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Impaired path integration in mice with disrupted grid cell firing

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Path integration (PI) is a highly conserved, self-motion-based navigation strategy. Since the discovery of grid cells in the medial entorhinal cortex, neurophysiological data and computational models have suggested that these neurons serve PI. However, more direct empirical evidence supporting this hypothesis has been missing due to a lack of selective manipulations of grid cell activity and suitable behavioral assessments. Here we report that selective disruption of grid cell activity in mice can be achieved by removing NMDA glutamate receptors from the retro-hippocampal region and that disrupted grid cell firing accounts for impaired PI performance. Notably, the genetic manipulation did not affect the activity of other spatially selective cells in the medial entorhinal cortex and the hippocampus. By directly linking grid cell activity to PI, these results contribute to a better understanding of how grid cells support navigation and spatial memory.

Path integration (PI) is a basic form of navigation. It involves continuous integration of self-movement so that global vectors connecting past and present locations can be continuously updated. Animals memorize these vectors to travel between locations using straight paths1–4. Experiments combining brain lesions and behavioral assays pointed to the hippocampus (HP) and the retro-hippocampal region (RH) as major players in PI1–6. The RH region (comprising the entorhinal cortex, subiculum, presubiculum, parasubiculum and parahippocampal cortex) contains different types of spatially tuned cell types, i.e., grid, head direction, border and speed cells, which are thought to contribute to space representation7–11. Grid cells display a stable periodic hexagonal firing pattern, and they are modulated by heading direction and running speed12,13. Together, these features can, in principle, enable the integration of self-motion-based information about traveled distance and running direction into a metric representation of space necessary for PI7,14,15.

Evaluating the link between grid cell activity and PI entails both adequate behavioral assays and selective manipulation of neuronal activity. Recently, we developed a behavioral assay, the L-maze, in which the percent infection in the HP region was 3% (Fig. 1d). In contrast, NMDAR-mediated currents were virtually abolished in NR1β−/− mice (mean ± s.e.m., WT: −240.54 ± 19.54 pA, n = 9; WT-GFP+: −204.2 ± 24.21 pA, n = 10; NR1β−/−: −230.45 ± 19.66 pA, n = 11; one-way ANOVA: F9,11 = 0.67, P = 0.52). As a result, the NMDAR/AMPAR ratio in cells of NR1β−/− mice was significantly smaller than in cells of WT and WT-GFP+ mice (Fig. 1b).

A second group of injected mice was subjected to behavioral tests and/or in vivo electrophysiological recordings after they reached adulthood (Fig. 1a). After completion of the experiments, the location and volume of the viral infection were determined for each mouse. As expected, the virus infection (indicated by the GFP signal) was not always confined to the MEC. In some mice, it spread to other structures within the RH region and to the HP. Using serial sections of each brain, we measured the extent of the viral infection in the RH and HP regions (henceforth, percent infection in the RH and HP regions, respectively; Fig. 1c and Supplementary Fig. 1). A threshold value of 3% was arbitrarily set to assign the mice to one of the two experimental groups: (i) NR1β−/− mice, in which the percent infection in the HP region was <3%, and (ii) NR1β0/0−/− mice, in which the percent infection in the HP region was >3% (Fig. 1d).

The control group included wild-type mice injected with AAV-Syn-Cre-GFP and NR1flox mice injected with AAV-Syn-Tomato. For further details about the viral infection quantification and its limitations, see Supplementary Fig. 1 and Methods.

Disruption of grid cell activity in NR1β−/− and NR1β0/0−/− mice. We conducted in vivo extracellular recordings to investigate

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the effect of region-specific NMDAR ablation on MEC neuronal activity. To do this, we unilaterally implanted five to six independently movable tetrodes in the MEC and recorded the activity of neurons as mice ran in a square arena. The number of recording sessions, the position of the tetrodes, the isolation distance of the recorded neurons and the running speed of the mice in the arena were similar across experimental groups (Supplementary Table 1 and Supplementary Fig. 2). We sorted the recorded neurons according to their mean firing rate into putative principal cells (<5 Hz) and interneurons (≥5 Hz) and found that the numbers of cells recorded per session, the percentages of cells and their firing rates did not differ across groups for both neuronal types (Supplementary Table 1). Moreover, rhythmic firing at theta frequency was preserved in NR1<sup>flx/flx</sup> and NR1<sup>flx/flx</sup><sup>−/−</sup> mice (Supplementary Fig. 3a–c). Theta rhythmicity in NR1<sup>flx/flx</sup><sup>−/−</sup> mice tended to be higher than in the other two groups, and theta modulated neurons in mutant mice had a lower peak frequency than neurons in control mice (Supplementary Fig. 3d,e).

Next, we characterized the spatial properties of the entire population of principal neurons (unless otherwise specified, all scores were computed using mice as experimental units). To estimate their spatial selectivity, i.e., the extent to which they fired in particular locations of the arena, we calculated the spatial information score. Principal cells in NR1<sup>flx/flx</sup> and NR1<sup>flx/flx</sup><sup>−/−</sup> mice showed significantly lower spatial information scores than in control mice (Fig. 2a). To measure how well multiple firing fields fitted to the typical hexagonal pattern of grid cells, we calculated the grid score. Principal cells in NR1<sup>flx/flx</sup> and NR1<sup>flx/flx</sup><sup>−/−</sup> mice had significantly lower grid scores than in control mice (Fig. 2a). In addition to the comparisons across groups, we took advantage of the variability in injection size (Fig. 1d) and correlated the percent of infection and the different electrophysiological variables. Notably, both spatial information and grid scores of principal cells in mutant mice were inversely correlated with the percent of infection in the RH region but not in the HP region (Fig. 2b and Supplementary Table 2). As expected, these correlations were absent in control mice, in which the virus did not lead to the ablation of NMDARs (Supplementary Table 2). The remaining firing features of principal neurons were similar across groups (Fig. 2a). These were: (i) the number of firing fields; (ii) the head direction score, indicating how well the firing was tuned to a preferred head direction; (iii) the border score, indicating the tendency to fire along a wall of the arena; and (iv) the speed score, indicating the extent to which the firing was modulated by the running speed of the animal. Similar results were obtained when statistics were performed using cells as experimental units (Supplementary Table 3). Therefore, MEC principal neurons in mutant mice exhibited disrupted grid spatial periodicity and spatial selectivity.

Within MEC principal neurons, we defined six mutually exclusive cell populations: grid cells, irregular spatially selective (ISS)
cells, border cells, head direction (HD) cells, speed modulated (SM) cells and nonspatially selective (NSS) cells (Fig. 2c; see Methods for details). We determined the percentage of each cell type per mouse and found that only the percentages of grid cells and ISS cells changed across genotypes, i.e., the percentages of grid cells and ISS cells were significantly lower in mutant than in control mice (Fig. 2c). Therefore, in mutant mice, disrupted grid periodicity and spatial selectivity (Fig. 2a) led to a decrease in the percentages of grid cells and ISS cells, which seemed to lead to a concomitant increase in the percentage of NSS cells, although the differences did not reach significance (Fig. 2c). The percentages of border cells, HD cells and SM cells were similar across genotypes (Fig. 2c). Furthermore, we found that in mutant mice the percentage of grid cells was inversely correlated with the percent infection in the RH region but not in the HP region (Fig. 2d, Supplementary Table 2). Such correlation was absent in control mice (Supplementary Table 2).

Next, we further characterized the firing properties of each of the six principal cell populations (Fig. 3, Table 1 and Supplementary Table 3). Grid cells were found in 86% of control mice (7 of 8) and in 79–72% of mutant mice (15 of 19 in NR1<sup>RIH</sup>−/− mice and 8 of 11 in NR1<sup>RIH&HP</sup>−/− mice). Critically, in the mutant mice in which grid cells were found, the percentage of grid cells was significantly smaller than in controls (mean ± s.e.m., control: 14.26 ± 4.25%, NR1<sup>RIH</sup>−/−: 4.69 ± 0.61%, NR1<sup>RIH&HP</sup>−/−: 4.61 ± 0.98%; one-way ANOVA followed by Tukey’s multiple comparisons: F<sub>2,27</sub> = 6.55, P = 0.005; control vs. NR1<sup>RIH</sup>−/−, P = 0.004; control vs. NR1<sup>RIH&HP</sup>−/−, P = 0.03; NR1<sup>RIH</sup>−/− vs. NR1<sup>RIH&HP</sup>−/−, P = 0.92). Moreover, in around 25% of mutant mice, only one grid cell was found (4 of 15 in NR1<sup>RIH</sup>−/− mice, 2 of 8 in NR1<sup>RIH&HP</sup>−/− mice), which was never the case in control mice (Supplementary Fig. 4). In sum, mutants had significantly fewer grid cells than control mice (Fig. 2c and Supplementary Fig. 4). The few grid cells recorded in mutant mice showed grid scores similar to those in controls (Fig. 3a and Table 1), but they were less spatially selective, i.e., grid cells in mutant mice showed significantly lower spatial information scores than those in control mice (Fig. 3a and Table 1). Also, grid cells in mutant mice had a lower peak firing rate (Table 1). These differences across groups were also evident when cells were used as experimental units (Supplementary Table 3). Additionally, grid cells in mutant mice were less stable than in controls, but only when cells were used as experimental units (Supplementary Table 3). In mutant mice, the spatial information score, the number of fields and the peak firing rate of grid cells were negatively correlated with the percent infection in the RH region but not in the HP region (Supplementary Table 2). All other firing features of grid cells were similar across groups (Table 1 and Supplementary Table 3). Overall, we conclude that mutant mice had fewer grid cells and that the remaining grid cells were less spatially selective, less stable, had a lower peak firing rate and tended to have fewer firing fields than those recorded in controls. Thus, ablation of NMDAR in the RH region disrupted the normal firing of grid cells.

