Arc/Arg3.1 mediates a critical period for spatial learning and hippocampal networks

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During early postnatal development, sensory regions of the brain undergo periods of heightened plasticity which sculpt neural networks and lay the foundation for adult sensory perception. Such critical periods were also postulated for learning and memory but remain elusive and poorly understood. Here, we present evidence that the activity-regulated and memory-linked gene Arc/Arg3.1 is transiently up-regulated in the hippocampus during the first postnatal month. Conditional removal of Arc/Arg3.1 during this period permanently alters hippocampal oscillations and diminishes spatial learning capacity throughout adulthood. In contrast, post developmental removal of Arc/Arg3.1 leaves learning and network activity patterns intact. Long-term memory storage continues to rely on Arc/Arg3.1 expression throughout life. These results demonstrate that Arc/Arg3.1 mediates a critical period for spatial learning, during which Arc/Arg3.1 fosters maturation of hippocampal network activity necessary for future learning and memory storage.

Arc/Arg3.1 | hippocampal oscillations | critical period | spatial learning | memory

During early postnatal development sensory regions of the brain undergo periods of heightened plasticity which sculpt and tune neural networks for adult sensory perception (1–3). It has long been debated whether critical periods exist for complex behaviors, such as orientation and learning and memory. Recent evidence unraveled a critical period for processing of emotional memories which depends on activity and plasticity mechanisms within the developing hippocampus (4, 5). These findings raise the question whether other hippocampal functions are subject to critical periods. Also, what consequences do these critical periods have for hippocampal network activity?

The hippocampus is essential for encoding spatial information and memories (6, 7), which might be achieved through linked neural representations of space and time (8–10). Hippocampal network oscillations in the theta, gamma, and ripple frequency bands are believed to play a central role in the generation and coordination of these representations, and their disruption diminishes spatial learning and memory retrieval (11–16). Hippocampal oscillatory activity emerges and develops within the first three postnatal weeks in rodents (17–20) whereupon the abilities to perform spatial learning and to store memories arise between postnatal day 20 (P20) and P24 (21–27). During this premnemonic period, hippocampal networks are built through neuronal growth and synaptogenesis, which provide the framework of the oscillatory activity (28, 29). Activity- and plasticity-dependent mechanisms shape network connectivity, but how they affect oscillatory activity and whether this has any consequences for spatial cognition are insufficiently understood.

Here, we addressed the role of the activity-regulated gene Arc/Arg3.1 in the development and maturation of spatial learning, explicit memory, and hippocampal network activity. Arc/Arg3.1 expression is rapidly induced by plasticity-producing stimuli (30–33) and following memory acquisition and retrieval (34). We previously demonstrated a profound loss of long-term memory and impaired synaptic plasticity in conventional Arc/Arg3.1 knockout (KO) mice, despite intact sensory perception and short-term memory, and normal brain morphology, neural activity, and synaptic transmission (35). These properties make Arc/Arg3.1 a particularly suited target for specific modulations of activity-dependent plasticity, distinct from neurogenesis and synaptogenesis.

Results

Deletion of Arc/Arg3.1 Before but Not After P21 Impairs Spatial Learning in Adult Mice. We used radioactive in situ hybridization (rISH) to examine natural Arc/Arg3.1 expression in the hippocampi of WT mice. Arc/Arg3.1 mRNA was detectable from P7 onwards, increased dramatically between P14 and P21, and subsequently decreased to a low baseline level in adulthood (Fig. 1*A*). This spontaneous up-regulation of Arc/Arg3.1, during a period in which mice do not yet store long-term memories, suggests an additional, novel role for Arc/Arg3.1 during the development of mnemonic circuitry. To investigate this role, we generated mice with a floxed Arc/Arg3.1 allele in which the open

Significance

Spatial learning and memory are hippocampal functions that emerge and mature during early postnatal development. The molecular mechanisms which shape this process are largely unknown. Here, we present evidence that the activity-regulated gene Arc/Arg3.1 is transiently up-regulated in the hippocampus of neonatal mice where it is required for establishing appropriate hippocampal network activity essential for spatial learning. Once established, network activity supports normal spatial learning in the absence of Arc/Arg3.1 while long-term memory storage continues to rely on Arc/Arg3.1 expression throughout life. These results demonstrate that hippocampal networks undergo a critical period of development mediated by Arc/Arg3.1 and open opportunities to investigate normal and pathological neurodevelopment of higher brain functions.

