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Arc/Arg3.1 Is Essential for the Consolidation of Synaptic Plasticity and Memories

Report

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Summary

Arc/Arg3.1 is robustly induced by plasticity-producing stimulation and specifically targeted to stimulated synaptic areas. To investigate the role of Arc/Arg3.1 in synaptic plasticity and learning and memory, we generated *Arc/Arg3.1* knockout mice. These animals fail to form long-lasting memories for implicit and explicit learning tasks, despite intact short-term memory. Moreover, they exhibit a biphasic alteration of hippocampal long-term potentiation in the dentate gyrus and area CA1 with an enhanced early and absent late phase. In addition, long-term depression is significantly impaired. Together, these results demonstrate a critical role for Arc/Arg3.1 in the consolidation of enduring synaptic plasticity and memory storage.

Introduction

Long-term potentiation (LTP) is a putative cellular correlate of learning and memory, (Bliss and Collingridge, 1993; Martin et al., 2000). Enduring forms of LTP and memory are dependent on mRNA and protein synthesis and rely on rearrangements in the structure and molecular composition of protein networks at synapses (Goelet et al., 1986; Sheng and Kim, 2002; Pocklington et al., 2006). Signaling from the synapse to the nucleus, which activates gene expression, may produce proteins that can alter the composition of these protein networks and provide a mechanism for translating synaptic activity into persistent changes of synaptic strength. A number of activity-regulated genes have been identified that may serve this function (Nedivi et al., 1993; Qian et al., 1993; Yamagata et al., 1993).

Among activity-regulated genes Arc/Arg3.1 is unique in that following LTP-producing stimulation its mRNA is robustly induced and transported to dendrites (Link et al., 1995; Lyford et al., 1995; Kuhl and Skehel, 1998). Within dendrites, Arc/Arg3.1 mRNA can be targeted to stimulated synaptic areas, suggesting that it may be translated on site (Steward et al., 1998). Both synaptic plasticity and induction of Arc/Arg3.1 expression depend upon activation of the N-methyl-D-aspartate receptor (NMDAR) and of the mitogen-activated/extracellular-regulated protein kinase (MAPK/ERK) signaling cascade (Waltereit et al., 2001). Importantly, changes in Arc/Arg3.1 expression have been directly linked to information processing in brain (Guzowski et al., 1999), and, correspondingly, Arc/Arg3.1-inducing stimuli trigger enduring changes in synaptic plasticity and behavior (Bock et al., 2005; Gusev et al., 2005; Guthrie et al., 2000; Kelly and Deadwyler, 2002; Montag-Sallaz et al., 1999; Ying et al., 2002). Arc/Arg3.1 expression has therefore been utilized extensively to map neuronal networks that underlie information processing and plasticity (Guzowski et al., 2001; Mahlke and Wallhausser-Franke, 2004; Burke et al., 2005; Zou and Buck, 2006;). Moreover, reducing basal Arc/Arg3.1 expression by less than 60% attenuated spatial learning and LTP (Guzowski et al., 2000). Because of these strong correlations it has been widely assumed that Arc/Arg3.1 plays a role in memory formation.

We have taken a genetic approach to specifically and completely abolish constitutive and induced Arc/Arg3.1

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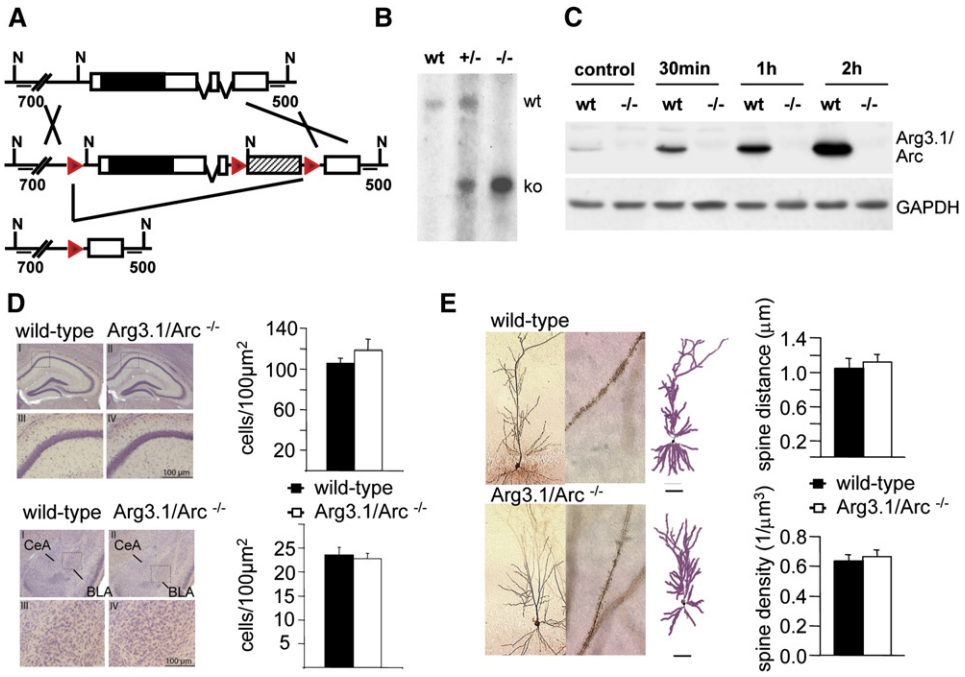

Figure 1. Generation and Neuroanatomy of *Arc/Arg3.1* KO Mice

(A) Gene-targeting approach. Top: *Arc/Arg3.1* gene locus (N, NheI restriction sites; open boxes, exons; black box, ORF; horizontal lines, homology regions used in targeting construct; 500, 700, Southern blot probes). Middle: Floxed KO gene locus (triangles, loxP sites; hatched box, neomycin-resistance cassette). Bottom: *Arc/Arg3.1* null mutation following Cre-mediated type I recombination.

