Published in final edited form as:

Hippocampus. 2011 April ; 21(4): 347–353. doi:10.1002/hipo.20789.

MATURATIONAL DYNAMICS OF HIPPOCAMPAL PLACE CELLS IN IMMATURE RATS

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Abstract

The ontogeny of neural substrates underlying episodic memory is not well described. Place cells are a surrogate for episodic memory and are important for spatial navigation in rodents. Although place cells are well described in mature brains, the nature of the maturation processes remains uncertain. We now report on the ontogeny of the place cell system in rats between P22 and P43, a time during which there is rapid improvement in spatial behavior. We found that place cells with adult like firing fields were observed at the earliest ages. However, at this age, adult like place cells were few in number and their place fields were not stable across multiple exposures to the same environment. Finally, independently of confounding factors such as the number of exposures to the environment, the proportion of adult-like place cells, their firing rate and their stability increased with age and the average spatial signal of all pyramidal cells improved. This finding could account for the poor spatial behavior observed at young ages (P20-P30) and suggests that a small number of adult-like place cells are insufficient to support navigation.

The notion that hippocampus plays a critical role in the formation and storage of spatial memory is supported by the existence in rats (O'Keefe and Dostrovsky, 1971) and humans (Ekstrom et al., 2003) of neurons called place cells that encode the spatial location of the individual in the environment (Nadel, 1991; Rolls and Xiang, 2006). The place cell system has been suggested as a surrogate for episodic memory in humans (Eichenbaum et al., 1999) and place cell discharge properties provide useful access to the integrity of the networks supporting episodic memory. For instance, the signal quality of place cells (spatial coherence; (Kubie et al., 1990)) and the information content of their firing (Skaggs et al., 1993) provide information on the computational properties of these networks. In addition, place field stability across multiple exposures to the environment reflects the ability of these networks to consolidate information in memory. Currently it remains uncertain whether maturational processes are required for the establishment of a mature place cell network and if the timeframe of this maturation correlates with the already known development of spatial cognition. Establishing normal ontogeny of place cells is important as it may shed light on the phenomenon of infantile amnesia (Ruffman et al., 2001). In addition, neurological disease may impact developmental processes with long term adverse consequences and in

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Author Contributions

All authors designed and carried out the experiments. R.C.S and P-P.L.S carried out the data collection and carried out the analyses. All authors wrote and approved the manuscript.

order to investigate these impacts it is essential that normal development is precisely described.

The development of behaviors supported by spatial memory has been previously investigated in rats. The rat spatial navigation systems are not functional prior to P20 at which point spatial navigation skills emerge. There is subsequent maturation up to approximately P45 (Rossier and Schenk, 2003; Brown and Kraemer, 1997) following which there seems to be little further behavioral development. On the basis of the behavioral data, we hypothesize that there is rapid change in the place cell system between P20 and P45, a period during which much of the development of spatial navigation occurs. To determine the characteristics and evolution of place cell quality, proportion and stability in young ages, we studied the properties of populations of hippocampal neurons in developing rats from P22 to P43 across multiple exposures to the same environment.

Rat pups were housed in standard conditions with their mother (10–12 rats per litter) until P19 at which point they were weaned and housed 2 per cage. Ensembles of pyramidal neurons were recorded from arrays of 3 independently drivable tetrodes aimed at the dorsal hippocampal area CA1. Data were obtained in 19 rats aged between P22 and P43. Three 12 minute recording sessions, separated by 5 and 30 minutes respectively, were performed. Recording methods were as previously described (Lenck-Santini and Holmes, 2008). Briefly, single neuronal firing was related to the animal position in the environment which was divided into 1.4 cm x 1.4 cm pixels.

Firing rate maps were generated by dividing the number of action potentials recorded in each pixel by the time spent in the pixel.

Data were obtained from a total of 313 pyramidal cells, which were determined by their large width waveform ($>0.3\text{ms}$), their low firing activity ($<5\text{Hz}$ for the whole recording session) and their characteristic “complex spike” discharge in bursts. Cluster cutting was carried out to identify single units and the quality of the clusters was evaluated using isolation distance, (Schmitzer-Torbert et al., 2005) which did not change as a function of age ($\rho = -0.09$ $p = 0.10$, See Figure 1A). In addition, the value of F did not depend on age (Session 1-Session 2: $\rho = -0.008$, $p = 0.89$; Session 2-Session 3 $\rho = 0.05$, $p = 0.44$). Therefore, well-isolated clusters could be cut at all ages and waveforms were stable across recording sessions.

