



## Reward modulates neuronal activity in the hippocampus of the rat

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### Abstract

In the hippocampus of freely moving rats, neurons have been recorded that fire predominately when the animal travels through a particular area while exploring the environment (so-called ‘place cells’). This study investigates if the neuronal firing characteristics of such cells are modulated by attention, expectation of reward or memory load. A total of 16 electrodes were implanted in the CA1 region of the hippocampus of 3-month-old Long–Evans rats. Using a tetrode recording system, single neurons were recorded while a rat explored an 8-arm maze and retrieved pellets at the end of each arm. It was found that 31 out of 67 neurons showed place cell characteristics, while the other cells either fired in more than one place or fired along whole arms of the maze. Interestingly, 11 of the 31 neurons showed enhanced firing activity when the animal entered a baited arm but did not fire when the arm was visited again after the bait had been retrieved. In a second experiment, only four out of eight arms were baited. Firing rates of 46 neurons were analysed, and all cells (spatial or non-spatial) fired more in baited arms than in non-baited ones ( $P < 0.001$ ). In a reversal task in which the previously unbaited four arms were subsequently baited, neuronal activity was increased in the newly baited arms (42 cells analysed,  $P < 0.001$ ). Since no alterations to the maze or cues have been made, we interpret the increased firing probability of neurons in baited arms compared to unbaited arms as a correlate for ‘attention’ or ‘expectation’.

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**Keywords:** Place cell; Place field; Spatial; Memory; Orientation; CA1; CA3; Electrophysiology

### 1. Introduction

The hippocampus is considered to be involved in spatial information processing and in memory processes. In recordings obtained from freely moving rats, neurons have been found that convey spatial information (‘place cells’; [29]). Numerous experiments have been conducted to characterise what type of information can influence place cell activity, and what role such cells might play in information processing [7,27,28,36,37]. Most single cell recording studies appear to show reproducible recordings of place fields if the environment is not altered, and such place fields appear unaffected by the targets of the animals or by variations in memory loads. However, hippocampal lesions or the application of receptor blockers that impair neuronal functionality in the hippocampus have been shown to affect short-term memory [1,2,8,17,35]. This apparent contradiction between the seemingly very stable place cell activity over time and the very dynamic functional role of the hippocampus in short- and long-term memory has puzzled many scientists. A num-

ber of studies have addressed this issue, and it was found that place cell activity is not as static as first assumed. For example, it was shown that landmarks that are observed by the rat to be mobile will have much less effect on place cell firing properties than landmarks that appear to be stationary [19,20]. Also, place cell activity can be related to the movements that the animal anticipates [10,16,46]. These modulations of neuronal activity have been interpreted as correlates for episodic memory, since they cannot be explained by changes in the environment that could induce remapping of place fields [5,25]. A different interpretation would be that the animals pay selective attention to different cues, and the over-representation of a target location as shown in the study by Hollup et al. [16] could be a correlate for increased selective attention for the life-saving platform in a water maze task. A difference in attention would then be reflected in the neuronal activity which would change depending on the animals’ actual activity even though no alteration of landmarks that drive place cells had occurred. This idea is supported by results from a number of different studies, e.g. in single cell recording from mice, where it was found that place cells were unstable during unbaited runs when there was no spatial task for the animals to solve. Only when a food reward was introduced did spatial firing of cells become

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65 more stable as the animal learned the spatial task [21]. This  
66 emergence of spatial information in neuronal activity could  
67 be due to shifted attention towards spatial information.

68 In order to test if there is an effect of selective attention  
69 or memory load on firing properties of single cells in the  
70 hippocampus, a task was employed that required animals to  
71 remember items and to selectively pay attention to specific  
72 items in order to be able to solve the task. In an 8-arm maze,  
73 arms were baited or unbaited to test whether a change of  
74 attention by baiting arms had any effect on place cell dy-  
75 namics. Also, since this task has both working memory and  
76 long-term memory components, it is possible to analyse if  
77 neuronal activity in the hippocampus shows firing modula-  
78 tion that could be the basis of memory.

## 79 2. Material and methods

80 Six naive male Long–Evans rats (300–400 g at implanta-  
81 tion stage) were housed individually in large transparent  
82 perspex cages with food and water available ad libitum. An-  
83 imals were kept on a reversed 12 h/12 h light/dark schedule  
84 and tested in the dark phase.

### 85 2.1. Electrode implantation

86 Tetrodes [30] were made of four twisted 25  $\mu\text{m}$  heavy for-  
87 mvar coated platinum–iridium (90%/10%) wires (Cal. Wire  
88 Company, USA). Electrodes were soldered to a headstage  
89 that allowed controlled lowering of tetrodes after implanta-  
90 tion. The impedance of electrodes were 500–800 k $\Omega$ .

91 Animals were anaesthetised with 75 mg/kg Ketamin  
92 (WDT, Germany) + 5 mg/kg Xylazine (Rompun, Bayer;  
93 s.c.). A local injection of lidocaine was given into the  
94 scalp, and the skin was cut and pulled back. Animals were  
95 mounted in a stereotaxic frame. A 1.5 mm diameter hole  
96 was drilled into the skull (4 mm post-bregma, 3 mm lateral  
97 midline) to allow the positioning of a microdrive with four  
98 tetrodes above the dorsal hippocampus. Four 1 mm stainless  
99 steel screws were inserted into the skull. One of the screws  
100 served as a ground electrode. The microdrive was fixed to  
101 the screws by dental acrylic cement. The sides of the scalp  
102 skin were treated with antibiotics. Animals were given 1  
103 week to recover before any recording was started.