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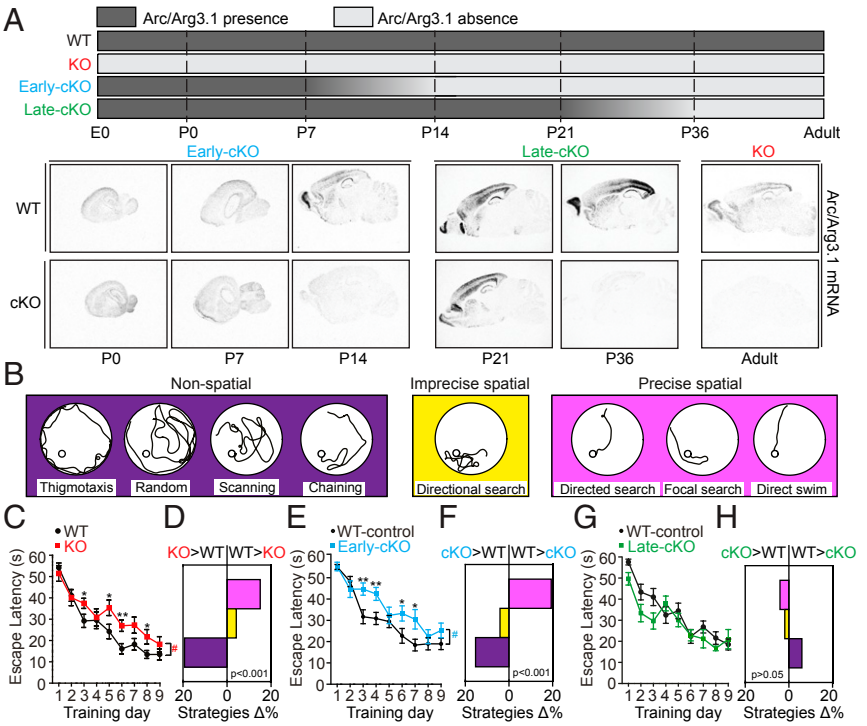

Fig. 1. Spatial learning depends on Arc/Arg3.1 expression during early postnatal development. (*A*) Spontaneous Arc/Arg3.1 up-regulation between P7 and P36 in WT mice; peak expression in the hippocampus is evident around P21. Generation of Arc/Arg3.1 KO (red), Early-cKO (blue), and Late-cKO (green) mice through terminal Cre-recombination in the germline (KO), starting after P7 and completed before P14 (Early-cKO), and after P21 and before P36 (Late-cKO). Coronal sections were subjected to rISH with an Arc/Arg3.1 probe. (*B*) Categories of search strategies in the MWM. Purple indicates nonspatial, yellow imprecise spatial, and magenta precise spatial strategies. (*C*, *E*, and *G*) Escape latencies to the hidden platform were scored for WT, KO, and cKO during the training days of the MWM. (*D*, *F*, and *H*) Search strategies during the last 4 d of training were analyzed in WT, KO, and cKO mice. (*C*) Significantly longer escape latencies in KO mice (genotype, $F_{1,22} = 6.066$, $^{\#}P < 0.05$; Bonferroni post hoc, $^{*}P < 0.05$, $^{**}P < 0.01$; $n = 12$, 12 mice). (*D*) KO mice employed more nonspatial and fewer spatial search strategies, $\chi^2_{(2)} = 14.435$, $P < 0.001$. (*E*) Significantly longer escape latencies in Early-cKO mice (genotype, $F_{1,29} = 5.507$, $^{\#}P < 0.05$; genotype x day, $F_{8,232} = 2.594$, $P = 0.010$, Bonferroni post hoc, $^{*}P < 0.05$, $^{**}P < 0.01$; $n = 17$, 14 mice). (*F*) Early-cKO mice employed more imprecise nonspatial, and fewer precise spatial, searching strategies, $\chi^2_{(2)} = 17.219$, $P < 0.001$. (*G*) Comparable escape latencies in Late-cKO mice (genotype and genotype x day, both $P > 0.05$; $n = 12$, 11 mice). (*H*) WT-control and Late-cKO mice adopted similar search strategies, $\chi^2_{(2)} = 1.115$, $P > 0.05$. The color code of search categories is as in *B*. Data shown are mean ± SEM. Two-Way ANOVA with repeated measures was performed in *C*, *E*, and *G*. A χ^2 independence test was applied in *D*, *F*, and *H*.

reading frame was flanked by LoxP sites (Arc/Arg3.1$^{f/f}$) (*SI Appendix*, Fig. S1*A*). Brain morphology, Arc/Arg3.1 expression, locomotor and exploratory behavior, pain sensitivity, and memory acquisition and persistence of the Arc/Arg3.1$^{f/f}$ mice were indistinguishable from those of WT littermates (*SI Appendix*, Fig. S1 *B–G*). We bred the Arc/Arg3.1$^{f/f}$ mice with two different CaMKIIα-Cre mice to generate either "Early-cKO" or "Late-cKO" mice, in which Arc/Arg3.1 was irreversibly removed after P7 or after P21, respectively (Fig. 1*A* and *SI Appendix*, Fig. S2*A*). We compared these to constitutive KO mice in which Arc/Arg3.1 was absent from the germline. Using immunostaining, immunoblotting, and rISH (*SI Appendix*, Fig. S2 *B–D*), we verified that near complete removal of Arc/Arg3.1 in adult forebrains was achieved in all mice.

