NMDA receptor dependent functions of hippocampal networks in spatial navigation and memory formation

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NMDA receptor dependent functions of hippocampal networks in spatial navigation and memory formation
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## Contents

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  Introduction</td>
<td>1</td>
</tr>
<tr>
<td>2  NMDA receptors in hippocampal area CA1 are essential for adaptive</td>
<td>23</td>
</tr>
<tr>
<td>spatial coding during navigation based on motor sequences, but not</td>
<td></td>
</tr>
<tr>
<td>external cues</td>
<td></td>
</tr>
<tr>
<td>3  Absence of NMDA receptors in hippocampal area CA1 disrupts</td>
<td>53</td>
</tr>
<tr>
<td>oscillatory patterns during a spatial navigation task</td>
<td></td>
</tr>
<tr>
<td>4  Phase locking of excitatory and inhibitory CA1 neurons in normal</td>
<td>75</td>
</tr>
<tr>
<td>and NMDAR knock-out mice during spatial navigation</td>
<td></td>
</tr>
<tr>
<td>5  Single-trial properties of place cells in Control and NR1-KO</td>
<td>99</td>
</tr>
<tr>
<td>mice</td>
<td></td>
</tr>
<tr>
<td>6  General discussion</td>
<td>125</td>
</tr>
<tr>
<td>Bibliography</td>
<td>143</td>
</tr>
<tr>
<td>Samenvatting in het Nederlands</td>
<td>179</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>185</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

In an increasingly sedentary world, where countless actions and goals can be accomplished sitting in front of a computer, the ability to navigate through an environment remains a crucial component of our daily life. In the animal world, survival depends strictly on the animal’s ability to locate and orient itself in varying circumstances and diverging goals, whether it is to escape a predator, find food or water or a safe place to sleep. For humans, this function is necessary in almost all tasks of a normal day, such as finding the car in the parking lot, food in the supermarket or getting from home to work and back.

These functions require the existence of a ‘cognitive map’, an implicit representation of the environment, its context and its value. This term, first introduced by Tolman in 1948 [Tolman, 1948], refers to an abstract representation, a map that goes beyond the simple navigation between two points A and B. Such a map would allow animals to choose a new, unexplored, alternative path when changes in its environment occur, a path that will bring them to the original goal, by cognitively creating associations between previously independent cues. Tolman proposed this hypothesis after observing rats that learned a path to a goal in a maze and then had to adjust their path, as part of the maze was blocked: rats would, when confronted with new possibilities in this new situation, chose the new path that would take them in the direction of the goal.

Despite the seemingly behavioral simplicity of this process, from a neural point view, it requires a complex computation, including self-localization, memory retrieval, route planning, spatial updating and goal identification. For this, different sources of information need to be integrated: on
the one hand, there is the information carried by external stimuli, mainly visual, which help us locate ourselves in the environment and allow us, by following landmark cues available, to go in the right direction (allothetic information). On the other hand, there is information carried by our proprioceptive system pertaining to self-motion (idiothetic information). This comprises a wide range of motion-related signals, such as head-direction (movement direction), velocity (distance moved per unit of time) and vestibular information (balance and orientation in the horizontal and vertical plane) [Wolbers and Hegarty, 2010]. Although allothetic and idiothetic information may support navigation independently of one another, it is the combination of both that produces an optimal strategy. At the core of the integration of both allo- and idiothetic information lies the hippocampus (HPC), a brain structure located in the medial temporal lobe, which plays a key role in memory formation, retrieval and spatial navigation. Due to the environmental dependency of this form of navigation, coupled to the relative independence of one's current viewpoint, this integrative form of spatial behavior has been termed place or allocentric navigation [Tolman, 1948]. Alternatively, animals may adopt a strategy based on the execution of a learned motor response, or sequence of responses [Packard et al., 1989, Rondi-Reig et al., 2006]. The dorsal striatum has been seen as the key player in this type of navigation [Packard and McGaugh, 1996, Packard and Knowlton, 2002], but more recent studies have shown that, when the motor response becomes more complex and requires learning of a sequence of motor responses, the hippocampus becomes involved [Rondi-Reig et al., 2006]. This type of navigation is termed sequence or (sequential) egocentric navigation.

In the following sections I will elaborate on the neural and behavioral basis of both types of navigation, place- and sequence-based, and then on the function and functioning of the HPC, as well as other brain structures coworking with it.
1.1 Place-based navigation

Place-based navigation can be defined as the ability to navigate in a familiar environment, independently of one’s current vantage point, using, for instance, landmarks (environmental cues which give information about the current location of the subject and/or the goal location) and also about the direction and distance relations between objects. These cues include discrete environmental objects, global orientation cues, the geometric structure of the environment, but also symbolic representations (such as maps or linguistic descriptions) [Wolbers and Hegarty, 2010]. In its simplest form, navigation is guided by a landmark directly associated with the goal location (‘target approaching’) [Tommasi et al., 2012]. In most cases, however, the goal is not immediately visible. To keep track of distance and direction of movement, as well as to keep track of one’s own relative position to environmental cues (spatial updating), a wide range of internal and external signals need to be processed and combined: sensory and vestibular inputs, head-direction and orientation information, motor efference copy and proprioceptive information [Taube, 2007].

A map-like representation of an environment can be, thus, formed by linking together discrete sensory and continuous idiothetic inputs (like optical flow, orientation, from the vestibular system, or movement, from proprioceptors in joints and muscles [Wolbers and Hegarty, 2010]), anchored to a common reference point, such as point of entry to the environment or distal salient landmarks. Simultaneously, these landmarks help recalibrating the position of the animal and orientation estimates. Different places can then be linked to the same reference point, allowing an animal to navigate from one place to the other [Wolbers and Hegarty, 2010, Lew, 2011]. Having such a map-like representation allows an animal to optimize its navigation, by adopting novel paths and/or compute directions to unseen goals.

Although each of these two information streams - idiothetic (self-motion based) and allothetic (guided by external cues) - can independently guide navigation, it is the combination of both that makes allocentric navigation efficient: allothetic navigation, by itself, relies on distance and angle estimation between subject and landmarks, which lacks accuracy. Relying
solely on idiothetic information, however, is prone to accumulate position 
estimation errors and needs allothetic feedback to keep the alignment 
[Wolbers and Hegarty, 2010].

1.2 Sequence-based navigation

The repetition of the same trajectory may lead to learning of the 
motor response associated with it, such that a particular starting stim-
ulus may trigger the execution of that motor response [Packard et al., 
1989, Packard and McGaugh, 1996], independently of spatial process-
ing. This type of navigation involves less neural computations, that is, 
it does not involve processing of external (distal) sensory and idiothetic 
inputs and can be, thus, seen as a unidimensional process: it involves 
the retrieval and execution of a stored memory of a sequence of body re-
sponses. Earlier studies of this type of navigation related it mainly to the 
striatum [Jarrard, 1993, Packard et al., 1989, Packard and McGaugh, 
1996]. These experiments involved, however, a very simple behavioral 
task, whereby the animal needed to learn only a single body-turn. More 
recent studies addressed egocentric navigation in a conceptually similar, 
but more complex task (e.g., the starmaze, [Rondi-Reig et al., 2006]). In-
creased complexity demands more intricate motor response. To memorize 
such responses, sequences of body movements must be linked together. 
Rondi-Reig’s study, using normal and KO mice lacking the NMDA recep-
tor (see Box 1) in the CA1 area, showed that a functional HPC is needed 
to perform a sequential egocentric response (mice needed to link the ade-
quate response in three choice points to reach the goal). In addition, it 
showed that the impairment was specific to the execution of a sequence 
of choices: no impairment was seen in the one-choice version of the task.

1.3 Hippocampus: the neural code of navigation

According to E.C.Tolman’s cognitive map theory [Tolman, 1948], a 
neural spatial representation of an environment would enable animals
to follow a place-based (allocentric) strategy. It remained a speculative observation for almost 25 years, until the seminal work of O’Keefe and Dostrovsky [O’Keefe and Dostrovsky, 1971]. The authors recorded single-unit activity from dorsal CA1 neurons and identified a subset of cells with a very specific pattern of activity: firing of an individual neuron was restricted to a specific location in the environment (later termed ‘place field’). Tolman’s cognitive map had found its neural substrate. The HPC became the research focus of learning and navigation, and later, memory processes.

**Box 1. The NMDA receptor**

The N-methyl-D-aspartate receptor (NMDAR) is an ionotropic glutamate receptor, critically involved in excitatory synaptic transmission and synaptic plasticity and, therefore, in memory formation. It contributes to the slow component of the EPSP (Excitatory Postsynaptic Potential) under depolarized membrane voltages [Herron et al., 1986]. At resting potentials, the receptor is blocked by Mg$^{2+}$ ions. To be activated, the postsynaptic element needs to be depolarized, which will release the blockade and, upon glutamate binding, allow the influx of Ca$^{2+}$ ions into the cell. This will trigger a cascade of events that may lead to a long-term strengthening of the connection between a pre- and postsynaptic neuron [Nowak et al., 1984, McBain and Mayer, 1994]. Its importance for spatial memory was shown for the first time in 1986, when Morris and his colleagues observed behavioral impairments in a spatial task in rats under NMDAR blockade by AP5, an antagonist of the receptor [Morris et al., 1986]. Under depolarized conditions, the slow time course of NMDAR conductances is also thought to be key for persistent activity models of working memory [Wang, 1999, van Wingerden et al., 2012]. One important development in the study of hippocampal NMDAR function was the creation of a knockout (KO) line, in which the gene of the NMDAR NR1 subunit is deleted in a temporally, spatially and cell type restricted way. In 1996, Tsien and colleagues [Tsien et al.,
1996] developed the cre/loxP system to create cell-type and spatially restricted mutations. Cre is a site-specific DNA recombinase derived from the P1 bacteriophage that recognizes specific 34 base-pair sequences termed loxP sites. It removes the DNA segment flanked by these two regions, resulting in a KO of the gene of interest. In the case of the NMDAR CA1-KO mouse (NR1-KO), first, one mutant mouse line carried the Cre gene under the control of the αCaMKII gene. The αCaMKII is mainly present in the forebrain and expressed only in pyramidal neurons. From the generated transgenic sublines, one showed a Cre expression mostly restricted to CA1 and was crossed with a second mutant mouse line, in which a loxP segment was inserted, by homologous recombination, on both sides of the NMDA receptor subunit-1 (NR1) gene. The result was a mutant mouse line, which expressed the Cre enzyme exclusively in this region, resulting in the local excision of the NR1 gene. The NMDAR has multiple subunits (NR1, NR2A-D, and NR3A and B), but only the NR1 is crucial for the stability of the NMDAR [Dunah et al., 1999, Cull-Candy et al., 2001]. As a consequence, the NMDAR is completely absent in CA1 [Tsien et al., 1996]. Subsequent studies have shown, however, that the mutation spreads to other brain areas in an age-dependent way, affecting other hippocampal regions (CA3 and dentate gyrus) and deep cortical layers in 4-months old mice [Fukaya et al., 2003]. NMDAR function has been associated to schizophrenia, a debilitating psychiatric disease, which affects almost 1% of the population, with a wide range of studies showing that NMDAR blockage (with phencyclidine, ketamine and MK801) [Neill et al., 2010, Inta et al., 2010] and KO lines lacking the NMDAR [Belforte et al., 2010, Mohn et al., 1999] elicit schizophrenia-like symptoms, both at the behavioral level and at the neurophysiological level [Ehrlichman et al., 2009, Lazarewicz et al., 2010]. Most notably, NMDAR antagonists lead to neural oscillatory and synchronization abnormalities in the hippocampus (under ketamine [Neymotin et al., 2011] and in modeling studies [Lisman and Buzsáki, 2008]), which are thought to play a crucial role in the communication between brain areas and integration of different sources of information [Gray et al., 1992, Fries et al., 2007, Colgin
et al., 2009, van Wingerden et al., 2012].

Since then, an exhaustive number of papers have shown behavioral deficits in spatial navigation tasks in animals with impaired hippocampal functioning [O’Keefe et al., 1975, Olton et al., 1978, Morris et al., 1982, Danysz et al., 1988, McLamb et al., 1990, Davis et al., 1992, Moser et al., 1993, McHugh et al., 1996]. Nonetheless, spatial navigation is a highly complex process, which may vary, depending on the exact task or situation.

1.3.1 Hippocampus: anatomy and connectivity

The rodent hippocampus is a bean-shaped structure, composed of two main subfields, the dentate gyrus (DG) and the Cornu Ammonis (CA). The latter region can be further divided into CA1 through 3. In addition, the subiculum, adjacent to the CA1, acts as a main exit point of the hippocampus. The hippocampus is organized into clearly distinguished layers of cell bodies and dendrites, in which the principal neurons are the excitatory granule cells in the DG and the pyramidal cells in the CA, which use glutamate as main transmitter. There are several classes of GABAergic inhibitory interneurons, which co-exist in the HPC and coordinate the activity of principal neurons [Klausberger et al., 2003] and play a crucial role in modulating network oscillations [Klausberger and Somogyi, 2008, Korotkova et al., 2010]. The flow of propagation of neural activity within the HPC is mostly unidirectional, starting in the DG, which propagates information to the CA3 via the mossy fibers, making strong and sparse synapses near pyramidal cell somata [Treves et al., 2008]. The CA3, characterized by its abundant recurrent connections, projects, in turn, via de Schaffer Collaterals (SC), to CA1 [Szirmai et al., 2012]. The main input structure to the HPC is the entorhinal cortex (EC), which projects both directly and indirectly to the CA1. The indirect route runs via the perforant path (PP), either relaying signals via the DG and CA3. The direct route is a monosynaptic entorhinal input to the CA1 and subiculum. In all cases entorhinal inputs to hippocampus arise from layer III, except for the DG, for which
projections come predominantly from the EC layer II [van Groen et al., 2003]. The importance of the EC inputs to the HPC is highlighted by the pivotal position of the EC: it receives most of its inputs from the peri- and postrhinal cortices and, indirectly, from most of the neocortex. The EC serves as the main entry point of neocortical inputs to the hippocampus. Outputs from the hippocampus are mainly relayed via the subiculum back to the EC, which projects back to the peri- and postrhinal cortices [Witter et al., 2000]. In addition to these inputs to the hippocampus, self-motion signals, such as vestibular inputs, have been shown to be important for the proper expression of a place field map [McNaughton et al., 1996]. The vestibular system carries angular and linear acceleration information.

1.3.2 A neural substrate for context

O’Keefe and Dostrovsky’s discovery of place cells [O’Keefe and Dostrovsky, 1971], pyramidal cells in the hippocampus, which fire in a particular location of the environment, led to the development of the ‘cognitive map’ theory [O’Keefe and Nadel, 1978]. This study differentiated two main types of navigation, one based on maps (the locale system) and

Figure 1.1: The Hippocampus
Schematic representation of the rat brain, with the hippocampus exposed, curving from the septal pole (S) to the temporal (T) one. Adapted from Amaral and Witter (1989) [Amaral and Witter, 1989]
one based on routes (the taxon system) and postulated that the latter one was anatomically located in the hippocampus. With technological advances, simultaneous recording of large sets of neurons became possible and since then place cells have been exhaustively studied and their properties carefully described (see Box 2). Place cells can be found in all subfields of the HPC, despite variation in their spatial selectivity [Park et al., 2011]. These advancements revealed that the ‘cognitive map’ represented in the hippocampus is more than just ‘place’: the formation of a place field involves the integration of multiple sources of information, such as sensory and internal (path integration, i.e., the ability to use self-motion cues to keep track of one’s position), but place cells are also shaped by context, showing changes in their pattern of activity, under different non-spatial manipulations to the task, such as planned navigation vs random foraging [Markus et al., 1995], prospective modulation by a future action [Wood et al., 2000, Ferbinteanu and Shapiro, 2003] or motivational salience [Lansink et al., 2009]. Context allows formation of a unique pattern of activity in each environment and contributes, therefore, to the establishment of an episodic memory [Smith and Mizumori, 2006]. Recent findings have triggered a renewed look at place cell firing, by showing that neurons in the hippocampus may encode distance [Pastalkova et al., 2008, Kraus et al., 2013] or time [MacDonald et al., 2011], when allocentric inputs are kept constant, such as treadmill running. ‘Time cells’ are also active during immobility [Macdonald et al., 2013], excluding the influence of running (on the treadmill) on its activity. These results suggest that hippocampal excitatory neurons, whether they are ‘place’, ‘distance’ or ‘time’ cells, may encode the relevant dimension, with its activity being modulated by that dimension.

**Role in memory** Remapping (see Box 2) properties of place cells suggest that the hippocampus might play a crucial role in the formation of episodic memories, given that space alone does not determine the activity of a given place cell: changes in various factors characterizing an episode might be enough to change the location or rate of firing of a place cell, such as changes in task contingencies, reward value and previous or upcoming events [Ferbinteanu and Shapiro, 2003, Ferbinteanu et al., 2011].
Each area of the HPC and its associated structures, has, however, its own particularities and functions.

### 1.3.3 Dentate Gyrus

The DG is at the starting point of the information processing line set up in the HPC. One of the main functions attributed to it is that of pattern separation [Yassa and Stark, 2011], the process which allows the orthogonalization of partially overlapping, often noisy, patterns of activation, so to retrieve one pattern, but not the other [Kesner et al., 2004]. This view has been supported by modeling studies showing that granule cells of the DG collectively act as a competitive learning network that removes redundancy from an input signal and produces a more orthogonal, sparse, and categorized set of outputs [Rolls et al., 2006]. Support has also come from electrophysiology studies, which, by introducing minimal changes to an environment, showed that activity patterns of place-modulated DG cells decorrelated substantially [Leutgeb and Leutgeb, 2007]. More recent studies, however, have suggested that only a very small subset of the granule cell population is active upon exposure to a given environment and that this activity is only short-lasting, showing no role in memory retrieval processes [Alme et al., 2010]. Moreover, that study suggests that recently generated new-born neurons represent the ‘active’ population and may either aid the formation of new representations to be formed in CA3 and CA1 or to the ‘updating’ of reinstated old memories (the process of ‘reconsolidating’ [Nader and Hardt, 2009]).

**Box 2 - Place cell properties**

**Rapid formation**

Typically, when an animal is placed in a novel environment, a place cell will rapidly adopt a seemingly random preferred firing location, displaying a sharp increase in spiking activity when the animal enters that place and very low or no spiking activity when the animal is
elsewhere.

**Stability**
Familiarization with an unchanging environment will stabilize a place field [Wilson and McNaughton, 1993].

**Position coding**
The ensemble of place cells covers the whole experimental environment, and even with a limited number of recorded neurons (<100), it is possible to accurately reconstruct the position of the animal by decoding the ensemble firing patterns at any given time, given pre-existing knowledge about place cell tuning properties [Wilson and McNaughton, 1993].

**Gradient of place field size along the hippocampal dorso-ventral axis**
Despite the similar basic intrinsic circuitry along the dorso-ventral axis [Anderson et al., 1971], inputs to the HPC differ along the axis. Along with these inputs, the size of place fields changes, with place cells of the ventral CA1 displaying larger fields [Kjelstrup et al., 2008].

**Modulated by theta (6 - 12 Hz) oscillations**
Place cells are strongly modulated by local theta oscillations, displaying a preferred firing phase near the trough of a theta wave. As the animal moves through its place field, firing phase advances, moving progressively forward on each theta cycle [O’Keefe and Recce, 1993]. This phase precession allows cells with adjacent place fields to fire in narrow temporal windows, which might enable the long-term strengthening (or weakening) of synapses via a process known as spike-time-dependent-plasticity (STDP) [Skaggs et al., 1996].

**Remapping**
When an environment undergoes changes, so does the firing of place
cells: subtle changes, like removal of a subset of cues, adding new objects to the environment or changing odor or tactile cues, may lead to an increase or a decrease in a cell’s in-field firing rate (rate remapping); significant changes to the environment, such as removal of salient cues, changing the shape of the experimental arena or displacing the animal to a different physical environment, may lead to a complete reorganization of the place code, in which place cells express a new field or even lose their field (global remapping) [Bostock et al., 1991, Markus et al., 1995, Fyhn et al., 2007].

*Place cells are present in several animal species*
Place cells were first discovered in rats [O’Keefe and Dostrovsky, 1971], but have since been discovered in several other species, such as mice [McHugh et al., 1996, Battaglia et al., 2009], bats [Ulanovsky and Moss, 2011], monkeys [Hori et al., 2003] and humans [Ekstrom et al., 2003].

*Lack of NMDAR function affects place cells*
Experiments with KO mice lacking the NMDA receptor have shown that, in the absence of this receptor, place fields are larger and less coherent among each other, suggesting that behavioral deficits following NMDAR dysfunction are, at least partly, a consequence of poor spatial representation [McHugh et al., 1996, Huerta et al., 2000].

1.3.4 *Cornus Ammonis 3*

Next in the hippocampal processing line is CA3. It receives inputs from the DG via the mossy fibers [Yassa and Stark, 2011] and from EC layer III (in the mouse) via the perforant path [van Groen et al., 2003]. A striking feature of this hippocampal subfield is its relatively dense recurrent network [Amaral, 1999]. Because of this feature, it has been associated with a large number of mnemonic processes, such as pattern separation and pattern completion (the capability of regenerating a full pattern of activity, given only a subset of this pattern) [Leutgeb and Leutgeb, 2007], rapid, one-trial learning of novel information [Lee
13

Chapter 1

and Kesner, 2004, Nakazawa et al., 2003], short-term memory [Kesner et al., 2004] and formation and retrieval of memory sequences [Jensen and Lisman, 1996]. Importantly, it has been suggested that CA3, endowed with this recurrent network, may drive CA1 patterns of activity during off-line states, such as during sharp wave-ripple complexes (EEG events associated with memory consolidation [Mizuseki et al., 2012, Carr and Frank, 2012, Jadhav et al., 2012]) and also when behavior is not under the influence of environmental or idiothetic cues, but follows self-organized internal mechanisms [Pastalkova et al., 2008]. This hints at an important role of this structure in complex egocentric navigation, where environmental influences are only weak and behavior is mainly internally driven.

1.3.5 Cornus Ammonis 1

The CA1 area is the ultimate integration site of the hippocampus, taking processed inputs from the DG and CA3 via the Schaffer Collaterals and combining them with layer III EC inputs arriving via the perforant path [Amaral, 1999]. The result is the development of a unique representation of a given environment, reflecting not only the spatial aspects of it, but also several non-spatial aspects that make up the context [Wilson and McNaughton, 1993, Eichenbaum et al., 1999]. It has been suggested to endow experiential information chunks with a temporal structure [Kesner et al., 2004], which would lead one to attribute an important role in the establishment of sequential memories to area CA1. CA1 controls most of the outgoing signal from the hippocampus, targeting many different neural regions, notably the subiculum, the prefrontal cortex and the EC [Amaral, 1999] and can, therefore, be seen as the functional output of the hippocampus.

One of the most important roles associated with area CA1 is the capacity to confer a temporal structure upon the spatial and non-spatial components of a context, encoding the temporal sequence of events [Kesner et al., 2004]. Indeed, studies in which CA1-lesioned rats needed to learn a sequence of choices suggested a role for this area in encoding sequences of events [Lee et al., 2005, Hoang and Kesner, 2008]. Area CA1 plays, furthermore, an important role in linking stimuli separated in time [Kesner et al., 2004]. In addition, theta phase precession [O’Keefe and Recce,
1993] enables the temporal compression of sequences, by bringing the firing of place cells with adjacent fields in a close time window [Skaggs et al., 1996]. In an offline behavioral state, such as sleep or awake immobility, CA1 changes activity mode and ‘replays’ patterns of neural activity emerging during a learning episode in a time compressed fashion [Wilson and McNaughton, 1994, Lee and Kesner, 2002] and is, therefore, believed to play an important role in memory establishment. Indeed, behavioral experiments disrupting sleep or directly targeting these replay events, which are nested in fast oscillatory events (about 150Hz) termed ripples, affect learning in hippocampal-dependent tasks [Girardeau et al., 2009, Ego-Stengel and Wilson, 2010, Jadhav et al., 2012].

1.3.6 Entorhinal Cortex

After the discovery of place cells, one of the pertinent questions in the field was: how are place fields formed? In early studies it was suggested that place field formation relied on a path integrator system located outside the HPC, but supporting spatial representation in the HPC [Samsonovich and McNaughton, 1997]. The EC was an obvious suspect, given its position in the parahippocampal system. However, previous studies had failed to show any spatial selectivity in (medial) EC neurons [Quirk et al., 1992]. It was not until 2005 that a groundbreaking study showed the striking spatial correlates of mEC principal neurons, when animals explored a large spatial arena: these neurons fire at regular spatial intervals, each cell displaying a hexagonal pattern of activity, tesselating the entire environment (therefore termed ‘grid cells’) [Hafting et al., 2005]. The lateral EC (IEC), on the other hand, shows only weak spatial specificity [Hargreaves et al., 2005]. Behaviorally, these two EC structures may subserve different functions, with the mEC highly involved in processing spatial information, while IEC relays non-spatial, sensory information to the HPC [Hunsaker et al., 2007]. It is thought that mEC grid cells act as the path integrator system supporting spatial representations in CA1 [McNaughton et al., 2006, Hafting et al., 2005]. Indeed, contrary to CA3 inputs, mEC inputs to the CA1 are necessary for the proper formation of place fields [Brun et al., 2002, 2008].

In addition, all layers of the EC, except for layer II, contain head-direction cells [Canto et al., 2012], which are thought to, in coordination with grid
Figure 1.2: The HPC-EC System

Horizontal section (interaural, 4.36mm) from the mouse (C57BL/6J) brain illustrating the subfields of the hippocampal formation [the dentate gyrus (DG), hippocampal fields CA3 and CA1] and the medial Entorhinal Cortex (highlighting layers II and III). EC layer II projects to the DG via the perforant path (orange arrow, PP), while layer III projects to the CA3 and CA1 (green arrows); DG outputs run through the Mossy Fibers (MF, purple) to CA3, which in turn projects to the CA1 via the Schaffer Collaterals (yellow). Adapted from Williams (2000) [Williams, 2000].

cells, support the firing of place cells in the hippocampus [McNaughton et al., 2006]. The vestibular system, which feeds linear and angular head acceleration information to the EC, plays a crucial role in the generation of head-direction firing [Taube, 2007].
1.3.7 Rhythms of the hippocampus: theta and gamma oscillations

Neural oscillations are believed to set temporal windows, coordinating exchange, processing, encoding and retrieval of information in and between different structures in the brain [Engel et al., 2001, Traub et al., 1999]. The theta rhythm (7-10 Hz oscillations) represents the most prominent neural oscillation in the hippocampus and is present whenever the animal is in motion (walking, exploring, running, swimming, etc) [Vanderwolf, 1969] and, to a lesser extent, during other awake behavioral states, such as grooming, whisking and rearing [Young and McNaughton, 2009]. Following the initial idea that changes in the firing rate of a place cell code for the position of the animal (rate coding) [O’Keefe, 1976], the observation that the theta phase, at which spikes emitted by a place cell in its place field fire, regresses [O’Keefe and Recce, 1993], showed that, in addition to rate information, precise spike timing information contributes to the coding of spatial aspects of the animal’s environment or behavior [Huxter et al., 2003]. Another prominent rhythm of the hippocampus is gamma (broadly, 30 - 100 Hz), which has been associated with various cognitive processes, such as sensory binding [Singer, 1993], memory [Fell et al., 2001], temporal ordering [Jensen, 2005], keeping the excitatory-inhibitory balance, preventing runaway excitation [Ray et al., 2013] and attentional selection [Fries et al., 2001]. In the hippocampus, it has been proposed that gamma mediates encoding and retrieval of memory traces [Bragin et al., 1995, Lisman and Idiart, 1995, Hasselmo and Wyble, 1997] and that it might coordinate the integration of idiothetic and allothetic information during navigation [White et al., 2012].

More recently, gamma has been subdivided into low gamma (25 - 40 Hz), high gamma (∼55 - 100) and very high gamma (∼100 - 150 Hz) [Colgin et al., 2009, Belluscio et al., 2012]. These gamma oscillations are nested into theta oscillations [Bragin et al., 1995, Buzsáki et al., 2003], a mechanism that may work as a time pacemaker in sequential memories [Lisman and Idiart, 1995]. Recent studies have shown that theta synchronizes with the low and high gamma bands independently and that they originate in different brain areas: CA3 appears to be at the origin of low gamma activity, while EC activity may underlie the genesis of high gamma activity. Furthermore, this synchronization takes place during different phases of
the theta cycle, with high gamma preceding low gamma [Colgin et al., 2009, Belluscio et al., 2012]. This may be a mechanism for CA1 to segregate information arriving from the CA3 and EC, and possibly, by switching tuning to one or the other, reflect different processing modes: one internally (CA3) and one externally (environment) driven (EC).

**1.3.8 Spatial representation and encoding of navigation strategies in CA1**

"...Each place cell receives two different inputs, one conveying information about a large number of environmental stimuli or events, and the other from a navigational system which calculates where an animal is in an environment independently of the stimuli impinging on it at that moment. The input from the navigational system gates the environmental input, allowing only those stimuli occurring when the animal is in a particular place to excite a particular cell.

One possible basis for the navigational system relies on the fact that information about changes in position and direction in space could be calculated from the animal’s movements. When the animal had located itself in an environment (using environmental stimuli) the hippocampus could calculate subsequent positions in that environment on the basis of how far, and in what direction the animal had moved in the interim.....In addition to information about distance traversed, a navigational system would need to know about changes in direction of movement either relative to some environmental landmark or within the animal’s own egocentric space...."


Evidence for integration of allothetic and idiothetic information by place cells first came from studies on animal navigation in darkness: if preceded by a light period, place representation of CA1 cells of rats remained unaffected [Quirk et al., 1990], showing that path integration (idiothetic) information is enough to maintain spatial representation, but landmark (allothetic) information is needed for its formation and alignment to the environment. One other very ingenious study took a different approach
to address the relevance of motion signals to place field quality: Terrazas et al. [Terrazas et al., 2005] trained rats to navigate on a track under two conditions. In one condition, rats ran in a circular track and in the other one, rats made an operant response to propel a car along in the same circular track, therefore excluding the influence from self-motion signals. They observed that in the latter condition fewer cells expressed place fields and, those that did, had substantially larger ones.

From an (allocentric) navigational point of view, two important conclusions can be drawn from these studies: first, place fields are anchored to visual landmark cues in the environment and, second, the development and maintenance of a proper spatial representation is dependent on self-motion signals, vital for position updating [McNaughton et al., 1996, 2006].