ISS cells constituted a heterogeneous group, including cells that almost reached the criteria that would classify them as grid cells (Fig. 3b). Like grid cells, ISS cells also tended to be less frequent in mutant mice, as they were found in 86% of control mice (7 of 8) and in 79–72% of mutant mice (15 of 19 in NR1<sup>RIH</sup>−/− mice and 8 of 11 in NR1<sup>RIH&HP</sup>−/− mice). In addition, ISS cells in mutant mice had significantly lower grid scores than those in control mice (Fig. 3b), while the other firing features remained similar across groups (Table 1). Comparable results were obtained when statistics were performed using cells as experimental units (Supplementary Table 3).

Border cells were found in all recorded mice and in similar proportions (Fig. 2c). The firing of border cells was affected only when the virus infection extended to the HP (NR1<sup>RIH&HP</sup>−/− mice), while it remained intact in NR1<sup>RIH</sup>−/− mice (Fig. 3c, Table 1, Supplementary Table 3 and Supplementary Fig. 5). Specifically, the border score and spatial information score of border cells were significantly lower in NR1<sup>RIH&HP</sup>−/− than in NR1<sup>RIH</sup>−/− and control mice (Fig. 3c, Table 1 and Supplementary Table 3). These differences were no longer detected when more conservative thresholds were used (Supplementary Table 4). When the analysis was performed using cells as experimental units, we found that border cells in NR1<sup>RIH&HP</sup>−/− mice also showed a significantly lower head direction score and stability than those in the other two groups (Supplementary Table 3). Additionally, in mutant mice the border score and stability of border cells were inversely correlated with the percent infection in the HP region but not in the RH region (Supplementary Table 2). These correlations were absent in control mice (Supplementary Table 2). These results suggest that hippocampal NMDAR ablation had a detrimental effect on the firing properties of MEC border cells.

HD cells were also found in similar proportions across groups (Fig. 2c), and their firing properties were similar across groups (Fig. 3d, Table 1 and Supplementary Fig. 6). However, HD cells in NR1<sup>RIH&HP</sup>−/− mice showed a significantly higher head direction score than those in the other two groups of mice when cells were considered as experimental units (Supplementary Table 3). Accordingly, head direction selectivity of mutant mice was positively correlated to the percent infection in the HP region but not in the RH region (Supplementary Table 2). Finally, firing properties of SM cells and NSS cells did not differ across genotypes (Fig. 2c, Table 1 and Supplementary Table 3).

Since the MEC is one of the major cortical inputs to the HP and grid cells have been shown to project directly to the HP, we also asked whether NMDAR ablation in the RH region affected the activity of hippocampal place cells. We bilaterally implanted six independently movable tetrodes in each HP of control and mutant mice and recorded the activity of place cells as the mice ran in a square arena. The firing rates of place cells were similar across groups (Table 1). The spatial information score and the stability of place cells remained intact in NR1<sup>RIH</sup>−/− mice and were reduced only when the virus infection extended to the HP (NR1<sup>RIH&HP</sup>−/− mice; Fig. 4, Table 1 and Supplementary Fig. 7). Similar differences across groups were observed when cells were used as experimental units (Supplementary Table 3). In addition, in mutant mice, the spatial information score and the stability of place cells were inversely correlated with the percent infection in the HP region but not in the RH region (Supplementary Table 2). Hence, place cell firing in NR1<sup>RIH</sup>−/− mice was indistinguishable from controls, while it was impaired in NR1<sup>RIH&HP</sup>−/− mice, which is in agreement with previous studies showing that normal place cell firing depends on hippocampal NMDAR signaling.

Altogether, our results demonstrate that NMDAR ablation in the RH region disrupted grid spatial periodicity and spatial selectivity of MEC principal neurons. This was reflected in a reduction in the number of grid cells and ISS cells, as well as a disruption of their normal firing. At the same time, head direction selectivity, border selectivity and speed modulation of MEC principal neurons, as well as place cell activity in the HP, remained unaffected. Hence, NR1<sup>RIH</sup>−/− mice constitute a valid tool to test whether normal grid cell firing is necessary for PI computation.

**Impaired path integration in NR1<sup>RIH</sup>−/− and NR1<sup>RIH&HP</sup>−/− mice.** We examined the performance of control, NR1<sup>RIH</sup>−/− and NR1<sup>RIH&HP</sup>−/− mice in the L-maze assay (Fig. 5). In this assay, mice first swim in complete darkness and reach a submerged platform at the far end of a corridor during an initial ‘sample’ trial. Subsequently, during the ‘test’ trial, the corridor is removed and they must find the platform. PI allows the mouse to swim along the corridor to build a global vector that, in its reversed form, enables them to quickly find the platform during a subsequent swim in the open tank, thereby minimizing the time spent in the water.

During the sample trials, mice were exposed...
to one of three corridors, referred to as Str. (straight), L-S (long–short) and S-L (short–long), in which the platform was located at 0°, 30° and 60° relative to the start box, respectively. Accordingly, the direction of the resulting global vector during the test trial should approach 0°, 30° and 60°, respectively (Fig. 5a). At the end of the experiment, each mouse swam between 8 and 10 times per condition, following a pseudorandom sequence. Calculation of individual mean global vectors from several test trials was performed as shown in Supplementary Fig. 8a. Performance did not improve with trial number (Supplementary Fig. 8b). In line with our previous study, control mice clearly exhibited different global vector directions during Str., L-S and S-L corridor swims (Figs. 5b, c). This was not the case for NR1Rh−/− and NR1RhNMDAR−/− mice, for which global vector directions in the Str. and L-S conditions overlapped (Figs. 5b,c). Vector directions across genotypes for each corridor configuration were also compared. In the Str. configuration, no differences were found across genotypes (Fig. 5c). Conversely, in the L-S and S-L configurations, vector directions differed across genotypes, i.e., the values were significantly smaller in mutant mice (Fig. 5c). The length of the resulting global vector within and across genotypes was also analyzed (Fig. 5d). Control mice showed similar vector length in all corridor configurations (Fig. 5d). In mutant mice, on the contrary, the vector length in the S-L configuration was shorter than in the Str. and L-S configurations (Fig. 5d). Comparison of the vector length across genotypes within each condition showed no difference for the Str. and L-S conditions (Fig. 5d). In contrast, in the S-L condition, the length of the global vector was shorter in mutants than in control mice (Fig. 5d). The differences across genotypes could not be accounted for by changes in swimming speed, since the mice from all groups swam at similar speeds during both sample and test trials in the three conditions (Fig. 5e). Therefore, the deficient performance of mutant mice in the L-S and S-L conditions (L-S: vector direction; S-L: vector direction and length) is consistent with a PI impairment (see also Supplementary Fig. 9).

