The development of the hippocampal neural representation of space
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The hippocampal formation (HF) contains a neural representation of the environment, based on the activity of several classes of neurons whose firing is tuned to an animal’s position and orientation in space. Recently, work has begun on understanding when and how this neural map of space emerges during development. Different classes of spatially tuned neurons emerge at different ages, some of them very early during development, before animals have started exploring their environment. The developmental timeline thus far uncovered has yielded insights into both the mechanisms of the ontogeny of the neural code for space, as well as how this system functions in the adult.

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Introduction
The rodent hippocampal formation (HF) contains several classes of neurons whose firing is tuned to an animal’s position and orientation in space, and which together constitute a neural ‘cognitive map’ \[1\] of the environment, by means of which an animal can locate itself and navigate to a goal. Four of these cell types, and their anatomical localisations, are illustrated in \textbf{Figure 1}: place cells, which code for a unique position in space \[2\]; head direction (HD) cells, which code for the current head direction of the animal \[3\]; grid cells, which fire in regularly repeating locations and may provide a distance metric \[4\] and boundary vector/border cells, which respond to barriers to movement \[5,6\].

Despite an intense research effort towards understanding how this neural code operates in the adult rat, until recently very little was known about how this neural map of space is assembled during development. During the first three weeks of post-natal life pronounced developmental changes take place in the rat HF at the cellular and physiological level \[7–9\]. Similarly, a repertoire of spatial behaviours emerges between two and three weeks of age: rats develop the capability for independent movement around post-natal day 13 (P13); followed by a drive to actively explore beyond their nest at P15–19; and from P20 onwards show learning on hippocampus-dependent spatial memory tasks (for a review, see \[10\]).

This article will review what is known about the development of the hippocampal neural code for space, focusing on work that ourselves and others have done mapping the emergence of spatially responsive neurons in the HF of immature rats, as well as the development of hippocampal network synchrony and oscillations. We will highlight what we have learned about the principles of hippocampal development, and how development has informed us about the functioning of this system in the adult.

\textbf{HD cells: the earliest spatial signal to emerge}  
The most striking aspect of the development of spatially tuned firing is the very early emergence of adult-like HD signalling. By P16, sub-populations of neurons in both pre-subiculum and the medial entorhinal cortex (mEC) are tuned to fire in a tight range of heading angles, as in the adult \[11\*\*\*\*\*\], see \textbf{Figure 2}a. The preferred direction of firing is stable between two sessions in the recording arena (separated by approx. 15 min), indicating that the HD system is capable of maintaining a directional ‘reference memory’ across at least this time span.

HD cells are adult-like before rats have extensively explored their environment \[10\], suggesting that some aspects of their development might be experience-independent. Consistent with this hypothesis, there is no correlation between the amount of experience in the recording arena and the quality of HD cell firing, and furthermore, HD cells appear to fire in an adult-like fashion from the very first exposure to the recording arena onwards, see \textbf{Figure 2}b \[11\*\*\*\]. The early maturation of the HD system, in comparison to the other spatially tuned cells investigated so far (place and grid cells, see below) also suggests that it may be a ‘primary’ spatial signal, not dependent on the input of any other hippocampal spatial cells for its development, possibly reflecting the origin of the HD signal outside the HF (see \textbf{Figure 1}b). This would also be consistent with the
Spatially tuned neurons and their anatomical localization in the hippocampal formation (HF). (a) Examples of (from left to right): a place cell; a head direction (HD) cell; a grid cell; a boundary vector cell (BVC). For HD cells, the polar plot represents the firing rate (action potentials/seconds of dwell time) for each directional heading; the peak firing rate is shown at the top-left corner. For all other cell types, the false-colour firing rate map represents an overhead view of the recording arena. When the rat visited those positions shown in hot colours, the firing rate was high. Peak rate (action potentials/seconds of dwell time) is shown in the top-left corner. Grid cell example thanks to Caswell Barry, BVC to Colin Lever. (b) Schematic of anatomical structures discussed in this review, showing the major connections and direction of information flow between them. See [64] for an extensive anatomical description of the HF. Colours represent the localization of spatially tuned neurons: green, HD cells; red, place cells; blue, grid cells; brown, boundary vector/border cells. Those structures within the green dashed-line ellipse lie outside of the HF, but represent the principal pathway for the generation of the HD cell signalling and its transmission to the HF, see [65].

