Molecular and Circuit Mechanisms for Hippocampal Learning

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The hippocampus is crucial for the formation of memories of facts and episodes (Scoville and Milner 1957; Jarrard 1993; Squire et al. 2004; Burgess et al. 2002). In storing the contents of a specific episode, the hippocampus must rapidly form and maintain representations of the temporal and spatial relationship of events and keep these representations distinct, allowing similar episodes to be distinguished, a property termed pattern separation. Furthermore, because specific episodes are rarely replicated in full, the hippocampus must be capable of using partial cues to retrieve previously stored patterns of representations, a phenomenon referred to as pattern completion. Based primarily on the anatomy (Fig. 1) and physiology of the hippocampus and its associated cortical structures, computational neuroscientists have suggested specific hippocampal subregions and circuits that may subserve these mnemonic requirements. These are the feedforward pathway from the entorhinal cortex (EC) to the dentate gyrus (DG) and on to CA3 for pattern separation, and the recurrent and highly plastic connections in CA3 for pattern completion (Marr 1971; McClelland and Goddard 1996; McNaughton and Nadel 1990; O'Reilly and McClelland 1994).

CA3 NMDA Receptors for Pattern Completion

CA3 pyramidal cells receive excitatory inputs from three sources: the mossy fibers of the DG granule cells (GC), the perforant path axons of the stellate cells in the superficial layers of the EC, and the recurrent collaterals (RC) of the CA3 pyramidal cells and, in return, provide output to CA1 pyramidal cells via Schaffer Collaterals (SC). The prominence of these RCs has led to suggestions that CA3 might engage these connections to serve as an associative memory network. Associative networks, in which memories are stored through modification of synaptic strength within the network, are capable of retrieving entire memory patterns from partial or degraded inputs (pattern completion; Marr 1971; Gardner-Medwin 1976; Hopfield 1982; McNaughton and Morris 1987; Rolls 1989; Hasselmo et al. 1995).

We set out to obtain evidence for this hypothesis by targeting the knockout of the NR1 gene, coding for the essential subunit of NMDA receptors, to postnatal CA3 pyramidal cells. Use of the Cre-loxP recombination system, in which the expression

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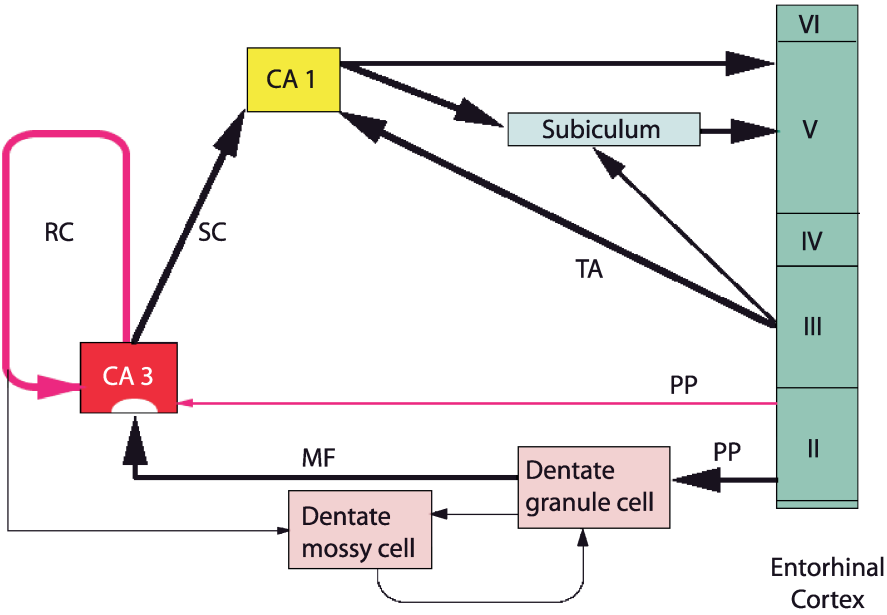

Fig. 1. Hippocampal excitatory pathway. PP = Perforant Path; MF = Mossy Fiber; RC = Recurrent Collaterals; SC = Schaffer Collaterals; TA = Temporoammonic Path. The feedforward pathway from entorhinal cortext to dentate gyrus and to CA3 is hypothesized to play a role in pattern separation while the RC in CA3 is hypothesized to be important for pattern completion

of the transgenic Cre gene was driven by the transcription-regulating elements of the KA-1 gene, permitted us to obtain such a cell type-specific knockout mouse (CA3-NR1 KO; Nakazawa et al. 2002). In situ hybridization, immunohistochemistry, and hippocampal slice electrophysiology data confirmed that the knockout is restricted to postnatal CA3 pyramidal cells.

