Goal-oriented searching mediated by ventral hippocampus early in trial-and-error learning
Ruediger, S, Spirig, D., Donato, F., Caroni, P.

Suppl. Information

Supplementary Figure 1. Strategy/latency analysis of individual mice during maze learning. a, Additional examples of strategy deployment by individual mice under control conditions. The second (nearly no strategy blocks) and third (extensive strategy 3 blocks) mouse were among the 20% slowest learners, whereas the fourth mouse did not reach a latency of 25sec at trial 36, and exhibited an obvious reduction in hippocampal volume. b, Examples of strategy/latency plots for mice with vH lesions, β-Adducin mutant mice, mice with vH or dH β-Adducin rescue, and for mice learning a maze with a 2A platform.
Supplementary Figure 2. Distinct filopodia/LMT baseline values correlated to LMT complexities in dH, iH and vH. Upper graph: Mean filopodia/LMT values along the dorso-ventral axis in the hippocampus of mice housed under standard cage conditions. Isolated hippocampi were sectioned transversally, and the dorsal and ventral ends (ca. 250µm and 460µm respectively) were excluded from the analysis. The connected bars indicate the regions along the dorso-ventral axis of the hippocampus that were defined as dH (20-30% of total length), iH (45-55%) and vH (70-80%) in this study17. Note how mean filopodia/LMT values increase substantially along the dorso-ventral axis, but are comparable within hippocampal subdivisions. The ventral end of vH exhibited mean filopodia/LMT values that were substantially higher than those in the rest of vH. Lower graph: Positive correlation between filopodia/LMT values and LMT complexities for individual LMTs (Methods). This finding suggests that at the level of individual LMTs filopodial numbers (which are proportional to FFI synapses onto parvalbumin interneurons22) are correlated to the numbers of feedforward excitatory synapses onto pyramidal neurons in CA3.

Nature Neuroscience: doi:10.1038/nn.3224
Supplementary Figure 3. vH FFI growth reflects behavioral learning of specific task-goal associations. 

**a.** Absence of FFI growth at vH and dH upon novel object recognition (NOR). Top: schematic of experimental protocol, involving habituation (10 min) on day 0, and discrimination test (5 min) on day 1. Familiar object in red. Mice explore identical objects equally (Habit.), but preferentially explore the novel object (NOR) on day 1. N=20. 

**b.** Converting an incidental NOR task into a reward-based one is sufficient to induce FFI growth specifically at vH upon learning. Top graphs: schematic of experimental protocols. Left, middle graph: training protocols with (red traces; Familiar Object Preference, FOP) and without (black traces; NOR) reward. Negative discrimination index values reflect longer exploration of familiar object. N=30. Left, lower graph: corresponding FFI growth values. N=3-7. Right: reference memory test (in the absence of reward) 1 day after last training day, as indicated; each dot represents an individual mouse, with (red) and without (black) reward. 

**c.** FFI growth in vH and dH upon contextual, but not upon explicit fear conditioning (FC). Left: schematic of FC protocols under light (contextual) and dark (explicit) conditions (top row), and corresponding freezing behavior 1d after learning (lower row). Center: representative camera lucidas of LMTs in CA3b of vH and dH. Bar: 10 µm. Right: contextual, but not explicit FC induces FFI growth (upper graphs) and enhanced CA3b pyramidal neuron c-Fos recruitment (lower graphs) in vH and dH. FC values 1 day after learning. N=20.
a) NOR 1d, Discrimination

- 10 min
- 24h
- 5 min

B) NOR / Reinforced FOP, Discrimination

- Reward
- Reward
- Reward

C) NOR / Reinforced FOP, FFI Growth

- Ventral H
- Dorsal H

D) Discrimination vs FFI Growth

Ruediger et al. Suppl. Fig.3
Supplementary Figure 4. Specific task-goal association reflected by vH FFI growth during maze learning. Left: Analysis of experimental conditions that produce FFI growth at vH. No platform: four 60sec swim trials in the maze; no trials: mice kept in home cage. Mean values; N= 5-8 mice. Right: Requirement for signaling by reward neurotransmitter dopamine during maze learning. Impaired maze learning, absence of FFI growth at ventral hippocampus, and impaired strategy progression in mice treated with D1/D5 antagonist. N=10 each.