We next examined the capacity of adult Arc/Arg3.1-deficient mice for spatial learning in the Morris water maze (MWM) (6, 36). During daily interspaced training sessions, mice searched for a hidden escape platform in the pool indicated by surrounding visual cues. As training proceeded, mice learned to locate the platform efficiently by progressively adopting spatially precise navigation strategies (Fig. 1*B*), thereby achieving shorter escape latencies, a process known as "incremental space learning" (37). We assessed this learning progression by performing an algorithm-based analysis of search strategies (*SI Appendix*, Fig. S3). As expected, WT and WT-control mice gradually reduced their escape latencies (Fig. 1 *C*, *E*, and *G*) and progressively increased the use of precise spatial strategies (*SI Appendix*, Fig. S4 *B–D*). In contrast, KO mice exhibited longer escape latencies and improved to a lesser extent and at a slower rate compared with their WT littermates (Fig. 1*C*). Strikingly, KO mice employed significantly different search strategies and adhered to nonspatial strategies even at training completion (Fig. 1*D* and *SI Appendix*, Fig. S4*B*). Similarly, Early-cKO mice exhibited a retarded learning curve, indicated by significantly longer escape latencies (Fig. 1*E*) and predominant use of non-spatial search strategies (Fig. 1*F* and *SI Appendix*, Fig. S4*C*). By strong contrast, Late-cKO mice learned to locate the platform as accurately and efficiently as their WT littermates, as indicated by the similarity of the escape latency curves (Fig. 1*G*) and by their ability to adopt precise spatial navigation strategies that were indistinguishable from those used by their WT littermates (Fig. 1*H* and *SI Appendix*, Fig. S4*D*). All KO and cKO mice exhibited low levels of thigmotaxis by the end of training (*SI Appendix*, Fig. S4 *B–D*) and performed the cued version of the MWM as well as their WT littermates (*SI Appendix*, Fig. S5 *A–D*), excluding motivational or motoric differences between them. Taken together, these data demonstrate that the KO and Early-cKO mice were impaired in spatial learning but had no deficit in nonspatial cued navigation. In contrast, Late-cKO mice exhibited both intact spatial learning and cued navigation.

Deletions of Arc/Arg3.1 Before and After P21 Impair Long-Term Memory in Adult Mice. To investigate the ability of the mice to consolidate long-term spatial memory, we performed a probe trial 1 wk after training completion. WT and WT-controls retained a stable and precise spatial memory, evident from their targeted swimming toward the platform (Fig. 2 *A*, *D*, and *G*), persistent

search within the target zone (Fig. 2 *B*, *E*, and *H*), and the significantly higher number of target-annulus crossings (Fig. 2 *C*, *F*, and *I*). In contrast, KO, Early-cKO, and Late-cKO mice failed to exhibit any preference for the platform zone (Fig. 2 *B*, *E*, and *H*) or target-annulus (Fig. 2 *C*, *F*, and *I*), disclosing impaired long-term spatial memory. KO and cKO mice remained at the release locus of the pool (in the opposite quadrant), thereby exhibiting an abnormal preference for this quadrant (O in Fig. 2 *A–H*). To examine the mnemonic capacity of the mice in a different hippocampus-dependent task, we subjected new groups of KO and cKO mice to contextual fear conditioning (CFC) (Fig. 2 *J–L*). Despite successful conditioning (*SI Appendix*, Fig. S5 *E–H*), KO, Early-cKO, and Late-cKO mice failed to remember the conditioning context, in contrast to their respective WT littermates, which maintained a strong and stable memory, as indicated by high levels of freezing (Fig. 2 *J–L*). Thus, consolidation of the long-term memory was dependent on expression of Arc/Arg3.1 in adulthood even when the learning ability was properly established during development.