(B) Southern blot (500 nt probe) of NheI-digested genomic mouse tail DNA of WT (+/+), heterozygous (+/−), and KO (−/−) mice carrying a type I recombination. Band sizes correspond to the calculated size of gene fragments (WT, 8.5 kb; KO, 4.1 kb).

(C) Immunoblot analysis of Arc/Arg3.1 protein in hippocampi of WT and KO mice under control conditions and 30 min, 1 hr, and 2 hr after kainic acid-induced seizures.

(D) HE stains of hippocampus (upper panel) and amygdala (lower panel). Bar plots indicate respective cell counts from hippocampus (upper panel) and amygdala (lower panel; Mann-Whitney and t tests, ns; n = 24 WT and KO).

(E) Left: Photomicrographs (10× and 160×) of biocytin-filled neurons and 3D reconstructions show normal appearing dendritic trees (black) and spines (magenta points) in KO neurons. Scale bars: 100 µm. Right: Comparable spine densities (t test, ns; n = 3 cells per group) and distance to nearest neighbor (t test, ns) in WT and KO CA1 neurons.

Errors in (D) indicate SEM and in (E) SD.

expression and here provide a demonstration that the protein is indeed critical for the expression of enduring memories. In a variety of behavioral paradigms, *Arc/Arg3.1* knockout (KO) mice fail to form long-lasting memories, while short-term memory is not affected. These changes in behavior are associated with marked alterations in LTP in vivo and in vitro. In the dentate gyrus (DG) and hippocampal subfield CA1 of KO mice, the early phase of LTP is greatly enhanced, whereas the late phase fails to consolidate and no potentiation remains after 60 min. In addition, we demonstrate that long-term depression is dramatically reduced in these animals.

Results

Generation of *Arc/Arg3.1*-Deficient KO Mice

We generated mice carrying a null mutation of *Arc/Arg3.1* by gene targeting (Figure 1A). In an earlier mouse genetic experiment in which a neomycin cassette remained and only a part of the coding region was removed from the genome, mutant animals died during early embryogenesis (Liu et al., 2000). In contrast, animals generated for the present study carry a deletion of the entire *Arc/Arg3.1* gene excluding dominant-negative effects from remaining cassettes or fusion peptides (Figures 1B and 1C). *Arc/Arg3.1*$^{-/-}$ mice are vital, breed normally according to the Mendelian law, and reach the same age and body weight as their littermates. Gross brain morphology of Arc/Arg3.1-deficient mice appears normal. Hematoxylin-eosin (HE) stains did not reveal detectable alterations in size, morphology, and neuron density of various brain regions (Figure 1D and see Figure S1 in Supplemental Data available with this article online). Dendritic trees of CA1 pyramidal cells show normal morphology and branching (Figure 1E). Spine densities were indistinguishable in wild-type (WT) and KO CA1 pyramidal cells (Figure 1E), and an electron microscopic examination of Arc/Arg3.1-deficient brains revealed normal appearing synapses and PSDs (Figure S1). Similarly, GFAP stains demonstrated normal distribution of glial cells in the DG of mutant mice (Figure S1).

Arc/Arg3.1-Deficient Mice Exhibit Impaired Spatial Learning Strategies

Arc/Arg3.1 expression is driven by exploration and positively correlated with spatial learning (Guzowski et al., 1999). We therefore tested *Arc/Arg3.1* KO animals in the Morris water maze. Following the acquisition phase