The CA3-NR1 KO mice, in contrast to CA1-NR1 KO mice (Tsien et al. 1996; McHugh et al. 1996), were normal in the standard hidden platform version of the Morris water maze task (Fig. 2). However, when the probe test was conducted under the conditions where only one of the four major visual cues (partial cue condition) was available after the training was performed with the four cues (full cue condition), the mutant mice exhibited a deficit compared to the control littermates (Fig. 2). These data indicate that the mutant mice are capable of acquiring this spatial memory and also retrieving it as long as the full set of cues are provided during the recall phase. However, the mutants are impaired in retrieving the memory using a partial set of cues (only one of the four major visual cues), conditions that are sufficient for recall in the control mice. These data suggest that the CA3-NR1 mice suffer from a specific impairment in a pattern completion-mediated recall.

This phenotype of the mutants observed at the behavioral level was corroborated at the level of neuronal ensemble activities in the CA1 area, which was shown by in vivo recording of CA1 pyramidal cells with the tetrode recording technique. The CA3-NR1 KO mice exhibited compact place fields that were indistinguishable from those of the

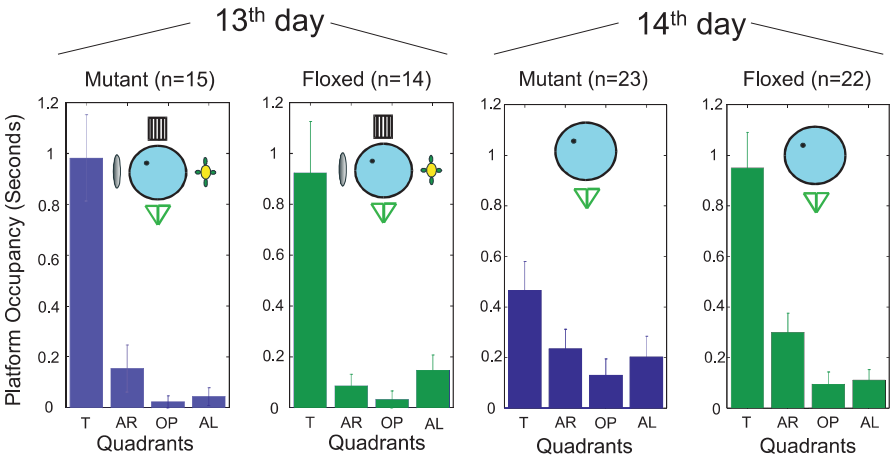

Fig. 2. The CA3-NR1 KO mice are defective in pattern completion. The mutants and control littermates (floxed) went through 12 day-long training in the hidden platform version of the Morris watermaze task under the full cue conditions (four visual cues surrounding the pool). The probe trial conducted on the 13th day under the same full cue condition indicated the mutant is normal in the acquisition and retrieval of the spatial memory under these conditions. However, the memory retrieval by the mutants was substantially diminished compared to the control mice in the probe trial conducted the next day (14th day) under the partial cue condition (only one of the four cues was available during this probe trial)

control mice under the familiar full cue conditions (four major visual cues; Fig. 3). When these mice were transferred to a home cage and after several hours returned to the same recording box with the full set of cues, the mutant place cells were reactivated as well as the control place cells. However, when the mice were returned to the recording box with a partial set of cues (only one of the four major visual cues), the extent of the reactivation of the place cells by the mutants was significantly diminished compared to the control mice (Fig. 3).

Thus, both behavioral and in vivo physiological data strongly support the hypothesis that the NMDA receptors in the CA3 pyramidal cells, and probably synaptic plasticity at the CA3-CA3 recurrent synapses, play a crucial role in pattern completion in the hippocampus.

