Bio Imaging Lab
University of Antwerp
Annemie Van der Linden

neuroplasticity

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2

3 MRI systems from Bruker

9.4T Biospec
7T Pharmascans (one J&J sponsored)
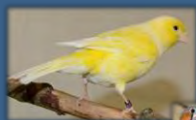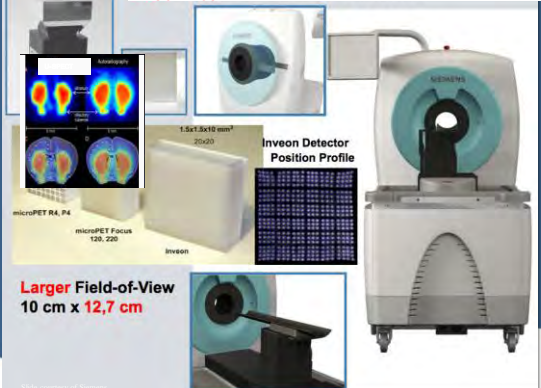
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3

Small animal imaging
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4

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Preclinical PET

microPET R4, P4

microPET Focus
120, 220

inveon

1.5x1.5x10 mm³
20x20

Inveon Detector
Position Profile

Larger Field-of-View
10 cm x 12,7 cm

neuroplasticity

- Definition
- Critical periods
- Health and disease
- Key mechanisms for (re)shaping neuronal circuits
  - Hebbian plasticity
  - Intracortical GABAergic inhibition
  - (Adult) neurogenesis
- In vivo Magnetic Resonance Imaging (MRI) to assess (re)shaping neuronal circuitry

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neuroplasticity

- Definition:

The brain's ability to reorganize its connections functionally and structurally in response to changes in environmental experience.

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neuroplasticity

- Critical periods:

Time windows / critical periods during which neural circuits display a heightened plasticity in response to external stimuli after which plasticity dramatically wanes

- Early postnatal live = critical period
- Postnatal/postcritical/Adult = high/intermediate/low

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neuroplasticity

- Health and disease

  - Plasticity as a natural process
  - Plasticity to recover from neuropathology
  - Plasticity as a cause of pathology

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neuroplasticity

- Key mechanisms for (re)shaping neuronal circuits

  1. Hebbian plasticity (pyramidal neurons)
  2. Intracortical GABAergic inhibition (GABAergic interneurons)
  3. (Adult) neurogenesis

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1. Hebbian plasticity

Hebb's postulate
"*When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased.*"

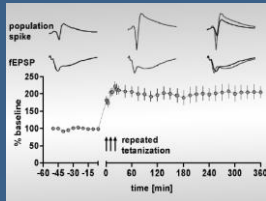D. O. Hebb, 1949

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repeatedly or persistently takes part in firing it
= Long Term Potentiation

Local Field Potential of the population
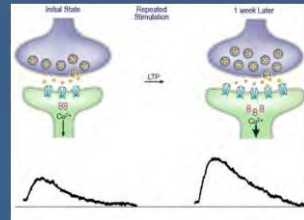excitatory postsynaptic potential EPSP

Experience dependent modula...

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growth process or metabolic change

LTP requires presynaptic glutamate
Release and postsnaptic polarization

Glutamate receptors : AMPA receptors
AMPA=Amino Methyl Propionic Acid

NMDA (N-Methyl-D-aspartic acid)
receptors

(number, subunit composition), …

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growth process or metabolic change, formation
of spines resulting in new excitatory synapses
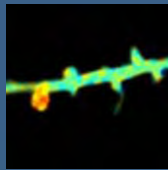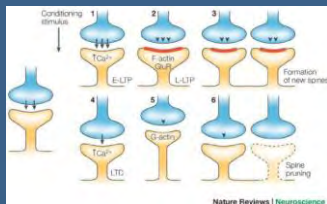
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The more often neural pathways fire, the
stronger the connections will become. This is
called long-term potentiation, and it is the basis
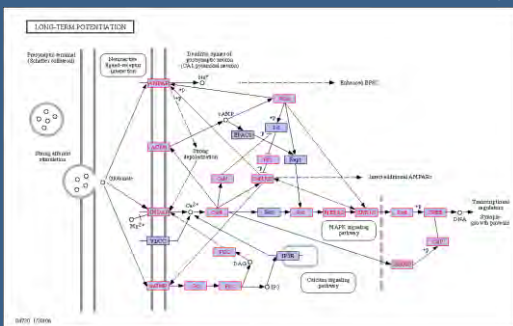of all learning and memory formation

Donald Hebb's famous quote, "neurons that fire
together wire together"

Best model is hippocampal learning

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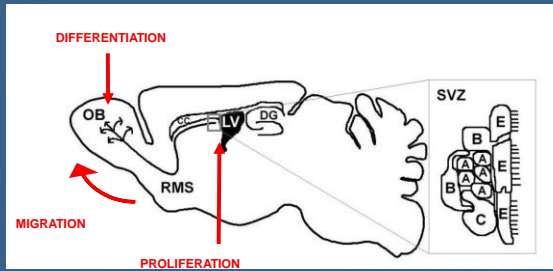
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Slide 17

Diseases linked to impaired hebbian plasticity
because one of the molecules in previous
schedule are down or upregulated?

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2. Intracortical GABAergic inhibition

By sculpting the pattern and timing of neuronal electrical activity, inhibitory GABAergic circuits are an ideal candidate for regulating the process of experience-dependent synaptic modifications

Prime model to investigate this is monocular deprivation early in development in kittens and mice

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Intracortical GABAergic inhibitory circuitry is THE KEY FACTOR for defining the bounderies (critical periods) of cortical plasticity

Figure 1.5: Perisomatic inhibition. Perisomatic inhibition on a pyramidal neuron by two types of PV-positive interneurons (PV = PV-positive basket neuron; PVc = PV-positive chandelier neuron). Another chandelier cell (green) is shown that expresses the cannabinoid receptor type 1 (blue) (see later). It is clear that axons of GABAergic neurons target distinct domains of pyramidal neurons. Adapted from Fish et al. (2011).

GABAergic interneurons

Inhibitory neurotransmission is required to enable neuroplasticity (critical minimum)

Resulting in a changing Balance between Excitatory and inhibitory Activity

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Fig. 1. The graphs illustrate a simple scenario in which changes in the rate of GABAergic maturation could affect the duration of the critical period. In this model, we assume a relatively constant rate of maturation [48,69] and a well-defined window of tolerance within which plasticity can occur. Cortical plasticity will be initiated when a minimal level of cortical inhibition is reached, will be terminated once a maximal level is reached, and it will peak somewhere in between. Manipulations that accelerate (green line) or attenuate (blue line) the maturation of GABAergic transmission (red line) will shorten and lengthen the duration of plasticity, respectively.

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Intracortical GABAergic inhibition is the key factor in developing pathological states characterized by severe intellectual disabilities

Developmental increase of brain GABAergic inhibition levels (normalized to the normal adult values; red curve) is paralleled by a progressive reduction of experience-dependent plasticity. Plasticity is high during early development (green block) and very low in the adult brain (yellow block). Anomalous increases in the strength of inhibitory neural circuits may lead to over-inhibition linked to permanent deficits in synaptic plasticity and neural development

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3. (Adult) neurogenesis

Shaping of the brain during early postnatal life

Migration and subsequent differentiation of neuroblasts

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adulte neurogenesis > two remaining niches

DIFFERENTIATION

MIGRATION

PROLIFERATION
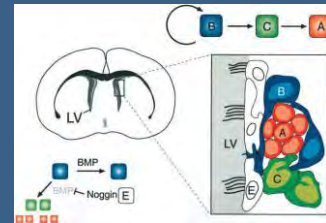
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SVZ = Sub Ventricular Zone

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- Neurogenesis - neuronal recruitment and survival are influenced by a variety of growth factors, cellular matrix proteins and of course pathologies (e.g. inflammation....)

- Impaired neurogenesis in SVZ in neurodegenerative diseases, depression etc

- Anti depressants upregulate neurogenesis in dendate gyrus

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neuroplasticity

- Definition
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- In vivo Magnetic Resonace Imaging (MRI) to assess (re)shaping neuronal circuitry

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In vivo imaging of neuroplasticity

Neuroplasticity is a natural phenomenon that seems impaired in several diseases

Gaining interest for imaging and quantifying it
- as diagnostic or 'treatment follow up' tool
- to understand the underlying mechanisms so that we can master it and use it for regeneration and treatment purposes
- MRI is best suited

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Example 1

Functional MRI Evidence for LTP-Induced Neural Network Reorganization

Measure synaptic plasticity with fMRI

Santiago Canals, Michael Beyerlein, Hellmut Merkle and Nikos K. Logothetis. Current Biology 19, 398–403, 2009

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Functional MRI: how does it work ?
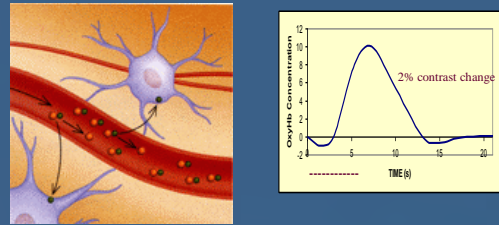Visualising the brain at work
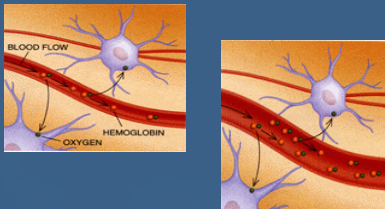
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Measure BOLD responses
Blood Oxygenation Level Dependent Contrast
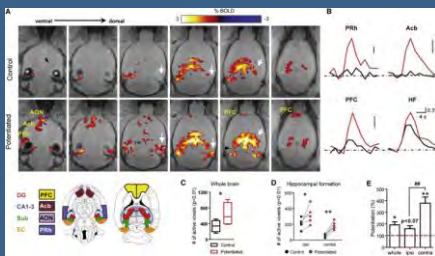

2% contrast change

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Long-lasting changes in network organisation after hippocampal LTP

A. fMRI showing brain active areas before and after perforant high frequency
   stimulation (potentiated) showing new structures recruitment after potentiation.
B. Bold from controls in black and after potentiation in red
Perforant path stimulation, NMDA channel anatgonists
> synaptic plasticity with fMRI

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Example 2

Examples of Magnetic resonance Imaging (MRI)
To assess the modulations in ocular dominance
in birds

Left eye in contact with body, right
eye with shell and thus can capture
light while in the egg.

This differential stimulation of the
eyes results in a natural visual
dominance of the right eye

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Right eye dominance,
left hemisphere highest activation
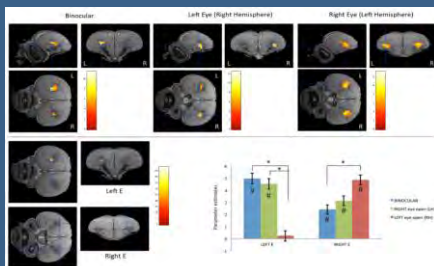
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Example 3

Diffusion Tensor Magnetic Resonance Imaging (DTI)
To assess the Seasonal rewiring of the brain in
seasonal songbirds

Songbirds represent a superior natural model for
neuroplasticity. Each spring the critical period
for neuroplasticity re-opens

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Songbird brain

The vocal behavior of songbirds is controlled by a circuitry of connected brain nuclei = Song Control System (SCS)

This system is highly plastic

The SCS displays a very pronounced sexual dimorphism

The SCS displays pronounced neural plasticity in adult birds:
volumes, activity and connectivity varies with season, steroid concentration and capacity for song learning
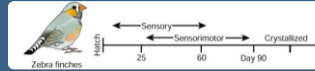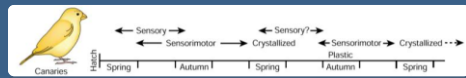
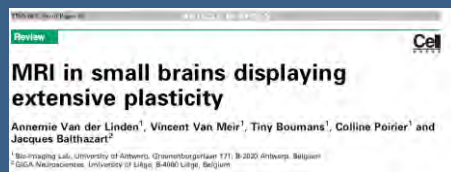Singing is a learned behaviour

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Song learning

Limited Learner

Zebra finches
Hatch · Sensory · Sensorimotor · Crystallized · 25 · 60 · Day 90

Open Ended Learner

Canaries
Hatch · Sensory · Sensorimotor · Crystallized · Sensory? · Sensorimotor · Crystallized · Plastic · Spring · Autumn · Spring · Autumn · Spring

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Review   Cell

MRI in small brains displaying extensive plasticity

Annemie Van der Linden[1], Vincent Van Meir[1], Tiny Boumans[1], Colline Poirier[1] and Jacques Balthazart[2]

[1] Bio-Imaging Lab, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium
[2] GIGA Neurosciences, University of Liège, B-4000 Liège, Belgium

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Songbird brain   Human brain

Cerebellum

Cerebrum
Thalamus
Midbrain
Spinal cord   Hindbrain

Cerebrum
Thalamus
Midbrain
Hindbrain
Cerebellum   Spinal cord

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NEUROPLASTICITY
in Song Control Circuit

Delicate balance between adult neurogenesis and cell death, cell volume and cell density changes : volume changes in song control nuclei

Creation of new axonal projections and dentrites: altered neuronal connectivity

Spring   Summer

RA   RA

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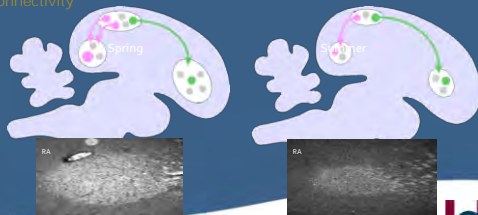
41

Diffusion Tensor Magnetic Resonance Imaging (DTI)

To assess the Seasonal rewiring of the brain in seasonal songbirds

Songbirds represent a superior natural model for neuroplasticity. Each spring the critical period re-opens and neuroplasticity occurs.

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Diffusion Tensor Imaging

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Distribution of protons gives us indirect information about tissue microstructures and connections

http://brainvat.wordpress.com/2007/10/27/the-beautiful-cerebellum/

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Principles Diffusion Tensor imaging

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Water:

Isotropic diffusion

Mean diffusion
= axial diffusion
= radial diffusion

Fractional anisotropy
FA = 0

radial Dif
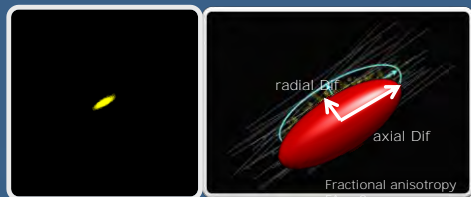
axial Dif

Courtesy of FMRIB Centre, Oxford University

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Principles Diffusion Tensor imaging

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Displacement due to diffusion in white matter tracts is ellipsoidal.

Diffusion Tensor has information about: myelination, axonal

density, axonal coherence,…   anisotropic diffusion

radial Dif

axial Dif

Fractional anisotropy

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Principles Diffusion Tensor Imaging

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Tensor has information about: myelination, axonal density, axonal coherence,…

cell body

free diffusion

restricted diffusion

terminus

axon (myelinated)

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Principles Diffusion Tensor Imaging

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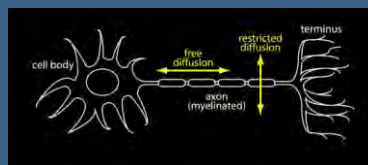
Calculate diffusion tensor for each pixel
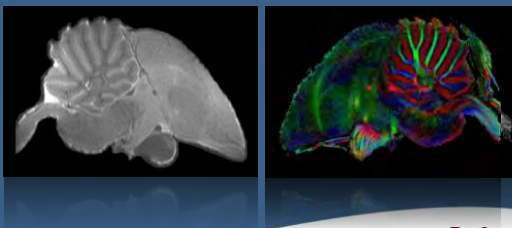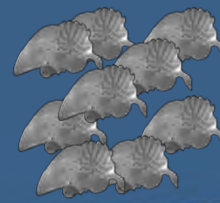
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Repeated DTI measurements in two seasons

47
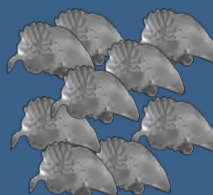
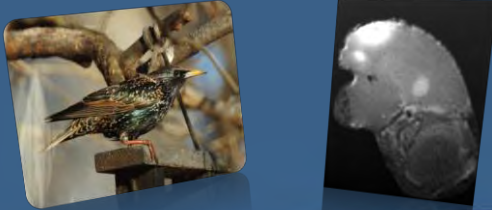9 birds in spring   9 birds in summer

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Example 4

Differential effects of testosterone on neuronal populations in HVC:
a Dynamic MEMRI study

Van Meir M. et al. NeuroImage , 21(2004), p. 914-923

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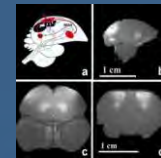

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Manganese Enhanced MRI
Pautler et al. (1998)

MnCl2

Mn^{2+} is transported along axons of a circuit

neuron

axon

Mn^{2+} is a biological Ca^{2+} analogue
Mn2+ is paramagnetic

synapse

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Dynamic ME-MRI

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Dynamic ME-MRI

MnCl₂

Accumulation of Mn in Area X and RA

Area X

male

female

RA

male

female
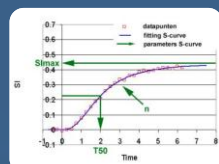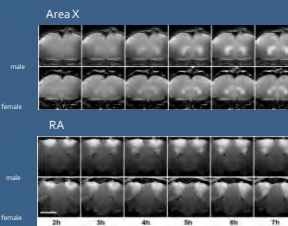
Van der Linden et al. *Neuroscience 122(2): 467–474 (2002)*

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Manganese-Enhanced MRI in a Rat Model of Parkinson's Disease

Manganese-enhanced magnetic resonance imaging of mossy fiber plasticity in vivo
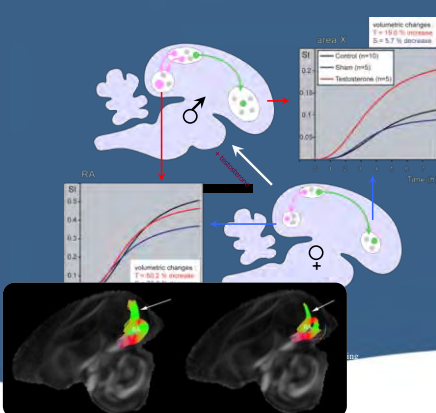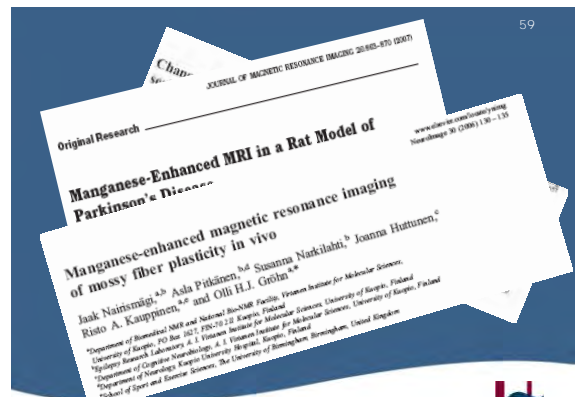
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Example 4

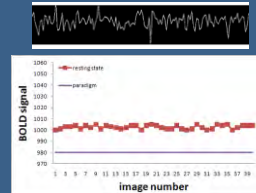Resting state fMRI: Functional connectivity

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Resting state fMRI

Resting state Functional MRI
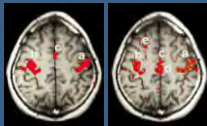
During rest spontaneous fluctuation in BOLD signal

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Functional connectivity

- Measured during rest (no task, stimulation)
- Low frequency fluctuations (LFF's) (0.01 – 0.1 Hz) in haemodynamic response measured with MRI
- Temporal correlation in intrinsic activity between different brain areas.

fMRI, motortask   rsfMRI, motornetwork
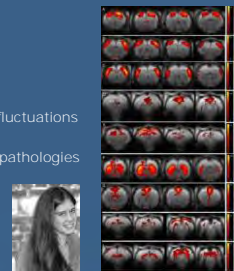Biswal et al. 1995

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Discern functional connectivity in the brain

Resting state fMRI

Components >
brain areas with similar LF BOLD fluctuations

Functional connectivity altered in pathologies
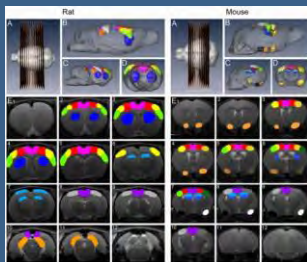(AD, PD, depression, etc..)

Optimized in rats and mice

Jonckers et al, on line, PlosOne

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Resting state networks
in rats and mice

Jonckers et al. 2010, PlosOne

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RsfMRI and learning/plasiticy

Changes occur in resting state network of motor system during 4 weeks of motor skill learning
Liangsuo Ma, Shalini Narayana, Donald A. Robin, Peter T. Fox, Jinhu Xiong

The Resting Human Brain and Motor Learning
Neil B. Albert[1,2], Edwin M. Robertson[3], and R. Chris Miall[1,*]

Functional connectivity between brain regions involved in learning words of a new language
Kim Veroude, David G. Norris, Elena Shumskaya, Marianne Gullberg, Peter Indefrey

Interhemispheric neuroplasticity following limb deafferentation detected by resting-state functional connectivity magnetic resonance imaging (fcMRI) and functional magnetic resonance imaging (fMRI)
Christopher P. Pawela, Bharat B. Biswal, Anthony G. Hudetz, Rupeng Li, Seth R. Jones, Younghoon R. Cho, Hani S. Matloub, James S. Hyde

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Example 5

Minotor cell migration in the RMS as a readout for neurogenesis in the SVZ using MRI

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MPIO = micron sized polystyreen particles with iron oxide (MRI Contrast) and a fluorophore (histo validation)
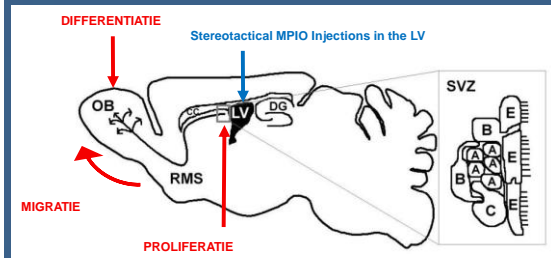
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MRI in mice
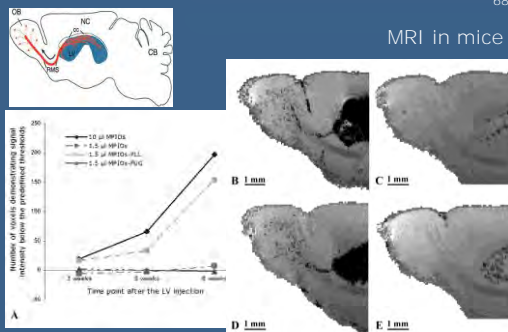
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In vivo bioluminescence imaging
SVZ transfection with lentivirale vectors expressing Luciferase

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SVZ transfection with lentivirale vectors

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SVZ transfection with lentivirale vectors expressing Luciferase and eGFP for histological validation
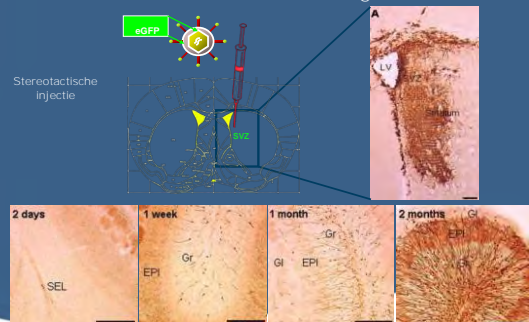
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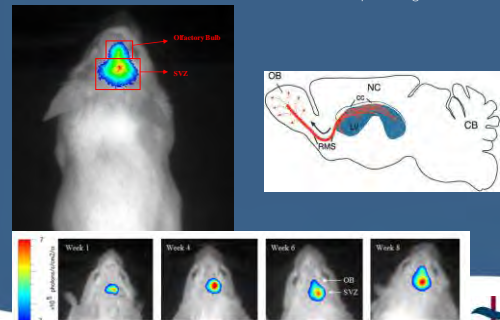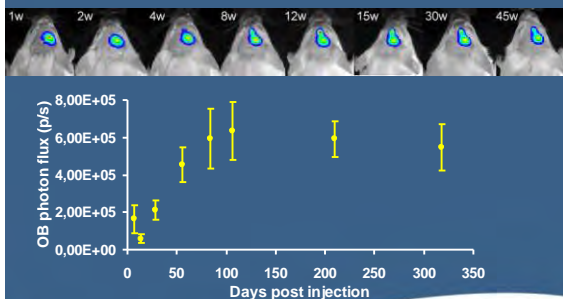In vivo monitoring of neuronal progenitor cell recruitment and survival is important for several reasons

- Neurogenesis is impaired in neurodegenerative diseases at the level of DG and or SVZ
- Anti depressants increase neurogenesis
- GABA also modulates neurogenesisis
- Upregulation of neurogenic capacity might foster neuro-regeneration

MRI has the highest spatial resolution

BLI allows to assess viability of the cells (BLI requires ATP)

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Thank you!