Initial exploratory statistical analyses were carried out using one-way ANOVA, Chi-square or Spearman correlation analyses. However, it is likely that the data from cells recorded from single animals is correlated (within – animal effects) and age bins created for ANOVA analyses were by necessity arbitrary and lead to artificial step changes in what is likely to be a continuous change in development. Therefore, for the definitive analyses we used generalized estimating equations (SPSS version 16; Chicago, Ill) in order to apply regression techniques to model the predictive value of age on place cell characteristics, adjusting for potential confounders and taking repeated measures across days into account. The first analyses investigated the relationships between age and the probability of a recorded pyramidal cell having characteristics of an adult-like place cell defined as a pyramidal cell with at least one place field (more than nine contiguous pixels with firing greater than the session global firing rate) and a spatial coherence greater than 0.3. We then investigated whether maturational changes occurred in pyramidal cells that do not have all of the characteristics of adult-like place cells with respect to coherence, size of the place field, and mean and peak firing rates. Other variables investigated as potential predictors were mean firing rate, isolation distance, number of days from implantation to first recording, whether the recording was during the first exposure to the environment or not, modal inter-spike

interval (ISI), bursting rate, burstiness and running speed. Burstiness and Modal ISI (the most prevalent ISI between 1 and 20ms) were computed on the basis of 1msec. bin ISI histograms. Burstiness was defined as the ratio of the number of action potentials belonging to a burst ($ISI \leq 10ms$) to the total number of action potentials emitted by a given cell (Harris et al., 2001).

Prior to carrying out stability analyses we investigated whether we could identify the same cell across sessions. To do this we developed a quantitative method allowing us to identify whether the same action potential (thus the same cell) has been recorded across recording sessions. The method is based on using the F-ratio derived from repeated-measures ANOVA analyses of the action potential amplitudes. We extracted waveform data (32 samples per action potential) from 50 randomly selected action potentials from the first session and then extracted data from another randomly selected 50 action potentials from the same recording. The waveforms from each wire of the tetrode were included in the analyses. The F ratio for the main effect of group was extracted for every cell at each recording session. This generates a distribution of F for waveforms that we know are from the same cell. The same procedure is then repeated for 50 action potentials at the first recording session and 50 action potentials at the following recording sessions. The F ratio is calculated as above (session 1 compared to session 2; session 2 compared to session 3). The highest observed F-value from the analysis in which we know the data are from the same cell is 9 and therefore putative cells which have an F ratio higher than 9 were excluded from the analyses (see Figure 3). The first stability analysis investigated the predictors of the proportion of adult-like place cells in one session but not in the other with emphasis on the effect of age. This was done comparing session 1 to session 2 and session 2 to session 3. The second analysis investigated the relationship between R_{max} and the R at 0° between session 1 and session 2, or between session 2 and session 3, as a function of age. We used circular statistics to compare the distribution of place field rotation angles between sessions S1–S2 or S2–S3. The mean rotation angle, its resultant length ($\bar{R}$), and concentration were computed. An ANOVA for concentration was then performed.

The proportion of cells which had characteristics of adult-like place cells increased with age (Chi-square = 6.8, $p = 0.03$). One of the most intriguing results of this study is that adult-like place cells were identified as early as P23. In addition, using a regression approach we found that pyramidal cells were more likely to be place cells with increasing age ($p < 0.001$, see Figure 1). Other factors found to independently predict the probability of whether a pyramidal cell was a place cell were increasing mean firing rate ($p < 0.001$) and increasing time from electrode implantation to initial recording ($p = 0.001$).