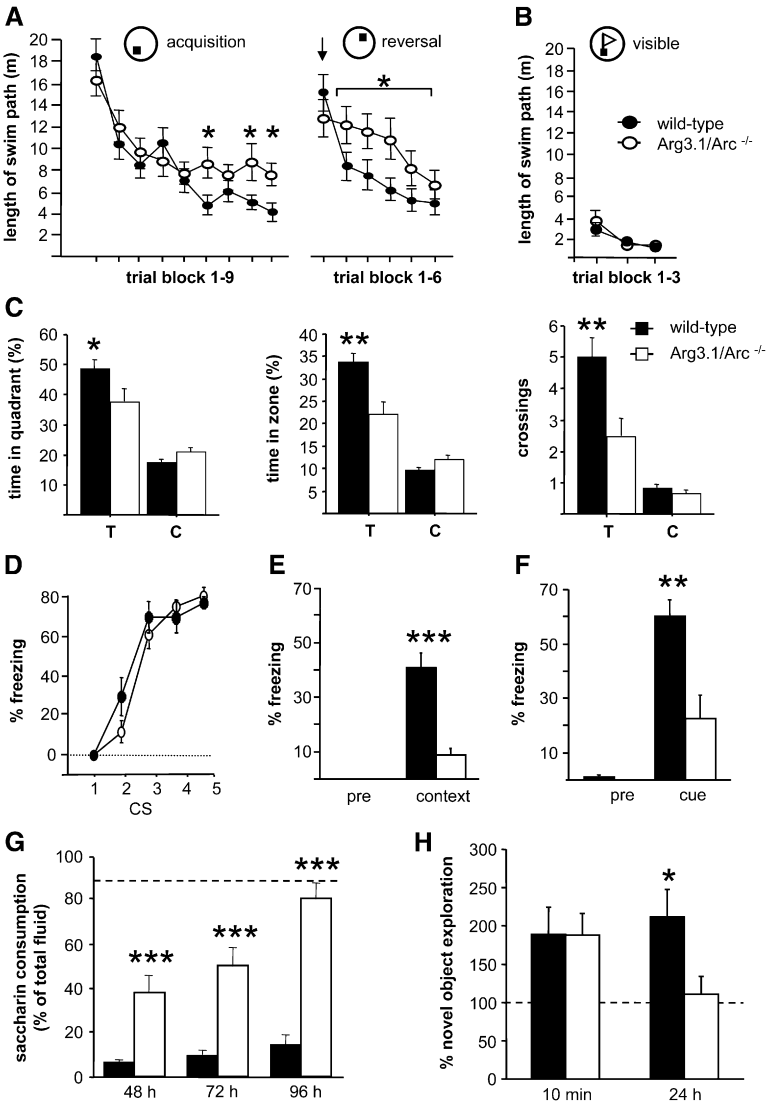

Figure 2. Disrupted Memory Consolidation during Spatial Learning, Fear Conditioning, Conditioned Taste Aversion, and Object Recognition in *Arc/Arg3.1*$^{-/-}$ Mice

(A–C) Spatial learning in the Morris water maze.

(A) Length of swim path to hidden platform during acquisition and reversal learning. Each point represents the average of two subsequent trials. KO mice displayed significantly longer swim paths during the late acquisition and the reversal phase (genotype $F_{(48,1)}$ = 2.5, ns; trial block $F_{(48,14)}$ = 16.8, $p < 0.0001$; interaction $F_{(48,14)}$ = 2.2, $p < 0.0066$; asterisks indicate significant post-hoc comparisons in single trial pairs; WT, n = 25; KO, n = 25).

(B) Length of swim path to visible platform (genotype $F_{(29,1)}$ = 0.6, ns; trial block $F_{(19,2)}$ = 12.2, $p < 0.0001$; interaction $F_{(29,1)}$ = 0.9, ns; WT, n = 16; KO, n = 15).

(C) Impaired spatial retention of *Arc/Arg3.1*$^{-/-}$ mice during the probe trial (arrow) according to comparison of time spent in trained (T) versus adjacent control (C) quadrants (left, interaction genotype-place $F_{(48,1)}$ = 5.6, $p < 0.0224$), time in circular zone around the trained platform location (12.5% of pool surface) (middle, interaction genotype place $F_{(48,1)}$ = 8.5, $p < 0.0054$), and annulus crossings over the trained position (right, interaction genotype place $F_{(48,1)}$ = 8.5, $p < 0.0053$; all two-way ANOVA).

(D–F) Contextual and cued fear conditioning.

(D) Averaged percentage of freezing to each CS during the acquisition phase.

(E) Reduced freezing of KO mice in the context 24 hr after training (WT, 40.5% ± 5.5%; KO, 9.2% ± 1%; $p < 0.0007$; pre, response before initial CS/US presentation).

(F) Reduced freezing of KO mice during tone presentation (WT, 60.2% ± 6.1%; KO, 22.4% ± 8.5%; $p < 0.003$; WT, n = 10; KO, n = 10). pre, baseline freezing before tone.

(G) Conditioned taste aversion. Saccharin consumption as percentage of total fluid uptake during saccharin/water choice tests 48, 72, and 96 hr after conditioning. *Arc/Arg3.1*$^{-/-}$ mice avoided saccharin significantly less during all choice tests (48 hr: WT, 6.3% ± 1.0%; KO, 38.9% ± 8.4%; 72 hr: WT, 10% ± 2.1%; KO, 52.1% ± 8.4%; 96 hr: WT, 14.7% ± 4.7%; KO, 83.1% ± 7.4%; all $p < 0.0001$; WT, n = 12; KO, n = 12). Moreover, mutant mice showed a significantly faster extinction rate ($p < 0.0002$), reaching levels of saline-injected WT mice (89.8% ± 1.5%, dotted line).

(H) Object recognition. Percent time spent exploring novel compared to familiar objects (dotted line). All mice spent significantly more time exploring the novel object 10 min after training (WT, $p < 0.05$; KO, $p < 0.01$). After 24 hr, however, only WTs showed preference for the novel object ($p < 0.05$; WT, n = 8; KO, ns, n = 8).