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Critical periods during sensory development

Nicoletta Berardi*, Tommaso Pizzorusso† and Lamberto Maffei‡

Recent studies have made progress in characterizing the determinants of critical periods for experience-dependent plasticity. They highlight the role of neurotrophins, NMDA receptors and GABAergic inhibition. In particular, genetic manipulation of a single molecule, brain-derived neurotrophic factor (BDNF), has been shown to alter the timing of the critical period of plasticity in mouse visual cortex, establishing a causal relation between neurotrophin action, the development of visual function, and the duration of the critical period.

Addresses
*†‡Istituto Neurofisiologia CNR, via San Zeno, 51 56125 Pisa, Italy
†‡Scuola Normale Superiore, Piazza Cavalieri, 7 56126 Pisa, Italy
‡e-mail: maffei@in.pi.cnr.it

Abbreviations

CREB	cyclic AMP response element binding protein
BDNF	brain-derived neurotrophic factor
DR	dark rearing
GABA	γ-aminobutyric acid
GAD	glutamic acid decarboxylase
LTP	long-term potentiation
MD	monocular deprivation
NGF	nerve growth factor
NMDA	N-methyl-D-aspartate
NMDAR	NMDA receptor

Introduction

Early in development, the existence, of critical periods for experience-dependent plasticity has been clearly demonstrated for the visual, auditory and somatosensory systems. Critical periods also exist for many other functions, including song in birds and language in humans — in this latter case, plasticity may even reach the extreme of allowing the interhemispheric transfer of language areas, as seen in the case of left hemisphere injury early in infancy [1–3]. Critical periods have been found to exist in virtually all species, from humans to *Drosophila* [4]. Here, we shall restrict ourselves mainly to studies focusing on the development of visual and auditory systems in mammals and birds.

Classical studies have shown that the effects of sensory deprivation (such as monocular deprivation or monaural plugging) and the effects of alterations in sensory input (such as caused by strabismus, rearing in a restricted auditory environment, or misalignment of the auditory and visual space) are evident only when the manipulation of the sensory input is made during the critical period; similar deprivations and alterations in mature animals have no effect. The duration of these critical periods depends on the function tested and on the way

plasticity is evaluated, as for instance, critical periods measured in terms of recovery from sensory deprivation are longer than critical periods measured in terms of the induction of sensory deprivation effects. In research on the visual system, the most extensively studied critical period is that for the effects of monocular deprivation (MD) on the ocular dominance of cortical neurons, which has been characterized in the monkey, cat, rat, mouse, ferret and human [5,6,7••,8–10] (Figure 1). In the auditory system, the most extensively studied critical period is that for the calibration of the auditory space map by visual input [3,11]. Critical periods for sensory plasticity resulting from increased sensory experience have also occasionally been reported: in humans, musical training in infancy leads to an expanded auditory cortical representation [12••], but only if practising began before the age of 9 (Figure 2).

As shown in Figure 1, the critical period is a definite portion of an animal's life devoted to the shaping of neural connections: the longer the life span, the longer the critical period. A relation also exists between the critical period and brain weight; if one assumes that brain weight is a rough measure of brain complexity, it follows that the more complex the brain, the longer the critical period. During the critical period, sensory functions reach maturity: for example, as shown in Figure 1b, the end of the critical period for MD roughly coincides with the completion of visual acuity development in a number of species. This indicates that experience-dependent plasticity during the critical period is closely interconnected with maturation of sensory functions.

It should be noted that some aspects of cortical organization are also modifiable, by experience, in the adult. This is the case for the frequency map in the auditory cortex [13] and the somatotopic map in the somatosensory cortex [1,14]. The search for the mechanisms determining the beginning and the end of critical periods has been progressing for some years. The possibility to exploit genetic manipulations in the mouse has given fresh impetus to the quest. In this review we shall highlight recent advances which have brought this quest to the cellular and molecular levels, calling into play neurotrophins, inhibitory circuitry and NMDA receptors.

Crossmodal plasticity following early sensory deprivation

Only during early development can total deprivation in one modality lead to compensatory changes in other modalities; namely, to 'crossmodal' plasticity. For example, it has recently been reported that early-blind human subjects localize sound sources better than sighted subjects [15•], particularly for peripheral locations [16•]. This

Figure 1

(a) Life span and critical period. The graph shows the relation between life span and critical period for monocular deprivation in mammals. The duration of the critical period is taken from references [5,6,7••,8–10]. The solid line is the linear correlation fitted to the data (r = 0.98; SD = 0.14; P < 0.001). It is evident that the longer the life span, the longer the critical period. This indicates that a definite portion of the life of an animal is allocated to experience-dependent shaping of neural connections. (b) Critical period and sensory function development. The development of visual acuity in man, monkey, cat and rat [9,57–59] reported as a function of age and compared with the critical period for monocular deprivation. The figure shows that visual acuity reaches its final value by the end of the critical period, indicating that the maturation of sensory functions and the decline of experience-dependent plasticity are two closely interconnected processes. A variation of critical period duration implies a variation in the rate of visual function development (see Figure 3). MD, monocular deprivation.

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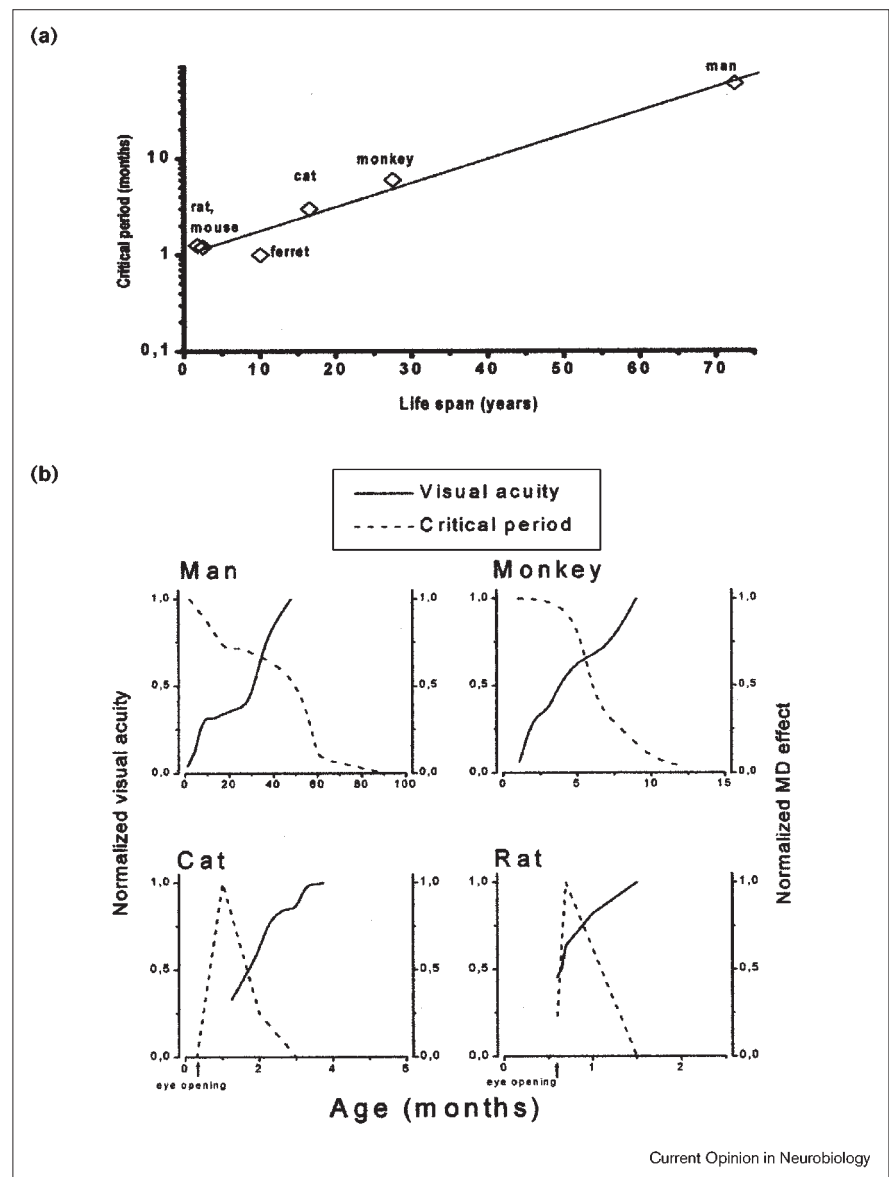
ability might be subserved by the expansion of auditory inputs to 'unused' visual areas and the improvement of auditory neuron spatial tuning, as observed in binocularly deprived cats [17]. An expansion of tactile inputs to primary visual cortex has also been demonstrated in early-blind, but not late-blind humans [18]. In addition, evidence for an improvement of movement detection has been obtained in congenitally deaf people, but only in the peripheral visual field [19].

Experience-related control of critical periods
Experience is a strong determinant for the duration of critical periods: total lack of experience usually prolongs critical periods and delays development of sensory functions. The clearest examples of this come from studies using dark rearing (DR), but evidence is also available from studies in early-deaf children who have been fitted with cochlear implants [13,20]; in addition, a lack of appropriate tutorial experience seems to prolong the critical period for the development of birdsong [2].

The issue of whether experience- and/or sensory-driven electrical activity has instructive or permissive roles for development during the critical period is much debated. It is often difficult to disentangle these two roles, and there are studies that suggest the occurrence of both. Even brief periods of exposure to light during DR can trigger the process of visual development; this would suggest a permissive role for sensory-driven activity. A permissive role for visually driven activity is also suggested by a recent

Figure 2
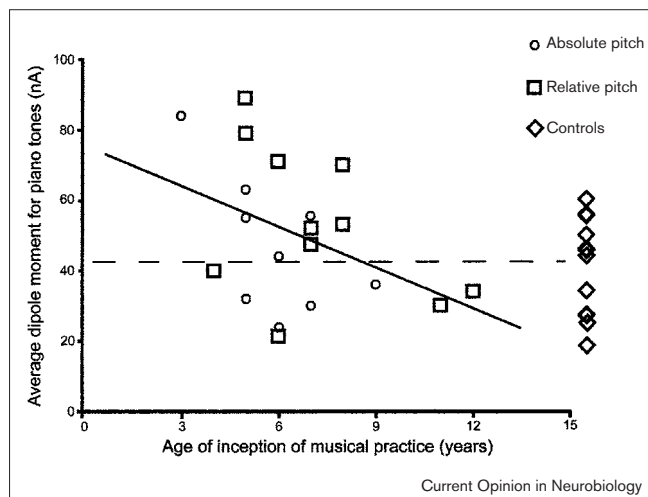
Strength of cortical activation in response to piano tones as a function of the age at which musical training began in musicians with absolute or relative pitch. Functional magnetic source imaging (single dipole moment) was used to measure the cortical representation of complex harmonic sounds, such as piano tones, in highly skilled musicians. Auditory evoked fields in response to piano tones were recorded above the left hemisphere. The dipole moment indicates the total strength of cortical activation (i.e. the number of neurons simultaneously active at the time of the auditory evoked field component measured). The solid line is the linear correlation fitted to the data of the combined groups of musicians. The broken line denotes the mean dipole moment in control subjects who had never played a musical instrument. These data indicate that the younger the subjects were when they started playing their instrument, the larger was their cortical reorganization in recognition of piano tones. Enhanced cortical representation was seen only in subjects who began practising before the age of 9 years (reproduced from [12••]). A similar correlation between enhanced somatosensory representation of the fingering digits and practice inception has been found in highly skilled string players [60].

experiment in binocularly deprived cats, where development of cortical orientation columns and ocular dominance maps is shown to proceed, initially, even in absence of patterned visual inputs [21]. Instructive roles for sensory-driven activity, on the other hand, are evident in several experimental paradigms: first, in experiments where the pattern of afferent electrical activity is artificially modified [22]; second, where sensory inputs from one modality are forcibly rerouted to cortical areas devoted to other modalities [23]; and third, when rearing animals in particular restricted visual environments [24•].

The finding that some aspects of cortical map development are independent of experience does not imply that they are independent of electrical activity. Spontaneous activity, which has been shown to be present prenatally, can clearly play an instructive role, as is the case for the development of ocular dominance columns in the monkey [25].

Determinants of critical periods
We shall now examine some factors whose status as determinants of critical periods has recently been established, including NMDA receptors, neurotrophins, and inhibitory circuitry.

NMDA receptors
The involvement of NMDA receptors (NMDARs) in developmental plasticity has been repeatedly suggested to take place in the somatosensory cortex [26], the auditory cortex (where new excitatory connections subserving auditory map plasticity are exclusively mediated by NMDA) [27], and the visual system (where block of NMDARs in the visual cortex blocks MD effects) [28]. A difficulty with pharmacologically blocking NMDARs in experiments on plasticity of the visual system is that it significantly affects visually driven activity. Recently, the use of different NMDAR antagonists [29] or antisense oligonucleotides [30] in order to reduce the expression of NMDAR1 has overcome this problem, showing that it is possible to block the effects of MD on plasticity without affecting visual responses.

Two interesting properties of NMDARs that make them likely candidates to be molecular determinants of critical periods are that the characteristics of NMDAR mediated synaptic transmission are developmentally regulated, and that their expression is modified by electrical activity [31,32]. In particular, their subunit composition varies in the visual cortex — from a dominant presence of receptors containing the subunit 2B to a high presence of receptors containing the subunit 2A — with a time course that parallels that of the critical period; the expression of 2A also correlates with the progressive shortening of the NMDA current [33,34]. DR delays the developmental shortening of NMDA currents [35], suggesting that the increase in 2A/2B ratio is related to visual cortical development and possibly to the closure of the critical period. It has now been shown that in dark-reared animals, 2A expression is indeed lower than in light-reared animals; interestingly, very brief visual experiences, which are sufficient to trigger the process of development, also trigger a rapid increase in the expression of NMDAR subunit 2A [36••]. In a very recent paper, the same group has shown that a reduction of 2A expression occurs also if animals are put in the dark at post-natal day 23 after a period of normal light rearing [37].

NMDA function is thought to be crucial for mechanisms of synaptic plasticity that follow Hebbian rules and rely on NMDA-dependent modifications of synaptic efficacy. LTP and LTD are such mechanisms, and both have been described in the visual (and somatosensory) cortex during their respective critical periods; no data are available for the auditory system. The critical period for the induction of LTP in the somatosensory cortex closely matches the developmental changes in NMDAR properties and the critical period for sensory deprivation [38]. Causal relationships, however, cannot be derived from such correlations, and many examples of research in which LTP inducibility and MD-induced plasticity do not correlate are known to exist [39,40].

Figure 3

BDNF regulates maturation of inhibition and the critical period of plasticity in mouse visual cortex. **(a)** Critical period for monocular deprivation and development of visual acuity are reported for wild-type mice and for transgenic mice with precocious expression of BDNF. In these transgenic mice, the postnatal rise of BDNF level in the forebrain has been genetically accelerated using the promoter α-CaMKII (the α subunit of calcium/calmodulin-dependent protein kinase II). By postnatal day (P7), BDNF mRNA is already at a level not reached in the wild type until P21. It is clear that for BDNF mice both the critical period (dotted line) and the visual acuity (solid line) curves are shifted to the left (see crossing points, dashed vertical lines). This indicates that precocious expression of BDNF induces both an accelerated development of visual function and an early closure of the critical period. **(b)** On the left, the amplitude of LTP induced in layers II–III by stimulation of the white matter (WM) is reported for wild-type mice and BDNF mutant mice as a function of age. The amplitude of LTP is expressed as a percentage of the baseline level of response. The developmental decline in LTP is clearly accelerated in BDNF mice. On the right, development of GABA inhibitory synapses is reported. The level of expression of the GABA precursor glutamic acid decarboxylase (GAD65) in the presynaptic boutons of GABAergic interneurons was quantified around the soma of the target neurons. In BDNF mice, there is an accelerated maturation of GABAergic synapses, which correlates with the early closure of the critical period (replotted from [7••]).
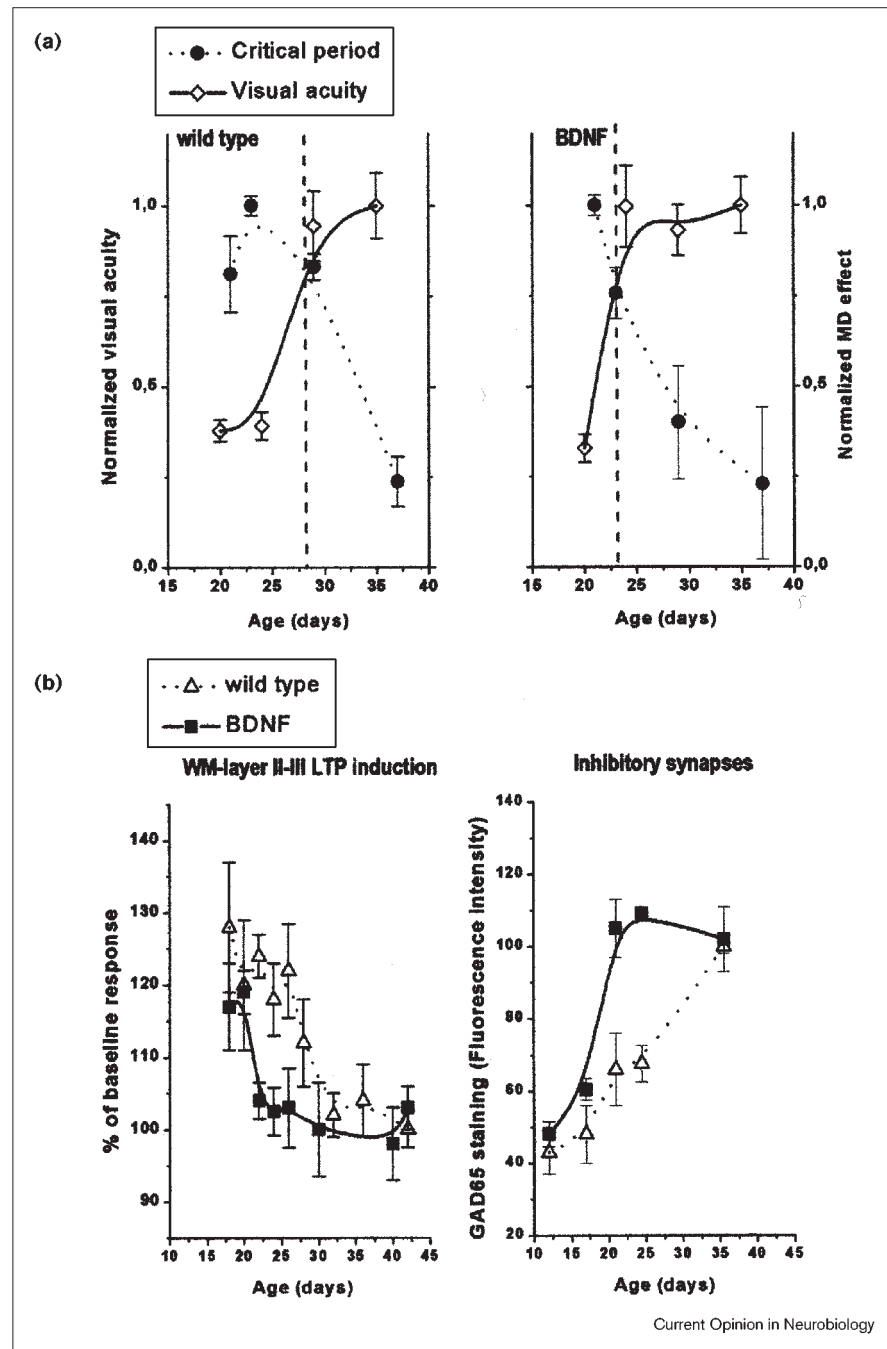
Neurotrophins

Several observations suggest that neurotrophins play important roles in the control of developmental plasticity; for instance, exogenous supply of neurotrophins counteracts the effects of MD and DR, and blocking their activity prevents the formation of ocular dominance columns. They can modulate synaptic transmission and, like NMDARs, their expression is developmentally regulated and dependent on electrical activity [41•,42]. This reciprocal regulation between neurotrophins and neural activity may provide a means by which active neuronal connections are selectively strengthened. Indeed, neurotrophins seem to require the presence of electrical activity in order to exert their effects [41•]. Particularly compelling evidence for this comes from Caleo *et al*. [43•], who have shown that nerve growth factor (NGF) signalling via the TrkA receptor must be coupled with afferent electrical activity to prevent the effects of MD.

In addition, there is clear evidence that neurotrophins control the duration of the critical period; in fact, they are the first molecules for which a causal relation has been established between their action and the duration of critical
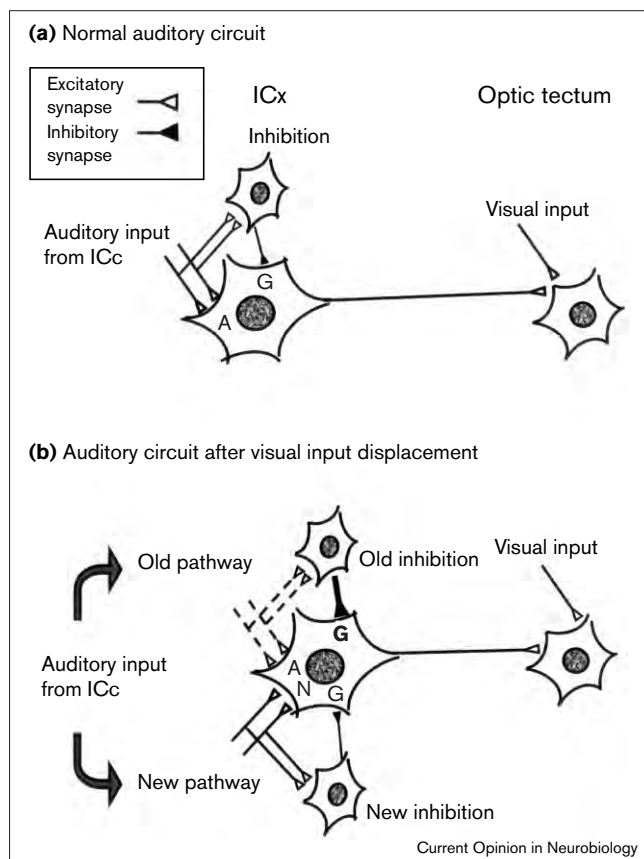
(a) Normal auditory circuit

Excitatory synapse
Inhibitory synapse

ICx   Optic tectum

Inhibition

Visual input

Auditory input from ICc

G
A

(b) Auditory circuit after visual input displacement

Old pathway   Old inhibition   Visual input

Auditory input from ICc

G
A
N
G

New pathway

New inhibition

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Development of selective inhibitory inputs is crucial for the plasticity of functional auditory maps during the critical period in barn owls. **(a)** Normal circuit subserving the connection of auditory and visual maps in barn owls. In the external nucleus of the inferior colliculus (ICx), neurons are sharply tuned for interaural differences in the timing of sound (interaural time differences or ITDs); ITDs are used to compute the left–right positioning of the sound source. The value of ITD to which ICx neurons are tuned varies topographically across the ICx, which thus contains a map of the auditory space according to the left–right positioning of a sound source. Neurons in the ICx receive excitatory input from neurons in the central nucleus of the inferior colliculus (ICc), which is tonotopically organized for sound frequency, at conventional AMPA receptors (A). A feed-forward GABAergic inhibition from these same neurons is also present (G). ICx neurons send their main output to the optic tectum, and activate neurons that are also driven by visual stimulation from the same point in space as the sound source: in this way, either the sight or the sound of a stimulus (such as prey) can stimulate the owl to orient itself toward it. **(b)** Horizontally displacing prisms (which displace the visual input to the left or to the right) mounted in front of the owl's eyes change the relationship between auditory spatial cues, such as ITDs, and their corresponding location in the visual field; auditory and visual maps are thus no longer in register. In juvenile owls reared with horizontal prism spectacles, a rearrangement occurs in the auditory space map in the ICx such that it becomes consistent with the visual map (i.e. adaptation). The shift in auditory space map involves the formation of new excitatory inputs from ICc to ICx; these new neural connections are mediated by NMDA receptors (N). The formation of these new excitatory connections explains how neurons in the ICx respond to stimulation corresponding to new positions in space (e.g. new ITD values) under the instruction of visual input. However, the old circuitry underlying the previous (unadapted) auditory map still exists. In order to eliminate these old and now inappropriate responses, a selective increase of GABAergic inhibition (**G**) occurs; this is activated by the same auditory stimuli that activated the original excitatory connections. This new inhibition (G) overcomes the excitatory signals from the old connections, which are thus functionally inactivated (dashed lines). In this way, the auditory and visual maps are once again in register, and the sound of prey once again causes appropriate orienting of the owl's gaze towards it. These findings clearly show that experience adjusts both excitation and inhibition [50**].

periods in mammals. The first evidence came from the finding that block of endogenous NGF through the use of antibodies prolongs the duration of the critical period — an effect similar to that of dark rearing [44]. Very recently, in an elegant study using transgenic mice that overexpress BDNF in the forebrain, Huang *et al*. [7**] have found accelerated visual development and early closure of the critical period. This is accompanied by a precocious development of inhibition, and by an early closure of synaptic plasticity that is usually enabled by LTP in the visual cortex (Figure 3). These findings suggest that BDNF controls the time course of the critical period by accelerating the maturation of GABAergic inhibition.