### 104 2.2. Recording procedure

105 The animal was connected to a headstage preamplifier  
106 (Axona, UK) which also contained three LEDs that were  
107 used by the tracking system to identify the location of the  
108 animal. The amplifier was connected via cables to a main  
109 AC coupled amplifier (10,000–25,000 amplification) and to  
110 the recording system (Axona, UK). The cables were sus-  
111 pended by rubber bands to prevent the animals trailing the  
112 wires during runs. Signals from each electrode were passed  
113 through bandpass filters 600 Hz to 6 kHz for the spike chan-

nels and low passed filtered >200 Hz for the EEG channel. 114  
Each channel of the tetrodes was recorded differentially to 115  
the reference electrode on the skull and to a different tetrode 116  
near by. Signals that crossed a pre-set trigger threshold were 117  
recorded. Waveforms were sampled at 48 kHz, time stamped 118  
and stored for off-line analysis. A video tracker recorded the 119  
position of the rat during each trial. The coordinates were 120  
stored off-line for later analysis. Positions were sampled at 121  
47 Hz at a resolution of 8 mm/pixel. 122

Animals were given trial runs through the radial-arm maze 123  
in order to identify neurons. Tetrodes were advanced daily 124  
about 50–150  $\mu\text{m}$  to optimise recording or to record from 125  
different cells. 126

### 2.3. Radial-arm maze 127

The 8-arm maze was constructed from chip board with 128  
white PVC coating. The length of each arm was 68 cm, 129  
width 17 cm and height 35 cm. The diameter of the central 130  
platform was 50 cm. At the end of each arm, a square black 131  
plastic weighing boat with 5 cm side length was attached. 132  
In this weighing boat, a 0.5 g cocoa flavoured cereal (Choco 133  
Krispies, Kellogs) was put during baiting. The experiments 134  
took place in a room (2.80 m  $\times$  4.50 m) with several salient 135  
landmarks such as a door, a bench and several black card- 136  
board figures (squares, triangles, strips, ca. 50 cm length) 137  
attached to the walls. These landmarks remained at a fixed 138  
position. The room was dimly lit by four 20 W lights, one 139  
in each corner. 140

#### 2.3.1. Pre-training 141

Animals were put on a diet of 80% of their free feeding 142  
diet 1 week before the experiments started. Animals were 143  
made familiar with the maze and the recording cable for 144  
several days. During this time, food pellets were scattered 145  
along the arms to encourage exploratory behaviour. 146

#### 2.3.2. Experiment 1 147

For 7 days, animals were given four trials daily where 148  
each arm was baited. Recording was conducted for 120 s 149  
during which all animals first retrieved the pellets and then 150  
explored the maze thoroughly. 151

#### 2.3.3. Experiment 2 152

For a further 8 days, only four of the eight arms were 153  
baited. Recording was conducted for 120 s during which 154  
all animals retrieved the pellets. After retrieving the pellets, 155  
animals continued to explore the maze. Four trials per day 156  
were given. 157

#### 2.3.4. Experiment 3 158

For further 7 days, the previously unbaited four of the 159  
eight arms were baited, while the previously baited arms 160  
were not. Recording was conducted for 120 s during which 161  
all animals retrieved the pellets. After retrieving the pellets, 162  
animals continued to explore the maze except for the last 163  
day. Four trials per day were given. 164

## 165 2.4. Spike isolation and analysis

166 Spikes were separated according to principal compo-  
 167 nent analysis using a software program (TINT, Axona,  
 168 UK). The slopes of waveforms recorded from each chan-  
 169 nel of a tetrode were compared with those from another  
 170 channel. ‘Clusters’ of waveforms with similar slopes were  
 171 identified and analysed further according to temporal and  
 172 waveshape criteria [14]. Interneurons were identified and  
 173 distinguished from pyramidal cells by the duration of  
 174 the extracellular action potential (>0.3 ms for pyramidal  
 175 cells), firing patterns (complex spikes), temporal firing pat-  
 176 terns (autocorrelation analysis of firing frequencies, eg.  
 177 theta firing patterns) and low firing average outside of the  
 178 place field. The quality of isolation was tested by auto-  
 179 correlation.

180 Average firing rate was expressed as the total number of  
 181 spikes divided by the total length of the recording period.  
 182 Cells with a firing rate of less than 1 Hz were not used for  
 183 analysis. The peak firing rate was calculated by dividing the  
 184 maze surface into  $48 \times 48$  bins. The number of spikes in each  
 185 bin was normalised to the time spent by the rat within the  
 186 space of each bin. The values in this array were smoothed  
 187 by replacing each value with the average of this value and  
 188 those of the adjacent eight neighbours. The peak rate was

## Working Memory errors exp. 1

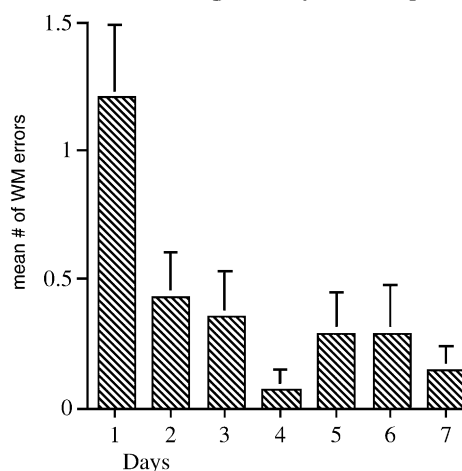


Fig. 1. Working memory errors (repeated visits of one arm within a trial) in experiment 1. The animals learned the task quickly. A difference in number of errors was found between days (ANOVA;  $P < 0.001$ ).

then taken as the maximum smoothed firing rate in any bin. Spike rates were separated into five groups: 0–20% of firing rate, 20–40, 40–60, 60–80 and 80–100% of the maximum firing rate. A place field was defined as a group of bins that