Error bars indicate SEM.

and transfer test, animals were trained to learn a new platform position to test their flexibility in handling spatial information ("reversal phase"). Both groups showed an overall decline in length of swim path during acquisition and reversal phases. However, in contrast to WTs, the KO mice failed to improve performance during the late acquisition phase (Figure 2A). In the transfer test, mutant mice showed a preference for the trained quadrant but spent significantly less time searching for the platform than WT littermates (Figures 2A and 2C). This impairment became even more pronounced with increased stringency of criteria used to assess spatial selectivity (time in goal vicinity, annulus crossings). Here, KO mice exhibited the most dramatic impairment when counting annulus crossings over the position of the trained platform (Figure 2C). During the reversal phase, mutants were significantly slower in learning the new platform location (Figure 2A). Hence, KO mice were not only less precise in processing spatial information, but also less flexible in relearning. A factorial analysis of predominant swimming strategies revealed that the learning impairment of *Arc/Arg3.1*$^{-/-}$ mice in the Morris water maze is due to a selective deficit in the formation of spatial memories, rather than aberrant performance of nonspatial strategies (Figures S2 and S3). No differences were observed in the cue version of the task (Figure 2B). Furthermore, swimming velocity and floating times were identical in KO and WT animals (data not shown), indicating normal sensory-motor function and coordination in *Arc/Arg3.1*$^{-/-}$ mice.

Arc/Arg3.1 Mutant Mice Display Disrupted Fear-Related Memory

Fear conditioning is a less complex behavioral test in which implicit (auditory cue) and explicit (context) memory can be tested within one experimental setup. During acquisition, both genotypes rapidly formed a short-lasting association (Figure 2D). When testing fear memory 24 hr later, WT mice showed high levels of freezing in the training environment and in a neutral environment when confronted with the tone. In contrast, KO animals responded with drastically lower levels of freezing to both stimuli, indicating impaired context- and cue-dependent long-term memory formation (Figures 2E and 2F). The absence of freezing before CS/US acquisition and the similar acquisition curves of mutant and control mice exclude impaired pain sensitivity or generalization as confounding factors. Moreover, normal performances in the O-maze, light/dark box, and open field revealed unaltered anxiety levels in *Arc/Arg3.1*$^{-/-}$ mice (Figure S4). These data demonstrate that Arc/Arg3.1 not only is required for explicit forms of memory (Morris water maze, contextual fear conditioning) but is also crucial for long-term memory of implicit learning tasks (cued fear conditioning).

Arc/Arg3.1 Is Required for Conditioned Taste Aversion Memory

To examine the role of Arc/Arg3.1 in hippocampus-independent learning, we subjected animals to conditioned taste aversion (CTA). During CTA, animals discover a novel taste (e.g., saccharin) followed by a LiCl injection, which causes transient gastrointestinal malaise. Conditioned mice subsequently avoid the novel taste. This association is remarkable for its single-trial acquisition, long duration, and moderate extinction (Welzl et al., 2001). In striking contrast to WT animals, *Arc/Arg3.1* KO mice failed to form a long-term CTA memory, as evidenced in a water/saccharin choice test performed 48 hr after conditioning (Figure 2G). Baseline saccharin uptake in the absence of aversive treatment was normal in KO mice excluding increased saccharin preference or reduced neophobia (Figure S5A). Thus, enhanced saccharin consumption of *Arc/Arg3.1*$^{-/-}$ mice during choice tests reflects a specific impairment in the formation of taste memory. Both genotypes avoided quinine, a naturally aversive taste for rodents, to the same extent (Figure S5B), indicating an essential role for Arc/Arg3.1 in the association of taste and malaise, but not normal gustatory processing. Furthermore, memory was stable in WT mice over consecutive choice tests (72, 96 hr), whereas mutant mice revealed a further dramatic increase of saccharin consumption. Whether increases in saccharin consumption are due to enhanced extinction or loss of long-term memory will be evaluated in future studies.

Intact Short-Term but Impaired Long-Term Object-Recognition Memory

To assess both short-term and long-term memory in the same cognitive test, we trained mice in the novel object recognition task. Following exposure to three objects, one was replaced by a novel object, and mice were reintroduced into the arena with a delay of either 10 min or 24 hr. After a delay of 10 min both genotypes spent significantly more time exploring the novel object, indicating intact short-term memory for this task (Figure 2H). After 24 hr, however, WTs still preferentially explored the novel object, whereas KO dwell time was hardly above chance level. This result demonstrates the ability of *Arc/Arg3.1*$^{-/-}$ mice to produce short-lasting memory traces and highlights their impairment in consolidating newly learned behavior into a long-lasting memory. Collectively, results from our behavioral studies demonstrate an essential role for Arc/Arg3.1 in the formation of long-lasting memories in implicit and explicit learning tasks.

Enhanced Early- but Impaired Late-Phase LTP in Arc/Arg3.1 Mutant Mice

Experience-dependent changes in behavior are thought to be encoded at a cellular level by changes in synaptic efficacy (Martin et al., 2000). To determine if LTP is affected by the absence of Arc/Arg3.1 expression, we examined LTP in the perforant pathway in vivo and the Schaffer collateral (SC) pathway in vitro. First, LTP was induced in the DG by tetanic peforant path stimulation. Both WT and *Arc/Arg3.1* KO mice developed an immediate increase in the EPSPs (fEPSP) slope (Figure 3A). Most remarkably, during the first 60 min after stimulation the magnitude of LTP in *Arc/Arg3.1*$^{-/-}$ mice exceeded that of WTs by 50%. However, responses decreased rapidly and returned to baseline after about 90 min. Thus, *Arc/Arg3.1*$^{-/-}$ synapses failed to consolidate an initial greatly enhanced potentiation into stable, long-lasting LTP.