One of the criteria for considering adult cells as place cells is that their spatial coherence is greater than 0.3. However, it is possible that cells with lower values also carry spatial information. We analyzed the spatial coherence value of the 199 pyramidal cells that had a place field (see detailed methods) during the first recording session on any given day. Using the regression approach described above we found that coherence increased with age ($p = 0.001$; see Figure 2), with increased mean firing rate ($p < 0.001$), with increasing time from implantation to recording ($p = 0.037$), whether the recording was from the animals first exposure to the environment ($p < 0.001$), with shorter ISI ($p < 0.001$), a higher bursting rate ($p = 0.002$) and with increasing burstiness ($p < 0.001$). These effects were independent of each other. Information content also changed with age ($p < 0.001$), increasing time from implantation to recording ($p < 0.001$), with shorter ISI ($p < 0.001$), a higher bursting rate ($p = 0.002$) and with increasing proportions of bursting ($p < 0.001$), consistent with the spatial coherence findings.

Similarly, field size increased with increasing age ($p = 0.001$; see Figure 2), increasing mean firing rate ($p < 0.001$), whether the recording was from the animals first exposure to the environment ($p = 0.012$), with shorter ISI ($p < 0.001$), a higher bursting rate ($p = 0.037$) and with increasing burstiness ($p < 0.001$). There were also increases in the in-field and peak firing rates with age ($p < 0.001$ for both; See Figure 2) and with increasing time from implantation to first recording ($p < 0.001$ for in-field rate and $p = 0.09$ for peak rate). In addition there were significant correlations between coherence and peak firing rate ($\rho = 0.51$; $p < 0.001$) and between coherence and in-field firing rate ($\rho = 0.57$; $p < 0.001$). Although, as expected, average running speed increased with age this was not a significant predictor of any place field characteristics.

To assess stability we identified those cells that showed adult-like place cell characteristics in any of the recording sessions and then evaluated whether these cells retained those adult-like place cell characteristics across sessions. Adult-like place cells were more likely to have a place field at both session 1 and session 2 ($p < 0.001$) and at both session 2 and session 3 ($p < 0.001$) with increasing age of the animal. There were no other significant predictors identified. We also show that the correlation between rate maps (R) at 0° between session 1 and session 2 increased with age ($p < 0.001$, see Figure 3) as well as between session 2 and session 3 ($p = 0.001$, see figure 4E). We also investigated the maximum correlation ($R_{\max}$) between rate maps from different sessions after rotation against each other. There was an increase in $R_{\max}$ between session 1 and session 2 ($p < 0.001$, see Figure 3) and between session 2 and session 3 ($p < 0.001$, see Figure 3) with increasing age. No other predictors of stability were identified. Between session 1 and session 2, although the mean rotation angle was close to zero, the resultant length of the vectors was smaller in the youngest animals (P20 to P30) when compared to the older ones (P30 to P45; data grouped at 5 days intervals), which is attributable to a greater dispersion around the mean of the rotation angles ($F_{4,306} = 5.463$, $p < 0.01$). The same trend was observed between session 2 and session 3 transitions but didn't reach statistical significance ($F_{4,271} = 1.584$, $p = 0.11$).

Our results show that the place cell system undergoes a progressive maturation that parallels previously described ontogeny in spatial behavior. Prior to post-natal day (P) 20 rats are able to swim efficiently in a water maze but are unable to reliably find the platform (Schapiro et al., 1970; Rudy et al., 1987) until approximately P25 (Brandeis et al., 1989). Rats can navigate to a goal marked by a beacon at P15, but fail to reach the goal in the absence of cues directly associated with it (Rudy et al., 1987; Schenk, 1985). These studies suggest that rat spatial navigation systems are not functional prior to P20 at which point spatial navigation skills emerge. Following the emergence of spatial skills there is a maturation of those skills until adult function is reached at approximately P45 (Rossier and Schenk, 2003; Brown and Kraemer, 1997).

There is only one other published study dealing with place cell ontogeny (Martin and Berthoz, 2002). In that study it is reported that adult-like place cells were first observed at P40 prior to which place cells had large fields with a noisy background, and that the place fields were not stable. Only 11 place cells were recorded and their recording system had a low spatial resolution. In the current study we observed place cells with adult-like firing fields as early as by P23, but they only represented a small proportion of all place cells at the early ages. Therefore, our ability to identify such cells and to precisely document developmental trajectories is a direct function of our much larger sample size.

