MATURATION FONCTIONNELLE DES RÉSEAUX NEURONAUX

MATURATION OF THE HIPPOCAMPAL NETWORK

Pr. Christophe Porcher
INMED INSERM U901 Marseille Luminy
Christophe.porcher@inserm.fr
STRUCTURE OF THE HIPPOCAMPUS AT P0-P5

CA1
CA3
DG
MATURATION OF THE HIPPOCAMPAL NETWORK

1. the **sequential events** that take place during the construction of the hippocampal network.

2. the maturation of **pyramidal neurons and interneurons**, and their transmitters **glutamate and GABA**

3. Relation between GABA receptors activity and **BDNF secretion** in immature neurons

4. The properties of **electrical activity** that result from this maturation

5. Integration of **newly generated neurons**
Unexpected observation

Electrical properties of immature tissue

Neonatal

Control | Bicuculline | Wash-out
Whole cell
Field

Adulte

Whole cell
Field

Ben-Ari et al, J Physiol. 1989
GABAergic neurons and GABAergic synapses develop prior to glutamatergic ones

- GABAergic interneurons divide and arborize prior to the principal neurons
- Interneurons are mature at an earlier stage than the bulk of principal cells

n.b. : In adults, GABAergic and glutamatergic signals equilibrate in order to prevent seizures
GABAergic synapses are established before glutamatergic ones on to pyramidal cells

P0 pyramidal cells

n.b.: These neurons express extra-synaptic receptors since bath applications of GABA or glutamate agonists evoke the usual currents (not shown) observed in more adult neurons, confirming that the expression of receptors precedes that of functional synapses

Synaptic markers of GABAergic terminals are first observed at birth at the level of the apical dendrites of pyramidal neurons.
Sequential expression of GABA and glutamate synapses is also observed in the hippocampus of subhuman primates in utero.

- Neurons, that have an axon but no dendrites, are **silent**
- Neurons that have axons and small apical dendrites express only $\text{GABA}_A$-mediated PSCs
- Neurons that have an arborized apical dendrite as well as basal dendrites exhibit both $\text{GABA}_A$- and glutamate-mediated PSCs
CONCLUDING REMARKS

1. Une règle universelle, respectée au cours de l’ évolution du ver de terre aux mammifères supérieurs

2. Cette séquence maturative est à mettre en parallèle avec tous les courants ioniques et activités de réseaux neuronaux
Schematic diagram of the different stages of maturation of pyramidal cell at P0

- GABAergic synapses between interneurons and the dendrites of pyramidal neurons are the first synapses to be established on pyramidal neurons of the hippocampus.

- Glutamatergic synapses are formed at a later stage.
1. Does the activation of GABA_A receptors in immature neurons evoke a current and a potential change identical to that in adults, or does the neonatal GABA_A synaptic response have different properties?

2. Is the other major inhibitory response, the metabotropic GABA_B-receptor-mediated IPSP, functional in immature neurons?

3. What are the consequences of these developmentally regulated features on the electrical properties of the immature network? How does the immature network discharge?

These observations in turn raise the following questions.
GABA$_A$- and GABA$_B$-mediated responses differ in developing and mature brains
Synaptic activation of GABA$_A$-receptors is depolarizing in neonatal rat hippocampus

(a) Cell-attached recordings of an interneurons in the presence of CNQX and APV

(b) GABA$_A$R antagonist bicuculline, silences ongoing activity in slices at an early developmental stage

(c) Electrical stimulation evokes a $[Ca^{2+}]$ increase in a pyramidal neuron

GABA depolarises and excites immature neurons
A DEVELOPMENTAL SEQUENCE

Neurones immatures → Development → Neurones matures

Expression de NKCC1
Expression de KCC2

0 6 13 Adulte
Transient perinatal loss of the GABA$_A$-mediated excitation: a major role during delivery
Transient perinatal loss of the $\text{GABA}_A$-mediated excitation: a major role during delivery

SSR126768A: OXT-R antagonist

Perinatal effects of GABA$_A$-Rs activation on [Ca$^{2+}$]i

A

Fura 2-AM

C

E18  P0  P5

D

Cells excited by GABA

E18  P0  P5

Cells inhibited or not responding to GABA

E18  P0  P5

*
Oxytocin causes a switch in $\text{GABA}_A$ signaling from depolarizing to hyperpolarizing.

MQAE: Cl- indicator
Blockade of oxytocin receptors decreases fetal brain resistance to anoxia-aglycemia at birth
Sequential development of NMDA and AMPA R-mediated glutamatergic synaptic transmission
Sequential development of NMDA and AMPA R-mediated glutamatergic synaptic transmission

Glutamatergic transmission is initially purely NMDA R-mediated, without any significant contribution of AMPA-R.

Since the voltage-dependent Mg2+ block of NMDA channels is as efficient in neonatal hippocampal neurones as it is in adults, these premature synapses are « silent » at resting membrane potential.
Synergistic excitatory actions of GABA<sub>A</sub> and NMDA receptors

(A) AMPA receptors cannot provide the depolarization sufficient to remove the Mg<sup>2+</sup> block and activate NMDA channels. This depolarization can be provided by GABA.