To summarise, BDNF regulates GABAergic inhibitory interneurons [45], potentiates GABA release [46•], and mediates the activity-dependent regulation of synaptic inhibition [47]. In addition to these actions, BDNF has been repeatedly found to affect excitatory transmission; also, modulation of NMDA responses by BDNF has recently been suggested [48,49].

Inhibitory circuitry

Experience-dependent plasticity is not limited to excitatory circuitry. In the tectum of young barn owls, the formation of new auditory maps in response to visual input displacement is accompanied by the development of selective inhibitory inputs; these inputs are thought to block the

excitation from the old map while leaving the neural circuitry in place (Figure 4). This inhibitory input is therefore crucial for the emergence of the new, appropriate visuo–audio coordination [50**]. Inhibitory connections also explain why the capacity for plasticity in adult owls is greater if they have experienced map rearrangement during the critical period [51•]: when the prism spectacles employed to displace the visual input in juvenile owls are removed, it is the new map, now inappropriate, which is overridden by inhibition. The presence of this 'hidden' auditory space map can be disclosed by a new prism spectacles experience (equal to that experienced as juveniles) even in adult owls, after the end of the critical period.

Two sets of experiments have addressed the role of the intracortical inhibitory circuitry in visual cortical plasticity during the critical period. The first shows that inhibitory interactions are necessary for the manifestation of experience-dependent plasticity. In transgenic mice lacking the 65 kDa isoform of the GABA-synthesizing enzyme GAD (GAD65), experience-dependent plasticity in response to MD is deficient. Normal plasticity in these animals can be

rescued if GABAergic transmission is enhanced in the visual cortex by means of benzodiazepines [52••].

The second set of experiments has a direct bearing on the possible role of inhibition as a critical period determinant. The development of inhibition lags behind that of excitation. This developmental mismatch between inhibition and excitation could provide a time window — a critical period — during which the organization of cortical circuitry can be particularly influenced by sensory experience. The results obtained in mice with precocious BDNF expression (Figure 3) point to this hypothesis: reducing the developmental mismatch between inhibition and excitation (by accelerating inhibition) results in an early closure of the critical period for plasticity in response to MD.

Other factors

Other factors have recently been suggested to have a bearing on critical periods. Intracortical blockade of tissue plasminogen activator selectively prevents the recovery from MD [53•]; this suggests that recovery of cortical function after a period of sensory deprivation is subserved by molecular mechanisms that are distinct from those involved in the induction of sensory deprivation effects. Two interesting factors, cpg 15 (*candidate plasticity gene* 15), and class I MHC (major histocompatibility complex), are currently under investigation in the laboratory of Carla Shatz [54•]: cpg 15 is expressed at high levels during postnatal cortical development, and its pattern of expression correlates with the timing of ocular dominance column formation; cpg 15 has also been shown to be regulated by activity. Class I MHC has been suprisingly involved in activity-dependent plasticity after its selection by differential display of mRNAs extracted from prenatal lateral geniculate cells after block of electrical activity. Class I MHC was found to be present at very high levels in the LGN during the activity-dependent process of layer formation and its expression is strictly regulated by electrical activity.

Furthermore, transcription factors such as CREB could play an important role in developmental plasticity. A link between CREB and the critical period is indicated by the observation that CRE-dependent transcription is activated in the visual cortex following brief monocular deprivation and this induction is strongly downregulated after the end of the critical period [56•].

Conclusions

It is becoming increasingly clear that critical periods result from changes in the expression of molecules; however, the identity of these putative molecular determinants is far from being established. For most candidate molecules correlative — but not causal — connections between expression of that molecule and duration of the critical period have been obtained. The first conclusion to be drawn from this survey is that neurotrophins are the only factors for which a causal link between expression of a single molecule and duration of the critical period has been established.

A second important conclusion is that inhibition plays a crucial role in the regulation of critical periods. Accelerating the development of intracortical inhibition through mutations that result in early expression of BDNF has been shown to cause accelerated visual development and early closure of the critical period. It is conceivable that control of the critical period involves the modulation of inhibitory circuits by other neurotrophins in addition to BDNF.

The third conclusion concerns the role of developmental regulation of NMDARs in control of the critical period. New evidence has reinforced the hypothesis that the switch in expression from NMDAR2B to NMDAR2A subunits plays a crucial role in critical period control. A causal link between this switch and critical period closure, however, is still missing. The study of transgenic mice that express the juvenile form of NMDARs into adulthood could make it possible to test this hypothesis in the future [56].

Note added in proof

Two papers have been published very recently [62,63]. The first is concerned with the acute effects of neurotrophins. BDNF has been found to excite isolated cortical, hippocampal and cerebellar neurons in culture just as rapidly as the neurotransmitter glutamate. The second is concerned with the development of ocular dominance columns in ferrets. Surprisingly, total removal of retinal influence early in visual development did not prevent segregation of geniculocortical axons into alternating stripes with periodicity normal for ocular dominance columns.

Acknowledgements

References and recommended reading

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

• of special interest
•• of outstanding interest

1.   Buonomano DV, Merzenich MM: **Cortical plasticity: from synapses to maps.** *Annu Rev Neurosci* 1998, **21**:149-186.

2.   Doupe AJ, Kuhl PK: **Birdsong and human speech: common themes and mechanisms.** *Annu Rev Neurosci* 1999, **22**:567-631.

3.   King AJ, Moore DR: **Plasticity of auditory maps in the brain.** *Trends Neurosci* 1991, **14**:31-37.

4.   Barth M, Hirsch HVB, Meinertzhagen IA, Heisenberg M: **Experience-dependent developmental plasticity in the optic lobe of *Drosophila melanogaster*.** *J Neurosci* 1997, **17**:1493-1504.

5.   Banks MS, Aslin RN, Letson RD: **Sensitive period for the development of human binocular vision.** *Science* 1975, **190**:675-677.

6.   Issa NP, Trachtenberg JT, Chapman B, Zahs KR, Stryker MP: **The critical period for ocular dominance plasticity in the ferret's visual cortex.** *J Neurosci* 1999, **19**:6965-6978.

7.   Huang ZJ, Kirkwood A, Pizzorusso T, Porciatti V, Morales B, Bear MF,
••   Maffei L, Tonegawa S: **BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex.** *Cell* 1999, **98**:739-755.
This paper shows, for the first time, that genetic manipulation of a single molecule, BDNF, accelerates the critical period and the development of the visual cortex. Transgenic mice that overexpress BDNF in the forebrain during postnatal life show a precocious development of inhibitory circuitry, an accel-

erated developmental decline of LTP in white-matter layers II and III, a premature closure of the critical period, and an accelerated development of visual acuity. The results suggest that BDNF controls the time course of the critical period by regulating the developmental increase of inhibition.

8. Olson CR, Freeman RD: **Profile of the sensitive period for monocular deprivation in kittens.** *Exp Brain Res* 1980, **39**:17-21.

9. Fagiolini M, Pizzorusso T, Berardi N, Domenici L, Maffei L: **Functional postnatal development of the rat primary visual cortex and the role of visual experience: dark rearing and monocular deprivation.** *Vision Res* 1994, **34**:709-720.

10. Harwerth RS, Smith EL3d, Duncan GC, Crawford ML, von Noorden GK: **Multiple sensitive periods in the development of the primate visual system.** *Science* 1986, **232**:235-238.

11. Brainard MS, Knudsen EI: **Sensitive periods for visual calibration of the auditory space map in the barn owl optic tectum.** *J Neurosci* 1998, **18**:3929-3942.

12. Pantev C, Oostenveld R, Engelien A, Ross B, Roberts LE, Hoke M:
•• **Increased auditory cortical representation in musicians.** *Nature* 1998, **392**:811-814.
Functional magnetic source imaging (single dipole moment) has been used to measure cortical representation in highly skilled musicians. Auditory cortex activation elicited by piano tones, but not by pure tones of similar fundamental frequency, was found to be stronger in musicians than in controls; enhanced cortical representation was seen only in musicians who began practising before age 9. This is a good example of a critical period for enhanced sensory experience.

13. Rauschecker JP: **Auditory cortical plasticity: a comparison with other sensory systems.** *Trends Neurosci* 1999, **22**:74-80.

14. Glazewski S, Fox K: **Time course of experience-dependent synaptic potentiation and depression in barrel cortex of adolescent rats.** *J Neurophysiol* 1996, **75**:1714-1729.

15. Lessard N, Pare M, Lepore F, Lassonde M: **Early-blind human**
• **subjects localize sound sources better than sighted subjects.** *Nature* 1998, **395**:278-280.
The authors report that early blind subjects, unlike sighted subjects, are able to correctly localize sound sources even under monaural conditions. Together with [16•], this work provides behavioral evidence that totally blind individuals have better auditory ability than sighted subjects.

16. Roder B, Teder-Salejarvi W, Sterr A, Rosler F, Hillyard SA, Neville HJ:
• **Improved auditory spatial tuning in blind humans.** *Nature* 1999, **400**:162-166.
The authors report that blind subjects display better spatial localization of sound sources with respect to sighted controls when attempting to localize sounds in peripheral auditory space. Sighted and blind subjects perform similarly when sounds originate in the central auditory space.

17. Rauschecker JP: **Compensatory plasticity and sensory substitution in the cerebral cortex.** *Trends Neurosci* 1995, **18**:36-43.

18. Sadato N, Pascual-Leone A, Grafman J, Ibanez V, Deiber MP, Dold G, Hallett M: **Activation of the primary visual cortex by Braille reading in blind subjects.** *Nature* 1996, **380**:526-528.

19. Neville HJ, Lawson D: **Attention to central and peripheral visual space in a movement detection task: an event-related potential and behavioral study. II. Congenitally deaf adults.** *Brain Res* 1987, **405**:268-283.

20. Ponton CW, Don M, Eggermont JJ, Waring MD, Kwong B, Masuda A: **Auditory system plasticity in children after long periods of complete deafness.** *Neuroreport* 1996, **8**:61-65.

21. Crair MC, Gillespie DC, Stryker MP: **The role of visual experience in the development of columns in cat visual cortex.** *Science* 1998, **279**:566-570.

22. Weliky M, Katz LC: **Disruption of orientation tuning in visual cortex by artificially correlated neuronal activity.** *Nature* 1997, **386**:680-685.

23. Angelucci A, Clasca F, Bricolo E, Cramer KS, Sur M: **Experimentally induced retinal projections to the ferret auditory thalamus: development of clustered eye-specific patterns in a novel target.** *J Neurosci* 1997, **17**:2040-2055.

24. Sengpiel F, Stawinski P, Bonhoeffer T: **Influence of experience on**
• **orientation maps in cat visual cortex.** *Nat Neurosci* 1999, **2**:727-732.
This paper shows that in kittens reared in striped cylinders (thus provided with a single-orientation environment), the cortical area devoted to the experienced orientation is twice that devoted to the orthogonal one. What is remarkable about this study is the fact that orientations never seen by the

animal still occupy a large proportion of cortical territory, suggesting a role for spontaneous activity.

25. Crair MC: **Neuronal activity during development: permissive or instructive?** *Curr Opin Neurobiol* 1999, **9**:88-93.

26. Schlaggar BL, Fox K, O'Leary DDM: **Postsynaptic control of plasticity in developing somatosensory cortex.** *Nature* 1993, **364**:623-626.

27. Bear MF, Kleinschmidt A, Gu QA, Singer W: **Disruption of experience-dependent synaptic modifications in striate cortex by infusion of an NMDA receptor antagonist.** *J Neurosci* 1990, **10**:909-925.

28. Feldman DE, Brainard MS, Knudsen EI: **Newly learned auditory responses mediated by NMDA receptors in the owl inferior colliculus.** *Science* 1996, **271**:525-528.

29. Daw NW, Gordon B, Fox KD, Flavin HJ, Kirsch JD, Beaver CJ, Ji Q, Reid SN, Czepita D: **Injection of MK-801 affects ocular dominance shifts more than visual activity.** *J Neurophysiol* 1999, **81**:204-215.

30. Roberts EB, Meredith MA, Ramoa AS: **Suppression of NMDA receptor function using antisense DNA block ocular dominance plasticity while preserving visual responses.** *J Neurophysiol* 1998, **80**:1021-1032.

31. Livingston FS, Mooney R: **Development of intrinsic and synaptic properties in a forebrain nucleus essential to avian song learning.** *J Neurosci* 1997, **17**:8997-9009.

32. Catalano SM, Chang CK, Shatz CJ: **Activity-dependent regulation of NMDAR1 immunoreactivity in the developing visual cortex.** *J Neurosci* 1997, **17**:8376-8390.

33. Flint AC, Maisch US, Weishaupt JH, Kriegstein AR, Monyer H: **NR2A subunit expression shortens NMDA receptor synaptic currents in developing neocortex.** *J Neurosci* 1997, **17**:2469-2476.

34. Roberts EB, Ramoa AS: **Enhanced NR2A subunit expression and decreased NMDA receptor decay time at the onset of ocular dominance plasticity in the ferret.** *J Neurophysiol* 1999, **81**:2587-2591.

35. Carmignoto G, Vicini S: **Activity-dependent decrease in NMDA receptor responses during development of the visual cortex.** *Science* 1992, **258**:1007-1011.

36. Quinlan EM, Philpot BD, Huganir RL, Bear MF: **Rapid, experience**
•• **dependent expression of synaptic NMDA receptors in visual cortex *in vivo*.** *Nat Neurosci* 1999, **2**:352-357.
This paper provides evidence that the expression of NMDAR subunit 2A (NMDAR2A) is reduced by dark rearing and is tightly regulated by brief visual experiences. This experience-dependent effect requires NMDAR activation and protein synthesis. The regulation of NMDAR expression and function by visual experience provides a mechanism by which sensory experience can modulate developmental plasticity in the visual cortex.

37. Quinlan EM, Olstein DH, Bear MF: **Bidirectional, experience-dependent regulation of N-methyl-D-aspartate receptor subunit composition in the rat visual cortex during postnatal development.** *Proc Natl Acad Sci USA* 1999, **76**:12876-12880.

38. Fox K: **The critical period for long-term potentiation in primary sensory cortex.** *Neuron* 1995, **15**:485-488.

39. Hensch TK, Gordon JA, Brandon EP, McKnight GS, Idzerda RL, Stryker MP: **Comparison of plasticity *in vivo* and *in vitro* in the developing visual cortex of normal and protein kinase A RIβ-deficient mice.** *J Neurosci* 1998, **18**:2108-2117.

40. Gordon JA, Cioffi D, Silva AJ, Stryker MP: **Deficient plasticity in the primary visual cortex of α-calcium/calmodulin-dependent protein kinase II mutant mice.** *Neuron* 1996, **17**:491-499.

41. McAllister AK, Katz LC, Lo DC: **Neurotrophins and synaptic**
• **plasticity.** *Annu Rev Neurosci* 1999, **22**:295-318.
An up-to-date and thoughtful review on the role of neurotrophins in plasticity. The actions of neurotrophins at cellular level are accurately correlated with their effects on visual cortex plasticity and development.

42. Berardi N, Maffei L: **From visual experience to visual function: roles of neurotrophins.** *J Neurobiol* 1999, **41**:119-126.

43. Caleo M, Lodovichi C, Maffei L: **Effects of nerve growth factor on**
• **visual cortical plasticity require afferent electrical activity.** *Eur J Neurosci* 1999, **11**:2979-2984.
It is known that the shift in ocular dominance caused by monocular deprivation can be prevented by administering NGF. The authors of this paper

report that NGF has no effect, however, if monocular deprivation is induced by injecting tetrodotoxin intraocularly, which blocks afferent activity.

44. Domenici L, Cellerino A, Berardi N, Cattaneo A, Maffei L:
     **Antibodies to nerve growth factor (NGF) prolong the sensitive
     period for monocular deprivation in the rat.** *Neuroreport* 1994,
     5:2041-2044.

45. Carnahan J, Nawa H: **Regulation of neuropeptide expression in the
     brain by neurotrophins. Potential role** *in vivo*. *Mol Neurobiol* 1995,
     10:135-149.

46. Sala R, Viegi A, Rossi FM, Pizzorusso T, Bonanno G, Raiteri M,
•   Maffei L: **Nerve growth factor and brain-derived neurotrophic
     factor increase neurotransmitter release in the rat visual cortex.**
     Eur J Neurosci 1998, **10**:2185-2191.
In a synaptosome preparation taken from the rat visual cortex during the crit-
ical period, both BDNF and NGF potentiate glutamate and acetylcholine
release, but only BDNF potentiates GABA release. These data suggest that
neurotrophins could act on plasticity by regulating neurotransmitter release.
This is also the first demonstration that neurotrophins can modulate inhibito-
ry transmitter release in the visual cortex.

47. Rutherford LC, Nelson SB, Turrigiano GG: **BDNF has opposite
     effects on the quantal amplitude of pyramidal neuron and
     interneuron excitatory synapses.** *Neuron* 1998, **21**:521-530.

48. Carmignoto G, Pizzorusso T, Tia S, Vicini S: **Brain-derived
     neurotrophic factor and nerve growth factor potentiate excitatory
     synaptic transmission in the rat visual cortex.** *J Physiol (Lond)*
     1997, **498**:153-164.

49. Levine ES, Crozier RA, Black IB, Plummer MR: **Brain-derived
     neurotrophic factor modulates hippocampal synaptic
     transmission by increasing N-methyl-D-aspartic acid receptor
     activity.** *Proc Natl Acad Sci USA* 1998, **95**:10235-10239.

50. Zheng W, Knudsen EI: **Functional selection of adaptive auditory
••   space map by GABA$_A$-mediated inhibition.** *Science* 1999,
     284:962-965.
The authors demonstrate that after adaptation to prisms, the excitatory
inputs from the old and new auditory maps coexist in the inferior colliculus
of the barn owl. However, excitation from the old pathways is selectively
over-ridden by increased GABAergic inhibition. This implies that inhibition
plays a crucial role in experience-dependent plasticity during the critical peri-
od of the auditory system.

51. Knudsen EI: **Capacity for plasticity in the adult owl auditory system
•   expanded by juvenile experience.** *Science* 1998, **279**:1531-1533.
Displacement of the visual input in owls causes a rearrangement of the
auditory space map only during the critical period. In owls that had expe-
rienced auditory map rearrangement as juveniles, visual input displace-
ment was also effective at rearranging the auditory map when they
became adults. Reference [49••] gives a possible explanation for
this phenomenon.

52. Hensch TK, Fagiolini M, Mataga N, Stryker MP, Baekkeskov S,
••   Kash SF: **Local GABA circuit control of experience-dependent
     plasticity in developing visual cortex.** *Science* 1998, **282**:1504-1508.
This study shows that adequate levels of inhibition are necessary for visu-
al cortical plasticity during the critical period. Transgenic mice lacking the
65 kDa isoform of the GABA synthesizing enzyme GAD (GAD65) are
deficient only in fast inhibitory transmission, even though basal GABA
release is impaired. Monocular deprivation is ineffective at inducing plas-
tic changes in the visual cortex of GAD65 knockout mice; however, this
plasticity can be rescued by intracortical administration of diazepam, a
GABA$_A$ agonist.

53. Muller CM, Griesinger CB: **Tissue plasminogen activator mediates
•   reverse occlusion plasticity in visual cortex.** *Nat Neurosci* 1998,
     1:47-53.
Intracortical blockade of tissue plasminogen activator selectively prevents
recovery of cortical functions and thalamic neuron size during reverse occlu-
sion without affecting the response to monocular deprivation. This suggests
that recovery plasticity is subserved by molecular mechanisms other than
those subserving monocular deprivation plasticity.

54. Corriveau RA, Shatz CJ, Nedivi E: **Dynamic regulation of cpg15
•   during activity-dependent synaptic development in the
     mammalian visual system.** *J Neurosci* 1999, **19**:7999-8008.
Localization and regulation of cpg15 expression in the cat visual system are
consistent with a presynaptic role for cpg15 in cortical plasticity. In search
of molecules involved in activity dependent refinement of connections, the
authors examine the regulation of the expression of an activity-regulated
gene, cpg15, during LGN layering and ocular dominance columns formation
in the visual cortex. They find that cpg15 expression is regulated by activity
in the LGN and in the cortex; localization and regulation of cpg15 expression
are consistent with a presynaptic role for cpg15 in cortical plasticity.

55. Corriveau RA, Huh GS, Shatz CJ: **Regulation of class I MHC gene
•   expression in the developing and mature CNS by neural activity.**
     Neuron 1998, **21**:505-520.
This paper demonstrates that the expression of class I MHC genes in the cat
visual system is developmentally regulated and is dependent on electrical
activity. This group had previously shown that the prenatal blockade of elec-
trical activity prevents the segregation of LGN layers. In this paper they per-
form a differential mRNA display between normal and activity-blocked LGN
to isolate genes involved in this activity-dependent process. Surprisingly, one
of these clones is the class I MHC gene, suggesting that molecules mediat-
ing cell–cell interactions in the immune function may play a novel role in
activity-dependent changes in synaptic connectivity.

56. Pham TA, Impey S, Storm DR, Stryker MP: **CRE-mediated gene
•   transcription in neocortical neuronal plasticity during the
     developmental critical period.** *Neuron* 1999, **22**:63-72.
Using transgenic mice in which the reporter gene lacZ is expressed under
the control of the CRE transcription sequence, the authors show that
monocular deprivation activates CRE-mediated gene expression in the visu-
al cortex contralateral to the non-deprived eye. This effect of monocular
deprivation is present only during the critical period.

57. Tang YP, Shimizu E, Dube GR, Rampon C, Kerchner GA, Zhuo M, Liu G,
     Tsien JZ: **Genetic enhancement of learning and memory in mice.**
     Nature 1999, **401**:63-69.

58. Boothe RG, Kiorpes L, Williams RA, Teller DY: **Operant
     measurements of contrast sensitivity in infant macaque monkeys
     during normal development.** *Vision Res* 1988, **28**:387-396.

59. Teller DY, McDonald MA, Preston K, Sebris SL, Dobson V:
     **Assessment of visual acuity in infants and children: the acuity
     card procedure.** *Dev Med Child Neurol* 1986, **28**:779-789.

60. Teller DY, Movshon JA: **Visual development.** *Vision Res* 1986,
     26:1483-1506.

61. Elbert T, Pantev C, Wienbruch C, Rockstroh B, Taub E: **Increased
     cortical representation of the fingers of the left hand in string
     players.** *Science* 1995, **270**:305-307.

62. Kafitz KW, Rose CR, Thoenen H, Konnerth A: **Neurotrophin-evoked
     rapid excitation through TrkB receptors.** *Nature* 1999, **401**:918-921.

63. Crowley JC, Katz LC: **Development of ocular dominance columns
     in the absence of retinal input.** *Nat Neurosci* 1999, **2**:1125-1130.

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Structural plasticity upon learning: regulation and functions

Pico Caroni[1], Flavio Donato[1] and Dominique Muller[2]

Abstract | Recent studies have provided long-sought evidence that behavioural learning involves specific synapse gain and elimination processes, which lead to memory traces that influence behaviour. The connectivity rearrangements are preceded by enhanced synapse turnover, which can be modulated through changes in inhibitory connectivity. Behaviourally related synapse rearrangement events tend to co-occur spatially within short stretches of dendrites, and involve signalling pathways partially overlapping with those controlling the functional plasticity of synapses. The new findings suggest that a mechanistic understanding of learning and memory processes will require monitoring ensembles of synapses *in situ* and the development of synaptic network models that combine changes in synaptic function and connectivity.

Synapse dynamics
Excitatory synapses at spines exhibit several forms of structural plasticity regulated by activity, including changes in the size of pre- and postsynaptic complexes, and spine disappearance and appearance events.