We have shown that the maturation of the place cell system is characterized by: 1) the presence of few adult-like place cells in young ages followed by an increase in their proportion with age, 2) an improvement in the average spatial signal with increasing age and 3) an increase of place field stability with age. It is important to note that these findings are

statistically independent of potential confounding factors such as multiple cells from individual animals being considered as independent observations, timing of surgical implantation with respect to when recordings began and whether a recording was from an initial exposure to the environment. Although firing characteristics of the recorded pyramidal cells were also important predictors of spatial coherence, field firing rates and field size, the age dependencies we report are independent of those firing characteristics. Nevertheless, these experimental factors did influence place cell characteristics and therefore they need to be taken into account in analyses and experiments that would minimize their effects need to be designed.

In order for an animal to build a cognitive map of its environment it needs multiple place cells to be active. At any given time, the animal's position within the environment is provided by the weighted average (weighted by firing rate) of the position of the field centers of active place cells (Muller, 1996). We have shown that during early development there are few active adult-like place cells which have small field sizes and low firing rates. This combination of findings would make spatial computation less accurate. It may be surprising that the size of fields increase with age. However, the observations that spatial behaviour improves with age suggests that the increase in field size is likely to be having a beneficial rather than harmful effect on normal development of spatial navigation. Nevertheless the mechanisms remain uncertain.

There is also an increase in the average spatial signal from all pyramidal cells with place fields, even if they do not meet the criteria for adult-like place cells. This would mean, at least at this age, that the place cell system is not binary (a cell either contributes to navigation or it does not), but rather a continuum. If this were true the immature rat would have access to information from many more cells than just adult-like place cells, but the information received would be of low quality. Overall, the poor spatial navigation ability of immature animals appears to be a combination of the lack of a critical mass of adult-like place cells, imprecision in the location specific firing of non adult-like place cells and low cell firing rates.

Another important aspect of the place cell system is that it is stable in the mature brain. In young rats place cells were less likely to behave as adult-like place cells in more than one session, and the inter-session stability in cells with place fields improved was poor. Thus, it appears that during development the networks subserving spatial memory become more able to accurately store and/or retrieve spatial representations.

Our precise characterization of place cell ontogeny is the first step in understanding the effects of childhood neurological disorders on the development of learning and memory abilities. Further studies addressing the effect of gender, sensory inputs and the relationships between place cells, head directions cells and grid cells are now required.

DETAILED METHODS

Place cell recordings

All rats underwent place cell recordings using methods previously described from this laboratory.

Electrode implantation—The multi-electrode arrays we used were manufactured in Robert U. Muller's Laboratory (State University of New York, Downstate Medical Center, Brooklyn, NY). The implant had 3 independently drivable tetrodes and one independently drivable EEG electrode. Electrode tips were surgically placed in the dorsal CA1 region of the hippocampus at different ages beginning at P20 using the appropriate co-ordinates

derived from Sherwood and Timiras (1970). Rats were anesthetized with 1.5% halothane in 1L/min of oxygen and then placed in a stereotaxic frame. The skull was exposed and three anchor screws were placed over the left olfactory bulb, the right frontal cortex and the right parietal cortex. A 2 mm hole was made in the left parietal bone and the dura was removed to expose the brain surface. The electrode tips were then placed immediately above the CA1 region. Sterile petroleum jelly was applied to the exposed brain surface and the electrode guide tube. Grip cement was applied onto the skull, anchor screws and around the electrode. Rats were given at least 24 hours to recover before recording.

Recording Chamber—The recording arena was a grey cylinder 50 cm in diameter and 51 cm high. It was placed on a plywood board painted grey. A sheet of white cardboard occupied 90 degrees on the inside arc of the cylinder. This apparatus was placed in a 2×2 m room separated from the experimenter and the electrophysiology set-up. It was illuminated by four 25 watt bulbs evenly spaced around the recording arena.

Electrophysiological and Position Recording—After a 24 hour recovery period the electrode arrays were checked four times a day for waveforms of sufficient amplitude while the rats were in their cage. Electrodes were advanced by 20 μm after each screening session until ripples and unit activity from CA1 started to appear. The electrodes were then advanced 10 μm or less, until one or more pyramidal cells with waveforms three times larger than the background ($>100 \mu\text{V}$) were isolated. It is not until pyramidal cells meeting these criteria were identified that recordings were started.