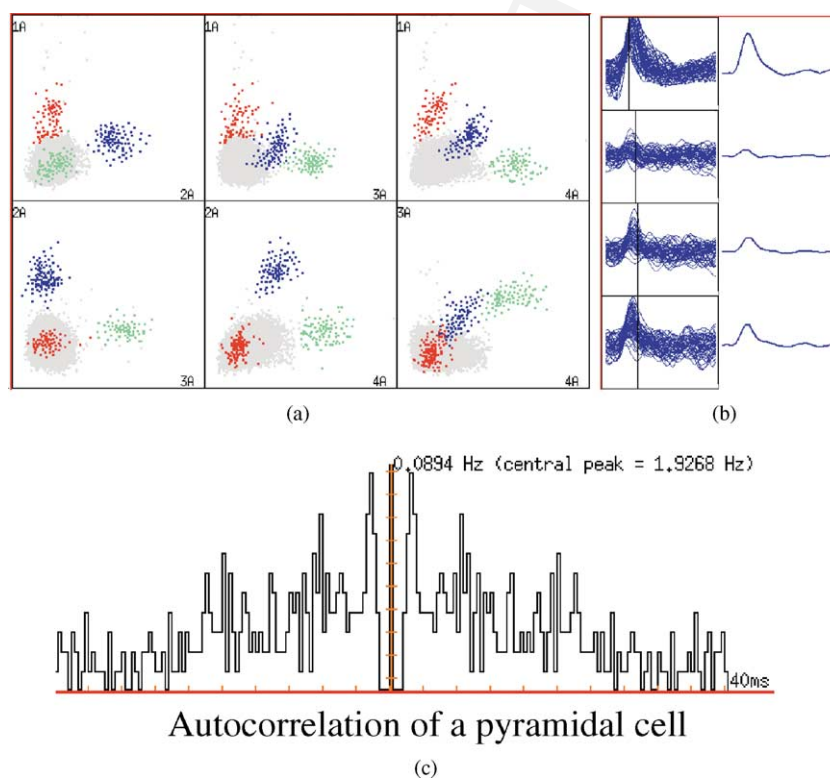


Fig. 2. Example of cell isolation. (a) Shown is the plot of spike slopes from electrode 1A of the tetrode compared with electrode 2A, 1A with 3A, etc. ‘Clusters’ of these dot clouds are selected and analysed further. (b) Traces of identified clusters are shown for each electrode. Due to distance differences from neuron to electrodes the signal strength differs for each electrode. Electrode 1 shows the highest spike amplitude, electrode 4 the second highest, while 3 and 4 show weak signals only. Left: overlay of traces, right: averaged trace. These traces are then further analysed and separated according to different parameters such as peak amplitude, valley amplitude, time of peak, and others. X-axis = 2 ms, Y-axis =  $280 \mu\text{V}$ . (c) An autocorrelation for a cell is shown. The time window between spikes is plotted, and the shortest delay from reference spike to the next spike is more than 2 ms (refractory period).

193 were in the maximum firing group. If more than one activity  
 194 field was found in the maze, the cell was not considered a  
 195 'place cell'. Cells were found that had several activity  
 196 fields within the maze. These cells were also included in the anal-  
 197 ysis of the firing activity difference in baited and unbaited  
 198 arms.

199 In the analysis of firing activity within each arm (Figs. 5b  
 200 and 7b), the highest activity was estimated. All arms were  
 201 evaluated for each cell. In this analysis, not only place cells  
 202 were included. More than one field of highest activity was  
 203 accepted, and a cell could have a high score in two arms or  
 204 more.

## 2.5. Behaviour

205

206 In experiment 1, the animals had to retrieve all pellets. 206  
 207 Visits of previously visited arms before all pellets were re- 207  
 208 trieved were counted as a working memory error. As the 208  
 209 animals were very explorative and continued to visit arms 209  
 210 after all eight arms had been visited, those visits were not 210  
 211 considered to be errors. In experiments 2 and 3, similar rules 211  
 212 applied. Repeated visits of previously visited arms before 212  
 213 the animals had visited all arms were considered working 213  
 214 memory errors. After day 1 of training in the new regime 214  
 (four arms baited, four unbaited), visits of arms that were