The contributions of brain networks to information processing and learning and memory are classically interpreted within the framework of Hebbian plasticity and the notion that synaptic weights can be modified by specific patterns of activity. However, accumulating evidence over the past decade indicates that synaptic networks are also structurally plastic, and that connectivity is remodelled throughout life, through mechanisms of synapse formation, stabilization and elimination[1]. This has led to the concept of structural plasticity, which can encompass a variety of morphological changes that have functional consequences. These include on the one hand structural rearrangements at pre-existing synapses, and on the other hand the formation or loss of synapses, of neuronal processes that form synapses or of neurons. In this Review we focus on plasticity that involves gains and/or losses of synapses. Its key potential implication for learning and memory is to physically alter circuit connectivity, thus providing long-lasting memory traces that can be recruited at subsequent retrieval. Detecting this form of plasticity and relating it to its possible functions poses unique challenges, which are in part due to our still limited understanding of how structure relates to function in the nervous system.

We review recent studies that relate the structural plasticity of neuronal circuits to behavioural learning and memory and discuss conceptual and mechanistic advances, as well as future challenges. The studies establish a number of strong links between specific behavioural learning processes and the assembly and loss of specific synapses. Further areas of substantial progress include molecular and cellular mechanisms

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that regulate synapse dynamics in response to alterations in synaptic activity, the specific spatial distribution of the synaptic changes among identified neurons and dendrites and the relative roles of excitation and inhibition in regulating structural plasticity.

The new findings provide exciting early vistas of how learning and memory may be implemented at the level of structural circuit plasticity. At the same time, they highlight major gaps in our understanding of plasticity regulation at the cellular, circuit and systems levels. Accordingly, achieving a better mechanistic understanding of learning and memory processes is likely to depend on the development of more effective techniques and models to investigate ensembles of identified synapses longitudinally, both functionally and structurally.

Molecular mechanisms of synapse remodelling

A remarkable feature of excitatory and inhibitory synapses is their high level of structural variability[2] and the fact that their morphologies and stabilities change over time[3]. This phenomenon is regulated by activity, and the size of spine heads correlates with synaptic strength[4], presynaptic properties[5] and the long-term stability of the synapse[6]. The morphological characteristics of synapses thus reveal important features of their function and stability. Most importantly, there is a continuity of regulatory processes relating synaptic activity to the strength, shape and long-term retention of existing synapses.

Synapse restructuring. Early electron microscopy studies provided the first evidence that the induction of synaptic plasticity could affect the size and shape of dendritic
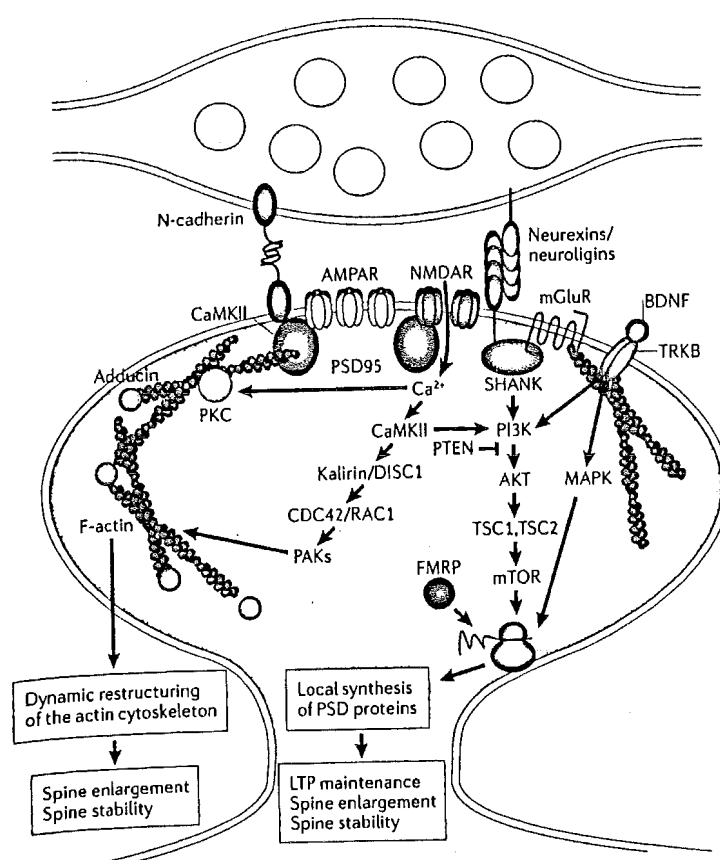
Figure 1 | Molecular mechanisms regulating activity-mediated stabilization of dendritic spines. Induction of synaptic plasticity at individual synapses is associated with a rapid enlargement of the spine head, an increase in synaptic efficacy and a switch in the stability of the synapse that could make them persistent. Recent findings implicate an important role of protein kinases (such as PKC (protein kinase C) and CaMKII (calcium/calmodulin protein kinase II)) contributing to long-term potentiation (LTP) maintenance, spine enlargement and in vivo spine stability (for PKC). In addition, local protein synthesis (for example of BDNF (brain-derived neurotrophic factor), TRKB (tyrosine kinase B), MAPK (mitogen-activated protein kinase), PI3K, (phosphoinositide 3-kinase), PTEN (phosphatase and tensin homologue), AKT, TSC1 (tuberous sclerosis 1), TSC2, mTOR (mammalian target of rapamycin) and FMRP (fragile X mental retardation protein)) contributes to LTP maintenance, spine enlargement and spine stability. Proteins implicated in the regulation of the actin cytoskeleton (such as DISC1 (disrupted in schizophrenia 1), CDC42 (cell division control protein 42), RAC1 (Ras-related C3 botulinum toxin substrate 1), PAKs (p21-activated kinases) and adducin) contribute to LTP maintenance and spine enlargement (and spine stability for PAK3). The actin cytoskeleton is indicated as F-actin. Moreover, adhesion molecules and molecules of the postsynaptic density (including PSD95 (postsynaptic density protein of 95 kDa), SHANKs (SH3 and multiple ankyrin repeat domains proteins), neuroligins, N-cadherins, AMPA receptors (AMPARs) and NMDA receptors (NMDARs)) are implicated in LTP maintenance, spine enlargement and spine stability.

spines[7]. Later, two-photon glutamate uncaging and imaging experiments demonstrated a close association between increased synaptic strength and an enlargement of the spine head[4]. The significance of this enlargement could reflect several important functional modifications of the synapse. It could be linked to the changes in receptor expression that are thought to account for the increase in synaptic strength at many synapses[8]. It could also result

from the mobilization of subcellular resources to potentiated synapses, such as ribosomes or additional cytoskeleton-associated proteins[9]. In addition, this restructuring could be part of a more global set of changes that promote the stabilization of the synapse[10]. Several recent studies have indeed highlighted the importance of synapse stabilization as a defined feature associated with behavioural learning. Novel sensory experience was shown to promote the stabilization of a new set of persistent spines in the somatosensory cortex in vivo[6]. Similarly, in motor skill learning experiments, new spines that grow on selective populations of neurons are preferentially stabilized during subsequent training, with the spines persisting long after training has stopped[11,12]. In birds, song learning by imitation during a juvenile sensitive period leads to a rapid stabilization and enlargement of dendritic spines that is correlated with an enhancement of synaptic activity[13]. These different studies support the idea that the stabilization of selective subpopulations of spines could represent a structural basis for memory storage. Although this stabilization process is often associated with the induction of plasticity, several important issues remain to be addressed. How does this stabilization relate to changes in synaptic strength or spine size? Are changes in synaptic strength required for the stabilization of a synapse? How stable is this mechanism? A recent study suggests that reconditioning following a procedure of conditioning and extinction preferentially eliminates dendritic spines formed and stabilized by extinction[14]. Accordingly, stabilization may be considered as a key reversible property of individual synapses that is linked to the induction of plasticity.

Molecular mechanisms of synapse stabilization. The molecular mechanisms accounting for synapse stabilization are likely to implicate a variety of factors, which have often been inferred from indirect analyses of either mechanisms contributing to long-term potentiation (LTP) maintenance or mechanisms implicated in activity-mediated spine enlargement. Relatively few studies have examined molecular mechanisms contributing to spine stabilization by directly measuring the persistence of dendritic spines in vivo. Current evidence, however, suggests that there is a significant overlap between the molecular pathways implicated in these different aspects of stability (FIG. 1), emphasizing the close link existing between induction of plasticity and synapse stability.

First, an important part is likely to be played by phosphorylation mechanisms. Both calcium/calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC) have been directly implicated in LTP maintenance and behavioural learning[15,16]. CaMKII activity is required for activity-mediated spine enlargement[17], and PKC contributes to in vivo spine stabilization[18]. Another central mechanism for spine stabilization involves the local regulation of protein synthesis, which includes the signalling cascades (such as the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways) downstream of receptor tyrosine kinase B (TRKB; also known as NTRK2) activation, the mammalian target of rapamycin (mTOR)

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signalling complex and the translation of mRNAs that encode proteins such as ARC or CaMKII. Interference with this signalling, with protein synthesis or with *ARC* translation have been strongly implicated in LTP maintenance and in spine enlargement[19-23], whereas *in vivo* blockade of protein synthesis results in synapse destabilization[18]. A third set of molecular factors critical for spine stabilization includes the various signalling pathways and actin-regulatory proteins that control the spine actin cytoskeleton. Interference with actin polymerization impairs LTP maintenance and changes in spine size[23-25]. Furthermore, phosphorylation of the cytoskeleton-stabilizing protein β-adducin through PKC is required for the stabilization of populations of synapses induced by environmental enrichment[18]. Additional evidence supporting a role of the cytoskeleton in spine stabilization comes from the implications of Rho GTPases and several upstream or downstream modulators of this pathway, such as kalirin 7, DISC1 (disrupted in schizophrenia 1) or PAKs (p21-activated kinases). Interference with this signalling affects LTP mechanisms and the capacity of spines to enlarge[26,27]. Finally, one important mechanism through which synapse stability could be improved is by changes in the organization of the postsynaptic density (PSD) that promote trans-synaptic adhesion and contact. Expression of PSD95 and/or AMPA receptors enhances synaptic strength and synapse stability[28,29]. Several adhesion molecule systems have also been linked to spine stability, including neuroligin 1 (REFS 29,30) and N-cadherin. Activity-mediated expression of N-cadherin correlates with, and is required for, the long-term stabilization of spines activated by theta-burst stimulation[31]. Secreted members of the C1q family have also been shown to rapidly induce changes in synapse numbers by, for example, stabilizing synapses in the mature cerebellum *in vivo* through the formation of trans-synaptic complexes[32]. Taken together, these data highlight how spine stabilization is regulated by a multiplicity of molecular mechanisms, probably reflecting the importance and complexity of the phenomenon.

Synapse turnover specificity in vivo

A comparatively small but significant fraction of synapses in the adult *in vivo* undergo a continuous turnover process, which may allow a continuous adaptation of synaptic networks to experience[1]. The magnitude of this turnover process varies strongly during development, decreasing significantly in adult brain[6,33,34], but a substantial capacity for circuit rewiring is maintained throughout life and can be reactivated by lesions[1]. As discussed below, processes known to involve enhanced plasticity also enhance the fraction of synapses that undergo turnover in the adult.

Remodelling of connectivity. An important feature of synapse turnover is its regulation by activity and sensory experience[33]. Whereas initial *in vitro* experiments mainly focused on spine growth and synapse formation in response to neuronal activation[34-36], more recent experiments have shown that activity also destabilizes

existing synapses[10,35]. Under *in vivo* conditions, training in motor skill learning tasks results in a rapid rewiring through the formation and elimination of spines in the primary motor cortex, affecting different sets of synapses for different motor skills[11,12]. Spine elimination and formation caused by fear conditioning and extinction, respectively, occur in a cue- and location-specific manner[14]. Similarly, a major correlate of environmental enrichment is a marked increase in synapse remodelling, including synapse formation and destabilization[18].

An interesting feature of activity-mediated spine dynamics is that it might be regulated locally: evidence suggests that induction of plasticity is facilitated in the vicinity of potentiated spines and that new spines preferentially form close to activated spines[10,37]. Two recent studies further support these results. Using a repetitive motor learning task, it has been shown that new spines formed during the acquisition of learning emerge in clusters as neighbouring spine pairs that are more likely to persist than non-clustered spines[38]. Another study carried out during development by monitoring synaptic activity through calcium imaging shows that neighbouring synapses are more likely to be co-active than synapses farther from each other[39]. Local regulation of spine dynamics may thus be an important mechanism to promote such clustering activity.

A different aspect of the regulation of spine turnover is that, in some cases, the effect may be more global and differentially affect spine formation and elimination, resulting in actual changes in spine density[40]. In the motor learning task experiments, the increase in spine formation and spine loss roughly cancelled each other out, resulting in no marked changes in spine density[11,12]. By contrast, the enriched environment protocols greatly promoted spine growth, leading to an increase in the absolute numbers of spines[18]. Regulation of spine dynamics thus not only promotes rewiring but also controls the level of connectivity of the network. Taken together, these observations suggest that the rewiring observed under behavioural learning conditions represents a structural correlate of learning (FIG. 2).

Regulation of spine and synapse turnover. One important factor controlling synapse turnover appears to be the balance between excitation and inhibition. Alterations of this balance during critical (or sensitive) periods — that is, developmental time windows of enhanced plasticity — strongly affect the capacity for structural plasticity[41]. Furthermore, several recent studies have shown that manipulations that reduce inhibition in adulthood are able to restore visual plasticity to levels comparable to those observed during development[42,43]. Although it remains unclear how exactly modulation of the excitatory–inhibitory balance can promote or reduce cortical plasticity, part of the effect could implicate changes in synapse dynamics. Consistent with this possibility, spine changes correlate with the capacity for visual plasticity *in vivo*[44] and, during development, short-term anaesthesia or administration of drugs that enhance GABAergic inhibition results in rapid and marked changes in spine growth and synapse gain[45].
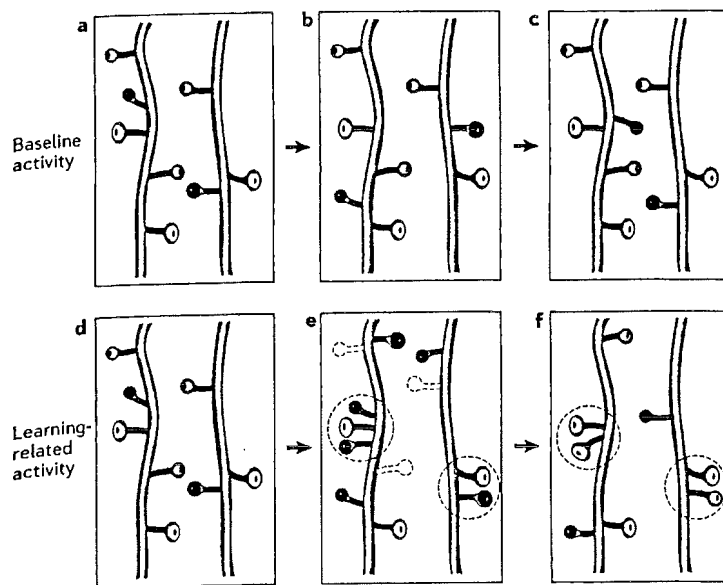
Figure 2 | **Learning-induced structural rewiring of synaptic networks. a–c |** Schematic showing a characteristic spine turnover sequence under baseline activity conditions, which includes both loss of existing spines and gain of new ones, and affects a small subpopulation of transient spines (small dark spines), leaving a larger population of more stable, persistent spines unaffected. **d–f |** Under conditions of behavioural learning, this turnover is markedly enhanced, leading to the formation of additional new spines (small dark spines), and the elimination of pre-existing spines (dashed spines). Although connectivity is modified, spine density can remain unchanged. The new spines formed following learning tend to occur in clusters (encircled areas) and exhibit a higher probability to become stabilized as persistent spines, introducing a lasting modification of the synaptic network.

Several additional molecular mechanisms have also been reported to modify spine numbers and dynamics. Oestrogens, for example, can rapidly shift the balance of spine turnover towards increased growth and stabilization, thus leading to an increase in spine density in the hippocampus[46,47]. The effect is reversible and probably accounts for the variations in spine density reported during the oestrous cycle. Brain-derived nerve growth factor (BDNF) also affects spine formation mechanisms by enhancing both destabilization of spines and spine formation in the cortex and hippocampus, and could thus contribute to some of the activity-dependent regulations of synapse dynamics[48,49]. The mechanisms through which BDNF influences spine growth are as yet unclear, but could be linked to a regulation of protein synthesis. Thus, PI3K, which interacts with AKT and has functional links with mTOR signalling, also regulates spinogenesis[50]. Furthermore, protein synthesis, mTOR signalling and spine turnover are affected in fragile X mental retardation protein (FMRP) knockout mice, a mouse model of fragile X syndrome[51]. A further group of molecular mechanisms affecting spine growth includes proteins implicated in the regulation of the cytoskeleton, such as Rho GTPases and their regulatory proteins. The extent to which some of these factors can diffuse locally could account for the mechanisms of clustered spinogenesis[52,53]. Notably, RAS, which is

Fragile X syndrome
X-linked syndrome caused by triplet repeat expansions (CCG) resulting in reduced expression of *FMR1* (fragile X mental retardation 1). The mutations are the most common single-gene cause of autism and intellectual disability.

Memory consolidation
The processes through which memory traces become long lasting. Synaptic consolidation mechanisms include protein synthesis-dependent long-term potentiation and structural plasticity.

activated by LTP induction, has been shown to diffuse locally and promote plasticity in neighbouring spines[37]. Through its activation of the MAPK pathway and its effects on protein synthesis, it could also locally modulate spine growth. Although substantial progress has been made recently, more work will be needed in order to better understand how precisely these molecular mechanisms control spine turnover.

Distribution of the structural plasticity
Circuit rearrangements can be confined to the neurons involved in the particular learning process, or to neuronal subpopulations within systems involved in the learning process. However, under different circumstances, structural rearrangements can also be induced in a broad range of systems in the brain (for example, upon environmental enrichment, see below). An issue that arises is whether the differences in plasticity distribution reflect different roles of structural plasticity or whether a common logic may underlie these distinct phenomena. In this context, it is useful to take into account that synapse gains and losses related to a particular learning process are mostly specified subsequent to the initial learning event. Accordingly, if memory consolidation upon learning involves the selective stabilization and strengthening of some synapses combined with the weakening and loss of other synapses, the different spatial scales of the structural plasticity may involve the distinction between the potential substrates of memory consolidation, which may be distributed locally or broadly, and the actual substrates of the consolidation, which may be specifically associated with the neurons involved in the particular learning process. Consequently, two crucial issues concern the specificity of the structural changes at the local level and whether more global structural alterations may serve as potential substrates for specific local modifications.

Plasticity within local microcircuits. A remarkable aspect of the recent studies relating learning to changes in dendritic spines and axon terminals is that the structural plasticity could be detected readily using sparse labelling approaches *in vivo*, provided that cortical areas relevant to the particular form of learning were analysed repeatedly during an appropriate time window. One might expect that changes in synapse numbers that correlate with new learning may only affect a very small fraction of the synapses within a relevant network, and for that reason methods that only sample 0.1–1% of the neurons of a given kind[1,6,35] may not be adequate to detect such changes. The dramatic detection sensitivity of these structural plasticity studies is probably owing to the fact that these experiments have involved longitudinal analysis of the same large ensembles of synaptic structures, an approach that is far superior to comparisons of synapse groups, which tend to underestimate the extent of the structural plasticity. In addition, the detection of structural changes was probably facilitated by the fact that behavioural learning initially increases the dynamics of a fraction of spine synapses that is larger than the fraction ultimately retained as a structural trace

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of learning[11,12,40]. Nevertheless, the detection of synapse remodelling events did not reflect a lack of specificity in the circuit elements involved in the structural plasticity. For example, in agreement with behavioural observations, structural plasticity in the motor cortex upon learning of a grasping movement was specifically confined to projection neurons driving distal limb muscles and did not affect those driving proximal muscles[54]. The specificity was particularly remarkable considering that the different projection neurons are locally intermingled within the primary motor cortex. Notably, the extent of the structural plasticity was correlated with the magnitude of the learned movement[54]. Evidence for specificity was also provided in experiments in which sensory deprivation in the adult produced specific patterns of growth and retraction in cortical axons and dendrites[55,56].

In support of the notion that the local structural plasticity was specifically associated with learning, re-learning the same task or a second occurrence of the same kind of sensory deprivation did not elicit further plasticity in the same neurons[11,12,40]. These findings suggest that learning-induced structural plasticity can initially affect a substantial fraction of the neurons involved in the learning, and that less abundant but more persistent alterations reflect 'lasting structural traces' of learning[40]. The number of structural traces of learning that become long lasting may depend on intrinsic processes that regulate plasticity and on the amount of repeated training that triggers memory consolidation and reconsolidation processes. Elucidating the extent to which the new synapses may truly mediate the encoding of memories (that is, whether they represent 'engrams') will require more sophisticated methods to combine structural and functional imaging of synapses *in vivo*[57] (see below). Nevertheless, two recent studies have provided some evidence that there may indeed be a direct correspondence between new synapses and engrams in learning. In one study, fear learning and its extinction affected the formation and disappearance of spines within two microns of distance on the same dendrites, suggesting that opposite changes in the numbers of spatially closely related synapses are associated with opposite behavioural outcomes[14]. Evidence for specificity was provided by the observation that learning–extinction cycles for different tones, which produced separate regulation behaviourally, were associated with distinct stretches of dendrites[14]. In a second study, new spines assembled upon repeated motor learning had a high probability to appear in the close vicinity of spines that had appeared at previous days during the same motor learning process, suggesting a striking correspondence between the gradual encoding of specific new memories and the spatial position of new spines along particular dendrites[38].

In addition to alterations at subsets of neurons and synapses, behavioural learning can produce more global alterations in the numbers of specific types of synapses within systems involved in the particular learning. For example, different forms of behavioural learning can lead to up to a doubling in the numbers of excitatory synapses onto fast-spiking inhibitory interneurons in

the hippocampus and/or cerebellar cortex (feedforward inhibitory (FFI) growth)[58]. Using targeted virus-mediated rescue experiments in a β-adducin mutant background deficient in learning-induced synaptogenesis, the same study provided causal evidence that this plasticity is critically important for the behavioural precision of the memory, but not for the memory of the learned association itself[58]. Although the high level of local prevalence of the FFI growth might suggest a lower circuit level specificity for this form of structural plasticity, this may in fact not be the case. Thus, fast-spiking interneurons are thought to detect local levels of circuit excitation through the convergence of large numbers of weak excitatory synapses onto them and to broadcast that signal to most excitatory neurons within their local environment. Accordingly, the broad FFI growth plasticity may be specifically adjusted to the connectivity properties of fast-spiking feedforward excitation targeting cell bodies and proximal dendrites. Whether learning produces additional broadly distributed alterations in defined elements of neuronal circuits remains to be determined.

Plasticity affecting multiple systems and neurons. Several factors have been shown to influence future learning and behavioural outputs by inducing major modifications in the numbers, arrangements and dynamics of synaptic connections. For example, environmental enrichment and oestrogen both produce large increases in synapse turnover and synapse numbers at multiple neuronal systems[18,46,47]. Conversely, stress can reduce synapse numbers in some systems (for example, in the hippocampus), while increasing them in other systems (for example, in the amygdala)[59]. Synapse dynamics and numbers are further influenced by seasonal changes and developmental age[60]. For environmental enrichment, the increased synapse turnover has been causally related to improved learning[18]. Common to these influences of external and internal contingencies on structural plasticity is the fact that they do not involve specific learning processes. The structural alterations related to experience, hormones and age are not confined to a few neuronal systems, but their distribution has not yet been investigated in sufficient detail to extract possible patterns. It is possible that these alterations may reflect the properties of the signals that induced them, such as the distribution of hormone receptors and the ways through which novel sensory experience influences circuit function.