The signal from the electrodes was preamplified directly from the rat's head by operational amplifiers mounted as followers (Gain = 1). The signal was transmitted via a cable, through a rotating commutator (Neuralynx, Bozeman, MT). Electrophysiological and position data were acquired on a DigitaLynx (Neuralynx, Bozeman, MT) recording system. A red light emitting diode (120° view angle) was mounted on the head stage in order to track the animal location, via a CCD camera and a frame grabber sampling at 30Hz (DataTranslation, Marlboro, MA). Recordings with more than 1% aberrant position detection (undetected LED) were discarded. Single unit recordings were filtered (300–6000 Hz), amplified (gain = 5–10K) and sampled at 32 K Hz whereas EEG signals were recorded wideband (0.1–5000 Hz), amplified (gain = 100–1000) and digitized at 10 KHz.

Rats were food deprived for 12–24 hours prior to recording. They were placed in the recording chamber with crushed chocolate-covered cereals on the floor in order to encourage them to homogeneously explore the environment. Recording sessions lasted 12 minutes and each rat underwent 3 recording sessions per day. The first session was 5 minutes after the first and the third session was 30 minutes after the second. Between sessions the rats were removed from the recording environment and placed in their home cage without being disconnected. Meanwhile, the apparatus floor was briefly wiped with a wet sponge to eliminate eventual uncontrolled cues (odours or droppings). At the completion of the session, electrodes were advanced approximately 10 μm to reach different neurons. Recordings were carried out daily until no waveform obeying our recording criteria was detected.

Data Analysis—Unit discrimination was carried out using a cluster-cutting software (Cluster 3D, Neuralynx, Bozeman, MT). To discriminate between pyramidal cells and interneurons, we used the same criteria as previously described.

All data analyses were performed using Matlab (Mathworks, Natick, MA, USA). Firing maps were created using methods described previously. The environment was divided into 1.4 cm × 1.4 cm pixels. To determine the firing rate of each cell as a function of the animal

location, the number of action potentials recorded in each pixel was divided by the time spent in the pixel. Rate maps were then displayed. If the animal had not explored at least 90% of the environment then the rate maps were discarded from further analysis. A pyramidal cell was considered a place cell if: 1) it has at least one place field (more than nine contiguous pixels with firing greater than the session global firing rate); and 2) it has a local smoothness greater than 0.3. Local smoothness consists of the correlation coefficient between the rate in every pixel and the average rate in its neighbors. Since it results from a correlation coefficient, local smoothness was normalized using the Fisher transform. Several measures were used to describe possible changes in positional firing patterns: (i) Field size defined as the number of pixels included in the firing field (each pixel has an area of about 2 cm²); (ii) Field firing rate defined as the number of spikes emitted by the cell while the rat is in the region of the firing field divided by the total time spent by the rat in that region; (iii) Peak firing rate defined as the number of spikes emitted by the cell in the 9 most active and contiguous pixels of the firing field divided by the total time spent by the rat in these pixels;

(iv) Spatial information content was calculated as: $I = \sum_i \left(\frac{\lambda(i)}{\lambda} \log_2 \frac{\lambda(i)}{\lambda} p(i) \right)$ where $p(i)$ is the probability of being in pixel (i) , $\lambda(i)$ is the rate in pixel (i) and λ is the overall rate. λ , here is defined as $\sum p(i) * \lambda(i)$.

Pixel-by-pixel cross-correlations between firing rate maps were calculated as the corresponding positional firing patterns were rotated against each other in 2° steps. R at 0° was the cross-correlation before any rotation had occurred. The rotation associated with the highest correlation ($R_{\max}$) was considered as the rotation of the field between the two sessions.