To confirm the specificity of the behavioral impairment, mice were also tested on two additional tasks: a win–shift task on an elevated T-maze and a beaconing task in the water maze (Supplementary Figs. 10 and 11). The performances of control and NR1Rh−/− mice in the win–shift task were similar, while NR1RhNMDAR−/− mice performed significantly worse (Supplementary Fig. 10b). The performance of mutant mice correlated with the percent infection in the HP region but not in the RH region (Supplementary Fig. 10c), which is in agreement with previous work showing that normal performance in this task depends on hippocampal NMDAR signaling. In the
Fig. 3 | Firing properties of grid cells, ISS cells, border cells and HD cells.

a. Firing rate maps (top) and mean (+ s.e.m.) grid and spatial information (inf.) scores (bottom) of grid cells for each genotype (one-way ANOVA followed by Tukey’s multiple comparisons, grid score: $F_{2,27} = 4.94; P = 0.01$; inf. score: $F_{2,27} = 0.7; P = 0.5$; control: $n = 7$, $NR1^{+/+}$: $n = 15$, $NR1^{-/-}$: $n = 8$). b, Firing rate maps (top) and mean (+ s.e.m.) grid and spatial information (inf.) scores (bottom) of ISS cells for each genotype (one-way ANOVA followed by Tukey’s multiple comparisons, grid score: $F_{2,27} = 0.07; P = 0.9$; inf. score: $F_{2,27} = 5.0; P = 0.01$; control vs. $NR1^{-/-}$: $P = 0.02$; control vs. $NR1^{-/-}$: $P = 0.005$; $NR1^{+/+}$ vs. $NR1^{-/-}$: $P = 0.31$; control: $n = 7$, $NR1^{+/+}$: $n = 15$, $NR1^{-/-}$: $n = 8$). c, Firing rate maps (top) and mean (+ s.e.m.) border and spatial information (inf.) scores (bottom) of border cells for each genotype. Numbers above each firing map correspond to the border score (left, black) and peak firing rate (right, red). d, Head direction firing rate polar plots (top) and mean (+ s.e.m.) HD and spatial information (inf.) scores (bottom) for each genotype. Numbers above each firing map correspond to the HD score (left, black) and peak firing rate (right, red). All tests were two-sided. **$P < 0.01$, *$P < 0.05$, ns: not significant after post hoc multiple comparisons. Yellow, red and blue symbols correspond to control, $NR1^{+/+}$ and $NR1^{-/-}$ mice, respectively.
### Table 1 | Firing properties of MEC and HP principal cells

|                          | Control | NRIPRM/- | NRIPHP/-/ |  
|--------------------------|---------|----------|-----------|
| **Grid cells (MEC)**     |         |          |           |  
| n (mice / cells)         | 7 / 207 | 15 / 83  | 8 / 33    |  
| Mean firing rate (Hz)    | 2.15 ± 0.05 | 1.97 ± 0.14 | 1.96 ± 0.27 |  
| Peak firing rate (Hz)    | 10.92 ± 1.10b | 7.85 ± 0.74a | 6.95 ± 1.29b |  
| Information score (bits/spike) | 0.56 ± 0.06a | 0.36 ± 0.04a | 0.29 ± 0.06a |  
| Grid score               | 0.74 ± 0.04 | 0.74 ± 0.02 | 0.73 ± 0.04 |  
| Number of fields         | 2.27 ± 0.33 | 1.46 ± 0.24 | 1.09 ± 0.36 |  
| Grid spacing (cm)        | 59.00 ± 1.91 | 56.70 ± 2.75 | 55.84 ± 3.01 |  
| Head direction score     | 0.15 ± 0.04 | 0.15 ± 0.03 | 0.23 ± 0.03 |  
| Border score             | 0.06 ± 0.07 | 0.19 ± 0.06 | 0.12 ± 0.09 |  
| Speed score              | 0.05 ± 0.01 | 0.06 ± 0.02 | 0.05 ± 0.01 |  
| Stability                | 0.59 ± 0.08 | 0.46 ± 0.06 | 0.63 ± 0.04 |  
| **ISS cells (MEC)**      |         |          |           |  
| n (mice / cells)         | 7 / 143  | 15 / 107 | 8 / 31    |  
| Mean firing rate (Hz)    | 1.35 ± 0.24 | 1.02 ± 0.10 | 1.02 ± 0.27 |  
| Peak firing rate (Hz)    | 9.39 ± 1.99 | 5.76 ± 0.69 | 7.82 ± 2.62 |  
| Information score (bits/spike) | 0.70 ± 0.03 | 0.66 ± 0.03 | 0.73 ± 0.08 |  
| Grid score               | 0.04 ± 0.02a | -0.05 ± 0.02a | -0.09 ± 0.03a |  
| Number of fields         | 1.14 ± 0.26 | 0.77 ± 0.16 | 0.99 ± 0.40 |  
| Head direction score     | 0.15 ± 0.003 | 0.19 ± 0.01 | 0.15 ± 0.02 |  
| Border score             | -0.25 ± 0.08 | -0.21 ± 0.09 | -0.40 ± 0.19 |  
| Speed score              | 0.02 ± 0.006 | -0.004 ± 0.01 | 0.008 ± 0.008 |  
| **Border cells (MEC)**   |         |          |           |  
| n (mice / cells)         | 8 / 390  | 19 / 414 | 11 / 236  |  
| Mean firing rate (Hz)    | 2.18 ± 0.08 | 2.20 ± 0.11 | 2.30 ± 0.12 |  
| Peak firing rate (Hz)    | 6.28 ± 0.47 | 6.44 ± 0.29 | 5.31 ± 0.36 |  
| Information score (bits/spike) | 0.27 ± 0.04a | 0.29 ± 0.03a | 0.17 ± 0.02b |  
| Grid score               | -0.02 ± 0.01 | -0.06 ± 0.01 | -0.03 ± 0.01 |  
| Head direction score     | 0.26 ± 0.02 | 0.24 ± 0.02 | 0.21 ± 0.03 |  
| Border score             | 0.58 ± 0.01a | 0.59 ± 0.01a | 0.56 ± 0.003a |  
| Speed score              | 0.03 ± 0.006 | 0.02 ± 0.008 | 0.03 ± 0.01 |  
| Stability                | 0.39 ± 0.06 | 0.44 ± 0.02 | 0.34 ± 0.03 |  
| **HD cells (MEC)**       |         |          |           |  
| n (mice / cells)         | 8 / 445  | 17 / 559 | 9 / 315   |  
| Mean firing rate (Hz)    | 1.52 ± 0.11 | 1.55 ± 0.09 | 1.82 ± 0.19 |  
| Peak firing rate (Hz)    | 6.08 ± 0.62 | 6.50 ± 0.38 | 6.61 ± 0.41 |  
| Information score (bits/spike) | 0.43 ± 0.04 | 0.46 ± 0.03 | 0.37 ± 0.04 |  
| Grid score               | -0.03 ± 0.01 | -0.03 ± 0.01 | -0.02 ± 0.04 |  
| Head direction score     | 0.58 ± 0.03 | 0.53 ± 0.02 | 0.56 ± 0.05 |  
| Border score             | -0.008 ± 0.03 | -0.04 ± 0.06 | 0.02 ± 0.06 |  
| Speed score              | 0.01 ± 0.005 | 0.03 ± 0.006 | 0.04 ± 0.01 |  
| **SM cells (MEC)**       |         |          |           |  
| n (mice / cells)         | 7 / 142  | 17 / 174 | 11 / 77   |  
| Mean firing rate (Hz)    | 2.17 ± 0.19 | 2.14 ± 0.21 | 2.05 ± 0.24 |  
| Peak firing rate (Hz)    | 9.42 ± 1.30 | 7.36 ± 0.67 | 6.19 ± 0.98 |  

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beaconing task, mice swam in an illuminated open tank and reached a tagged platform located at 0°, 30° or 60° relative to the start box (Supplementary Fig. 11a). This control experiment served to test for sensorimotor, motivational or learning deficits that might account for the differential performance in the L-maze. Control, NR1RH−/− and NR1RH&HP−/− mice successfully solved this task, i.e., global vectors differed across conditions within each genotype but not across genotypes within each condition (Supplementary Fig. 11b,c). Thus, the performance of mutant mice in the beaconing task was indistinguishable from that of controls. Beaconing swims represented optimal vectors for each condition, and we used them to quantify the directional deviation from optimal performance in all corridor configurations of the L-maze assay. Accordingly, we calculated the directional deviation from optimal performance in all corridor configurations of the L-maze assay. Place cells in the HP have been addressed before, but its contribution to MEC neurophysiological properties of MEC and HP neurons (Supplementary Table 5). Altogether, our results demonstrate that the severity of grid cell activity disruption correlated with the extent of PI impairment.

**Discussion**

Here we demonstrate that NMDAR ablation in the RH region disrupts grid cell firing and PI performance. The link between the degrees of grid cell and PI impairment provides strong experimental support for the hypothesis that grid cell activity underlies PI navigation.