Surprisingly little, however, is known about egocentric navigation and corresponding forms of spatial memory, and most of this knowledge is based on Packard’s early studies [Packard and McGaugh, 1996]. In these studies, Packard et al. trained rats in two different versions of a plus-maze, one requiring the animals to travel from one arm to a goal arm, which remained fixed to the environmental cues (place-based navigation) and the other requiring the animals to always execute the same turn in order to reach the goal regardless of the spatial starting location (e.g., left turn - ‘response’ navigation). Selectively inactivating either the Caudate Putamen (CP) or the HPC, they were able to show that, while place-based navigation was hippocampus-dependent, response navigation relied on the CP. Furthermore, inactivating one of these structures would lead to an increased use of the strategy supported by the other structure. Recordings of CA1 place cells in this task revealed that switching from one strategy to the other would lead to rate and global remapping of its place fields [Eschenko and Mizumori, 2007].

Since in this task, egocentric navigation was not dependent on the HPC, spatial representations, found in hippocampus during this type of navigation, may not have had any causal implications for behavior. In these cases, the hippocampus may be automatically encoding ongoing processes [Nadel and Moscovitch, 1997], which suggests that these results may not reveal the behavioral and causal relevance of the CA1 network in a hippocampus-dependent egocentric task. Given the role of the hip-
pocampus, notably CA3 and CA1, in memory sequences and temporal structuring of sequences [Kesner et al., 2004, Jensen, 2005], how does the CA1 process sequences of egocentric actions? Another pertinent question, based on Rondi-Reig’s results [Rondi-Reig et al., 2006], is how a spatial representation changes when navigation strategy is spontaneously changed: their results show that, in a starmaze swimming task [Rondi-Reig et al., 2006] mice made use of both sequential egocentric and allocentric navigation in similar proportions. Importantly, contrary to Packard’s results, which showed that animals initially rely mainly on place-based navigation and with experience switch to a stimulus-response strategy, animals showed no such pattern and in most cases switched back and forth between strategies, which in addition suggests that the egocentric strategies used in the Packard vs Rondi-Reig tasks may be fundamentally different: Packard’s egocentric navigation may be more habit based, whereas Rondi-Reig’s task relies more on sequence memory. Several studies have shown the coexistence of different reference frames in the same behavioral setting [Bures et al., 1998, Gothard et al., 1996b, Jackson and Redish, 2007, Fenton et al., 2010], suggesting that spatial representation in CA1 may switch between different modes according to the computations driving behavior and memory. In these studies, however, the different reference frames are thought to work alternately, switching spontaneously, not allowing to analyze them both separately.

1.4 Outline of the thesis

In our study, we have recorded the activity of ensembles of neurons from the CA1 area of the hippocampus, in normal and NR1-KO mice. Our goal was two-fold: to characterize CA1 place cell and network patterns of activity during different behavioral states in a navigation task and to characterize how the lack of the NMDAR affects CA1 functioning. Given that the different behavioral states expressed during the learning of the starmaze task recruit disparate processes, we further sought to investigate whether the mutation in NR1-KO mice elicited distinct behavioral and neuronal impairments in these disparate processes. In Chapter 2 we made a comprehensive analysis of behavior and place cell spatial rep-
representation in the starmaze task. Rondi-Reig et al. [Rondi-Reig et al., 2006] showed that NR1-KO mice were impaired at using either place- or sequence-based (learning the series of choice points leading to the goal) strategy to solve the starmaze task. To make it suitable for electrophysiological recordings, we transformed the original, water-based task into a land-based appetitive version, intensifying also the training protocol. In this chapter, we report that NR1-KO mice were capable of learning the task, despite showing an early deficit in the learning curve. They did, nonetheless, retain some impairments throughout experiments, specifically related to the use of the sequence-based strategy. Our main goal was to study place cell spatial representation during the use of the different navigation strategies in probe trials (trials, in which the departure point was changed). Given the different processes governing each strategy (spatial and sensory inputs are the relevant dimension in place-based navigation, while memory of a spatial sequence drives sequence-based navigation), we asked ourselves how place cells would react to the changed departure point: in control mice, place cells retained the same location of firing, in agreement with a navigation strategy which is anchored to the layout of the environment. During sequence-based navigation, however, place cells rotated along with the changed departure point, reflecting a behavior which is anchored to the start of the navigation route and paralleling the expression of a sequence of body responses. NR1-KOs showed the most accentuated impairments in sequence-based navigation. These results suggested that the mutation may affect differently the distinct inputs driving the different (behavioral) processes. Network oscillations offer a window into the communication entrained by different brain areas [Fries, 2005] and, as such, may allow to disentangle different input paths supporting place- and sequence-based navigation. Recent findings have suggested that different gamma bands (low, 25-45Hz and high, 55-95Hz gamma) may mediate integration of inputs from CA3 (low gamma) and EC (high gamma) in CA1 [Middleton et al., 2008, Colgin et al., 2009, Belluscio et al., 2012]. We therefore asked ourselves whether the balance between these two gamma bands would be a function of the strategy being employed. And how does coherence between theta and gamma vary? In Chapter 3 we looked into the different rhythms prevalent during training in the starmaze. Theta, low and
high gamma were present during the task and we report that the balance between the two gamma bands differed during place-based and sequence-based probe trials, shifting to greater high gamma influence during the former and to greater low gamma influence in the latter one, thus suggesting that EC may play a greater role in driving place-based navigation and CA3 during sequence-based navigation. Theta nested both gamma oscillations at different phases (as in [Colgin et al., 2009, Belluscio et al., 2012]), possibly a mechanism to reduce interference between both types of input. NR1-KO mice showed increased power in gamma oscillations and did not show the balance shift observed in control animals, possibly due to a saturation state of the network.

In Chapter 4 we analyzed the relation between place cell activity and network oscillations. Modulation of neuronal firing by oscillations are essential for its coherent activity [van Wingerden et al., 2010] and, thus, to spatial representation. Place cells are strongly locked to theta oscillations and the relation between place cell firing and theta may subserve the coordination of ensemble activity. The results reported point to a link between theta locking and sequence-based navigation in control mice and to a shift in the preferred spiking phase during place-based navigation, which may reflect a preferential tuning to the EC. Interneurons also showed behavior-dependent phase locking strength, which may reflect the role they have in modulating pyramidal cell activity, by gating inputs arriving to CA1[Klausberger et al., 2004, Leão et al., 2012].

One caveat of the starmaze task is the relative low number of trials, which makes an in-depth analysis of the trial-by-trial evolution of spatial representation difficult. We, therefore, recorded the activity of CA1 neurons of control and NR1-KO mice in a circular maze task, in which animals needed to shuttle back and forth across an interrupted circular track and collect a reward. The behavior in this task has no explicit learning component and allows the repetition of a stereotypical behavior. In Chapter 5 we report that single-trial place fields of NR1-KO mice have a similar size as place fields of control mice and that the larger, session-average place field observed (in our experiments and elsewhere [McHugh et al., 1996]) are the consequence of intertrial firing variability and may reflect an impairment these mice have in integrating CA3 and EC inputs to CA1.
Chapter 2

NMDA receptors in hippocampal area CA1 are essential for adaptive spatial coding during navigation based on motor sequences, but not external cues

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2.1 Abstract

The hippocampus is a structure central to spatial navigation. Despite being classically associated with allocentric (or place-strategy) navigation, recent evidence [Rondi-Reig et al., 2006] indicates that it is also important for the execution of sequences of egocentric responses (sequence-strategy). Furthermore, the N-methyl-D-aspartate receptor (NMDAR), crucial for inducing long-term changes in synaptic strength, is a key player in the hippocampal circuitry and the lack of this receptor leads to impairments in the ability to learn spatial tasks. To study patterns of activity in hippocampal area CA1 during the use of different navigation studies, and the cellular effects of the deletion of the NMDA receptor in this region, we trained and recorded CA1 single-cell activity in control and knock-out mice lacking this receptor in CA1 (NR1-KO) in a spatial navigation task (starmaze) allowing the disentanglement of the behavioral strategy adopted by the mice. Our results show that NR1-KO mice are capable of learning the task, but show specific impairments related to the use of a sequence-strategy. At the neuronal level, we report that the pattern of activity of place cells in control (CTR) mice is shaped by behavioral training on the starmaze task, is re-expressed during sequence navigation, but not place-based navigation, with the place cells expressing their fields according to the sequence of body-turns, with NR1-KO showing heavy impairments in the latter situation. These findings point to a crucial role of the CA1-NMDAR in linking different events in a motor sequence and show that, in the normal animal, place cell activity is modulated by the relevant dimension driving behavioral navigation, therefore following the type of information being processed.

2.2 Introduction

Accumulating evidence suggests that the hippocampus (HPC) is one of the crucial brain structures supporting navigation to a goal site. Pyramidal neurons in CA1 fire at a particular location in the environment and are thought to create a representation of a spatial map (a ‘cognitive map’) [O’Keefe and Nadel, 1978]. This firing behavior is stable in a cer-
tain environment and task context, but changes when either one or both of these two factors is altered - a place cell (PC) can lose or adopt a new firing location (place remapping) or keep its place field; and in this case, it may increase or decrease its firing rate (rate remapping) [Muller and Kubie, 1987, Fyhn et al., 2007, Leutgeb et al., 2005].

The HPC has long been pointed out as a key player in so-called place-based (or allocentric) navigation [Packard and McGaugh, 1996, Packard, 1999, Suthana et al., 2009], a strategy supported by a viewpoint independent map of the environment, based on converging evidence from lesion [Morris et al., 1982, Lavenex et al., 2006] and inactivation studies [Packard and McGaugh, 1996, Rogers and Kesner, 2006] and studies using transgenic animals [Rondi-Reig et al., 2006, McHugh et al., 1996, Nakazawa et al., 2004]. Nonetheless, several studies have shown that, depending on task details, animals may learn to use place-based navigation without a functional HPC [Winocur et al., 2005, Bannerman et al., 1995, 2012], indicating that it does not uniquely account for coordinating place-based navigation.

Another prominent navigation strategy employed by animals is egocentric navigation, whereby a memorized sequence of movements is retrieved and executed, disregarding the use of external information for guiding purposes [Rondi-Reig et al., 2006, Packard and McGaugh, 1996]. The fact that a functional HPC is not necessary for a simple egocentric trajectory (e.g. one body turn, [Packard and McGaugh, 1996]), but becomes necessary with increasing complexity (e.g. sequence of body turns, [Rondi-Reig et al., 2006]), highlights one of the functions attributed to CA1: that of temporal organization of memories and linking events in a sequence [Wallenstein et al., 1998, Eichenbaum, 2000].

Spatial representation in CA1 depends on its two main input sources, CA3 and the entorhinal cortex (EC) [Witter et al., 2000, Brun et al., 2002, 2008]. Both structures show, similarly, striking spatial functional correlates: CA3 also contains place cells [Mizuseki et al., 2012] and despite its similarities with CA1 place cells, shows important differences in the firing rate and pattern of its principal neurons, spike-LFP properties and network connectivity [Mizuseki et al., 2012]. These different properties and its location in the EC-HPC hub endows it also with different functions. Most notably, CA3 is seen as an autoassociator of memories, capable of
completing memories, for instance, in their proper sequential order, given only a subset of cues [Treves, 1995, Leutgeb and Leutgeb, 2007], these functions being supported by its extensive recurrent network. In addition, CA3 is crucial for one-trial learning [Nakazawa et al., 2003]. The EC, notably its medial section, contains principal neurons with highly regular spatial firing properties: firing of such neuronal populations tessellates the entire environment in regular hexagons [Hafting et al., 2005]. Contrary to CA3 inputs, mEC inputs, which project to CA1 from its layer III, are crucial for place field formation [Brun el al., 2002, 2008]. The lateral portion of the EC has lower spatial specificity [Hargreaves et al., 2005] and is believed to relay non-spatial, sensory information to the HPC, while the mEC provides the spatial inputs [Deshmukh and Knierim, 2011, Hunsaker et al., 2007].

Navigational strategies based on place and sequence memory are not mutually exclusive, and typically they will be jointly employed [Gothard et al., 1996b]. However, when the initial conditions are changed, the animal will have to opt for an optimal strategy to reach its target. Based on this principle, Packard et al. developed a behavioral task to distinguish between the two strategies: the plus-maze, where animals are trained to find a reward in, e.g., one of the east-west oriented arms, when departing from one of the north-south oriented arms. Upon a change in the departure point to one of the alternative north-south oriented arms (e.g. north, instead of the usual south arm), the animal will have to either use the cues surrounding the maze to reach the goal arm (place-based, or cue-guided strategy), or execute the same body-turn as previously (response strategy). Packard showed that, whereas the latter one depends on the Caudate Putamen (CP), the former one depends on the HPC [Packard and McGaugh, 1996]. The fact that the animal was only required to learn one body-turn suggests that navigation, in this case, may bypass the HPC by not requiring the use of one of its main functions: that of endowing a complex behavioral action with a temporal structure.

Following up on these studies, Rondi-Reig et al. (2006) developed a conceptually similar, but more complex task, the starmaze, involving several choice points between departure and goal. With this, they were able to demonstrate that learning of a sequence of body-turns (sequential egocentric navigation) demands a functional CA1 (mice, where the N-
methyl-D-aspartate (NMDA) receptor had been knocked-out in this area, were impaired in the task). Importantly, this impairment was limited to the multiple choice-points version of the task: in the single choice-point version, KO animals performed at the same level as control ones.

While hippocampal spatial representation during place-based navigation has been exhaustively studied over the past decades [Wilson and McNaughton, 1993, Gothard et al., 1996b, Fenton and Muller, 1998, Quirk et al., 1990, Muller and Kubie, 1987], it remains unknown how sequential egocentric trajectories are encoded. Given that CA1 is capable of switching between different reference frames [Gothard et al., 1996b, Jackson and Redish, 2007, Jezek et al., 2011], how does CA1 spatial representation change, when the animal spontaneously changes strategy (i.e., strategies are not imposed by the experimental protocol)? This becomes more relevant, given the fact that, in the starmaze, mice usually switched back and forth between the two strategies (which can be identified in probe trials in the starmaze, in which the departure arm is changed). For this, we recorded place cell activity during learning of the starmaze task in normal mice and CA1 NMDAR-KO mice (NR1-KO). The NMDA receptor is a crucial molecule in synaptic plasticity [Bliss and Collingridge, 1993, McHugh et al., 1996] and its absence or blockade leads to severe behavioral [Morris et al., 1986, Rondi-Reig et al., 2006] and neuronal [McHugh et al., 1996, Korotkova et al., 2010] deficits.

Here we demonstrate how NR1-KO mice are capable of learning both place-based and sequence-based trajectories, but show specific impairments in the latter one, dependent on the complexity of the trajectory. Furthermore, we show that CA1 spatial representations dynamically change during the use of place- and sequence-based navigation, reflecting the basis of the strategies guiding behavior in both cases. While spatial representation in the former case was largely unaffected in NR1-KO mice, it was severely disrupted in the latter one. These results suggest a crucial role for CA1-NMDA receptors in storing and retrieving sequential memories, which may be linked to the different inputs to area CA1 supporting both types of navigation.
2.3 Methods

Subjects. Male C57BL/6 mice lacking the NMDAR1 gene in the CA1 subregion of the hippocampus, originally created at the MIT (Cambridge, Massachusetts) [Tsien et al., 1996], were inbred in and obtained from Université Paris VI. The KO is specific to the CA1 subfield until 2.5 months of age [Fukaya et al., 2003]. Control (CTR) mice were ‘floxed’ littermates of KOs, not carrying the NR-1 deletion. Animals were maintained on a reversed day/night cycle (lights on/off: 20h/08h), single-housed and food restricted to 90% of their free feeding weight. All experiments were carried out in accordance with Dutch National Animal Experiments regulations (Wet op Dierproeven) and approved by the Universiteit van Amsterdam. Fifteen mice (8 KO and 7 CTR), were used for electrophysiological experiments, and implanted at the age of 41±5 days, weighing approximately 20 grs. All mice were between 42 (minimum) and 63 (maximum) days of age during the recording phase. At all times, a KO and a CTR mouse were studied in parallel, with all procedures counterbalanced by genotype. Electrophysiology data from 6 mice were excluded, because histology did not yield a reliable reconstruction of the probe’s position, or other technical problems.

Apparatus. Training took place in a custom made starmaze (segment dimensions 7 x 20 x 35 cm (37 cm) - for central alleys (radial arms)), with lexan glas walls. The experimental room had black curtains at the walls with large geometrical cues on the four sides, and 40 watts light-bulbs at each corner. Two exemplars of each wall cue were presented to ensure that radial arm identification could not be accomplished by seeing a single visual cue.

Behavioral Protocol. In training trials, animals had to navigate to the goal arm leaving from a fixed departure arm (Fig. 2.3a). Probe trials were administered to assess the behavioral strategy used by the animals. In these trials, the animal was set to depart from a different arm, no effort being made to disorient the animals. Depending on the trajectory described by the mouse, each trial was attributed to a different strategy: a probe trial resulting in a run directly to the same arm, that was
rewarded in training trials, was classified as a place-strategy trial, consistent with the animal identifying the goal arm with reference to a map of the environment. Finally, if the animal used the same sequence of turns as in training trials (e.g. left-right-left turn) this was interpreted as the animal executing a sequence of egocentric movements to reach the goal (sequence-strategy trial) (Fig. 2.3b). During probe trials, both place- and sequence-based choices were rewarded.

Four days after arrival at the lab (average age at arrival: 29.8±1.7 days), mice were introduced to a 2-days habituation protocol to the maze. After that pre-training started, consisting of two daily sessions for 5 days (one in the morning, one in the afternoon), each session containing seven training trials. From day 3 until the end, a probe trial was introduced between trials 4 and 5 (Fig. 2.1a).

After pre-training, mice underwent drive implant surgery (at all times a CTR/NR-1 KO pair was studied in parallel). Eight days after implantation, recordings in the starmaze started with two daily sessions during the first 7 days and a single session on the 8th day (total = 15 sessions).
Each session contained 15 training trials; probe trials were introduced in session 2 (one probe trial in session 2 and 3 probe trials thereafter). Each session was flanked by two rest periods (at least 20 min long). For the recording series of experiments, a new configuration of departure and goal arms was chosen, and a new set of environmental cues was used. In any given trial, a visit to an unrewarded arm was considered a mistake. After two mistakes, the trial was terminated and the mouse was returned to its home cage. During a 1 minute inter-trial interval, the experimenter wiped the maze with a 20% ethanol solution. Each recording session was flanked by 20 minutes sleep periods that were used to assess recording stability.

Drive, Surgery and Tetrode positioning. Six independently moveable tetrodes were loaded into a custom made microdrive [Battaglia et al., 2009] and implanted over the dorsal hippocampus (AP: -2.0 mm, ML: -2.0 mm). In the week after surgery, tetrodes were gradually lowered until they reached CA1 pyramidal layer, before recordings began. Tetrode position was adjusted between recording sessions to maintain them in CA1 pyramidal layer, which was identified by the presence of strong ripple events and the presence of bursty excitatory neurons.

Histology. After recordings, electrolytic lesions were made at the recording sites by passing 20 µA of current for 10s through one lead of each tetrode. After perfusion with formal saline, coronal brain sections (40µm) were cut on a Vibratome and Nissl-stained for verification of tetrode tracks and end points. Only animals with clear lesions in CA1 pyramidal layer and/or clear sharpwave-ripple complexes and ripple-modulated cell firing were included in the analysis.

Data acquisition. Tetrode signals were unit-gain amplified by the head-stage pre-amplifiers (Neuralynx, Bozeman, MT) and relayed to amplifiers for single-unit and local field potentials (LFP) recordings. The signal was amplified 2000 times, bandpass filtered (0.6-6.0 kHz for single-unit; 1-475 Hz for LFP), acquired (sampling rate: every time the signal exceeded a manually selected threshold at 32 kHz for single-unit; continuously at 2 kHz for LFP) and time-stamped. A tetrode (targeted to a location devoid
of units and near the area of interest, like the corpus callosum) was used as a reference.

Single-unit data were pre-processed with KlustaKwik [Harris et al., 2000] for automated spike clustering. Spike sorting results were manually refined using Klusters [Hazan et al., 2006]. Mouse position and orientation on the maze were extracted from video footage (using the full animal silhouette as filmed by a camera placed directly on top of the maze) with Ethovision XT image analysis software (Noldus, Wageningen, The Netherlands), which was synchronized with the electrophysiology data acquisition system.

Data analysis: Behavior. To quantify performance in the starmaze, we used the localization score [Fouquet et al., 2011] calculated by evaluating the animal’s choice at each intersection: a choice bringing it closer to the goal was awarded a value of 100 (0 otherwise). Values from every intersection in each trial were averaged and the result of all training trials in a session was averaged, to obtain a score for that session. Because

![Figure 2.2: Speed profiles of CTR and NR1-KO mice](image)

(a) Speed profiles along the normalized trial length (from departure point to goal point), per trial type. ‘Short’ paths are shown in the top row, ‘long’ paths in the bottom one. Local minima correspond to the intersections in the maze.

(b) Average velocity per trial type. There was no difference in the average velocity in the different trial types (ANOVA, n.s.).
there are two possible paths to goal (short and long, see (Fig. 2.3a)), the choice at the first intersection was always awarded 100. For the strategy identification in probe trials, only probe trials that were preceded by at least 2 equivalent correct training trials (e.g., for a short sequence-strategy trial, a minimum of 2 short training trials) since the previous probe trial, were considered. There was no significant difference between genotypes, or trial types in the average running speed (ANOVA P(genotype) n.s., P(trial type) n.s., P(interaction) n.s.; Figure 2.2b).

Data analysis: electrophysiology. All data used for analysis were from periods in which the animal was moving at speeds above 3 cm/sec. Neuron Classification: Clusters with more than 0.5% spikes during the first 2 msec of the interspike interval (refractory period), or a firing rate during the run period of the recording session lower than 0.25 Hz were excluded from analysis. The remaining clusters were separated in putative interneurons and pyramidal neurons, using a fuzzy-clustering algorithm (see: Fuzzy Clustering and Data Analysis Toolbox, http://www.fmt.vein.hu/softcomp/logystoolbox [Bezdek, 1981]), based on the firing rate, the mean of the autocorrelogram and the initial slope of valley decay (ISVD). The ISVD was calculated as follows:

\[
ISVD = -100 \times \frac{V_v - V_{0.26}}{A_{PV}}
\]

where \(V_v\) is the most negative value (valley point) of the spike waveform, \(V_{0.26}\) the voltage at 0.26 msec after \(V_v\) and \(A_{PV}\) the peak to valley amplitude [Lansink et al., 2010]. Only neurons that were included in the pyramidal or the interneuron cluster with more than 70% certainty of belonging to one of them, were used for analysis (Fig. 2.1 right panel).

Firing map construction: Firing maps were constructed by dividing the maze area into 2x2cm bins. The number of spikes fired by each cell in each pixel was divided by the occupancy time in that pixel to obtain the firing rate.

Place Field definition: For each cell, its place field was defined as all pixels with a firing rate above 30% of the maximum firing rate, as long
as there were at least 10 spikes fired within the place field.

Spatial information and sparsity. Spatial information is a measure of the extent to which the firing of a cell can be used to predict the position of the animal. The estimate of the rate of information Spatial Information [Skaggs et al., 1993] pertaining to firing rate $F$ and location $x$ is as follows:

$$\text{Spatial Information} = \frac{\sum p(x)f(x)\log_2 \left( \frac{f(x)}{F} \right)}{F},$$

where $p(x)$ is the probability of the animal being at location $x$, $f(x)$ is the firing rate observed at $x$, and $F$ is the overall firing rate of the cell. Sparsity [Skaggs et al., 1993] measures the fraction of the environment in which a cell is active and is given by:

$$\text{Sparsity} = \frac{(\sum p(x)f(x))^2}{\sum (p(x)f(x)^2)}$$

Place ($P_{idx}$) and sequence ($S_{idx}$) indices. To address the similarity of the firing rate maps between training and probe trials, we calculated two different indices. For place-strategy trials, we extracted the common occupancy area between a training and a place-strategy trial. For cells with at least two thirds of their training trial place field in the common area, we calculated the Pearson’s correlation between its firing map in the two conditions in that area.

The $S_{idx}$ was computed similarly, but first the mouse’s occupancy map in sequence-strategy trials was rotated by the angle difference between the departure arms of training trials and for probe trials ($360° / 5 = 72°$) and aligned to match the occupancy map of the training trials. Sequence-strategy trials making use of ‘short’ and ‘long’ trajectories were compared with their training trial equivalents (Fig. 2.3a,b).

To normalize the correlation, accounting for the small differences in place field size across genotypes, we formed a shuffled data set by calculating the correlation between the firing map in training trials for a given cell with the firing map in probe trials for a different cell. This procedure was repeated for all possible cell pairs of mice of the same genotype and the
average value was used for the normalization.

2.4 Results

**NR1 deletion particularly affects sequence-based navigation**

We trained control (CTR) and NR-1 CA1 KO mice to run on a complex, pentagon-shaped maze with five radial arms (the ‘star-maze’ [Rondi-Reig et al., 2006]; Fig. 2.3a), and find food rewards placed at the end of a radial ‘goal’ arm. In training trials mice left from a fixed departure arm, but in probe trials (1 to 3 per session, see Fig. 2.3a for the task protocol), they were started from a different radial arm, placed at a 72 degrees angle with respect to the regular departure arm (Fig. 2.3b). In pre-training sessions preceding surgical implant, localization score ([Fouquet et al., 2011], see Methods), a measure of how well the animal is capable of orienting itself towards the goal location, of both CTR and NR-1 KO mice climbed steadily (Fig. 2.3c).

NR1-KO were, however, significantly slower than CTR in acquiring the task (repeated measures ANOVA n(CTR) = 15, n(NR1-KO) = 13, P < 0.05), confirming previous results in the same task with a water-based maze and a more spaced training schedule [Rondi-Reig et al., 2006]. Nevertheless, in a second series of trials with a different choice of departure and goal arms and set of cues and a more intense protocol, during electrophysiological recordings, learning proceeded at similar rates for the two genotypes (Fig. 2.3c, repeated measures ANOVA n (CTR) = 6, n (NR1-KO) = 8, P = 0.96). The same was observed, when using the percentage of correct trials as a measure of performance (data not shown).

In most probe trials, mice spontaneously followed one of two routes (Fig. 2.3a). The first one ended in the same goal arm as in training trials, as if mice were finding the reward based on a ‘place’ reference frame, using external landmark information. The second route reproduced the same, memorized sequence of left and right turns as in training trials, but from a different starting point, and therefore ending in a different arm, disregarding distal sensory inputs. This is compatible with animals using a ‘sequence’ reference frame. Both CTR and NR1-KO mice showed a sim-
Figure 2.3: Learning in the starmaze task in CTR and NR1-KO mice.
(a) Scheme of the starmaze, displaying the ‘long’ (solid line) and ‘short’ (dashed line) paths used by mice to reach the reward site during a training trial.
(b) The short paths used by mice during place- (gray) and sequence-strategy (black) probe trials.
(c) Learning performance for training trial expressed as the ‘localization score’ (see Methods), for CTRs (filled squares) and NR-1 KOs (empty squares). Data is separated into a pre-training period (15 CTR and 13 NR1-KO) and the recording period (6 CTR and 8 NR1-KO). In pre-training, KO performed learned slower than CTRs (repeated measures ANOVA $P < 0.05$); during the recording period there was no difference.
(d) Fraction of probe trials in which mice used, respectively, sequence-based, place-based, ‘serial’ (defined as visiting adjacent alleys one after the other), and ‘random’ strategies during the recording period, divided into 5 blocks (3 sessions per block). No significant difference between CTR and NR1-KO mice (Fisher’s 2x4 exact test (per block), n.s.) was detected.

ilar profile in the evolution of strategy usage (Fig. 2.3d; Fisher’s exact test: n.s. in each stage block): at early stages of training, random and serial (visiting adjacent arms sequentially until finding the rewarded one) search strategies predominated, however, with learning, both place- and sequence-based strategies prevailed over the former two. Furthermore, place- and sequence-based strategies evolved similarly along the training protocol (Binomial Test, n.s. in each block), suggesting that mice are equally likely of using either one.

In training and probe trials, mice traversed the maze either via a ‘short’ route spanning two sides of the central pentagon or a ‘long’ route span-
Figure 2.4

Training three sides (Fig. 2.3a). In training trials, CTR mice were as likely to use either path. NR1-KO, however, relied mainly on the short path (Fig. 2.4a, $\chi^2$-test: $P < 0.001$). In place-strategy trials, there was no difference between genotypes, both making use predominantly of the short path, while during sequence-strategy trials, CTR mice used both paths in similar proportions, but NR1-KO used almost exclusively the short path ($\chi^2$-test: $P < 0.001$). A closer analysis showed that, at the second intersection along the long training path, both genotypes made the same
Figure 2.4: NR1-KO are impaired at using the long path
(a) Fraction of trials in which mice used the short vs long trajectory to the goal, per trial type. NR1-KOs used significantly less often the long trajectory to the goal in training and sequence-strategy trials ($**\chi^2$ test, $P < 0.001$).
(b-d) Ratio of correct choices at the intersections of short and long paths during training (b), place- (c) and sequence-strategy (d) trials ($\chi^2$ test (between genotypes) *$P<0.05$, ***$P<0.001$).
(e) Outcome probability of short and long-path trials during the recording period. There was no difference in the outcome probability in short trials ($\chi^2$ test (between genotypes) n.s.), but the success rate in long-path trials was lower in NR1-KO ($\chi^2$ test (between genotypes) $P<0.05$). In addition, short-path trials had an overall higher success rate than long-path trials ($\chi^2$ test (between genotypes) $P < 0.01$).
(f) Percentage of long path choices during pretraining and recording phases. Both genotypes made use of the long trajectory in similar proportion and less often than the short one (Binomial Test (short x long), #: $P < 0.01$). While NR1-KO mice reduced the use of the long trajectory during the recording period, CTR increased it (in both: $\chi^2$-test (pretraining vs recording), *$P < 0.01$), to level with short path choices (Binomial Test (short x long): n.s.). During recording NR1-KO chose the long path less often than CTR mice ($\chi^2$ test (between genotypes), *$P < 0.01$); dashed line indicates 50% change level.
(g) Localization Score in short or long-path trials. During pretraining, NR1-KO mice performed worse than CTR in short-path trials, while during recording the opposite pattern emerged (in both cases: ANOVA, Post-Hoc Tukey’s HSD test: $P < 0.05$).

number of correct turns, higher than chance (Fig. 2.4b, Binomial-test: $P < 0.001$; $\chi^2$-test between genotypes: $P < 0.05$), but only CTR were above chance at the third one. This suggests that NR-1 KO mice are impaired in following a route composed of more than one choice point.