Pairing-induced LTP in SC-CA1 synapses had a time course similar to that seen in the DG. KO mice exhibited an early enhanced LTP (1.5× WT) that decayed back to baseline within 60 min (Figure 3B). Because these experiments were conducted in the presence of GABA-receptor blockers, they demonstrate that the LTP impairment results from modifications at excitatory synapses and not from an altered excitation/inhibition balance. Early LTP in *Arc/Arg3.1* KOs, like LTP in WT mice, was synapse specific (Figure 3C). Because LTP in the KO declined to baseline, specificity could not be assessed beyond 30 min. Early LTP in the DG and CA1 of KO and WT synapses was NMDAR dependent, as it was completely blocked by application of CPP or D-APV (Figure S6).

Long-term depression (LTD) of synaptic strength is considered important for information storage and network stabilization and shares some common mechanisms with LTP (Malenka and Bear, 2004). We tested if a form of NMDAR-mediated LTD was altered in *Arc/Arg3.1* KOs. Low frequency stimulation of WT SC-CA1 synapses produced a pronounced decrease in fEPSP amplitudes which lasted for at least 60 min. The same stimulation in *Arc/Arg3.1* KO slices resulted in a smaller LTD, which gradually declined to baseline levels within 40 min (Figure 3D). These results indicate that LTD, like LTP, requires Arc/Arg3.1 for consolidation.

Unaltered Baseline Synaptic Transmission in Arc/Arg3.1 Mutant Mice

AMPA and NMDA receptors are essential for induction and expression of plasticity. We therefore measured their properties in naïve slices. AMPAR and NMDAR I–V

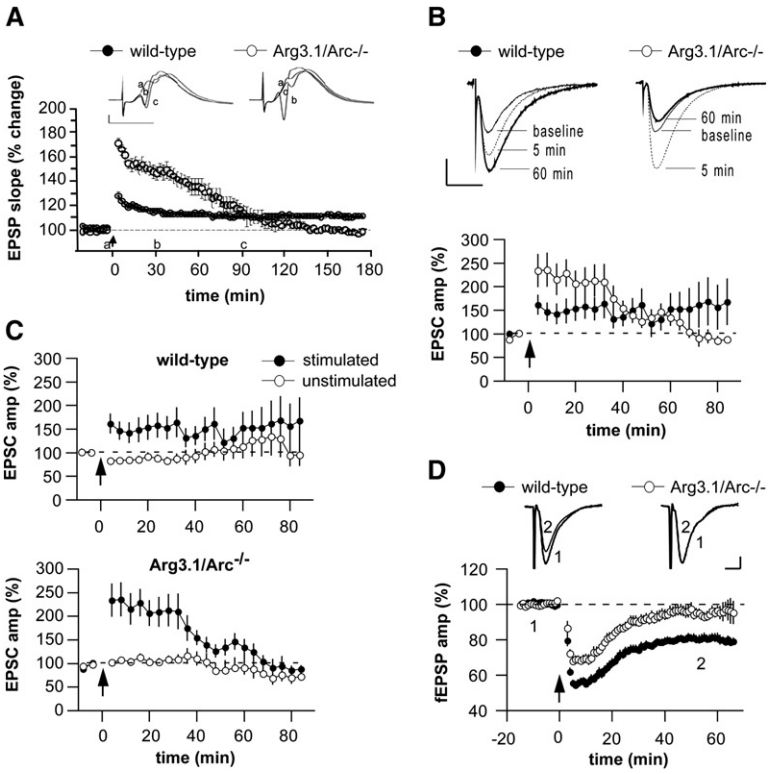

Figure 3. Impaired Hippocampal Plasticity in *Arc/Arg3.1*$^{-/-}$ Mice

(A) LTP at perforant path/granule cell synapses in vivo. Mean normalized fEPSP slope is plotted as a function of time (WT, n = 5; KO, n = 6). A high-frequency tetanus (arrow) induced a strongly enhanced early LTP in KO mice (0–5 min: WT, 126.2% ± 2.6%; KO, 169.8% ± 4.2%, $p < 0.0005$) that subsequently decayed to baseline (175–180 min: WT, 111.2% ± 2.0%; KO, 97.5% ± 1.6%, $p < 0.0001$; Mann-Whitney test). Representative fEPSP traces measured at indicated time points are shown.

(B and C) Altered LTP at Schaffer collateral/CA1 pyramidal cell synapses revealed by in vitro whole-cell recordings.

(B) Upper panel: Representative EPSCs from individual WT and KO neurons. Scale bars: vertical bar 50 pA and 100 pA for WT and KO, respectively; horizontal bar 10 ms. Lower panel: Mean normalized EPSC amplitudes in WT (n = 13) and KO mice (n = 12). Pairing stimulation (arrow) induced significantly larger early LTP in KO mice as compared to WT ($p < 0.01$), which declined to baseline by 70 min ($p > 0.05$). In contrast, LTP was maintained in the WT (150%, $p < 0.05$, baseline versus last 20 min).