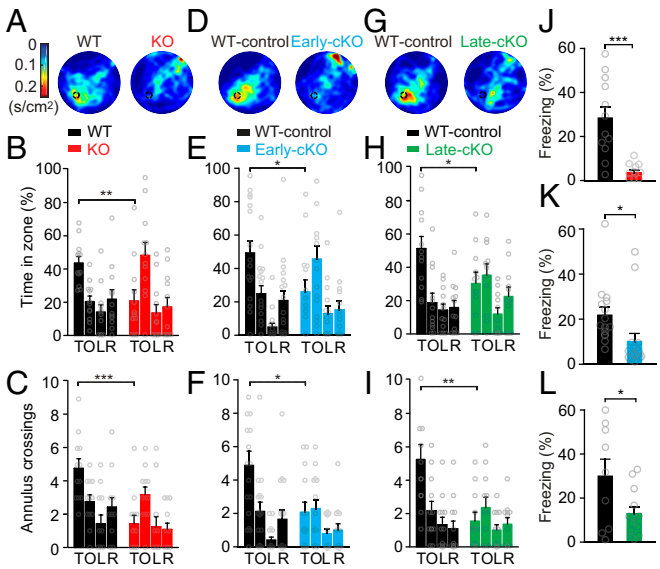

Fig. 2. Arc/Arg3.1 expression is required for the consolidation of long-term spatial and contextual memories throughout life. (*A–I*) Long-term memory in the MWM test, assessed during a probe trial 7 d after training completion. (*A*, *D*, and *G*) Mean occupancy density plots per group. (Scale bar in *A* applies to *D* and *G*.) (*B–I*) Percent time spent in the defined zones and number of annulus crossings were scored in WT, KO, and cKO. Axis labeling refers to zones as follows: L, left; O, opposite; R, right; T, target. (*A*) Off-target occupancy plot of KO mice. (*B*) KO mice demonstrated no preference for the target zone (genotype x quadrant, $F_{3,66} = 5.573$, $P < 0.01$; WT-T vs. KO-T, $^{**}P < 0.01$; $n = 12$, 12). (*C*) Reduced target-annulus crossings in KO mice (genotype x quadrant, $F_{3,66} = 4.368$, $P < 0.01$; WT-T vs. KO-T, $^{***}P < 0.001$; $n = 12$, 12). (*D*) Off-target occupancy plot of Early-cKO mice. (*E*) Loss of preference for the target zone in Early-cKO mice (genotype x quadrant, $F_{3,87} = 4.093$, $P < 0.01$; WT-control-T vs. Early-cKO-T, $^{*}P < 0.05$; $n = 17$, 14). (*F*) Significantly reduced target-annulus crossings in Early-cKO mice (genotype x quadrant, $F_{3,87} = 2.939$, $P < 0.05$; WT-control-T vs. Early-cKO-T, $^{*}P < 0.05$; $n = 17$, 14). (*G*) Off-target occupancy plot in Late-cKO mice. (*H*) Late-cKO spent significantly less time in the target zone (genotype x quadrant, $F_{3,63} = 3.055$, $P < 0.05$; WT-control-T vs. Late-cKO-T, $^{*}P < 0.05$; $n = 12$, 11). (*I*) Fewer target-annulus crossings in Late-cKO mice (genotype x quadrant, $F_{3,63} = 4.834$, $P < 0.01$; WT-control-T vs. Late-cKO-T, $^{**}P < 0.01$; $n = 12$, 11). (*J–L*) Long-term (7 d) contextual fear memory was scored as percent of time freezing in the conditioning context. (*J*) Freezing levels in WT and KO ($t_{21} = 4.551$, $^{***}P < 0.001$; $n = 12$, 11). (*K*) Freezing levels in WT-control and Early-cKO ($t_{29} = 2.355$, $^{*}P < 0.05$; $n = 15$, 16). (*L*) Freezing levels in WT-control and Late-cKO mice ($t_{20} = 2.343$, $^{*}P < 0.05$; $n = 9$, 13). All data are mean ± SEM. Open gray circles indicate data of individual mice. Two-Way ANOVA with Bonferroni post hoc test was performed in *B*, *C*, *E*, *F*, *H*, and *I*. One-Way ANOVA with Dunnett post hoc test was also performed in *B*, *C*, *E*, *F*, *H*, and *I* to test the preference for the target quadrant within groups (*SI Appendix*, Table S1). Two-tailed two-sample *t* test was applied in *J–L*.