We found that NMDAR ablation in the RH region reduced spatial selectivity and grid spatial periodicity of MEC principal cells. The manipulation led to a strong reduction in the number of grid cells and ISS cells (Fig. 6 and Supplementary Table 5). No correlation was found between the DOP score and the remaining electrophysiological properties of MEC and HP neurons (Supplementary Table 5). Altogether, our results demonstrate that the severity of grid cell activity disruption correlated with the extent of PI impairment.

|                         | Control       | NR1RH−/−     | NR1RH&HP−/−  |
|-------------------------|---------------|--------------|--------------|
| Information score (bits/spike) | 0.35 ± 0.06   | 0.25 ± 0.03  | 0.19 ± 0.04  | H = 5.69, P = 0.06 |
| Grid score              | 0.01 ± 0.02   | 0.0002 ± 0.03| -0.03 ± 0.03 | F = 3.32, P = 0.69 |
| Head direction score    | 0.12 ± 0.01   | 0.12 ± 0.02  | 0.11 ± 0.01  | F = 1.37, P = 0.32 |
| Border score            | 0.07 ± 0.05   | 0.16 ± 0.05  | 0.22 ± 0.08  | H = 4.51, P = 0.11 |
| Speed score             | 0.12 ± 0.01   | 0.16 ± 0.02  | 0.13 ± 0.01  | H = 3.05, P = 0.22 |

NSS cells (MEC)

- **n (mice / cells)**: 8 / 350, 19 / 486, 11 / 214
- **Mean firing rate (Hz)**: 1.46 ± 0.14, 1.75 ± 0.08, 1.45 ± 0.18
- **Peak firing rate (Hz)**: 4.84 ± 0.19, 5.70 ± 0.28, 3.84 ± 0.41
- **Information score (bits/spike)**: 0.18 ± 0.006, 0.17 ± 0.006, 0.16 ± 0.01
- **Grid score**: -0.02 ± 0.008, -0.02 ± 0.01, -0.01 ± 0.01
- **Head direction score**: 0.13 ± 0.006, 0.12 ± 0.007, 0.11 ± 0.01
- **Border score**: 0.10 ± 0.03, 0.12 ± 0.02, 0.17 ± 0.04
- **Speed score**: 0.008 ± 0.007, -0.007 ± 0.004, -0.0001 ± 0.001

Place cells (HP)

- **n (hemispheres / cells / mice)**: 8 / 206 / 4, 13 / 185 / 9, 7 / 148 / 6
- **Mean firing rate (Hz)**: 0.95 ± 0.16, 0.99 ± 0.16, 1.04 ± 0.14
- **Peak firing rate (Hz)**: 6.58 ± 0.88, 7.23 ± 1.19, 4.46 ± 0.82
- **Information score (bits/spike)**: 0.96 ± 0.07*, 1.03 ± 0.12*, 0.47 ± 0.14* |
- **Stability**: 0.59 ± 0.05*, 0.57 ± 0.05*, 0.31 ± 0.07* |

Mean ± s.e.m. using mice (MEC) or hemispheres (HP) as experimental units. One-way ANOVA or Kruskal–Wallis tests were used. Values with different superscripted letters (a, b) are statistically different (P < 0.05 after two-sided post hoc multiple comparisons) and values sharing the same letter are not.
One limitation of previous pharmacological and genetic approaches, is the lack of confinement to the grid cell network, thus precluding a direct test of the link between grid cell activity and PI. Here we overcame this limitation by ablating NMDARs in the RH region. The genetic manipulation disrupted grid periodicity while leaving intact the activity of the remaining MEC neurons and that of hippocampal cells. Our manipulation affected also other subregions within the RH region (subiculum, presubiculum, postsubiculum and parasubiculum) that contain spatially tuned cells\(^8,11,12\). We did not investigate the firing properties of these neurons. However, it is reasonable to assume that the effect of NMDAR ablation in these regions would be similar to the one found in the MEC, i.e., it is unlikely that the activity of spatially tuned cells other than grid cells is affected in these areas but not in the MEC upon virus infection. Although further experiments are required to confirm this assumption, NR1\(^{-/-}\) mice represent a powerful tool for testing the function of grid cells in general and the role of grid cell function in PI in particular.

MEC neurons in NR1\(^{-/-}\) and NR1\(^{B10HOP/-}\) mice exhibited similar firing properties, except for border cells and HD cells. On one hand, border cells of NR1\(^{B10HOP/-}\) mice had a lower border score and spatial selectivity (although this effect was no longer detectable when more conservative thresholds were applied). This finding points toward a putative contribution of the HP to the firing of MEC border cells. A previous study reported that HP inactivation did not affect MEC border cell firing, but the number of recorded border cells was small (\(n = 7\)). Further experiments would be necessary to determine whether or not normal HP activity is required for normal firing of MEC border cells. On the other hand, HD cells in NR1\(^{B10HOP/-}\) mice showed higher head direction selectivity, which is in line with previous reports showing that HP inactivation results in an increase in head direction modulation of MEC neurons\(^8\).

Notably, NMDAR ablation in the RH region also spared the firing of place cells in the HP. This is in agreement with previous studies showing that grid cell spatial periodicity is not essential for normal place cell firing\(^26,27,29\) and also with the observation that place cells mature (acquire adult-like firing properties) earlier than grid cells\(^5,11\). Hence, our results do not support models proposing grid cells as the primary source of spatial information for the formation of place cells\(^8\). One alternative, however, is that residual spatial information of MEC neurons suffices for the generation of normal place fields in the HP.

In rats, grid cell activity emerges postnatally after eye opening and a short period of exploratory behavior\(^3,5,7,11,31\), suggesting that the system might not be hardwired and that its development might require synaptic plasticity\(^5,8\). We removed functional NMDARs in young mice before eye opening and exploratory experience, most likely before grid cells emerged. It has been shown that NMDARs are necessary for the development of modularly arranged neural circuits in the visual and somatosensory cortices\(^34–36\). Grid cells in the RH region are also organized in a discretized manner\(^3\), and our results indicate that proper development of this network depends on NMDAR signaling. Hence, our findings support the hypothesis that an NMDAR-dependent learning process is required for normal development and/or functioning of the grid cell network\(^8\). Our results also suggest that network interactions between grid cells are likely to be necessary to maintain their periodic firing. Previous experimental data indicate that such network interactions could work either via direct connections between grid cells in layer III\(^19\) or via a recurrent network of inhibitory interneurons present in layer II of the MEC\(^9,40\). It is noteworthy that NMDAR ablation, and most likely the developmental plasticity conferred by the receptor, affected grid cells specifically.

Irrespective of the underlying mechanism leading to grid cell firing disruption, NMDAR ablation in the RH region allowed us to investigate the link between grid cell firing and PI. We used the L-maze assay, which has several advantages compared to the
Fig. 5 | NR1−+/− and NR1−−/− mice show impaired path integration. a, Schematics of the L-maze assay consisting of one sample trial (corridor swim) and a test trial (open tank swim) with intertrial intervals of 15 min. The experiment was performed in complete darkness and compass information was provided only by the position of the start box. b, Polar plots depicting individual mean global vectors (blue arrows) and average global vectors (red arrows) for each genotype and condition. c, Average global vector direction (circular mean ± s.e.m.). Top: comparisons within genotypes (Watson-Williams F-tests, control: F Str=22.44, P=1.32 × 10−3, Str vs. L-S: P=0.006, Str vs. S-L: P=8.07 × 10−4, L-S vs. S-L: P=0.0009; NR1−+/−: F Str=19.12, P=1.58 × 10−3, Str vs. L-S: P=0.03, Str vs. S-L: P=8.35 × 10−3, L-S vs. S-L: P=2.04 × 10−3; NR1−−/−: F Str=16.28, P=1.14 × 10−4, Str vs. L-S: P=0.86, Str vs. S-L: P=1.69 × 10−5, L-S vs. S-L: P=1.6 × 10−5). Bottom: comparisons across genotypes (Watson-Williams F-tests, Str: F Str=1.02, P=0.37, L-S: F Str=4.21, P=0.02, control vs. NR1−+/−: P=0.01, control vs. NR1−−/−: P=0.009, NR1−+/− vs. NR1−−/−: P=0.82; S-L: F Str=3.54, P=0.03, control vs. NR1−+/−: P=0.02, control vs. NR1−−/−: P=0.02, NR1−+/− vs. NR1−−/−: P=0.67). d, Global vector length (mean ± s.e.m.) for each genotype and condition. The top and bottom panels show comparisons within and across genotypes, respectively (two-way repeated-measures ANOVA, interaction: F Str=2.45, P=0.04, condition effect: F Str=2.22, P=0.11, genotype effect: F Str=1.39, P=0.25; top: Tukey’s multiple comparisons, control: Str vs. L-S: P=0.88, Str vs. S-L: P=0.71, L-S vs. S-L: P=0.44; NR1−+/−: Str vs. L-S: P=0.74, Str vs. S-L: P=0.03, L-S vs. S-L: P=0.03; NR1−−/−: Str vs. L-S: P=0.80, Str vs. S-L: P=0.01, L-S vs. S-L: P=0.03; bottom: Tukey’s multiple comparisons, control vs. NR1−+/−: P=0.90, control vs. NR1−−/−: P=0.74, NR1−+/− vs. NR1−−/−: P=0.38; L-S: control vs. NR1−+/−: P=0.89, control vs. NR1−−/−: P=0.72, NR1−+/− vs. NR1−−/−: P=0.93; S-L: control vs. NR1−+/−: P=0.0007, control vs. NR1−−/−: P=0.009, NR1−+/− vs. NR1−−/−: P=0.59). e, Mean (± s.e.m.) swimming speed during sample (top) and test (bottom) trials of the L-maze assay (two-way repeated-measures ANOVA, sample trial: interaction: F Str=0.18, P=0.95, condition effect: F Str=2.17, P=0.08, genotype effect: F Str=0.43, P=0.65; test trial: interaction: F Str=0.94, P=0.44, condition effect: F Str=2.51, P=0.08, genotype effect: F Str=0.64, P=0.53). f, Mean (± s.e.m.) DOP score for each genotype (one-way ANOVA followed by Tukey’s multiple comparisons, F Str=6.86, P=0.002, control vs. NR1−+/−: P=0.02, control vs. NR1−−/−: P=0.0004, NR1−+/− vs. NR1−−/−: P=0.012; control: n=17, NR1−+/−: n=28, NR1−−/−: n=28). Mice were used as experimental units. All tests were two-sided. *P<0.05, **P≤0.01, ***P≤0.001, ns: not significant. White, light gray and dark gray bars correspond to the Str, L-S and S-L conditions, respectively. Yellow, red and blue bars correspond to control, NR1−+/− and NR1−−/− mice, respectively.
Fig. 6 | Impaired PI integration correlates with altered grid cell firing in mutant mice. a. Relationship between DOP score and percentage of grid cells and NSS cells (Pearson correlation, percent grid cells: $r = -0.42$, $P = 0.02$; percent NSS cells: $r = 0.29$, $P = 0.08$; $n = 23$). b. Relationship between DOP score and the spatial information (inf.) score and grid score of MEC principal neurons (Pearson correlation, inf. score: $r = -0.43$, $P = 0.02$; grid score: $r = -0.52$, $P = 0.005$; $n = 23$). c. Relationship between DOP score and the spatial information (inf.) score and grid score of grid cells (Pearson correlation, inf. score: $r = -0.21$, $P = 0.20$; grid score: $r = -0.47$, $P = 0.02$; $n = 18$). d. Relationship between DOP score and the spatial information (inf.) score and grid score of ISS cells (Pearson correlation, inf. score: $r = -0.29$, $P = 0.11$; grid score: $r = -0.41$, $P = 0.03$; $n = 20$). All other correlations between DOP score and the remaining electrophysiological properties of MEC neurons were not significant (Supplementary Table 5). Mice were used as experimental units. All tests were two-sided. *$P < 0.05$, **$P \leq 0.01$, ns: not significant.

