Exploring the triad of behaviour, genes and neuronal networks: Heritability of instrumental conditioning and the Arc/Arg3.1 gene in hippocampal coding
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Chapter 4. The role of Arc/Arg3.1 in spatial coding and rhythmic synchronization of hippocampal ensembles in the awake state

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In preparation to be submitted as: The activity-regulated cytoskeletal-associated protein (Arc/Arg3.1) controls rhythmic synchronization and sharp wave–ripple activity of hippocampal CA1 neurons during spatial behavior and sleep: candidate mechanisms for deficient memory formation and consolidation
Abstract

Arc/Arg3.1 is an immediate early gene whose mRNA is localized in dendrites of principal cells in various areas of the brain. It is prominently found in hippocampal pyramidal neurons, where its expression is rapidly increased upon exploration of a novel environment. Loss of Arc/Arg3.1 function has been previously linked to impaired late-phase acquisition of spatial learning and spatial reversal learning, as well long-term synaptic plasticity and memory consolidation.

To study the role of Arc/Arg3.1 in neural coding and oscillatory activity in the hippocampus during spatial exploration and navigation, we recorded hippocampal CA1 single unit and local field potential activity in Arc/Arg3.1 knockout and wildtype mice in different novel and familiar environments.

We found significant changes neither in behavioural activity nor general firing properties of pyramidal cells. Place fields of hippocampal cells in Arc/Arg3.1 KO mice were intact. However, we found a significant attenuation of power in the higher frequency range of local field potential oscillations in KO animals during exploration, particularly in the beta-2 (20-35 Hz) and gamma frequency bands (35-100 Hz). Furthermore, we found that locking of spiking activity to LFP oscillations in theta, beta and gamma bands was decreased in KO mice.

Enhancement of beta-2 activity in mouse hippocampus has been previously implicated in exploration of novel environments on a rapid time scale. Gamma oscillations have been linked to cognitive functions including working memory as well as formation and retrieval of long-term memory, and their attenuation may contribute to the previously observed learning deficits in Arc/Arg3.1 knockout animals. In addition, the decrement in phase-locking of spiking may help explain their impairment in encoding and retrieving spatial information.
1. Introduction

Arc/Arg3.1 is an immediate early gene that is rapidly expressed upon an animal’s exposure to a novel environment (Vazdarjanova et al., 2006). It is expressed in 30-40% of hippocampal pyramidal neurons, which is around the same proportion of hippocampal pyramidal neurons showing spatially selective firing rate changes (Guzowski et al. 1999). Following an exposure to a novel environment, the Arc/Arg3.1 gene is transcribed within minutes and the mRNA is transferred from the nucleus to cytoplasm and further to activated synaptic sites within 30 minutes (Guzowski et al., 1999; Steward and Worley, 2001). In support of a causal role of Arc/Arg3.1 in learning, knockout mice were significantly impaired both in the late acquisition phase and reversal learning of the Morris water maze task, despite normal basal behavioral characteristics (i.e. swimming velocity and floating times; Plath et al. 2006).

So far, studies on Arc/Arg3.1 function have been mainly restricted to the molecular machinery underlying synaptic plasticity (Chowdhury et al., 2006; Huang et al., 2007; Messaoudi et al., 2007; Peebles et al., 2010). Recent studies have shown Arc/Arg3.1 to be crucial for experience-dependent plasticity in mouse visual cortex (Gao et al., 2010; McCurry et al., 2010). However, the role of Arc/Arg3.1 in the coding of more complex, relational information, pertaining to configurations of objects in space and to navigation, is unknown. Given that Arc/Arg3.1 is expressed in hippocampus particularly during environmental exploration and is strongly involved in synaptic plasticity — which may be important in shaping and stability of place fields (McHugh et al., 1996; Mehta et al., 1997; Kentros et al., 1998) — we investigated the role of Arc/Ag3.1 in hippocampal coding while mice ran a circular-track task.

First, we assessed whether a genetic knockout of Arc/Arg3.1 function affects place cell characteristics such as place field size and information transmitted per spike, or alters the firing rate of spatially selective cells. Second, we studied whether Arc/Arg3.1 is involved in regulating oscillatory activity associated with spatial learning, such as theta, beta and gamma power. Place cells have a tendency to fire at specific phases of the theta oscillation cycle. This preferred phase advances as the animal moves through the place field, a phenomenon known as theta phase precession (O’Keefe and Recce, 1993). As precession results in a time-compressed representation of sequentially visited locations by place cells, it may subserve functions in memory encoding (Skaggs et al., 1996; Lisman, 2005; Maurer et al., 2006). Both hippocampal
theta power and strength of spike–field phase locking are correlated with improved learning outcomes (Berry and Thompson, 1978; Rutishauser et al., 2010; cf. Klimesch et al., 1996).