Ruediger et al., Suppl. Fig. 4
Supplementary Figure 5. Ibotenic acid lesions of hippocampal subdivisions. 

a, Representative examples of dH, iH and vH lesions, and of corresponding vehicle-treated hippocampi. Nissl staining. b, Examples of dH, iH and vH lesions. Representative low-magnitude views of Nissl stained sections from lesioned mice at dH, iH and vH levels. c, d, Representative analysis of hippocampal subdivision lesions. c, vH lesions. Left: Higher magnification views of Nissl stainings. Note near to complete absence of intact cells in dentate gyrus and CA3 of vH, and absence of detectable damage in iH and dH. Right: Analysis of excitotoxic damage spread along the hippocampus of ventrally lesioned mice. Schematic of hippocampal regions along dorso-ventral axis, and fraction of sections with at least 10% damaged cells in CA3. N=20 mice (five 50µm sections each per hippocampal region). d, Analysis of iH and dH (complete and partial (D1)) lesions, as described in c. N=10 (iH), 20 (dH, complete) and 10 (dH partial) mice.
Ruediger et al., Suppl. Fig.5
Supplementary Figure 6. Single trial latency deviations from the means for explorative strategies.

Extent to which the latencies of individual trials involving the exploration of alternative strategies differed from the mean latencies for that phase of the training process (see methods). Data analysis as described for Fig. 1g.
Supplementary Figure 7. Local rescue of β-Adducin expression and FFI growth in vH or dH granule cells of β-Adducin\(^-\) mice. a, Lentivirus-mediated expression of GFP-β-Adducin specifically in dH granule cells of β-Adducin\(^-\) mouse. The panels show representative examples of GFP-β-Adducin signal in granule cells (note labeled dendrites in the dentate gyrus, and labeled mossy fibers in the hilus and along CA3) in dH, but not vH. Lentivirus-mediated rescue led to GFP-β-Adducin expression in 18-25% of the granule cells in dH. Bar: 100μm. b, Rescue of learning-related FFI growth upon GFP-β-Adducin expression in vH or dH. Data from water maze learning experiments. As previously reported\(^{21}\), lentivirus-mediated GFP-β-Adducin transduction rescued FFI growth specifically in those (GFP+) granule cells that exhibited virus-mediated expression. N=10 mice each.

Ruediger et al., Suppl. Fig. 7
Supplementary Figure 8. Sequence of subtasks involving ventral-to-dorsal hippocampal subdivisions in complex trial-and-error navigation learning. The schematic summarizes how an hippocampus-dependent trial-and-error learning process such as maze navigation is structured stereotypically into subtasks, which are implemented by individual hippocampal subdivisions (e.g. dH for spatial search). The subtasks build upon previously learned relationships (arrows), and lead to learning about increasingly defined features of the particular task. FFI growth upon learning focuses subsequent searches, supporting the deployment of strategy habits. Our results suggest that mice may: 1) learn on day 1 that there is a goal (platform), leading to a global search strategy; 2) learn on days 1-2 (vH) that the platform is consistently at a certain distance from the wall, supporting the deployment of local search strategies and local search habits; 3) learn on days 3-5/6 (iH) that the platform is consistently at the same position within the pool, supporting the deployment of spatial search strategies and spatial search habits; 4) learn on days 6-9 (dH) a fine scale spatial map of the task, leading to direct swim from any position in the maze.

Maze navigation learning structure: Subtask sequence involving ventral-intermediate-dorsal hippocampal subdivisions

---

<table>
<thead>
<tr>
<th>Strategy</th>
<th>supporting learning</th>
<th>when</th>
<th>FFI growth</th>
<th>What learned</th>
<th>Cues used and learned</th>
</tr>
</thead>
<tbody>
<tr>
<td>escape</td>
<td>?</td>
<td>day 1</td>
<td>?</td>
<td>Invariant goal</td>
<td>Circular</td>
</tr>
<tr>
<td>global search</td>
<td>vH</td>
<td>days 1-2</td>
<td>vH</td>
<td>Invariant goal-context</td>
<td>Circular</td>
</tr>
<tr>
<td>local search</td>
<td>iH</td>
<td>days 3-6</td>
<td>iH</td>
<td>Invariant place ?</td>
<td>Circular, Dot</td>
</tr>
<tr>
<td>spatial search</td>
<td>dH</td>
<td>days 6-9</td>
<td>dH</td>
<td>Invariant spatial map</td>
<td>Grid</td>
</tr>
</tbody>
</table>

Ruediger et al., Suppl. Fig. 8