Deletion of Arc/Arg3.1 Before but Not After P21 Alters in Vivo Hippocampal Oscillatory Activity in Adult Mice. Hippocampal oscillations in the theta and gamma frequency bands and sharp wave ripples in CA1 are believed to be important for encoding spatial information and for consolidating memory (8, 11–16). We hypothesized that these activity patterns would be modified in KO and cKO mice. To test this hypothesis, we performed multichannel depth recordings from the hippocampi of urethane-anesthetized mice and measured local field potentials (LFPs) (Fig. 3 and *SI Appendix*, Fig. S6). During rapid eye movement (REM)-like phases, WT mice exhibited clear theta (4 to 6 Hz) accompanied by gamma (20 to 50 Hz) oscillations in the CA1 pyramidal layer (Fig. 3 *A* and *C*). Slow wave sleep (SWS)-like phases were characterized by large amplitude LFPs in the stratum radiatum [sharp waves (SPWs)], accompanied by high frequency ripples in the pyramidal layer of CA1 [CA1 stratum pyramidale (CA1 str. pyr.)] (Fig. 3 *B* and *C*). Strikingly, KO mice exhibited significantly reduced theta and gamma power in the CA1 str. pyr. (Fig. 3*D*) and significantly fewer ripples (Fig. 3*E*). Remaining ripples that were still detected in the KO CA1 str. pyr., as well as co-occurring SPWs in the stratum radiatum, were similar in amplitude to those of their WT counterparts (Fig. 3*E*). Notably, the frequency of the remaining ripples was higher in KO mice (Fig. 3*E*). Early-cKO mice showed a reduction in theta but not in gamma power in the CA1 str. pyr (Fig. 3*F*). Ripple numbers in CA1 of Early-cKO mice were WT-like, but their frequency and amplitudes were significantly lower, as was the amplitude of SPWs (Fig. 3*G*). In stark contrast, Late-cKO mice were WT-like in all measures of network activity in the hippocampus (Fig. 3 *H* and *I*). Thus, genetic ablation of Arc/Arg3.1 in the germline or before P14 (KO and Early-cKO) impairs adult oscillatory network activity in the hippocampus whereas removal of Arc/Arg3.1 after P21 (Late-cKO) leaves network activity intact. Our LFP measurements reflect sleep-like activity and are complemented by a previous report of altered hippocampal oscillations and a desynchronization of place cell activity in the Arc/Arg3.1 KO mice during running (38).

Deletion of Arc/Arg3.1 in Adult Mice Impairs Spatial Memory but Not Learning. The behavior and LFP analyses suggest that the maturation of spatial learning requires Arc/Arg3.1 expression only during a limited time window of postnatal development. To verify this, we genetically removed Arc/Arg3.1 from the hippocampi of adult mice (Adult-cKO) through stereotactic delivery of rAAV-Cre (Fig. 4*A* and *SI Appendix*, Fig. S7). Adult-cKO mice performed in the MWM as well as their control littermates, exhibiting rapidly decreasing escape latencies (Fig. 4*B*) and indistinguishable utilization of spatial strategies (Fig. 4 *C* and *D*). Thus, adult removal of Arc/Arg3.1 from the hippocampus does not affect spatial learning. Similar to KO, Early-cKO, and Late-cKO mice, Adult-cKO also exhibited a profound loss of long-term spatial memory (Fig. 4 *E–G*).

Discussion

Oscillatory network activity in the rodent hippocampus develops postnatally, starting with the generation of SPWs at P2 to P4, followed by increased gamma (17) and theta power (18), and finally the emergence of ripples (39) in CA1 at P10 to P12. In agreement with this timeline, the presence of Arc/Arg3.1 until the second postnatal week in the Early-cKO mice may have supported the development of CA3 and the emergence of theta/gamma oscillations and sharp waves in CA1, but not their subsequent maturation. This interpretation is in line with an activity-dependent sequential maturation of the hippocampal regions (40). Impaired spatial encoding and loss of theta/gamma entrainment may be mechanisms contributing to the profound behavioral deficits in both KO and