DG NMDA Receptors for Rapid Pattern Separation

The key data that support the hypothesis that the feedforward EC → DG → CA3 pathway may be responsible in pattern separation are that 1) the number of DG GCs is substantially greater than the numbers of EC layer II stellate cells and CA3 pyramidal cells, 2) the connection between DG and CA3 is two orders of magnitude more sparse than the connections between other regions, including EC and DG, and 3) the DG GC spiking activity is lower compared to other regions. It is therefore possible that relatively overlapping memory engrams present in EC are separated (orthogonalized)

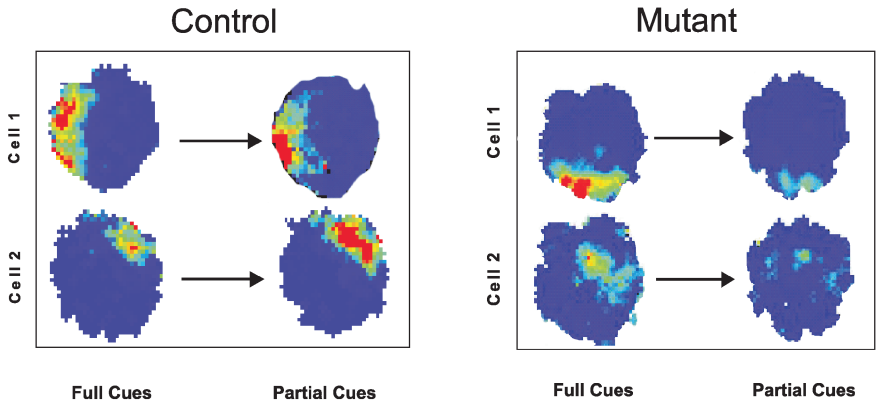

Fig. 3. CA1 place cells are reactivatable under full cue condition but not under partial cue condition in CA3-NR1 KO mice. The CA3-NR1 KO mice formed compact CA1 place fields in a familiar environment under full cue conditions. Upon a reexposure these place cells were reactivated well under full cue conditions (four cues), but only poorly under partial cue conditions (one cue)

as the information is transferred through the EC → DG → CA3 pathway. Since NMDA receptors in DG GCs are expected to modulate the activity of DG GCs in an experience-dependent manner, it is possible that we may see a deficit in an experience-dependent pattern separation in mutant mice in which the NR1 gene knockout is targeted to postnatal GCs.

We generated such NR1 knockout mice (DG-NR1 KO mice) fortuitously by employing the transcriptional regulatory elements of the proopiomelanocortin (POMC) gene as the driver of the Cre expression and crossing the Cre transgenic mice with the same "floxed" NR1 gene mice that we previously used for the CA3 (Nakazawa et al. 2002) and CA1 (Tsien et al. 1996; McHugh et al. 1996) studies (McHugh et al. 2007). Again, in situ hybridization in these mice, immunohistochemistry, and synaptic electrophysiology confirmed that the NR1 knockout is well restricted to postnatal DG GCs.

The performance of the DG-NR1 KO mice was normal in the standard Morris water maze task as well as in the standard contextual fear conditioning. However, in an incremental context discrimination fear conditioning task, the mutant mice exhibited a deficit in the early phase of the trials, although their ability to discriminate the contexts developed slowly to the normal level as the trials were repeated. Thus, the mutant mice were normal in spatial and contextual learning per se but had a problem in being able to rapidly distinguish similar contexts with just a few trials, which the control littermates accomplished with no problem. These results suggest that the NMDA receptors in DG GCs and probably NMDA receptor-dependent synaptic plasticity at the perforant path-DG GC synapses play an important role in fast (with one or two trials) pattern separation. However, the fact that the mutant mice can catch up to the control mice with more trials suggests that the hardwiring in the EC → DG → CA3 pathways permits slow, multitrial-dependent acquisition of pattern separation.

To detect a pattern separation deficit of the DG-NR1 KO mice at the neuronal ensemble activity level, we recorded with the tetrode technique the spiking activities in

CA1 and CA3 as the mice explored two distinct contexts (low-walled white circular box vs. black square box) at the same site in the same room. Earlier studies with normal rats had shown that, under these conditions, individual pyramidal cells in CA1 exhibited similar firing rates in the two contexts whereas those in CA3 displayed context-specific firing rates (Leutgreb et al. 2005). Thus, in the latter case, there was a "remapping" of the firing rates as the animals were shifted from one context to the other. Like rats, our control mice showed significant rate remapping in CA3 but no remapping in CA1. In contrast, the DG-NR1 KO mice exhibited a significant deficit in rate remapping in CA3 but no remapping in CA1. These results corroborate the behavioral deficit of contextual discrimination and reinforce the conclusion that DG NMDA receptors play a role in rapid pattern separation.