commonly used food-carrying task. First, the L-maze does not require previous training. Naïve mice swim proficiently and minimize the time in the water from the very first trial, which reduces interindividual variations arising from learning and motivation components. Second, due to the initial corridor swim, the outbound path is imposed by the experimenter instead of the animal. This allows experimenters to control the accumulation of errors inherent to PI, reduces the variability among animals and allows clear predictions about the direction and length of the expected global vectors. Last, the assay allows control of mouse-borne olfactory cues.

Our results clearly showed that mutant mice were unable to compute proper global vectors in the L-maze (they performed vectors of altered direction in the L-S condition and vectors of altered direction and length in the S-L condition). This was true even when the viral infection did not affect the complete RH region. Thus, NMDAR ablation in just a part of the RH network was enough to induce a significant PI impairment. Taken together, our data demonstrate that NMDAR ablation in the RH region disrupts both grid cell firing and PI performance. Notably, we were able to confirm the link between these two variables by using the same set of mice for behavioral and in vivo electrophysiological recordings. While our findings are, to our knowledge, the first to demonstrate an important role of grid cell firing in PI, they do not exclude the possibility that other, yet-unknown, cell types within and outside the RH region also contribute to this function.

Our results are relevant for several reasons. First, they offer new insight into the function of NMDARs in the RH area, both at the cellular and behavioral level. Second, they support the idea that NMDAR-dependent plasticity is required for the normal development and/or functioning of the grid cell network. Third, they suggest that grid cell firing is not essential for the development or functioning of hippocampal place cells. Fourth, ever since the discovery of grid cells, theoretical considerations based on an overwhelming amount of neurophysiological and computational data have led to the hypothesis that these spatially selective neurons support PI. Here we provide experimental evidence supporting this hypothesis.

Methods
Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41593-017-0039-3.

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References
1. Mittelstaedt, M. L. & Mittelstaedt, H. Homing by path integration in a mammal. Naturwissenschaften. 67, 566–567 (1980).
2. Wehner, R. Desert ant navigation: how miniature brains solve complex tasks. J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol. 189, 579–588 (2003).
3. Allen, K. et al. Impaired path integration and grid cell spatial periodicity in mice lacking GluA1-containing AMPA receptors. J. Neurosci. 34, 6245–6259 (2014).
4. Maaswinkel, H., Jarrard, L. E. & Whishaw, I. Q. Hippocampectomized rats are impaired in homing by path integration. Hippocampus. 9, 553–561 (1999).
5. Perron, C. & Save, E. Evidence for entorhinal and parietal cortices involvement in path integration in the rat. Exp. Brain. Res. 159, 349–359 (2004).
6. Van Cauter, T. et al. Distinct roles of medial and lateral entorhinal cortex in spatial cognition. Cereb. Cortex. 23, 451–459 (2013).
7. Blackstad, T. W. Commissural connections of the hippocampal region in the rat, with special reference to their mode of termination. J. Comp. Neurol. 105, 417–537 (1956).
8. Taube, J. S., Muller, R. U. & Ranck, J. B. Jr. Head-direction cells recorded from the postsubiculum in freely moving rats. I. Description and quantitative analysis. J. Neurosci. 10, 420–435 (1990).
9. Fyhn, M., Molden, S., Witter, M. P., Moser, E. I. & Moser, M.-B. Spatial representation in the entorhinal cortex. Science 305, 1258–1264 (2004).
10. Solstad, T., Boccara, C. N., Kropff, E., Moser, M.-B. & Moser, E. I. Representation of geometric borders in the entorhinal cortex. *Science* **322**, 1865–1868 (2008).

11. Lever, C., Burton, S., Jeeves, A., O'Keefe, J. & Burgess, N. Boundary vector cells in the subiculum of the hippocampal formation. *J. Neurosci.* **29**, 9771–9777 (2009).

12. Boccara, C. N. et al. Grid cells in pre- and parasubiculum. *Nat. Neurosci.* **13**, 987–994 (2010).

13. Kropff, E., Carmichael, J. E., Moser, M.-B. & Moser, E. I. Speed cells in the medial entorhinal cortex. *Nat. Neuroscience* **523**, 419–424 (2015).

14. Hafling, T., Fynh, M., Molden, S., Moser, M.-B. & Moser, E. I. Microstructure of a spatial map in the entorhinal cortex. *Nature* **436**, 801–806 (2005).

15. Sargolini, F. et al. Conjunctive representation of position, direction, and velocity in entorhinal cortex. *Science* **312**, 758–762 (2006).

16. McNaughton, B. L., Battaglia, F. P., Jensen, O., Moser, E. I. & Moser, M.-B. Path integration and the neural basis of the ‘cognitive map’. *Nat. Rev. Neurosci.* **7**, 663–678 (2006).

17. Moser, E. I., Kropff, E. & Moser, M.-B. Place cells, grid cells, and the brain's spatial representation system. *Annu. Rev. Neurosci.* **31**, 69–89 (2008).

18. Monyer, H. et al. Heteromeric NMDA receptors: molecular and functional distinction of subtypes. *Science* **256**, 1217–1221 (1992).

19. Zhang, S. J. et al. Optogenetic dissection of entorhinal-hippocampal functional connectivity. *Science* **340**, 1232627 (2013).

20. McHugh, T. J., Blum, K. I., Tisen, J. Z., Tonegawa, S. & Wilson, M. A. Impaired hippocampal representation of space in CA1-specific NMDAR1 knockout mice. *Cell* **87**, 1339–1349 (1996).

21. Nakazawa, K. et al. Hippocampal CA3 NMDA receptors are crucial for memory acquisition of one-time experience. *Neuron* **38**, 305–315 (2003).

22. Cabral, H. O., Fouquet, C., Rondi-Reig, L., Pennartz, C. M. & Battaglia, F. P. Single-trial properties of place cells in control and CA1 NMDA receptor subunit 1-KO mice. *J. Neurosci.* **34**, 15861–15869 (2014).

23. McHugh, S. R., Niewoehner, B., Rawlins, J. N. & Bannerman, D. M. Dorsal hippocampal N-methyl-D-aspartate receptors underlie spatial working memory performance during non-matching to place testing on the T-maze. *Behav. Brain. Res.* **186**, 41–47 (2008).

24. Tisen, J. Z., Huerta, P. T. & Tonegawa, S. The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. *Cell* **87**, 1327–1338 (1996).