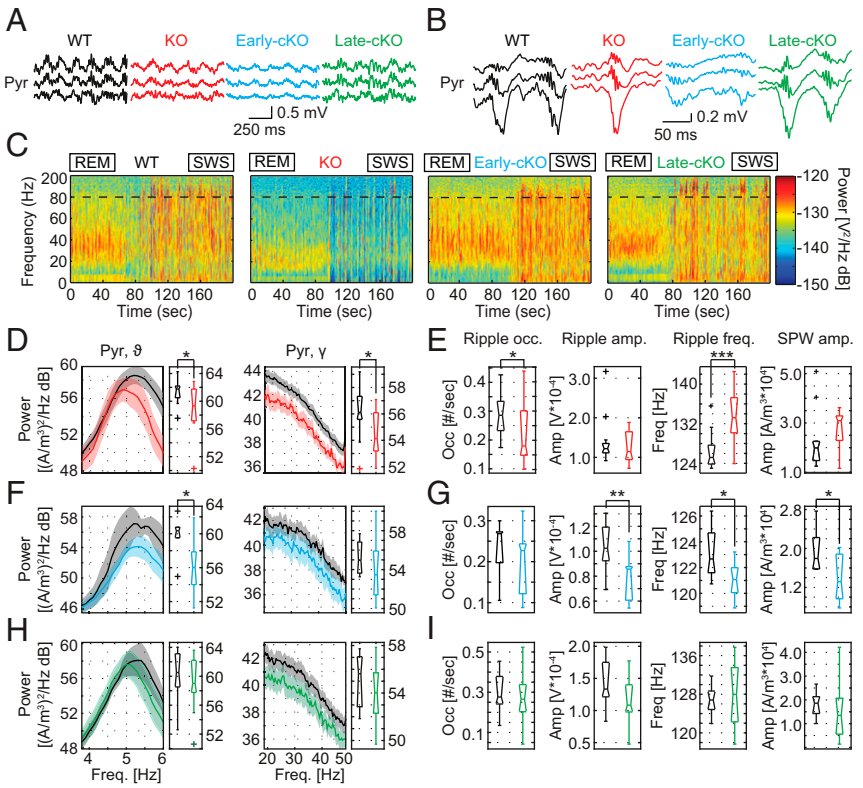

Fig. 3. Genetic deletion of Arc/Arg3.1 before P21, but not afterward, impairs oscillatory network activity in the hippocampus. (*A* and *B*) Exemplary raw LFP traces were recorded in hippocampus CA1 (three electrodes shown: located above, within, and below str. pyr., respectively) during (*A*) paradoxical/REM-like sleep and (*B*) SWS-like states. Genotype is color coded throughout the figure: black, WT/WT-control; red, constitutive KO; blue, Early-cKO; green, Late-cKO. (*C*) Exemplary spectrograms from CA1 str. pyr of WT, KO, and cKO mice indicated by power per dB. Frequency bands >80 Hz (above the dashed line) were displayed at a lower resolution. (*D*, *F*, and *H*) Power spectra and box plots display the mean power (in dB) in the theta (ϑ) and gamma (γ) frequency bands recorded during paradoxical/REM-like states. A significant reduction in ϑ power is observed in KO mice (*D*, $U = 25$, $^*P < 0.05$; $n = 14, 8$) and Early-cKO (*F*, $U = 50$, $^*P < 0.05$; $n = 6, 9$) but not in Late-cKO mice (*H*, $U = 60$, $P > 0.05$; $n = 10, 11$). Reduced γ power was significant only in the KO mice (*D*, $U = 26$, $^*P < 0.05$; $n = 14, 8$). (*E*, *G*, and *I*) SPW-ripple complexes were analyzed from SWS-like states. Ripple occurrence (occ.) (s^{-1}), amplitude (amp.) (V^{-4}), frequency (freq.) (Hz), and SPW amplitude (A/m^3*10^4) were significantly impacted in KO (*E*, Ripple occ.: $U = 44$, $^*P < 0.05$; Ripple freq.: $U = 145$, $^{***}P < 0.001$; $n = 14, 12$) and Early-cKO mice (*G*, Ripple amp.: $U = 55$, $^{**}P < 0.01$; Ripple freq.: $U = 53$, $^*P < 0.05$; SPW amp.: $U = 44$, $^*P < 0.05$; $n = 7, 9$). Indistinguishable SPW-ripple activity in WT-control and Late-cKO mice (*I*, all parameters, $P > 0.05$; $n = 9, 10$). Hour glass markers represent the median and 25th and 75th percentiles. Crosses mark outliers. Mann–Whitney–Wilcoxon *U* test was applied in *D*–*I*.

Early-cKO mice. Our findings extend previous reports linking theta/gamma oscillations and ripples to spatial learning (11–16) and indicate that their developmental maturation is a prerequisite for the emergence of reference-based navigation. During hippocampus-dependent spatial learning, Arc/Arg3.1 expression is induced in more than 30% of CA1 neurons (34, 41, 42). Navigation-relevant oscillations engage a similarly large network of CA3-CA1 neurons (43, 44). We therefore think it is unlikely that the very low amount of residual Arc/Arg3.1 in the hippocampus of Late-cKO mice (<7% in dentate gyrus (DG), undetectable in CA1) can be responsible for the intact spatial learning and oscillations observed in these animals. The absence of long-term memory in the Late-cKO mice, despite intact oscillatory network activity and ripples, suggests that Arc/Arg3.1 is continuously required for linking these activity patterns to memory consolidation. The loss of contextual fear memory and spatial memory in all KO and cKO mice after a hiatus of 1 wk confirms previous findings (35, 45) that Arc/Arg3.1 is essential for the maintenance of explicit memories. However, in contrast to KO and Early-cKO, Late-cKO mice exhibited normal incremental spatial learning where daily-interspaced training in the same environment incrementally enhances space learning and improves reference-based navigation (6, 46). We hypothesize that the intact oscillatory activity in the Late-cKO enables precise encoding of spatial representations by the hippocampal–entorhinal cortical network (8, 47) and may recruit short-term plasticity, sufficient to carry the spatial representations from day to day. The induced expression of Arc/Arg3.1 observed during MWM training (41, 48) is then required not for incremental learning itself but for the consolidation of memory at later stages.