Theta phase covaries with gamma power (35-100 Hz), both in hippocampus and other brain areas such as the neocortex (Buzsáki et al., 1983; Sirota et al., 2003; Canolty et al., 2006; Colgin et al., 2009) and striatum (Tort et al., 2008). Unlike gamma-band activity, beta-2 activity (23-30 Hz) has not been shown to covary with theta, suggesting it has an independent role in information processing, for instance, during exploration of novel environments (Berke et al., 2008).

2. Methods

2.1. Mice

Arc/Arg3.1 KO and wildtype mice were bred at the Center for Molecular Neurobiology, University of Hamburg (Germany) and arrived at the local animal housing facility at the age of 5-7 weeks. After arrival, mice were habituated to the colony rooms set on a reversed day–night cycle (light off/on at 9.00/21.00 hrs) for at least 3 weeks prior to surgery. During the habituation period, mice were also offered sucrose pellets (14 mg, Bioserv, Frenchtown, NJ) in the home cage in addition to regular lab chow.

Before implantation, mice were housed in pairs with ad libitum access to food, except during pretraining, when animals were subjected to food restriction prior to training to achieve about 5% weight loss (see Chapter 2). Water was provided ad libitum in the home cage at all times. Two weeks before surgery, the experimenters started handling the animals and carried out pretraining sessions, during which mice learned to collect sucrose pellets while exploring a T-maze (not used for the recordings described here). Mice that did not learn to reliably explore the maze and consume the sucrose pellets were excluded from further experiments. All experimental procedures were approved by the institution’s Animal Welfare Committee and were in compliance with the European Council Directive (86/609/EEC) and Principles of laboratory animal care (NIH publication No. 86-23, revised 1985).
2.2. Behavioral paradigm

Each session, mice ran 20 laps unidirectionally on two different circular tracks (Fig. 1), motivated by sucrose pellets which were dropped on the tracks on random locations, about 10 pellets per running episode of 20 laps. Before and after track running, mice were allowed to rest or sleep for about 30 minutes in their home cage which was placed in the middle of the circular track. Prior to recordings, mice were food-restricted to about 5% weight loss. After recordings, mice had *ad libitum* access to food in the home cage.

Behavior and neuronal activity during task running and during preceding and subsequent rest phases were monitored in the same experimental room to maintain constant electrophysiological recording conditions and minimize environmental differences not under the control of the experimenter. To assess remapping of CA1 place fields in different environments, we created two distinct environments in the same room. Within this room, the circular tracks were placed at two non-overlapping locations. Multiple measures were taken to ensure that the mice would treat each condition as a different environment (Table 1): The circular tracks on which the mice ran were identical in shape and colour, but differed in odour, auditory noise, lighting level and surrounding cues, including the round arena around which the circular track was positioned (Fig. 1). A given cue on the wall of the recording enclosure was always repeated elsewhere on the same enclosure to prevent visual cuing of track locations. Aqueous odorized solutions were lightly sprayed on the circular track about half an hour before the beginning of each recording session. Noise was applied via a speaker placed beneath the arena. Furthermore, mice were transported from the colony rooms to experiments in different conditions via different routes. The order in which each environment was presented to the animal was recorded on a given day was alternated on consecutive days. Both conditions were presented once on a given day, meaning that each mouse had two novelty sessions (one for each session) followed by three sessions in a familiar environment.

Table 1. Recording environments. 'Transport route' pertains to the path from colony room to recording lab.

<table>
<thead>
<tr>
<th>Environment</th>
<th>odour</th>
<th>inner arena colour</th>
<th>walls</th>
<th>cues</th>
<th>noise</th>
<th>lighting</th>
<th>transport</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Grass'</td>
<td>grass</td>
<td>chocolate brown</td>
<td>black</td>
<td>white</td>
<td></td>
<td>dim</td>
<td>short</td>
</tr>
<tr>
<td>'Hay'</td>
<td>hay</td>
<td>light brown</td>
<td>white</td>
<td>black</td>
<td></td>
<td>bright</td>
<td>long</td>
</tr>
</tbody>
</table>
2.3. Electrodes, microdrive and surgery

All recordings were carried out using a custom-made, light-weight (1.6 gr) mouse microdrive with 6 independently moveable tetrodes (the "Lantern"; Battaglia et al. 2009). After loading the microdrive, tetrodes, each consisting of four polyimide coated nichrome wires (ø17.8 μm; Kanthal, PalmCoast, FL) twisted together, were gold-plated electrolytically in gold cyanide solution (Select Plating, Meppel, the Netherlands) to achieve an impedance of 600-1000 kΩ per lead.