25. Bonnevie, T. et al. Grid cells require excitatory drive from the hippocampus. *Nat. Neurosci.* **16**, 309–317 (2013).

26. Koenig, J., Linder, A. N., Leutgeb, J. K. & Leutgeb, S. The spatial periodicity of grid cells is not sustained during reduced cortical oscillations. *Science* **332**, 592–595 (2011).

27. Brandon, M. P. et al. Reduction of theta rhythm dissociates grid cell spatial periodicity from directional tuning. *Science* **332**, 595–599 (2011).

28. Winter, S. S., Clark, B. J. & Taube, J. S. Spatial navigation. Disruption of the head direction cell network impairs the parahippocampal grid cell signal. *Science* **347**, 870–874 (2015).

29. Miao, C. et al. Hippocampal remapping after partial inactivation of the medial entorhinal cortex. *Neuron* **88**, 590–603 (2015).

30. Langston, R. E. et al. Development of the spatial representation system in the rat. *Science* **328**, 1576–1580 (2010).

31. Wills, T. J., Cacucci, F., Burgess, N. & O’Keefe, J. Development of the hippocampal cognitive map in preweanling rats. *Science* **328**, 1573–1576 (2010).

32. Bush, D., Barry, C. & Burgess, N. What do grid cells contribute to place cell firing? *Trends. Neurosci.* **37**, 136–145 (2014).

33. Wldoski, J. & Fiete, I. R. A model of grid cell development through spatial exploration and spike time-dependent plasticity. *Neuron* **83**, 481–495 (2014).

34. Simon, D. K., Prusky, G. T., O'Leary, D. D. & Constantine-Paton, M. N-methyl-D-aspartate receptor antagonists disrupt the formation of the mammalian neural map. *Proc. Natl Acad. Sci. USA* **89**, 10593–10597 (1992).

35. Iwasato, T. et al. NMDA receptor-dependent refinement of somatotopic maps. *Neuron* **19**, 1201–1210 (1997).

36. Iwasato, T. et al. Cortex-restricted disruption of NMDAR1 impairs neuronal patterns in the barrel cortex. *Nature* **406**, 726–731 (2000).

37. Stensola, H. et al. The entorhinal grid map is discretized. *Nature* **492**, 72–78 (2012).

38. Dhillon, A. & Jones, R. S. Laminar differences in recurrent excitatory transmission in the rat entorhinal cortex in vitro. *Neuroscience* **99**, 413–422 (2000).

39. Cousey, J. J. et al. Recurrent inhibitory circuitry as a mechanism for grid formation. *Nat. Neurosci.* **16**, 318–324 (2013).

40. Pustoll, H., Solanka, L., van Buren, M. C. & Nolan, M. F. Feedback inhibition enables nested oscillations and grid firing fields. *Neuron* **77**, 141–154 (2013).

41. Valero, S. & Taube, J. S. Path integration: how the head direction signal maintains and corrects spatial orientation. *Nat. Neurosci.* **15**, 1445–1453 (2012).

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Author contributions

H.M. conceived the study; R.J.D.M. designed the L-maze assay, beaconing assay, behavioral data analysis and viral infection quantification; M.G. performed viral injections, tetrode implantations, behavioral assays, MEC in vivo electrophysiological recordings, histology, brain imaging and data analysis; M.A. performed MEC in vivo electrophysiological recordings and data analysis; M.S. performed HP in vivo electrophysiological recordings, the corresponding tetrode implantations and data processing; A.N. performed whole-cell patch-clamp recordings; K.A. developed software for in vivo electrophysiological data analysis; M.G. and H.M. wrote the paper with contributions from all coauthors.

Competing interests

The authors declare no competing financial interests.

Additional information

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Articles

**Methods**

**Animals.** Experiments were performed using NR1fl/fl−/− mice (henceforth; NR1fl−/− mice) and wild-type littermates with a C57BL/6 background (64 males and 36 females; the male/female ratio was 1.63 for control, 1.85 for NR1fl−/− and 1.83 for NR1fl+/− mice). Mice were kept on a 12:12 light/dark schedule with all testing performed during the light phase. All experiments were approved by the Regierungsspräsidium Karlsruhe in compliance with the European guidelines for the care and use of laboratory animals (licenses G-24/14 and G-50/14).

**Virus-mediated NR1 gene ablation in young mice.** Four-day-old (P4) NR1fl−/− mice received bilateral injections into MEC of AAV-Syn-GFP-T2A-Cre. In this vector, the green fluorescent protein (GFP) and the protein Cre-recombinase (Cre) are expressed under the control of the neuron-specific synapsin promoter (Syn). Anesthesia was induced and maintained with isoflurane (1–2.5%). We injected 1 µL of the viral construct in each MEC (±5 mm lateral from bregma, 0.1 mm anterior from the transverse sinus, at a 3° angle in the sagittal plane, with the tip pointing in the anterior direction). Following virus injection, Cre-recombinase excises the loxP-flanked exons 11–18 of the NR1fl+/− alleles and transforms them to NR1fl−/− alleles, causing ablation of NR1 specifically in the area of injection. The group included (i) wild-type littermate mice injected with AAV-Syn-GFP-T2A-Cre and (ii) NR1fl−/− mice injected with AAV-Syn-Tomato (in which the synapsin promoter directs the expression of the fluorescent protein Tomato). Viral constructs were produced in-house. After virus injection, mice stayed with their mother until weaning (~4 weeks), at which time they were separated from the mother and divided by gender (4 mice per cage). Mice were housed in Plexiglas home cages (25 × 19 × 13 cm) with free access to food and water.

**Electrophysiological recordings in brain slices.** Whole-cell patch-clamp recordings were performed in slices obtained from a subgroup of NR1fl+/−−/− mice 4–8 weeks after injection, (WT; n = 9 cells from 3 mice, WT-GFP; n = 10 cells from 3 mice, NR1fl+/−−/−; n = 11 cells from 4 mice). The experimenter was not blinded to the genotype of the mice. Mice were deeply anesthetized with isoflurane, followed by transcardial perfusion with 30 mL sucrose solution containing (in mM) 212 sucrose, 26 NaHCO3, 1.25 NaH2PO4, 3 KCl, 7 MgCl2, 10 glucose and 0.2 CaCl2, cooled to 4 °C and oxygenated with carbogen gas (95% O2/5% CO2, pH 7.4). Acute sagittal sections (300 µm) containing the MEC were prepared using a vibratome (Slicer HR2, Sigmann Elektronik, Germany), and the tissue was incubated at room temperature for 1 h in oxygenated extracellular solution containing (in mM) 125 NaCl, 2.5 NaHCO3, 0.125 NaH2PO4, 0.25 KCl, 2 CaCl2, 1 MgCl2 and 25 glucose. For recording, individual slices were transferred into a submerged chamber superfused (perfusion rate = 3.0 mL/min) with oxygenated extracellular solution (heated to 30–32 °C). Fluorescently labeled (GFP+) cells in the MEC were visualized with epifluorescence and DIC optics. Agonist-induced currents, pulled from horizontal pipettes, were filtered at 3 kHz and digitized at 20 kHz. Data were analyzed offline with HEKA software. The presence of synaptic activity was evaluated using the ratio of NMDAR/AMPAR, the stimulus intensity and amplitude of AMPAR-mediated currents were recorded at a holding potential of −70 mV in presence of the AMPA/kainate receptor blocker CNQX (10 µM) and gabazine. NMDAR-mediated currents were verified using d-AP5 (10 µM), a potent NMDAR blocker. To evaluate the ratio of NMDAR/AMPAR, the stimulus intensity and amplitude of AMPAR-mediated currents were kept constant (8–10% of 1 mV and 200–230 pA, respectively). For data analysis, an average of 15 sweeps was used, and only monosynaptic inputs with latencies lower than 2 ms were considered. All recordings were obtained using HEKA PatchMaster EPC 10, and signals were filtered at 3 kHz and digitized at 20 kHz. Data were analyzed offline with HEKA software. Statistics were performed using R as experimental units.

**Behavioral tests.** Behavioral experiments started between 11 and 20 weeks after virus injection. The experimenter was blind to the genotype of the mice. In all experiments, mice were first video-recorded at 25 frames per second and their movements subsequently analyzed using a position tracking system (Ethovision XT9, Noldus). After task completion, mice rested for at least 10 days before being exposed to a new task. Half of the mice were first tested in the maze assay and next in the win–shift task, while the other half was first tested in the win–shift task and next in the L-maze assay. The beaconing task was always performed after the L-maze assay.