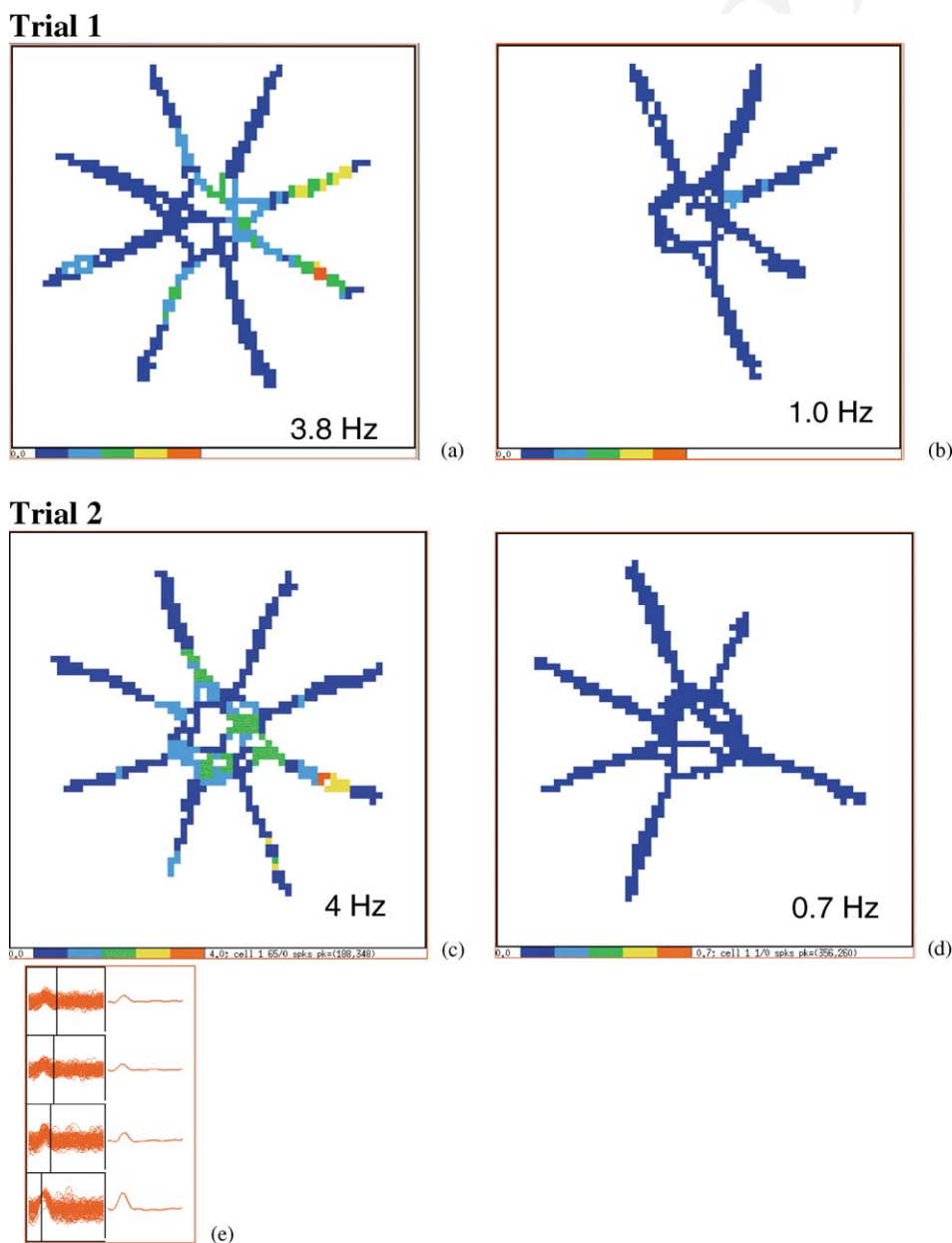


Fig. 3. Example of a cell that is active when the animal retrieves baited arms (a, first 60 s) and when the arms explores arms that are not baited any longer (b, second 60 s). Cell activity ceases in (b). This effect was reproducible in a second trial (c, first 60 s; d, second 60 s). (e) Spike traces for cell, overlay of traces left, average trace right. X-axis = 2 ms, Y-axis = 280 μV. The number shown in the boxes refers to the maximum activity of that cell in Hertz.

215 not baited before visiting all baited arms were considered  
216 long-term memory errors.

## 217 2.6. Statistics

218 The activities in the arms were identified and grouped  
219 into five bins: (1) 0–20% of firing rate, (2) 20–40%, (3)  
220 40–60%, (4) 60–80% and (5) 80–100% of the maximum  
221 firing rate. The scores for the baited arms in all five groups  
222 were compared with the scores for the unbaited arms. A  
223 one-way repeated measures ANOVA was performed with  
224 a post hoc corrected Bonferroni test to identify which of  
225 the five groups showed a significant difference between the  
226 baited and unbaited arms.

## 227 2.7. Histology

228 Animals were anaesthetised with urethane and the  
229 brains removed. The brains were fixed in a buffered 8%  
230 paraformaldehyde solution for 12 h. Brains were cut on a  
231 vibratome, mounted and stained with cresyl violet. Sections  
232 were then examined under a light microscope to identify  
233 the locations of the tetrodes.

234 All experiments were licensed under German and EC law.

## 235 3. Results

### 236 3.1. Experiment 1

237 All animals learned the task of retrieving the pellets after  
238 1 day (Figs. 1 and 2). An overall difference between work-  
239 ing memory errors was found by a one-way repeated mea-  
240 sures ANOVA ( $P = 0.0003$ ). The number of working mem-  
241 ory errors dropped to almost zero after the first day. During  
242 the 7 days of recording, a total of 67 cells were identified  
243 in six rats. Of these cells, 31 fit the criterion of a place cell  
244 as defined in this study. A place cell cannot have more than  
245 one area of highest activity (the place field) in our defini-  
246 tion, since the spatial location would not be unambiguously  
247 defined if there are several place fields per cell. A total of  
248 34 additional cells were found having more than one place  
249 field. Furthermore, cells were found that fired along the en-  
250 tire length of one or more arms. Again, such cells were not  
251 defined as place cells because the spatial selectivity would  
252 be too poor to be useful for orientation. Eleven place cells  
253 were found that fired in correlation with the location of the  
254 animals, but the activity ceased when all pellets had been  
255 retrieved and some arms were visited again. This effect was  
256 reliably reproduced up to four times (Fig. 3). To test for  
257 the effect of smell on the activity of these cells, arms were  
258 re-baited after the animal had retrieved the pellets. Again,  
259 the firing activity of the cells was low and did not increase  
260 after retrieval of a pellet, making it unlikely that neuronal  
261 activity was driven by the smell of the pellets or the feed-  
262 ing activity (maximum activity of 11 cells in first 60 s while

retrieving baits 7–17 Hz, in the second 60 s when no baits 263  
remained 0.7–1 Hz, after re-baiting the arms 0.8–1.2 Hz). 264

### 265 3.2. Experiment 2

266 The animals learned the new task in which only four 266  
arms were baited after 1 day (Fig. 4). Working memory 267  
errors were low, and no overall difference of working mem- 268  
ory errors over time was found in a one-way repeated mea- 269  
sures ANOVA ( $P > 0.05$ ). Long-term memory errors were 270  
high on day 2 but decreased subsequently. An overall differ- 271  
ence between long-term memory errors committed over time 272  
by the groups was found by a one-way repeated measures 273  
ANOVA ( $P < 0.001$ ) (Fig. 4). During the course of 8 days, 274  
46 cells were identified. Of these cells, 29 cells fitted the cri- 275  
terion of place cells. Of the remaining cells, 12 cells were ac- 276  
tive along the whole length of one or several arms (Fig. 5a). 277

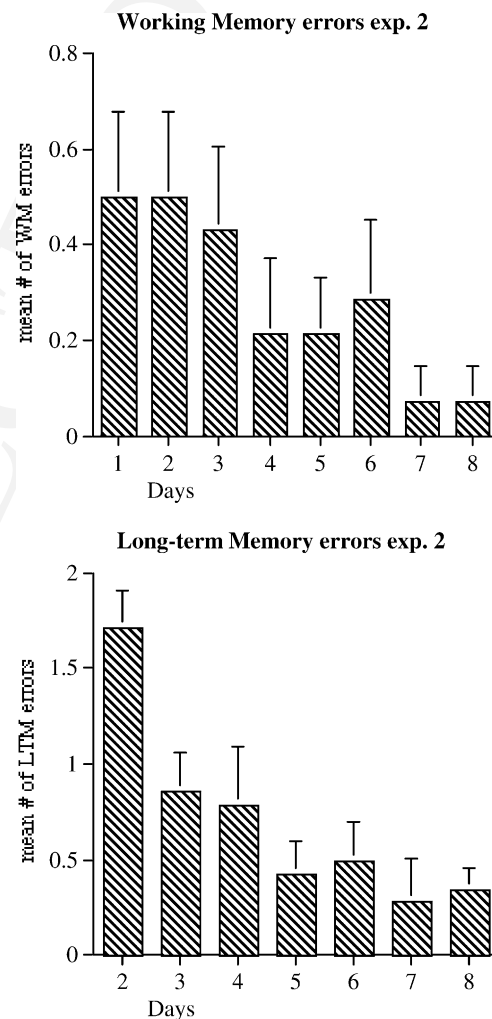


Fig. 4. Results of learning the experiment 2 test with four arms baited. The animals committed few working memory errors (no difference overall in a one-way ANOVA;  $P > 0.05$ ). On days 2–8, long-term memory errors were analysed. Animals committed more errors on day 2 compared with the other days (one-way ANOVA;  $P < 0.001$ ) with post hoc Bonferroni test,  $P < 0.01$  (day 1).