Prior to implantation, mice were given a subcutaneous injection of buprenorphine (3 mg/kg; Temgesic, Schering-Plough, Kenilworth, NJ) for sedation and analgesia. Thirty minutes after injection, anesthesia was induced by 3% isoflurane in 100% oxygen, upon which the animal was placed into a stereotact (David Kopf Instruments, Tujunga, CA). Anesthesia was maintained with 1-2% isoflurane. Body temperature was maintained around 36.5 °C with a thermal pad. Six stainless steel screws were inserted into the exposed skull to support the microdrive. One of the supporting screws placed contralaterally to the implant served as ground.
A craniotomy of about 1.5 mm in diameter was made over the right hemisphere, -2.00 mm lateral and -2.00 mm posterior to bregma (Paxinos and Franklin, 2004). After removing the dura mater, the exit grid of the drive was placed on top of the brain. The connection was sealed with silastic elastomer (Kwik-Sil, World Precision Instruments, Berlin, Germany) and the drive was anchored to the supporting screws and skull with dental acrylic. Immediately after surgery, tetrodes were lowered by about 500 um and then gradually turned down to the CA1 pyramidal cell layer across several days, as indicated by sharp wave–ripple oscillations and pyramidal cells exhibiting complex spiking activity. As functional criteria, only data from tetrodes with negative-going sharp wave–ripple complexes and place-active cells were included in further analysis.

After implantation, mice were kept in a cylindrical recovery cage having an elevated ceiling. During electrophysiological recordings, mice were 12-20 weeks of age. There was no significant difference in the average age of KO and WT mice (p = 0.27).

2.4. Data acquisition

The Lantern was connected to two 16-channel headstage preamplifiers via two omnetic connectors (Omnetics Connectors Corporation, Minneapolis, MN; type: NPD-18-FF-GS, Nano Dual Row Male, 18 contacts). The pre-amplifiers were in turn connected to the amplifiers via a commutator and tether cable (Neuralynx, Bozeman, MT). Spiking activity was referenced to one of the tetrodes which was positioned in the corpus callosum. For local field potentials (LFPs), we used either this reference electrode or the ground screw located in the contralateral hemisphere.

For single units, the signal was band-pass filtered to 600-6000 Hz. When the voltage signal exceeded an assigned threshold, the spiking activity was sampled at 32 kHz during a 1 ms time window. Local field potentials were sampled continuously at 2 kHz and band-pass filtered to 1-475 Hz.

Electrophysiological recordings were complemented with videotracking data acquired with Ethovision XT 5.1 software (Noldus, Wageningen, The Netherlands). These data were synchronised by transistor–transistor logic (TTL) pulses sent from the Ethovision system to the Neuralynx recording system. Automatically tracked coordinates of the mouse’s body center
position were manually inspected and corrected. Position data from Ethovision were visually inspected, corrected and exported to MATLAB for further analysis.

2.5. Histology

After the end of each experiment, end positions of tetrodes were marked by a lesion induced by 10 µA current through one lead per tetrode for 10 seconds. Mice were sacrificed the following day with an overdose of sodium pentobarbital (Euthasol; 80 mg/kg, ASTfarma BV, Oudewater, The Netherlands), after which a cardiac perfusion with saline, followed by paraformaldehyde (4%), was carried out. Brains were removed and further fixated in paraformaldehyde for at least a week before slicing them in 40 µm coronal sections with a vibratome. Brain slices were mounted on gelatin-coated object glasses and Nissl-stained.

Recordings of hippocampal neurons were made from locations between approximately 1.8 mm and 2.3 mm posterior and 1.2 mm and 2.4 mm lateral to bregma (Paxinos and Franklin, 2004). The anterior-posterior or medial-lateral positioning of tetrodes was very similar for WT and KO mice. For reference tetrodes, endpoints of the tetrode tracks were found in the corpus callosum. Endpoints of recording tetrodes were mostly in stratum pyramidale and sometimes in stratum radiatum, approximately 1/3 on the way to stratum lacunosum-moleculare (See Fig. 1D for examples).

2.6. Behavioral analysis

Locomotion speed was calculated based on the time-stamped location data of the animal as recorded by Ethovision XT. In addition to assessing the locomotion speed per session, these data were used in order to exclude spiking activity during periods of immobility from place field analysis (see 2.8.). All results were averaged over 27 (WT) and 19 (KO) track running episodes.

2.7. Analysis of local field potentials

For all LFP analyses, high-amplitude artifacts (>2000 µV, with margin of 25 ms) were discarded, and 50 Hz oscillation and its harmonics were removed by notch filtering. For power analysis, we used Fast Fourier Transform (FFT) with the Hamming tapering method (Kalenscher et al., 2010). Spectral power in a given frequency range was normalized to the average power between 1-
250 Hz. Average power was computed across all tetrodes in a given session. Hippocampal ripples were detected and analyzed as described in Chapter 5. Wilcoxon’s rank sum test was used for testing significance of LFP analysis.