Abbreviations: ADN, antero-dorsal nucleus of the thalamus; CA, cornu ammonis; DG, dentate gyrus; DTN, dorsal tegmental nucleus; mEC, medial entorhinal cortex; LMN, lateral mammillary nucleus; Para, parasubiculum; Pre, pre-subiculum; Sub, subiculum.

The development of grid cell firing

The adult mEC contains both HD and grid cells, potentially encoding the two different spatial parameters (direction and distance) thought to be necessary to support path integration [18]. It is noteworthy, therefore, that there is a marked delay between the developmental emergence of these two cell types: the very earliest age at which stable, adult-like grid cell firing is consistently observed is P20 [11**,12**], see Figure 2e; roughly a week after adult-like HD cells can be recorded. This corresponds to an age when exploratory behaviour is present, and animals are starting to show learning in hippocampus-dependent spatial memory tasks [10]. Before this age, some spatially localized firing can be observed in the mEC, including some multi-peaked firing fields. However, this spatial firing is not arranged with the regularity and hexagonal symmetry of adult grid cells, and, just as importantly, the location of the firing fields is not stable, either within or between recording sessions [11**,12**], and therefore could not support path integration. Other adult-like characteristics of mEC firing, such as the existence of ‘conjunctive’ (combined grid and HD tuning) cells, and speed-modulation of grid cell firing [18], also emerge at around P20 [19*], suggesting a relatively rapid emergence of an adult-like grid cell network.

The development of grid network connectivity

Adult grid cells are arranged in functional ‘modules’ [4,20,21]: within each module all grid cells share the same wavelength and orientation, and the relative spatial phases of the grid fields remains fixed, even though the absolute position of their firing peaks (phase) can change [22]. This coherent spatial structure is indicative of grid cells (or their inputs) being recurrently connected with a pattern of excitation and inhibition whereby cells excite those other cells with similar spatial phases, and inhibit those with dissimilar phases. This finding has served as important supporting evidence in favour of continuous attractor models of grid cell network structure [23,24*,25,26]. A recent study found no evidence of direct excitatory connections between mEC layer II stellate cells (putative grid cells), but did find disynaptic inhibitory connections between them: such connections could create a network with a continuous attractor structure [27*].

When does this network connectivity emerge during development? It appears to be present concurrently with the first stable grid cells: simultaneously recorded grid cells share the same wavelength and orientation already at P20 [11**]; furthermore, when pups are transferred between different recording arenas, absolute spatial phases change, but all relative spatial phases are preserved, as in the adult [19*,22], see Figure 2d,e. In light of this, it is also interesting that increased network synchrony in the mEC in vitro emerges from P22 onwards, roughly coincident...
with the emergence of grid cells in vivo [12**], see also [27*]. These data suggest that a stable grid cell network emerges during development as a coherent unit, and that recurrent connectivity might be a pre-requisite of stable grid firing. A further test of whether adult-like recurrent connectivity exists in young pups would be whether the relative spatial offsets between grid cells’ firing are preserved even when individual grid fields are deformed and less symmetric, such as when rats encounter a novel environment for the first time [28], as is the case in adult rats [29].

Interestingly, whilst recurrent connectivity in the grid cell circuit was traditionally a feature of continuous attractor models of grid cell firing, several groups working on the other major class of grid cell model (‘oscillatory interference’ models, see ‘Oscillations and timing,’ below) have also incorporated this type of recurrent connectivity into their models [30,31; Bush & Burgess, abstract P2-D-118 at British Neuroscience Association 2013]. Importantly, whilst the existence of recurrent connectivity between grid cells is consistent with continuous attractor models, it still does not speak to the existence of the additional, necessary component of these models, namely a set of asymmetrically weighted connections matching the animal’s speed and direction of movement which shift neural activity in line with changes in the animal’s position, see [24*], for example, for further details.

Modelling the development of the grid cell network has so far received less attention than modelling the adult state of the system. It has been proposed that recurrent connectivity could be created intrinsically, through synchronous waves of coordinated neural activity [24*]. However, this assumes a topographical mapping between grid cell phase and anatomical position at some point in development, for which there is currently no evidence. Other attempts at modelling the emergence of grid firing posit that the required network connectivity could be learnt during the initial exploration of the environment, and therefore be experience-dependent. In these models, the learning process depends on some pre-existing spatially tuned input, either place cells [32; Widloski & Fiete, abstract 100.4/KKK20 in Soc. Neuro. Abstr 2010], or repeating unidirectional bands [33*], see also [34]. It should be noted that an analogous model of HD cell development has been proposed, according to which the connections in a continuous attractor network are learnt using an instructive input from stable visual landmarks [35*].