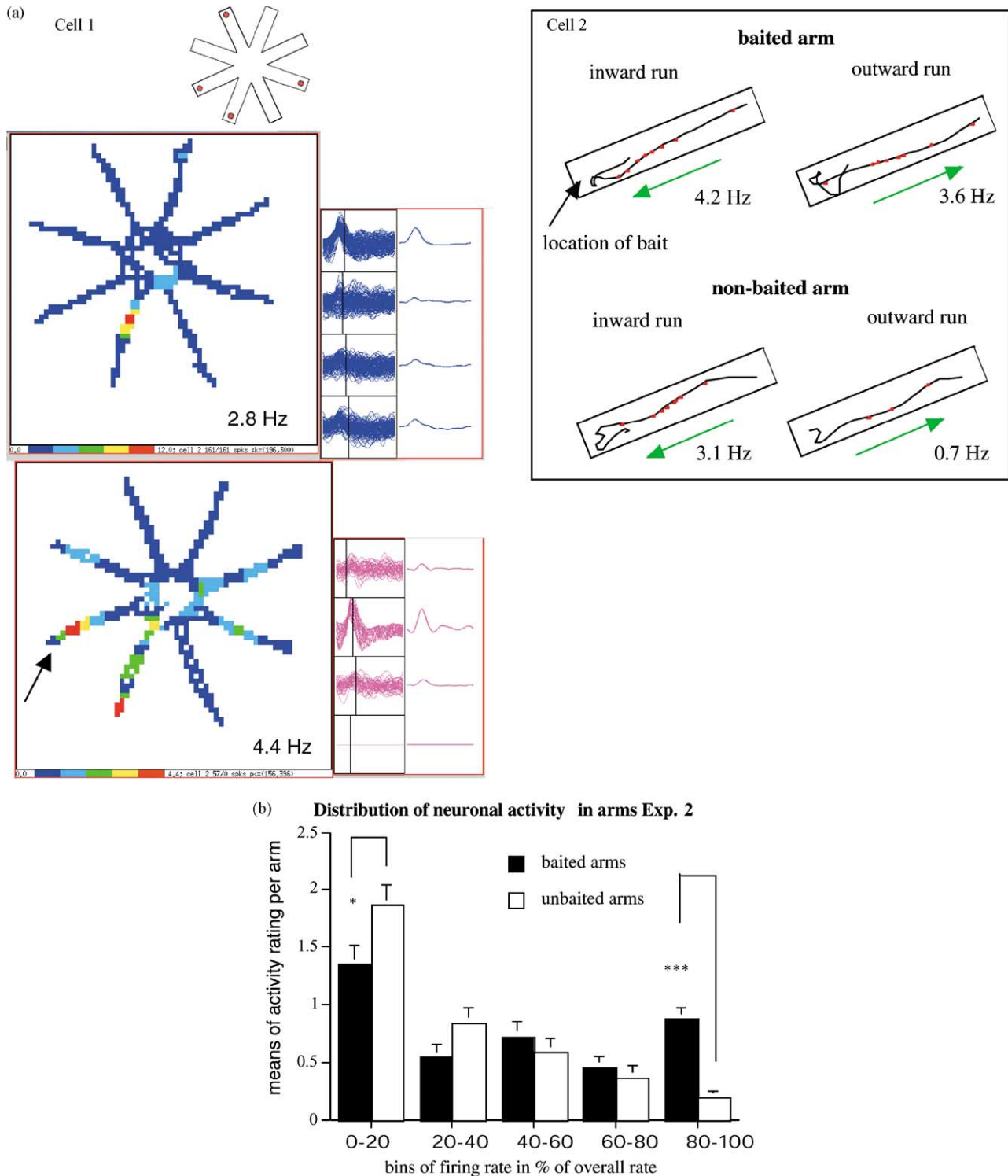


Fig. 5. (a) The baited arms are indicated by red dots in the small drawing of the maze. The number shown in the boxes refer to the maximum activity of that particular cell (top). Cell 1: example of a place cell in experiment 2. Shown is the normalised cell firing activity during the run in the 8-arm maze. Firing activity was binned in five colour-coded sections: 0–20% of maximum activity (blue), 20–40% (light blue), 40–60% (green), 60–80% (yellow) and 80–100% (red). The cell fired predominately in one area only (place field). The traces are shown on the right. X-axis = 2 ms, Y-axis = 280  $\mu$ V. Cell 2: shown is another cell with a less spatially defined firing field. The traces are shown on the right. Electrode 4 was the EEG channel and did not contain spike data. X-axis = 2 ms, Y-axis = 280  $\mu$ V. Samples of trajectories of visits of baited and non-baited arms are given. The traces are from arm 6, as indicated by the black arrow. The speed and direction of movement of the animal is similar, but the cell activity (red dots) when leaving the baited arm is reduced when leaving the unbaited arm. (b) Analysis of activity of all cells in each of the eight different arms. The neuronal activity of a given cell in each of the eight arms was normalised and binned in five different categories of activity (see Section 2 or (a)). Cells were included that had a distinct place field (place cells) and others that showed activity in more than one arm of the maze. Such cells could have high activity firing fields in more than one arm. A one-way ANOVA ( $P < 0.001$ ) with a post hoc Bonferroni test showed a significant difference between baited and unbaited arms. \* $P = 0.05$ , \*\*\* $P = 0.001$ .