2.8. Spike-sorting and single unit analysis

Spike data were pre-processed by a custom-made Python script (http://www.python.org/), which uses the waveform of each tetrode lead to compute the first three principal components of the spike waveforms. The resulting 12-dimensional vectors, describing each spike, were classified into clusters by KlustaKwik (Harris et al., 2000). These clusters were manually assessed and corrected using Klusters 1.6.4 (Hazan et al., 2006) running on Kubuntu 11.04, a free open source Ubuntu operating system distribution package to ensure that each cluster was well isolated from other clusters recorded on the same tetrode. Examples of isolated clusters are shown in Fig. 2A-F.

We only included clusters with a minimum of 100 spikes during the track-running episode and a maximum of 0.5% of interspike intervals shorter than 2 ms. Furthermore, we assigned these clusters to putative pyramidal cells or interneurons based on their waveform features, namely, initial slope of valley decay and half-decay time (Lansink et al., 2010). Clusters that could not be assigned to either class were excluded from all analyses presented in the results.

For putative pyramidal cells, rate maps were generated as described in Battaglia et al. (2009). Briefly, the square encompassing the circular track (Fig. 1) was divided into 100x100 position bins and the mouse’s location was determined for each videoframe to assign the location of the mouse to one of these bins for every time point. Data from epochs during which the animal was moving below a speed of 2 cm/s were excluded from place field analysis. The average firing rate was computed for each bin and Gaussian smoothing was applied to produce rate maps. A place field was classified as an area of at least 20 continuous bins where a cell’s firing rate was at least 30% of its peak firing rate. Place fields which were separated by less than 10 bins were merged and counted as a single place field; place fields smaller than 20 bins were excluded.

Measures used to quantify sharpness of place field tuning of spike trains were computed according to Skaggs et al. (1996). First, we calculated the spatial information per spike, which indicates how many bits of information each spike conveys:
where the environment was divided into non-overlapping spatial bins $i=1,...,N$ (100x100 bins, as above for the rate-maps), $p_i$ being the probability of bin $i$ being occupied, $\lambda_i$ the mean firing rate for bin $i$ and $\lambda$ the mean firing rate of the cell.

The second measure we quantified was sparsity, which indicates the extent to which spatially selective firing stands out relative to the mean firing rate.

$$Sparsity = \frac{\langle \lambda \rangle}{\langle \lambda^2 \rangle} = \frac{(\sum p_i \lambda_i)^2}{\sum p_i \lambda_i^2}$$

Sparsity is bounded between 0 and 1, 0 being maximally sparse and 1 meaning firing equally over the arena.

Finally, we computed the selectivity, which is defined as the maximal firing rate across spatial bins, divided by the mean firing rate. Student’s independent t-test was used for testing significance, unless otherwise specified.

**2.9 Spike–field phase locking and theta phase precession**

Phase-locking analysis was carried out using the methods as described in Vinck et al. (2012). We calculated pairwise phase consistency, a measure of spike–field locking which is not biased by spike count and is not affected by history effects within spike trains. Instead of comparing individual spikes to the LFP around them, pairwise phase consistency is based on the cross-spectra between spike trains and concurrent LFPs. Significance estimates were corrected for multiple comparisons following the randomization test of Westfall and Young (1993).

We analyzed phase precession for place cells with a sparsity (Skaggs et al., 1996; see above) of $< 0.25$. For a given cell, we defined the place field as running from the location of the (smoothed; Taylor, 2008) peak firing density to the locations where the firing density had reached 50% of the peak firing density. We then fit a linear-circular regression model to this data (Fisher and Lee, 1992; Schmidt et al., 2009).
3. Results

Four wildtype and three knockout mice were recorded in the circular track task, each for up to 8 sessions. A survey of animals, sessions and cell counts is given in Table 1. WT and KO mice exhibited a similar locomotion speed during track running: $7.29 \pm 0.33$ cm/s (WT) and $7.62 \pm 0.49$ cm/s (KO; $p=0.56$; all values are mean $\pm$ SEM unless otherwise noted). The amount of time they spent running on the track was also similar ($69 \pm 3\%$; WTs) vs. $70 \pm 5\%$; KOs; $p = 0.93$). The mice were also otherwise indistinguishable to the experimenter during recordings. Only putative pyramidal cells that fired at least 100 spikes during track running were included in the analyses presented here. Mean firing rates of putative pyramidal cells were very similar (WTs $1.36 \pm 0.26$ Hz; $N = 37$ cells; KOs $1.39 \pm 0.19$ Hz; $N = 73$ cells; $p = 0.92$; Fig. 2G). Firing rates of putative interneurons showed a difference, which was however not significant (WTs $4.87 \pm 1.70$ Hz; $N = 26$ cells; KOs $2.72 \pm 0.53$ Hz; $N = 21$ cells; $p = 0.29$).