Statistical Analysis—Initial exploratory analyses were carried out using one-way ANOVA for normally distributed data, Chi-square for categorical data or Spearman correlation analyses for non-normally distributed data. In these analyses the data from each cell was considered as an independent observation. However, it is likely that the data from cells recorded from single animals is correlated (within – animal effects) and that the changes with age are continuous (rather than falling into unique groups) in nature. Therefore, for the definitive analyses we used generalized estimating equations in SPSS version 16 (Chicago, Ill) in order to apply regression techniques to model the predictive value of age on place cell characteristics, adjusting for potential confounders and taking repeated measures into account. A robust estimator for the covariance matrix and unstructured working correlation matrix were used for all analyses. For binary outcomes (is an individual cell an adult-like place cell or not) a logit link function was used and for continuous outcomes a linear link function was used. Square root or logarithmic transformations of the data were used to ensure normal distributions of the continuous variables. Goodness of fit for the linear models was assessed by; (1) confirming the distribution of residuals was normal (visual and single sample Kolmogorov-Smirnov test) and (2) that when the residuals were plotted against the predicted values from the model they were randomly distributed around $y = 0$ and that the variance was constant. The model with the minimal quasi-likelihood under independence model criterion (QIC) was used as the final model.

Acknowledgments

This work was supported by grants from NIH (NINDS) NS044295, NS041595

Reference List

Brandeis R, Brandys Y, Yehuda S. The use of the Morris Water Maze in the study of memory and learning. *Int J Neurosci*. 1989; 48:29–69. [PubMed: 2684886]

- Brown RW, Kraemer PJ. Ontogenetic differences in retention of spatial learning tested with the Morris water maze. *Dev Psychobiol.* 1997; 30:329–341. [PubMed: 9142508]
- Eichenbaum H, Dudchenko P, Wood E, Shapiro M, Tanila H. The hippocampus, memory, and place cells: is it spatial memory or a memory space? *Neuron.* 1999; 23:209–226. [PubMed: 10399928]
- Ekstrom AD, Kahana MJ, Caplan JB, Fields TA, Isham EA, Newman EL, Fried I. Cellular networks underlying human spatial navigation. *Nature.* 2003; 425:184–188. [PubMed: 12968182]
- Harris KD, Hirase H, Leinekugel X, Henze DA, Buzsaki G. Temporal interaction between single spikes and complex spike bursts in hippocampal pyramidal cells. *Neuron.* 2001; 32:141–149. [PubMed: 11604145]
- Kubie JL, Muller RU, Bostock E. Spatial firing properties of hippocampal theta cells. *J Neurosci.* 1990; 10:1110–1123. [PubMed: 2329371]
- Lenck-Santini PP, Holmes GL. Altered phase precession and compression of temporal sequences by place cells in epileptic rats. *J Neurosci.* 2008; 28:5053–5062. [PubMed: 18463258]
- Martin PD, Berthoz A. Development of spatial firing in the hippocampus of young rats. *Hippocampus.* 2002; 12:465–480. [PubMed: 12201631]
- Muller R. A quarter of a century of place cells. *Neuron.* 1996; 17:813–822. [PubMed: 8938115]
- Nadel L. The hippocampus and space revisited. *Hippocampus.* 1991; 1:221–229. [PubMed: 1669368]
- O'Keefe J, Dostrovsky J. The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Res.* 1971; 34:171–175. [PubMed: 5124915]
- Rolls ET, Xiang JZ. Spatial view cells in the primate hippocampus and memory recall. *Rev Neurosci.* 2006; 17:175–200. [PubMed: 16703951]
- Rossier J, Schenk F. Olfactory and/or visual cues for spatial navigation through ontogeny: olfactory cues enable the use of visual cues. *Behav Neurosci.* 2003; 117:412–425. [PubMed: 12802871]
- Rudy JW, Stadler-Morris S, Albert P. Ontogeny of spatial navigation behaviors in the rat: dissociation of “proximal”- and “distal”-cue-based behaviors. *Behav Neurosci.* 1987; 101:62–73. [PubMed: 3828056]
- Ruffman T, Rustin C, Garnham W, Parkin AJ. Source monitoring and false memories in children: relation to certainty and executive functioning. *J Exp Child Psychol.* 2001; 80:95–111. [PubMed: 11529670]
- Schapiro S, Salas M, Vukovich K. Hormonal effects on ontogeny of swimming ability in the rat: assessment of central nervous system development. *Science.* 1970; 168:147–150. [PubMed: 5417059]
- Schenk F. Development of place navigation in rats from weaning to puberty. *Behav Neural Biol.* 1985; 43:69–85. [PubMed: 3994625]
- Schmitzer-Torbert N, Jackson J, Henze D, Harris K, Redish AD. Quantitative measures of cluster quality for use in extracellular recordings. *Neuroscience.* 2005; 131:1–11. [PubMed: 15680687]
- Skaggs, WE.; McNaughton, BL.; Gothard, KM.; Markus, E. An information-theoretic approach to deciphering the neural code. In: Hanson, S.; Cowan, J.; Giles, G., editors. *Advances in neural information processing systems*. Vol. 5. San Mateo: Morgan Kaufmann; 1993. p. 1030-1037.