(C) Only stimulated synapses potentiated during the first 30 min after pairing ($p < 0.001$ in both WT and KO, all two-way ANOVA).

(D) Reduced LTD at Schaffer collateral/CA1 pyramidal cell synapses in *Arc/Arg3.1*$^{-/-}$ mice revealed by in vitro field recording. Upper panel: Representative fEPSPs from individual WT and KO slices at indicated time points. Scale bars: vertical bar 0.1 mV and horizontal bar 10 ms. Lower panel: Mean normalized fEPSP amplitudes in WT (n = 13) and KO (n = 15) littermates. Low frequency stimulation (arrow) induced smaller LTD in KO slices which decayed to baseline by 60 min after stimulation (ANOVA: $p < 0.001$, WT versus KO).

Error bars indicate SEM.

curves were unaltered in KO mice (Figures 4A and 4B). Further, the ratio of synaptic NMDAR to AMPAR currents was not significantly different in the two genotypes (Figure 4C). We conclude that synaptic conductances are largely unaltered.

We tested whether the failure of LTP consolidation might be related to a difference in basal synaptic properties. Paired-pulse facilitation, an index of release probability, was identical between *Arc/Arg3.1* KO and WT mice over a wide range of interpulse intervals (Figures S7A and S7B). The amplitude distributions of miniature EPSCs (mEPSCs) were indistinguishable and their frequency was not significantly different (WT—0.39 ± 0.37 Hz, n = 14; KO—0.23 ± 0.12 Hz, n = 12, t test—$p = 0.14$; Figure 4D). Likewise, the fEPSP input/output function was unchanged in KO mice (Figure S7C).

Taken together, these experiments demonstrate four important points. First, Arc/Arg3.1 is not required for the induction of synaptic potentiation but limits the magnitude of early LTP. Second, Arc/Arg3.1 is critically important for consolidation of an early initial potentiation of synaptic transmission into a lasting form of LTP. Third, synaptic pathway specificity of early LTP is independent of Arc/Arg3.1. Fourth, Arc/Arg3.1 is not required for baseline synaptic transmission in the hippocampus.

Discussion

In this study, we generated a mouse line carrying a null mutation for Arc/Arg3.1 and demonstrate that Arc/Arg3.1 plays a critical role in the consolidation of synaptic plasticity and memories.

Arc/Arg3.1 and the Formation of Long-Term Memories

Early, nonspatial stages of training in the Morris water maze mainly challenge behavioral flexibility and the capacity to cope with stress, whereas the ability to integrate and remember spatial information becomes increasingly important as learning progresses. The vast majority of genetically modified animals with learning impairments have deficits associated with early nonspatial learning stages (Wolfer et al., 1998). In contrast, the behavior of *Arc/Arg3.1* KO animals is comparable to that of WTs during early acquisition and the mutants showed normal nonhippocampal procedural strategies. Nevertheless, *Arc/Arg3.1* KO mice are characterized by severe and selective deficits in later, spatial search strategies. Dramatic impairments were also observed for long-term memory of auditory and context-dependent fear conditioning. These two forms of learning depend upon the amygdala for cue-dependent processes and on both hippocampus and amygdala for context-dependent processes (Kim et al., 1993; LeDoux, 2000). Results demonstrating that the mutants also exhibit complete loss of (1) long- but not short-term memory for object recognition, a function that is partly dependent on the hippocampus (Broadbent et al., 2004), and (2) long-term memory for single trial conditioned taste aversion, which involves insular cortex and amygdala

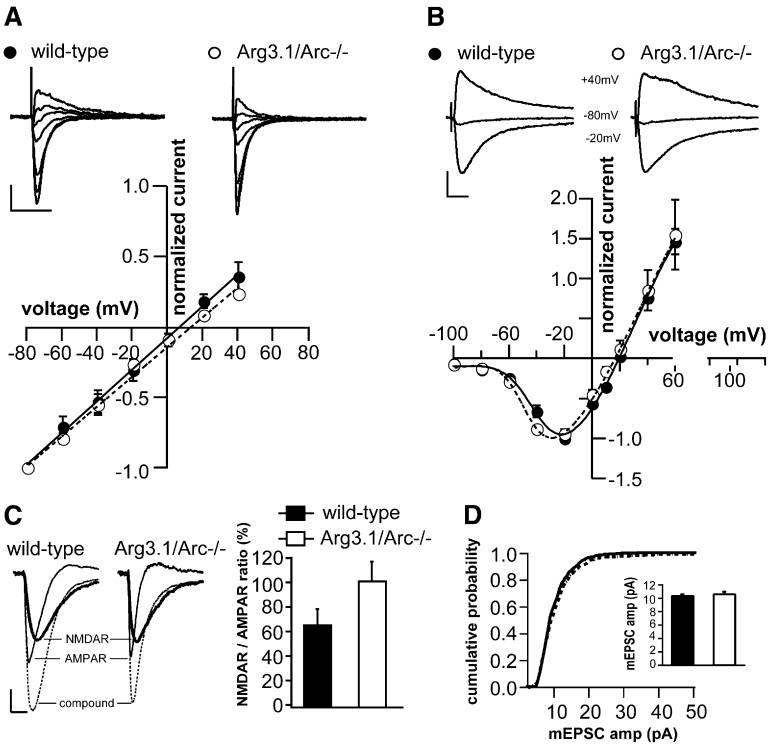

Figure 4. Unaltered Baseline Synaptic Properties in *Arc/Arg3.1*$^{-/-}$ Hippocampal Area CA1