A similar pattern was observed in sequence-strategy trials (Fig. 2.4c, $\chi^2$-test between genotypes: $P < 0.001$), but no differences were found in place-strategy trials (Fig. 2.4d). Note that a ‘correct’ choice towards a long place-strategy trials means taking the central alley (i.e. the side of the pentagon) at the third intersection. The opposite choice (i.e., taking the radial arm, for the purpose of this analysis marked as an error) results in a successful short sequence-strategy trial, which is the most probable occurrence for both genotypes. A similar ambiguity as in the previous
case exists between long sequence-strategy and short place-strategy trials, resulting in a fraction of correct choices below 50%. This explains why both genotypes show a relatively low number of ‘correct’ choices. These results highlight the deficit of NR1-KO mice in using the long sequence-strategy trajectory.

The long path, by adding one extra choice point, may represent a significant increase in task complexity. To quantify this, we calculated the probability of a successful outcome, upon choosing for the short or long paths at the first intersection during training trials and found that it was significantly lower in the latter case for both genotypes (Fig. 2.4e, \( \chi^2 \)-test: \( P < 0.01 \)), suggesting that, indeed, this route is more complex than the short one. Successful outcome probability for the long paths was, in addition, lower in NR1-KO mice (\( \chi^2 \)-test, Bonferroni corrected: \( P < 0.05 \)), raising the possibility that this impairment in the long path to the goal may be leading to the impaired performance seen in the pretraining stage and that NR1-KO mice adapt their strategy, by choosing less often this path, during the recording stage. We, therefore, calculated the percentage of choice for either the short or long path in training trials during the pretrained and the recording period. During pretraining, mice from both genotypes opted for the long path (irrespective of outcome of that trial) slightly less often than for the short path (Fig. 2.4f, Binomial test: \( P < 0.01 \), no difference between genotypes: \( \chi^2 \)-test: n.s.). During the recording period, however, CTR mice increased their choices for the long path, to level with the short path choices (Binomial test: n.s.), while NR1-KO mice decreased it (\( \chi^2 \)-test: \( P < 0.001 \)), suggesting that the unimpaired learning observed in Figure 2.3c is mainly due to the performance during short training trials.

When analyzing both paths separately, we observed that, during pretraining, both CTR and NR1-KO mice had a lower performance in long-path trials (Fig. 2.4g Post-Hoc Tukey’s HSD \( P < 0.01 \)). In short-path trials performance was greater than in long ones in both cases, but NR1-KO were still below that of CTR mice (Post-Hoc Tukey’s HSD \( P < 0.05 \)). During the recording phase, both genotypes showed an increase in performance for both short and long paths (Post-Hoc Tukey’s HSD \( P < 0.01 \)), presumably due to the longer training. But while NR1-KO leveled the CTR’s performance during short trials, they were impaired in long-path
Figure 2.5: NR1-KO place fields have lower spatially tuning
Place field of CTR and NR1-KO pyramidal neurons: NR1-KO place cells had larger
place fields, a lower firing rate increase within the place field, more place fields per
cell, carried less information per spike and had sparser firing (Student’s t-test: ***P
< 0.001). There was no difference in the maximum firing rate.

trials (Post-Hoc Tukey’s HSD P < 0.01). These results suggest that, in
both genotypes, learning of the long path is slower than the short one,
in agreement with its increased complexity, and that NR1-KO mice are
slower at learning both paths.

Place field map switching to sequence reference frame is impaired
in NR1-KO place cells

We recorded the activity of 952 cells from CTR mice (n=6) and 739
cells from NR1-KO mice (n=8), which were classified as putative in-
terneurons or pyramidal cells (see Methods and Fig. 2.1b). This sorting
procedure yielded 357 and 247 pyramidal neurons from the CTR and
NR1-KO groups, respectively, with a place field (see Methods) that were
included in the following analyses. Similar to previous studies [McHugh
et al., 1996], place field coding in NR1-KO mice was impaired (Fig. 2.5,
t-test, *: P < 0.01). This was reflected in the number of place fields per
cell, the firing rate ratio within the place field to outside it, the proportion
of the maze area covered by the place field, the spatial information per
spike and sparsity of firing.

In CTR mice, hippocampal place fields appeared to span the same lo-
cations as in training trials during place-strategy trials (thus remaining
anchored to a place-based reference frame), but, in sequence-strategy tri-
als, they rotated so as to keep a consistent relationship with a sequence
reference frame (Fig. 2.6a).
To quantify these effects, we calculated two similarity indices: a place ($P_{idx}$) and a sequence index ($S_{idx}$). The first was calculated as the Pearson’s correlation between firing rate maps in training trials and probe trials, using the overlapping portion of the routes. $S_{idx}$ is the Pearson’s correlation between the firing rate maps in training trials and the firing rate map in sequence-strategy trials, rotated by 72° to make training and probe departure arms coincide. Both correlations were normalized by means of a shuffled condition (see Methods). Both CTR and NR1-KO mice showed elevated $P_{idx}$ values, several-fold higher than shuffled con-
Figure 2.6: Place cell behavior during place and sequence-strategy trials.  
(a) Left: Examples of place cells of CTR mice during training trials (actual mouse trajectory outlined in cyan), place- (green outlines) and sequence-strategy (orange outlines) trials. The peak firing rate is indicated next to each display. Right: same as left, for NR-1 KO mice.  
(b) Top: diagram of two example trajectories, respectively from training trial (cyan) and a place-strategy trials (green) that may be used for $P_{ids}$ calculation. Parentheses indicate the overlapping portion of the trajectory that may be used for the index calculation. Bottom: average $P_{ids}$, for the two genotypes: NR-KO mice showed a significantly lower value than CTRs (*: t-test, $P < 0.05$). Dashed line indicates chance level (see Methods).  
(c) Same as (b), but for $S_{ids}$. Top: diagram of two example trajectories from, respectively, training (cyan) and sequence-strategy trials (yellow) that entered $S_{ids}$ calculation. Before the correlation between firing rate maps is computed, the map for the sequence-strategy trials is rotated 72° clockwise so that it superimposes on the map for training trials. NR-1 KOs had strongly reduced $S_{ids}$ values as compared to CTR mice (**: t-test, $P < 0.01$).  
(d) Average firing rate of pyramidal neurons during the different trial types. NR1-KO mice showed a lower firing rate during sequence-strategy trials (ANOVA: $P$ (trial type) < 0.05, *: Post-hoc Tukey’s HSD, $P < 0.05$).  
(e) $P_{ids}$ and $S_{ids}$ of place cells of CTR mice recorded in both trial types show a negative correlation (Spearman’s $R$, $P < 0.05$).  

Contrasts, with NR1-KO mice showing moderately, but significantly, lower values than CTR (t-test $P < 0.05$, Fig. 2.6b).  
During sequence-strategy trials, CTR place cells displayed on average a high $S_{ids}$ (Fig. 2.6c), suggesting that they can maintain spatial selectivity and rotate their place fields with the departure arm. Strikingly, NR1-KO place cells had a strongly reduced $S_{ids}$ (t-test $P < 0.01$) compared to CTR, only showing remnants of a rotation effect. In sequence-strategy trials, NR-1 KO place cells showed a disrupted activity pattern, as can be seen from the reduced firing rates (Fig. 2.6d, ANOVA $P$ (trial type)<0.05, Post-Hoc Turkey’s HSD (NR1-KO training trial vs sequence): $P < 0.05$), hinting at a critical role for CA1 NMDA receptors in the expression of a sequence-based place field map.
Fifty-six cells in CTR and 29 in NR1-KO mice were recorded during both place- and sequence-strategy trials, and for those the respective indices could be compared. Interestingly, for CTR place cells there was a negative correlation (Fig. 2.6e; Spearman: r = -0.32, P < 0.05), compatible with a gradient of tuning of CA1 pyramidal cells to the two references frames. This variability in cell behavior was lost in NR1-KO mice, in which the distributions for $P_{idx}$ and $S_{idx}$ were much more concentrated, and no correlation between the two measures was observed (between genotypes: Fisher’s test of correlations $P < 0.05$).

Dividing the $S_{idx}$ of CTR neurons in short and long sequence-strategy trials (Fig. 2.7) revealed that during the latter ones, pyramidal neurons were more likely to accurately rotate their place fields. This observation offers further support to the hypothesis that the long trajectory, by demanding the memorization of a more complex trajectory, requires a stronger hippocampal involvement. NR1-KO mice never made use of the long sequence-strategy route, thus making the comparison not possible.

**Experience-dependent changes in the behavioral modulation of place cell firing**

We analyzed the improvement in sequence- and place-based coding, as training progressed (Fig. 2.8). Interestingly, $P_{idx}$ in CTR animals increased along the session blocks (left, ANOVA, $P < 0.01$). The $S_{idx}$ (right), despite a rising trend over the first three blocks, did not reveal a significant effect. NR1-KO place cells did not show any increase in their place or sequence index.

We also compared the spatial information per spike carried by each place cell in the first half of the recording experiments with the second half (Fig.

Figure 2.7: Sequence index in CTR in short vs long sequence-strategy trials

*CTR showed a higher $S_{idx}$ in long trials than in short ones (Student’s t-test: $P < 0.01$).*
Average $P_{idx}$ (left) and $S_{idx}$ (right) indices per session block. **CTR** showed an experience-dependent increase in the $P_{idx}$ (Spearman’s $R = 0.3$, $P < 0.001$), an effect absent in NR1-KO mice (Fisher’s test between correlations: $P < 0.05$) and for the $S_{idx}$ in both genotypes.

2.9), showing that in all cases, including CTR and NR1-KO groups, the distribution was bimodal (Lillietest, $P < 0.001$). This analysis further revealed that, in CTR mice, there was a group of cells with lower spatial information values and one with higher values, the latter one shifting to even higher values in the second half of the recordings (KS-test: $P < 0.001$). NR1-KO place cells also showed a lower and higher SI group, but it was the former one that shifted to higher SI values (KS-test: $P < 0.05$), maybe reflecting an improvement of the overall worse spatial representation. These results suggest that, in CTR, there is a group of cells which are more ‘task-engaged’ and refine their spatial tuning with experience.

In CTR mice, approximately half of the cells (Fig. 2.10, fraction $= 0.48$, $n = 19$ trials), recorded simultaneously during sequence-strategy trials (in sessions with 3 or more cells) had an $S_{idx}$ above the 95th percentile of the shuffled data. This proportion was significantly higher than that of NR1-KO mice (Fig. 2.10, fraction $= 0.16$, $n = 12$ trials, t-test $P < 0.01$). This suggests that in CTR mice, once they perform a sequence trial, not all cells in the population follow the sequence framework and that only a residual subset of NR1-KO cells follow this framework. It is possible, however, that these numbers (in particular in the CTR case) are an underestimation of the true values (see next section and discussion).
Spatial Information (spikes/bin)
CTR
KO
sessions 1-7
sessions 8-15

fraction of place cells

0.04
0.08
0
1 2 3 4 5 6 7
1 2 3 4 5 6 7

Figure 2.9: Experience-dependent population changes in spatial tuning. Distribution of spatial information (SI) values for place cells recorded during the first (green) and last (orange) halves of each recording session, for CTR (left) and KO (right). In all cases were the distributions bimodal (Lillietest, $P < 0.001$). In CTRs, the group showing higher SI values in the first half shifted to even higher values (KS-test: $P < 0.001$). NR1-KO showed a blurrier picture, in which it is the group with the lower SI values in the first half that shifts to higher values (KS-test: $P < 0.05$).

Inconsistencies in the use of strategies

A significant subset of cells (Binomial Test: $P < 0.001$ in both genotypes) showed a firing pattern during sequence-strategy trials that matched that of a typical place-strategy trials: instead of rotating their fields, they maintained the same firing location (Fig. 2.11 top, black bars indicate units, that significantly rotated their place fields), which may represent cases of a within-trial strategy shift, where the animal starts the trial repeating the same sequence of body turns, but during the run, switches to a place navigation strategy, maybe triggered by the sight of one of the cues surrounding the maze.

Similarly, a significant subset of cells in CTR (Binomial-test: $P < 0.001$)

Figure 2.10: Ensemble behavior during sequence-strategy trials
Fraction of place cells per session (with a minimum of 3 place cells with a place field in sequence-strategy trials) with a significant $S_{st}$. CTR had a higher fraction than NR1-KO mice (t-test: $P < 0.05$).
Figure 2.11: Inconsistencies in the use of strategies.
A subset of place cells in both genotypes showed a pattern of activity inconsistent with the behavior employed: a significant number remained locked to the place framework during sequence-based navigation (threshold: 95th percentile of shuffled data; top: Bonferroni corrected Binomial Test: P (CTR) < 0.01, P (NR1-KO) = 0.05), while others rotated their place fields during place-strategy trials (bottom: Bonferroni corrected Binomial Test: P (CTR) < 0.05, P (NR1-KO) n.s.).

rotated its firing fields during place-based navigation (Fig. 2.11 bottom). In this case, either the ensemble adopts a sequence-strategy firing pattern and, like in the previous case, does not follow the actual behavioral pattern of the animal, or represents cases, in which the animal switched from a sequence-strategy to a place-strategy within the trial (this situation is unlikely in the previous case, given that a sequence-based navigation is, by definition, anchored to departure, and it is unlikely that an animal would switch from a place- to a sequence-strategy in the course of a trial). NR1-KO mice also showed a small number of cells with a significant effect (although with a much lower \( S_{idx} \)).
2.5 Discussion

The starmaze task allowed us the identification of two different strategies used by animals in route finding, one (‘place-based’ strategy) driven by environmental cues and the other requiring retrieval of a sequence of body turns, disregarding distal sensory information (‘sequence-based’ strategy). While a hippocampal involvement in place strategy [Packard and McGaugh, 1996] is to be expected based on its role in forming a cognitive map of an environment [McNaughton et al., 2006], the response strategy is commonly linked to basal ganglia function [Packard and McGaugh, 1996]. However, the hippocampus, and CA1 in particular, may become more and more important for this type of behavior as the complexity of the sequence increases [Lee et al., 2005, Rondi-Reig et al., 2006], or when disambiguation is needed between conflicting information [Bannerman et al., 2012]. This hypothesis is supported in this study by the very low likelihood of NR1-KO mice to successfully take the more complex long path to the goal during sequence-strategy trials. While there are still signs of memory retention for the first and second turn on that path, these KO animals perform at chance level at the third intersection. In our experiment, hippocampal involvement is most remarkably signaled by the slower initial learning of NR-1 KO mice, which confirms previous results in a water-based, spaced learning version of the same task [Rondi-Reig et al., 2006]. Asymptotic behavior of NR-1 KOs is, however, indistinguishable from that of control animals, in terms of training trial performance, running speed (Fig. 2.2) and strategy selection during probe trials, a favorable circumstance for comparing electrophysiological correlates of maze exploration in the two genotypes.

A combination of factors in our task is likely to facilitate learning, of which the most important are the training intensity of the task and the change of a water- to a land-based version. Compared to the training protocol, the recording protocol contains more trials per session and more sessions, which may facilitate learning. The increased steepness of the learning curve in the recording phase also suggests that, despite changing cues and rotating the maze, mice are able to efficiently transfer knowledge from the pretaining to the recording phase. In addition, navigation
in a dry maze may facilitate integration of allocentric and idiothetic sig-
als, given the increased accuracy of proprioceptive and motor efferent
information in land over water [Whishaw and Pasztor, 2000] (in water,
animals rely mainly on dynamic visual information [Sautter et al., 2008]).
This may improve self-localization and position updating and enable the
learning of the task.
Nonetheless, NR1-KO show, throughout the experimental protocol, spe-
cific behavioral, path-complexity dependent impairments. This complex-
ity dependency seems to be different in the water-based version of the
starmaze, where NR1-KO mice failed to learn even the short path to the
goal [Rondi-Reig et al., 2006]. In this study, however, neither CTR nor
NR1-KO mice made use of the long trajectory to the goal, likely due to
the aversive and effortful nature of the task. Rondi-Reig et al. [Rondi-Reig
et al., 2006] showed that these KO mice were unable to learn the two first
choice points, an impairment attributed to the deficit in linking the two
intersection choices required to reach the goal into a sequence. Contrary
to the original water-based version of the task, in our land-based version
mice made extensive use of both short and long paths to the goal and,
thus, either choice in the first intersection was considered correct. Since
the correct choice probability in the last intersection was extremely high
(90% in both genotypes), it follows that the short path carries one main
choice point, while the long one carries two. The behavioral results (Fig.
2.4) suggest that, similar to Rondi-Reig et al., NR1-KO mice in our task
are impaired at linking sequences of actions that will lead to the goal.
During the pretraining phase, CTR and NR1-KO mice used the long
trajectory in similar proportions, but, while CTR mice leveled the use
of short and long paths during the recording phase, NR1-KO mice de-
creased the use of the latter one, adopting a preference for the short path.
While this could suggest that the impaired learning behavior during pre-
training was due to lower performance in long-path trials, this was not
the case. Both CTR and NR1-KO showed a low overall performance in
these long-path trials (Fig. 2.4g) and the difference between genotypes
could be attributed to the performance in short-path trials. During the
recording phase, the opposite pattern was observed: CTR and NR1-KO
leveled at an overall high short-path trial performance and, despite the
improvements in both cases, NR1-KO mice were impaired during long-
path trials. These results show that learning the short path to the goal occurs faster, in agreement with its higher simplicity and highlights the importance of a fully functional hippocampus in learning complex spatial sequences [Rondi-Reig et al., 2006].

Place cells dynamics in probe trials provide some further hints about the possible mechanisms of hippocampal involvement in route finding: when mice spontaneously select a place strategy, place cells fired at the same location as in training trials (Fig. 2.6). During sequence-strategy trials, CTR place cells preserved the firing sequence observed during training trials, but that sequence is expressed at spatial locations that are rotated with respect to the training trial path. Thus, the same place field map is expressed in distinct reference frames. Previous work has already shown how the place field map can be dynamically switched to a new configuration [Jackson and Redish, 2007], or as it is the case here, shift to a different reference frame translated [Gothard et al., 1996a] or rotated with respect to the original one [Gothard et al., 1996b, Kelemen and Fenton, 2010]. Different reference frames may be maintained based on different sets of visual cues, path integration or sequence memory. Notably, in the present data the reference frame shift depended on the behavior spontaneously selected by the animal, resulting in a differential weight for one or the other mechanism. In place-strategy trials cells are likely driven by the interplay between place memory, landmark information and path integration [McNaughton et al., 2006]. Distal visual cues are more likely to play a role in this task than local ones [Rondi-Reig et al., 2006]. Despite thorough odor neutralization following each trial (see Methods) it is possible that remaining olfactory cues play a role as well. During sequence-strategy trials however the hippocampal representation appears less susceptible to external cues (and to disregard distal polarizing cues). Rather, the activity pattern observed in sequence-strategy trials may be driven by path integration [McNaughton et al., 2006]. Path integration, however, is likely to play less of a role in complex mazes, where each maze intersection acts as a path integrator resetting cue. This resetting behavior was indeed observed in Medial Entorhinal grid fields (supposedly strongly involved in path integration [Derdikman et al., 2009]) as well as in CA1 [Royer et al., 2010, Mizuseki et al., 2012]. Another possibility is
that place cell firing during sequence-strategy trials reflects the retrieval of memorized sequences by cell assemblies [Pastalkova et al., 2008, MacDonald et al., 2011], possibly stored in CA3, and paced by self-motion information or by local landmarks (e.g. maze intersections) segmenting the trajectory. Interestingly, in a multi-intersection ‘hairpin’ maze, CA3 activity was not reset at each intersection, while CA1 was [Royer et al., 2010, Mizuseki et al., 2012], suggesting that sequence information, under those conditions, is present in CA3. The CA3 input, as we will argue below, may be especially important in this situation.

Place cells in NR-1 KO mice display somewhat reduced spatial information [McHugh et al., 1996], expressed in terms of the place field’s area, specificity (measured as the firing rate increase inside the place field) and its spatial information and sparsity. Here, we show that, while they behave to a large extent like their CTR counterparts in most conditions, NR1-KO cells show deeply disrupted activity in sequence-strategy trials, with a decreased overall firing rate and a reshuffled place field map. This suggests that, while sensory-based spatial processing is relatively unaffected by NMDAR channel dysfunction in CA1, these receptors are specifically crucial for the emergence of internally-driven, memory based space and/or motor-sequence representations. This latter type of spatial representation may be especially sensitive to the disruption of synchronized firing that was shown by McHugh et al. [McHugh et al., 1996]. This specific impairment may provide a novel explanation for the moderately blurred place fields observed with this transgenic model under 'normal' conditions, when multiple sources of information are combined [McHugh et al., 1996] (but see Chapter 5). Spatial learning deficits [Tsien et al., 1996, Rondi-Reig et al., 2006] in NR1-KO mice could thus be interpreted as a failure to integrate distal sensory information with memory and internal state information into a relatively intact spatial map. That map remains however relatively intact in training and place-based trials, and can be anchored to visual landmarks, a function for which CA1 is ideally suited, based on its place in the hippocampal circuitry. Notably, this defective integration of different information streams was measured under conditions in which task performance was basically unimpaired, suggesting that this particular deficit affects mostly route learning rather than its successive expression.
Associative long-term potentiation in CA1 is impaired in NR1-KO mice [Tsien et al., 1996], and it is possible that memory storage and retrieval of a spatial sequence is affected by reduced synaptic plasticity, e.g. in Schaffer collaterals [Nakazawa et al., 2004]. This may explain why sequence spatial maps are the most disrupted in our task. Furthermore, CTR place cells showed a higher $S_{idx}$ during long sequence-strategy trials, compared to short ones (Fig. 2.8), suggesting that this more complex sequence of egocentric responses is more hippocampus dependent and highlighting the role of CA1 in linking events into a sequence [Eichenbaum, 2000]. This is in agreement with the specific behavioral impairments NR1-KO mice show, not making use of this long sequence-strategy trajectory. This suggests also that taking the short path to the goal may rely on a behavioral process similar to that observed in the plus-maze task, where animals need to learn only one choice. Making selective lesions in either the dorsal striatum or the HPC, Packard et al [Packard and McGaugh, 1996] demonstrated that the former one is relevant for this form of simple egocentric navigation, while the latter one is crucial for place-based navigation and may explain why NR1-KO mice are able of using the short sequence-strategy trajectory in our task.

The $P_{idx}$ in CTR mice showed a clear increase as learning progressed, while this was not the case for the $S_{idx}$. The former case is suggestive of an improvement of spatial representation as a function of experience, which has been previously shown [Cacucci et al., 2007]. In that study, while place cells of normal mice showed an increase in spatial information per spike across recording days, $\alpha$CamKII KO mice (which lack NMDA receptor-dependent LTP in area CA1) did not show any changes, similar to what we observed in our NR1-KO mice. In addition, it has been shown that the CA1 place cell population undergoes an experience-dependent, self-organized triage into cells with a highly selective spatial tuning and those with a lower spatial tuning [Karlsson and Frank, 2008]. We show that this triage process is also present in CTR mice (Fig. 2.9) and that the high spatial tuning group further refines its spatial tuning in the second half of the recordings. NR1-KO mice showed an accentuation of the separation into low and high spatial information groups, but not an increase of the latter one. Interestingly, Karlsson and Frank report that
CA3 cells do not show this increase in the fraction of high spatial information place cells. Given the key role that this area plays in the rapid encoding of novel information [Nakazawa et al., 2003], the absence of experience-dependent changes in the $S_{idx}$ (Fig. 2.10) may reflect this quick, sustained degree of spatial tuning of CA3 place cells.

The $P_{idx}$ in NR1-KO mice did not change over the course of training, supporting the hypothesis that precision of spatial representation during place-based navigation is tightly coupled to the strength of spatial tuning (measured in the spatial information per spike) of place cells (which suffered only little change, affecting the low spatial tuning group). Across training, the $S_{idx}$ remained close to chance level, reflecting the poor capability of NR1-KO place cells to adopt this framework.

Looking at the ensemble behavior of place cells during sequence-based navigation, we observe that roughly half of the cells recorded simultaneously in CTR mice show a significant rotation of their place field in each sequence-strategy trial. This is in agreement with recent reports showing that about half of the recorded CA3 place cells show path-equivalent firing in environments with repeating elements [Singer and Frank, 2009]. We believe, however, that this number may be underestimated, due to the presence of trials with inconsistent spatial strategy representation (Fig. 2.10) and also due to the low number of probe trials: in many sessions, place fields in training trials are matched to a single sequence-strategy trial and, while in most cases, a single run is enough to accurately define a cell’s place field, in some cases firing in these single trials may be too variable or residual to address the rotation effect. In congruence with the results reported so far, only about a quarter of NR1-KO place cells showed a significant rotation effect in sequence-strategy trials.

In a subset of the place-strategy trials, the neural activity pattern did not match the actual behavioral trajectory of the animal (Fig. 2.11). Igloi et al. (2010) recorded fMRI brain activity in humans navigating in a virtual starmaze and observed that in some (<20%) of the probe trials, where the departure arm was very similar to the training trial’s departure arm (thus encouraging sequence-based navigation), subjects started the trial by repeating the previously realized body-turns (sequence-based navigation), but reoriented themselves later on the path by using environmental
cues (i.e., they shifted to a place-based navigation). Consistently, during visits to the departure arm, hippocampal activity in these trials was similar to that seen in pure sequence-strategy trials. Our results on the suspected use of a mixed strategy in place-strategy trials parallel Igloi’s study very well and suggest a within-trial strategy shift in some place-strategy trials.

Similarly, in a subset of sequence-strategy trials, place cells remained anchored to the place framework, while the animal executed the same sequence of body movements. Mixed sequence-strategy trials are unlikely, given the assumption that this navigation is anchored to departure. Instead, we believe that these sequence-strategy trials represent trials, in which the mouse intended to perform a long-path place-strategy trials, but made a mistake at the third intersection, which led it to end in the sequence-strategy rewarded goal arm. Unfortunately our dataset does not allow to confirm this possibility.

In conclusion, we have shown that CA1 NR1-KO mice are capable of learning a complex navigation task, despite showing a learning deficit during the early stages of training and path-complexity specific impairments. Furthermore, we have shown that spatial representation in CA1 closely follows the strategy adopted by the animal, based on the main dimension guiding its behavior: environmental cues during place-based navigation and a spatial memory sequence during sequence-based navigation. In particular, the latter one is severely affected by the absence of NMDA receptors in pyramidal cells of CA1, suggesting that Hebbian learning in the CA3-CA1 pathway is particular sensitive to the absence of this receptor, consistent with the role of CA3 in retrieval of spatial memories. Place-based navigation is likely to rely more on perforant path inputs from the entorhinal cortex and is affected less in NR1-KO mice, both at the level of the spatial representation and behavior. Strategy-related signals may be present e.g. in the prefrontal cortex [Rich and Shapiro, 2009], which gates the flow of information between perirhinal and entorhinal cortex [Paz et al., 2007]. Similarly, the prefrontal cortex may shift CA1 from a mainly EC to a CA3-driven state, by shutting down EC throughput to yield dominance to CA3, therefore determining the strategy used by the animal.
Chapter 3

Absence of NMDA receptors in hippocampal area CA1 disrupts oscillatory patterns during a spatial navigation task

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3.1 Abstract

Neural oscillations are key processes for cognitive processing, synchronizing the activity of the neuronal population in a given brain area, but also enabling communication between different structures via coherent rhythmicity [Fries, 2005]. The hippocampus, and notably the CA1 area, which is at its output end, integrates and processes different types of inputs to produce a unique representation of a given environment and form a memory combining space and context (the what and when). The different types of input are relayed by different brain structures, such as CA3 and the entorhinal cortex, and it has been suggested that different oscillatory rhythms facilitate communication between one area and the other. We analyzed, therefore, CA1 network activity in mice during learning of a spatial task, enabling the use of two different spatial navigation strategies: one ‘space-dependent’ (‘place-based navigation’) and one ‘sequence-dependent’ (‘sequence-navigation’), the former based mainly on visuospatial inputs and the latter on the retrieval of a memory of a body movement sequence. We report an important role for theta rhythms throughout learning and link the different gamma bands to the strategy used, reflecting increased influence of area CA3 during sequence-based strategy (low gamma-mediated) and of the entorhinal cortex during place-based strategy (high gamma-mediated) on CA1 activity. By also recording from knock-out mice lacking the NMDA receptor in area CA1, we confirm the importance of this receptor in modulating oscillatory patterns, reflected in a disruption of strategy-dependent coding patterns present during the different types of navigation in normal mice.
3.2 Introduction

"In the course of learning, something like a field map of the environment gets established in the rat’s brain. (...) And it is this tentative map, indicating routes and paths and environmental relationships, which finally determines what responses, if any, the animal will finally release."

*Tolman, 1948* [Tolman, 1948]

Tolman, by observing behavior of rats that were running in different mazes, suggested that, during learning, a spatial representation of the environment is formed somewhere in the brain, which would help the animal navigate. Almost 25 years later, O’Keefe and Nadel showed that the locus for this representation is likely to involve the hippocampus [O’Keefe and Dostrovsky, 1971]: the discovery that principal neurons in the hippocampus, notably in the dorsal CA1 subfield, show place selective activity (place cells [O’Keefe and Dostrovsky, 1971]) and that, together, they can represent the animal’s location in the environment [Wilson and McNaughton, 1993], definitely turned the spotlight of spatial learning research to the hippocampus.

Today, research has extended to other brain areas and considers how they interact with the hippocampus to effectively support spatial navigation. Several brain areas, with a role in spatial navigation, receive and/or project information directly or indirectly to the hippocampus, such as the medial prefrontal cortex (mPFC, involved in, a.o. strategy switching/selection [Rich and Shapiro, 2009]) or the dorsal striatum (dStr, involved in response navigation [Packard and McGaugh, 1996]). The entorhinal cortex (EC), which is the main gateway and exit station of the hippocampus [Witter, 1993], is the region that has received the most attention over the past years. This became the case particularly after the discovery that principal neurons in the medial portion of the EC also display a strong spatial modulation of activity: each cell’s firing pattern forms regular hexagons that cover the entire environment [Hafting et al., 2005]. The EC can be divided into a medial (mEC) and lateral (lEC) portion, each one conveying different functional inputs to the HPC, with the former being the main source of spatial information and the latter of non-spatial
information [Van Cauter et al., 2012, Hunsaker et al., 2007].
Rhythmic oscillatory patterns reflect the coordinated activity of the neu-
ronal population of a given area. In the hippocampus, two oscillatory pat-
terns prevail during behavior: theta (6-12 Hz) and gamma (25-140 Hz).
Theta rhythms are generated in the medial septum [Petsche et al., 1962],
but are also modulated by EC inputs [Buzsáki, 2002]. The hippocampus
shows robust theta activity during locomotion [Vanderwolf, 1969], syn-
chronizes place cell activity and also pyramidal cell firing in other brain
structures (mPFC [Siapas et al., 2005], mEC [Mizuseki et al., 2009],
ventral striatum [van der Meer and Redish, 2011]) and nests faster oscil-
lations within [Tort et al., 2009] and across brain regions, such as mEC,
in its cycles [Colgin et al., 2009]. Gamma oscillations in the hippocampus
are thought to arise from two sources, one in CA3 and another one in the
EC. Crucial players in the generation and maintenance of gamma rhythms
are interneurons [Buzsáki and Wang, 2012, Korotkova et al., 2010], but
they likely act in a coordinated fashion with pyramidal neurons (PING -
Pyramidal-Interneuron-Network-Gamma mechanisms) [Gonzalez-Burgos
and Lewis, 2012]. Among the vast populations of interneurons, parval-
bumin (PV)-positive basket cells appear to play a predominant role in
controlling gamma oscillations in the HPC/EC formation [Wulff et al.,
2009, Korotkova et al., 2010].
An important systems function is the capability of two different brain
areas to synchronize with each other, such as the examples mentioned
for theta. This synchronization and coherence may subserve interac-
tions between different brain areas [Schoffelen et al., 2005, Siapas et al.,
2005, Benchenane et al., 2010]. The CTC theory (Communication By
Coherence, [Fries, 2005]) suggests that this opens a common temporal
window for inputs and outputs to interact between brain areas. One of
the ways this is achieved is when one area entrains to oscillations in-
trinsically generated in a different one, or is even simply driven by the
oscillation generated by an upstream region on the receiving side. Recent
studies have shown that differential entrainment of gamma frequencies in
CA1 may allow synchronization (and therefore communication) between
CA1 and CA3, on the one hand, and CA1 and EC on the other hand
[Colgin et al., 2009]. Gamma has been subdivided into three different
bands, slow (25-45 Hz), high (50-90 Hz) and very high (90-150 Hz) and
its modulation by theta cycles in CA1 occurs in a mutually exclusive way, such that low and high gamma tend not to occur at the same time. Furthermore, slow gamma is highly coherent between CA1 and CA3 and fast gamma between CA1 and mEC [Montgomery and Buzsáki, 2007, Colgin et al., 2009], suggesting that the two different gamma rhythms subserve routing of different sources of information to CA1 [Colgin et al., 2009, Carr and Frank, 2012].