In the adult mEC, a topographic mapping exists between the anatomical position of grid cells along the dorso-ventral axis, and the scale of space that is encoded by their grid fields; grid cells belonging to modules with short wavelengths lie towards at the dorsal tip of the mEC, and those with long wavelengths lie more ventrally (though there can be considerable anatomical overlap between different modules) [4,20]. Little is currently known about which anatomical features underlie this modular functional arrangement, or how they emerge during development [20]. During development, almost all grid cells recorded between P20 and P30 were of a similar, short, wavelength (approximately 30 cm) [11**,12**]. It is tempting to speculate that short wavelength grid modules develop first, allowing the animal to map out small-scale space at the beginning of its independent life, and that the neural code for larger spaces develops later, as rats become capable of moving over larger distances.

Place cells
The maturation of the CA1 place cell network follows a slow and gradual trajectory. Spatially localised, stable place fields exist as early as P16, but the quality of spatial tuning and stability continue to improve until much later in life, at least P45 [11**,12**,36**,37], see Figure 2f. This developmental programme contrasts with that of HD and grid cells, which appear to emerge in adult-like form relatively abruptly (within a few days). However, it is important to stress that, at any given age, the CA1 region will contain a heterogeneous set of place cells at different developmental stages [36**], and that fully adult-like place cells can be seen as early as P16 [11**], Figure 2g. Similarly to HD and grid cells, the maturity of place cell firing is not correlated with the amount of experience of the recording arena [11**], suggesting that the differences between cells are due to intrinsic factors rather than experience-dependent learning.

The existence of adult-like place responses as early as P16, in the absence of grid cell firing in the mEC, was the first demonstration that grid cells are not a necessary input for place cell firing (see also [38,39] for further evidence in adult rats). The question then arises as to what are the inputs driving early place cell firing. One intriguing possibility is that this input is provided by cells which fire in response to the presence of boundaries at a specific distance and direction from the animal, such as border cells in mEC [5] or boundary vector cells in the subiculum [6]. Preliminary evidence indicates that such responses can be recorded early during development, at least as early as P17 in both mEC and subiculum [Bjerknes et al., abstract 702.09/ DDD25 in Soc. Neurosci. Abstr 2012; Cacucci et al., abstract in Soc. Neurosci. Abstr 2013]. If boundary-responsive cells are providing the main input to place cells early in development, it should be possible to detect differences in place cell firing near to boundaries, as opposed to in the middle of an open field, in young pups. In support of this hypothesis, preliminary evidence indicates that in pre-weanling pups, the spatial stability of place field position is correlated with
The development of spatially responsive firing in rat HF neurons. (a) Three HD cells recorded from mEC at P16 (left), and from adults (right). Each row represents one cell, each column a separate recording trial (separated by 15 min). Note the similarity between P16 and adult HD cells, in terms of directional selectivity and the stability of the preferred direction of firing. (b) A HD cell recorded at P16, during the first exposure to the recording arena.
the distance of the field from the nearest wall of the recording arena [Cacucci et al., abstract in Soc. Neurosci. Abstr 2013]. The boundaries of an environment may therefore provide a foundational input for hippocampal place cells, consistent with the boundary vector cell model [40], which originally proposed that environment boundaries are a critical determinant of place cell firing.

**Oscillations and spike timing**

The rodent HF exhibits several prominent synchronised and rhythmic signals, which are related to the concurrent behaviour of the animal [41]. As discussed below, these signals all develop early, before animals are capable of self-locomotion, suggesting that they may be a pre-requisite for the development of spatially tuned firing.

The earliest adult-like activity in the local field potential (LFP) are large, slow deflections which reverse polarity across the CA1 pyramidal cell layer, seen from P2 onwards [42,43]. These are consistent with the Large Irregular Activity (LIA) state of the adult hippocampus, seen during rest and immobility [41], and may also correspond to the Giant Depolarising Potentials [44] seen in immature hippocampal slices in vitro [42]. These large, synchronous activity bursts are thought to be required for the development of network connectivity in the HF [45]. The 140–200 Hz fast oscillations (‘ripples’), which accompany LIA sharp waves in the CA1 layer [1,46], emerge later, at around P7 [43].