(A) Upper panel: Representative AMPAR-EPSCs from individual WT and KO neurons recorded at increasing membrane potentials (−80 mV to +40 mV at 20 mV increments). Scale bars: vertical bar 50 pA, horizontal bar 50 ms. Lower panel: Normalized (to −80 mV) AMPAR I–V curves were linear and nearly identical in WT (n = 5, filled circles and solid line) and KO neurons (n = 6, open circles and dashed line) (ANOVA: p = 0.2).

(B) Representative NMDAR-EPSCs from individual neurons are shown in the upper panel. Scale bars: vertical bar 100 pA, horizontal bar 50 ms. The normalized (to maximal current) averaged (n = 6 WT and KO) NMDAR I–V curves shown in the lower panel were fitted with a second-order Boltzman function. Symbols are as in (A).

(C) Left panel: Representative compound-, isolated NMDAR-, and calculated AMPAR-EPSCs recorded in the absence of extracellular Mg^{2+}. Scale bars: vertical bar, 60 and 20 pA for WT and KO, respectively; horizontal bar, 20 ms. Right panel: Bar plot of the averaged NMDAR/AMPAR ratios from Arc/Arg3.1 KO mice were not statistically different from WT synapses (WT, 67% ± 13%, n = 11; KO, 103% ± 16% SEM, n = 18; t test: p = 0.14).

(D) Cumulative probability histograms of mEPSC amplitudes recorded from WT (solid line) and KO (dashed line) CA1 pyramidal neurons were indistinguishable (Kolmogorov-Smirnov significance: p = 0.15). Inlay shows bar graph of the mean mEPSC amplitudes (10.3 ± 0.15 pA, n = 814 and 10.6 ± 0.25 pA, n = 540) in WT and KO mice, respectively. Error bars indicate SEM.

(Welzl et al., 2001), extend these findings to nonspatial tasks. The results obtained using several behavioral paradigms, single and repeated training events, different sensory modalities, and different types of reinforcement, indicate that Arc/Arg3.1 is required for the formation of explicit hippocampus-dependent as well as implicit hippocampus-independent forms of long-term memory. Nevertheless transient storage of new information during acquisition, within task recall, i.e., working memory, and short-term memory can be formed independently of Arc/Arg3.1. More generally, these results suggest that similar molecular mechanisms are recruited for the consolidation of long-term memories in different brain regions and that Arc/Arg3.1 is a central component of this process. In addition, Wang et al. (2006) demonstrated that Arc/Arg3.1 plays an essential role in experience-dependent plasticity in the visual cortex.

Arc/Arg3.1 and Synaptic Plasticity

To our knowledge, the initially enhanced but rapidly declining LTP seen at Arc/Arg3.1-deficient synapses has not been reported in other pharmacological or gene-targeting experiments. A simple explanation for enhanced early LTP in *Arc/Arg3.1* KO animals might lie in altered metaplasticity (Abraham and Bear, 1996; Perez-Otano and Ehlers, 2005). Since Arc/Arg3.1-deficient synapses cannot consolidate prior potentiation, they are constitutively at a more plastic state and hence exhibit a larger early LTP. Our findings that early LTP is enhanced and LTD is reduced in *Arc/Arg3.1* KO animals might be the consequence of decreased activity-dependent AMPAR endocytosis. Three accompanying articles in this issue using organotypic or primary cultures of hippocampal neurons indicate that Arc/Arg3.1 might be involved in this process (Chowdhury et al., 2006 [this issue of *Neuron*]; Rial Verde et al., 2006 [this issue of *Neuron*]; Shepherd et al., 2006 [this issue of *Neuron*]). Shepherd et al. (2006) demonstrate that the effect of Arc/Arg3.1 on AMPAR trafficking and mEPSC amplitudes strongly depends on activity. Importantly, at conditions of low synaptic activity (under TTX in culture) mEPSCs are not different between WT and KO cultures. This is in accordance with our finding that in the hippocampal slice, which has a low constitutive level of synaptic activity, deletion of Arc/Arg3.1 has no effect on mEPSC amplitudes. However, our finding that Arc/Arg3.1 is also required for maintenance of plasticity and memory is not easily explained by altered AMPAR insertion but is likely to entail additional mechanisms. It will be important to investigate how AMPAR-trafficking relates to loss of LTP and memory consolidation in *Arc/Arg3.1*$^{-/-}$ animals. Functionally, our finding that *Arc/Arg3.1*$^{-/-}$ mice fail to consolidate LTP could explain the impairment in long-term memory formation. Additionally, their inability to refine spatial memory may also reflect the loss of LTD consolidation, in line with the possibility that hippocampal place fields are tuned by concurrent NMDAR-dependent LTP and LTD (Blum and Abbott, 1996; Kentros et al., 1998).