**L-maze assay for path integration.** The test was carried out as previously reported, with slight variations. The experiment was performed in a circular tank (120 cm in diameter and 60 cm tall) filled with water (21 ± 1 °C) to a depth of 22 cm. The water was made opaque by the addition of milk. An L-shaped maze was present inside the tank during sample trials (see below). The maze was made of Plexiglas and consisted of a corridor that had a long (82.5 × 10 × 35 cm) and a short segment (42.5 × 10 × 35 cm) joined at a 90° angle. Start and end boxes (13.3 × 10 × 35 cm) were located at the end of each segment. The end box had a small platform to confine the mouse at the beginning of each trial. The end box contained a circular platform (6.5 cm diameter) submerged 1 cm below the water surface. The entire experiment (including the pretraining phase, see below) was performed in darkness. The tank was illuminated with infrared light and a video camera with an infrared filter was located above its center. Two loudspeakers producing white noise were located below the tank to mask potential directional auditory cues. First, mice underwent a pretraining phase consisting of 9 trials. At the beginning of each trial, a mouse was placed inside the start box for 10 s before the sliding door was removed, and it would swim in the open tank to find one of three hidden platforms, whose distance from the start box increased gradually across trials. All mice successfully found one of the three platforms at the end of the pretraining phase. In the main phase, mice learned the basic rules of the task, i.e., to swim and find a hidden platform in a dark pool to escape from the water. After this preparatory phase, the L-maze assay was performed. It consisted of one sample trial (corridor swim) and a test trial (open tank swim) with intertrial intervals of 15 min. At the beginning of the sample trial, the mouse was placed inside the start box (10 s before the sliding door was removed) and had to swim along the corridor to find the hidden platform located in the end box. During sample trials, the corridor could be arranged according to one of three configurations: (i) straight (Str.): the start box and the end box were both connected to opposite ends of the long segment; thus, mice had to swim in a straight line to find the platform at its end. (ii) long–short (L-S): the start box was connected to the long segment followed by the short segment, thus mice had to swim along the long segment first, then turn 90° and swim along the shorter segment to find the end platform; and (iii) short–long (S-L): the start box was connected to the short segment followed by the long segment; in this case, mice had to swim first along the short segment, then turn 90° and swim along the long segment to find the platform. The position of the platform relative to the long axis of the start box was at 0°, 30° and 60° in the Str-, L-S and S-L corridors, respectively. After the sample trial, the corridor was removed, leaving in place only the start box and the hidden platform. During the test trial, the mouse was placed again inside the start box, but this time it had to swim in the open tank to find the hidden platform. Both sample and test trials lasted until the mouse found the platform or 1 min elapsed. If the mouse failed to find the platform within 1 min, it was placed onto it by the experimenter. The mouse was left on the platform for 20 s before it was returned to its home cage. Mice performed 4–5 sample trials of a given condition each day, and this was repeated twice following a pseudorandom sequence. Test trials with a vector length shorter than 28 cm and/or a mean swimming speed lower than 5 cm/s were excluded from the analysis.

**Beaconing task.** The experiment was performed in the same circular tank filled with opaque water, but this time it was illuminated with visible light and the position of the platform was tagged with a flag that extended above the water surface. As before, at the beginning of each trial, mice were placed inside the start box and had to swim in the open tank to find the platform located at 0°, 30° or 60° relative to the long axis of the start box. The start box and the platform were moved around the tank in each trial. In this task, the flag attached to the platform was the only conspicuous cue reliably signaling the location of the goal. Each mouse swam 6–8 times per condition following a pseudorandom sequence.

**Win–shift task.** The experiment was performed in a T-maze (95 × 135 cm) illuminated with visible light and surrounded by conspicuous visual cues. Mice were maintained at 80–85% of their normal body weight with free access to water. Each trial consisted of a sample run and a choice run. On sample runs, the mouse was forced to turn either left or right by blocking access to one goal arm with a wooden block. Food reward was available at the end of the goal arm. The block was then removed, and the mouse was placed back at the beginning of the start arm to begin the choice run. During the choice run, the mouse was rewarded for choosing the previously unvisited arm. The delay between the sample run and the choice run was 10–15 s. Mice ran 10 daily trials (intertrial interval: 30 min) for 7 d.

**Data analysis.** Circular statistics were used to compare individual mean global vectors from 5 to 8 swims performed by each mouse in each condition during the L-maze and beaconing assays. First, a trial vector (tv) connecting the start and the end of each trajectory was calculated. The end of the trajectory was defined as the position of the mouse when: (i) it touched the wall of the pool, (ii) its trajectory accumulated a turn ≥95° or (iii) it reached the platform. Next, for each mouse and condition, the mean vector (dagv) was calculated as the mean vector weighted by the length of each tv. The individual mean global vectors (avg) were then used to calculate an average global vector (agv) for each condition and genotype. The direction and lengths of average global vectors were compared within and across genotypes.

The directions of the average global vectors obtained in the beaconing task (dagv) were used to quantify the directional deviation from optimal performance in the L-maze. The Dop (deviation from optimal performance) score was calculated for each mouse as follows:
DOP score $= \frac{[d\bar{v}_{\text{bin},0}][d\bar{v}_{\text{bin},1}][d\bar{v}_{\text{bin},0}][d\bar{v}_{\text{bin},1}]}{[d\bar{v}_{\text{bin},0}][d\bar{v}_{\text{bin},1}][d\bar{v}_{\text{bin},0}][d\bar{v}_{\text{bin},1}]}$ where $d\bar{v}_{\text{bin},0}$ and $d\bar{v}_{\text{bin},1}$ are the directions of the individual mean vector along the sampling period for all bins. The DOP score was calculated for each bin for each cell and was then averaged across all bins to obtain the DOP score for each cell. The DOP score was used to characterize the directionality of the firing pattern for each cell.

$\theta = \frac{\pi}{2} - \frac{\pi}{2}$

where $\theta$ is the mean power at theta frequency range (6–10 Hz) and $\beta$ is the mean power within two frequency intervals (3–5 and 11–13 Hz). A score higher than zero indicates more power at theta frequency than for adjacent frequencies. For neurons with a theta rhythm between 0.5, the peak theta frequency was calculated as the frequency with the highest power within the frequency range between 6 and 10 Hz.

4) The border score was calculated as:

$$\text{Border score} = \frac{CM - DM}{CM + DM}$$

where $CM$ and $DM$ are the mean shortest distances to a wall for a firing field and a background map, respectively.

5) The speed score was obtained as the Pearson product–moment correlation coefficient between the speed profile and the firing rate profile for each cell.

6) The speed score was calculated using the power spectrum of the instantaneous firing rate of each unit as:

$$\text{The theta rhythmicity score} = \frac{\theta - \beta}{\theta + \beta}$$

where $\theta$ is the theta rhythm and $\beta$ is the mean power at beta frequency range (11–20 Hz) and $\gamma$ is the mean power within two frequency intervals (20–30 Hz).

7) The grid score was calculated using spatial autocorrelation matrixes calculated from the firing rate map for each cell. Each bin of a spatial autocorrelation matrix represented a Pearson correlation coefficient between all possible pairs of bins in a firing rate map with a given spatial lag. The spatial lag associated with a particular bin of the matrix was determined by the location of that bin relative to the matrix center. Pearson correlations at spatial lags for which < 20% of firing rates were available were not considered. A peak in the autocorrelation matrix was defined as more than 10% adjacent bins with a correlation coefficient higher than a peak detection threshold, which was set to 0.1. To determine the periodicity of the spatial autocorrelation matrix, a circular region of the spatial autocorrelation matrix was defined containing up to six peaks but excluding the central peak. Pearson correlation coefficients ($r$) were calculated between the circular region of the matrix and a rotated version of it by 30°, 60°, 90°, 120°, and 150°. Then the grid score was calculated as:

$$\text{Grid score} = \frac{r_{30°} + r_{120°} - r_{60°} - r_{90°} - r_{150°}}{2}$$
is because we performed bilateral recordings and injections varied between hemispheres within single animals.

**Histology.** After the electrophysiological recordings, mice were perfused transcardially with phosphate buffered saline, followed by 4% paraformaldehyde. Brains were removed and sliced sagittally in 50-µm sections on a vibratome (Leica VT1000S, Heidelberg, Germany). Free-floating sections were permeabilized in 0.2% Triton-PBS for 30 min, washed three times with PBS and incubated for 5 min with DAPI (4′,6-diamidino-2-phenylindole; 1:1,000). Sections were mounted in Mowiol. Visualization of viral spread and location of the tetrodes was performed using a confocal microscope (Zeiss LSM 700) and a slide scanner (Zeiss Axio Scan Z1). The experimenter was blind to the genotype of the mice.