Indeed, both CA3 and mEC are involved in spatial learning, but their functions are very different. In the mouse, the latter area projects to all subareas of the HPC via the perforant path (PP), with layer III neurons projecting directly to CA1 and CA3 (in the mouse) sub-fields [Ahmed and Mehta, 2012]. Lesions of this layer result in impaired spatial representation in CA1 area, indicating that the direct projections are crucial for proper CA1 place cell functioning [Brun et al., 2008]. In addition, lesions of the mEC result in impairments in spatial information processing [Hunsaker et al., 2007], more specifically in the processing of allocentric and idiothetic (path integration) inputs [Van Cauter et al., 2012]. The lateral portion of the EC, on the other hand, shows little spatial specificity [Hargreaves et al., 2005] and is, instead, involved in the processing of non-spatial inputs and purely sensory stimuli [Hunsaker et al., 2007, Deshmukh and Knierim, 2013]. Together, these two structures are the likely key players in driving CA1 during allocentric navigation [McNaughton et al., 2006]. On the other hand, disruption of CA3 inputs to CA1 (the Schaffer collaterals) impairs CA1 place fields only marginally and acquisition of a hippocampus-dependent spatial task is unaffected, but spatial recall is impaired [Brun et al., 2002], giving strong evidence for an important role of this area in spatial memory retrieval. CA3 is characterized by a relatively dense recurrent network and has been suggested to carry out heteroassociative processes, particularly relevant for sequence recall [Treves, 1995, Lisman and Otmakhova, 2001] and, indeed, several lesion and KO studies strengthen this view (involvement in retention, but to a lesser extent, formation of a spatial memory [Steffenach et al., 2002], temporal-spatial pattern completion [Hoang and Kesner, 2008] and rapid encoding of novel information [Nakazawa et al., 2003]).

This brings forth the possibility that CA1 integrates both EC and CA3 inputs, such that CA1 place cells can be activated either by 'external'
(distal sensory) drive from the EC during place coding or by internal CA3 inputs during memory replay [Carr and Frank, 2012]. What are the weights of the inputs from CA3 and EC to CA1 during the use of the different navigation strategies? What oscillatory bands serve as communication channels between CA1 and area CA3 and EC? And how does the absence of the NMDA receptor in CA1 affect these processes? To address these issues, we have made in vivo recordings from the dorsal CA1 pyramidal layer from mice, during learning of a complex navigation task (the ‘starmaze’), allowing the distinction of different navigation resources used. The starmaze [Rondi-Reig et al., 2006] is a labyrinth with multiple intersections, which allowed us to disentangle the use of place-based navigation from sequence-based behavior. Given the relevance of theta and gamma oscillations in spatial learning and the dual origin of slow and fast gamma rhythms, we investigated changes in LFP patterns during the use of different navigation strategies. In addition we also trained and recorded from KO mice, lacking the NMDA receptor subunit 1 in area CA1(NR1-KO) [Tsien et al., 1996]. In light of the crucial role of the NMDA receptor in spatial learning and spatial representation [Morris et al., 1986, Rondi-Reig et al., 2006, McHugh et al., 1996] and its role in hippocampal oscillatory rhythms [Whittington et al., 1995, Korotkova et al., 2010, Lazarewicz et al., 2010], we sought to understand how it affects LFPs in this task and if its impact is dependent on the behavioral strategy used to guide behavior.

### 3.3 Methods

For a detailed description of the experimental subjects, the starmaze task, drive, surgery and tetrode positioning, as well as histology and data acquisition parameters, refer to Chapter 2.

**Data analysis.** All data used for analysis were from periods in which the animal was moving at speeds above 3 cm/sec.

**Spectral power analysis.** A notch filter was applied to the LFP trace, around 50 Hz and its 2\textsuperscript{nd} and 3\textsuperscript{rd} harmonic. Power spectra were con-
structured on a trial-by-trial basis, to allow comparison with probe trials, using the function mtspectrum.m from the chronux toolbox (www.chronux.org). To circumvent differences arising from various trial lengths, power was calculated in windows of 1 sec, which were then averaged for each trial. For the construction of the normalized power plots in Figure 3.2b and c, the power spectrum of each trial was divided by the mean power of the entire session between 4 and 140 Hz.

For the construction of the log power ratio plots in Figure 3.4a and b, the power spectra of training, place- and sequence-trials trials were divided by the average of the power spectra of the correct trials of that session. The 5th and 95th percentiles of the shuffled data were obtained by bootstrapping 2000 times the ratio between a trial taken randomly among the correct, place- and sequence-trials trials of each session and dividing it by the average power of the correct trials of that session. The low to high gamma ratios in Figure 3.4c-e were obtained from the raw power spectrum.

*Locomotor speed* - *Power correlations.* For each trial, a time spectrogram was constructed using the mtspecgramc.m function of Chronux (www.chronux.org) with a time window of 1sec in 0.5sec steps. Instant velocity was averaged for each time window and the correlation between speed and power was taken for each frequency bin.

### 3.4 Results

We trained control (CTR) and NR1 CA1 KO mice to run on a complex, pentagon-shaped maze with five radial arms, (the ‘star-maze’ [Roudi-Reig et al., 2006]; Fig. 3.1) and find a food reward placed at the end of a ‘goal’ radial arm. In training trials mice left from a fixed departure arm, but in probe trials (1 to 3 per session), they were started from a different radial arm, placed at a 72° angle with respect to the regular departure arm (Fig. 3.1b). In these trials, a direct run to the same arm being rewarded in training trials was considered a place-based probe trial, while a repetition of the same sequence of body movements was considered a sequence-based probe trial.
In chapter 2, we showed that CA1 place cell representation changes according to the strategy being employed: in place-based navigation, the spatial representation remains as in training trials, while during the use of a sequence-strategy trajectory, place cells rotate in a manner consistent with the different start arm, thus remaining anchored to the departure point of the animal. This difference may reflect a predominant influence from different brain areas on CA1: CA1 representations during place-strategy trials, more dependent on the processing of distal spatial information of the environment, might be predominantly shaped by mEC and IEC inputs, conveying spatial and non-spatial (e.g. sensory) inputs, while CA1 representations during sequence-based navigation, involving the retrieval and execution of a stored spatial memory, may more strongly depend on CA3 inputs. While spatial representation in NR1-KO was only mildly impaired in place-strategy trials, it was massively disrupted in sequence-strategy trials (Chapter 2).

Lack of NMDARs leads to different power changes in the low and high frequency range

During running periods, CA1 pyramidal layer local field potential (LFP) was characterized by three distinct bumps in the power spectrum: theta (6 - 12 Hz) and low (LG) and high (HG) gamma (Fig. 3.2b, arrows). We calculated the normalized power spectrum in each trial for
Figure 3.2: NR1-KO mice show disrupted power in the low and high frequency range.

(a) LFP spectrograms for two example training trials from, respectively, a CTR (left) and a NR1-KO (right) mouse.

(b) Average power spectrum normalized by total power in the 4–140 Hz frequency range, during all trials. Except in the theta range, NR1-KO (red) LFPs showed higher power, starting in the beta or low gamma range (around 20 Hz) and extending to all analyzed higher frequencies. Note also the separation of the two gamma bands (LG, 23–40 Hz and HG, 55–95 Hz).

(c) Boxplot distributions of average power values within the three main frequency bands (whiskers indicate 25th and 75th percentiles, line separating the box the median).

both genotypes: compared to CTR, NR-1 KO mice showed decreased power in the theta range, and larger power in LG and HG (Fig. 3.2c), similar to what has been shown under pharmacological NMDA receptor blockage [Lazarewicz et al., 2010].
NMDAR knock-out effects on speed modulation of low and high frequencies

Position updating and predicting future position is dependent on encoding of running speed. Given recent reports showing that not only theta, but also gamma amplitude increases with locomotion speed in mice [Chen et al., 2011], we looked at power amplitude modulation by running speed. We observed that both genotypes follow a similar pattern, with clear positive correlation bumps in the theta, low and high gamma range (Fig. 3.3a). Congruent with what we observed in the power spectra (Fig. 3.2b), speed modulation of theta was higher in CTR mice, while that of HG was higher in NR1-KO animals (Bonferroni corrected t-test: P < 0.05; LG: n.s.). This difference could not be attributed to different

![Graphs showing speed modulation](image)

Figure 3.3: Speed positively modulates theta, low and high gamma power in training trials
(a) Speed-power modulation spectrum. Pearson’s correlation between speed and power at different frequencies. NR1-KO mice showed a lower modulation of theta power, but a higher modulation of HG (black dots: Bonferroni corrected t-test: P < 0.05)
(b) Speed-power modulation in different maze sections. Degree of low and high gamma modulation was dependent on the section of the maze, with NR1-KO showing an altered pattern. Colors of the vertical bars indicate the different arm segments: departure (blue), middle (green) and goal (orange) arms (horizontal bars: Post-hoc Tukey, P < 0.05).
(c) Speed profile in the different maze sections. There was a significant effect of maze section, but no difference between genotypes (ANOVA: P < 0.05).
speed profiles: as can be seen in Fig. 3.3c, mice from both genotypes ran at similar speeds in the different portions of the maze. Navigation along a trajectory comprises different computations, such as self-localization, strategy selection, integration of action-outcome feedback and also the comparison of current sensory inputs to retrieved memories. Therefore we split the star maze into three different sections: departure arm, middle arms and goal arm (Fig. 3.3b). Theta power modulation by speed did not vary along these three sections, with CTR mice showing a stronger modulation in all of them. Interestingly, in both low and high gamma power in CTR, modulation increased sharply after the departure arm, remaining high throughout the rest of the trajectory (Post-hoc’s Tukey, $P < 0.05$; difference between departure and goal arm for LG was n.s., but trending ($P = 0.09$)). NR1-KO mice failed to show this pattern, with only modulation of HG in goal arm being significantly above that of the departure arm (Post-hoc Tukey, $P < 0.05$). This difference could not be attributed to different speed profiles in the three maze sections, as can be seen in in Fig. 3.3c. These results suggest that the computational processes carried out during the different sections of the navigation to the goal in training trials may change the way low and high gamma frequencies are modulated by speed, which is not the case for theta. NR1-KO mice only partially showed this effect (Anova: $P(\text{genotype}) < 0.01$). Noteworthy, LG modulation in these mice was lower in the central sections of the maze, where intersection choices are made, compared to CTR, which may underlie some of the impairments shown by these mice in the task.

Differential recruitment of low and high gamma oscillations during different probe trial types

The computations involved in expressing place fields in the place-or sequence-based reference frames may need different input streams, as suggested by the differences in place cell behavior (see Chapter 2). For example, more sensory information about environmental cues may be needed for the place-based frame, and more memory-based inputs for the sequence-strategy frame. Thus, it is possible that the former depends more on perforant path inputs from entorhinal cortex, and the latter on Schaffer collateral inputs from CA3 [Lee and Kesner, 2003, Parron et al.,

We further restricted our power analysis to the different probe trial types, in order to discern different power signatures underlying place and sequence-based navigation in CTR and NR1-KO mice. Remarkably, during sequence-strategy trials, NR1-KO mice showed increased power with respect to the already abnormally high value of training trials, encompassing both the LG and HG range (30-100 Hz; Fig. 3.4b). CTR mice showed a pattern in both cases similar to the one of training trials (Fig. 3.4a). To quantify the changes across trial types, we calculated the log-ratio between LG and HG power (Fig. 3.4c). This revealed a change in the balance between LG and HG amplitude in CTRs (Kruskal-Wallis one-way ANOVA P (trial type) < 0.05), dependent on the strategy being employed: while sequence-strategy trials showed a similar balance as training trials, during place-strategy trials this balance was shifted towards HG (Post-Hoc Tukey test P < 0.05). No significant place-sequence difference was detected, however, in NR1-KO. Furthermore, NR1-KO showed a global lower LG/HG ratio (Kruskal-Wallis One-way ANOVA P (between genotypes) < 0.001), suggesting a generally higher HG influence. In summary, in CTRs, but not NR1-KOs, the balance between LG and HG power
Figure 3.4: Behavior-dependent changes in low and high gamma power. 
(a) and (b) Log power ratio between probe and training trials of, respectively, place-
(light green area: 95% confidence interval) and sequence-strategy trials (orange) to
correct training trials (yellow area represents bootstrap 95% confidence interval com-
puted from all trials, excluding errors; see Methods). In CTRs (a) probe trial power 

did not differ from what is observed in correct training trials. In NR1-KOs (b) 
power in sequence-strategy trials was significantly higher in LG and HG. Black bars 
denote frequency range for which a significant ($p < 0.05$, Bonferroni corrected) differ-
ence between sequence-strategy trials and training trials was observed.

(c) Ratio of LG to HG gamma power in training trials, place- and sequence-based 
trials. CTRs showed higher LG to HG ratio in sequence-strategy trials compared to 
place-strategy trials (*: t-test $P < 0.05$), which were also lower than the ratio observed 
in training trials.

(d) Low-to-high gamma ratio as a function of session block and trial type. In CTR, 
the ratio in training and sequence-strategy trials decreased with learning (ANOVA, $P$
(block) < 0.001). In NR1-KO this was only the case in training trials. During train-
ing and sequence-based trials, there was a significant difference between genotypes 
(ANOVA: $P$(geno) < 0.01).

(e) Log-ratio of LG to HG gamma power in training trials, place- and sequence-trials 
trials, discriminating between short and long paths. NR1-KO mice showed a lower 

cratio when using the long vs. short path in training trials (Kruskal-Wallis: $P < 0.05$), 
while CTR showed an increase, when using the long path during place-strategy trials 
(Kruskal-Wallis: $P < 0.001$).

changed as a function of the strategy adopted during probe trials, com-
patible with a greater influence of CA3 inputs in sequence-strategy trials, 
and of EC inputs in place-strategy trials.

To look at learning-dependent changes in EEG power patterns in CA1, we 
divided the recording sessions into blocks of three sessions and calculated the 
low-to-high gamma ratio per block for each trial type (Fig. 3.4d). This analysis 
revealed a decrease in the ratio in training trials in both genotypes (ANOVA, $P$
(block) < 0.001 in both), while in place-strategy trials the ratio did not exhibit suffer any changes throughout learning.

While CTR mice also showed a decrease in the ratio in sequence-strategy 
trials (ANOVA, $P$ (block) < 0.001), NR1-KO mice failed to show such a 
pattern. Instead, the ratio increased over the first 3 blocks (though not
Figure 3.5: Theta-Gamma synchronzation is disrupted in NR1-KO mice. 
(a) Example LFP traces (CTR) highlighting co-occurrence of theta and, respectively, LG (top) and HG (bottom).
(b) Example cross-frequency comodulogram for a single trial (CTR), showing average spectral power in gamma ranges as a function of theta phase. Superimposed is the average theta cycle for the trial. Note different preferred phases for HG and LG.
(c) and (d) Polar histogram of trial-wise preferred theta phase for LG (blue shaded area) and HG (pink shaded area) for all trials in CTRs (c) and NR1-KO mice (d). Arrows represent the mean resultant vector across all trials (in arbitrary units). There is a clear separation between the two distributions, with HG peaking at an earlier theta phase than LG (circular ANOVA, P < 0.001). There was no difference between genotypes (circular Anova: P(geno) n.s.).
(e) Theta modulation of higher (>20Hz) frequencies in CTR mice, per trial type. During place-strategy trials, coherence in the HG band was augmented: inset shows average coherence in the HG band (Anova: P (trial type) < 0.05, Post-Hoc: P (place vs Training/sequence-strategy trials) < 0.05).
(f) Same as (e), but for NR1-KO mice. There was no trial type effect (ANOVA n.s.).
Given our results showing that the long path is more complex and more difficult to learn (see Chapter 2), we sought to analyze how this affects the balance between the different gamma frequencies (Fig. 3.4e). Interestingly, in CTR mice, this balance was affected only during the use of place-based navigation: during long place-strategy trajectories there was a shift in the balance towards more LG influence (Fig. 3.4e middle plot: Post-Hoc Tukey test P < 0.05). The added intersection might make this path more memory-dependent, which could explain this increased low gamma component, as seen during sequence-strategy trials. In addition, there was a significant decrease in the ratio during long paths in training trials in NR1-KO animals, in agreement with the behavioral impairments shown in Chapter 2.

**Increased high gamma modulation by theta oscillations during place-based navigation in CTRs**

The trial-type dependent changes in the spiking activity in CA1 (see Chapter 2) may derive from different effectiveness of EC and CA3 inputs. These two structures have different preferred spiking phases [Mizuseki et al., 2009] and communicate with the hippocampus via HG and LG, respectively, which peak at different theta phases [Colgin et al., 2009, Belluscio et al., 2012] (Fig. 3.5a). Here, both CTR and NR1-KO LFPs show an earlier peak phase of modulation by theta for HG than LG (Fig. 3.5c&d), following the peak of the theta wave. We next calculated the cross-frequency theta modulation in each trial type (Fig. 3.5e&f) and show that during place-based navigation, HG-theta coherence in CTR mice was increased (Fig. 3.5e, inset: ANOVA: P (trial type) < 0.05; Post-Hoc P (place vs training and sequence) < 0.05), consistent with the results showing a higher contribution of HG oscillations during this type of trials (Fig. 3.4c). As in the LG/HG ratio, NR1-KO failed to show this effect.

To investigate strategy-dependent differences in preferred theta phases, we calculated Kappa values, a phase concentration parameter, for each trial type (Fig. 3.6). High gamma modulation showed a higher phase concentration in CTR (Circular Kuiper-test: P < 0.05, non significant in sequence-strategy trials, but trending). This pattern applied to NR1-KO mice as well, except in sequence-strategy trials.
Figure 3.6: High gamma is more strongly modulated by theta oscillations than low gamma. Concentration of modulation phases as Von Mises kappa. Preferred modulation phases were more concentrated for HG, compared to LG. This was the case for the training and place trials in CTR (Circular Kuiper-test: **P < 0.001, *P < 0.01; n.s. in sequence-strategy trials). NR1-KO showed a similar pattern, except in sequence-strategy trials, where the concentration of phases was similar for LG and HG.

Figure 3.7: Low and high gamma carry different spatial content. (a) The log ratio between low and high gamma was calculated for each theta cycle and the lower and higher quartiles were used to form a spatial firing map of low or high gamma periods.

(b) Example of a CTR neuron: The firing map of a single CA1 cell was calculated for all periods of a given trial type (‘long’ training trial, in this case; left) and correlated with the firing map during high LG or HG periods of the matching probe trial (sequence-strategy trial in this case; middle and right, respectively); color scale indicates firing rate.

(c) An overlap index (see Fig. 2.6, Chapter 2) for LG periods revealed that the firing map in sequence-strategy trials in CTR neurons is significantly more similar to the overall firing map in the respective training trial than that during HG periods. This effect was absent in NR1-KOs (Multiple t-test, **P < 0.005, ****P < 0.0001).

LG and HG convey different contributions to CA1 place cell spatial representations

Inspired by the trial type-dependent differences observed at the LFP and LFP-LFP level, we looked at the spatial representation of single neurons during periods of predominant LG or predominant HG. For this, we
took the LG-to-HG ratio per theta cycle (Fig. 3.7 a, left) and used the lower and upper quartile cycles as HG and LG-dominated periods, respectively (see Methods; Fig. 3.7 a, right).

We constructed spatial firing maps of individual cells for each probe trial, only using spikes emitted during respectively HG and LG periods, and correlated those with the firing map calculated over all periods for the corresponding training trial (similar to the overlap index calculated in Chapter 2, Fig. 2.6 b&c, Fig. 3.7b). This analysis revealed that, in

![Figure 3.7](image-url)
CTR mice, the spatial firing map constructed in LG periods in sequence-strategy trials showed an increased similarity with the overall spatial firing map of corresponding training trials (Fig. 3.7 c, Multiple t-test: P < 0.005), further strengthening the link between LG oscillations and the use of sequence-based navigation. NR1-KO mice did not show the same effect, in agreement with a selective disruption of CA1 patterns of firing activity during this type of navigation. Interestingly, despite the relatively strong influence of HG during place-based navigation, as shown by a decrease in the LG/HG ratio (Fig. 3.4 c) and the higher theta-HG coherence (Fig. 3.5 e) during these probe trials, spikes emitted during LG and HG periods contributed equally to the overall spatial representation.

3.5 Discussion

Using the star maze task, we were able to analyze oscillatory patterns of activity in the dorsal CA1 area of the hippocampus during different behavioral states. The main feature of this task is that it enables distinguishing different navigation strategies spontaneously employed by animals. We focused our analysis on two strategies: a ‘place’-based strategy, dependent on the configuration of the recording environment, and a ‘sequence-based’ strategy, independent on the configuration of the environment and based on a stored memory of a sequence of egocentric movements.

CA1 acts as a hub for spatial processing and given the different nature of these two strategies, different brain areas may be more or less involved in either strategy. The study of EEG oscillatory patterns is a powerful analytical tool to get insights into the importance of different brain regions for each behavioral state: the EEG represents the cumulative activity, mainly derived from synaptic potentials of neurons in CA1. The oscillatory, synchronous patterns observed are, however, the result of the response of CA1 neurons to external inputs and may open temporal windows for communication between receiving and sending brain areas [Fries, 2005].

We looked, therefore, at changes in power across frequencies, during the use of different strategies, as well as at intrahippocampal cross-frequency coherence in CTR and NR1-KO mice. We found evidence for an increased
LG role during sequence-strategy trials, while place-strategy trials appear to have a stronger HG component. NR1-KO mice showed a similar pattern during place-based navigation, but showed profound differences in sequence-strategy trials, consistent with our previous results showing impairments at the behavioral and single-cell level in the same animals and task.

Three main EEG rhythms prevailed during running in the starmaze: theta and low and high gamma (Fig. 3.2b). All three were affected by the NR1-KO deletion in CA1: these animals showed decreased theta power, but increased low and high gamma power, which replicates previous results from pharmacological and transgenic studies [Whittington et al., 1995, Korotkova et al., 2010, Lazarewicz et al., 2010]. This duality in the effects of the NMDAR deletion may be related to the slow dynamics of the receptor current: absence of the slow EPSP component may depress slow oscillations and boost fast oscillations [Rodriguez-Molina et al., 2007], possibly through increased spike time precision of pyramidal neurons. Interestingly, while previous work ascribed the effect on gamma to NMDARs on interneurons [Carlén et al., 2012, Korotkova et al., 2010, Lazarewicz et al., 2010], a similar result is reproduced here by knockout of those receptors on principal cells only. There are two types of mechanisms underlying gamma generation processes: ING (Interneuron-Network-Gamma) mechanisms depend on the mutual inhibition between reciprocally connected interneurons, rhythmically synchronizing pyramidal neurons, which are not directly involved, whereas a PING (Pyramidal-Interneuron-Network-Gamma) mechanism depends on the interplay between inhibitory interneurons and excitatory pyramidal neurons [Gonzalez-Burgos and Lewis, 2012]. Given the interconnectivity of pyramidal neurons and interneurons in the hippocampus [Freund and Buzsáki, 1996], our results showing that NMDAR deletion in pyramidal neurons disrupts gamma oscillations suggests an important role of PING mechanisms in the generation of gamma rhythms in CA1, as has been previously suggested [Horowitz, 1972, Leung, 1982].

Computing running speed is a crucial component of navigation, as it facilitates position updating [Chen et al., 2011]. Confirming previous
studies [Chen et al., 2011], we observed that all three rhythms were posi-
tively modulated by the running speed of the animal (Fig. 3.3a). The
differences in the degree of locomotor modulation between genotypes are
likely to be a consequence of the power differences observed. Interest-
ingly, CTR showed increased speed modulation after the first intersection
(Fig. 3.3b). It is likely that in the departure arm the predominant navi-
gational processes occurring are related to self-localization and action-
selection, processes less dependent of the integration of running speed.
Position-updating presumably becomes increasingly relevant as naviga-
tion progresses and may explain this increased speed modulation of all
three rhythms after leaving the departure arm. This pattern is largely
absent in NR1-KO mice, with modulation reaching its highest level as
far as in the goal arm, which may contribute to the impaired spatial repre-
sentation shown by us and previous studies [McHugh et al., 1996] at
the place cell level: increased modulation indicates “keeping-up” of firing
synchrony with increasing speed. Not being able to do so may result in
lower firing precision of pyramidal neurons, leading to larger place fields
and more widespread firing.

During normal navigation, both memory-based and environment-based
processes occur in parallel. Maybe for this reason we did not find any
changes in power during place or sequence-strategy trials of CTR mice,
compared to training trials (Fig. 3.4a left). Interestingly, the ratio of
low over high gamma power was reduced in place-strategy trials of CTR
mice, suggesting a stronger influence of HG activity during this type of
navigation. This effect, however, was only present during the use of the
short path in place-strategy trials (short path was used approx. 80% of
the time). When mice used the long path, the ratio was strongly in-
creased to the low gamma side (Fig. 3.4e middle). The necessity of
performing two “high-cost” choices (a wrong choice will take the mouse
to an unrewarded arm) in the long path (as compared to only one in the
short path) and linking them in a sequence is likely to make this path
more memory-demanding, conferring a pattern more similar to sequence-
strategy trials. NR1-KOs showed a different pattern specifically during
the use of the long path: in training trials they had a reduced low-to-
high gamma ratio and failed to show the increase seen in CTR during
place-based navigation. These results further support the possibility that
the lack of the NMDAR in CA1 leads to specific deficits in establishing
and/or retrieving sequential spatial memories.
Not only behavior, but also experience in the maze affected the gamma
ratio: in both genotypes it decreased with training evolution (Fig. 3.4d).
CTR mice additionally showed the same pattern in sequence-strategy
trials. Decreasing hippocampal involvement as training progresses has
been reported in a study looking at fMRI signals during learning of a
virtual starmaze in humans [Iglói et al., 2010]. They reported a decrease
only during training and sequential navigation, which may be related to
reduced hippocampal involvement as sequential tasks become familiar
[Schendan et al., 2003, ?] and learning rate is reduced [Wolbers and
Büchel, 2005]. The decreased ratio may, thus, be a consequence of a de-
creased hippocampal (especially CA3-dependent) influence, whereas the
continuous EC inputs [Sybirska et al., 2000] persist as important elements
throughout the experiment.

Given the prominence of theta activity in CA1 in the behaving rodent
and the strong modulatory effect it has on the firing of both pyramidal
and interneurons [Vanderwolf, 1969, Buzsáki and Eidelberg, 1983, Fox
et al., 1986, O’Keefe and Recce, 1993], oscillatory inputs arriving must
be synchronized with theta for efficient information integration [Colgin
et al., 2009]. More specifically, gamma nesting in theta cycles has been
proposed to play a role in sequential memory organization and working
memory maintenance [Lisman and Grace, 2005, Chrobak et al., 2008].
Confirming previous studies [Colgin et al., 2009, Tort et al., 2010, Bel-
luscio et al., 2012], we show that theta modulates low and high gamma
frequencies at different phases, with the latter consistently preceding the
former one (Fig. 3.5c&d). This phase separation might be crucial to re-
duce interference by different incoming information, so that different, but
related processes may be efficiently encoded (such as ‘sensory-driven pro-
cessing’ - mEC - and ‘memory retrieval’ - CA3) [Hasselmo et al., 2002].
HG modulation by theta was increased during place-based navigation in
CTR mice (Fig. 3.5e), suggesting increased coherence between CA1 and
the HG-generating mEC [Colgin et al., 2009, Belluscio et al., 2012]. This
effect was absent in NR1-KO mice, suggesting that poor communication
between these two structures may underlie the deficits in spatial representation these animals show (see Chapter 2) and may be a consequence of the increased power observed in the gamma range, which puts the network in a saturation mode.

The link between LG, HG, and respectively sequence- and place-strategy computations is supported more directly by showing that place field maps change depending on the currently dominating gamma frequency (Fig. 3.7). In particular, the increased $S_{size}$ shown by CTR cells during sequence-strategy trials (see Chapter 2, Fig. 2.6c) was mainly supported by spikes emitted during dominating LG. While it is unlikely that the hippocampus ever completely "switches" from one computational mode to the other, fluctuations in oscillatory activity provide us a handle to disentangle, at least partially, the effect of different dynamic regimes directly on the place field map.