During development, Arc/Arg3.1 might modify hippocampal network architecture via its known effects on homeostatic synaptic scaling (49–51) and possibly through hitherto unexplored mechanisms. Potential candidate mechanisms might include changes in the microarchitecture of excitation/inhibition connectivity, which affect ripple oscillations and memory (52–54) and mark the opening and closure of critical periods (55). In adult animals, Arc/Arg3.1 could regulate memory consolidation through mechanisms of long-term plasticity (35, 56).

We propose that our findings reflect a critical period for spatial learning mediated by Arc/Arg3.1 expression between P7 and P21. The existence of critical periods for human learning and memory has been debated (57) but has gained strong support by recent rodent research (5, 58). A critical period (P17 to P24) for the acquisition of latent episodic memories (reflected by infantile amnesia) was identified, which is mediated by BDNF and the NMDA receptor (NMDAR) subunit switch, two molecular mechanisms involved in sensory cortex critical periods (1). Of note, since Arc/Arg3.1 is part of the NMDAR complex (59), is up-regulated by BDNF (60, 61), and mediates consolidation of BDNF-dependent synaptic plasticity (56), it potentially may also be involved in sculpting this critical period.

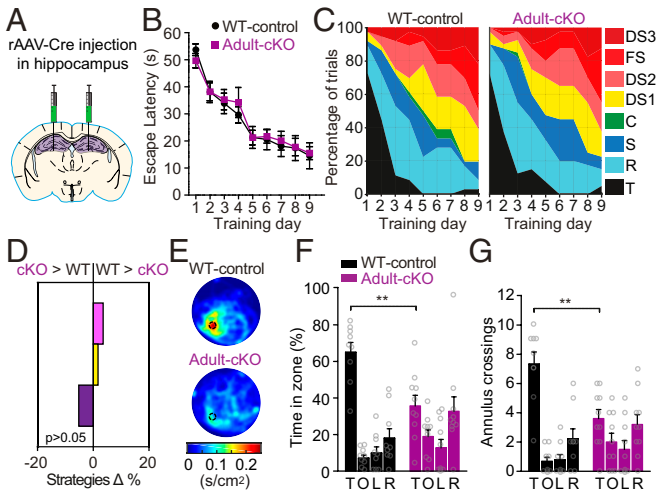

Fig. 4. Deletion of Arc/Arg3.1 in adult mice impairs spatial memory but not learning. (*A*) Illustration of bilateral rAAV-Cre injection in the hippocampus to achieve Arc/Arg3.1 ablation in the adult mice. (*B*) Escape latencies to the hidden platform during nine training days of the MWM were scored for control and Adult-cKO mice (genotype and genotype x day, both $P > 0.05$; $n = 9, 10$). (*C*) Analysis of search strategies in WT-control and Adult-cKO mice scored as percentage of trials for each defined strategy following 9 d of training. Similar progression of navigational strategy was observed in both groups [statistics for day 6 to 9: χ^2 test of independence over individual strategies, $\chi^2_{(6)} = 9.835$, $P > 0.05$; $n = 9, 10$]. Color-coded individual strategy is as in *SI Appendix*, Fig. S4. C, chaining; DS1, directional search; DS2, directed search; DS3, direct swim; FS, focal search; R, random; S, scanning; T, thigmotaxis. (*D*) Strategies grouped into precise-spatial (magenta), imprecise-spatial (yellow), and nonspatial (purple) were analyzed for days 6 to 9 in WT-control and Adult-cKO mice [χ^2 test of independence for grouped strategies as color coded, $\chi^2_{(2)} = 1.826$, $P > 0.05$; $n = 9, 10$]. Color code for grouped search categories is as in Fig. 1*B*. (*E–G*) Analysis of a probe trial performed 7 d after training completion. Occupancy plot, the percent time spent in the defined zones, and number of annulus crossings were scored in WT-control and Adult-cKO mice. Axis labeling refers to zones as follows: L, left; O, opposite; R, right; T, target. (*E*) A diffuse occupancy plot of Adult-cKO in contrast to a focal plot of WT-control littermates. (*F*) Significantly reduced percent time spent in target zone in Adult-cKO mice (genotype x quadrant, $F_{3,51} = 5.916$, $P < 0.01$; WT-control-T vs. Adult-cKO-T, $^{**}P < 0.01$; $n = 9, 10$). (*G*) Decreased number of target-annulus crossings in Adult-cKO mice (genotype x quadrant, $F_{3,51} = 6.957$, $P < 0.01$; WT-control-T vs. Adult-cKO-T, $^{**}P < 0.01$; $n = 9, 10$). Data are presented as mean ± SEM in *F* and *G*. Open gray circles indicate measurements of individual mice. Two-way ANOVA with Bonferroni post hoc test was performed in *B*, *F*, and *G*. One-way ANOVA with Dunnett post hoc test was also performed in *F* and *G*, to test the preference for the target quadrant within groups (*SI Appendix*, Table S1). WT-controls showed a preference for the target quadrant, but not Adult-cKO.