Widespread dynamics followed by confined consolidation. How can the presence of broadly distributed structural alterations upon experience and learning be reconciled with the specificity necessary for the structural modifications to selectively reflect learned relationships? It is possible that some of the broad changes in circuit structure affect function in ways that are unrelated to mechanisms of learning. However, many of the alterations as a result of experience, hormones and ageing are likely to affect learning and memory by acting on the same cellular and molecular processes. As
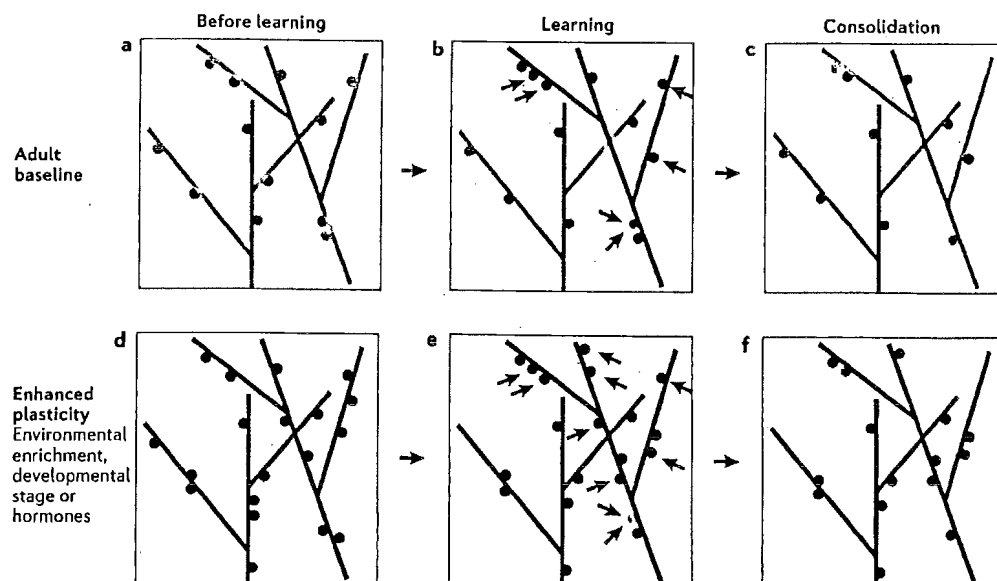
Figure 3 | **Global and local synapse turnover regulation processes affecting learning and memory.** The schematics represent dendrites of two excitatory neurons and their spine synapses. Increasing plasticity is represented as darker grey tones. Dynamic spines are green (gains) and red (losses); spine changes upon learning are indicated by green and red arrows; orange spines appear upon learning, but do not persist during consolidation; and structural traces of learning upon consolidation include spine gains (blue) and spine losses. **a–c** | Learning-induced structural plasticity enhances the turnover of subpopulations of new and pre-existing synapses specifically in excitatory neurons involved in the learning (a versus b), and leads to the selective stabilization of some learning-induced spines (c). **d–f** | Enhanced baseline levels of synapse turnover as a consequence of enrichment, developmental stage or hormones (d versus a) may augment the magnitude of learning-induced spine gains and losses (e versus b), and may lead to more robust structural traces of learning (f versus c). The enhanced structural plasticity baseline levels underlie improved behavioural learning upon enrichment[18], and improved song learning in the presence of a tutor during zebra finch development[13].

discussed in previous sections, LTP and learning are accompanied by enhanced rates of synapse assembly and disassembly events[10,61]. Several studies of learning-related synapse dynamics *in vivo* have provided strong evidence that enhanced dynamics is specifically correlated with new learning in intact birds, rodents and primates, and with recovery after stroke in the human adult[13,44,59,62,63]. Similar studies have further shown that a subpopulation of new synapses is subsequently stabilized during a process depending on repeated training, which lasts for many days and even weeks[11–13,63]. A study of how zebra finches learn to sing from a tutor provides a particularly compelling case for the relationship between behavioural learning and synapse turnover[13]. Thus, at the appropriate developmental stage, enhanced spine turnover was detected on sensorimotor neurons involved in the learning, and the learning experience stabilized some of these spines. An age-related decline in spine dynamics was delayed if the birds were raised without a tutor[13]. Furthermore, enhanced learning upon environmental enrichment was dependent on increased gains and losses of synapses[18]. These were, in part, provided by the population of additional dynamic synapses that were induced upon enrichment[18]. Similar principles seem to apply to the increase in labile synapses induced by oestrogen[46,47]. It is likely that several types of signals, some acting locally and directly related to new learning,

and others acting more globally and related to experience, hormones and age, may all produce alterations in synapse turnover and in the numbers of dynamic synapses that provide potential substrates for learning. The presence of larger numbers of dynamic synapses before learning may facilitate learning, whereas the selective stabilization of small subsets of dynamic synapses upon repeated learning may provide structural traces of learning (FIG. 3). As enhanced learning upon environmental enrichment also depends on synapse loss[18], it is likely that learning also involves the selective elimination of synapse subpopulations.

It is conceivable that learning and memory, under a regime of previously enhanced (for example, after environmental enrichment) or reduced widespread synapse dynamics, might be subject to regulation that differs, in part, from that involving synapse dynamics specifically induced during learning. That may, for example, involve distinct molecular compositions and stabilization mechanisms at synapses involved in learning. Such differences could have important implications for how experience (for example, stress) influences internal states and learning, but an adequate investigation of these phenomena will probably depend on the establishment of more sensitive experimental paradigms to study specific relationships between the structure and function of neuronal networks in living animals (see below).

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Plasticity regulation

What mechanisms regulate the potential for structural plasticity (metaplasticity) in the brain? Much of the current knowledge and concepts about plasticity regulation are derived from studies of juvenile animals in which time windows of enhanced plasticity facilitate adjustments that are important for adult function[41,42,64-66]. Whereas most of the studies have investigated plasticity to adjust for malformations such as strabism or monocular deprivation, a recent study revealed that within the binocular visual cortex, critical period plasticity produces

a matching of the orientation preferences of individual neurons in response to each eye[67]. Critical period studies in the visual and auditory system have provided evidence for profound structural plasticity during learning, including the assembly and long-term retention of alternative extra circuits that can be recruited in the adult under appropriate conditions[64,65,67-69]. Studies in barn owls have revealed that the additional learned circuits that had been assembled during a sensitive period in juvenile birds were turned on and off in the adult through mechanisms distinct from those that turn innate natural circuits on and off (disinhibition versus AMPA/NMDA ratios for the innate and learned circuits, respectively), suggesting that innate and acquired circuit arrangements can be distinguished functionally[64,65]. At the mechanistic level, the studies of critical periods have uncovered a major role for the maturation of inhibitory circuits, and in particular those established by parvalbumin-positive (PV+) fast-spiking interneurons, in opening and closing plasticity windows[41,66,70]. Recent findings suggest that similar mechanisms may regulate plasticity in the adult, and that the regulatory mechanisms may in part involve structural plasticity at inhibitory interneurons.
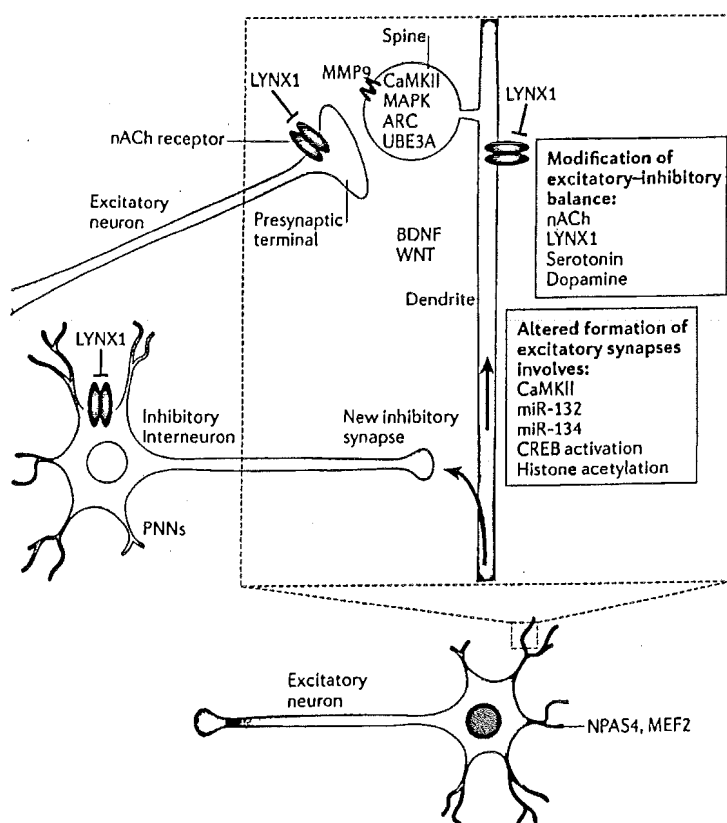
Factors promoting and inhibiting plasticity. Some of the molecular pathways known to regulate plasticity are illustrated in FIG. 4. In most cases, plasticity regulation involves signalling pathways relating neuronal activity to the expression of key activity-regulated genes[71-73]. Consistent with its central roles in mediating signalling downstream of synaptic activity, calcium has prominent roles in activity-regulated gene expression. One of the genes regulated by calcium is the transcription factor *MEF2* (myocyte enhancer factor 2), which reduces excitatory synapse numbers. Genes regulated through MEF2 include the synaptic components *ARC* and *HOMER1*, and the neurotrophin *BDNF*, which augments inhibitory synapse numbers[71]. Although many growth factors can enhance plasticity when applied to cultured neurons or *in vivo*, only a few of them, particularly BDNF, have been related conclusively to endogenous plasticity regulation under physiological conditions[74]. Strong evidence supports the notion that BDNF signalling has a key role in promoting plasticity, and that this signalling pathway is recruited upon enhanced excitation[48,49]. Intracellular signalling molecules and pathways relating excitation and BDNF signalling to plasticity include: ARC, MAPK, CaMK, CREB (cAMP response element-binding) activation, histone acetylation and the microRNA miR-132 (REFS 19, 75-82). Mechanisms through which age influences plasticity regulation can involve chromatin remodelling pathways[81]. Extracellular factors that facilitate plasticity include the proteases matrix metalloprotease 9 and urokinase-type plasminogen activator[83]. In addition, WNT signalling can enhance synapse numbers[84]. Further important signalling molecules with a major role in regulating plasticity include the neuromodulators acetylcholine, noradrenaline, serotonin and dopamine. Among them, a particularly strong case has been made for a link between nicotinic cholinergic transmission and enhanced plasticity. Thus, cholinergic transmission is

Figure 4 | **Mechanisms of structural metaplasticity regulation.** The capacity for structural plasticity can be regulated at various levels. Alterations in expression of certain genes in target neurons (shown in purple) and their transport into dendrites and to synapses (straight arrow) results in structural plasticity by mechanisms that may enhance the formation of excitatory synapses (such as calcium/calmodulin kinases (CaMKs), miR-132, CREB (cAMP response element-binding), UBE3A (ubiquitin protein ligase E3A) and histone acetylation) or reduce the formation of such synapses (such as MEF2 (myocyte enhancer factor 2) and miR-134). Expression of the transcription factor NPAS4 (neuronal PAS domain-containing protein 4) promotes the formation of inhibitory synapses (indicated by a curved arrow). Structural plasticity can result from neuromodulatory modifications of the excitatory–inhibitory balance (including the cholinergic system, LYNX1 (Ly-6/neurotoxin-like protein 1), serotonin and dopamine). LYNX1 inhibits nicotinic acetylcholine (nACh) receptors, which can be found presynaptically, on dendrites and around somas. Furthermore, structural plasticity can be achieved through diffusible factors (including brain-derived neurotrophic factor (BDNF) and WNT, indicated by the green shading) that can affect synaptic signalling pathways (such as CaMKII, MAPK (mitogen-activated protein kinase), ARC and UBE3A) or through alterations of the extracellular matrix (matrix metalloprotease 9 (MMP9) and perineuronal nets (PNNs)).

critically important for skill learning and for functional recovery after brain injury[85–87].

In addition to enhanced excitation, reduced inhibition augments plasticity under a number of different conditions, including environmental enrichment, the effects of fluoxetine treatments and the reduction of perineuronal nets around the cell body and proximal dendrites of PV+ interneurons[41,88–90]. Several lines of evidence have directly related reduced inhibition to enhanced plasticity during critical periods and in the adult in rodents[41,62,90].

Finally, important recent studies have introduced the notion that the potential for plasticity in the adult may be as robust as that detected in juvenile animals, but that adult plasticity is effectively prevented through 'brake' mechanisms[62]. The reduced plasticity in the adult may prevent aberrant plasticity after the formation of lesions and may ensure the transmission of adaptive behaviours learned from conspecifics across generations. In addition to perineuronal nets and myelin-associated inhibitors, which may in part have structural roles, LYNX1 (Ly-6/neurotoxin-like protein 1) has been identified as a specific inhibitor of nicotinic cholinergic signalling that suppresses plasticity in the presence of widespread cholinergic innervation in the adult[43]. An important transcriptional pathway involving NPAS4 (neuronal PAS domain-containing protein 4) also specifically links excitation to the establishment of a higher number of inhibitory synapses onto activated neurons[91]. Furthermore, miR-134 has been identified as a major negative post-transcriptional regulator of plasticity downstream of SIRT1 (NAD-dependent protein deacetylase sirtuin 1) and upstream of CREB[92].

Inhibitory circuit rearrangements. Whereas most studies of structural plasticity initially focused on excitatory neurons, several recent studies have revealed that structural plasticity by inhibitory neurons[93] precedes that by excitatory neurons and may have a critical role in regulating plasticity during learning. An initial series of studies documented structural plasticity of dendritic tips by GABAergic neurons in adult mouse cortex, with most of the plasticity contained within a superficial strip of layer 2/3 (REFS 94–96). A subsequent study documented pronounced structural plasticity of inhibitory axons upon sensory deprivation, which preceded sprouting by excitatory axons, and several-fold enhanced spine and axonal bouton turnover[55,56]. Changes in structural plasticity were detected within hours following peripheral lesions, suggesting that they might account for rapid changes in functional plasticity of receptive fields. Furthermore, dramatic changes in structural plasticity by fast-spiking striatal inhibitory neuron axons that specifically target the indirect striatal pathway were detected following lesions that result in dopamine deprivation[97]. Finally, two recent studies in sensory-deprived visual cortex provided evidence that regulation of structural plasticity by inhibitory interneurons may provide permissive conditions for subsequent plasticity by excitatory neurons. One study reported an early loss of spines, thus reducing excitatory

inputs onto a subpopulation of inhibitory interneurons (mainly neuropeptide Y-positive), and a subsequent loss of axonal boutons, thus reducing inhibitory output by the same interneurons upon sensory deprivation[98]. The second study reported a loss of excitatory inputs onto inhibitory neurons in layer 2/3 upon visual deprivation[99]. Together, the studies suggest that early structural plasticity in sensory-deprived cortex may lead to a diminished excitatory drive onto inhibitory interneurons, suggesting a possible structural basis for disinhibition and enhanced excitation.

Is it inhibition or excitation? The recent discovery of early structural plasticity at inhibitory interneuron subpopulations preceding plasticity at excitatory neurons suggests a possible conceptual framework to account for how excitation–inhibition balances may regulate short- and long-term structural plasticity in the adult. The mechanisms involved appear to resemble those regulating plasticity during circuit maturation, consistent with the notion that plasticity is controlled in similar ways in young animals and in adults. Instead of focusing on excitatory or inhibitory neurotransmitter levels, or on global levels of excitation and inhibition, this emerging framework addresses plasticity regulation at the circuit level, thus offering possible mechanistic solutions to account for fine-tuned regulation and specificity in learning-related plasticity. Findings discussed in previous sections that may be particularly relevant are: at the level of individual neurons, structural plasticity is augmented by enhanced excitation; reducing inhibition is sufficient to enhance plasticity in the adult; and salient activity (for example, exposure to light after dark rearing) can produce disinhibition of excitatory neurons by activating 'second layer' (disinhibiting) inhibitory interneurons, partly through structural plasticity. Accordingly, signals that trigger plasticity may initially reduce the activation of GABAergic neurons, such as PV+ interneurons that target excitatory neurons; depending on the extent of the plasticity, this may involve recruitment of disinhibitory interneurons and/ or structural plasticity to reduce the connectivity of PV+ interneurons, in turn leading to enhanced excitation and structural plasticity of excitatory neurons (FIG. 5). Targeting inhibitory neuron networks first might have a plasticity-facilitating effect at the network level. The enhanced potential for plasticity could then serve as a basis for more specific synapse remodelling processes at the level of individual excitatory neurons. The validity of the model, the identity of the particular interneuron subpopulations and the circuit mechanisms involved in short- and long-term plasticity regulation processes remain to be determined.

From structural plasticity to memories
It is generally assumed that structural plasticity provides a mechanism for long-term storage of memory traces upon learning[100]. However, the temporal sequences of events and the regulatory mechanisms relating learning and structural plasticity to long-term memory are still poorly understood. An important aspect involves

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the temporal delay between the early potentiation of pre-existing synapses, spine growth and synaptogenesis upon learning. Such delays may differ among learning protocols and systems involved. Thus, some studies have suggested that synapses involving new spines or filopodia are assembled within the first 1–3 hours after potentiation[101], whereas other studies have provided evidence for delays of 12–18 hours[102]. The longer delays provide a potential mechanism to relate learning to the consolidation of memories, for example, during sleep. Such scenarios may enhance the specificity of synapse remodelling processes upon learning by uncoupling contingencies present during learning from the consolidation of new synapses and their integration into memory networks. Further structural plasticity may occur during longer lasting system-level consolidation processes, but experimental evidence for such plasticity is not available yet. Likewise, whether and how memory retrieval and reconsolidation processes involve structural plasticity remains to be determined. Addressing

these fundamental issues in learning and memory at the structural level will require the development of more specific and sensitive approaches to investigate circuit and network remodelling processes *in vivo*, at the level of identified synapse ensembles.

Synapse remodelling and mental health

The important contribution of structural plasticity to various behavioural learning situations highlights the importance of connectivity remodelling and synapse stabilization as substrates for learning processes and memory retention. Accordingly, any defect in synapse dynamics can be expected to have a significant impact on the development, organization or specificity of synaptic networks. Indeed, the mechanisms regulating synapse dynamics have been implicated in several developmental psychiatric disorders as discussed below.

Synapse rearrangements in disease and upon lesions.
Analyses of the synaptic defects associated with a number of synaptic proteins implicated in intellectual disability, autism spectrum disorders or schizophrenia show alterations of synapse structure or numbers (TABLE 1). Consistent with a key role for structural plasticity and the excitation–inhibition balance in controlling circuit maturation, many of the psychiatric conditions manifest during early life. SHANK3 (SH3 and multiple ankyrin repeat domains protein 3), PSD95, synapse-associated protein 97 and ubiquitin protein ligase E3A are involved in excitatory synapse stabilization. FMRP, PTEN (phosphatase and tensin homologue), TSC1 (tuberous sclerosis 1; also known as hamartin) and TCS2 (also known as tuberin) regulate local protein synthesis, possibly affecting mechanisms of synapse stabilization. Several molecules (such as DISC1, kalirin, EPAC2 (also known as RAPGEF4), PAK3 and ARHGEF6 (Rho guanine nucleotide exchange factor 6)) are implicated in signalling through Rho GTPases, and could perturb cytoskeletal functions that regulate spine and synapse dynamics. Finally, MECP2 (methyl-CpG-binding protein 2) and molecules of the neuroligin–neurexin complex appear to be important for regulating the balance between excitation and inhibition and could therefore interfere with spine formation and dynamics[103]. All these observations point to the possibility that alterations of structural plasticity mechanisms may have an important role in these diseases. Consistent with this notion, defects in connectivity between layer 5 cortical neurons have been reported in a mouse model of Rett syndrome[104], and this is associated with important alterations of spine dynamics[105]. Other recent evidence from *in vivo* imaging in a mouse model of fragile X syndrome suggests that synapse dynamics could be exaggerated, leading to an increased proportion of unstable synapses and an excessive remodelling of synaptic circuits[106,107]. Similarly, mutation of the intellectual disability gene *PAK3*, which is an effector of the Rho GTPases RAC1 (Ras-related C3 botulinum toxin substrate 1) and CDC42 (cell division control protein 42 homologue), results in excessive
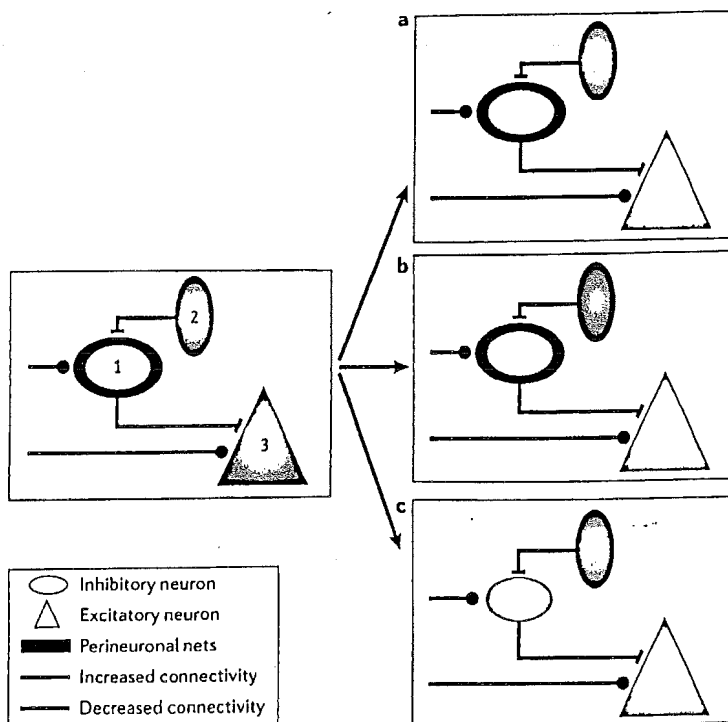
Figure 5 | **Circuit mechanisms of plasticity regulation.** Left: schematic representing a local circuit arrangement involving two inhibitory neurons (ovals 1 and 2, perisomatic and disinhibiting, respectively) impinging onto one excitatory cell (triangle 3). Circles: excitatory inputs; bars: inhibitory inputs. Right: circuit mechanisms leading to enhanced plasticity. Decreased connectivity (decreased synapse numbers (a,b) or decreased synapse function (c)) is represented by red colours; increased connectivity is represented by green colours. Structural plasticity in the excitatory cell is enhanced (shown in purple) under conditions of decreased excitatory connectivity (a), increased inhibitory connectivity (b) or perineuronal net reduction (c) on the perisomatic interneuron that directly inhibits the excitatory cell. Whereas the three scenarios involving structural plasticity at inhibitory interneurons lead to broad disinhibition of excitatory cells, a direct increase of the excitatory drive onto the excitatory neuron can also enhance plasticity.

Legend:
- Inhibitory neuron
- Excitatory neuron
- Perineuronal nets
- Increased connectivity
- Decreased connectivity

Table 1 | Synaptic proteins with genetic defects that have been associated with developmental psychiatric disorders

Protein	Function	Synaptic contribution	Disease
ARHGEF6	RAC GEF, regulation of actin cytoskeleton	Synapse formation and maturation	Intellectual disability
CYFIP1	Protein synthesis	Unknown	Fragile X syndrome
DISC1	Scaffold protein	Synapse formation and maturation	Schizophrenia
EPAC2	RAP GEF	Spine maturation	ASD
ERBB4	Receptor tyrosine kinase	Regulation of excitatory transmission	Schizophrenia
FMRP	Protein synthesis	Synapse stabilization	Fragile X syndrome
GABRB3, GABRA5, GABRG3	GABA receptor subunits	Excitation–inhibition balance	ASD
IL1RAPL1	Scaffold protein	Synapse formation	Intellectual disability
Kalirin	RAC GEF, regulation of actin cytoskeleton	Synapse formation and maturation	Schizophrenia, ASD
LIMK1	Protein kinase, actin skeleton	Spine maturation	Williams syndrome, intellectual disability
MINT2	Presynaptic adaptor protein	Neurosecretion	ASD, schizophrenia
Neuregulin 1	Trans-synaptic modulator of ERBB4	Regulation of excitatory transmission	Schizophrenia
Neurexin 1	Presynaptic adhesion molecule	Synapse stabilization	ASD
Neuroligin 3, neuroligin 4	Adhesion molecules	Synapse stabilization	ASD
Oligophrenin 1	RhoA GAP, regulation of receptor trafficking	Spine maturation	Intellectual disability
PAK3	Protein kinase, actin cytoskeleton	Synapse formation and stabilization	Intellectual disability
Protocadherins	Adhesion molecules	Unknown	ASD
PSD95	Scaffold protein	Synapse plasticity and stabilization	ASD, schizophrenia
PTEN	Tyrosine phosphatase, protein synthesis	Synapse stabilization	ASD, macrocephaly
RSK2	Protein kinase	Neurosecretion	Intellectual disability
SAP97	Scaffold protein	PSD protein trafficking	ASD, schizophrenia
SHANK2, SHANK3	Scaffold protein	Synapse stabilization	ASD
srGAP3	RAC1 GAP	Unknown	Intellectual disability
SSCAM (also known as MAGi2)	Scaffold protein	Receptor trafficking	Intellectual disability
SynGAP	RAS/RAP/RAC-GAP	Receptor trafficking and actin cytoskeleton	ASD, intellectual disability
TSC1, TSC2	Protein synthesis	Synapse stabilization	Intellectual disability
UBE3A	Protein degradation	Synapse formation	Angelman syndrome, intellectual disability

Synaptic proteins for which genetic defects (single point mutations, deletions, translocations or copy number variations (CNVs)) have been associated with autism spectrum disorders (ASDs), intellectual disability or schizophrenia. Supporting references can be found in recent reviews[72,103,114,115]. ARHGEF6, Rho guanine nucleotide exchange factor 6; CYFIP1, cytoplasmic FMR1-interacting protein 1; DISC1, disrupted in schizophrenia 1; EPAC2, Rap guanine nucleotide exchange factor 4; FMRP, fragile X mental retardation protein; IL1RAPL1, interleukin-1 receptor accessory protein-like 1; LIMK1, LIM domain kinase 1; MINT2, MUNC18-interacting protein 2; PAK3, p21-activated kinase 3; PSD, postsynaptic density; PTEN, phosphatase and tensin homologue; RSK2, ribosomal S6 kinase 2; SAP97, synapse-associated protein 97; SHANK, SH3 and multiple ankyrin repeat domains protein; srGAP3, SLIT-ROBO Rho GTPase-activating protein 3; SSCAM, membrane associated guanylate kinase, WW and PDZ domain containing 2; SynGAP, Ras GTPase-activating protein; TSC, tuberous sclerosis; UBE3A, ubiquitin protein ligase E3A.

spine growth and defects in activity-mediated spine stabilization[53]. Alterations in synapse dynamics, either through excessive or insufficient rewiring or defects in synapse stabilization, could perturb the specificity of the mechanisms through which learning shapes the formation of synaptic networks.