In summary, our results show that place and sequence-based navigation, two strategies based on fundamentally different mnemonic and multisensory processes, the former more related to information encoding and the latter one relying on memory retrieval, appear to be related to different gamma frequencies: the place-strategy has a stronger high gamma involvement, while low gamma activity is more prevalent in sequence-strategy trials. NR1-KO mice showed increased low and high gamma oscillations and a disturbed balance of both oscillatory bands during place- and sequence-based navigation. The results in CTR mice strongly suggest a link between CA1 and either CA3 or EC with the strategy being employed, with CA1 mainly driven by one of these two structures (via the two gamma "channels"), according to the behavioral strategy adopted: an externally oriented, spatial information processing, EC dependent state during place-based and an internally oriented, memory-based, CA3 dependent state during sequence-based navigation.
Chapter 4

Phase locking of excitatory and inhibitory CA1 neurons in normal and NMDAR knock-out mice during spatial navigation

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4.1 Abstract

The relation between neuronal firing and network oscillations is a complex one and may subserve several functions, from facilitating synaptic plasticity, as may be the case for phase precession to theta rhythm [Tukker et al., 2007], to coordinating activity between different brain areas, as has been shown for the spiking of prefrontal cortex neurons to hippocampal oscillations [Siapas et al., 2005]. We have shown (see Chapter 3) that, in a complex navigation task requiring the use of different strategies, three main types of oscillation prevail: theta and low and high gamma. We have proposed that the latter two may facilitate communication between area CA1 and either CA3 or the entorhinal cortex (EC), depending on the type of information driving behavior. To follow up on this, we analyzed in depth the relationship between locking of pyramidal neurons and interneurons during the learning of the starmaze task, in normal mice and knock-out mice lacking the NMDA receptor in area CA1 (NR1-KO). We show that locking of pyramidal neurons to theta oscillations is strategy-dependent and that the spiking phase suggests that CA1 neurons are being driven by different brain areas during the use of different navigation strategies. Interneurons, which have a vital role in modulating firing of pyramidal neurons, may gate the flow of information from EC and CA3 to CA1. These results further support a differential contribution of these two brain areas afferent to CA1, during different computations being conducted in CA1 and, moreover, highlight the importance of locking to theta rhythms during active behavior. NR1-KO mice showed impaired relationship between phase locking and behavior, despite the stronger locking of its pyramidal neurons as compared to control mice to, notably, low and high gamma oscillations.

4.2 Introduction

Departing from the original view that firing rate alone codes the spatially oriented activity of place cells, the discovery of a dynamic relationship between place cell firing within its place field and theta oscillations (6-12 Hz oscillations) revealed that the timing of each spike refines the
precision with which spiking defines the position of the animal [O’Keefe and Recce, 1993, Skaggs et al., 1996, Jensen and Lisman, 2000]. Despite the fact that a hippocampal place cell in area CA1 will fire at a preferred phase of theta [Buzsáki and Eidelberg, 1983, Fox et al., 1986], when a rat runs through a place field, firing of that particular place cell will show a gradual advancement of the theta phase at which it spikes, with phases precessing between 180° and 360° [O’Keefe and Recce, 1993]. A consequence of this phenomenon is that action potentials of place cells with adjacent fields will be fired in a short temporal window, which may allow strengthening of the synapses through spike timing dependent plasticity, preserving also the order of firing in a sequence of consecutively active place cells [Foster and Knierim, 2012]. This is shown in replay events, which occur throughout sleep or pausing periods during or following a behavioral task. During there fast oscillatory events (150-200Hz), place cells firing in a sequence during behavior replay these firing sequences offline and in a compressed time window [Lee and Kesner, 2002].

In addition to the possible role of theta phase precession described above, phase locking may also indicate a mechanism by which two different brain regions communicate: support for this hypothesis comes from studies demonstrating that neurons of a particular brain region are phase locked to the oscillatory rhythms of a different one, such as medial prefrontal cortex (mPFC) neurons [Siapas et al., 2005, Benchenane et al., 2010] or entorhinal (EC) neurons to hippocampal theta oscillations [Mizuseki et al., 2009]. This relationship between single cell activity and the local EEG is not restricted to theta oscillations. Although weaker, principal cells in the hippocampus also fire at preferred phases of higher frequency oscillations, such as gamma [Csicsvari et al., 2003, Colgin et al., 2009, Chen et al., 2011].

We have previously shown that intrahippocampal theta to low (23-40 Hz) and high (55-95 Hz) gamma coherence was similar, when behavior was guided by different information sources (Fig. 3.5). We speculate that theta-gamma coherence represents a dynamic state in the behaving rodent, establishing a flexible communication between CA1 and the two brain regions generating low and high gamma activity (CA3 and medial EC (mEC), respectively). One possibility, however, is that firing of CA1 place cells (and, therefore, spatial representation) tunes more strongly
to one or the other type of gamma oscillation, such that their activity patterns become more dependent on the inputs of one of the two brain regions mentioned above [Carr and Frank, 2012]

Hippocampal interneurons also display strong locking to a wide range of network oscillations [Freund and Buzsáki, 1996, Kuang et al., 2010]. These inhibitory cells comprise a wide range of sub-classes [Kuang et al., 2010] and are believed to play a crucial role in coordinating pyramidal cells and in the timing of their activity, according to the current behavioral state of the animal [Klausberger et al., 2003, Freund and Buzsáki, 1996]. Most interestingly, different sub-classes of interneurons, such as bistratified [Klausberger et al., 2004] and oriens lacunosum-moleculare (OLM) cells [Leão et al., 2012] may play an important role in the gating of inputs to CA1, facilitating information flow from CA3, while minimizing the influence of EC inputs. Our previous results (see Chapter 2 and 3) suggest that CA1 tunes to either CA3 or mEC, basing spatial representation on an internally driven or externally guided state. Specific sub-classes of interneurons may, thus, be responsible for triggering this behaviorally dependent switch and changing who CA1 "listens to".

We have recorded CA1 single-unit activity and local EEG in control mice and KO mice lacking the NMDA receptor in CA1 area (NR1-KO [Tsien et al., 1996]), a receptor which is critically involved in the proper functioning of networks [Whittington et al., 1995, Middleton et al., 2008, Korotkova et al., 2010]. Recordings were made while mice learned a spatial navigation task allowing the disambiguation of different navigation strategies: one based on the layout of the environment (place-based strategy) and one based on an internal, memory-based representation of motor sequences (sequence-based). In this task (the 'starmaze' [Rondi-Reig et al., 2006]), mice spontaneously chose which strategy to follow, making it ideal to study neural correlates of behavioral states based on different cognitive processes.

In addition, we employed analytical techniques, which allow determining the degree of phase locking of spikes to rhythmic LFPs, based on just a few trials and even in a single-trial analysis ( PPC measure [Vincx et al., 2011]). This was a crucial step to address phase locking strength on trials, in which mice changed navigation strategy. Given the different patterns of activity of network oscillation (Chapter 3) and differences
in spatial representation by CA1 place cells (Chapter 2), depending on
the navigation strategy employed, we sought to investigate whether the
relationship between place cell firing and the EEG could modulate the
different computation modes taking place in CA1.
Our results point to a crucial role of theta oscillations in assisting place cell
activity, dependent on navigation strategy employed and we show further
evidence linking CA3 and mEC to sequence- and place-based navigation
strategies. The absence of the NMDA receptor strongly changed patterns
of activity, highlighting its importance in maintaining stable network dy-
namics. Our results give further insight into how CA1 integrates different
streams of information, weighing the influence of brain regions afferent
to CA1 according to behavioral needs.

4.3 Methods

For a detailed description of the experimental subjects, the starmaze
task, drive, surgery and tetrode positioning, as well as histology and data
acquisition parameters, refer to Chapter 2.

Data analysis. All data used for analysis were from periods in which
the animal was moving at speeds above 3 cm/sec. LFP signals were
always taken from a tetrode located in the pyramidal cell layer. $0^\circ$ was
taken as the trough of the theta wave.

Spike-LFP PPC analysis. The circular concentration of spike-LFP phases
was quantified using the pairwise phase consistency (PPC) [Vinck et al.,
2011]. For a given frequency $f$, we determined the instantaneous spike-
LFP phases by Fast Fourier Transforming a Hann-tapered LFP segment
around the spike, with length $5/f$ seconds, thereby maintaining a con-
stant frequency resolution at any frequency $f$. We denote the spike-LFP
phase for the $i_{th}$ spike in the $m_{th}$ trial at frequency $f$ by $\theta_{i,m}$. For single
trial analysis, we computed the PPC as in [Vinck et al., 2011] by eq. 1

$$ppc_0 = \frac{\sum_{j=1}^{N_m} \sum_{k\neq j}^{N_m} \left( \sin(\theta_{j,m}) \sin(\theta_{k,m}) + \cos(\theta_{j,m}) \cos(\theta_{k,m}) \right)}{N_m(N_m-1)}$$
where $N_m$ denotes the number of spikes in trial $m$. If multiple trials are available, we use the following equation:

$$\text{eq. 2} \quad \text{ppc}_1 = \frac{\sum_{m=1}^M \sum_{i \neq m} \sum_{j=1}^{N_m} \sum_{k=1}^{N_m} \left( \sin(\theta_{j,m}) \sin(\theta_{k,l}) + \cos(\theta_{j,m}) \cos(\theta_{k,l}) \right)}{\sum_{m=1}^M \sum_{i \neq m} N_m N_l}$$

The PPC considers one pair of spike-LFP phases at a time and determines to what degree this pair of spike-LFP phases coincides in phase or not, using the dot product. Because PPC is a pairwise measure, it is not biased by the number of spikes. Furthermore, $\text{ppc}_1$ is not affected by non-Poissonian history effects within spike trains, such as bursting, autorhythmicity or a refractory period [Vinck et al., 2011]. The expected value of the PPC equals the squared phase-locking value (i.e., the resultant length of the spike phases) [Vinck et al., 2011]. We only considered spike-LFP pairs recorded on two different electrodes. The significance of phase locking (18-100 Hz) was assessed using cluster-mass based permutation statistics [Bullmore et al., 1999, Maris et al., 2007] The Rayleigh test’s p-value (at $P < 0.05$) was used as criterion for significance of individual frequencies and the cluster-mass of aligned, significant PPC (eq. 2) values was used as the test statistic, thereby correcting for multiple comparisons across frequencies.

**Single trial phase precession analysis.** Phase precession was calculated on a trial-by-trial basis [Schmidt et al., 2009]. Mouse paths were linearized and the firing rate calculated as a function of distance from departure. Single-trial place fields were determined by detecting adjacent bins (bin=4cm) where cell activity was above 1/3 of the trial’s maximum firing rate and where at least 4 spikes were fired. The theta firing phase for each spike was determined using the Hilbert transform of the filtered (7 - 10Hz) LFP signal. Taking $\theta_i$ and $x_i$ as respectively the theta phase and the position of the mouse on the track for the $i_{th}$ spike, an estimate of the precession slope, $\hat{a}$, was calculated by maximizing the resultant length eq. 3

$$R(a) = \sqrt{\left( \frac{1}{n} \sum_{j=1}^n \cos \theta_j - 2\pi a x_j \right)^2 + \left( \frac{1}{n} \sum_{j=1}^n \sin \theta_j - 2\pi a x_j \right)^2}$$
where \( n \) is the number of spikes and \( a \) the range taken for the slope \([-4 \pi : 0]\). Restricting the values of \( a \) to this range avoids fitting lines with arbitrarily high values or with positive slopes. The phase range of the precession \( R_\phi \) is then calculated from the slope and the ‘place field size’ \( R_p \), defined as the distance between the 1st and the last spike: eq. 4

\[
R_\phi = \hat{a} R_p
\]

For the correlation strength of the phase precession fit (between position and phase), a multiple of \( 2\pi \) was added to each \( \theta_i \) in order to minimize the residues of the linear-circular fit and the (linear) Pearson’s correlation coefficient between \( \theta_i \) and \( x_i \) was then taken (Fig. 4.6(b)). Circular analysis statistics were performed using the Circular Statistics Toolbox for Matlab [Beren, 2009].

### 4.4 Results

We trained control (CTR) and NR1 CA1 KO (NR1-KO) mice to run on a complex, pentagon-shaped maze with five radial arms, (the ‘star-maze’ [Rondi-Reig et al., 2006]), which allowed us to distinguish between two different navigation strategies: a place-based, environment-dependent strategy and a sequence-based strategy, supported by an internal representation of the motor sequence necessary to reach the goal. This strategy identification was made by evaluating the trajectory mice took during probe trials, where the departure arm was changed. Previously we showed (Chapter 2 and 3 ) strong evidence, at the single-cell and at the network level, for a dual commitment of CA1 neurons of control mice to either a place- or sequence-based navigation framework, respectively: place fields remained anchored to the layout of the environment during the former case, while their place fields rotated along with the changed departure arm in the latter one. We highlighted the importance of three oscillatory bands during this task: theta (6-12 Hz) and low (23-45 Hz) and high gamma (55-95 Hz), with low gamma more associated with sequence-based navigation and high gamma with place-based navigation. We proposed that the latter two frequency bands may allow CA1 cells to
shift their tuning to EC or CA3, with the balance between low and high gamma influence determining whether spatial representation in CA1 is externally (environment)-oriented or internally, memory-oriented. In the former case, place cells kept their place fields anchored to the environmental cues, while in the latter case, place cells rotated their fields, such that the location of firing remained anchored to the departure point.

Increased power in a certain frequency band, or increased intra-CA1 coherence between two different LFP frequencies (e.g. theta with low or high gamma) may be indicative of a stronger tuning of CA1 populations to different input structures, but ultimately we want to know what is pacing the activity of individual, task-related neurons. For this, we analyzed in depth the relationship between single-cell firing and ongoing oscillatory patterns. Phase locking of single neuron spike trains was quantified by the pairwise phase consistency (PPC) measure [Vinch et al., 2011] (see Methods), a measure of phase locking which, contrary to traditional measures, remains unbiased independent of the number of spikes in the train, and is therefore applicable even to small spike samples.

The phase locking spectrum of individual neurons (Fig. 4.1) revealed a

![Figure 4.1](image)

**Figure 4.1:** NR1-KO neurons show an abnormal phase locking spectrum of spikes of putative pyramidal cells and putative inhibitory neurons to LFP oscillations.

(a) *Phase locking spectrum (PPC) of pyramidal cells in CTR (blue) and NR1-KO (red).*

(b) *Similar plot, but for interneurons.*
stronger phase locking of NR1-KO pyramidal neurons to theta and LG oscillations (at Raleigh’s test $P < 0.05$, see Methods), while in interneurons there was only a significant difference in the theta range, in this case with CTR mice showing a stronger phase locking power. To address the relation between phase locking and navigation behavior, we analyzed locking profiles for each oscillatory band individually.

Robust theta phase locking of CTR and NR1-KO pyramidal cells

Hippocampal theta rhythm is present very consistently throughout active behavior, such as running, rearing or grooming [Vanderwolf, 1969], strongly modulating the activity of neurons [Fox et al., 1986]. In our task, pyramidal cells of both CTR and NR1-KO mice were strongly phase locked to the trough of local theta waves (Fig. 4.2a). The mean preferred phase of CTR neurons was slightly more advanced than NR1-KO, but this difference did not reach significance (Watson-Williams (WW) multi-sample test: $P = 0.06$). There was a small, but significantly higher fraction of NR1-KO cells modulated by theta (Fig. 4.2b, top; $\chi^2$-test: $P < 0.05$), but the locking strength (PPC) of significantly modulated neurons was similar between genotypes (Fig. 4.2b, bottom; $t$-test: n.s.).

We also found that locking to theta increased as training progressed in CTRs (Fig. 4.2c, solid lines; Spearman’s Rho (session vs PPC) = 0.16, $P < 0.05$; ANOVA: $P$ (geno) $< 0.005$, $P$ (session) $< 0.001$, $P$ (interaction) $< 0.05$). This increase followed behavioral performance very closely (shaded area, learning measure used was the localization score [Fouquet et al., 2011]), suggesting that theta locking plays a role in performance. If this would be the case, then one might expect locking to theta oscillations to be lower in incorrect trials. We separated each session into correct and incorrect training trials and indeed found that in CTR pyramidal cells, theta locking was stronger in the former case (Fig. 4.2d, Permutation test [Womelsdorf et al., 2012] correct vs incorrect $P$ (CTR) $< 0.01$, $P$ (NR1-KO) n.s.).

These results show that more NR1-KO pyramidal neurons were modulated by theta, but this state was very different from CTR neurons, where locking was not a constant state throughout exposure to the maze, but varied according to experience and performance. In contrast, NR1-KO pyramidal cells did not show any significant performance-dependent vari-
Figure 4.2

ations in locking strength.

We next asked ourselves whether theta locking varied as a function of strategy used. We have already shown (see Chapter 2) that place cells are anchored to different frameworks (place- and sequence-based), used during execution of different strategies (see Chapter 2, Fig. 2.6e). Hence, we correlated the place and sequence indices (P_{idx} and S_{idx}, respectively; a measure of how well a place cell follows one of these patterns during the respective probe trial) with theta locking strength. Given the difficulty in addressing phase locking when too few spikes are available, we measured the PPC over the entire session, pooling together all training trials. This
Figure 4.2: Theta phase locking of pyramidal cells.

(a) Polar histogram of preferred theta phase locking of pyramidal cells (0° represents theta trough, see inset). Radial axis indicates fraction, arrow direction the mean preferred phase and arrow length the mean resultant length. Here, as in the other panels in this figure, blue and red represent CTR and NR1 mice, respectively. There was a small, but significant difference in the distribution of the preferred phases (Kuiper-test, $P < 0.01$).

(b) top: fraction of modulated cells: NR1-KO mice showed a small, but significantly higher fraction ($\chi^2$-test: $P < 0.05$); bottom: mean PPC value of significantly modulated cells (t-test: n.s.).

(c) Mean and SEM of PPC to theta value (lines) and behavioral performance (shaded area); performance measure used was the localization score [Fouquet et al., 2011] as a function of training session. PPC and performance follow a similar pattern, both increasing with experience (ANOVA: $P$ (geno) $< 0.005$, $P$ (session) $< 0.001$, $P$ (interaction) $< 0.05$). While CTR mice showed a positive correlation between theta locking and session number (Spearman’s $R = 0.16$, $P < 0.05$), NR1-KO did not show a significant effect.

(d) Mean resultant length (radial axis) and preferred theta phase (arrow direction) of all spikes pooled together, separated into correct and incorrect training trials. CTR, but not NR1-KO mice, showed a three-fold decrease in PPC in incorrect trials (Permutation test [Womelsdorf et al., 2012]: correct vs incorrect $P$ (CTR) $< 0.01$, $P$ (NR1-KO) n.s.).

(e) and (f) Scatter plot of $P_{\text{idx}}$ (e) and $S_{\text{idx}}$ (f) (measures of how well a place cell follows either a place-based or a sequence-based, respectively, framework) vs theta peak PPC (measured over all trials), for all recorded pyramidal neurons. For CTR cells, theta PPC correlated negatively with $P_{\text{idx}}$ and positively with $S_{\text{idx}}$ (Spearman’s rho = -0.19 and 0.26, respectively, $P < 0.05$).

analysis revealed that the peak PPC in CTR pyramidal cells correlated negatively with the $P_{\text{idx}}$, but positively with the $S_{\text{idx}}$ (Spearman’s rho = -0.19, $P < 0.05$ and rho = 0.26, $P < 0.01$, respectively; Spearman’s rho for NR1- KO: n.s.), suggesting that theta locking may act as a switch between the two modes.
Theta phase locking of interneurons

Interneurons play a crucial role in controlling the timing of pyramidal cell activity [Freund and Buzsáki, 1996, Korotkova et al., 2010] and have recently been shown to modulate information flow to CA1 [Leão et al., 2012]. We therefore analyzed in greater depth locking properties of interneurons \((n(CTR) = 75, n(NR1-KO) = 95)\) to the three oscillatory bands and their relation with the different navigation strategies.

Fig. 4.1b shows that CTR putative interneurons, contrary to pyramidal neurons, are more phase locked to theta than NR1-KO interneurons. The preferred spike phases were, in both cases, clustered around the trough of the theta wave (Fig. 4.3a) and there was no difference in the frac-
tion of interneurons that were significantly modulated (Fig. 4.3b, top). Nevertheless, the PPC of these cells in CTR mice was higher than in NR1-KO mice (Fig. 4.3b, bottom). Given the different characteristics of different interneuron subclasses [Kuang et al., 2010] and the difficulties in identifying these classes based solely on firing properties and tetrode location, we cannot, however, say whether this difference is attributed to the lack of the NMDAR in KO mice or to sampling of different classes of interneurons in different mice: the three known classes of interneurons in the pyramidal layer of CA1 all show different degrees of theta phase modulation [Kuang et al., 2010].

Interneurons do not share the same place-modulated firing patterns as place cells, but since they are key players in modulating activity of pyramidal neurons, we correlated interneuron theta locking with the av-

![Graphs showing fraction of modulated cells, mean peak PPC, LG Locking, and HG Locking](image)

Pyramidal neurons are locked to low and high gamma-band oscillations.

Figure 4.4: (a) Fraction of pyramidal neurons significantly locked to LG and HG ($^*$ $\chi^2$-test: $P < 0.05$). Here, as in other figures, blue and red represent CTR and NR1 mice, respectively.

(b) Mean of peak PPC values in the LG and HG range for significantly locked pyramidal neurons; for each cell the peak value of PPC within the LG and HG range was taken. NR1-KO locking was stronger in the LG and HG range ($^**$ t-test, $P < 0.01$).

(c) Polar histogram (as in Fig. 4.1a) of preferred LG phase locking of pyramidal cells. CTR showed a higher concentration of phases (Circular K-test: $P < 0.05$) and the two mean directions differed from one another (Watson-Williams test for circular data: $P < 0.01$).

(d) Same as (c), but for HG. Both genotypes showed a similar concentration of phases, but the two mean directions differed (Watson-Williams test for circular data: $P < 0.01$).
verage place and sequence indices of the simultaneously recorded place cells (Fig. 4.3c). There was no significant correlation of theta locking with the $P_{\theta}$ in either genotype, but CTR interneuron theta locking was positively correlated with the average $S_{\theta}$ (Spearman’s rho = 0.41, $P < 0.05$), similar to what CTR place cells show (Fig. 4.2f), indicating a stronger involvement of interneurons in this type of navigation.

**NR1-KO neurons are individually more locked to LG, but less synchronous with each other**

Locking to a certain LFP frequency band can be analyzed at the individual level or at the network level. The former one is expressed through locking strength of individual neurons, here measured as the PPC value. The latter one, however, can be assessed as the synchrony between the different neurons, i.e., whether or not they share the same preferred phase with respect to an LFP oscillation. Though weaker, pyramidal cells from both CTR and NR1-KO were also modulated by low and high gamma-band locking activity (Fig. 4.1a). In the case of LG, NR1-KO had a higher fraction of significantly modulated cells (Fig. 4.4a, $\chi^2$-test: $P < 0.05$; HG: n.s.) and the PPC of significantly modulated cells was higher for the NR1-KO in both LG and HG (Fig. 4.4b, t-test: $P < 0.01$). Preferred LG phases were, however, more concentrated in CTR neurons (Fig. 4.4c, Circular K-test: $P < 0.05$). The two phase distributions also differed in the mean preferred phase (W-W test for circular data: $P < 0.01$). The concentration of preferred HG phases was similar in CTR and NR1-KO (Fig. 4.4d), but the average preferred phase was still different (WW-test: $P < 0.01$). These data show that NR1-KO pyramidal cells are, overall, more phase locked to the local LG and HG rhythms, but, for LG, there is a higher variability in the preferred phase between cells, indicating an asynchronous LG modulation of these cells. This decreased synchrony during LG oscillations in NR1-KO may underlie the impaired spatial representation during sequence-based navigation (see Fig. 3.7).
Figure 4.5: Low gamma locking of interneurons
(a) Scatter plot of LG locking of interneurons and the average $S_{hde}$ measured from simultaneously recorded place cells (CTR: Spearman’s rho = 0.35, P < 0.05, KO: n.s.; Fisher’s test between correlations: n.s.).
(b) Scatter plot of LG locking of interneurons in place- and sequence-strategy trials (CTR: Spearman’s rho = -0.63, P < 0.05; KO n.s.; Fisher’s test between correlations: P < 0.01).

**LG Locking of interneurons is particularly tuned to sequence-based strategy**

We have previously shown (Chapter 3) that the balance between high and low gamma LFP activity varies according to the strategy being employed, with this balance being shifted to the LG side in sequence-strategy trials and to the HG side in place-strategy trials (in CTR). We therefore asked whether LG locking would also vary as a function of strategy. Similar to the analysis we performed before (Fig. 4.3c), we correlated interneuronal LG locking to the average of the $S_{hde}$ of the pyramidal cells simultaneously recorded (Fig. 4.5a). This analysis showed a small, but significant positive correlation in CTRs, suggesting that phase locking correlates with the ability of pyramidal cells to rotate their place fields during sequence-strategy trials (Fig. 4.5a, CTR: Spearman’s rho = 0.35, P < 0.05, KO: n.s.; Fisher’s test between correlations: n.s.). In addition, we found a strong negative correlation between LG locking of interneurons of CTR mice in sequence- and place-strategy trials (CTR: Spearman’s rho = -0.63, P < 0.05; Fisher’s test between correlations: P < 0.01). These results indicate that the interneuron population of CTR mice closely follows the strategy being employed.
Behavioral dependency of single-trial theta phase precession

Phase locking does not fully characterize the relationship between the firing of hippocampal place cells and the theta rhythm. A negative correlation ('phase precession' [O’Keefe and Recce, 1993]) exists between animal position within the cell’s place field and the instantaneous firing theta phase of a place cell. While classically this phenomenon has been analyzed by pooling together spikes from multiple passages through the place field, analytical techniques have been recently devised [Schmidt

Figure 4.6
et al., 2009, Reifenstein et al., 2012] to quantify theta phase precession on a trial-by-trial basis. This allowed us to compare phase precession patterns across different trial types of our task.

\hspace{1cm} Figure 4.6: Single-trial phase precession analysis highlights faster place field dynamics in sequence-strategy trials of control mice.

(a) Example of single-trial phase precession of pyramidal cells from one session (CTR). Top: five trajectories for correct training trials (long path, same session) are shown. Dots represent positions at which a place cell fired during those runs. Yellow dots denote spikes that were fired in the cell’s place field, gray dots denote spikes fired elsewhere on the track. Bottom, trial-by-trial position (x-axis) to theta firing phase (y-axis) diagram for all spikes in the place field. Theta phases are repeated over 2 cycles, for clarity (0.0 \pm 360°). The circular-linear regression line is also displayed.

(b) Average linear-circular Pearson correlation coefficient per genotype and trial type. There was an effect of trial type (2-way ANOVA, p (trial type) < 0.05), with correlation in sequence-strategy trials trending to more negative values, however this did not reach significance in post-hoc tests.

(c) Average slope of the phase precession per genotype and trial type. There was a significant genotype x trial type interaction (2-way ANOVA, p (interaction) < 0.05). Post-hoc tests revealed a significant difference between sequence-strategy trials versus training and place-strategy trials in CTR place cells.

(d) Average single-trial PF size per genotype and trial type; place fields were smaller in sequence-strategy trials in CTR mice, compared to training trials (2-way ANOVA, p (interaction genotype x trial type) < 0.05). *: Post-hoc’s Tukey, p < 0.05.

(e) Phase Range of a precession cycle per genotype and trial type. There was no significant difference between the amount of precession between genotypes and trial types.

(f) Theta phase distribution of spikes included in the phase precession analysis, per trial type, in CTR (left) and NR1-KO (right) mice. There was a significant trial type effect in both genotypes (circular ANOVA, p (trial type) < 0.01). In CTRs, this difference was due to place-strategy trials, where the spike phases were more concentrated around the descending phase of theta. Training trials and sequence-strategy trials showed a preferred phase lagging that of place. NR1-KO mice showed a similar pattern in place-strategy trials, but spike phases were more shifted to the early ascending theta cycle.
Most trials showed robust theta phase precession, even when as few as 4 spikes (which we took as the threshold for inclusion in the analysis) were fired within a cell’s place field (Fig. 4.6a).

Following [Schmidt et al., 2009, Reifenstein et al., 2012], precession can be described by using a linear-circular model fit, with a modified Pearson's R measure used to assess goodness of fit (see Methods). Phase precession was present across genotypes and trial types (Fig. 4.6b). However, CTRs displayed a larger negative slope (hence, a faster precession rate) in sequence-strategy trials, compared to place-strategy or training trials (Fig. 4.6c; post-hoc Tukey’s HSD, P < 0.05), while NR1-KO place cells had a similar slope in all trial types.

The faster precession was, however, balanced by a decrease in place field size (Fig. 4.6d, CTR training trial vs Sequence: post-hoc Tukey’s HSD, P < 0.05), such that the range of the phase precession per place-field passage (see Methods) was maintained in all conditions (Fig. 4.6e). While complete, 'unitary' place fields, corresponding to one cycle of phase precession [Maurer et al., 2006], are thus expressed under all conditions, these results suggest that in CTRs this precession takes place at a more compressed pace during sequence-strategy trials.

Interestingly, when looking at the theta phase of phase-precessing spikes, we observed that spikes emitted during place-strategy trials were advanced in relation to spikes emitted during training and sequence-strategy trials, occurring most often before the trough of the theta wave (0°, Fig. 4.6f, left). This coincides with the phase of incoming inputs from mEC layer III principal neurons [Mizuseki et al., 2009]. NR1-KO mice also showed a similar effect, though attenuated (Fig. 4.6f, right). During sequence-strategy trials, spikes fired by NR1-KO mice were delayed, compared to CTRs and also training trials (but not significant, Kuiper-test, P < 0.01).
4.5 Discussion

In this study we have recorded single-unit and network activity from the dorsal hippocampal area CA1 of normal mice and KOs lacking the NMDAR in this hippocampal sub-field. Our goal was to study in depth the functioning of the CA1 network during spatial navigation based on different strategies. Specifically, we asked how the synchronization between CA1 neurons and the LFP varies, when the system processes external, sensory-based information during place-based navigation, or internal, memory-based information during sequence-based navigation. We found that NR1-KO pyramidal neurons displayed a stronger modulation by local low and high gamma rhythms than control. Theta phase locking of CTR neurons on the other hand, showed strong correlations with behavior and behaviorally-modulated single-cell activities, in particular during sequence-based trials. In addition, we showed that in CTR mice, behavioral strategy modulated the rate of theta phase precession and that the phases of precessing spikes gave further support for a distinction of the different brain regions supporting CA1 during the different navigation strategies. Phase locking of interneurons was also influenced by the type of navigation driving behavior, opening the possibility that this type of neuron plays an important role in the gating of information to CA1.

Theta is the most prominent rhythm in the hippocampus [Buzsáki, 2002], with the vast majority of the pyramidal neurons strongly locked to the trough of the theta wave [Csicsvari et al., 1999]. Not surprisingly, this was also the case for both genotypes in our task. On the other hand, both LG and HG locking of significantly modulated neurons was higher in NR1-KO mice (Fig. 4.4b). The NMDA receptor is characterized by a slow current and, in the absence of this channel, the EPSCs of CA1 pyramidal neurons show reduced duration [Tsien et al., 1996] and an almost three-fold decrease in the EPSP time decay constant [Burgard and Hablitz, 1993]. Action potentials arising from shorter EPSPs show increased spike time precision (STP, [Rodriguez-Molina et al., 2007]), which may explain the increased locking of pyramidal neurons in NR1-KO mice. This effect will likely be most prominent at higher LFP frequencies, where an oscillatory
cycle is very short. This may explain why the mean PPC value of theta-modulated cells was similar across genotypes.