Table 1  
Activity of cells in baited arms vs. unbaited arms in experiment 2 and after reversal of baited arms in experiment 3

Cells	Experiment 2		Experiment 3 (after reversal of arms)	
	Maximum activity in baited arms	Maximum activity in unbaited arms	Maximum activity in baited arms	Maximum activity in unbaited arms
1	2.2	1.7	3.0	1.2
2	4.1	1.9	4.2	2.8
3	1.5	3.7	2.1	3.3
4	0.2	2.0	1.7	2.1
5	3.1	2.4	2.2	1.3
6	2.0	0.3	3.4	2.2
7	1.1	2.7	0.5	1.5
8	2.8	2.0	4.5	1.1
9	4.5	1.0	0.3	1.8
10	3.2	0.7	1.7	2.9
11	1.1	2.2	3.6	3.0
12	4.0	1.6	3.1	1.7
13	1.8	2.7	1.3	2.1
14	3.4	0.2	1.4	2.4
15	4.6	2.3	5.7	1.5
16	1.1	3.2	1.8	2.4
17	7.2	1.0	0.3	2.1
18	0.3	1.8	3.1	0.4
19	3.1	0.7	2.5	3.2
20	1.7	3.1	1.8	0.2
21	0.4	1.9	2.1	1.7
22	1.2	2.3	1.2	2.0
23	4.8	1.1	2.7	1.7
24	2.0	2.7	4.6	1.0
25			2.3	1.2
26			0.4	2.1
Firing rate mean/S.E.M. of place fields	3.7 ± 0.34	2.6 ± 0.18	3.3 ± 0.29	2.3 ± 0.19
No. of place fields	13	11	14	12

Shown are the results from cells that had their main place fields in arms (other cells had place fields in the centre of the maze). Some of the cells shown here had place fields in several arms. Highest firing rates per category are shown here for comparison. While the number of place fields was not increased in baited arms, the firing rate of cells (normalised for numbers of place fields) was higher overall compared to unbaited arms ( $P < 0.05$ ,  $t$ -test). A more detailed analysis is shown in Figs. 5b and 7b.

278 No cells were found that fired exclusively in all baited arms  
 279 and never in the unbaited arms ('goal cells'). Furthermore,  
 280 the distribution of place fields of the identified place cells  
 281 was not biased for the locations at the end of the arms where  
 282 the food wells were located. Also, it was not found that  
 283 more place cells fired in baited arms only (11 cells fired  
 284 in baited arms, 13 in unbaited arms). The remaining place  
 285 fields were located on the central platform (Table 1). How-  
 286 ever, when analysing the firing activity of all cells (place  
 287 cells and cells that fired along entire arms) for all baited  
 288 arms (binned in five groups of increasing activity) com-  
 289 pared with activity in all unbaited arms, a clear bias for the  
 290 baited arms was visible (for details see Section 2; Fig. 5b).  
 291 Firing rates of cells are also given in a table for compari-  
 292 son (Table 1). Examples of trajectories and firing rates of  
 293 a cell when visiting a baited or non-baited arm are shown  
 294 in Fig. 5a. Running speed of animals was not different  
 295 when comparing speed in baited arms with speed in un-  
 296 baited arms (baited:  $64 \pm 19$  cm/s, unbaited:  $56 \pm 13$  cm/s;  
 297  $P > 0.05$ ).

### 3.3. Experiment 3

298  
 299 After changing the pattern of baited arms and unbaited  
 300 arms, animals again learned the new pattern after 1 day.  
 301 Working memory errors were low, and no overall difference  
 302 between working memory errors was found in a one-way  
 303 repeated measures ANOVA ( $P > 0.05$ ). Long-term memory  
 304 errors were high on day 2 but were reduced in numbers in  
 305 subsequent days. An overall difference of long-term mem-  
 306 ory errors committed over time was found by a one-way  
 307 repeated measures ANOVA ( $P < 0.005$ ) (Fig. 6). Over the  
 308 course of 7 days, 42 cells were identified; 22 cells fitted  
 309 the criterion for place cells (Fig. 7a). Firing rates of cells  
 310 are also given in a table for comparison (Table 1). As in  
 311 experiment 2, no bias for the baited arms was found when  
 312 analysing the locations of place fields. When pooling all  
 313 cells (place cells and non-place cells) and estimating the  
 314 cell activity in baited arms compared with activity in un-  
 315 baited arms, a clear bias for the baited arms was again vis-  
 316 ible (Fig. 7b).

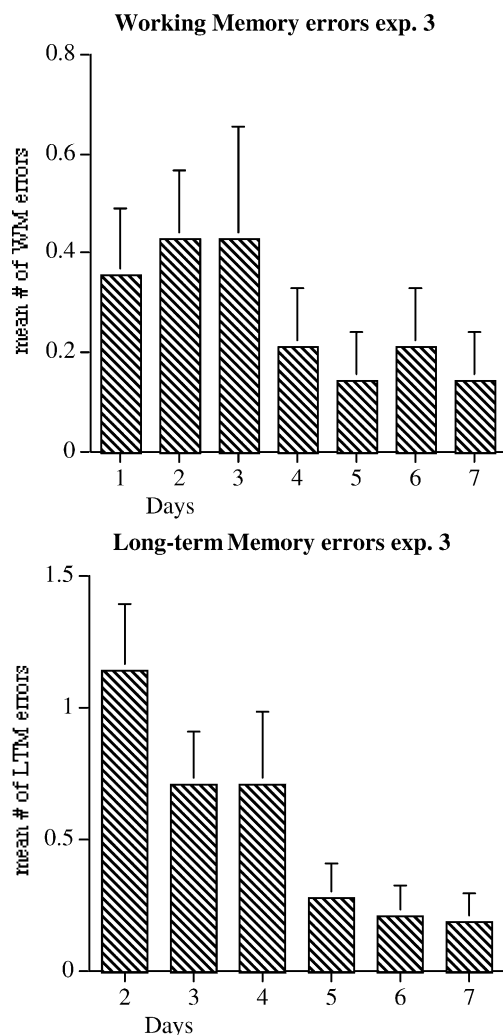


Fig. 6. Numbers of errors during learning the experiment 3 test with four arms baited. The animals committed few working memory errors (no difference overall in a one-way ANOVA;  $P > 0.05$ ). On days 2–7, long-term memory errors were analysed. Animals committed more errors on day 2 compared with the other days (one-way ANOVA;  $P < 0.001$ ) with post hoc Bonferroni test,  $P < 0.01$  (day 1).