Structural plasticity is also important to restore function following lesions. Several recent studies have highlighted the extensive remodelling of both dendritic spines and axons in cortical tissue recovering from stroke or in the visual cortex following lesions[53,98,108]. Synapse-restructuring-associated growth and pruning correlates with functional changes recapitulating the structural plasticity seen in early development.

Outlook: network structure-function

Studies of structural plasticity related to learning and memory have led to major advances during the past couple of years. First, specific synapse assembly and synapse loss processes have been related conclusively to animal learning, and to structural traces of the learning. How the new synapses contribute to memory is not yet clear[57], but the current evidence favours the notion that the new synapse arrangements do have specific roles in memory encoding. Second, causality relationships could be established between the new assembly of identified synapses upon learning and the behavioural expression of the learned memories. Third, important mechanisms and principles underlying the regulation of synapse

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remodelling upon enhanced synaptic activity and learning are being defined at the molecular and cellular level. Among them, an important new insight involves the assembly of new synapses in spatial clusters, suggesting mechanisms of local co-regulation for synapses that may involve the same or connected learning-related memories. Finally, recent results suggest first conceptual frameworks to account for plasticity regulation mechanisms at the circuit level.

The emergence of structural plasticity as a growing research area in learning and memory raises new immediate and long-term challenges. Major unresolved mechanistic issues include: defining the relationships between gains and losses of identified individual synapses upon learning and the memory of what was learned at the microcircuit and systems level; identifying causal sequences of events that relate experience and learning to alterations in structural plasticity and the balance between excitation and inhibition, which includes elucidating how structural remodelling of identified inhibitory and excitatory neuron microcircuits impinge on long-term plasticity regulation during development, in the adult and in disease; and relating genes involved in psychiatric conditions to synapse and microcircuit maturation and remodelling and to the functional consequences of these remodelling processes for system function and animal behaviour.

What will be the probable impact of these new findings for research in neuroscience? The recent advances suggest that structural plasticity processes may be integral components of most aspects of learning and memory. Accordingly, this field of research is likely to have an increasing impact on cognitive neuroscience. The main limitations going forward are of a technical nature.

Although functional imaging techniques in intact animals are extremely valuable for uncovering volume alterations in grey matter or axonal projections upon learning or in disease models, they still lack the resolution required to detect structural plasticity at the microcircuit level. Nevertheless, future research will have to tackle network functions at the level of ensembles of individual identified synapses and neurons in vivo. Further progress will probably depend on the development of methods to image synapses and their molecular components with high sensitivity and spatiotemporal resolution in situ[109-111]. Exciting recent developments mainly, but not exclusively, based on calcium imaging have achieved sufficient resolution to monitor function at the level of ensembles of spines in the neocortex[112,113]. Combining such methods in vivo and in slice preparations should allow neuroscientists to bridge important gaps between the anatomy of microcircuits, their plasticity and their function. In parallel, modelling efforts will probably be important for the development of testable conceptual frameworks that take into account specific structural rearrangements within realistic neuronal networks. The addition of structural plasticity rules to current functional plasticity models may reveal new behaviours or properties that are important for learning capacity. Finally, targeted manipulations in situ — for example, through cellular, but possibly even subcellular, compartment-specific optogenetic methods — will be key in order to establish causal relationships between defined structural alterations in network architecture and network function in behaving animals. Combining cell- and synapse-specific imaging, modelling and optogenetic methods should allow neuroscientists to tackle learning, memory and cognition at the level of defined neuronal circuits.

1. Holtmaat, A. & Svoboda, K. Experience-dependent structural synaptic plasticity in the mammalian brain. Nature Rev. Neurosci. 10, 647–658 (2009).
2. Bourne, J. N. & Harris, K. M. Balancing structure and function at hippocampal dendritic spines. Annu. Rev. Neurosci. 31, 47–67 (2008).
3. Kasai, H. et al. Learning rules and persistence of dendritic spines. Eur J. Neurosci. 32, 241–249 (2010).
4. Matsuzaki, M., Honkura, N., Ellis-Davies, G. C. & Kasai, H. Structural basis of long-term potentiation in single dendritic spines. Nature 429, 761–766 (2004).
5. Tokuoka, H. & Goda, Y. Activity-dependent coordination of presynaptic release probability and postsynaptic GluR2 abundance at single synapses. Proc. Natl Acad. Sci. USA 105, 14656–14661 (2008).
6. Holtmaat, A. J. et al. Transient and persistent dendritic spines in the neocortex in vivo. Neuron 45, 279–291 (2005).
7. Yuste, R. & Bonhoeffer, T. Morphological changes in dendritic spines associated with long-term synaptic plasticity. Annu. Rev. Neurosci. 24, 1071–1089 (2001).
8. Malinow, R. & Malenka, R. C. AMPA receptor trafficking and synaptic plasticity. Annu. Rev. Neurosci. 25, 103–126 (2002).
9. Ostroff, L. E., Fiala, J. C., Allwardt, B. & Harris, K. M. Polyribosomes redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. Neuron 35, 535–545 (2002).
10. De Roo, M., Klauser, P. & Muller, D. LTP promotes a selective long-term stabilization and clustering of dendritic spines. PLoS Biol. 6, e219 (2008).
    This study provides evidence that new spines, upon LTP, tend to be established in clusters, providing a potential mechanism for clustered plasticity specifically upon learning.

11. Xu, T. et al. Rapid formation and selective stabilization of synapses for enduring motor memories. Nature 462, 915–919 (2009).
    This study, and those in references 12, 13 and 40, show that new spine synapses assembled upon behavioural learning and were maintained for many months in the adult, providing long-lasting traces of learning.
12. Yang, G., Pan, F. & Gan, W. B. Stably maintained dendritic spines are associated with lifelong memories. Nature 462, 920–924 (2009).
    See comments to reference 11.
13. Roberts, T. F., Tschida, K. A., Klein, M. E. & Mooney, R. Rapid spine stabilization and synaptic enhancement at the onset of behavioural learning. Nature 463, 948–952 (2010).
    See comments to reference 11.
14. Lai, C. S., Franke, T. F. & Gan, W. B. Opposite effects of fear conditioning and extinction on dendritic spine remodelling. Nature 483, 87–91 (2012).
    This study provides evidence that learning with opposite behavioural outcomes has opposite consequences on spines (for example, on their appearance and disappearance) within a short stretch of the same dendrite. This suggests a specific relationship between changes in spines and specific aspects of the learning.
15. Lisman, J., Yasuda, R. & Raghavachari, S. Mechanisms of CaMKII action in long-term potentiation. Nature Rev. Neurosci. 13, 169–182 (2012).
16. Sacktor, T. C. How does PKMζ maintain long-term memory? Nature Rev. Neurosci. 12, 9–15 (2011).
17. Yamagata, Y. et al. Kinase-dead knock-in mouse reveals an essential role of kinase activity of Ca²⁺/calmodulin-dependent protein kinase IIα in dendritic spine enlargement, long-term potentiation, and learning. J. Neurosci. 29, 7607–7618 (2009).

18. Bednarek, E. & Caroni, P. β-Adducin is required for stable assembly of new synapses and improved memory upon environmental enrichment. Neuron 69, 1132–1146 (2011).
    This paper provides evidence that enhanced learning upon environmental enrichment depends on both enhanced synapse assembly and disassembly processes.
19. Shepherd, J. D. & Bear, M. F. New views of Arc, a master regulator of synaptic plasticity. Nature Neurosci. 14, 279–284 (2011).
20. Costa-Mattioli, M., Sossin, W. S., Klann, E. & Sonenberg, N. Translational control of long-lasting synaptic plasticity and memory. Neuron 61, 10–26 (2009).
21. Tanaka, J. et al. Protein synthesis and neurotrophin-dependent structural plasticity of single dendritic spines. Science 319, 1683–1687 (2008).
22. Minichiello, L. TrkB signalling pathways in LTP and learning. Nature Rev. Neurosci. 10, 850–860 (2009).
23. Bramham, C. R. Local protein synthesis, actin dynamics, and LTP consolidation. Curr. Opin. Neurobiol. 18, 524–531 (2008).
24. Honkura, N., Matsuzaki, M., Noguchi, J., Ellis-Davies, G. C. & Kasai, H. The subspine organization of actin fibers regulates the structure and plasticity of dendritic spines. Neuron 57, 719–729 (2008).
25. Cingolani, L. A. & Goda, Y. Actin in action: the interplay between the actin cytoskeleton and synaptic efficacy. Nature Rev. Neurosci. 9, 344–356 (2008).
26. Hayashi-Takagi, A. et al. Disrupted-in-Schizophrenia 1 (DISC1) regulates spines of the glutamate synapse via Rac1. Nature Neurosci. 13, 327–332 (2010).
27. Xie, Z. et al. Kalirin-7 controls activity-dependent structural and functional plasticity of dendritic spines. Neuron 56, 640–656 (2007).

28. Ehrlich, I., Klein, M., Rumpel, S. & Malinow, R. PSD-95 is required for activity-driven synapse stabilization. *Proc. Natl Acad. Sci. USA* 104, 4176–4181 (2007).

29. Ripley, B., Otto, S., Tiglio, K., Williams, M. E. & Ghosh, A. Regulation of synaptic stability by AMPA receptor reverse signaling. *Proc. Natl Acad. Sci. USA* 108, 367–372 (2011).

30. Wittenmayer, N. *et al.* Postsynaptic Neuroligin1 regulates presynaptic maturation. *Proc. Natl Acad. Sci. USA* 106, 13564–13569 (2009).

31. Mendez, P., De Roo, M., Poglia, L., Klauser, P. & Muller, D. N-cadherin mediates plasticity-induced long-term spine stabilization. *J. Cell Biol.* 189, 589–600 (2010).

32. Yuzaki, M. Cbln1 and its family proteins in synapse formation and maintenance. *Curr. Opin. Neurobiol.* 21, 215–220 (2011).

33. Trachtenberg, J. T. *et al.* Long-term *in vivo* imaging of experience-dependent synaptic plasticity in adult cortex. *Nature* 420, 788–794 (2002).

34. Grutzendler, J., Kasthuri, N. & Gan, W. B. Long-term dendritic spine stability in the adult cortex. *Nature* 420, 812–816 (2002).

35. Holtmaat, A., Wilbrecht, L., Knott, G. W., Welker, E. & Svoboda, K. Experience-dependent and cell-type-specific spine growth in the neocortex. *Nature* 441, 979–983 (2006).
This study provides evidence for enhanced spine turnover and stabilization of some labile spines upon sensory deprivation in the mouse barrel cortex.

36. Engert, F. & Bonhoeffer, T. Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature* 399, 66–70 (1999).

37. Harvey, C. D., Yasuda, R., Zhong, H. & Svoboda, K. The spread of Ras activity triggered by activation of a single dendritic spine. *Science* 321, 136–140 (2008).

38. Fu, M., Yu, X., Lu, J. & Zuo, Y. Repetitive motor learning induces coordinated formation of clustered dendritic spines *in vivo. Nature* 483, 92–95 (2012).
Repeated motor learning leads to the formation of new spines adjacent to spines that had formed during training on the same task. By contrast, 'non-related spines' avoid forming in the vicinity of previous spines. Furthermore, spine neighbours are selectively stabilized. Like reference 14, this study supports the notion that spatially close spines encode functionally related memories.

39. Kleindienst, T., Winnubst, J., Roth-Alpermann, C., Bonhoeffer, T. & Lohmann, C. Activity-dependent clustering of functional synaptic inputs on developing hippocampal dendrites. *Neuron* 72, 1012–1024 (2011).

40. Hofer, S. B., Mrsic-Flogel, T. D., Bonhoeffer, T. & Hubener, M. Experience leaves a lasting structural trace in cortical circuits. *Nature* 457, 313–317 (2009).
See comments to reference 11.

41. Hensch, T. K. Critical period plasticity in local cortical circuits. *Nature Rev. Neurosci.* 6, 877–888 (2005).

42. Bavelier, D., Levi, D. M., Li, R. W., Dan, Y. & Hensch, T. K. Removing brakes on adult brain plasticity: from molecular to behavioral interventions. *J. Neurosci.* 30, 14964–14971 (2010).

43. Morishita, H., Miwa, J. M., Heintz, N. & Hensch, T. K. Lynx1, a cholinergic brake, limits plasticity in adult visual cortex. *Science* 330, 1238–1240 (2010).
The endogenous negative regulator of nicotinic cholinergic transmission LYNX1 functions as an inhibitor of cortical plasticity in the adult.

44. Keck, T. *et al.* Massive restructuring of neuronal circuits during functional reorganization of adult visual cortex. *Nature Neurosci.* 11, 1162–1167 (2008).
This study provides evidence for a major enhancement of structural plasticity leading to replacement of most spine synapses during functional reorganization in a deprived visual cortex.

45. De Roo, M. *et al.* Anesthetics rapidly promote synaptogenesis during a critical period of brain development. *PLoS ONE* 4, e7043 (2009).

46. Kramar, E. A. *et al.* Cytoskeletal changes underlie estrogen's acute effects on synaptic transmission and plasticity. *J. Neurosci.* 29, 12982–12993 (2009).

47. Mendez, P., Garcia-Segura, L. M. & Muller, D. Estradiol promotes spine growth and synapse formation without affecting pre-established networks. *Hippocampus* 21, 1263–1267 (2010).
In hippocampal slice cultures, oestradiol reversibly promotes the assembly of new spine synapses without influencing the turnover of pre-existing synapses.

48. Horch, H. W., Kruttgen, A., Portbury, S. D. & Katz, L. C. Destabilization of cortical dendrites and spines by BDNF. *Neuron* 23, 353–364 (1999).

49. Horch, H. W. & Katz, L. C. BDNF release from single cells elicits local dendritic growth in nearby neurons. *Nature Neurosci.* 5, 1177–1184 (2002).

50. Cuesto, G. *et al.* Phosphoinositide-3-kinase activation controls synaptogenesis and spinogenesis in hippocampal neurons. *J. Neurosci.* 31, 2721–2733 (2011).

51. Sharma, A. *et al.* Dysregulation of mTOR signaling in fragile X syndrome. *J. Neurosci.* 30, 694–702 (2010).

52. Cerri, C. *et al.* Activation of rho GTPases triggers structural remodeling and functional plasticity in the adult rat visual cortex. *J. Neurosci.* 31, 15163–15172 (2011).

53. Dubos, A. *et al.* Alteration of synaptic network dynamics by the intellectual disability protein PAK3. *J. Neurosci.* 32, 519–527 (2012).

54. Wang, L., Conner, J. M., Rickert, J. & Tuszynski, M. H. Structural plasticity within highly specific neuronal populations identifies a unique parcellation of motor learning in the adult brain. *Proc. Natl Acad. Sci. USA* 108, 2545–2550 (2011).
Structural plasticity associated with learning of a forelimb skilled grasping task in adult rats is restricted to upper motor neurons controlling distal but not proximal forelimb musculature within the same area of the primary motor cortex.

55. Yamahachi, H., Marik, S. A., McManus, J. N., Denk, W. & Gilbert, C. D. Rapid axonal sprouting and pruning accompany functional reorganization in primary visual cortex. *Neuron* 64, 719–729 (2009).

56. Marik, S. A., Yamahachi, H., McManus, J. N., Szabo, G. & Gilbert, C. D. Axonal dynamics of excitatory and inhibitory neurons in somatosensory cortex. *PLoS Biol.* 8, e1000395 (2010).
Patterns of excitatory and inhibitory structural plasticity within deprived and non-deprived barrels upon whisker plucking in mice suggest a structural basis for topographic remapping upon sensory deprivation.

57. Hubener, M. & Bonhoeffer, T. Searching for engrams. *Neuron* 67, 363–371 (2010).

58. Ruediger, S. *et al.* Learning-related feedforward inhibitory connectivity growth required for memory precision. *Nature* 473, 514–518 (2011).
The study establishes a causal relationship between increased structural connectivity with PV interneurons by mossy fibres in hippocampal CA3 and the precision of memory upon behavioural learning.

59. McEwen, B. S. The ever-changing brain: cellular and molecular mechanisms for the effects of stressful experiences. *Dev. Neurobiol.* 26 Aug 2011 (doi:10.1002/dneu.20968).

60. De Groof, G. *et al.* Structural changes between seasons in the songbird auditory forebrain. *J. Neurosci.* 29, 13557–13565 (2009).

61. Becker, N., Wierenga, C. J., Fonseca, R., Bonhoeffer, T. & Nagerl, U. V. LTD induction causes morphological changes of presynaptic boutons and reduces their contacts with spines. *Neuron* 60, 590–597 (2008).

62. Tropea, D., Majewska, A. K., Garcia, R. & Sur, M. Structural dynamics of synapses *in vivo* correlate with functional changes during experience-dependent plasticity in visual cortex. *J. Neurosci.* 30, 11086–11095 (2010).

63. Yu, X. & Zuo, Y. Spine plasticity in the motor cortex. *Curr. Opin. Neurobiol.* 21, 169–174 (2011).

64. Knudsen, E. I. Instructed learning in the auditory localization pathway of the barn owl. *Nature* 417, 322–328 (2002).

65. Knudsen, E. I. Sensitive periods in the development of the brain and behavior. *J. Cogn. Neurosci.* 16, 1412–1425 (2004).

66. Yazaki-Sugiyama, Y., Kang, S., Cateau, H., Fukai, T. & Hensch, T. K. Bidirectional plasticity in fast-spiking GABA circuits by visual experience. *Nature* 462, 218–221 (2009).
This study provides evidence for inhibitory circuit plasticity that is consistent with spike-time-dependent plasticity involving PV interneurons in the deprived visual cortex.

67. Wang, B. S., Sarnaik, R. & Cang, J. Critical period plasticity matches binocular orientation preference in the visual cortex. *Neuron* 65, 246–256 (2010).
This study provides evidence for a key role of critical period plasticity to adjust binocular receptive field properties in the visual cortex.

68. Linkenhoker, B. A., von der Ohe, C. G. & Knudsen, E. I. Anatomical traces of juvenile learning in the auditory system of adult barn owls. *Nature Neurosci.* 8, 93–98 (2005).
This study and the one in reference 69 provide evidence for the establishment and long-lasting retention of adaptive extra circuits upon juvenile learning in barn owls and mice.

69. Hofer, S. B., Mrsic-Flogel, T. D., Bonhoeffer, T. & Hubener, M. Prior experience enhances plasticity in adult visual cortex. *Nature Neurosci.* 9, 127–132 (2006).
See comments to reference 68.

70. Southwell, D. G., Froemke, R. C., Alvarez-Buylla, A., Stryker, M. P. & Gandhi, S. P. Cortical plasticity induced by inhibitory neuron transplantation. *Science* 327, 1145–1148 (2010).
Transplanted immature interneurons produce a second critical period window in the visual cortex of young mice, arguing for cell-intrinsic mechanisms of critical period regulation driven by interneuron maturation.

71. Flavell, S. E. & Greenberg, M. E. Signaling mechanisms linking neuronal activity to gene expression and plasticity in the nervous system. *Ann. Rev. Neurosci.* 31, 563–590 (2008).

72. West, A. E. & Greenberg, M. E. Neuronal activity-regulated gene transcription in synapse development and cognitive function. *Cold Spring Harb. Perspect. Biol.* 3, a005744 (2011).

73. Leslie, J. H. & Nedivi, E. Activity-regulated genes as mediators of neural circuit plasticity. *Prog. Neurobiol.* 94, 223–237 (2011).

74. Berardi, N., Pizzorusso, T., Ratto, G. M. & Maffei, L. Molecular basis of plasticity in the visual cortex. *Trends Neurosci.* 26, 369–378 (2003).

75. Dityatev, A. & Rusakov, D. A. Molecular signals of plasticity at the tetrapartite synapse. *Curr. Opin. Neurobiol.* 21, 353–359 (2011).

76. Jourdain, P., Fukunaga, K. & Muller, D. Calcium/calmodulin-dependent protein kinase II contributes to activity-dependent filopodia growth and spine formation. *J. Neurosci.* 23, 10645–10649 (2003).

77. McCurry, C. L. *et al.* Loss of Arc renders the visual cortex impervious to the effects of sensory experience or deprivation. *Nature Neurosci.* 13, 450–457 (2010).

78. Mellios, N. *et al.* miR-132, an experience-dependent microRNA, is essential for visual cortex plasticity. *Nature Neurosci.* 14, 1240–1242 (2011).

79. Tognini, P., Putignano, E., Coatti, A. & Pizzorusso, T. Experience-dependent expression of miR-132 regulates ocular dominance plasticity. *Nature Neurosci.* 14, 1237–1239 (2011).

80. Peleg, S. *et al.* Altered histone acetylation is associated with age-dependent memory impairment in mice. *Science* 328, 753–756 (2010).

81. Putignano, E. *et al.* Developmental downregulation of histone posttranslational modifications regulates visual cortical plasticity. *Neuron* 53, 747–759 (2007).

82. Foscarin, S. *et al.* Experience-dependent plasticity and modulation of growth regulatory molecules at central synapses. *PLoS ONE* 6, e16666 (2011).

83. Michaluk, P. *et al.* Matrix metalloproteinase-9 controls NMDA receptor surface diffusion through integrin β1 signaling. *J. Neurosci.* 29, 6007–6012 (2009).

84. Gogolla, N., Galimberti, I., Deguchi, Y. & Caroni, P. Wnt signaling mediates experience-related regulation of synapse numbers and mossy fiber connectivities in the adult hippocampus. *Neuron* 62, 510–525 (2009).

85. Conner, J. M., Chiba, A. A. & Tuszynski, M. H. The basal forebrain cholinergic system is essential for cortical plasticity and functional recovery following brain injury. *Neuron* 46, 173–179 (2005).

86. Conner, J. M., Kulczycki, M. & Tuszynski, M. H. Unique contributions of distinct cholinergic projections to motor cortical plasticity and learning. *Cereb. Cortex* 20, 2739–2748 (2010).

87. Ramanathan, D., Tuszynski, M. H. & Conner, J. M. The basal forebrain cholinergic system is required specifically for behaviorally mediated cortical map plasticity. *J. Neurosci.* 29, 5992–6000 (2009).

88. Maffei, A., Lambo, M. E. & Turrigiano, G. G. Critical period for inhibitory plasticity in rodent binocular V1. *J. Neurosci.* 30, 3304–3309 (2010).

89. Maya Vetencourt, J. F. *et al.* The antidepressant fluoxetine restores plasticity in the adult visual cortex. *Science* 320, 385–388 (2008).

90. Sale, A. *et al.* Environmental enrichment in adulthood promotes amblyopia recovery through a reduction of intracortical inhibition. *Nature Neurosci.* 10, 679–681 (2007).

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91. Lin, Y. et al. Activity-dependent regulation of inhibitory synapse development by Npas4. Nature 455, 1198–1204 (2008).
This study identifies the transcription factor NPAS4 as a key activity-dependent regulator of inhibitory synaptogenesis onto excitatory neurons.