CA1 for Novelty Detection?

Our earlier study, carried out by applying the same interdisciplinary strategy to CA1 pyramidal cells, demonstrated that a knockout of NMDA receptors in this "outpost" of the excitatory hippocampal trisynaptic pathway leads to a severe impairment in hippocampus-dependent learning tests, such as the Morris water maze and trace fear conditioning (Tsien et al. 1996; McHugh et al. 1996). This finding is in contrast to the knockout of the same NR1 gene in CA3 or DG, but it is not surprising because, in the CA1-NR1 KO mice, the NMDA receptors are knocked out not only at the SC-CA1 synapses, the most downstream of the trisynaptic pathway, but also at the temporoammonic (TA) path-CA1 synapses, an integral part of the direct EC→CA1→(subiculum)→EC pathway. There has been a suggestion that inputs from these two circuits (trisynaptic and temporoammonic) are "compared" at CA1 to generate a "novelty signal" that may be necessary to convert the hippocampus to a "learning mode" (Fig. 4; Vinogradova 2001; Lisman 2005). After all, we may learn something when we encounter novelty whereas we cannot learn from something we already know. To address this postulated function of CA1, we need a new genetic manipulation technique that will allow us to block the SC input to CA1 specifically while keeping the TA input intact or vice versa. Such a technique is under development.

The genetic technology that permits a cell-type specific and postnatal knockout of a gene (such as the NR1 gene) and multidisciplinary analyses of these conditional mutant mice are allowing us to test a number of hypotheses regarding the distinct functions of hippocampal subregions and their circuits in various aspects of hippocampus-dependent learning and memory. In the future, this general strategy could be extended to brain systems and circuits outside of the hippocampus to uncover mechanisms underlying memory and other cognitive functions.

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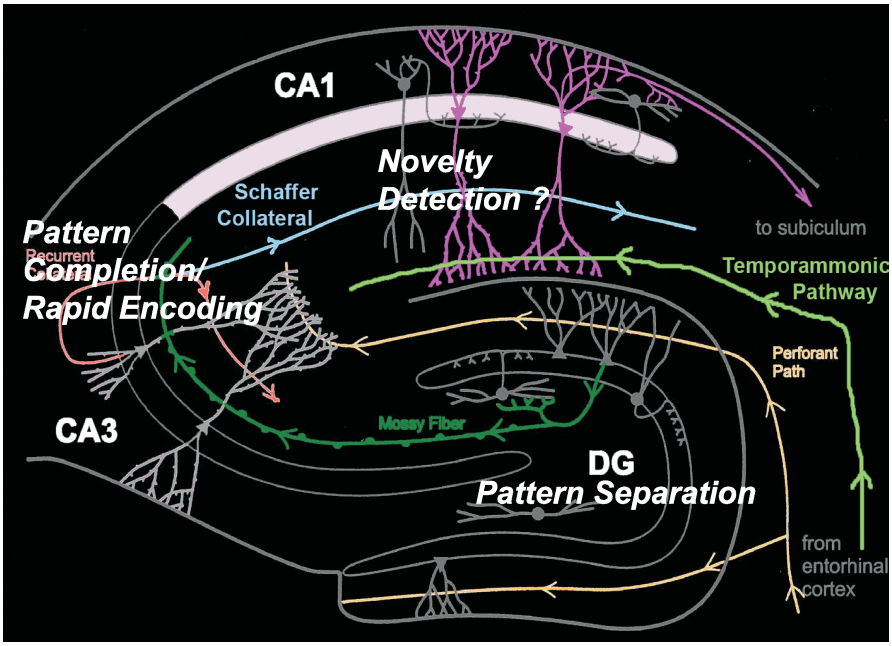

Fig. 4. Distinct mnemonic functions of hippocampal excitatory circuits. The cell type-restricted knockout technology revealed that the NMDA receptors in CA3 pyramidal cells and DG granule cells are important for pattern completion and pattern separation, respectively. The CA3 NMDA receptors also play a role in rapid encoding of one trial/experience memory (Nakazawa et al. 2003). It is hypothesized that CA1 pyramidal cells may compare the SC input which may be loaded with previously acquired memory information and the TA input which conveys on-line sensory input and, thereby, provides novelty/familiarity signal to the downstream areas like subiculum. The novelty signal is thought to be important to convert the hippocampal to a "learning mode"

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