Theta locking was not constant throughout recordings in the starmaze: in both genotypes, the increase in the PPC of theta modulation of pyramidal cells paralleled the increase in behavioral performance in the maze (Fig. 4.2c), highlighting the important role of theta in coordinating spatial navigation. A similar link has also been shown in humans performing a spatial navigation task [Cornwell et al., 2008, Kaplan et al., 2012, Watrous et al., 2013], where theta power was strongly correlated with performance. In addition, we did not observe the same pattern when analyzing data collected while CTR or NR1-KO mice ran on a circular maze, which requires no learning (data not shown).

We showed previously, however, that theta is strongly modulated by running speed (see Chapter 3, Fig. 4.3a), which also increases as training progresses (data not shown, Pearson’s R for session vs speed: r (CTR) = 0.34, P (CTR) < 0.001, r (NR1-KO) = 0.32, P (NR1-KO) < 0.001). It is possible, thus, that this correlation between theta locking and performance is a consequence of the increased theta power resulting from the increased speed. A relationship between locking strength and locomotion speed has (to the best of our knowledge) not yet been shown and, thus, the higher theta locking, which is observed only in CTR pyramidal neurons in correct against incorrect trials (Fig. 4.2d), argues, in favor of a relation between locking and performance.

Probe trials in our task allowed distinction of two different dynamic states in CA1, related, respectively, to sequence- and place-based reference frames, most importantly with a more dominant role played by theta oscillations during sequence-strategy trials, as seen by the positive correlation between theta phase locking and the $S_{tdx}$ (Fig. 4.2e and f). Theta locking, on the other hand, was inversely correlated with the $P_{tdx}$. This effect may be explained by the stronger role that external sensory information plays during place-strategy trials, when the influence from the less theta-modulated lateral entorhinal inputs [Deshmukh et al., 2010], carrying the sensory information that anchors place fields to external landmarks [Hunsaker et al., 2007], is presumably more marked.

NR1-KO neurons, despite showing stronger phase locking to LG oscil-
lation (Fig. 4.4), had more spread preferred phases, indicating lower synchrony among them. This finding may relate to our results showing that, in CTR mice, spikes fired during LG periods contribute more information to the overall spatial representation during sequence-based navigation (see Fig. 3.7). Spatial representation in NR1-KO mice was most affected during the use of this type of strategy (see Fig. 2.6d), which may be a consequence of decreased synchrony of firing relative to LG oscillations.

In each run through their place fields, pyramidal cells of both genotypes showed a robust phase precession of its spikes relative to theta oscillations (Fig. 4.6). When going through its field, a place cell typically showed approximately 180° advancement of its spikes. This shift remained constant across trial types, despite the steeper precessing slope observed in sequence-strategy trials in CTR, which is explained by the finding that the single-trial place field was also reduced (Fig. 4.6c-e). The reduction in place field size may be a direct reflection of the more compact place fields observed in area CA3 [Mizuseki et al., 2012]. This balance between slope and place field size ensures that a 'unitary' field is completed [Terrazas et al., 2005], when the animal passes through a place field. The completion of a precession cycle may be a vital process: phase precession is believed to facilitate synaptic plasticity between place cells with neighboring fields, by bringing the spikes of both cells temporally close enough to allow STDP [Maurer and McNaughton, 2007].

In CTR, theta phases of phase-precessing spikes showed a different distribution depending on the strategy being employed: during place-based navigation, spikes were advanced to the descending phase of theta, while in training and sequence-strategy trials they were centered around the trough. Principal cells in layer III of mEC, which monosynaptically project to CA1 place cells [Witter et al., 2000], are also locked to theta oscillations and fire approximately half a theta cycle earlier than CA1 pyramidal neurons [Mizuseki et al., 2009], suggesting that the shift in the phase of spikes during place-based navigation that we observe, corresponds to the timing of incoming EC inputs.

Many mEC neurons fire in a regular hexagonal pattern ("grid") and are crucial for spatial navigation processes, as has been shown in lesion
[Van Cauter et al., 2012, Hunsaker et al., 2007] and electrophysiological [Brun et al., 2008, 2002] studies. In these later studies, either CA3 or EC inputs to CA1 were lesioned. The results indicate that direct EC inputs to CA1 are both necessary and sufficient to produce a sharp spatial representation in area CA1, indicating that layer III EC neurons are fundamental for the map/path integration-based place activity. The advanced spiking of place cells of CTR mice during place-based navigation, in our task (Fig. 4.6f), may, therefore, reflect a direct response of place cells to incoming mEC inputs. This advancement may reflect a change in the computation mode of the CA1 network, where encoding of incoming sensory and spatial information from EC prevails over retrieval mechanisms, presumably mediated by CA3 [Huxter et al., 2008, Douchamps et al., 2013].

Interneurons are key players in coordinating pyramidal cell activity [Freund and Buzsáki, 1996, Klausberger et al., 2004, Leão et al., 2012] and their locking to theta (in all trials) in CTR mice was indicative of how well the pyramidal cell population followed the sequence-based framework in sequence-strategy trials (Fig. 4.3c, bottom). This positive relation between theta locking and sequence spatial representation is the same as for pyramidal neurons (Fig. 4.2f) and underscores the importance of theta oscillations during this type of navigation. Note that theta power did not vary as a function of trial type (see Chapter 3, Fig. 3.4a), showing that this is an effect at the single-cell level, rather than at the network level. The high firing rate of interneurons make it feasible to reliably address single-trial phase locking. In sessions in which both place- and sequence-strategy trials were present, interneurons of CTR mice showed a negative correlation between LG locking in both trial types (Fig. 4.5b), suggesting that interneurons may act as a switch between the two modes. However, without proper techniques, it is difficult to identify the different types of interneurons. Nonetheless, different classes of interneurons show very different firing activity patterns, varying in their firing rate, autocorrelogram, locking to oscillatory rhythms (especially theta) and locking to high frequency ripples [Kuang et al., 2010, Klausberger and Somogyi, 2008]. Fig. 4.3a shows that the vast majority of the recorded interneurons in both genotypes are strongly locked to theta and to the same phase of
theta as the pyramidal neurons, the trough. From previous work [Kuang et al., 2010, Klausberger et al., 2003], we may thus assume that our population of interneurons belongs to either type-2 bistratified cells or type-4 OLM (orien-lacunosum moleculare) cells. Given that all analyses were done using tetrodes located in the pyramidal cell layer and that most interneurons showed a robust firing rate increase during ripples (74% of all interneurons showed an increase in firing rate during ripples, average increase (ratio firing rate inside/outside ripple period) = 2.22 ± 0.01, one-sided t-test: P < 0.0001), it is likely that we mainly recorded from bistratified cells [Kuang et al., 2010]. Output synapses of bistratified interneurons are co-aligned with Schaffer collateral inputs [Klausberger et al., 2004] and are therefore an ideal candidate to modulate CA3 inputs on to CA1 pyramidal cells. Given the evidence presented here and elsewhere (see Chapters 2 and 3), attributing to CA3 a strong role in sequence-based navigation, increased theta locking of these interneurons during sequence trials of CTR mice (Fig. 4.3c, bottom) may phase-modulate excitatory inputs from CA3 during theta oscillations [Klausberger et al., 2004]. This is in line with the hypothesis that CA1 processing during sequence trials is strongly driven by CA3 activity associated with the internal regeneration of memory traces. Interestingly, this interneuron sub-class shows the strongest gamma phase locking [Tukker et al., 2007] and it has been suggested that these interneurons, by synchronizing the activity of CA1 pyramidal neurons, can phase excitatory inputs arriving from CA3, opening temporal windows on gamma time scales consistent with a role in regulating spike timing-dependent plasticity (STDP) [Tukker et al., 2007].

This study provides novel insight into how the interplay between single neurons and network oscillations, notably theta, may assist different behavioral states of the animal, here reflected in the use of different navigation strategies. We have shown that theta oscillations play an important role in modulating both pyramidal cell and interneuronal firing: increased theta locking was associated with a better spatial representation during sequence-based navigation and lower during place-based navigation. The theta phase, at which spikes were fired, suggests that CA1 place cells shift their tuning to different inputs, reflecting current
behavioral strategy. In addition, the differential locking of interneurons to LG - across sequence- and place-based trials (Fig. 4.5) - suggests that this locking may be important in switching CA1 spatial representations between the two types of strategy. The results further strengthen the association between place- and sequence-based navigation with the EC and area CA3, respectively.
Chapter 5

Single-trial properties of place cells in Control and NR1-KO mice

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5.1 Abstract

The NMDA receptor plays a key role in synaptic plasticity and its disruption leads to impaired spatial representation in the CA1 area of the hippocampus, with place cells exhibiting larger place fields [McHugh et al., 1996]. Place fields are defined by the spatial and non-spatial inputs of a given place and context, by intrinsic network processes, such as phase precession, but also by the matching of these inputs to a pre-existing spatial representation. Larger place fields may be a consequence of spatially widened firing upon a single crossing of a place field, or of increased variability in place field positions across traversals. We addressed this question by monitoring CA1 place cell activity, with tetrodes, in control and KO mice lacking the NMDA receptor in this region. In individual crossings of the field, we found no difference between genotypes in place field size; the larger, overall place field size turns out to be a consequence of jitter across trials. We suggest that this jitter reflects a deficit in the matching of current spatial inputs to the stored spatial representation of the track. This is supported by the finding that deficits in place field size and spatial information are rescued by extensive exposure of the mouse to the track, which may echo an increased influence of memory retrieval processes in CA3 on firing in CA1.

5.2 Introduction

Since the landmark discovery of place cells [O’Keefe and Nadel, 1978], the hippocampus (HPC) has become the focus of research of the neural mechanisms underlying spatial navigation and episodic memory. In particular, the CA1 subfield, which is the main output station of the HPC, is thought to assemble a ‘cognitive map’ of a given environment, expressed by the activity of place cells, with their highly specific location and context-dependent firing [Wilson and McNaughton, 1993, Eichenbaum, 2000]. Place fields (PFs) are plastic and a wide range of context and experience dependent factors influence place field properties: extended exposure to the same context leads to an asymmetric expansion of the place field [Mehta et al., 2000], changes to the environment may
lead to a reorganization of the place map [Leutgeb et al., 2004, Fyhn et al., 2007], behavioral context modulates spatial representation [Griffin et al., 2007, Lansink et al., 2012] and running direction changes firing of a place cell within its place field [McNaughton et al., 1983, Battaglia et al., 2004]. These properties endow the CA1 with the capability to develop a unique and plastic spatial representation for each environmental context, with the additional capacity to adapt to ever changing situations.

A vital player for the correct functioning of the CA1 map is the N-methyl-D-aspartate receptor (NMDAR). This membrane receptor, critically involved in long-lasting synaptic plasticity [Bliss and Collingridge, 1993, Malenka and Nicoll, 1999], regulates oscillatory network dynamics [Whittington et al., 1995, Korotkova et al., 2010, Middleton et al., 2008] and the formation of a proper spatial representation in CA1 [McHugh et al., 1996]. Tsien et al. (1996) developed a knockout (KO) mouse line lacking the NMDAR-subunit 1 (NR-1) in pyramidal neurons of the CA1 area, but not the rest of the hippocampus. Pyramidal neurons in these mice do not exhibit LTP [Tsien et al., 1996], which is likely to impair the integration of inputs arriving to the CA1 from CA3 and the entorhinal cortex, its two main input structures [Witter, 1993]. These mice show behavioral impairments linked to spatial and temporal processing [Tsien et al., 1996, Huerta et al., 2000, Rondi-Reig et al., 2006], as well as impaired spatial representation. A classic measure for the quality of hippocampal spatial representation is the size of place field: if enough cells are sampled, the smaller the field, the more accurately the position of the animal can be reconstructed. Indeed, in CA1-NR-1KO mice, place fields are larger and place cells with overlapping place fields show less coherence [McHugh et al., 1996].

Place field size is determined jointly by the spatially and idiothetic informative inputs to the respective cells, and by intrinsic cellular and network processes, defining the temporal boundaries of a firing episode. For example, it has been argued [Maurer and McNaughton, 2007] that the size of a place field is determined by the complex interactions between spatial and idiothetic inputs and oscillatory phenomena, that determine theta phase precession [O’Keefe and Recce, 1993], so that a place field is best defined (for a 1-dimensional track) by the portion of the animal’s track run covered by a sweep of the theta cycle as apparent from the cell’s pre-
ferred firing phase. However, an important open question is whether the increase in place field size is a consequence of the spatial widening of this 'canonical place field', or whether it is a result of increased variability in the position where the cell begins and ends firing, each time the animal crosses the place field. These two possibilities depend on very different processes: the latter one relies mainly on the integration of spatial inputs, i.e. the alignment of visual and idiothetic information with the pre-existing spatial representation, stored as a spatial memory possibly in area CA3 [Nakazawa et al., 2004]. The former option, an increase in the length of the portion of the track on which a place cell is firing in any given passage, possibly extends to include the hippocampal-medial entorhinal loop [McNaughton et al., 2006, Moser et al., 2008, Buzsáki and Moser, 2013], which suffices to produce sharp place fields [Brun et al., 2008].

To disambiguate between these two possibilities, we recorded CA1 place cell activity in control and NR1-KO mice running on a circular track and by analyzing spatial properties on a trial-by-trial basis. Confirming previous studies with the same KO mice [McHugh et al., 1996], we show that these animals have larger place fields, when calculated over the entire session, but not when place fields are analyzed on a single-trial basis. The increased session place field was a consequence of a greater variability in firing maps across trials. This increase in variability subsided with increasing experience on the track, such that some of the impairments were rescued towards the end of the recording. In addition, KO mice failed to express some of the plastic changes affecting place fields in CTR over the course of a session, such as a reduction of place field size and a precession of the PF’s center of mass.

5.3 Methods

Subjects. Male mice lacking the NMDAR1 gene in the CA1 subregion of the hippocampus, originally created at MIT (Cambridge, Massachusetts) [Tsien et al., 1996], were inbred at and obtained from Université Paris VI. Control (CTR) mice were ‘floxed’ littermates of KOs, not carrying the NR-1 deletion. Animals were maintained on a reversed
day/night cycle (lights on/off: 20h/08h), single-housed and food restricted to 90% of their free feeding weight. All experiments were carried out in accordance with Dutch National Animal Experiments regulations and approved by the Committee on Animal Experiments of the Universiteit van Amsterdam. Fifteen mice (8 KO and 7 CTR), were used for electrophysiological experiments, and implanted at the age of 41 ± 5 days, weighing approximately 20 grs. All mice were between 51 (minimum) and 77 (maximum) days of age during the recording phase, an age range which delimits the mutation to the CA1 region [Fukaya et al., 2003]. At all times, a KO and a CTR mouse were studied in parallel, with all procedures counterbalanced by genotype.

**Apparatus.** Training took place in a custom made circular track with a 30cm radius. The track was made out of aluminium, had 7cm wide and with 5cm high walls. The experimental room had black curtains at the walls with large geometrical cues on the four sides, and 40 watts light bulbs at each corner.

**Behavioral Protocol.** Mice ran on the circular track, which was interrupted at one point by a barrier, so that mice had to shuttle back and forth alternating clockwise and counter-clockwise directions and collect two sucrose pellets placed at each end. Each session comprised a maximum of 30 trials, 15 in each direction. Each mouse ran 2 daily sessions (one in the morning, the other in the afternoon) over the course of 5 days and each session was flanked by two 20 min periods of rest, in which the animal was placed in his home cage in the center of the circular track, surrounded by a black cardboard enclosure, while baseline electrophysiological signals were recorded. The first recording session was also the first time the animals were exposed to the environment.

Trials, in which mice changed direction during running, were excluded from analysis.

**Drive, Surgery and Tetrode positioning.** Six independently moveable tetrodes (polyimide-insulated,13 μm diameter nichrome wire (Kanthal, PalmCoast, FL) ) were loaded into a custom-made, ultralight (1,8 gr) "Lantern” Microdrive [Battaglia et al., 2009], their impedances were low-
erated to 0.5-1MΩ and were implanted over the dorsal hippocampus (AP: -2.0 mm, ML: -2.0 mm). In the week after surgery, tetrodes were gradually lowered until they reached the CA1 pyramidal layer. Tetrode position was adjusted between recording sessions to maintain them in the CA1 pyramidal layer, which was identified by the presence of strong sharp-wave ripple events and the presence of spike trains fired in bursts.

**Histology.** After recordings, electrolytic lesions were made at the recording sites by passing 20μA of current for 10 s through one lead of each tetrode. After perfusion with formaldehyde (buffered in PBS pH 7.4) coronal brain sections (40μm) were cut on a Vibratome and Nissl-stained for verification of tetrode tracks and end points. Only animals with clear lesions or presence of tetrode tracks in the CA1 pyramidal layer and/or clear sharpwave-ripple complexes and ripple-modulated cell firing were included in the analysis.

**Data acquisition.** Tetrode signals were unit-gain amplified by the headstage pre-amplifiers (Neuralynx, Bozeman, MT) and relayed to amplifiers for single-unit and local field potentials (LFP) recordings. The signal was amplified 2000 times, bandpass filtered (0.6-6.0 kHz for single-unit; 1-475 Hz for LFP), acquired and time-stamped. For single units, the sampling rate was set at 32 kHz every time the signal exceeded a manually selected threshold; LFPs were sampled continuously at 2 kHz. One of the 6 tetrodes was targeted to a location devoid of single unit signals and near the area of interest (in the corpus callosum or its close proximity) and was used as a reference.

Single-unit data were pre-processed with KlustaKwik [Harris et al., 2000] for automated spike clustering. Spike sorting results were manually refined using Klusters [Hazan et al., 2006]. Mouse position and orientation on the maze were extracted from video footage (using the full animal silhouette as filmed by a camera placed directly on top of the maze) with Ethovision XT image analysis software (Noldus, Wageningen, The Netherlands), which was synchronized with the electrophysiology data acquisition system. All data used for analysis were from periods in which the animal was moving at speeds exceeding 3 cm/sec. Unless otherwise indicated, the bin size used was 4 cm.
**Neuron Classification.** Clusters with more than 0.5% spikes during the first 2 msec of the interspike interval (refractory period), or a firing rate during the run period of the recording session lower than 0.25 Hz were excluded from analysis. The remaining clusters were separated in putative interneurons and pyramidal neurons, using a fuzzy-clustering algorithm (see: Fuzzy Clustering and Data Analysis Toolbox, http://www.fmt.vein.hu/softcomp/clusttoolbox [Bezdek, 1981], based on the firing rate, the mean of the autocorrelogram and the initial slope of valley decay (ISVD). The ISVD was calculated as follows:

\[
ISVD = -100 \times \frac{V_v - V_{0.26}}{A_{PV}},
\]

where \(V_v\) is the most negative value (valley point) of the spike waveform, \(V_{0.26}\) the voltage at 0.26 msec after \(V_v\), and \(A_{PV}\) the peak to valley amplitude [Lansink et al., 2010]. Only neurons that were included in the pyramidal or the interneuron cluster with more than 70% certainty of belonging to one of them, were used for analysis.

**Place field analysis.** Position on the circular track was linearized and place field maps were constructed by dividing the number of spikes falling within a 4 cm bin along the track by the total time spent within each bin. The resulting firing map was smoothed using an eight cm moving window. Place fields were defined as the spatial regions where firing exceeded a threshold of 1/3 of the maximum firing rate. Place fields larger than one bin were included in the analysis. Since place cells are known to fire differently depending on running direction [McNaughton et al., 1983], clockwise and counterclockwise runs were analyzed separately. All analyses were repeated using a smaller bin size (2cm), which yielded similar results. Unless stated otherwise, normalized distance was calculated as distance from departure divided by the maximum distance recorded in that session.

**Trial-by-Trial analysis.** For each cell, trials with less than 5 spikes were excluded from analysis. To investigate how place fields evolve across a session (Fig. 5.5), the session-wide place fields were computed as explained above. Similarly, the trial-by-trial place fields were found, and
those intersecting a session-wide place field were retained for analysis.

Population analysis. For the construction of population firing rate maps (Figure Fig. 5.6a), the linearized firing maps in both clock- and counterclockwise runs were stacked and sorted according to the temporal order of the bin of maximum firing of each cell in counter clockwise runs. This population matrix was then used to calculate the spatial matrix of crosscorrelations (Fig. 5.6b): for each bin i, the population vector for runs in one direction was taken and correlated (using Pearson’s correlation coefficients) with the population vector of bin i of runs in the opposite direction. Bins on the positive diagonal correspond to bins situated at the same distance relative to the stop point reached from the opposite running direction, while bins on the negative diagonal correspond to bins situated at the same physical location.

The crosscorrelation of the population vector as a function of distance (Fig. 5.6d) was calculated as the Pearson’s correlation coefficient of the population vector in one bin with all other bins, for both the ‘same-distance’ condition (population matrix for clock- and counter clockwise trials calculated in the running direction) and the ”same (physical) location” condition (population matrix in clockwise runs mirrored, such that the same bin corresponds to the same physical location in both directions). In the case of same-distance, negative lags correspond to shorter distances from departure in clockwise, as compared to counterclockwise, runs and in the case of same-location, positions occupied in clockwise runs preceding those in counterclockwise runs.

5.4 Results

NR1-KO single-trial place fields have similar size as control fields

We recorded the activity of 405 cells from CTR and 386 cells from NR1-KO mice. After exclusion of units that did not fulfill the criteria (see Methods and Materials), we clustered units into putative pyramidal neurons (n = 273 and 242, for CTR (n=4) and NR1-KO (n=4) mice, respectively; 10 CTR pyramidal neurons were further excluded from analysis for not having enough spikes on the track; except otherwise mentioned,
Figure 5.1: NR1-KO single-trial place fields have similar size to controls. 
(a) Clockwise (left) and counter-clockwise (right) place field examples of a CTR (top) and a NR1-KO (bottom) place cell. Numbers at bottom right are maximal firing rates. 
(b) Example place fields of a CTR (left) and a NR1-KO place cell. Rows show the linearly binned firing rate for each trial. Only trials running in the same direction are shown. Hot colors indicate the firing rate normalized to that trial’s maximum (color bar as in A). Gray curve depicts the session’s average.
(c) Place field size computed for the entire session: NR1-KO place cells have larger place fields (t-test: ***: P < 0.001).
(d) Trial-by-trial place field size: no difference between genotypes was found (t-test n.s.).

263 CTR and 242 NR1-KO pyramidal neurons were included for analysis) and interneurons (n = 32 and 42; see Methods and Materials). As expected, most pyramidal neurons showed place-related activity across the entire session (68.4% of CTR and 52.9% of NR1-KO cells had two or less place fields). In agreement with previous results [McHugh et al., 1996], NR1-KO cells showed a lower spatial resolution, with place fields of increased size (Fig. 5.1c, t-test: P < 0.001) and more place fields per place cell (not shown; mean ± SEM: CTR = 2.4±0.06, NR1-KO = 2.8±0.07, t-test: P < 0.001). When analyzing place field sizes on a trial-by-trial basis, however, we found no difference between genotypes (Fig. 5.1d, t-test, n.s.).
This suggests that the increased session place field size, observed in NR1-KO mice, is a consequence of between-trial variability: if a given cell fires with the same spatial precision on each run through its place field, but less reliably at the same place across trials, the resulting session place field will be broader. Differences in locomotion speed could
influence such parameters as place field size. NR1-KO mice did show a higher per-trial average speed (CTR: 14.1 $\pm$ 0.17 cm/sec vs NR1-KO: 15.8 $\pm$ 0.17 cm/sec; t-test: $P < 0.01$). Speed may influence place field size by increasing place cell average activity and the frequency of theta oscillations: because the limits of a place field will be determined by the completion of a theta phase precession cycle (around 1 theta cycle), higher speeds should lead to smaller place fields [Geisler et al., 2007]. To exclude the influence of speed, we repeated the per trial place field size analysis using only trials with an average speed within the 20 and 80 percentiles of CTR mice, which did not change the results presented in Figure 5.1 (place field size: CTR: mean $\pm$ SEM: CTR = 41.8 $\pm$1.02, NR1-KO = 43.9 $\pm$ 0.9, t-test: n.s.).
Figure 5.2: NR1-KO place cells display more spatial jitter than control cells
(a) Example place field of a CTR (top, mean firing rate = 1.98Hz) and a NR1-KO (bottom, mean firing rate = 1.88Hz) place cell across all trials of a session, in the same direction. (layout as in fig.1B). Gray curve depicts the session’s average. The bin of maximum firing rate in each trial is marked with a blue dot. The jitter across trials can be gauged from the left- and rightward shifts of the blue line.
(b) Distribution of the average jitter between bin of maximum firing rate of each trial with respect to the session average per place cell (t-test: \( P < 0.001 \)).
(c) Distribution of the average Pearson’s correlation coefficient between each trial’s firing map and the session average per place cell (t-test: \( P < 0.001 \)).
(d) Average jitter across trials: NR1-KO cells showed a significant decrease in jitter along the course of a session (linear regression: * \( P < 0.05 \)). CTR showed a similar trend, which was however not significant. (ANOVA: \( P(\text{genotype}) < 0.001 \), \( P(\text{trial}) < 0.005 \), \( P(\text{interaction}) \text{ n.s.} \)). Jitter values above 50cm were removed from analysis to exclude spurious observations.
(e) Average Pearson’s correlation across trials: both genotypes showed an increase along the course of a session (linear regression: * \( P < 0.05 \), ** \( P < 0.01 \); ANOVA: \( P(\text{genotype}) < 0.001 \), \( P(\text{trial}) < 0.001 \), \( P(\text{interaction}) \text{ n.s.} \)).

Increased variability in NR1-KO spatial representations between trials

To test the hypothesis brought forward in the previous section, we calculated the absolute value of the distance between the bin of maximum firing rate in each trial to that of the session average, yielding a "jitter" value for each trial and place cell (Fig. 5.2b). NR1-KO place cells showed significantly higher jitter than CTR (t-test: \( P < 0.01 \)). We also conducted a similar analysis by calculating the Pearson’s correlation between each trial’s spatial firing map and the session average (Fig. 5.2c). This analysis has the advantage of taking the entire firing map into account rather than only the location of peak firing. In agreement with the "jitter" results, we found that NR1-KO place fields showed a decreased correlation (t-test: \( P < 0.01 \)), further supporting the hypothesis that the increase in session place field size is a consequence of increased inter-trial variability.

The examples in Figure 5.2c seem to suggest that the jitter decreases
as the session progresses. Hence, we averaged the jitter per trial across all sessions (Fig. 5.2d): both genotypes showed a negative trend, which however reached significance only for NR1-KO (simple linear regression (SLR): $P < 0.05$). There was, in addition, a genotype effect (ANOVA $P < 0.001$), as jitter was lower for CTR (Fig. 5.2b). Similarly, we averaged Pearson’s R along trials and across sessions (Fig. 5.2e). Both genotypes showed a significant increase in the correlation along a session (SLR, CTR: * $P < 0.05$, KO: ** $P < 0.01$; ANOVA: $P$ (genotype) $< 0.001$). These results show that in both cases, trial-by-trial variability decreases with trial or experience on the track within a session. This effect was more salient in NR1-KOs, as suggested by the jitter and Pearson’s correlation measures, suggesting that CTR place cells may reach an asymptotic value earlier on. We correlated the jitter values between pairs of simultaneously recorded neurons across trials, to investigate whether if the deviation of place field firing reflects individual processing errors, or rather network errors. We found that, in both genotypes, there was a weak positive, but significant correlation between jitter values, favoring the latter possibility (data not shown, average Pearson’s R, mean±sem: CTR = 0.026 ±0.005, NR1 − KO = 0.022±0.005; One-sample t-test: $P < 0.001$ in both cases).

**NR1-KO mice have normal ‘phase precession-bound’ place fields**

Theta rhythm, a prominent oscillation in the hippocampus in the active rodent [Vanderwolf, 1969], has a strict relationship with place cell firing: spikes tend to occur in the trough of the oscillation, but during a place field crossing, they will gradually advance their firing phase (‘phase precession’ [O’Keefe and Recce, 1993]). This process has also been used to define a place field as being the distance necessary for a cell to complete a phase precession cycle [Maurer et al., 2006]. If NR1-KO place cells have normal place fields and it is the across-trials variability that underlies impaired spatial representation, than spikes fired by NR1-KO PCs, within its field, should show a similar amount of theta phase precession as CTR PCs. We analyzed, therefore, phase precession at the single-trial level. Phase precession was robust in both genotypes (Fig. 5.3a, examples of single trial phase precession slopes for a CTR (top) and a NR1-KO (bottom) PC). We calculated three parameters related
to phase precession: slope (Fig. 5.3b - the rate of precession), phase precession range (Fig. 5.3c - the amount of precession) and the distance covered (Fig. 5.3d - distance between first and last spike). In all three measures, NR1-KO PCs showed similar values to CTR PCs (t-test, n.s.). These results further support our claim that NR1-KO PCs show normal, 'unitary' place fields.

![Figure 5.3](image_url)

Figure 5.3: NR1-KO mice have normal 'phase precession-bound' place fields.

(a) Examples of a CTR (left) and NR1-KO (right) place cell. Phase precession events are represented as the phase advancement (normalized to first spike phase) over the spike (distance) range for each trial (left Y-axis). The session-wide place field is depicted in the light gray curve (right Y-axis).

(b) Slope of phase precession. There was no difference between genotypes (t-test, n.s.).

(c) Phase range of a precession event. There was no difference between genotypes (t-test, n.s.).