92. Gao, J. et al. A novel pathway regulates memory and plasticity via SIRT1 and miR-134. Nature 466, 1105–1109 (2010).

93. Chen, J. L. & Nedivi, E. Neuronal structural remodeling: is it all about access? Curr. Opin. Neurobiol. 20, 557–562 (2010).

94. Lee, W. C. et al. Dynamic remodeling of dendritic arbors in GABAergic interneurons of adult visual cortex. PLoS Biol. 4, e29 (2006).
The first live imaging study of inhibitory dendritic arbor structural plasticity in the adult mouse visual cortex.

95. Lee, W. C. et al. A dynamic zone defines interneuron remodeling in the adult neocortex. Proc. Natl Acad. Sci. USA 105, 19968–19973 (2008).

96. Chen, J. L., Flanders, G. H., Lee, W. C., Lin, W. C. & Nedivi, E. Inhibitory dendrite dynamics as a general feature of the adult cortical microcircuit. J. Neurosci. 31, 12437–12443 (2011).

97. Gittis, A. H. et al. Rapid target-specific remodeling of fast-spiking inhibitory circuits after loss of dopamine. Neuron 71, 858–868 (2011).
See comment to reference 98.

98. Keck, T. et al. Loss of sensory input causes rapid structural changes of inhibitory neurons in adult mouse visual cortex. Neuron 71, 869–882 (2011).
This study, and the studies in references 97 and 99, provide evidence for rapid and pronounced structural plasticity of inhibitory circuitry upon deafferentation in the adult cortex and striatum.

99. Chen, J. L. et al. Structural basis for the role of inhibition in facilitating adult brain plasticity. Nature Neurosci. 14, 587–594 (2011).
See comment to reference 98.

100. Bailey, C. H. & Kandel, E. R. Synaptic remodeling, synaptic growth and the storage of long-term memory in Aplysia. Prog. Brain Res. 169, 179–198 (2008).

101. Zito, K., Scheuss, V., Knott, G., Hill, T. & Svoboda, K. Rapid functional maturation of nascent dendritic spines. Neuron 61, 247–258 (2009).

102. Nägerl, U. V., Köstinger, G., Anderson, J. C., Martin, K. A. & Bonhoeffer, T. Protracted synaptogenesis after activity-dependent spinogenesis in hippocampal neurons. J. Neurosci. 27, 8149–8156 (2007).

103. Penzes, P., Cahill, M. E., Jones, K. A., VanLeeuwen, J. E. & Woolfrey, K. M. Dendritic spine pathology in neuropsychiatric disorders. Nature Neurosci. 14, 285–293 (2011).

104. Dani, V. S. & Nelson, S. B. Intact long-term potentiation but reduced connectivity between neocortical layer 5 pyramidal neurons in a mouse model of Rett syndrome. J. Neurosci. 29, 11263–11270 (2009).

105. Landi, S. et al. The short-time structural plasticity of dendritic spines is altered in a model of Rett syndrome. Sci. Rep. 1, 45 (2011).

106. Pan, F., Aldridge, G. M., Greenough, W. T. & Gan, W. B. Dendritic spine instability and insensitivity to modulation by sensory experience in a mouse model of fragile X syndrome. Proc. Natl Acad. Sci. USA 107, 17768–17773 (2010).

107. Cruz-Martin, A., Crespo, M. & Portera-Cailliau, C. Delayed stabilization of dendritic spines in fragile X mice. J. Neurosci. 30, 7793–7803 (2010).

108. Brown, C. E., Li, P., Boyd, J. D., Delaney, K. R. & Murphy, T. H. Extensive turnover of dendritic spines and vascular remodeling in cortical tissues recovering from stroke. J. Neurosci. 27, 4101–4109 (2007).

109. Lang, S. B., Bonhoeffer, T. & Lohmann, C. Simultaneous imaging of morphological plasticity and calcium dynamics in dendrites. Nature Protoc. 1, 1859–1864 (2006).

110. Komiyama, T. et al. Learning-related fine-scale specificity imaged in motor cortex circuits of behaving mice. Nature 464, 1182–1186 (2010).

111. Brown, C. E., Aminoltejari, K., Erb, H., Winship, I. R. & Murphy, T. H. In vivo voltage-sensitive dye imaging in adult mice reveals that somatosensory maps lost to stroke are replaced over weeks by new structural and functional circuits with prolonged modes of activation within both the peri-infarct zone and distant sites. J. Neurosci. 29, 1719–1734 (2009).

112. Chen, X., Leischner, U., Rochefort, N. L., Neiken, I. & Konnerth, A. Functional mapping of single spines in cortical neurons in vivo. Nature 475, 501–505 (2011).
This live calcium imaging study in a mouse cortex provides a first view of spine ensemble function in situ.

113. Jia, H., Rochefort, N. L., Chen, X. & Konnerth, A. In vivo two-photon imaging of sensory-evoked dendritic calcium signals in cortical neurons. Nature Protoc. 6, 28–35 (2011).

114. Miles, J. H. Autism spectrum disorders — a genetics review. Genet. Med. 13, 278–294 (2011).

115. Mitchell, K. J. The genetics of neurodevelopmental disease. Curr. Opin. Neurobiol. 21, 197–203 (2011).

MRI in small brains displaying extensive plasticity

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Manganese-enhanced magnetic resonance imaging (ME-MRI), blood oxygen-level-dependent functional MRI (BOLD fMRI) and diffusion tensor imaging (DTI) can now be applied to animal species as small as mice or songbirds. These techniques confirmed previous findings but are also beginning to reveal new phenomena that were difficult or impossible to study previously. These imaging techniques will lead to major technical and conceptual advances in systems neurosciences. We illustrate these new developments with studies of the song control and auditory systems in songbirds, a spatially organized neuronal circuitry that mediates the acquisition, production and perception of complex learned vocalizations. This neural system is an outstanding model for studying vocal learning, brain steroid hormone action, brain plasticity and lateralization of brain function.

Introduction

Modern neuroscience research devotes a substantial amount of attention to changes in brain function and structure including neural plasticity associated with cognition. In adult animals, including humans, recent studies have confirmed the remarkable plasticity accompanying normal daily processes such as learning but also recovery from neurological insults. Integrated brain studies should, thus, simultaneously consider cellular function and morphological changes. This requires repeated visualization of neural activity associated with an analysis of morphological changes and interactions between brain regions. This is only possible by *in vivo* techniques based on a whole-brain approach that enable the simultaneous assessment of activity in defined neuronal populations and determination of structural changes.

We describe here how this was accomplished by applying multiple recently developed magnetic resonance imaging (MRI) techniques in studies of small songbirds; this is a model system exhibiting vocal learning associated with an extreme brain plasticity linked to quantifiable behavioral changes. Similar MRI approaches were also incorporated in studies of other small animals such as rats and mice. The extensive neural plasticity and cognitive capacities of songbirds, however, provide a very suitable model for illustrating the capacity of these imaging tools. These imaging techniques should in the near future lead to

major conceptual advances in the study of how the brain changes behavior and how behavior changes the brain, in both health and disease. These advances will be due mainly to the inherent capacity of MRI for repeated measures over the course of longitudinal studies of both brain structure and activity.

Plasticity in the song control system

Songbirds (members of the order Passeriformes) share with humans, a few other mammalian taxa (e.g. cetaceans, possibly bats and elephants) and birds from two other orders (some parrots and hummingbirds) the ability to learn their vocalizations. They are probably the only model system exhibiting learned vocalizations that can be easily studied in the laboratory. Vocal communication probably emerged independently in songbirds and humans [1], but brain circuits underlying learned vocal communication in these two taxa display substantial similarities (whether these similarities represent homologies or constraints in evolution is not known at present [1,2]). There are additional interesting parallels between song and human speech, including the existence of crucial periods for learning, dependence on auditory experience and feedback, and lateralization of sound production [3].

During evolution, several forebrain regions of songbirds became part of a spatially organized circuitry that mediates this ability to learn and produce songs: the so-called song control system (SCS) [4] (Figure 1a).

In seasonally breeding species, the SCS displays a high degree of seasonal plasticity including changes in the volume of neuronal cell groups (nuclei) and in their connectivity [5]. This discovery led to one of the most important paradigm shifts in modern neuroscience: the realization of the extent to which dynamic changes in brain function and structure are the norm rather than the exception. Nuclei such as HVC (acronym now used as a proper name; formerly high vocal center), nucleus robustus arco-

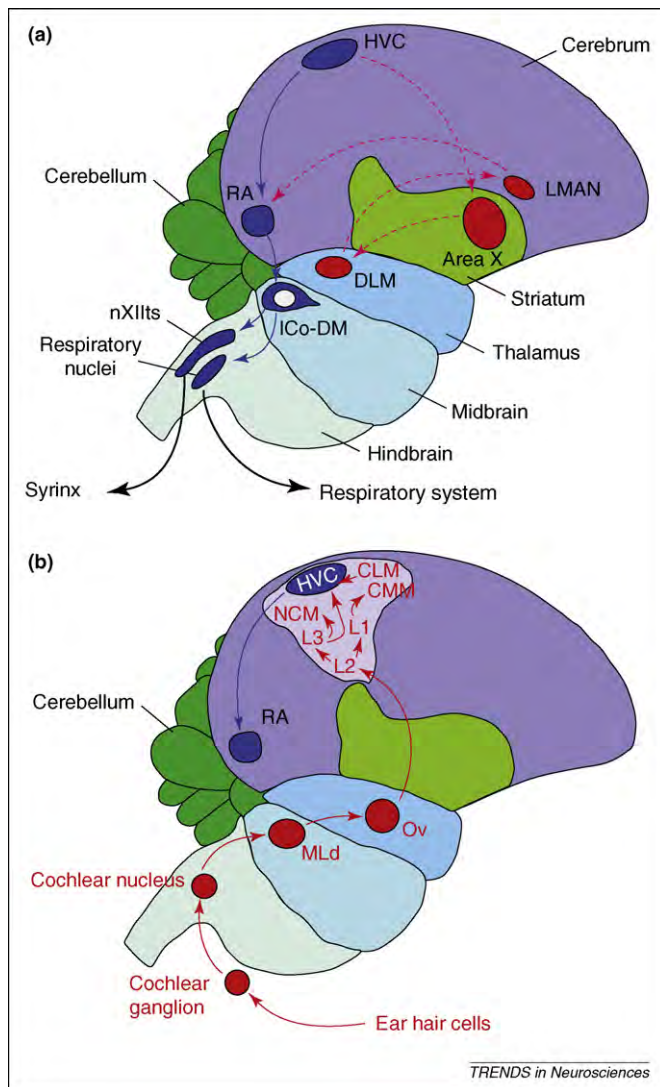*Corresponding author:* Van der Linden, A. (annemie.vanderlinden@ua.ac.be)

Figure 1. The song and auditory systems in songbirds: a unique model of vocal learning and production. **(a)** Song control system (SCS). Songbirds have evolved a suite of neural specializations that mediates learning and production of song, the so-called SCS [67]. The SCS is schematically divided into two main parts. The caudal motor-control pathway (dark blue nuclei and arrows) is made up of the nucleus HVC, the nucleus robustus arcopallialis (RA), the dorso-medial portion (DM) of the nucleus intercollicularis (ICo), medullary nuclei that modulate respiratory motor neurons and the tracheosyringal portion of the hypoglossal nucleus (nXIIts) that controls muscles of the syrinx, the avian vocal organ [4,68] (see Ref. [69] for the recent revision of the avian brain nomenclature). In the more rostral, anterior forebrain pathway (AFP; red nuclei and arrows), another population of HVC neurons projects to area X of the medial striatum (homolog of the caudate and putamen), which in turn projects to the medial dorsolateral thalamic nucleus (DLM). DLM projects to the lateral magnocellular nucleus of the anterior nidopallium (LMAN), and LMAN projects (predominantly) to the RA. This pathway is organized as follows: HVC→X→DLM→LMAN→RA [67]. Thus, there are two pathways from HVC to RA: the caudal pathway, which is essential for song production, and the indirect AFP, which is crucial for song learning [70]. The AFP is also involved in the maintenance of adult song stereotypy. **(b)** Auditory system. HVC receives direct auditory inputs required for acquisition of a song model to be reproduced and for auditory feedback enabling the match of the bird's vocal production with the template stored during learning. Afferents from the inner ear project to various hindbrain nuclei, including the cochlear nucleus, then to the auditory midbrain (nucleus mesencephalicus lateralis pars dorsalis; MLd) and the thalamic nucleus ovoidalis (Ov). Ov, in turn, sends projections to the primary auditory area in the pallium, called field L, a structure analogous to the mammalian primary auditory cortex. Field L has been subdivided into L1, L2a, L2b and L3 based on differences in cytoarchitecture and connectivity [71]. L1 and L3 make bi-directional connections with two secondary auditory areas: the caudal lateral and medial mesopallium (CLM/CMM) and the caudal medial nidopallium (NCM). Auditory information reaches HVC, partially from field L and mainly from CM, which projects directly and indirectly to HVC via the interfacial nucleus of the nidopallium. NCM has no direct connection with HVC. Redrawn from figures in Ref. [2].

pallialis (RA) and area X are twice as large in the spring as in the fall. The cellular bases of these volumetric differences relate to variation in cell size, branching and spacing but also in the case of HVC to an active process of neurogenesis and new neuron incorporation [5]. Young neurons born in the ventricular zone migrate and are incorporated into functional circuits throughout most of the dorsal telencephalon, especially in the HVC of songbirds. However, only neurons projecting to the RA are replaced at a high rate and there is no replacement of area X projecting cells in the HVC. This cellular plasticity is controlled by testosterone acting directly and indirectly on the SCS but also by changes in the social environment or by activity-dependent changes associated with vocal production (i.e. increased metabolic activity in HVC and RA, and activity-dependent production of brain-derived neurotrophic factor) [6].

Auditory information reaches the SCS at the level of HVC after transiting through the auditory pathway that includes the cochlear ganglion and nucleus, the nucleus mesencephalicus lateralis pars dorsalis (MLd) in the midbrain, the thalamic nucleus ovoidalis (Ov) and various telencephalic nuclei (Figure 1b).

Plasticity in the song system has been intensively studied, but histology, by definition performed postmortem, only enables a single determination of morphological features, and electrophysiology usually captures the activity of just a few neurons. It is, therefore, difficult to correlate the overall status of the song and auditory systems with dynamic behavioral data. MRI, a non-destructive microscopic tool, now enables investigation of these issues *in vivo* (Box 1). We have explored this approach intensively for the past six years.

MRI and neuroplasticity in songbirds: the proof of principle

The first analyses of the brain in a live songbird provided images with high anatomical resolution (section thickness 58 μm, in plane resolution 78 μm) that identified a large number of structures including fiber tracts but failed to reveal SCS nuclei [7]. The very dense projections from HVC to RA and area X subsequently enabled visualization of these targets by manganese enhanced MRI (ME-MRI; see Glossary) (Box 1). Stereotaxic injection of $MnCl_2$ into the HVC labeled, within a few hours via anterograde transport, RA and area X in images collected in live subjects so that these nuclei could be delineated and their volume measured (Figure 2a–c). This experimental approach was first validated by comparing the brains of male and female European starlings (*Sturnus vulgaris*) [8], which confirmed the previously established sex differences in area X volume. Subsequently, RA and area X volumes were repeatedly measured in the same subjects (starlings or canaries, *Serinus canaria*) before and after experimental manipulations, enabling for the first time the performance of longitudinal studies on these brain nuclei.

Female starlings that had received a six-week treatment with testosterone displayed RA and X volumes that were numerically larger than before treatment; however, this difference was not statistically significant, apparently because the effect of testosterone was confounded by a

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Box 1. Different MRI techniques: what they are and what they can tell us?

Manganese-enhanced MRI
Mn^{2+} is a paramagnetic Ca^{2+} analog that enters cells through L-type voltage-gated Ca^{2+} channels. It is then transported by fast axonal transport to synapses, released and picked up by postsynaptic neurons. Mn^{2+} can thus be visualized as hyperintense regions in T1-weighted MRI in neuronal circuits [75] including several synapses [54,76]. The blood–brain barrier is almost impermeable to Mn^{2+}, which mainly enters the brain through the choroid plexus–cerebrospinal fluid interface or through the olfactory and retinal pathways. Mn^{2+} can also enter the brain after a transient pharmacological opening of the blood–brain barrier or by stereotaxic injections in the area of interest (for review, see Ref. [77]). Because Mn^{2+} cellular uptake is activity dependent [75], manganese-enhanced MRI (ME-MRI) can be used to monitor brain activation even in awake moving small animals: after being injected with Mn^{2+}, subjects are allowed to express a given behavior for several hours and accumulation of Mn^{2+} is then visualized by MRI [39,44].

Diffusion tensor imaging
Diffusion tensor imaging (DTI) determines the preferential directionality of water-diffusion movements that is constrained by the local micro-architecture of brain structures [78]. The resulting measure

called fractional anisotropy (FA) is determined by the structure of interest (e.g. neurons versus fiber tracts) and the fine organization of fiber tracts (i.e. number of axons, their more or less parallel organization, their degree of myelination). Damage to axonal structures and demyelination affect the observed anisotropy.

Functional MRI
Neuronal activity increases glucose and oxygen consumption, resulting in local decreases in oxygenated hemoglobin (HbO_2) and increases in deoxy-hemoglobin (Hb) that are rapidly over-compensated by increased blood flow and cerebral blood volume. Owing to its paramagnetic properties, Hb disturbs the magnetic fields, a property not shared by the diamagnetic HbO_2. The changes in HbO_2:Hb ratio are recorded by the so-called blood oxygen-level-dependent (BOLD) MRI contrast [79]. BOLD responses reflect the synaptic and postsynaptic activity of neurons and are, thus, best correlated with local field potentials [80]. The time scale of BOLD responses (a few seconds) is constrained by hemodynamic events. Its spatial resolution is mainly constrained by the field strength of the MRI scanner ($\sim$2 mm at 1.5–3 tesla and 0.2 mm at 7 tesla). BOLD functional MRI (fMRI) provides indirect measures of neuronal activity within small voxels but these still contain hundreds to thousands of neurons.

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parallel decrease of the corresponding volumes in control birds. Because control and testosterone-treated subjects were imaged before and after treatments, it was, however, possible to identify this volume decrease in controls and detect a significant interaction between treatments and repeated measures, therefore confirming an effect of testosterone that would have gone unnoticed in a single histological measure [9].

The repeated images provided by ME-MRI can also be compared with the specific behavior displayed by subjects before and between imaging sessions, which enables an analysis of individual correlations between behavior and brain plasticity potentially driven by singing activity. We explored, for example, relationships between seasonal changes of the SCS, song output and intrinsic properties of specific nuclei. Singing was recorded and quantified and Mn^{2+} uptake was analyzed in the same female starlings (female starlings sing contrary to females in many other species) when sexually active and singing a lot (March) or inactive and silent (July). Besides prominent decreases in RA and X volumes between March and July (Figure 2d), significant correlations were also discovered between brain structures, their changes and singing activity (e.g. individual song rate and song bout length in March predicted seasonal changes in telencephalic volume), correlations that could only be obtained by repeated brain measures via MRI [10].

Functional studies by dynamic ME-MRI
Mn^{2+} is a biological calcium analog and MRI contrast agent that enters activated cells such as neurons through voltage-gated calcium channels. When combined with MRI, Mn^{2+} can be used to assess neuronal function and highlight specific brain areas that are active (Box 1).

In songbirds, HVC neurons are known to exhibit auditory responsiveness [11]. This responsiveness is well documented in some species (including canaries, starlings and sparrows) and usually stronger under anesthesia. Based on electrophysiological evidence, both RA-projecting and

area-X-projecting neurons seem to be responsive to auditory stimuli [12]. To generalize this conclusion to large populations of HVC neurons, we stereotaxically injected Mn^{2+} in the HVC of anesthetized male canaries and then measured the dynamic accumulation of Mn^{2+} in RA and area X while birds were or were not exposed to conspecific songs playbacks [13]. This accumulation depends on the activity of HVC projecting neurons and the density of their projections. Accumulation of Mn^{2+} was significantly affected by the exposure to conspecific songs in an anatomically specific manner (different effects in RA and X), thus confirming that broad populations of RA- and area-X-projecting neurons in the HVC are auditory responsive and that these two populations react differentially to the same auditory stimuli as previously demonstrated [12] (Figure 2e).

Comparison of Mn^{2+} uptake in male and female starlings also revealed sexually differentiated kinetics of Mn^{2+} transport from HVC to specific targets. More Mn^{2+} was transported to area X in males than in females (Figure 2c) and the dynamics of this transport was also sexually differentiated (different shape of the sigmoid accumulation curve modeling the process) in area X but not RA [8].

In another experiment, a six-week treatment of female starlings with testosterone increased the total amount of Mn^{2+} transported to area X (increased maximal signal intensity) and altered the rate of this accumulation, but these parameters were not changed in RA, indicating that testosterone differentially affects X- and RA-projecting HVC neurons [9]. The non-invasive nature of this technique enabled imaging of the same birds before and after exposure to testosterone, which markedly improved the sensitivity of the study.

Dynamic ME-MRI (DME-MRI) thus provides an anatomically discrete indirect assessment of electrical activity (Mn^{2+} uptake by neurons depends on their action potentials) in neurons of a brain nucleus specifically connected to a specific target. Changes in Mn^{2+} accumulation in this target are directly related to electrical activity of neurons at the origin of the projection when comparing the same

Review
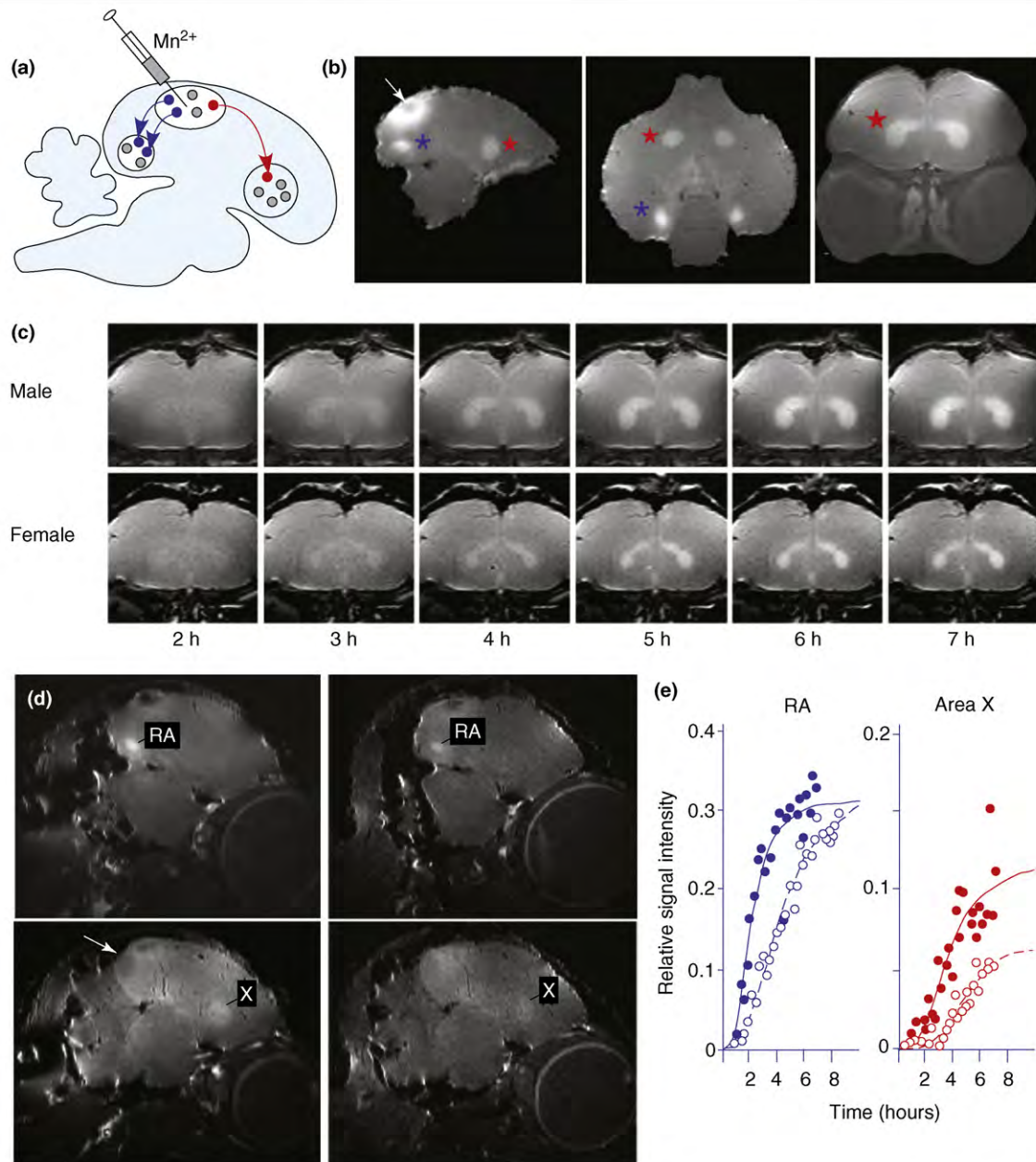
Figure 2. Brain plasticity and function as assessed by manganese-enhanced MRI protocols. **(a)** Manganese (Mn^{2+}) stereotaxically injected into HVC is taken up by the two types of projection neurons and anterogradely transported to the two targets of these neurons, RA (blue) and area X (red). **(b)** This uptake, transport and accumulation of Mn^{2+} visualizes RA (blue asterisks) and area X (red stars) that are shown here in sagittal (left) horizontal (middle) and transversal (right) sections. The injection site in HVC is also visible in the sagittal view (arrow). **(c)** The accumulation of Mn^{2+} in HVC targets, illustrated here in area X, takes place over a period of 7 hours, and the dynamics of this accumulation reflects the properties of the projections in addition to the activity of the projection neurons in HVC. The accumulation is shown here to be different in a male and female starling. These images also confirm the larger size of area X in males than in females. **(d)** Mn^{2+} uptake was monitored in female starlings during the spring breeding season (March) and then again in July, when females had stopped all reproductive activities including song production. RA and area X volumes were much smaller in July with area X being even undetectable in most subjects. The larger HVC in spring producing a bump in the dorsal surface of the brain is also clearly visible (arrow in bottom panel). The data clearly show that, at least in starlings, seasonal plasticity also affects the female brain. Unexpectedly, individual song output in March was not correlated with the volumes of the song control nuclei but, rather, with activity of the RA projecting HVC neurons (assessed by rate of Mn^{2+} accumulation) in both seasons (i.e. also in the summer when birds are not singing). Correlations were also detected between stable X-projecting and replaceable RA-projecting neurons, indicating unexpected stable relations between the activity in the caudal motor pathway (HVC→RA) and anterior forebrain pathway (HVC→area X). **(e)** Indirect measures of the activity of HVC neurons are obtained through the quantification of the dynamics of Mn^{2+} uptake in RA and area X after injection in HVC. Mn^{2+} accumulation in RA and area X was accurately modeled by a sigmoid function that could be summarized by its basic parameters (asymptotic maximal signal intensity or [SI_{max}], time to half SI_{max} [$T_{1/2}$] and shape of the curve [n] providing an indirect measure of the complexity of underling process; see Ref. [8]). Mn^{2+} uptake was quantified for 8 hours in male canaries that were either exposed to the playback of conspecific songs (filled symbols) or kept in the control condition with no sound playback (open symbols). The two types of HVC projection neurons were differentially activated by the auditory stimuli. In RA-projecting neurons exposure to songs modified the shape of the sigmoid curve (n parameter) resulting in a faster accumulation of Mn^{2+} in the target nucleus, whereas in area X the shape of the curve (n) was not affected but the total amount of Mn^{2+} accumulated was markedly increased (larger SI_{max}). Redrawn from figures in Refs [8] (b,c), [10] (d) and [13] (e).

subject under different conditions (e.g. male canaries hearing or not conspecific song). When comparing different subjects (e.g. males and females) or during longitudinal studies over a long period, changes in the structure of the neural circuit might of course also have a potential role in generating differences in Mn^{2+} accumulation.