#### 317 4. Discussion

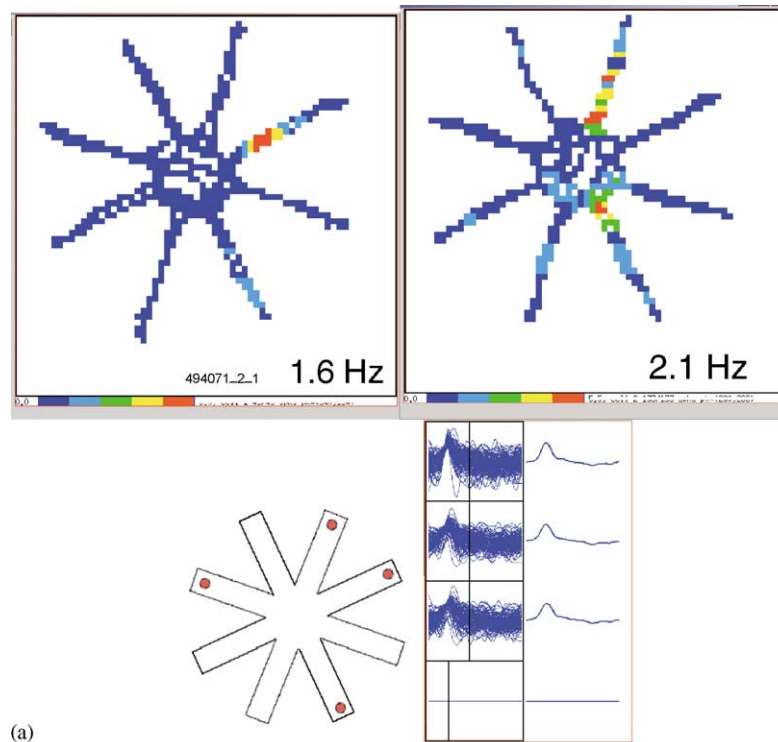
318 The experiments showed that neuronal activity in the hip-  
 319 pocampus was affected by the presence or absence of food  
 320 bait in the arms. When analysing the distribution of place  
 321 fields, it was not found that more place fields were found  
 322 in baited arms compared to unbaited arms. The main dif-  
 323 ference found was that firing rates increased when the arms  
 324 were baited compared to unbaited arms. In addition, cells  
 325 were found that fired in baited arms which stopped firing  
 326 when the bait had been retrieved. These results suggest that  
 327 the hippocampus does not receive specific information of  
 328 targets or food sources ('goal cells'). Otherwise, cells that  
 329 fired exclusively in baited arms but never in unbaited arms  
 330 should have been observed.

331 These results are in agreement with previous studies of  
 332 single cell recording in animals that explored radial-arm  
 333 mazes. Firstly, place fields were found that are very similar  
 334 to the place fields described by other researchers in  
 335 4-, 6- or 8-arm mazes [23,31,32,38,39,41]. Furthermore,  
 336 those studies never reported an obvious bias of place field  
 337 distribution towards baited arms. This is in agreement  
 338 with other studies that did not find a bias of place field  
 339 distribution towards baited arms or towards goal areas  
 340 [32,38,45].

341 Some place cells were found that were active when the  
 342 animal entered a baited arm but not when the arm was sub-  
 343 sequently re-entered without bait. Such cells are not classic  
 344 place cells as they appear to code a condition (baited versus  
 345 unbaited) in addition to the location of the animal in space.  
 346 These cells are comparable to cells found in a water maze  
 347 study [12] in which cells were described that fired when the  
 348 animals found the platform that was in the location where  
 349 they expected it, but that did not fire when the platform  
 350 had been removed yet the animals expected it to be there  
 351 ('match cells'). Such match cells have been found also in  
 352 a different study which used a very different set-up. In a  
 353 delayed non-match-to-sample task, cells were found that  
 354 fired when an expected cue had been presented, but did  
 355 not fire when this cue was missing [13]. This suggests that  
 356 information in the hippocampus is actively used for the de-  
 357 velopment of goal-directed behaviour, and not just for the  
 358 representation of space.

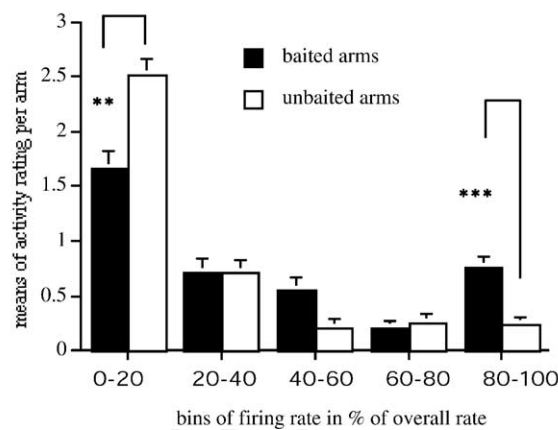
359 It is unlikely that smell was responsible for the observed  
 360 change in neuronal activity in the present study, as the pres-  
 361 ence of pellets alone did not activate the cells. It was shown  
 362 previously that some neurons in the hippocampus fired in  
 363 correlation to smell [37,43]; however, such cells did not  
 364 code spatial information and were not selective for partic-  
 365 ular locations of the rat. Also, motor related activity of  
 366 cells while chewing/picking up the pellet, etc. is not likely.  
 367 When adding pellets in the maze during the task to test  
 368 for olfactory coding of neuronal activity, no increased neu-  
 369 ronal activity was elicited when the animals picked up the  
 370 pellets.

371 Running speed also affects the firing frequency of place  
 372 cells [4]. If the running speed of animals is higher in  
 373 baited arms, then cells coding for those arms should fire  
 374 at a higher frequency. However, running speed was not  
 375 significantly increased. When including the activity of all  
 376 cells (place cells and non-place cells), it was found that  
 377 there was an increased firing probability of cells when  
 378 the animal entered baited arms. No cells were found that  
 379 fired in all baited arms, so that a selective coding for 'goal  
 380 arms' or a direct association between reward and loca-  
 381 tion within single cells is unlikely. Instead, it appears that  
 382 the firing probability of cells increased when the arm was  
 383 baited and decreased when the arm was not baited. This  
 384 is a modulatory effect, i.e. a shift towards more activity  
 385 rather than a shift of place cell distribution towards a target  
 386 area.