**Quantification of brain area/volume infected by the virus and estimation of tetrode location.** To estimate the boundaries and volume of single injections, we took pictures (5x) of each brain serial section (~100 sections per mouse) using a slide scanner (Zeiss Axio Scan Z1). The images were subsequently analyzed using the software Zen 2Lite (Zeiss Microscopy). In each brain section, we first defined two areas of interest: (i) the retro-hippocampal region (which includes the entorhinal area, subiculum, parasubiculum, presubiculum, and postsubiculum) and (ii) the hippocampal region (which includes Ammon’s horn and the dentate gyrus). Next, we measured the area covered by the GFP signal (i.e., area of viral expression) within each region. All regions were manually defined. The percentage of infection (GFP expression) inside each region was calculated as:

\[
\text{% infection RH} = \frac{\sum_{i=1}^{N} r_i \times 100}{R_H} \quad \text{and} \quad \text{% infection HP} = \frac{\sum_{i=1}^{N} h_i \times 100}{H_P}
\]

where \(r_i\) and \(h_i\) are the areas expressing GFP in the RH and HP regions of the brain sections, respectively, and \(R_H\) and \(H_P\) are the areas covered by the RH and HP regions in the brain section, respectively. The percent infection in RH and HP regions was calculated per hemisphere and per mouse for the electrophysiological and behavioral experiments, respectively. To calculate the percent infection per mouse the following formulas were used:

\[
\text{% infection RH (per mouse)} = \frac{\left( \frac{\text{% infection RH (Right) } \times 50}{100} \right) + \left( \frac{\text{% infection RH (Left) } \times 50}{100} \right)}{2}
\]

\[
\text{% infection HP (per mouse)} = \frac{\left( \frac{\text{% infection HP (Right) } \times 50}{100} \right) + \left( \frac{\text{% infection HP (Left) } \times 50}{100} \right)}{2}
\]

A threshold value of 3% was arbitrarily set to assign hemispheres/mice to the experimental groups. Hemispheres/mice with more than 3% infection in the HP region were included in the \(NR1^{11−/−}\) group. Hemispheres/mice with more than 3% infection in the HP region were included in the \(NR1^{11−/−}\) group. This quantification is a coarse measure that is invariant to superinfection (since fluorescence level does not add to its magnitude). Quantifying the actual fraction of infected neurons would increase the exactness of our estimate. However, this would not improve the subsequent analysis as inaccuracies in estimation of viral infection are unlikely to be greater than interindividual variations.

Tetrode location was estimated using the same brain sections and software. The dorsoventral position of the tetrode tips was defined as the distance between the dorsal border of the MEC and the tetrode tip. The mediolateral position was obtained from a stereotaxic atlas (Allen Brain Atlas). Misimplanted animals were excluded from the analysis. The experimenter was blind to the genotype of the mice.

**Statistical analysis.** No statistical methods were used to predetermine the sample size, but our sample sizes are similar to (or larger than) those generally employed in the field. Statistical analysis was performed using Prism 6 (GraphPad Software) and Oriana 4.02 (Kovach Computing). Data were tested for normality and homoscedasticity using the KS normality test and the Brown–Forsythe test, respectively. We used t tests, one-way ANOVA with Tukey’s multiple comparison tests (with Bonferroni adjustments for multiple comparisons) and two-way repeated-measures ANOVA when data passed normality and homoscedasticity assumptions. Kruskal–Wallis tests and Dunn’s multiple comparison tests (with Bonferroni adjustment for multiple comparisons) were used when this was not the case. Watson–Williams F-tests (with Bonferroni adjustments for multiple comparisons) for circular data were used to compare the directions of global vectors. Pearson correlations were used to analyze the relationship between variables. All tests were two-sided. Unless otherwise specified, mice were used as experimental units. During the experiments, we used 73 \(NR1^{11−}\) mice injected with AAV-Cre-GFP. Of these, four mice were used for whole-cell patch-clamp recordings, and the remaining 69 mice for behavioral tests and in vivo electrophysiology (\(NR1^{11−/−}\) n = 37, \(NR1^{11−/−}\) \(\times\) n = 33). In addition, 27 control mice were injected (19 WT mice injected with AAV-Cre-GFF and 8 \(NR1^{11−}\) mice injected with AAV-Syn-Tomato). Of the control mice, 4 (plus 3 noninjected WT mice) were used for whole-cell patch-clamp recordings, and the remaining 24 mice were used for behavioral tests and in vivo electrophysiology. Some mice were used in either the behavioral tests or electrophysiological recordings, while others were used in both types of experiments. L-maze assay: control: n = 17, \(NR1^{11−/−}\): n = 28, \(NR1^{11−/−}\) \(\times\) n = 28. Beaconing task: control: n = 13, \(NR1^{11−/−}\): n = 11, \(NR1^{11−/−}\) \(\times\) n = 10 (all these mice also performed the L-maze assay). Win–shift task: control: n = 14, \(NR1^{11−/−}\): n = 19, \(NR1^{11−/−}\) \(\times\) n = 20 (all these mice also performed the L-maze assay). MEC electrophysiological recordings: control: n = 8, \(NR1^{11−/−}\): n = 19, \(NR1^{11−/−}\) \(\times\) n = 11 (some of these mice also took part in the L-maze assay: control: n = 4, \(NR1^{11−/−}\): n = 13, \(NR1^{11−/−}\) \(\times\) n = 10). HP electrophysiological recordings: control: n = 8 hemispheres from 4 mice, \(NR1^{11−/−}\) \(\times\) n = 13 hemispheres from 9 mice, \(NR1^{11−/−}\) \(\times\) n = 7 hemispheres from 6 mice (some of these mice also took part in the L-maze assay: control: n = 3, \(NR1^{11−/−}\) \(\times\) n = 4, \(NR1^{11−/−}\) \(\times\) n = 5).

**Life sciences reporting summary.** Further information on experimental design is available in the Life Sciences Reporting Summary.

**Data and code availability.** Codes for characterization of the in vivo recorded units can be provided by the corresponding author upon reasonable request. The data that support the findings of this study are available from the corresponding author on reasonable request.

**References**

42. Niewoehner, B. et al. Impaired spatial working memory but spared spatial reference memory following functional loss of NMDA receptors in the dentate gyrus. *Eur. J. Neurosci.* 25, 837–846 (2007).

43. Csicsvari, J., Hirase, H., Czurkó, A., Mamiya, A. & Buzsáki, G. Oscillatory coupling of hippocampal pyramidal cells and interneurons in the behaving *Rat. J. Neurosci.* 19, 274–287 (1999).

44. Schmitzer-Torbert, N., Jackson, J., Henze, D., Harris, K. & Redish, A. D. Quantitative measures of cluster quality for use in extracellular recordings. *Neuroscience.* 131, 1–11 (2005).

45. Skaggs, W. E., McNaughton, B. L., Wilson, M. A. & Barnes, C. A. Theta phase precession in hippocampal neuronal populations and the compression of temporal sequences. *Hippocampus.* 6, 149–172 (1996).
Experimental design

1. Sample size
Describe how sample size was determined.

No statistical methods were used to predetermine the sample size, however, our sample sizes are similar (or even higher) to those generally employed in the field.

2. Data exclusions
Describe any data exclusions.

Cells in which serial resistance changed more than 20% or was higher than 40 M\(\Omega\) were discarded from the whole-cell patch clamp analysis. Miss-implanted animals were excluded from the electrophysiological analysis. In the L-maze and beaconing assays, test trials with a vector length shorter than 20 cm and/or a mean swimming speed lower than 5 cm/s were excluded from the analysis. These exclusion criteria were pre-established and are reported in the Methods section.

3. Replication
Describe whether the experimental findings were reliably reproduced.

The experiments were conducted with different litters of mice and were always reliably reproduced.

4. Randomization
Describe how samples/organisms/participants were allocated into experimental groups.

Experimental groups were defined at posteriori upon analysis of the brain injections.

5. Blinding
Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

The experimenter was blind to the genotype of the mice since experimental groups were defined at posteriori upon analysis of the brain injections.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters
For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a | Confirmed
---|---

- [ ] The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- [ ] A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- [ ] A statement indicating how many times each experiment was replicated
- [ ] The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
- [ ] A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- [ ] The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted
- [ ] A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- [ ] Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.
7. Software

Describe the software used to analyze the data in this study.

Electrophysiological raw data was processed using custom written C++ programs, shell scripts and R scripts.
Behavioral data raw data was processed using Ethovision XT9 (Noldus).
Statistical analysis was performed using Prism 6 (GraphPad Software) and Oriana 4.02 (Kovach Computing).
Quantification of brain area/volume infected by the virus and estimation of tetrode location were analyzed using the software Zen 2lite (Zeiss microscopy).

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

All unique materials are readily available from the authors.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

No antibodies were used in this study.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No eukaryotic cell lines were used in this study.

b. Describe the method of cell line authentication used.

NA

c. Report whether the cell lines were tested for mycoplasma contamination.

NA

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

NA

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Experiments were performed using four day old (P4) NR1flox mice and wild-type littermates with a C57BL/6 background (64 males and 36 females).

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The study did not include human research participants.