplementary Table 1** online). There were no significant differences between the two genetically modified groups. The rescue was specific to the hippocampal-dependent SWM tasks.

These results demonstrate genetic rescue of a high-level cognitive ability and provide a new link between hippocampal GluR-A–dependent LTP and SWM, a rapid, flexible form of memory[7–9]. The partial behavioral rescue suggests that the mosaic of rescued and dysfunctional neurons may act like a neural network that shows graceful degradation after partial deletion of key connections. The rescue was specific to the SWM impairment. This is consistent with the hypothesis that the learning impairment in the knockout mice results from GluR-A deletion in the dorsal hippocampus and that emotional or non-cognitive effects of the knockout are unlikely to contribute to the memory deficit.

Note: Supplementary information is available on the Nature Neuroscience website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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