Arc/Arg3.1 has also been implicated in the critical period of ocular dominance in the visual cortex (62), by mediating potentiation of the preserved-eye visual response and depression of the deprived-eye visual response. Recently, Jenks et al. (63) showed that artificially enhancing Arc/Arg3.1 expression can extend this critical period and reintroducing Arc/Arg3.1 into KO visual cortex can restore it. These reports and our findings demonstrate a role for Arc/Arg3.1 in cortical/sensory and hippocampal/memory critical periods. They also raise the intriguing possibility that local differences in Arc/Arg3.1 regulation could generate different time windows and forms of plasticity unique to each region. We speculate that misregulation of Arc/Arg3.1 during critical periods might result in neurodevelopmental disorders, such as schizophrenia (64), fragile X mental retardation syndrome (65), and autism spectrum disorders (66), in which Arc/Arg3.1 has been implicated.

Materials and Methods

Animals. Mice aged 3 to 6 mo were used in all experiments. All of the experiments were conducted in accordance with the German and European Community laws on protection of experimental animals and approved by the local authorities of the City of Hamburg. Experimenters were blind to the mice genotype until conclusion of experiments and analysis.

Floxed Arc/Arg3.1 mice were generated together with the conventional KO line previously described (35). A type II recombination clone was injected into C57BL/6J blastocysts. Male chimeras were backcrossed into C57BL/6J. Arc/Arg3.1 loxP flanked mice (Arc/Arg3.1$^{f/f}$) were normal and fertile. The F1 generation of Arc/Arg3.1$^{f/f}$ was backcrossed into C57BL/6J for at least 10 generations. Conditional Arc/Arg3.1 KO (cKO) mice were obtained by breeding the Arc/Arg3.1$^{f/f}$ mice with the following Cre recombinase transgenic mice: (*i*) Tg(CaMKIIα-cre)1Gsc (67) to obtain Early-cKO mice; (*ii*) Tg(CaMKIIα-cre)T29-1Stl (68) to obtain Late-cKO mice; and (*iii*) Tg(CMV-cre) (69) to rederive germline KO mice [these were not different from the conventional KO line (35), and their results were pooled together]. Additional information can be found in *SI Appendix, SI Materials and Methods*. Detection and quantification of Arc/Arg3.1 were performed with immunoblotting, immunohistochemistry, and radioactive in situ hybridization. Further details are available in *SI Appendix, SI Materials and Methods*.

Virus Injection. rAAV$_{1/2}$-CaMKIIα-CreER^{T2}-2A-Venus were injected into the dorsal and ventral hippocampus of adult mice (3 to 6 mo old), bilaterally. Experiments began 7 d after injections. At the end of experiments, brains of all mice were collected, sectioned, and immunostained to evaluate the extent of Cre expression and Arc/Arg3.1 ablation. Only animals with >80% ablation were included in the results. Details are available in *SI Appendix, SI Materials and Methods*.

Behavioral Analysis. Open field, Zero maze, and flinch-jump tests were used to characterize behavior and sensory sensitivity of Arc/Arg3.1$^{f/f}$ mice. The Morris water maze was used to assess spatial learning and memory. Contextual fear conditioning was used to test long-term memory. A custom-made algorithm was written in MATLAB to analyze spatial navigation strategies of mice, based on their swim tracks. Further details are available in *SI Appendix, SI Materials and Methods* and Fig. S3.

In Vivo Electrophysiology. Adult mice were recorded under urethane anesthesia using linear 16-site silicon probes inserted into the dorsal hippocampus, as previously described (70, 71). SWS- and REM-like epochs were identified from the multitaper spectrograms calculated from the LFP. Ripples during SWS-like epochs were automatically detected and analyzed, together with their co-occurring sharp waves (SPWs). Power spectra were computed from the current source density (CSD) (72), and theta and gamma power were calculated for the selected REM-like epochs by integrating the area below their respective frequency bands. Details are available in *SI Appendix, SI Materials and Methods*.

Statistics. Various statistical tests were used for analysis of different datasets, as indicated in the figure legends. These are also described in detail in *SI Appendix, SI Materials and Methods* and Table S1.

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