Table 1. Table includes cells that fired > 100 spikes during circular track running and could be assigned to either putative pyramidal cells or interneurons.

<table>
<thead>
<tr>
<th></th>
<th>WTs</th>
<th>KOs</th>
</tr>
</thead>
<tbody>
<tr>
<td># Animals</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td># Sessions</td>
<td>27</td>
<td>19</td>
</tr>
<tr>
<td># Putative pyramidal cells</td>
<td>37</td>
<td>73</td>
</tr>
<tr>
<td># Putative interneurons</td>
<td>26</td>
<td>21</td>
</tr>
</tbody>
</table>

3.1. Place fields and ripple activity

While exploring the environment, both WT and KO animals showed place fields with variable sizes and discreteness (Fig. 2B and 2D). Comparing WT and KO mice, median place field areas were similar (Fig. 2H; Wilcoxon’s rank sum test, $p = 0.78$; median values are used here to avoid a strong bias imposed by outliers) and there was also no significant difference in the number of place fields in WT and KO mice (a mean of $2.31 \pm 0.27$ and $2.11 \pm 0.16$, respectively; independent t-test, $p = 0.51$). Spatial information (WT = $0.63 \pm 0.06$; KO = $0.63 \pm 0.05$ bits/spike; independent t-test, $p=0.99$), sparsity (WT = $0.54 \pm 0.05$; KO = $0.55 \pm 0.03$; independent t-test, $p = 0.74$) and selectivity (WT = $7.23 \pm 0.55$; KO = $7.26 \pm 0.52$; independent t-test, $p=0.97$)
Figure 2. Cluster isolation, rate maps and place fields.

A | Examples of isolated clusters from WT mice; X and Y axis indicate different principal components from different leads. B | Examples of rate maps corresponding to the two clusters from WT mice shown in (A). Colour bar indicates firing rate in Hz. C | Examples of isolated clusters from KO mice. D | Examples of rate maps of two cells from KO mice shown in (C).

E-F | Average waveforms and inter-spike interval (ISI) histograms of the above clusters. Shaded outlines around average waveforms on different tetrode leads indicate standard deviations. In the Interspike-interval (ISI) histograms, the dotted line indicates a 1 ms time interval. G | Mean firing rates for putative pyramidal cells in WT and KO. Error bars indicate standard error of the mean. H | Median place field size. I | Mean ripple rates during immobile periods on the track. * : p < 0.05, data normally distributed.
measures were also nearly identical. Place cells also showed remapping across the different environments in both WT and KO mice (Fig. S1). However, when we focused on immobile periods of the mouse pausing on the track, we found the incidence of hippocampal ripples to be decreased (WT = 1.15 ± 0.21 ripples / min; KO = 0.51 ± 0.17; independent t-test, p = 0.03; Fig. 2I). None of these measures showed a significant change over the course of the sessions, while mice were progressively familiarized to the environments, so data from all the sessions were pooled.

4.2. Local field potentials and rhythmic synchronization

An overview of LFP spectra of WT (N = 27 sessions) and KO (N = 19 sessions) mice during circular track running revealed that WT mice show higher power in several higher frequency bands as normalized to the mean power in the frequency range (1-250 Hz; Fig. 3A).

In the lower frequency bands (Fig. 3B), KOs expressed a slightly increased power in the delta range (1-4 Hz; Fig. 3C) and WTs showed a trend towards higher power in theta range (6-10 Hz; Fig. 3C), but these differences were not significant (p = 0.07 and p = 0.33, respectively; Wilcoxon’s rank sum test). There was also no significant change in the low beta (10-20 Hz) frequency range (p = 0.16; data not shown).

Oscillatory activity was significantly weaker in KOs in the beta-2 (20-35 Hz, p = 0.003; Fig. 2E), low gamma (35-45 Hz, p = 0.0008; Fig. 2F) and high gamma (60-100 Hz, p = 0.0005; Fig. 2G) ranges. In these ranges, 3 out of 4 WT mice showed clearly increased power as compared to all 3 KO mice. There were no significant changes from novel to familiar (1st to 4th) sessions in either WT or KO mice so all sessions were pooled together.

4.3. Spike–field phase locking

To test whether mass synaptic activity, as expressed in the LFP, affects hippocampal output patterns, we examined locking of hippocampal spiking to different oscillatory rhythms. Overall, locking of hippocampal CA1 neurons to LFP was weaker in KO as compared to WT animals across multiple frequency ranges (Fig. 4B; corrected for multiple comparisons). WTs showed a significantly stronger (p < 0.05) pair-wise phase consistency than KO mice particularly in the theta, beta-2 and high gamma range.
Figure 3. Local field potential spectra during track running in wildtype (WT) and knockout (KO) mice.