In the adult, movement and exploratory behaviour are accompanied by a 7–12 Hz (theta) oscillation in the hippocampal LFP [41], which is first observed during development at P8 [47,48]. The frequency of theta is lower in the youngest pups (5 Hz), and gradually increases to adult levels [11*,12*,47,48]. Gamma oscillations (30–100 Hz), which co-occur with theta in the adult HF, are first observed at P2, and increase sharply in power after P8 [48]. Immature rats spend a large proportion of their time in a behavioural state known as ‘active sleep’ characterised by twitches of the limbs, head and eyes [49]. Active sleep muscle twitches are associated with neuronal firing in the hippocampus, and with the first bouts of theta and gamma oscillations between P2 and P8 [48], see Figure 3a, highlighting the importance of peripheral input from the sensory-motor system in HF development [50*].

The firing of adult place cells is modulated at theta frequency, but at a slightly higher frequency than the ongoing LFP theta, with the result that the timing of spikes, relative to LFP theta phase, changes as animals traverse each place field (‘phase precession’) [51]. As the theta phase of spiking correlates better with distance travelled through the field than with other variables, it may provide a temporal code for space, in addition to the rate code provided by the place field [52]. Theta-band modulation and phase precession are features of grid cell, as well as place cell, firing in rodents, and these phenomena originally inspired the ‘oscillatory interference’ models of spatial firing [30,31,53,54]. The common theme of these models is that the frequency difference between two theta-band oscillators, one of whose frequency is modulated by velocity, serves to track the distance travelled by the animal and thereby generate spatially stable firing fields.

During development, both grid and place cell firing are consistent with these models: both are modulated within the theta band, at a slightly higher frequency than the LFP theta, at the earliest ages at which these spatial responses can be observed [11*,12*, see Figure 3b,c. Phase precession appears at least as early as P17 in place cells [11*,12*], indicating that this temporal aspect of spatial coding, in the hippocampus, is adult-like during earliest exploration (see Figure 3d).

Intriguingly, recent evidence shows that, during laboratory foraging tasks, some species of bats show very little theta modulation in the HF, both in the LFP and in neuronal spiking. They nevertheless display the same spatially tuned neurons as those described in rodents [55,56], at odds with the idea that these responses would be generated via theta-band oscillators (but see [57]). A cross-species, comparative approach may therefore be fruitful to fully understand the development of spatial coding in mammals.

(Figure 2 Legend Continued) The central firing rate map shows all data from the recording trial, the four firing rate maps at the corners show the directional firing filtered by which quadrant of the recording arena (in 2D space) the rat was occupying. As for adult HD cells, the cell always codes for the heading direction of the animal, regardless of 2D position. (c) mEC cells recorded at P16–17, P20–21 and from adult rats. Each row shows a cell, each pair of columns a trial. For each trial, the left column shows the firing rate map, the right column a spatial auto-correlogram of the firing rate map, which highlights hexagonal grid structure. Note the absence of stable and regularly symmetrical grid firing fields at P16–17. Note also that, for grid cells only, adult recording arenas are larger than those for immature rats: rate maps are shown to scale. (d, e) Simultaneously recorded grid cells have a coherent spatial structure, which is maintained in all environments. (d), left column, shows three grid cells simultaneously recorded at P22. All cells share the same wavelength (spacing between fields) and orientation. In a different environment (right column, ‘Recording Arena B’), the absolute position of the grid fields changes, but the relative offsets between grid fields remains the same, as highlighted in the cartoon version of the same data (e). Coloured circles indicate correspondence between cells in (d) and (e). (f) Three place cells recorded at P16–17, P20–21 and from adult rats. Note the gradual improvement in the specificity of spatial tuning, and the stability of the place field position. (g) Four examples of adult-like place cells, recorded at P16–17.

All data adapted from [11**] except (d), adapted from [19].
Conclusions

The development of neural systems relies on a variety of different mechanisms, including molecular cues, intrinsic neural activity and environmental experience [58–61]. Studying the developmental timeline of spatially tuned neurons (Figure 4) has shown which spatial responses occur before the first active exploration of the environment, and may therefore not require this experience (e.g. HD cells) and those which instead emerge later and may therefore require experience-dependent learning (e.g. grid cells). Further work will be required to understand how these different mechanisms interact during development.

