Developmental neuroscience: neural activity drives maturation of hippocampal neural circuits for navigation.

Francesca Cacucci[1], Patricia Salinas[2], Thomas J. Wills[2]

New research reveals that neural activity is required for the post-natal maturation of hippocampal neural circuits underlying memory and navigation. Activity-dependent maturation occurs sequentially along the classic 'tri-synaptic' pathway, following the direction of information flow found in the adult hippocampus.

During development, billions of neurons are generated making thousands of synaptic connections that are assembled in complex circuits. Understanding how these circuits form remains a key research frontier in neuroscience. Two main mechanisms guide circuit development: some require neural activity (e.g. the firing of action potentials) while others proceed independently of it. A new study by Donato et al[1] delineates for the first time the role of neural activity in the maturation of hippocampal circuits during post-natal development.

The hippocampal formation is known to support spatial memory and navigation in vertebrates. It contains neurons whose activity is modulated by an animal's position and orientation in space, such as place cells (coding for position), head direction cells (coding for orientation) and grid cells (whose regularly repeating firing may code for distance travelled)[2]. Anatomically, the hippocampus can be subdivided into hippocampus proper (dentate gyrus [DG], the cornu ammonis [CA] fields 1-3, and subiculum), and the parahippocampal complex, which includes the medial and lateral entorhinal cortices (mEC and lEC, respectively). Information flow through the adult hippocampal formation is classically portrayed as uni-directional, moving from the periphery through the superficial layers of the EC, to the DG, CA3, CA1, subiculum, before finally reaching the deep layers of the EC from where information is broadcast to the rest of the cortical mantle (figure 1A)[3].

Donato et al report that neuronal maturation in the mouse hippocampus occurs in a sequential manner, which (almost) faithfully follows the direction of information flow through this circuit (Figure 1B). The authors use the levels of doublecortin (DCX), a micro-tubule associated protein, as a marker of immature neurons. Stellate neurons in mEC-L2 are the first to silence DCX expression around postnatal day 14 (P14), followed by L2 pyramidal cells and the CA3 (P20), then CA1 (P23), subiculum, DG, mEC and lEC L5 (all P26). Finally, the lEC L2 matures from P30. Consistent with this sequence of neuronal maturation,

the authors also report that levels of Bassoon (a pre-synaptic marker) and parvalbumin (PV, which labels a subset of GABAergic neurons) increase sequentially along the transverse hippocampal axis, suggesting that synaptogenesis and the maturation of interneurons also proceed in the same unidirectional pattern. The article also confirms previous reports of the existence of a dorso-ventral gradient of maturation within the mEC[4], with glutamatergic (stellate and pyramidal cells) and PV+ neurons in the most dorsal part of mEC being the first to mature. An interesting question that remains to be addressed is whether the morphological maturation of neurons (dendritic branching and spine formation[5]) also follows the same step-wise pattern observed by Donato et al. Surprisingly, the timing of emergence of the spatial tuning of hippocampal neurons, which occurs concurrently to the wave of maturation described by Donato et al (2^{nd}-3^{rd} post-natal week) does not follow this entorhinal-to -subiculum maturation pattern. Indeed, parahippocampal and sub-cortical head direction cells emerge first, followed by place cells in CA1, with grid cell in superficial mEC emerging latest of all[6, 7]. It would therefore be of great interest to understand how changes in DCX, Bassoon and PV expression levels, reported by Donato et al, tie in to the broader picture of hippocampal development.

There are two notable exceptions to the parallels between the direction of the maturational cascade and information flow observed in the adult hippocampus. The first is the DG, whose maturation is completed much later than in CA3 (its post-synaptic target). These data confirm the distinct and protracted nature of DG development in the rodent [8]. The second exception is the lEC which contributes as much as the mEC to the perforant path input to the hippocampus proper, and yet matures last (compare figure 1A, 1B). The late maturation of the lEC is even more remarkable given that, during embryogenesis, its neurons are born *before* those in the mEC, and the rest of the hippocampal formation[9]. This dissociation between neurogenesis and maturation across the mEC and lEC might reflect their different embryonic origin, as the mEC (similarly to the rest of the hippocampus proper) derives from the medial pallium, whereas the lEC is likely to derive from the dorsolateral caudal pallium[10]. It is tempting to speculate that the different developmental origins of mEC and lEC might at least partially account for their distinct input patterns and cognitive functions in the adult[11]

Notably, the study by Donato et al reveals a more general dissociation between neurogenesis and maturation. In the rat, a conspicuous 'rhinal to dentate' neurogenetic progression has been observed[12], which proceeds from entorhinal cortex through subiculum to CA1-3 and DG, in the opposite direction to the maturation gradient observed by Donato et

al. Furthermore, the reported maturation of superficial and deep neurons in mEC is at odds with the classic 'inside-out' pattern of cortical development.

Taken together, these findings indicate that the wave of maturation through the hippocampal circuit is unlikely to be driven by genetically determined factors, which are likely to be specified during neurogenesis, but rather by neural activity. A prominent phenomenon during early post-natal development of the brain is the presence of waves of neural activity, which are known to proceed from peripheral to central areas of the nervous system. The most classic example of this are retinal waves, spontaneous activity patterns observed in the immature retina, occurring well before vision onset, and known to shape the development of visual thalamo-cortical circuits[13]. Similarly, within the hippocampal formation, several patterns of activity have been described during the first weeks of life in the rat (both in vivo and in vitro) [14, 15]. Synchronous plateau assemblies and giant depolarising potentials are prominent during the first two weeks of life. These are progressively replaced by brain rhythms characteristics of adult hippocampal circuits (theta, gamma oscillations and sharp waves-ripples), over the second and third weeks of life[16].