A | Normalized power spectra during circular track running in wildtype (dark gray) and KO (light gray) mice. Solid lines show the spectra averaged across sessions, while bands below and above indicate the 95% confidence intervals. Abscissa indicates the frequency range on a logarithmic scale. The small peak around 50 Hz is a remnant of the original 50 Hz peak.

B | As above, but abscissa now indicates the frequency range on a linear scale to illustrate the spectra in the lower frequency range.

C | Mean power in delta (1-4 Hz) range.

D | Theta (6-10 Hz).

E | Beta-2 (20-35 Hz).

F | Low gamma (35-45 Hz).

G | High gamma (60-100 Hz). Power in the various frequency ranges of interest was normalized to the average power in the whole spectrum (1-250 Hz). *** : p < 0.005.
In beta-2 and gamma ranges, phase-locking was consistently higher in all WT mice as compared to all KO mice. In the theta range, phase-locking was higher in 2 out of 3 WT mice as compared to all 3 KO mice; therefore the significance of this effect should be considered somewhat less certain than for beta-2 and gamma activity. When looking at pyramidal cells only, the KOs showed significantly reduced phase locking in the theta and beta-2 range, but not throughout the whole gamma range (Fig. 4C). In the interneuron population, the reduced phase locking effect was significant only in the theta and beta-2 range (Fig. 4D). The overall reduction in spike–field phase locking in KO mice may be due to an extremely strong form of theta phase precession, or to a general dysorganization of spike activity relative to LFP oscillations. We therefore tested whether WT and KO mice differ in theta phase precession.

We found theta phase precession to be significant for 4 out of 19 WT cells (p < 0.01, binomial test; two examples shown in Fig. 5) and 3 out of 8 KO cells (p < 0.01, binomial test) with an average T-statistic of the regression of -0.89 ± 0.36 (median ± SE median; n.s., Wilcoxon signed rank test) and -1.09 ± 1.04, respectively. The average theta phase difference between beginning and end of the place field amounted to -1.46 ±1.01 (median ± SE) radians (p = 0.15, Wilcoxon signed rank test) for WT and -1.56 ±1.05 radians for KO (difference between conditions n.s.). In conclusion, the strong reduction in spike–field phase locking in KO mice could not be ascribed to a notable difference in phase precession, as this phenomenon was only modestly manifested in both groups of mice.

5. Discussion

In line with an earlier study (Plath et al., 2006), Arc/Arg3.1 KO mice were indistinguishable from WT mice in terms of navigation behaviour. In this previous study, no changes in anxiety levels or locomotor activity were reported. Also in our study, WT and KO mice did not differ significantly in locomotion speed or the amount of time which they spent running.

Basic firing properties of hippocampal CA1 neurons (Fig. 2G) did not differ between WT and KO mice and pyramidal cells in both WT and KO animals exhibited similar place fields (Fig. 2B, D and H). Furthermore, place field size (Fig. 2H) and spatial information per spike appeared intact. However, the rate at which hippocampal ripples were generated during immobile periods on the track was significantly decreased in KO mice (Fig. 2I; see also Chapter 5 for discussion).
Figure 4. Spike–field phase locking.
A | Examples of spike-to-field locking in WT and KO animals in theta (6-10 Hz), beta-2 (20-35 Hz) and high gamma (60-100 Hz) ranges. Individual spikes are shown above filtered LFP traces. B | Pairwise phase consistency, an unbiased measure of spike-to-field locking, is plotted as a function of LFP frequency. Bands indicate SEMs. Significant differences between WT and KO mice were found in the theta, beta-2 and high gamma frequency bands. Both putative pyramidal cells and interneurons were included in this graph. C | Pairwise phase consistency for putative pyramidal cells only. Significant differences between WT and KO mice were found in the theta, beta-2 range, around 65-70 Hz and 95-100 Hz. D | Pairwise phase consistency for putative interneurons only. Significant differences between WT and KO were found in the theta and beta-2 range.

Interestingly, hippocampal LFP in KO mice showed attenuated power selectively in higher frequency ranges (Fig. 3A): KOs had significantly lower power in the beta-2, low and high gamma range (Fig. 3D-F) while relative delta power was slightly increased in KOs, reflecting an overall shift from high to low frequencies in Arc/Arg3.1 KO mice (Fig. 3B-C). Although there was no significant difference in power in the theta range, KO mice failed to show strong phase-
locking to theta rhythm, as opposed to WT mice. KO mice also showed decreased locking to oscillations in higher frequency ranges.