(a)

## Distribution of neuronal activity in arms Exp. 3



(b)

Fig. 7. (a) Example of a typical place cell in experiment 3. Shown is the normalised cell firing activity during the run in the 8-arm maze (for details see Fig. 5a). The cell fired predominately in an area of  $15\text{ cm} \times 15\text{ cm}$  (place field). The maximum activity of this cell is shown in Hertz (left). Shown is a cell that is active along a whole arm and has not developed a restricted firing field within one area (right). The baited arms are indicated by red dots in the small drawing of the maze. The traces are shown for the cell shown in the top right drawing. Electrode 4 was the EEG channel and did not contain spike data. X-axis = 2 ms, Y-axis =  $280\text{ }\mu\text{V}$  (bottom). (b) Analysis of activity of all cells in each of the eight different arms. Activity was normalised and binned in five different sections (see Section 2 or Fig. 5a). A one-way ANOVA ( $P < 0.001$ ) with a post hoc Bonferroni test showed a significant difference between baited and unbaited arms. \* $P = 0.01$ , \*\*\* $P = 0.001$ .

## 387 4.1. Memory correlates?

388 A number of studies in recent years have dealt with the  
 389 question whether cells in the hippocampus are involved in  
 390 memory formation. Since lesion studies have emphasised  
 391 the role of the hippocampus in learning and memory pro-  
 392 cesses [8,17,18,24,33–35], it is likely that single cell activity  
 393 would reflect such memory processes. In two studies, firing

activity of single cells in the hippocampus was found to be 394  
 dependent on which route the animal chose in a modified 395  
 T-maze [46] or in a W- or U-shaped maze [10]. In another 396  
 study mentioned before, a large percentage of place cells 397  
 coded for the location of a submerged platform in a water 398  
 maze [16]. The activity of such cells cannot be explained 399  
 by spatial information coding alone. The authors interpret 400  
 such cell activity as a correlate for episodic memory [5,25]. 401

402 The animals keep the information that was required to per-  
 403 form the tasks in memory. The increased cellular activity in  
 404 the study presented here could be interpreted similarly as a  
 405 memory correlate. An increase of neuronal activity has been  
 406 described in memory tasks before [6,22,26]. This could be  
 407 interpreted as a form of dynamic memory, in which infor-  
 408 mation is stored by active neuronal loops that retain the in-  
 409 formation. The rats in this study and in others rarely visit an  
 410 arm twice during one run when retrieving pellets [15,17,32],  
 411 showing that the animals keep in memory which arms have  
 412 been visited during the run. However, since the neurons only  
 413 showed increased firing in baited arms, it is unlikely that it  
 414 is a correlate for purely spatial memory, since all arm visits  
 415 should be kept in memory during the task. However, it is  
 416 feasible that the increased firing rate is the result or a combi-  
 417 nation of the 'reward' information with the spatial informa-  
 418 tion (the defined arms) within an episodic memory system.  
 419 The small increase of firing activity and the lack of selectiv-  
 420 ity of neuronal activity for baited arms only (no cells were  
 421 found that exclusively fired in baited arms only) makes this  
 422 hypothesis less likely.

#### 423 4.2. Correlates for attention or expectation?

424 The observed effect of an increase in firing probability in  
 425 neurons is consistent with previous studies that have inves-  
 426 tigated the effect of attention on firing rates. In these single  
 427 cell recording studies performed in primates, it was found  
 428 that neurons in the visual cortex which are driven by specific  
 429 visual stimuli will increase their firing rate when the stimu-  
 430 lus is of importance for solving a task, as compared to firing  
 431 rates evoked by the same stimulus in a different task where  
 432 it is of no relevance [3,11,42]. The neuronal mechanism for  
 433 this effect is presumed to be a suppression of non-relevant  
 434 stimuli that compete for activational influence of the neuron  
 435 (most likely via a top-down process) while at the same time  
 436 the relevant stimuli are amplified in their activational influ-  
 437 ence (perhaps via top-down processes but also by activation  
 438 from basal brain nuclei of the Ascending Reticular Arousal  
 439 System; see also [40] for a discussion). In the present study,  
 440 there was a comparable firing increase of cells that fired  
 441 preferentially in baited arms. Hence, the results presented in  
 442 the present study are consistent with the concept that the an-  
 443 imals paid increased attention to the baited arms, or that the  
 444 animals had an expectation of what they would encounter,  
 445 and that this modulating influence increased the firing rates  
 446 of neurons that process spatial information for those arms.  
 447 It has been shown that theta rhythm in the hippocampus in-  
 448 creases in amplitude during sensory stimulation [9,44]. This  
 449 suggests that hippocampal activity is modulated by arousal  
 450 and attention towards novel information. Furthermore, the  
 451 results presented here are in line with the results by Jef-  
 452 fery [19,20] who showed that not all salient landmarks were  
 453 equally effective in driving place cells, and that the efficacy  
 454 of landmarks in driving cells could change according to ex-  
 455 perience. Landmarks that were moved around in the pres-

ence of the animals and that were recognised by the animals 456  
 as mobile appear to be 'ignored' when orienting themselves 457  
 in space, while stationary landmarks continued to influence 458  
 place cell activity and appeared to receive more attention. 459  
 Interestingly enough, it has been shown in another study 460  
 that neurons in the hippocampus of mice do not necessari- 461  
 ly fire in a spatially selective manner if the animals move 462  
 around without having to solve a spatial problem. Only when 463  
 a reward was given in a defined location did neuronal ac- 464  
 tivity in the hippocampus become more spatially correlated 465  
 and place fields become more stable [21]. The authors in- 466  
 terpreted this finding as a shift of attention towards spatial 467  
 information. In a different study, place cells that coded spa- 468  
 tial information that was of importance for solving a spatial 469  
 task showed more stable and organised firing activity than 470  
 cells that coded spatial information that was not essential for 471  
 performing the task [47]. Again, these findings suggest that 472  
 attention or perhaps memory processes play an important 473  
 role in establishing and stabilising place cell activity. 474

In conclusion, it appears that the information which is 475  
 processed in the hippocampus is not exclusively spatial and 476  
 that neurons in the hippocampus are not 'hard wired' for 477  
 spatial information but are instead flexible to compute in- 478  
 formation that is task relevant and that the animals attend 479  
 to at the time. Attentional processes appear to support and 480  
 strengthen the activity of neurons that represent information 481  
 that is crucial for solving tasks. 482

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