Experimental Procedures

Generation of Arc/Arg3.1-Deficient Mice

Genomic fragments of *Arc/Arg3.1* were isolated from a λ phage genomic library (AB-1) prepared from 129/Sv(ev) embryonic stem (ES)

cells. A 4 kb fragment encompassing the promoter and 5′UTR was subcloned into pBLUESCRIPT (Stratagene), and a 3.7 kb fragment covering the whole open reading frame (ORF) and 3′UTR was subcloned into pZErO-1 (Invitrogen). These plasmids were used for generation of a targeting construct (Figure 1A). See Supplemental Data for additional information on targeting, genotyping, and anatomical analysis.

Animal Preparation and Electrophysiology

In Vivo Recording

Male mice were anesthetized with urethane (1.8 g/kg i.p.). Recordings and stimulation were made in the hilus of the DG (2.1 mm posterior and 1.7 mm lateral to bregma) and in the medial perforant path (3.1 mm lateral to lambda, respectively). LTP was induced by six series of six trains of six stimuli at 400 Hz, 200 ms between trains, 20 s between series. 3-((RS)-2-carboxypiperazin-4-yl)-propyl-1-phosphononic acid (CPP) was injected i.p. (10 mg/kg) 1 hr prior to the tetanus.

In Vitro Intracellular Recordings

Transversal hippocampal slices (300 μm) were prepared from male and female mice (2–6 months). Intracellular- and field-recording experiments were performed in a submerged recording-chamber under constant ACSF perfusion at 34°C ± 2°C. LTP experiments were conducted in ASCF containing 4 mM $MgCl_2$ 4, 4 mM $CaCl_2$, 0.02 mM Bicuculline, 0.01 mM Glycine. Recordings were made in voltage clamp whole-cell mode. Data were sampled at 10–20 kHz and filtered at 3 kHz. Two extracellular stimulating electrodes were placed in the stratum radiatum, and LTP was induced by pairing 200 synaptic stimuli (at 1.5 Hz) with postsynaptic depolarization (0 mV). mEPSCs were measured in the presence of 2 μM gabazine and 1 μM TTX. NMDAR I–V was measured in 25 μM NBQX. AMPAR I–V was measured in 100 μM APV and with 100 μM spermine in the pipette solution. Liquid junction of 20 mV was subtracted from the membrane voltage.

In Vitro fEPSP Recordings

Recordings were made in the stratum radiatum without GABAR blockers. Signals were amplified 1000× and filtered at 1 KHz. The LTD-inducing stimulus consisted of 900 pulses (at 5 Hz for 3 min).

See Supplemental Data for additional information on electrophysiology.

Behavioral Experiments

Morris Water Maze, Spatial Reference Memory, and Reversal Learning

Water maze experiments were performed as previously described (Minichiello et al., 1999) using male and female animals of the same age (3–5 months). No gender-specific differences were observed. Animals were video-tracked using a Noldus EthoVision 1.96 system (Noldus Information Technology). Raw data were transferred to public domain software Wintrack 2.4 (www.dpwolfer.ch/wintrack) for further analysis (Supplemental Data).

Fear Conditioning

Following habituation (2 days) animals were placed into the conditioning chamber (Box A; 25 × 17 × 23 cm, opaque walls, dim red light < 5 lux) and received five temporally overlapping tone pairings (15 s, 2000 Hz, 98 dB)/shock (2 s, 0.26 mA) with 215 s intervals. Animals remained in the chamber during the whole training procedure. Twenty-four hours after training, freezing was assessed in Box A for 2 min and subsequently in a new environment (Box B) for 1 min without (pretone) and 1 min with tone. Box B differed from Box A in size (20 × 10 × 23 cm), floor (flat with bedding material), light (white), and odor. Freezing was defined as complete lack of mobility and observed by video analysis.

Conditioned Taste Aversion

Animals adapted to two daily drinking sessions of 15 min each over 4 days. In the acquisition, water was replaced by saccharin (0.5% solution) during the first drinking session followed by a LiCl injection (i.p., 0.14 M solution, 2% bw) 60 min later. Control mice were injected with saline (2% bw). Water/saccharin choice tests were performed 48, 72, 96 hr after conditioning during the first daily drinking session. Saccharin preference tests were performed in three choice tests excluding LiCl injections. Taste sensitivity was measured in a water/quinine solution (0.04%) choice test.

Novel Object Recognition

Mice were habituated to a 35 cm wide circular arena (3 days). Training consisted of two 10 min exploration trials per day (2 days) with an ITI of 10 min. In each trial, mice were allowed to freely explore three differently shaped and colored objects (made of Lego) positioned equidistantly from each other on the arena floor. Object memory was tested 10 min or 24 hr later by placing mice back into the arena, with one of the objects replaced by a novel object. To overcome possible object preference, training and novel object varied. Discrimination index was calculated as (time spent exploring novel object/mean time exploring familiar object) × 100.

Statistics

If not stated otherwise, error bars represent SEM. Comparison of groups and effects were performed with ANOVA using Statview 5.0 or SYSTAT 11 for Windows. If necessary, learning effects were assessed using repeated designs including time as within and gender as between subject factor. Mann-Whitney tests were conducted if data were not normally distributed. Single comparisons were performed with a two-tailed t test.

Supplemental Data

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/52/3/437/DC1/.

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