5.1 Single unit data and place field properties

Consistent with previous studies, which report that lack of Arc/Arg3.1 does not cause major changes in brain or cellular architecture and baseline synaptic signaling (Plath et al., 2006; McCurry et al., 2010), we found that firing rates of both putative pyramidal cells and interneurons were similar in WT and KO animals.

In KO mice, hippocampal cells expressed place fields that did not differ significantly from WT place fields in terms of size, number of place fields per cell or information content per spike (Skaggs et al., 1996). That Arc/Arg3.1 knockout animals display normal place fields is not too surprising taken that they acquire context-dependent fear conditioning as WT mice do (Plath et al., 2006). Considering another protein implied in long-term synaptic plasticity, knocking out the NR-1 subunit of the NMDA-receptor likewise did not abolish place fields (McHugh et al., 1996).

Previously, Plath et al. (2006) showed KO mice to have be impaired in the late acquisition phase of the Morris water maze task, which may suggest less precision in processing spatial information. However, in their study the training was spread out across multiple days and the significant difference between KO and WT mice emerged only around day 2-3, in line with the idea that the difference is independent of place field formation. Poor stability of place fields across days, and poor consolidation and retrieval of spatial information, may thus play a more important role in the observed learning deficit.

In line with a previously suggested consolidation deficit (Plath et al., 2006; Ploski et al., 2008), KO mice showed a reduced density of ripples during the immobile periods on the track. If ripples, that take place during pauses in between track running epochs, can be taken as markers of replay of behavioral experiences on the track (Foster and Wilson, 2006; Diba and Buzsáki, 2007; Davidson et al., 2009; Carr et al., 2011), decreased ripple activity during these periods may indicate that replay of past sequences is impaired, which may affect further strengthening of the synaptic matrices underlying these firing patterns and decrease the likelihood of them being consolidated during subsequent rest. Because of the close correlation between ripple
activity and replay, this is a plausible scenario which, however, must be tested further by studying actual replay of place-cell sequences.

On the other hand, Plath et al. (2006) showed that KO mice were impaired in the reversal phase of the Morris water maze task. In this case, the difference between WT and KO animals reached significance already on the first training day. Interestingly, heterozygous deletion of vesicular glutamate transporter type 1 (VGLUT1) in mice has been shown to cause both a specific deficit in spatial reversal learning in the water maze as well as impaired hippocampal LTP (Balschun et al., 2010). The observed spatial reversal learning deficit in Arc/Arg3.1 KO mice might thus be a result of their long-term synaptic plasticity deficit. When juxtaposed to our current data, an early reversal deficit is compatible with the decrease in ripple activity observed during pauses in track running. Indeed, disrupting such awake-state ripple activity by hippocampal stimulation hampers subsequent memory (Jadhav et al., 2012).

5.2. Local field potentials and rhythmic synchronization

Phase locking analysis indicated that theta phase precession was present (Fig. 5), but the phenomenon was not robust enough to make a quantitative comparison between WT and KO mice. Thus, while there was some tendency for phases to precess to earlier theta phases along the place field, larger sample sizes are required to reliably detect this effect, and to potentially detect differences between WT and KO mice. Nonetheless, the strong reduction in spike–field phase locking in KO mice (Fig. 4) cannot be accounted for by an extreme form of theta phase precession, because this would have been detectable in our data.

High frequency (>20 Hz) oscillatory activity was significantly reduced in Arc/Arg3.1 KO animals. Given that oscillatory activity in the beta-2 range has been connected with spatial exploration particularly in novel environments (Berke et al., 2008), and gamma range activity has been linked with cognitive functions, power attenuation in these ranges may lead to deficits in memory storage, retrieval and memory separation (for reviews, see e.g. Colgin et al., 2009; Colgin and Moser, 2010). In rats, low gamma has been associated with coupling of CA1 to CA3 whereas high gamma has been suggested to couple CA1 to entorhinal cortex. Low and high gamma would thus appear to have dissociable roles, possibly facilitating retrieval and intake of information, respectively.
Under normal conditions, place fields are formed rapidly – within minutes - upon exploration of a novel environment and are stabilized within around 30 minutes (Kentros et al., 1998; Knierim, 2002; Cheng and Frank, 2008). Interestingly, beta-2 activity peaks in the early stage of novel environment exploration (Berke et al., 2008). Beta-2 oscillations have been suggested to serve a role in organizing place cell activity in order to facilitate place field stabilization (Berke et al., 2008), although a causal relationship has not been demonstrated to date. In our data we did not find a significant difference in beta-2 activity between novel versus familiar sessions neither for WT nor Arc/Arg3.1 KO mice, although it must be added that the statistical power of session observations may not have been sufficient to detect modest differences. There were also some differences in the experimental paradigm. For instance, in the experiment by Berke et al., the animal started running laps immediately after being placed on the track, whereas in our experiments, the mouse was allowed to sleep in an enclosed cylinder in the middle of the arena for about half an hour before the beginning of each recording session. This may have resulted in a difference in stress levels. Further experiments are needed to examine whether loss of Arc/Arg3.1 affects differences in beta-2 oscillatory activity between novel and familiar environments.