(d) Distance covered between the first and last spike of a phase precession event. There was no difference between genotypes (t-test, n.s.); a phase precession event was detected as a minimum of 4 spikes occurring within a single-trial place field.
**NR1-KO spatial representation improves with experience**

To quantify how much a firing map changes within a session, we split the session into two halves and calculated, as a stability index, the Pearson’s R between the two firing maps (Fig. 5.4a). NR1-KO place fields showed lower stability than CTRs (KS-test: $P < 0.001$, t-test: $P < 0.001$). Next we calculated the number of trials needed for a place cell to acquire a stable place field. First, we constructed, for a designated trial, the firing map using all trials up until the designated one and calculated the Pearson’s R with the overall session’s firing map. To demonstrate that this effect does not depend on an arbitrary split of the trials in two halves, we used a variable threshold for the Pearson’s R (between 0 and 1), and then calculated the number of trials it took the place cell to reach that threshold. NR1-KO place cells took significantly more trials to stabilize their place fields for thresholds at or above 0.45 (Fig. 5.4b; ANOVA: $P($genotype$) < 0.001$). The stability index in NR1-KO mice improved with familiarity to the circular maze across sessions (Fig. 5.4c, NR1-KO: Spearman’s Rho $= 0.12$, $P < 0.01$; Fisher’s test between Spearman’s Rho for NR1-KO versus CTRs: $P < 0.05$), showing that spatial representations are less subject to change within a session in later training days. If NR1-KO place fields become more stable as sessions progress, increased familiarity with the environment is expected to lead to a more stable spatial representation. To address this question, we compared two place field parameters, size and spatial information, across the two first and two last (sessions 9 and 10) sessions of the recording protocol (Fig. 5.4d, # units: CTR $= 67$ and NR1-KO $= 46$). In both cases, NR1-KO mice were impaired, showing a larger place field size and lower spatial information (Post-Hoc $P < 0.05$) than CTR mice in the first two sessions. Towards the end of the recording, however, NR1-KO mice leveled with CTR mice (for place field size and spatial information: ANOVA, $P($genotype$) < 0.05$, $P($session$) < 0.05$, $P($interaction$)$ n.s.). These results confirm the above-described results, showing that NR1-KO place fields become more consistent with experience on the track. CTR mice also showed a very mild (non-significant) decrease in place field size and increase in spatial information (therefore the lack of an interaction effect in the ANOVA).
Figure 5.4: NR1-KO spatial representation improves with experience.
(a) Stability index, calculated as the Pearson’s correlation between the firing map of a given place cell during the 1st and the 2nd half of a session. NR1-KO cells showed a lower stability index (KS-test, $P < 0.001$, t-test, $P < 0.001$).
(b) Number of trials necessary for the cumulative place field map to cross a Pearson’s R-based threshold-similarity value to the PF of the entire session (ANOVA: $P$(geno) < 0.0001; * Post-Hoc: $P < 0.05$).
(c) Stability index per session: NR1-KO showed a significant positive Spearman’s correlation between stability index ($R$) and session (Spearman’s $r$: $r = 0.12$, $P < 0.001$; CTR: n.s.; Fisher’s test between correlations: $P < 0.05$).
(d) Place field size (left) and Spatial information (right) calculated over the first and last two sessions (sessions 9 and 10) in NR1-KO mice were reduced in the former and increased in the latter, to level with the values of CTR (Post-Hoc $P < 0.05$).

NR1-KO place cells fail to undergo experience-dependent changes

In the previous analysis we focused on differences in the accuracy of place field firing and in the similarity of firing across trials. But place fields are plastic and change over the first runs on a track, showing an expansion that is backwards relative to the direction of locomotion [Mehta et al., 1997, 2000]. We therefore looked at three parameters which define a place field and analyzed how they change over the course of a session: CTRs showed an increase in the maximum in-field firing rate (Fig. 5.5a, simple linear regression (SLR): $P < 0.01$; ANCOVA: $P$(genotype) < 0.001, $P$(trial) < 0.001, $P$(interaction) < 0.001), an increase in place field size (Fig. 5.5b, SLR: $P < 0.05$; ANCOVA: $P$(genotype) < 0.001, $P$(trial) < 0.001, $P$(interaction) = 0.1) and a displacement of the place field’s Center Of Mass (COM), towards the point of departure (Fig. 5.5c, SLR: $P < 0.005$; ANCOVA: $P$(genotype) < 0.001, $P$(trial) < 0.001, $P$(interaction) < 0.001).
P(interaction) = 0.1). NR1-KOs showed a similar trend in all three measures, but failed to reach significance. We also looked at the skewness of the place fields: place fields tend to show a negative skewness [Mehta et al., 2000], that is, to have most spikes concentrated at the end of the place field. Session place fields of CTR mice were more negatively skewed (Fig. 5.5d, t-test: P < 0.05). These results highlight how the shape of the place field of CTR mice changes as a function of experience within a session, as previously described [Mehta et al., 2000], adopting an asymmetrical shape. Remarkably, NR-1 KO mice failed to generate such plasticity of place field shape.

Figure 5.5: NR1-KO place cells fail to undergo experience-dependent changes.
(a) In-Field maximum firing rate per trial normalized to the first trial. CTRs showed an increase over trials (linear regression, CTR: ** P < 0.01, NR1-KO n.s.; ANCOVA: P(genotype) < 0.001, P(trial) < 0.001, P(interaction) < 0.001).
(b) Place field size per trial normalized to the first trial. CTRs showed an increase over trials (linear regression, CTR: * P < 0.05, NR1-KO n.s.; ANCOVA: P(genotype) < 0.001, P(trial) < 0.001, P(interaction) = 0.1).
(c) COM shift per trial. CTRs showed a precession of the COM over trials (linear regression, *** P < 0.005; ANCOVA: P(genotype) < 0.001, P(trial) < 0.001, P(interaction) = 0.1).
(d) Average skewness of the session’s place field: NR1-KOs had a less negative skewness (t-test, * P < 0.05).
NR1-KO place fields in the two running direction are less precisely aligned to both physical location and distance run from departure point

The collection of place fields spanned the entire circular track in both genotypes (Fig. 5.6a). This track tessellation reveals additional structure, most strikingly in CTR, where a mirror contour of the place field map is obvious in the clockwise trials. ‘Bidirectional’ place fields in this display represent place cells [Battaglia et al., 2004] that have place fields located at the same physical location in both clock- and counter clockwise runs. 'Same-distance' place cells have fields at the same distance traveled from departure point in the two directions. To analyze how firing is influenced by location and distance run, we constructed a spatial correlation matrix of the population vectors at each spatial bin in both running directions (Fig. 5.6b). In this display, bins falling on the positive diagonal represent path integration-supported (reset at each departure) firing ('same distance place fields'), while those falling on the negative diagonal represent firing primarily guided by environmental cues ('same-location'). Same-location spatial representations predominated in both genotypes, but were weaker in NR1-KOs than CTRs. To quantify this, we calculated the correlation between the population matrices (Fig. 5.6c) in both directions (for the same-location condition, the clockwise population matrix was mirrored): same-location and same-distance maps showed a stronger correlation in CTRs than KOs (Fisher’s test between correlations: $P < 0.01$). In both genotypes, same-location mapping was, indeed, more salient than same-distance mapping (Fisher’s test between correlations: $P < 0.01$), indicating that place cells are more likely to be anchored to distal cues, rather than relying on path integration. All four correlation values exceeded the value taken from a shuffled condition (calculated as the correlation between a stacked place field matrix in one direction and the stacked place field matrix in the opposite direction (mirrored, in the same-location case) with shuffled rows; mean $+ 2$STD was taken as the upper bound of the shuffled distribution).

Previous studies have shown that bidirectional place cells fire earlier in runs in one direction, as compared to the opposite direction, an effect attributed to prospective coding [Battaglia et al., 2004, Resnik et al., 2012]. We looked at this by calculating the Pearson’s correlation for
each bin in one direction with bins at a given lag with respect to the corresponding bin in the opposite direction for the same-distance (Fig. 5.6d top) and same-location (Fig. 5.6d bottom) conditions. We observed a similar effect as previously described, with the correlation peaking at negative lags, i.e., at shorter distances from departure (same-distance condition) and at earlier locations (same-location condition), when mice ran in one direction, compared to running in the opposite direction. The effect was most salient in CTRs and in both genotypes was most striking in the same-location condition. In the latter case, NR1-KOs did not show a clear peak, nor a difference in the Pearson’s R values between positive and negative lags (t-test (NR1-KO) R(pos) vs R(neg): n.s.; t-test (CTR) in both conditions and t-test (NR1-KO) in same-location condition: P < 10^-5). The differences between genotypes suggest a deficit in anticipatory firing upon place field approach in NR1-KO mice. For the
Figure 5.6: NR1-KO place fields follow both path-integration and allocentric frameworks less precisely.

(a) Distribution of place fields along the two running directions. Each row displays the linearized firing map of a neuron, computed over the entire session and normalized to its maximum. Neurons were aligned according to the bin of maximum firing in the counterclockwise direction.

(b) Spatial bin-by-bin correlation. Bins at the same physical location in both directions (‘same-location’ condition) are located along the negative diagonal, while bins representing distance from departure are located along the positive diagonal (‘same-distance’ condition left bottom panel). CTR mice (top) show areas of high correlation for both cases, while in NR1-KO (bottom) the pattern was much more blurred.

(c) Pearson’s correlation between counter-clockwise and clockwise (same-distance) or mirrored clockwise (same-location) stacked firing map matrix (see A). In both cases, CTR showed a higher correlation than NR1-KO (Fisher’s test between correlations: ***$P < 0.001$). Dashed lines show the significance level, calculated as the mean + 2STD of a shuffled dataset. In addition, the correlation values of both genotypes were higher in the same-location than in the same-distance condition (Fisher’s test between correlations: ***$P < 0.001$).

(d) Average population vector correlation (see Experimental Procedures) as a function of normalized distance for the firing maps in the same-distance (top) and same-location (bottom) conditions. In the same-distance condition for CTR mice and in the same-location condition for both genotypes, correlation in the negative lags, that is, distance (location) preceding the distance (location) in the reference run, was higher than in the positive ones (t-test negative vs positive lags: $P < 10^{-5}$).

’same-distance’ condition, the trajectory used as a reference will determine whether the peak occurs in negative or positive lags. Importantly, in all cases, the first trial was a counter-clockwise run, so that the clockwise run was the “return” run, suggesting that this effect may indeed reflect prospective coding of distance ran.

Higher density of place fields near goal location

The collection of place fields spanned the entire track, but they showed an unequal distribution: there was a higher density of place fields near the goal location (Fig. 5.7a): CTR place cells showed a significant
increase in the number of place fields near the end of the track, but a decrease in the last bin, near the goal (Binomial test (expected value: 1 / #bins) corrected for multiple comparisons: P < 0.05), indicating a tendency for a denser representation with place fields at goal-site approach. NR1-KO, in addition, showed an increase also near the departure point, balanced by a decrease of the fraction of place fields with a COM around the center of the running track (Binomial-test, corrected for multiple comparisons, P < 0.05). Place fields occurring at the very beginning or very end of the track may encode a framework relying more on path integration and/or local cues such as the wall marking the stopping and

Figure 5.7: Higher density of place fields near the goal location on the track.
(a) Histogram of the distribution of the location of a place field’s COM along the track. There was no significant difference in the overall distribution between genotypes (KS-test n.s.); arrows indicate bins significantly higher (upward arrows) or lower (downward arrows) than chance level (0.1). Both genotypes showed an increase in the place field proportion at locations close to arrival at the goal site. NR1-KOs, in addition, also showed a significant increase at locations traversed just after departure, and a decrease at the center of the track (Binomial test (expected value: 1 / #bins = 0.1) corrected for multiple comparisons.
(b) Place field size as a function of location of the place field’s COM. Place fields with a COM close to the center of the track were larger (ANOVA: P(geno) n.s., P(COM) < 0.001, P(interaction) < 0.05). Place fields with edges in the first or last bin of the track were excluded from analysis.
departure point [Gothard et al., 1996b], rather than on salient distal cues in the environment. We, therefore, wondered whether place fields of KO and CTR mice would be different as a function of distance to goal location (Fig. 5.7b). Indeed, both in CTR and NR1-KO mice place fields with a COM at the departure or arrival site were smaller in size compared to place fields situated at the center of the track, but we found no difference between genotypes (ANOVA: \(P(\text{genotype}) \text{ n.s.}, \ P(\text{COM}) < 0.001, \ P(\text{interaction}) < 0.05\)). The proximity to departure and goal sites thus correlates with greater place cell precision. Interestingly, in both genotypes, the distribution was bimodal, with place field size peaking just before and after the center of the maze. Although not significant, this trend was present in both genotypes.

5.5 Discussion

It is widely accepted that the lack of the NMDA receptor leads to impaired spatial representation [McHugh et al., 1996, Mehta et al., 2000, Ekstrom et al., 2001], but the nature of this deficit is not clear. By doing an in-depth single-trial analysis of place cell firing in NR1-KO mice, we show that these animals have 'unitary' (i.e., from a single crossing) place fields of normal size and that the larger, session-wide place fields are, rather, a consequence of increased across-trial firing variability. While CTR place cells underwent changes over the course of a session, in their maximum firing rate and position of COM, NR1-KO cells did not, showing that the lack of the NMDAR impairs some aspects of neural plasticity.

Genetic deletion of NMDARs in hippocampal area CA1 [McHugh et al., 1996], but also other manipulations affecting hippocampal function [Kentrys et al., 1998, Brun et al., 2008, Hussain et al., 2011], have been reported to result in an increase in place field size. We reproduced such an increase in our experiments, but only when the place field was defined over the entire session: at the single-trial level PFs in KO mice had a similar size as in CTR mice. This raises the possibility that the increased place field size is a consequence of inter-trial variability, rather than larger "unitary" place fields: a place cell that fails to fire precisely at the same
location over trials will cumulatively show a broader place field.
In each run through a place field, a unique set of inputs providing spatial
(sensory and self-motion) information will define that place cell’s firing.
This process appears to be normal in NR1-KO mice, which suggests that
the higher between-trial variability is due to a failure to properly inte-
grade this information with other inputs arriving to area CA1. Spatial
and non-spatial (e.g. object-related) inputs are thought to be relayed
to CA1 by the medial and lateral EC, respectively [Witter and Moser,
2006]. The other main structure projecting to the CA1 is area CA3,
which is crucial for one-trial learning and memory retrieval [Nakazawa
et al., 2002]. The impairments may be due to a failure of NR1-KO mice
to properly integrate inputs from both streams [Cabral et al., 2014], as it
has been suggested that the development of a stable spatial representa-
tion in CA1 requires the reconciliation of CA3 and EC inputs [Carr and
Frank, 2012]. CA3 is characterized by a dense recurrent network and is
crucially involved in one-time learning processes [Nakazawa et al., 2003]
and in the formation and retrieval of spatial memory [Jensen and Lisman,
1996]. The EC, on the other hand, has a more "real-time" role, providing
the CA1 with information about the current environment. In this sense,
NR1-KO mice may not be able of properly storing the precise location of
firing upon a place field crossing into a stable spatial representation and,
therefore, fail to properly match the current place field to an existent
spatial representation [Lee and Kesner, 2004].
Despite the significantly higher running speed of NR1-KO mice, this
difference was likely too small to explain the smaller place field size on a
trial-by-trial basis. Indeed, the effect reported by Geisler et al. (2007),
which shows that theta frequency increases with speed, thereby accelerat-
ing the rate of phase precession, which, likely, determines the boundaries
of a place field, depended on differences in average running speed of the
order of 24 cm/sec, well above the difference in our study (<2cm/sec).
Furthermore, we replicated the same results for the place field sizes using
only running periods falling within the same velocity range (between the
20th and 80th percentiles of the velocity of CTR mice).
The inter-trial variability decreased over the course of the session in both
genotypes and the decrease was present throughout recordings (data not
shown; see also Mehta et al. (1997, 2000) and Lee et al. (2004)), sug-
sugging that multiple exposures to the track are required for the formation of a stable spatial representation. Indeed, in rats, the CA1, but not CA3, network undergoes gradual changes over the course of several days of track exposure, expressed as a decrease in population firing rate and in percentage of active cells [Karlsson and Frank, 2008]. CA3 neurons, on the other hand, are deemed crucial for one-trial learning and pattern completion [Nakazawa et al., 2003] and may, therefore, assist CA1 in its development of a stable place field map. NR1-KO mice showed lower stability within a session, but the stability index increased over sessions, such that their place fields leveled those of CTR in two key place field quality measures in the last sessions: size and spatial information. This effect may be due to either CA1 NMDAR-independent plasticity [Moosmang et al., 2005] or CA1 spatial representations being increasingly driven by representations in CA3, which is spared from the mutation, as training proceeds. By lesioning direct EC projections to area CA1, Brun et al. (2008), showed that, in a novel environment, CA1 place cells were still capable of developing normal place fields with extended experience, suggesting that the contribution of Schaffer Collateral inputs to CA1 place cells may evolve with prolonged, multi-session exposure to the track and, thereby, assist in the improvements in spatial representation seen in NR1-KO mice. Hussaini et al. (2011) showed, previously, an increase in spatial memory in HCN1 KO mice, despite also showing larger (session-wide) place fields. Crucially, they also report increased place field stability, which is probably an essential process underlying spatial memory. This suggests that the decreased stability we observe in our NR1-KO mice may be, in part, responsible to the deficits in spatial memory present in these mice [Rondi-Reig et al., 2006, Cabral et al., 2014].

The theta rhythm endows place cells with a temporal structure and is thought to be a crucial process for synaptic plasticity [Skaggs et al., 1996]. As we had previously reported [Cabral et al., 2014], NR1-KO mice showed intact phase precession during normal navigation. The rate of precession was similar to CTR mice and the distance covered by the animal between the first and last spike of a phase precession event was also the same, suggesting that a place field may be bounded by a fixed
amount of theta phase precession of spikes as the animal runs through its place field [Maurer et al., 2006].

Mehta et al. (1997, 2000) first showed that place fields (in rats) are asymmetrical, exhibiting a shape negatively skewed, which develops gradually over trials. We found, similarly, that place fields of both genotypes were negatively skewed, but do not show a gradual skewness increase (data not shown). They did, however, show a progressive increase in size, maximum firing rate and negative shift in the lap-based place field COM. Lee et al. (2004) reported that COM shifts can occur over the session in the absence of skewness changes, suggesting that a backward shift of the place field’s COM is not necessarily accompanied by the development of negative skewness and that the former parameter may be a more reliable measure of PF plasticity. The backward shift of a place field is NMDA-dependent [Ekstrom et al., 2001]. Mehta et al proposed that LTP/LTD in CA1 could induce the PF changes, rendering them with an asymmetric shape and their model predicts that blocked of NMDA should abolish the negative skewness. Here we show that impaired NMDA functioning in CA1 does, indeed, affect skewness of PFs, although it does not abolish it completely, suggesting that this receptor is not the sole responsible for this plastic change to PFs.

Some place cells exhibit a different firing pattern according to the direction of movement [McNaughton et al., 1983]. Other place cells, however, may fire at the same location of the environment regardless of movement direction (bidirectional place cells [Battaglia et al., 2004]), or at similar distances from the departure point, when running in both directions [Mizuseki et al., 2012]. Our results were predominated by the same-location cells, but both types were present. As in previous studies in rats [Battaglia et al., 2004, Resnik et al., 2012], firing in runs in one direction occurred at earlier moments than in the opposite direction, suggestive of prospective coding, which may signal to the animal it is approaching a particular point in space. NR1-KOs, despite also having same-location and same-distance place cells, showed a much weaker consistency in same-location and same-distance mapping than CTRs, a result similar to that reported by Resnik et al. (2012), who recorded from GluA1 mice, which
lack this subunit of the AMPA receptor and show both impaired synaptic transmission and long-term plasticity. These results highlight the importance of long-term plasticity for prospective coding mechanisms.

CTR place cells showed an increased probability of having a place field near the goal location. This may be a reflection of non-spatial factors acting on place fields: the added motivational value of this location, signaling the upcoming delivery of reward, makes it more susceptible to having a place field [Hollup et al., 2001, Lansink et al., 2012, Ziv et al., 2013], which may improve reward-driven behavior [Dupret et al., 2010]. We did not, however, find a gradual increase in the proportion of place fields near the end of the track across the sessions (data not shown), as has been described previously [Dupret et al., 2010], possibly due to the simplicity of our task, which had no explicit learning component. In such a situation, the development of place fields around the goal-site may take place immediately, in the first few trials. In addition NR1-KO mice showed a predominance of place fields near the departure point. At these departure and arrival sites, place fields were smaller in size, which suggests that the salience and reward-predictive value of local cues allow encoding of place fields with increased precision, also in NR1-KOs.

This set of results shows that NR1-KO animals are capable of producing spatially restricted firing of place cells carrying information about the layout of the environment and path integration, not unlike that of CTR mice (as shown by the normal single-trial place fields). We suggest that the building of a coherent, stable spatial representation in the CA1 is hindered in NR1-KOs due to a failure to consolidate single-trial information into a long-lasting representation that is stable across trials. This failure may be caused by the lack of plasticity at the Schaffer Collaterals, likely involved in the storage of a spatial memory trace [Gruart et al., 2006, Madroñal et al., 2010, Nakazawa et al., 2004]. In normal conditions, the formation of a stable spatial memory in CA1 and its integration with spatial and non-spatial inputs conveyed by the EC, may mask inherent noise that the latter signals are subjected to, caused by trial-by-trial variations in neural responses to the same set of stimuli [Deneve et al., 2001]. This may suggest that, in addition to the consequences of the lack of plasticity
in the Schaffer Collateral synapses in NR1-KO mice, CA1 spatial representation may be more susceptible to the influence of noise, as caused by e.g. incomplete sensory sampling of environmental cues, introducing the across-trial jitter we report.

With extensive experience on the track, NR1- KO mice showed a reduction in deficits, such that by the end of the recordings their place fields were similar in quality as those of CTRs, as illustrated by parameters such as size and spatial information per spike, suggesting that prolonged experience may facilitate the establishment of a proper spatial representation in CA1 in NR1-KO, possibly reflecting increased influence from the CA3 network with its intact NMDARs. An alternative account of this finding may depend on adaptive changes already at the level of EC inputs (an hypothesis needing direct experimental testing).

Besides disruption of plasticity, NMDAR deletion causes also changes in neural circuit dynamics affecting persistent (Wang 1999) as well as oscillatory activity [Korotkova et al., 2010, Middleton et al., 2008, van Wingerden et al., 2012, Cabral et al., 2014]. These modifications may also contribute to disrupted communication between CA1 and its afferent structures (which depends on gamma oscillations; [Colgin et al., 2009, Montgomery and Buzsáki, 2007] and may disturb integration of memory and sensory inputs in CA1 [Cabral et al., 2014]. These results reinforce the importance of single-trial based analyses, to obtain a complete dynamical picture of place cell activity, as pooling multiple trials together may cache some effects of the manipulation being tested (see, e.g., single-trial phase precession studies [Schmidt et al., 2009, Kempter et al., 2012, Reifenstein et al., 2012]).
Chapter 6

General discussion

In this study, we have assessed neural patterns of activity in the CA1 region of the dorsal hippocampus (HPC) in mice learning a task requiring the integration and processing of different information streams: external (sensory and spatial) and internal (spatial sequence memory) inputs. This task, the starmaze [Rondi-Reig et al., 2006], allows, by changing the departure point in a given trial, to separate these different information streams, thus enabling the analysis of each of them individually. Specifically, the starmaze task may be solved by either learning a route in an environment using the available cues (place-based navigation) or by switching to an ‘internal’ mode, where navigation is based on the repetition of a sequence of body turns. In this latter case, rather than path-integration supporting navigation (which “resets” at each intersection [Derdikman et al., 2009, Royer et al., 2010]), it is likely that this sequence is founded on the intersections of the maze, which may act as discrete events composing the sequence. The HPC has long been attributed a crucial role in supporting place-based navigation, a role in which it enters an ‘externally-oriented’ mode, processing spatial and sensory information [O’Keefe and Dostrovsky, 1971, Packard and McGaugh, 1996, McNaughton et al., 2006], but only more recently has it been associated with the learning of a sequence of body turns [Rondi-Reig et al., 2006]. Earlier studies [Packard and McGaugh, 1996] had shown that the HPC is not involved in the acquisition of a simple egocentric strategy, where, e.g. a single body turn suffices to reach a goal. However, when reaching the goal involves the linking of multiple motor responses, the HPC, in agreement with its role in encoding and retrieving information
about complex events with a common context, of a temporal structure [Kesner et al., 2004], appears to be fundamental for the expression of the sequence of responses [Rondi-Reig et al., 2006].

In addition, we sought to investigate the functional role of the NMDA receptor of the CA1 region of the hippocampus. We recorded neuronal activity in mice with a spatially restricted mutation, which lack the receptor specifically in this region. The NMDAR plays a crucial role in synaptic plasticity [Bliss and Collingridge, 1993, Tsien et al., 1996] (but also in regular synaptic transmission at depolarized membrane potentials, due to its contribution to the slow EPSP component [Wang, 1999]) and the lack of NMDAR function in the HPC impairs spatial learning [Morris et al., 1986, McHugh et al., 1996, Rondi-Reig et al., 2006]. In what way the absence of NMDAR function leads to the observed behavioral impairments is, however, unknown, but, given its role in synaptic plasticity and oscillatory dynamics [Whittington et al., 1995, Middleton et al., 2008, Korotkova et al., 2010], deficits at both the single-neuron and at the network level are to be expected.

A technological development that was key to the work in this thesis was the application of an electrophysiological technique to monitor activity of large groups of neurons and the network in mice (with tetrodes [Battaglia et al., 2009]). This was combined with the use of a highly specific mutant mouse line (NR1-KO mice [Tsien et al., 1996]) and a complex behavioral paradigm. In this way we could link, in detail, behavioral impairments and neural processing deficits resulting from the mutation, as well as establish detailed correlates between specific behavioral states and neural patterns.

I will now summarize, for each chapter, the most relevant results, focusing first on behavior and single-cell correlates, followed by an analysis of network oscillations and its relation with single-cell activity and finish with basic, single-trial place cell properties in both genotypes, while mice ran a stereotypical circularmaze task, which allowed us to closely monitor the spatial representations by CA1 place cell populations.
CA1 place cells follow different frameworks during the use of different navigation strategies

Previous studies by our collaborators [Rondi-Reig et al., 2006] had shown that NR1-KO mice show impaired learning in the starmaze task. In our study, replicating basic behavioral findings by Rondi-Reig et al., we found some interesting differences too: despite showing an impaired initial learning of the task, NR1-KO mice leveled performance with CTR mice with extensive and more intensive training. While different factors may have contributed to this, we highlight here the transformation of the original watermaze-based task into an appetitive, land-based one and the increased number of trials, with shorter intertrial-intervals. Despite slower learning during pretraining, NR1-KO mice may have learned some task-related rules at this stage, which were positively transferred to the recording stage (where the maze had a different layout) [Bannerman et al., 1995]. NR1-KO mice showed similar performance indices and similar usage of the different navigation strategies as CTR mice, but they also displayed specific, relevant deficits: they were unable to learn the long route to the goal, when their behavior was guided by a sequence-based strategy (Fig. 2.4), an effect that could be narrowed down to an impairment in correctly performing the second main choice in this route, which suggests a disruption in linking multiple events in time. Interestingly, we found that place cells obey different frameworks, depending on the strategy being employed by the animal: during place-based navigation, place fields remain anchored to the environment, such that a given place cell continues firing in exactly the same physical location, while during sequence-based navigation, place fields become anchored to the departure, such that the place field will rotate along with the changed departure arm. In this situation, the animal may not acknowledge the different departure arm, disregarding the allothetic inputs: as a consequence, place fields reflect the expression of a sequence of body responses. This pattern of activity is consistent with a behavior which follows 'external', environment-based inputs during place-navigation and 'internal', memory-based inputs during sequence-based navigation. In agreement with the behavioral deficits observed, NR1-KO mice showed the heaviest and most striking impairments specifically during the use of the sequence-based navigation.
Interestingly, a subset of place cells showed a pattern of activity not consistent with the behavior expressed, e.g., a place cell that rotated its field along with the changed departure arm, but the mouse executed a place-strategy (Fig. 2.11). These may represent cases, in which the strategy was changed over the course of the run, maybe triggered by the sight of a salient cue.

**Low (23-40 Hz) and high (55-95 Hz) frequency oscillations predominate during sequence- and place-based navigation, respectively**

Underlying the processing of different information streams is the assumption that there is a differential contribution of different brain structures, depending on what information is processed or is more relevant. There are several structures supporting spatial representation in the HPC: the EC is thought to provide it with path-integration (via its medial portion [Hafting et al., 2005]) and sensory (via its lateral portion [Deshmukh and Knierim, 2011]) inputs, the parietal and prefrontal cortices may play an important role in strategy selection [Whitlock et al., 2008, Rich and Shapiro, 2009] and the peri- and post-rhinal cortices, which, respectively, provide the HPC with information regarding object representation and the spatial organization of objects [Norman and Eacott, 2005]. Even as we are limited to recording from a single brain area, analysis of local oscillatory patterns opens a window to cross-structural communication processes taking place [Fries, 2005, Schoffelen et al., 2005] and indicates which brain areas are contributing most to the formation of spatial representations in CA1: synchronization between brain structures is achieved by coherent oscillations between them and different types of oscillation may enable communication of a given area with different brain areas. Gamma oscillations may present such a case: these broadband oscillations (20-120Hz) have been subdivided into low (LG, about 25-40Hz) and high gamma (HG, about 55-100Hz) [Colgin et al., 2009] (or, alternatively, into low, medium and fast gamma [Belluscio et al., 2012]). They are all present in CA1 region and are nested into local theta oscillations, but different brain structures projecting to CA1 are involved in their generation [Colgin et al., 2009, Montgomery et al., 2008]: LG oscillations originate in CA3, while HG originates in the EC and it is thought that CA1, by coherently oscillating with one or the other frequency, may
"shift" its tuning to either of the two brain structures [Colgin et al., 2009, Middleton et al., 2008, Carr and Frank, 2012].

In chapter 3, we analyzed trial-dependent changes in EEG power, as well as modulation of higher frequency oscillations (>20Hz) by the theta rhythm. To quantify the relative contribution of the two gamma bands described above (LG and HG) during the use of different navigation strategies, we took the ratio between them. We found that, specifically during place-strategy trials, the ratio of LG/HG was decreased (Fig. 3.4), suggesting a stronger influence of HG oscillations during this type of trials. NR1-KO mice did not show a difference in the LG/HG ratio between trial types. Power in both gamma bands was strongly increased, compared to CTR mice (Fig. 3.2), suggesting that the network may be in a saturation mode, thus masking the influence of behavioral state on EEG power. We showed, in addition, that theta oscillations nest both LG and HG at different phases, with the latter preceding the former, confirming previous results in rats [Belluscio et al., 2012] and mice [Chen et al., 2011] (Fig. 3.5). The strength of the theta modulation of HG was increased during place-based navigation in CTRs, further adding to the LG/HG ratio results in suggesting a heavier role of HG oscillations in place-based probe trials. We finally asked ourselves whether the spiking activity of CA1 place cells during periods of LG and HG (which are mutually exclusive [Colgin et al., 2009]) varied and whether the spatial information conveyed from spiking activity during one or the other period contributed most to the overall spatial representation. Hence, we constructed firing maps during both periods for each type of probe trial and compared them with the spatial maps over all periods for training trials (Fig. 3.6). This analysis revealed that, in CTR mice, the map in sequence-strategy trials, conveyed during LG periods, accounts for most of the rotation effect place cells show during this type of navigation. The firing map conveyed during HG periods did not show differences, compared to the spatial maps over all periods for training trials, in both place- and sequence-based trials. NR1-KO place cells failed to show the selective increase in similarity of firing maps during LG periods in sequence-based trials and overall periods in training trials.