In a series of painstaking and meticulous experiments, Donato proceeded to demonstrate that it is indeed neural activity, spreading from the mEC to the hippocampus proper, that sustains the maturation of the entire network. By inducing the expression of inhibitory DREADDs ('Designer Receptors Exclusively Activated by Designer Drugs') in different neuronal subpopulations, the authors demonstrate that silencing layer II mEC excitatory neurons during the 3rd week of life (P14-20) halts the development of the rest of the hippocampal circuit. Silencing the hippocampus proper has a similar effect on mEC deep layers and the entirety of lEC. These results show that blocking the flow of neural activity in any of the fields of the hippocampal circuit suppresses further maturation in downstream areas (figure 1B, 1C). The authors speculate that maturation signals may be transmitted synaptically, but further research is needed to explore their identity and propagation mechanism. Although the study by Donato et al examined the levels of c-fos as a read out of DREADD activation, it would be of great interest to study whether and to what extent DREADD silencing affects the normal activity patterns in the hippocampus at this developmental stage, by performing electrophysiological recordings. Another interesting observation by Donato et al is the absence of a "critical period" for maturation of this circuit during the 3rd week of life. Once silencing is relieved at the beginning of the fourth week of life, maturation of the mEC network resumes. Thus, the requirement for neural activity to spur maturation in entorhinal circuits is not time-sensitive.

Donato et al further demonstrate that specifically silencing of layer II stellate cells suppresses maturation of downstream areas in the hippocampus. Moreover, when DREADD expression is selectively targeted to an early born stellate cell cohort (E12 ±24hr), silencing a much smaller proportion of excitatory neurons is now sufficient to stop maturation of pyramidal and PV+ cells in the mEC. This raises the possibility that coordinated activity of sub-populations of stellate cells, which are born within a tight temporal window, drives the maturation of hippocampal circuits. This result is in line with accumulating evidence that hippocampal neurons, whose birth occurs within similar timeframes, are preferentially connected in sub-networks[17, 18] and could therefore act synergistically to promote neural maturation.

Strikingly, Donato et al show that the maturation of mEC stellate cells (but not that of pyramidal cells in mEC-L2) is well correlated with birthdate, suggesting that stellate neurons' maturation may exclusively involve cell-autonomous processes. However, more research is needed to establish whether this is the case. The mEC receives strong direct projections not only from parahippocampal areas and post-rhinal cortex but also from association cortices[11]. The effect of silencing these areas on stellate maturation needs to be assessed before a role for activity in stellate cell maturation can be definitively excluded. Indeed, evidence suggests that spontaneous waves of neural activity in the somatosensory cortex[19] and EC/hippocampus[20] are triggered by motor twitches during early post-natal development, indicating that neural activity propagates from the periphery to the hippocampus and could therefore affect stellate cell maturation.

Lastly, an unexplored question raised by this study is when and how neurons in mEC layer III, which provide direct connections to CA1 and subiculum through the temporo-ammonic pathway, mature. Their activity may also contribute to the maturation of the rest of the hippocampal network. Future studies should address this important question.

In conclusion, the paper by Donato and colleagues demonstrates that a wave of maturation, triggered by neural activity, moves through the transverse axis of the hippocampus during postnatal development. These results raise the exciting possibility that this developmental mechanism might also operate in other brain areas.

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Affiliations:

[1] Department of Neuroscience, Physiology and Pharmacology, and [2] Department of Cell and Developmental Biology, University College London, London, WC1E 6BT, UK.

Correspondence: f.cacucci@ucl.ac.uk

Figure 1. Activity-dependent maturation of the hippocampal circuit proceeds in a step-wise unidirectional fashion through the network.

A) Schematic horizontal slice through the hippocampus depicting: hippocampus proper (dentate gyrus [DG], cornu ammonis [CA] fields CA3 and CA1, and subiculum [SUB]), and the medial and lateral entorhinal cortices (mEC and lEC, respectively). Superficial and deep layers of mEC and lEC are marked by 'su' and 'dp' respectively. Neurons shown in superficial mEC and lEC represent stellate cells. Coloured arrows highlight some of the principal connections of the hippocampal circuit. The colour coding of arrows (in the order red-orange-yellow-green-blue) represents the unidirectional flow of information through the circuit. The 'perforant-path' input (red) to the DG and CA3 arises in superficial mEC and lEC, followed by a series uni-directional connections which propagate information along the transverse axis, finally terminating in the deep layers of mEC and lEC (blue).

B) Schematic horizontal slice of hippocampus (as in A), colour-coded to indicate the order of maturation reported in Donato et al. Stellate neurons in mEC mature first (red), followed by pyramidal cells in mEC and CA3 (orange), then CA1 (yellow), followed by SUB, deep layers of mEC and lEC, and DG (green). The superficial layers of the lEC are the latest to mature. Note overlap between the direction of information flow (A) and maturational sequence. Two notable exceptions are the DG and superficial lEC (see text for further details).

C) Left panel depicts synaptic connections between mEC stellate cells and CA3, and between CA3 and CA1, during early post-natal development. Colour-coding of neurons matches that of 1B. Top right panel depicts the progression of normal development, whereby neuronal activity in stellate cells results in the release of maturation signals onto CA3 neurons, promoting the formation of synapses (1). In turn, activity in CA3 neurons promotes the formation of synapses in region CA1 (2). Lower right panel depicts how silencing stellate cells by driving expression of inhibitory DREADDs halts maturation of downstream targets: lack of activity in stellate cells prevents the normal development of CA3. In turn, lack of activity in CA3 blocks maturation and synaptogenesis in CA1 neurons.