**Figure 5: Theta phase precession.**  
A-B: Examples of theta phase precession displayed by two neurons from WT mice. Abscissa indicates the position of the mouse relative to the place field center (marked as 0) in radians and ordinate shows the preferred theta phase of the cell in radians. Phases are plotted across multiple cycles to reveal precession more clearly. In higher frequency ranges (>20 Hz), loss of phase locking may be at least partially ascribed to a loss of power in that frequency range (see Fig. 3 and Fig. 4). Although theta power in KO animals did not differ significantly from WT animals, we did find a significant loss of phase-
locking in the theta-band, which cannot be reasonably attributed to the subtle changes we found in theta power. The strong reduction in locking of spiking activity to theta oscillations points to a temporal dysorganization of neural activity, which may impair encoding of spatial information.

Attenuated power in the 20-45 Hz range may be attributed to Arc/Arg3.1–dependent long-term plasticity. Rat studies have shown that oscillations in the low gamma band originate from CA3-CA1 connectivity (Montgomery and Buzsáki, 2007; Colgin et al., 2009), which is likely regulated by Hebbian LTP and LTD. Loss of Arc/Arg3.1-dependent long-term plasticity may therefore explain why knocking out Arc/Arg3.1 reduces oscillatory activity in that range.

The attenuation of LFP power and spike–field phase locking in the gamma range (Fig. 3F-G) is particularly intriguing given that coherence in the gamma band has been suggested to serve as a communication mechanism for cell assemblies processing spatial information, and to play an important role in temporally structuring information for episodic memory storage (Lisman and Idiart, 1995; Jensen and Lisman, 2005; Montgomery and Buzsáki, 2007). The attenuation of both power and phase-locking in the gamma range, observed in KO mice, may thus contribute to previously demonstrated deficits in the late phase of spatial learning and reversal learning in the Morris water maze. Given that locking of neuronal firing to gamma rhythms is impaired in KO mice (Fig. 4), the capacity to organize and coordinate neuronal activity both within hippocampus and across brain areas might be hampered. However, one should be cautious in attributing a general importance of gamma-band activity to all phases of spatial learning, because the early acquisition phase of the Morris water maze task was not impaired (Plath et al., 2006).

In the rat hippocampus, activating the cannabinoid receptor CB1 has been shown to disrupt spike timing while population firing rates remain nearly intact. It also decreased hippocampal oscillations, particularly in the theta, gamma and ripple ranges. These findings were accompanied by an impairment of hippocampus-dependent memory (Robbe et al., 2006). Similar mechanisms could underlie the memory impairment of Arc/Arg3.1 KO mice as well, taken the decreased power in higher frequency ranges and decreased correlated firing (Chapter 5) against a background of normal tracking-running firing rates.
Exploratory behaviour has been shown to induce Arc expression only in CaMKII-positive principal neurons. Generally, Arc/Arg3.1 is not thought to be expressed in interneurons under behaviorally naturalistic circumstances, although pathological conditions such as seizures or electroconvulsive shocks may trigger its expression also in interneurons (Vazdarjanova et al., 2006). The lack of Arc/Arg3.1 expression in interneurons by no means implies that it has no effect on LFP oscillatory activity. In Arc/Arg3.1 KO mice, the deletion is already present during embryonic development, meaning that synapses may not be subject to normal long-lasting modifications during maturation, which may in turn affect the development of normal network activity, including oscillatory activity. The attenuation in high frequency oscillations observed in Arc/Arg3.1 KO mice is likely to be dependent on altered pyramidal cell–interneuron afferent connections, either intrinsic to hippocampal circuitry or present in upstream neocortical circuits including entorhinal cortex (Colgin et al., 2009).

To conclude, although the effects of loss of Arc/Arg3.1 function may appear subtle when assessed at the level of single cell activity, abolished Arc-mediated long-term synaptic plasticity during development likely has severe system-level consequences. It may affect pyramidal cell–interneuron connections and related circuitries that generate LFP rhythms, thus impairing beta-2 and gamma oscillations, which may lead to deficits in memory storage, retrieval, and memory separation. Furthermore, plasticity deficits, resulting from aberrant rhythmicity, may affect fine-tuning of spatial encoding, capability of spatial reversal learning and impair long-term stabilization of place fields as well as consolidation and retrieval of spatial memory.

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Supplementary Figure 1. Remapping.
A | Example of a WT mouse place cell that exhibits a change in place field when the mouse was exposed to two different environments. B | Same for a KO mouse.