The distinction between LG and HG is not novel [Middleton et al., 2008, Colgin et al., 2009, Belluscio et al., 2012]. Our results add to these
studies by linking each of the gamma bands with a different behavioral state (as suggested in Carr et al. [Carr and Frank, 2012]) and offer strong support for a role in oscillations in mediating communication between different brain areas. Despite the predominance of either LG or HG oscillations in sequence- or place-strategy trials, respectively, neither oscillation ever subsides completely (Fig. 3.2), which suggests a continuous dialogue between CA1 and CA3 and EC during spatial navigation. It is the balance between both that determines the relative weight of the inputs carried by the different brain areas that support LG and HG in CA1. Previous pharmacological and transgenic studies showing increased gamma power ascribed the effect to NMDARs on interneurons [Korotkova et al., 2010, Lazarewicz et al., 2010, Carlén et al., 2012]. Here we show that in a mouse line, in which the NR1 deletion is restricted to pyramidal neurons, network oscillations are strongly affected. This result favors a PING (Pyramidal-Interneuron-Network-Gamma) model of gamma generation in CA1, according to which gamma is sustained by the interplay between pyramidal neurons and interneurons (as has been suggested previously [Horowitz, 1972, Leung, 1982]).

**Phase and strength of theta locking of place cells is dependent on specific navigation behavior**

In chapters 2 and 3 we looked at single-cell properties of CA1 pyramidal neurons and oscillatory network patterns of activity. In the third and last starmaze-related chapter, we looked at how spatial behavior influences the interaction between single neurons and network oscillation patterns of activity. Notably, we report for CTRs that, while during place-based navigation theta locking strength of place cells was inversely correlated to their ability to retain 'place-anchoring', during sequence-based navigation locking strength was positively correlated to the hippocampal system’s ability to rotate the place field (remaining 'departure-anchored'), hinting that theta oscillations might work as a switch between the two modes, a process dependent on NMDAR in CA1, as shown by the absence of this pattern in NR1-KO mice. The negative relation between the place index (P_{sdz}) and theta locking may be a reflection of an increased influence from the lateral EC, which is thought to relay sensory information (most relevant during place-based navigation) to the HPC
[Hunsaker et al., 2007] and contains neurons only weakly modulated by theta oscillations [Deshmukh et al., 2010]. We showed that, in CTR mice, theta phase precession during sequence-strategy trials occurs at a faster pace, which is paralleled by a decreased place field size in this trial type (Fig. 4.6). During a single crossing through a place field, spike theta phase precesses, such that the interplay between place field size and slope of precession maintain a similar amount of precession, defining a complete 'unitary' field [Maurer et al., 2006]. But is it the smaller place field size in sequence-strategy trials that determines the steeper slope, or is it the faster precession rate that determines a narrower place field? While our study does not allow us to answer this question, the fact that CA3 place cells exhibit smaller place fields [Mizuseki et al., 2012] may imply that during sequence-strategy trials, CA1 place fields are a more direct reflection of CA3 place fields and lead to a steeper phase precession slope.

In CTR mice, spiking during place-based navigation was also concentrated at more advanced theta phases than during sequence-strategy and training trials. This differential preferred phase coincides with the arrival times of spikes from EC layer III principal neurons [Mizuseki et al., 2009, Douchamps et al., 2013], suggesting that it reflects a more direct tuning of CA1 place cells to EC activity. NR1-KOs showed a generally blurrier picture, failing to show any correlation between a single-cell ability to follow either frameworks and theta locking (Fig. 4.6).

Phase locking may, therefore, act as a mechanism to coordinate groups of neurons engaged in similar processing mechanisms. By becoming 'released' from the local synchronization phase (through the theta oscillation), neurons may tune to external inputs. This ‘release’ may be brought about by interneurons (such as bistratified or oriens lacunosum-molecular interneurons), which modulate pyramidal cell firing [Klausberger and Somogyi, 2008] and are in a position to facilitate the flow of information from intrahippocampal regions, such as CA3, and extrahippocampal regions, like the EC, to CA1 [Klausberger et al., 2004, Leão et al., 2012].
Intertrial firing variability may explain some of the deficits NR1-KO place cells show in spatial representation

In the last chapter of the thesis we report a thorough analysis of place cell properties on a trial-by-trial basis, recorded while mice shuttled in a circular maze track, thus allowing a large repetition of stereotypical behavior, necessary for this sort of analysis. Interestingly, we show that the increased place fields observed in NR1-KO mice were a consequence of higher intertrial variability and that a 'unitary', singularly crossed place field was of similar size as CTRs. This observation suggests that the place field deficits observed in KOs may be due to an impairment in matching the currently processed spatial information to the stored spatial memory of that particular environment or, alternatively, that there is a mismatch between the path integration inputs and allothetic (visual) inputs. Our results in the starmaze, showing a stronger impairment at putatively CA3-mediated low gamma oscillations favors the former hypothesis. Although we report these results for this specific KO line, we believe that similar observations may be made for other types of KO lines, which have shown impaired spatial representation in the HPC (such as GluA1 [Resnik et al., 2012] or NR1PV [Korotkova et al., 2010] KOs) and reinforces the importance of analyzing results on a single-trial basis, as pooling multiple trials together may cache some features of the manipulation being tested (see, e.g., single-trial phase precession studies [Schmidt et al., 2009, Kempter et al., 2012, Reifenstein et al., 2012, Resnik et al., 2012]). We also report that, with extensive experience on the track, NR1-KOs showed a reduction in the deficits within a session, such that by the end of the recordings their place fields had leveled those of CTRs in such parameters as area and spatial information, suggesting that prolonged experience eventually allows the establishment of a proper spatial representation in CA1 in NR1-KO, possibly reflecting increased influence of CA3 inputs, where the mutation is unaffected, or alternatively, NMDAR-independent mechanisms in CA1 and elsewhere.
Putting it all together...

The link between spatial navigation and the hippocampus is not a novel one: it dates back to the ‘cognitive map’ theory postulated by Tolman in 1948 [Tolman, 1948], gained solid ground with the discovery of place cells in the hippocampus, which, together, form this ‘cognitive map’ of any given environment [O’Keefe and Dostrovsky, 1971]. More than creating a spatial representation of an environment, however, the hippocampus forms a spatial memory representation of a context, establishing a memory trace for a given episode [Wood et al., 2000, Eichenbaum, 2000]. Place cell and ensemble patterns of activity have also been widely studied, with its properties well described (phase precession [Skaggs et al., 1996, Maurer et al., 2006], remapping [Bures et al., 1997, Fyhn et al., 2007], theta-gamma coupling [Tort et al., 2009, Colgin et al., 2009, Belluscio et al., 2012]). Nonetheless, the link between hippocampal single-cell, network activity and spatial navigation, in particular when behavior requires the switch between different computation modes, both dependent on the HPC, is still largely unknown. The starmaze task [Rondi-Reig et al., 2006] has a clever design, allowing the use and identification of two different navigation strategies, both hippocampus dependent. Importantly, mice are not forced to adopt one particular strategy and, instead, choose which strategy to use, making use of both, switching flexibly between them. This allowed us to study how CA1 circuitry spontaneously switches between the two behavioral states.

Our results support the idea that different brain structures are driving CA1 during these two behavioral states, as has been previously suggested [Carr and Frank, 2012]. During place-based navigation, behavior is externally driven and CA1 is likely to follow mainly the EC. HG oscillations may form a tuning mode, which facilitates communication between these two structures during this strategy. Indeed, theta-gamma coherence has been postulated as being a way for linking separate codes, encoded by different cell assemblies, acting, for instance, at the gamma cycle temporal resolution [Lisman and Jensen, 2013]. Applied to our task, it may represent a way for CA1 to coherently organize place cell coding for different locations, linking different episodes that together form the context, not unlike how the association of the different paragraphs in this chapter is necessary for its comprehension.
During sequence-based navigation, place cell behavior is relatively detached from the influence of external factors, such as the layout of the environment, and place cell behavior is internally driven, reflecting a repetition of the same order of place cell activations as during training trials. It is the other main input structure to CA1, CA3, which may now take the lead in driving CA1 activity. This 'detachment' from external factors can also be seen in a non-theta mode of activity, viz. during sharp wave/ripple complexes: during sleep or awake immobility, CA1 displays irregular bursts of activity in very short time windows [Buzsáki, 1989], which have been linked to memory consolidation [Kudrimoti et al., 1999, Girardeau et al., 2009]. Place cell firing is strongly locked to ripples and reflect similar patterns of activity as seen during behavior: they 'replay' the same sequence of place cell activation as was seen, e.g., while the animal ran on a linear track [Lee and Wilson, 2002, Foster and Wilson, 2006], but in a compressed time window (250ms). Crucial to the interpretation of our results is the fact that the successful replay of place cell activity depends on CA3 inputs [Nakashiba et al., 2009], suggesting that they are necessary for the repetition of a stored spatial sequence in CA1. In contrast to the previous case, LG oscillations may entrain the communication between CA3 and CA1 [Montgomery et al., 2008, Colgin et al., 2009, Belluscio et al., 2012].

What regulates the strength of CA3 and EC inputs to CA1? As mentioned before, there are other brain areas supporting spatial representation in CA1. Two of these, the prefrontal cortex (PFC) and the parietal cortex (PC), have been suggested to play a role in strategy selection. Rich et al. [Rich and Shapiro, 2009] showed that activity of prelimbic (PL) neurons in the PFC changed as rats switched between an egocentric, dorsal striatum (DS) dependent and a hippocampus-dependent task. In our task, a sequence-based strategy can be seen as the execution of a series of egocentric responses [Packard and McGaugh, 1996, Rondi-Reig et al., 2006], with the hippocampus coordinating its activity with the DS. In addition, PFC neurons are modulated by hippocampal theta activity [Siapas et al., 2005] and theta oscillations of both HPC and PFC are coherent, with peak coherence occurring at choice locations [Bchenane et al., 2010], indicating increased dialogue between these two areas at decision-making moments. A recent study [Kitamura et al., 2014] reported the
existence, in rats, of patches of pyramidal neurons (island cells) in EC layer II, which project to stratum lacunosum interneurons. The latter ones, in turn, project to the same CA1 synapses receiving inputs from EC layer III neurons. This finding shows the existence of a feed-forward inhibitory mechanism, whereby island cells may modulate the strength of EC inputs onto CA1 pyramidal neurons. By exciting or inhibiting these neuron population in vivo during a trace fear memory task, the authors conclude that the direct inputs by island cells and EC layer III neurons support the temporal association of episodic events in CA1, while EC inputs carried via the trisynaptic pathway primarily processes context and spatial information. Assuming the existence of similar neuron patches in

Figure 6.1: Different information routes support place- and sequence-based navigation.
Place- (green) and sequence-based (orange) navigation may be supported by different kinds of information, conveyed by either the EC or CA3, respectively. The predomiance of either low or high gamma oscillations in CA1 may entrain CA1 firing activity to CA3 or the EC, respectively, switching between an internal (memory based) or external (environment based) navigation mode. Different subclasses of interneurons (such as bistratified or oriens lacunosum-moleculare interneurons; red) are likely to modulate inputs arriving from both structures, facilitating or reducing the drive of CA1 place cells (blue) by area CA3 (orange) or EC (green).
the mouse, we may, however, expect the feed-forward inhibition by island
cells to be important during sequence-based navigation, by dampening
the input strength of EC projections and facilitating the integration of
CA3 inputs to CA1.
A conclusive study would require simultaneous recordings between the
three structures, but current recording techniques do not allow such large-
scale monitoring of single-cell and network activity in the mouse, although
recent advances in developing smaller and more powerful recording de-
vices for mice [Voigts et al., 2013] or the rise of available rat transgenic
lines [Sato et al., 2004] may enable such studies. It is our belief, however,
that the results presented in this dissertation are solid enough to estab-
lish a link between the different structures driving CA1 during different
behavior states.

The role of NMDARs in CA1 during spatial navigation

The vast majority of studies on the role of the NMDAR in spatial
navigation focused on place-based navigation [Morris et al., 1986, Davis
et al., 1992, Bannerman et al., 1995, Nicholls et al., 2008]. This may
be largely due to earlier studies showing that body-centered (egocentric)
navigation is HPC independent [Packard and McGaugh, 1996, Packard,
1999]. More recently, and inspired by the relevant role the HPC plays
in tasks with a temporal sequence component [DeCoteau and Kesner,
2000, Lee et al., 2005], Rondi-Reig et al. have shown that mice with a
knock-out of NMDARs in CA1 are impaired during allocentric navigation,
but also in egocentric navigation, if this required the execution of a series
of body turns [Rondi-Reig et al., 2006]. When recording from this same
line of KO mice (NR1-KO), our goal was to discern neural deficits, which
could explain the behavioral impairments during normal navigation, but,
especially, when place- and sequence-based navigation are disentangled
(in probe trials).
Confirming previous studies [McHugh et al., 1996], we report that NR1-KO
mice have impaired spatial representation, as shown by the increased
place field size, lower spatial information per spike, lower in-field/out-
field firing rate ratio, amongst others (Chapter 2, Figure 2.5). Strikingly,
while the deficits these animals showed during place-based navigation,
both behaviorally and at the neuronal level, were relatively mild, they
Figure 6.2: NMDAR KO hampers integration of CA3 and EC inputs in CA1.

During spatial navigation, EC and CA3 input, conveying retrieved and encoded information, respectively, are integrated at CA1 synapses. In NR1-KO mice (right-hand panel), long-term potentiation in CA1 pyramidal neurons is absent, which leads to a defective spatial representation in CA1. SC, Schaffer Collaterals; PP, Perforant Path; DG, Dentate Gyrus; CA1 & 3, Cornu Ammonis 1 & 3; EC, Entorhinal Cortex.

were very accentuated during sequence-based navigation. This was expressed behaviorally, in the animal’s inability to follow the long sequence-strategy trajectory, which requires the memorization of a complex sequence of body-turns and has a stronger HPC involvement, compared to the short sequence-strategy trajectory (see Chapter 2, Figure 2.7). At the single-cell level, we observed that place cells utterly failed to follow the body-centered place field rotation that CTR mice showed, a defect mainly due to the poor spatial content transmitted during periods of LG (Chapter 3, Figure 3.7) At the network level, both LG and HG oscillations were strongly increased during sequence-strategy trials (Chapter 3, Figure 3.4), suggesting a saturation state of the network [Gruart et al., 2006].

These results suggest that the impairments seen in the NR1-KO line are most strongly caused by deficits in the processing of CA3 inputs at the Schaffer Collateral (SC) synapses. One possible interpretation is that, because sequence-based navigation is memory-based, the lack of
NMDARs prevents LTP and LTD from occurring [Bliss and Collingridge, 1993, McBain and Mayer, 1994], hindering the storage of the spatial sequence memory trace at CA3-CA1 synapses [Gruart et al., 2006, Madroñal et al., 2010, Nakazawa et al., 2004]. Place-based navigation, on the other hand, may be "backed-up" by the (unaffected) EC, but also related structures, such as the parietal cortex [Whitlock et al., 2008] and the peri- and postrhinal cortices [Norman and Eacott, 2005].

Our results in the circularmaze may give further insight into the nature of the larger place fields that NR1-KO mice exhibit: we report that single-trial place fields are normal in these mice and that the larger place fields observed at the session level are the result of across-trial variability. We suggest that EC inputs, which are sufficient to elicit spatially "sharp" place field activity (as in [Brun et al., 2008]), permit the expression of single-trial place fields and that the NR1-KO mice suffer from an impairment in integrating these high-level sensory inputs, with the stored spatial representation. Indeed, EC direct projections, via the perforant path (PP) and CA3 projections, via the Schaffer Collaterals, terminate in the same CA1 neurons [Kajiwara et al., 2008]. This impairment then leads to across-trials variability on the exact place field location.

One alternative explanation, not yet discussed, involves the lateral EC. The EC has two main subdivisions, a medial and a lateral one: the former plays a prominent role in spatial navigation [Hunsaker et al., 2007, Brun et al., 2008] and contains neurons that are sharply space-oriented (grid cells, [Hafting et al., 2005]), but the lateral EC shows only weak spatial modulation, with the firing of its neurons being mainly driven by the influence of sensory cues, such as discrete objects [Deshmukh and Knierim, 2011]. Thus, the EC is believed to relay spatial information via its medial portion and non-spatial (visual object) information via its lateral segment [Hunsaker et al., 2007, Van Cauter et al., 2012]. Interestingly, projections relayed by the medial and lateral perforant path (mPP and IPP, respectively) rely on different neurotransmitter receptors: while LTP at the mPP synapses is NMDAR-dependent, this is not the case at the IPP synapses [Breindl et al., 1994, Do et al., 2002, Villarreal et al., 2002]. This brings forward the possibility that, due to the impaired synaptic transmission at the mEC-CA1 synapse in NR1-KO mice, during place-based navigation, when behavior is mainly driven by environmental
cues, spatial representations in CA1 are still partially sustained by IPP inputs. Support for this hypothesis may come from a recent study modeling the dorsoventral changes in place field size in the hippocampus, in which the authors suggest that the increased place field size seen in more ventral HPC regions may reflect the processing of primarily non-spatial information [Lytte et al., 2013]. Formation of place fields in the dorsal HPC of NR1-KO mice in place-based navigation could, therefore, be formed in a process similar to that used in the ventral HPC place fields in normal mice.

The balance between encoding and retrieval mechanisms

During probe trials, the behavior of the animal was biased towards one of the two strategies. But during training trials, it is likely that behavior is driven by the integration of both CA3 and EC inputs. Indeed, during learning there is a continuous interplay between encoding and retrieval processes [Carr and Frank, 2012, Douchamps et al., 2013]. During training trials, thus, a CA1 spatial representation (output) is likely to be the result of matching incoming spatial information (from the EC) to the stored representation of that environment (from CA3, [Kesner, 2007]). Spatial representation in CA1 is, therefore, constructed from the delicate balance between these two inputs, modulated by the interneural population [Klausberger et al., 2004, Leão et al., 2012] and also by the EC-CA1 synapses, as it has been shown that EC inputs arriving via the PP at CA1 modulate SC collaterals, reducing or increasing excitability at CA3-CA1 synapses [Remondes and Schuman, 2002].

Conclusion

In this dissertation I aimed to offer compelling evidence for a flexible, two-state spatial representation in CA1: in correspondence to the computations driving behavior, CA1 place cells adopt strikingly different patterns of activity, shaped by different network oscillations. CA1 switches between an outwards-oriented mode, where spatial representation is a reflection of the environment and an inward-oriented mode, in which spatial representation is the result of the retrieval of a spatial sequence memory and is, just like the animal’s behavior, a replay of a previous episode.
These two states are likely to be supported by different input structures to CA1, the EC and CA3. Although we do not present a direct link between the role of these two structures and the navigation strategy being employed, place cell patterns of activity and different oscillatory patterns offer us a window into the inter-structural communication of CA1. Based on several studies linking high gamma oscillations to EC and low gamma oscillations to CA3, and the role these two gamma bands may play during place- and sequence-based navigation, respectively, and together with the suspected role of CA3 and EC in spatial navigation, we postulate that the two-state, behavior-dependent spatial representation of CA1 represents differential tuning of the network to either one of its two main input structures. It is likely that an upstream structure plays a key role in triggering the transition from one state to the other, such as the prefrontal cortex, which may be central to coordinating multiple memory systems [Rich and Shapiro, 2009].

In addition, we show that the lack of the NMDA receptor in CA1 strongly affects spatial coding, despite only mildly affecting behavior, in a hippocampus-dependent task [Rondi-Reig et al., 2006]. We believe that this deficit is due to a failure to properly integrate EC and CA3 inputs, either independently, as is the case in probe trials, or with each other, during general navigation.

The previous two paragraphs answer two of the three questions we had posed ourselves at the beginning of the project: how does CA1 encode different aspects of navigation strategies and which impairments at the neuronal level in NR1-KO mice can explain the impairments in behavior. The third main question we wanted to address was whether we could identify the strategy being employed solely by looking at neural patterns of activity. This question remains open, mainly due to technical challenges. Doing in vivo recordings in mice, while they perform a complex navigation task, is challenging and, unfortunately, the available techniques to do tetrode recordings in mice do not allow sampling of a large enough ensemble of neurons to do the population analyses necessary to answer this question as has been done with rats, where the larger size and weight of the animal allows an up to 5-fold increase in the yield of the neuronal sampling [Wilson and McNaughton, 1993, Foster and Wilson, 2006, Dragoi and Tonegawa, 2013]. Recent developments, however, may
allow to unravel such processes in this or similar tasks in mice, all requiring a shift between different computational modes [Voigts et al., 2013]. In addition, they add the possibility of modulating subset of neurons or neuron types by means of optogenetic techniques [Cardin et al., 2010]. This attractive possibility would allow, for instance, to stimulate or suppress the activity of a single class of interneurons, such as bistratified or oriens lacunosum-moleculare interneurons, which, as we and others [Klausberger et al., 2004, Leão et al., 2012] suggest, may modulate the flow of information between the EC and CA3 to CA1. One could, thus, attempt to impair or promote the expression of one specific strategy. In addition, by recording simultaneously from CA1 and EC and/or CA3 [Middleton et al., 2008, Colgin et al., 2009, Mizuseki et al., 2009], one may acquire a better insight into the dialogue between CA1 and its two main input structures during different behavioral states.
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Marijn van Wingerden, Martin Vinck, Vincent Tijms, Irene R. S. Ferreira, Allert J. Jonker, and Cyriel M. A. Pennartz. Nmda receptors control cue-outcome selectivity and plasticity of orbitofrontal firing patterns


Samenvatting in het Nederlands

**NMDA-receptor afhankelijke functies van hippocampale netwerken in ruimtelijke navigatie en geheugenvorming**

Ruimtelijke navigatie is een essentieel onderdeel van ons dagelijks leven. Efficiënte navigatie is voor de meeste dieren essentieel voor het vinden van voedsel of onderdak, jagen of het voorkomen dat het dier ten prooi valt. Voor mensen is ruimtelijke navigatie essentieel voor het uitvoeren van onze dagelijkse taken, zoals het naar de supermarkt gaan, terug naar huis gaan, naar het werk gaan, of de auto in de parkeergarage vinden.

Er zijn grofweg twee typen informatiestromen die we gebruiken om te navigeren in een omgeving. Ten eerste, de structuur van de omgeving, met al haar sensorische eigenschappen. Ten tweede, het gevoel van beweging en richting dat gegeven wordt door ons path-integration system (het vermogen om te navigeren door gebruik te maken van de richting van het hoofd en berekening van afgelegde afstand, gebruikmakend van vestibulaire informatie en informatie over de stand van de spieren), waardoor dieren (sommige meer efficiënt dan andere) in staat zijn om hun weg te vinden in het donker. De combinatie van deze twee informatiestromen maakt navigatie efficiënt. Buiten deze vorm van navigatie, genaamd place-based navigation, kan gedrag ook gestuurd worden door het onthouden van een sequentie van bewegingen. In plaats van het in de gaten houden van bewegingen door je positie ten opzichte van de omgeving bij te houden, is deze strategie gebaseerd op acties die ondernomen worden bij specifieke gebeurtenissen: Bijvoorbeeld vanuit de slaapkamer naar het toilet gaan in het midden van de nacht met je ogen dicht; je kunt de weg vinden door links af te slaan bij de uitgang van je kamer, de gang op te
lopen, naar rechts te gaan en een tweede deur naar links te nemen. Dit type navigatie noemen we sequence-based navigation. De hippocampus is een hersenstructuur die kritiek betrokken is bij hogere orde processen, zoals het vormen van herinneringen, het ophalen van herinneringen, en ruimtelijke navigatie. Met name het CA1 veld van de hippocampus, dat de uitgangssignalen van de hippocampus genereert, is een punt van convergentie van meerdere informatiestromen, zoals emoties (amygdala), beweging en beloning (striatum), ruimtelijke informatie (entorhinal cortex) en geheugensporen. De integratie van deze informatiestromen vormt de basis voor het vormen van een unieke ruimtelijke representatie (een 'cognitive kaart') van een specifieke omgeving.

In deze studie hebben we een analyse gemaakt van activiteitspatronen in CA1 gedurende place- en sequence-based navigation. Muisen konden gebruik maken van een van deze twee strategieën. Het gebruik van deze strategieën werd niet opgelegd door de regels van de taak en kon geïdentificeerd worden in speciale trials (een gedragstaak wordt onderverdeeld in zogenaamde trials met een begin en een einde die een aantal keren herhaald worden) waarin het vertrekpunt was veranderd. Als de muis nu naar dezelfde plaats rende als voorheen liet dat zien dat de muis een place-based navigation strategie gebruikte, aangezien de locatie van de beloning ten opzichte van de omgeving bekend was. Echter, als de muis dezelfde sequentie van lichaamsbewegingen maakte, bijv. links-rechts-links, dan eindigde de muis op een andere locatie als voorheen (die nog steeds beloond werd) en werd de trial als een sequence-based navigation trial geclassificeerd. Tijdens de training van de taak hebben we de activiteit gemeten van grote groepen individuele neuronen en het EEG, dat grofweg de gesommeerde activiteit van alle cellen in een gebied representeren.

We hebben niet alleen normale (controle, CTR) muisen gebruikt, maar hebben ook trainen en metingen verricht met zogenaamde knock-out muisen, die geen N-methyl-D-aspartaat receptor (NMDAR) bezitten in het CA1 gebied (NR1-KO muisen). Dit molecuul speelt een centrale rol in het moduleren van synaptische verbindingen doordat het in staat is om de efficiëntie van communicatie tussen verbonden neuronen te versterken of te verzwakken, en is sterk gerelateerd aan geheugenprocessen en geïmpliceerd in cognitieve stoornissen zoals schizofrenie. Het was reeds
bekend dat knock-out muizen een verzwakte ruimtelijke representatie in het CA1 gebied hebben en niet in staat zijn om de zogenaamde starmaze (de maze heeft de vorm van een ster) taak te leren. Op het niveau van gedrag hebben we laten zien dat NR1-KO muizen langzamer leren, maar na intensieve training toch hetzelfde niveau bereiken als controle muizen. Als we een vergelijking maken met voorgaande studies waarin NR1-KO muizen niet in staat waren om de taak te leren, dan denken we dat het gebruik van een land-versie van de starmaze (in de oorspronkelijke taak moesten muizen leren om te zwemmen in de starmaze en een verborgen platform te vinden om te ontsnappen aan het water) en het grotere aantal trials het leren van de taak vereenvoudigd heeft. Desalniettemin hebben we toch specifieke gebreken geobserveerd: de muizen waren niet in staat om de sequence-based navigation strategie te gebruiken bij de langere weg naar het doel, waarbij de muizen twee opeenvolgende keuzes moesten leren waaraan hoge kosten waren verbonden. Bovendien was dit gebrek het meest pregnant bij de tweede van de high-cost keuzen, hetgeen suggereert dat NR1-KO muizen een gebrek hadden in het verbinden van de twee keuzen in een sequentie.

Excitatoire cellen in CA1 hebben de bijzondere eigenschap dat ze bij voorkeur in een beperkt deel van een omgeving actief worden: ieder van deze neuronen, genaamd plaatscellen, wordt typisch actief (door het 'vuren' van actiepotentiaal) in een enkel, uniek gebied van de omgeving. Toen het vertrekpunt van de muizen was veranderd, om te onderzoeken welke strategie ze gebruikten om de navigeren, veranderde ook het gedrag van deze cellen: tijdens place-based navigation werden ze nog steeds actief in hetzelfde gebied van de maze (een cel vurde dan bijv. altijd dicht bij het witte bord op de gordijnen die langs de maze hingen). Echter, tijdens sequence-based navigation gingen de cellen op een andere plek vuren. Die plek werd dan bepaald door het uitvoeren van de sequentie van lichaamsbewegingen en was daarom verankerd in het vertrekpunt van het dier (de cel vurde bijv. altijd op het tweede kruispunt). Zoals reeds eerder was aangetoond, vertoonden NR1-KO muizen een licht verzwakte ruimtelijke representatie, waarbij het vuurgebied van een afzonderlijk neuron groter was dan normaal. En, net zoals bij de gedragsresultaten, lieten ze specifieke gebreken zien tijdens het uitvoeren van sequence-based navigation, waarbij de plaatscellen niet in staat
waren om de sequentie van lichaamsbewegingen te weerspiegelen. Op het niveau van het EEG leggen onze resultaten een verbinding tussen place-based navigation en een hoog-frequente band van hersengolven (55-95 Hz - high gamma, HG), en sequence-based navigation en een laag-frequente band van hersengolven (23-45 Hz - low gamma, LG). Dit kwam tot uitdrukking in de veranderde balans van EEG power (d.w.z, de energie in een signaal) in de twee oscillaties en de mate waarin het zogenaamde theta ritme, de meest voorkomende oscillatie in het CA1 gebied (7-10 Hz) die altijd aanwezig is in bewegende dieren, de LG en HG hersengolven moduleerde ('nesting'). Deze nesting vond plaats op verschillende fasen van de theta cyclus, hetgeen mogelijke twee verschillende 'kanalen' voor de transmissie van informatie naar CA1 weerspiegelt.

Onze interpretatie van deze resultaten is dat CA1, door sterker in de een of de andere gamma frequentie-band te oscilleren, in staat is selectief te communiceren met verschillende hersengebieden die de verschillende vormen van navigatie ondersteunen: tijdens place-based navigation met de enthorinale cortex (EC), dat de ruimtelijke en niet-ruimtelijke (sensorische) informatie doorgeeft aan de hippocampus, wordt communicatie gemedieerd door HG oscillaties, en tijdens sequence-based navigation met CA1, dat een belangrijke rol speelt in het vormen en ophalen van geheugensporen voor sequenties, loopt de communicatie via LG oscillaties.

Door dezelfde muizen te trainen op een taak zonder leercomponent, waren we in staat om CA1 activiteit te analyseren tijdens 'normale' navigatie op een circular track (een cirkelvormige loopbaan), waar de muizen heen en weer renden vanaf een muur die de track onderbrak. We hebben daarbij gekeken naar activiteitspatronen in afzonderlijke trials, d.w.z in afzonderlijke runs van de ene plaats op de circular track naar een andere plaats. Ofschoon de gemiddelde vuurgebieden (gemeten over sessies) van NR1-KO plaatscellen groter waren dan in controle muizen, waren ze in afzonderlijke trials even groot. Dit wordt verklaard doordat er variatie was in de voorkeursplaats van vuren over trials heen, zodat het gemiddelde vuurgebied over trials groter was. We stellen dat plaatscellen in NR1-KO muizen in staat zijn om EC inputs te volgen, maar er niet in slagen om inputs uit CA3 te volgen of te integreren, die informatie dragen over de
bestaande ruimtelijke representaties.

We hopen dat met deze thesis voortschrijdend inzicht wordt gegenereerd over hoe het CA1 gebied de relevante inputs die de huidige gedragsstaat van het dier ondersteunen integreert en verwerkt. Bovendien hebben we laten zien welke rol het NMDAR molecul in CA1 speelt bij deze processen en hoe het bijdraagt aan een juiste ruimtelijke representatie voor plaatscellen en een coherente functie van het netwerk.
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