Figure 1. Predicted probabilities that a pyramidal cell has adult-like place cell characteristics as a function of age. Each dot represents the mean predicted probability that a pyramidal cell, within any individual animal, has adult-like place cell properties. The lines join the dots from sequential recordings in the same animal.

Figure 2. Ontogeny of place cells. A). Examples of rate maps from different animals at ages varying from P22 to P35. B) Mean coherence (± 1 standard error) shown in age groups. There is a rapid increase in coherence up to approximately P30 following which the mean change in behavior across animals stabilizes. C–G) Mean predicted spatial coherence (C), field size (D), information content (E), in-field firing rate (F) and peak firing rate (G) within individual animals. Each dot represents the unadjusted mean predicted value in an individual animal on the day of recording and the lines join the dots from sequential recordings in single animals. Note that the y-axes of D–F are on a logarithmic scale. H) Isolation distance as a function of age. Each dot represents data from a pyramidal cell. Cells with an Isolation distance $< X$ were not included in the study.

Figure 3.

Maturation of stability in place cells. A) Tetrode activity recorded in a P23 rat. Rate maps, corresponding waveforms and F values from 4 simultaneously recorded units (Cells 1–4) from 2 consecutive sessions (Session 1 and Session 2). The rate maps across the sessions look remarkably different in cells 1,2 and 4. However, the waveforms remain stable as evidenced by their visual similarity and the low F values (see Methods). The waveforms from each wire of the tetrode are shown. Cell 5 shows a waveform excluded from subsequent analyses as the F-value was >9 (see statistical analysis section). This demonstrates that it is possible to reliably record the same units across sessions in immature rats. B) Examples of rate maps from 5 simultaneously recorded cells in a P24 rat and 5 simultaneously recorded cells in a P28 rat. Each cell is shown across the three recording sessions. The only cell in the younger rat that was stable was cell 3 between session 1 and session 2. In contrast cells 6, 9 and 10 are stable across 3 sessions, and cell 8 is stable across 2 sessions even though this animal is only 4 days older. C) 3-dimensional bar charts of the proportion of cells that have adult-like place cells at both session 1 and session 2 (top), and at both session 2 and session 3 (bottom) showing an increase in the proportion in the older ages. D) Changes in stability measures with age in individual animals; Mean predicted R at

0^0 in individual animals comparing session 1 with session 2 (top left) and session 2 with session 3 (top right). Mean predicted $R_{\max}$ in individual animals comparing session 1 with session 2 (bottom left) and session 2 with session 3 (bottom right). Each dot represents the mean predicted value in an individual animal on the day of recording and the lines join the dots from sequential recordings in single animals.

Table 1

Characteristics of the 19 rats included in the study.

ID	Age of Implantation	Age at Recording	Number of Cells
1	21	22,23,24,28,30,31,34,35,38	61
2	21	23,28,29,30,31,34,35	28
3	20	23,27	24
4	20	23,24	7
5	21	23,24,25,26	20
6	21	24,25,26,27,28,29	33
7	22	27,29,30,34	5
8	21	27,28	3
9	19	28,29	5
10	20	28,30,33	9
11	22	29,36	15
12	28	29,31,36,38,42	22
13	30	32,33,35,36,37	27
14	28	33	16
15	34	38	1
16	39	41,43	13
17	39	42	5
18	38	43	1
19	40	43	18