(B) A combined activation of GABA<sub>A</sub> and NMDA receptors resulted in significant increase of [Ca<sup>2+</sup>]i fluorescence.

D600: voltage-gated Ca<sup>2+</sup>-channel blocker
Part 1: Summary
Sequential maturation of GABAergic and glutamatergic synaptic transmission

GABA_A R, which provide membrane depolarization leading to activation of Na^+ and Ca^{2+} VDC, are the only source of synaptic excitation of hippocampal pyramidal cells at P0–P2.
Sequential maturation of GABAergic and glutamatergic synaptic transmission

Glutamatergic synapses, which initially lack functional AMPA-Rs, appear after P2 and act in synergy with GABAergic synapses, leading to the depolarizing effect of GABA releasing the voltage-dependent Mg2+ block of NMDA channels.
Sequential maturation of GABAergic and glutamatergic synaptic transmission

At the end of the first postnatal week, the main excitatory drive is provided by the AMPA receptors whereas GABA, acting via GABA$_A$ and GABA$_B$-Rs, takes its classical inhibitory role.
Synergy between $\text{GABA}_A$ and NMDA-R in the immature hippocampus

Major developmental changes in the GABA-glutamate interactions
Excitatory action of GABA on immature neurons

Ben Ari & Holmes, 2006
Developmental switch in the GABA_A signaling

Asterisks: GDPs
Part 2: Spontaneous neuronal activity in developing networks
• **Cortical early network oscillations** (cENOs; cortex): require action potentials and are driven by NMDA and AMPA-Rs

• **Giant depolarizing potentials** (GDPs; hippocampus) are the earliest synapse-driven network pattern: require GABAergic transmission and disappear with the shift in GABA depol/hyperpol
Network patterns in immature neocortex

• Immature neocortex produces at birth:

  – cENOs and **synchronous plateaus assemblies** (cSPAs, i.e. synchronous Ca$^{2+}$ plateaus associated with intrinsic membrane potential oscillations in restricted groups of neurons)

  – **GABA driven GDPs** (similar to hippocampus GDPs)
Multibeam two-photon imaging of the four maturation steps of spontaneous neuronal activity in somatosensory cortical slices from embryonic stages to first postnatal days.

Calcium Plateaus (SPAs) Are Temporally Correlated and Selectively Affected by Gap Junction Blockers

A

Active cells (%)

SPA-cells (%)

30 s

B

Non-synaptic activity

+Gap-junction blocker
Cortical ENOs and GDPs display two distinct spatiotemporal dynamics.
Differential role of glutamate in the generation of cortical ENOs and GDPs
cENOs peaks around birth (P0-P3) and disappear when GDPs dominate the network (P6-P8)
A general sequence for the maturation of coherent electrical activity patterns
Network patterns in immature hippocampus: a triphasic sequence

Three Dominant Forms of Primary Activity in the CA1 Region from Embryonic Stages to First Postnatal Days (A1)
GDPs result from the synchronous discharges of pyramidal cells and GABAergic interneurons

(A) Bursts of action potentials in the interneurone are synchronous with GDPs in the pyramidal cell.

(B) Dual whole-cell voltage-clamp recordings of CA3 pyramidal neurone and stratum radiatum interneurone, showing that GDPs are synchronously generated in both cells

Khazipov et al, J. Physiol. 1997
GABAergic component of the GDPs

Khazipov et al, JN 2001
GDPs and associated \([\text{Ca}^{2+}]_i\) oscillations

GDPs are associated with synchronous \([\text{Ca}^{2+}]_i\) oscillations in pyramidal neurons of neonatal hippocampal slices
GDPs and associated [Ca2+]i oscillations

1. GDPs result from the synchronous discharges of pyramidal cells and GABAergic interneurons

2. GDPs are associated with synchronous Ca2+ oscillations mediated by VDCC and NMDA-Rs

3. GDPs provide synchronous activity of presynaptic afferents and postsynaptic [Ca2+]i increases: they are likely candidates to mediate both Hebbian activity dependent plasticity of developing synapses and formation of the hippocampal neuronal network.
Spontaneous synaptic activity in the developing rat hippocampus

**In vivo Field**

Leinekugel et al, Science, 2002

**SPW**

Leinekugel et al, Science, 2002

**Acute slice**

Ben-Ari et al, J. Physiol., 1989

**GDPs**

Ben-Ari et al, J. Physiol., 1989

**Pyr. Cell**

Ben-Ari et al, J. Physiol., 1989

*In vivo*
How ongoing synaptic activity translates into functional and/or morphological development?

Patch-Clamp (whole cell) in NBQX & D-APV P0-P6 slices

Pre-DPs

20 DPs @ 0.1Hz

20 min post-DPs

CA3

sGABA

P0-P6 slices

20 DPs @ 0.1Hz

20 mV

0.4 nA

500 ms

40 pA

600 ms

sGABA_A-PSCs frequency

% of pre-DPs

0 20 40 60 80 100 120 140

control

sGABA_A-PSCs amplitude

% of pre-DPs

0 20 40 60 80 100 120 140 160

control

Gubellini et al., J. Neurosci 2005
How ongoing synaptic activity translates into functional and/or morphological development?

- Spontaneous network activity triggers a release of BDNF
- BDNF secretion induces synaptic plasticity