Studying the development of the hippocampus has also yielded important insights into the organisation of spatial firing in the adult rat. One clear example is the finding that adult-like place cell firing precedes the emergence of grid cell firing during development by at least one week, demonstrating that other types of input are sufficient to support place cell responses during this developmental window. Further studies are required to substantiate the intriguing first indications that place fields may be built on the foundations of responses to the borders of the environment. If this were the case, it would represent a striking confirmation, and extension, of the boundary vector cell theory, which was proposed even before the
The experimental description of border and boundary vector responses in the adult HF [40].

The developmental pattern of grid cell emergence also speaks to the ongoing debate regarding how the grid cell signal is generated in adults. At present, the developmental data argue for the importance of elements from both the continuous attractor and oscillatory interference classes of models (recurrent connectivity and theta-band modulation of spiking, respectively), and is consistent with a current generation of models that include both of these elements [26,30,31]. We would hope that the experimental study of the development of spatially tuned neurons in the HF will also encourage theoreticians to incorporate biologically plausible developmental mechanisms into their models.

A striking aspect of the development of the neural map of space is the dissociation between the developmental programmes of different cell types. Grid cell networks emerge abruptly (over a small number of days), in an all-or-none fashion [11**,12**,19]. CA1 place cells, by contrast, exhibit a protracted development (over the course of weeks), and different cells mature at different ages [11**,12**,36**]. The underlying reasons for this dissociation are not currently understood. Most physiological and anatomical characteristics of the CA1 are mature by three weeks of age [7,8,62,63]; therefore there is no obvious candidate mechanism to explain the protracted functional development of place cell firing. Furthermore, little is known of the anatomical and physiological development of the parahippocampal regions where grid cells are found. One speculative hypothesis would be that abrupt or gradual developmental programmes are determined by the network architectures underlying these systems in the adult brain: the recurrent attractor network of the grid cells might necessarily form in an ‘all-or-none’, abrupt fashion, whereas in the CA1, the lack of extensive recurrent connections means that different place cells can mature independently, at different ages.

Finally, it is also tempting to speculate on the functional links between the development of spatial firing and spatial behaviour: as discussed, the emergence of adult-like HD firing precedes the onset of active exploration, possibly supporting directional path integration during the crucial first forays outside the nest; whilst it is also intriguing that the onset of hippocampus-dependent spatial learning coincides with the emergence of grid cells (for further discussion, see [10]). We believe that the studies outlined in this review provide the first steps towards an integrated understanding of the development of spatial cognition, from the neural circuit to the behavioural level, and will inspire further work in this field.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

● of special interest
●● of outstanding interest


This study maps the developmental emergence of HD, place and grid cells. By staggering the starting age of testing, the authors were also able to dissociate developmental changes from effects of experience.


This study also reveals the development of spatially tuned neurons in the HF. The authors also showed that mEC network synchrony, when tested in vitro, develops during the same developmental period.


Demonstrates that rat pups can use path integration to return to their nest, even during the very earliest exploratory forays.


A more detailed examination of the emergence of grid cells, showing that important characteristics of adult-like firing emerge at P20-P22. Also includes data from mEC cells as they make the developmental transition to becoming grid cells.


Comprehensive review of the functional principles of continuous attractor networks, as they apply to grid and HD cell firing. Proposes a mechanism for grid cell network development based on intrinsic activity early in development.


In vitro study investigating the nature of connectivity between mEC layer II stellate cells (putative grid cells). Authors report the absence of direct excitatory connections between these cells, but demonstrate the existence of disynaptic inhibitory connections, and suggest that these may also support a continuous attractor network. This pattern of connectivity emerges after P16.


33. Pilpy PK, Grossberg S: Spiking neurons in a hierarchical self-organizing map model can learn to develop spatial and temporal properties of entorhinal grid cells and hippocampal place cells. PLoS ONE 2013, 8:e60599.

Network model proposing that input from repeating unidirectional bands could combine to form grid cells during development.


Network model of HD cell development, proposing that the connectivity underlying the various attractor networks could be learnt by combining vestibular signals with input from a stable visual landmark.


Shows that the first developmental emergence of theta and gamma rhythms between P2 and P12, as well as HF neuronal spiking, occurs concurrently with bouts of whole-body muscular activity during active sleep.