Seasonal rewiring of the brain detected by diffusion tensor imaging

In vivo diffusion tensor imaging (DTI) is an MRI method that focuses on the preferential directionality of water movements in tissue. Specifically, it is ideally suited for visualizing fiber tracts and their changes (Box 1). DTI is classically used to study connectivity changes in the human brain during development or neurodegeneration [14–16] but a high-resolution variant of the technique was recently implemented for studying songbirds [17]. Longitudinal studies comparing a group of male starlings between the spring (the reproductive season) and summer (the sexually quiescent period) confirmed changes in the HVC to RA projection previously identified by histology (Figure 3). However, this study also revealed the existence of a marked seasonal plasticity affecting connectivity in multiple fiber pathways including the occipito-mesence-

phalic tract, the optic chiasm and the posterior commissure [18].

Functional MRI of auditory processing: a new window on cognitive neurosciences in songbirds

It has been known for ~25 years based on electrophysiological recordings in song control nuclei that they exhibit auditory responsiveness and a specialization characterized by cells tuned to respond selectively to the bird's own song (BOS) as compared with other conspecific songs [11]. More recently, analysis of immediate early gene (IEG) expression in songbird brains highlighted the central role of the caudal telencephalon, which comprises the primary and secondary auditory regions, in auditory processing [19]. This molecular technique revealed the specialization of secondary auditory regions (caudal medial mesopallium [CMM] and caudal medial nidopallium [NCM]) in conspecific song processing (as compared with artificial sounds and heterospecific songs) (for a review, see Ref. [19]). This specialization was further confirmed by electrophysiological methods [20].

Blood oxygen-level-dependent (BOLD) functional MRI (fMRI) detects the global hemodynamic changes in response to synaptic activity of a large number of neurons
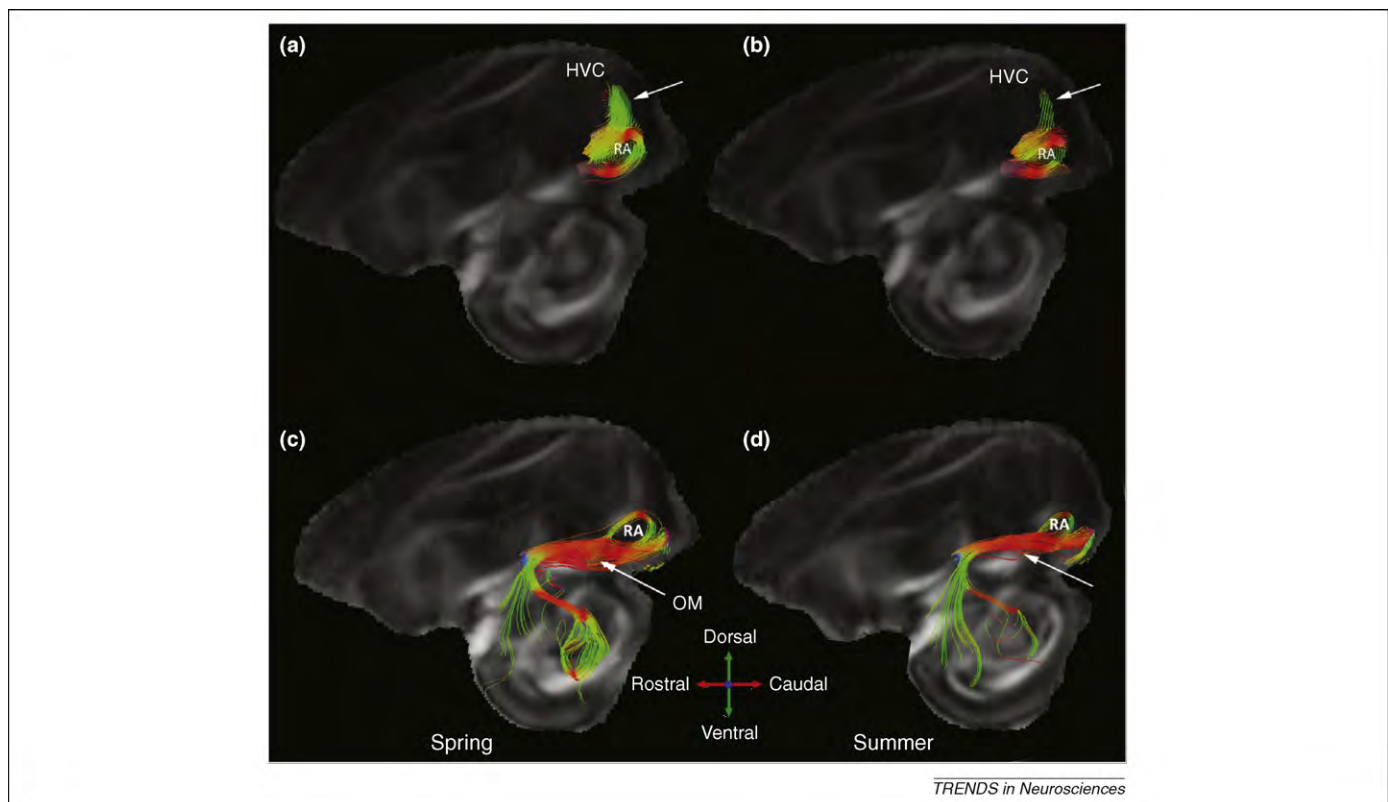

Figure 3. Analysis by diffusion tensor imaging (DTI) of brain connectivity. Analysis by DTI of the starling brain during the spring reproductive season (April) **(a,c)** and in the summer (July) **(b,d)** when birds are photorefractory and their testes are completely regressed demonstrates extensive plasticity in connectivity. This MRI approach first confirms the important variation of the connection of HVC to RA between April and July. This projection is denser during the spring (increased axonal density and myelination; see arrows in parts a and b), which is reflected in DTI in an increased fractional anisotropy (FA). Repeated analysis by DTI of the entire brain additionally revealed the presence of massive seasonal changes in connectivity. These changes concerned the song system (e.g. increased myelination during the breeding season in the medial telencephalic part of the occipito-mesencephalic tract (OM) that contains RA fibers projecting towards the dorsomedial nucleus of the intercollicular complex [DM] of the mesencephalon; see arrows in part c and d) [72] but also many other fiber tracts such as the optic chiasma displaying in spring an increased myelination (resulting in larger FA values; not shown here). The color-coded arrows (centre bottom) indicate the predominant direction of fiber tracts; the same color code is used in the entire figure (e.g. red: rostro-caudal tract). Repeated *in vivo* DTI thus identified an unexpected degree of plasticity, resulting in optimized connections in April (larger numbers of axons and/or increased myelination) that might prepare birds for the demanding behavioral tasks conditioning successful reproduction. Unpublished images describing results published in Ref. [18].

(Box 1). In addition to studies investigating the neurophysiological bases of the BOLD signal, fMRI has, until now, only been sparsely used to study cognition in non-human species. Owing to the intense noise produced by the MRI scanner, BOLD fMRI is particularly challenging but feasible in the auditory domain. It is now beginning to be exploited to study cognitive issues in macaques and birds. In macaques, auditory fMRI has been used to map the auditory regions of the brain and their tonotopy [21], investigate multi-sensory integration in the auditory cortex [22] and identify the neural substrates of conspecific voice recognition [23].

Establishment and methodological developments of BOLD fMRI in songbirds

The feasibility of auditory fMRI in songbirds was first demonstrated in 2005 [24] (Figure 4a) on starlings and has now been extended to a smaller songbird species, the zebra finch [25,26]. The first starling fMRI study established the existence of a hemodynamic response and demonstrated that the temporal pattern of the auditory BOLD response is remarkably similar to auditory evoked BOLD responses in human subjects [24]. The occurrence of a BOLD response was recently confirmed with near-infrared spectroscopy in birds exposed to hypercapnic conditions [27].

Although it is measuring a different neuronal signal from electrophysiological and IEG studies, fMRI has confirmed many results obtained by these techniques. Two independent research groups [24–26] have confirmed the specialization of the caudal telencephalon for processing conspecific songs (over artificial sounds and modified conspecific songs). It was also established that increasing background noise progressively decreases in a very similar manner behavioral responses to conspecific song and activation of the secondary auditory region NCM, as measured
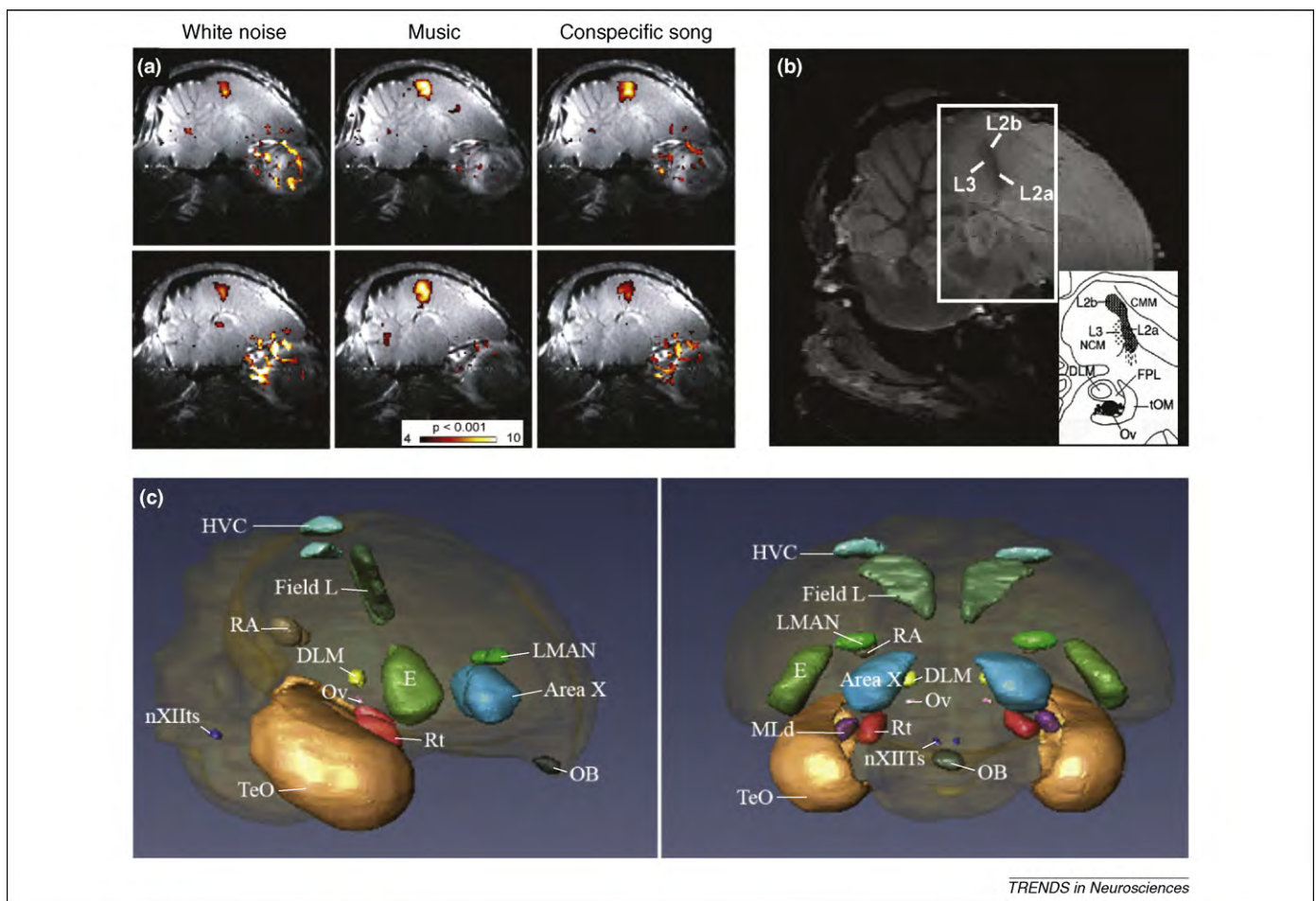

Figure 4. Assessment of brain function by BOLD fMRI. **(a)** Z-score maps illustrating the differential brain activation in male starlings as observed by BOLD fMRI in response to three types of auditory stimuli, namely white noise (sounds with no organized structure), music (organized sounds with no biologically relevant meaning for the species) and conspecific song (songs of a male starling recorded during the breeding season that has, therefore, an obvious signification for the species). Images were collected in two parasagittal slices positioned in the right hemisphere at increasing distances from the midline (top: more medial; bottom: more lateral). White noise activated the auditory field L but more complex sounds such as music or songs extended activation caudally to secondary auditory areas such as NCM. Activation is also observed in the thalamus and cerebellum in addition to field L and NCM. The highlighted pixels around the eye are due to eye movements. This differential activation by different sounds was recently suggested to depend on the previous auditory experience of the subject [73]. **(b)** High-resolution MRI of the male zebra finch auditory system illustrating subregions L2a and L2b of field L as dark areas. The boxed area is schematically represented in the insert with the name of all regions as identified in Ref. [71]. Analysis of the BOLD fMRI responses induced by conspecific songs distinguishes subregions of field L [32]. These subregions (L2a and L2b) were not distinguished by IEG analysis owing to the absence of expression in subfield L2 [74]. **(c)** Rendering of the whole male zebra finch brain as reconstructed in a 3D stereotaxic atlas and of a selection of structures that could be delineated on the MRI images, including all major song control nuclei; (left) right sagittal view, (right) frontal view. Abbreviations: E, entopallium; FPL, lateral forebrain bundle; OB, olfactory bulb; TeO, tectum opticum; tOM, tractus occipitomesencephalicus; Rt, nucleus rotundus. Modified from data in Ref. [24] (a), [32] (c) and [31] (b).

by IEG expression [28]. This was confirmed by fMRI [29]. Successful recognition of biologically relevant information (conspecific song) within noise, the so-called cocktail party effect [30], is thus reflected in the fMRI BOLD response.

These fMRI studies were all based on the analysis of regions of interest (ROI) and had, therefore, limited effective spatial resolution. Although statistically powerful (signal intensity is averaged over all voxels in the ROI, thus removing between-voxel variability), this approach decreases spatial resolution in the results and implicitly assumes that effects are identical over all voxels in the ROI. This can be problematic if the ROI is large and heterogeneous (e.g. auditory region including the primary auditory region field L, and the secondary auditory regions NCM and caudal mesopallium, CM in Ref. [26]). Voxel-based analyses increase effective spatial resolution but require a precise co-registration of individual fMRI datasets to a reference anatomical frame. To facilitate this approach, we recently developed an MRI atlas of the male zebra finch brain [31] that can now be used as the reference for super-imposing statistical results of fMRI experiments as is commonly done in humans (Figure 4c).

New discoveries on auditory processing in the songbird brain
Besides confirming results provided by traditional methods, BOLD fMRI has started to provide novel contributions to the understanding of auditory processing. When compared with a normal conspecific song, spectrally and temporally filtered conspecific songs trigger a differential neural activation in the frontal and caudal regions of a cluster covering the primary auditory cortex (and possibly small parts of surrounding secondary auditory regions) [25] (Figure 4b). The first identified functional difference between sub-regions L2a and L2b of the primary auditory cortex was revealed in terms of BOLD signal intensity [32], an important contribution of fMRI because field L2 does not display increased IEG expression after exposure to song and electrophysiology only enables recording of a limited set of neurons.

Selective responses to BOS initially observed based on electrophysiological methods in the SCS are usually expected to emerge at least at an intermediary level in the ascending auditory system [33,34], and numerous studies were designed to determine where this selectivity exactly emerges. Because electrophysiology requires an *a priori* hypothesis about the location of the selectivity, previous studies focused on the primary and secondary auditory regions [34] but without success (see Ref. [35] for a recent study indicating BOS selectivity in some excitatory neurons of the secondary auditory region CM). Using the zebra finch MRI atlas and voxel-based statistics, we recently investigated this question with BOLD fMRI on the whole zebra finch brain [36]. Comparison of neural activation elicited by the BOS and a familiar conspecific song confirmed BOS selectivity in large neuronal populations of HVC (and the HVC shelf) and part of area X. This differential activation would be impossible to detect by the IEG studies because these genes are not enhanced by hearing songs in these song control nuclei. This study also revealed the presence of activity-based selectivity to the

BOS within the ascending auditory pathway at the level of the midbrain (MLd; homolog to the inferior colliculus) where it had never been investigated before.

Surprisingly, this selectivity was lateralized with a bias towards the right side, a finding reminiscent of the neural right lateralization of self-voice and self-face recognition in human beings [37,38]. A previous fMRI study [26] already suggested that activation by songs could be lateralized because some effects were observed on the right side of the brain only (this study, however, did not report any direct comparison of selectivity between both hemispheres). Interestingly selectivity for conspecific over heterospecific songs was also observed in the MLd but in this case it was lateralized to the left side of the brain [36]. Future electrophysiological and IEG experiments should, therefore, systematically measure and report results from each hemisphere separately.

Advantages and limitations of MRI measures for brain activity compared with other approaches
The scarcity of fMRI experiments completed in small animals to date possibly relates to the fact that they require anesthesia or sedation to achieve the complete immobilization of the subjects. This restricts the type of questions that can be addressed and also disturbs the stability of the hemodynamic parameters challenging the fMRI outcome. Solutions to these problems are progressively identified, as indicated in Table 1, which summarizes the main advantages and limitations of MRI techniques as compared with other approaches. Most importantly, the non-invasiveness of MRI enables repeated longitudinal measures on the same subjects, which opens a large range of new possibilities.

Conclusions and future perspectives
Multiple MRI techniques have now been adapted for use in small animals such as songbirds with body weight as low as 15–20 g. They have also been used in studies on rats (>200 hits for titles including MRI and rat brain in PubMed) and to a smaller extent in mice that have a body weight similar to songbirds such a zebra finches (Table 2).

These techniques were originally validated in songbirds by showing that they identify changes in brain structure or function consistent with previous results obtained by histological, tract-tracing or electrophysiological approaches. Recently, they started to reveal new brain characteristics such as the prominent plasticity affecting not only the SCS but also large areas of the songbird brain and the unexpected lateralization in a mesencephalic auditory nucleus of neural activity related to BOS, conspecific or heterospecific song hearing. The tools have been sharpened and are now available for exploring a long list of unresolved issues in songbird neuroscience such as the respective roles had by testosterone, social stimuli and singing activity itself in the control of neural plasticity in the SCS [6] and the specific mechanisms that promote neuronal recruitment in the SCS and the exact function of these new neurons.

The growing awareness within the neuroscience community of the capabilities of MRI tools as illustrated here in songbirds (an unusually auspicious model for studying neuroplasticity, cognition, learning and memory) should

Table 1. Comparison of the advantages and limitations of two MRI techniques[a] (BOLD fMRI and ME-MRI) applied to a small animal brain compared with electrophysiology or mapping of brain activity by the IEG technique

Features	fMRI	ME-MRI	Electrophysiology	IEG
Spatial resolution	+ Discrete neuronal populations ($\pm$ 100 μm) no cellular resolution	+ Discrete neuronal populations ($\pm$ 100 μm) no cellular resolution	++ Individual cells	++ Cellular resolution
Brain coverage	++ Whole-brain approach	++ Whole-brain approach	− Single cells or small numbers	+/− Whole-brain approach feasible but time consuming[b]
Temporal resolution (compared with neural activity)	+/− Seconds	− Minutes to hours	++ milliseconds	− Minutes to hours
Connectivity (possibility of analyzing functional circuits)	+ Through functional correlation	++ Direct monitoring of projecting neurons	+ Through functional correlation	− Only via pre-injection of tracer
Possibility of studies with freely moving animals	− Anesthesia or immobilization limits types of tasks[c]	+ Brain can also be studied after specific behavioral task[d]	+/− Recording on freely behaving subjects possible but difficult	+ Brain studied after specific behavioral task
Possibility of repeated measurements	++ Easy	+ Easy but requires re-injection of Mn^{2+}	+/− Possible but technically challenging	− Impossible[e]
Possibility of multiple stimuli or conditions in one experiment	++ Easy	+ Possible at two weeks intervals[f]	++ Possible	− Impossible

[a]Limitations of MRI techniques relate to spatial and temporal resolution (first 4 rows of the table) and the need for anesthesia or immobilization during imaging with the potentially associated stress (row 5). The most prominent advantages concern the possibility of repeated measures that enable longitudinal studies of the same individuals and the analysis of brain responses to multiple stimuli (rows 6 and 7).

[b]Importantly, the expression of specific IEG is not ubiquitous. For example Zenk (also called egr-1 in mammals) is not expressed in field L2 or HVC of songbirds, which limits its use for studying auditory processing.

[c]This limitation can be circumvented by using awake subjects habituated to the imaging protocols (e.g. studies on rat maternal behavior [41,42]) or studying neural processes that remain active under anesthesia such as auditory processing in songbirds [34], even if some aspects are affected (e.g. modification of attentional or motivational processes [43]).

[d]Some DME-MRI protocols permit assessment of brain activation in awake behaving animals through the quantification of Mn^{2+} uptake in a target between the injection and the imaging session. Mn^{2+} is injected, behavior is expressed or sensory experience endured by awake subjects for 8–24 hours and then imaging is performed [44].

[e]Double fluorescence *in situ* hybridization potentially enables visualization of neuronal populations that reacted to two different stimuli at different times before sacrifice in the same animal through the analysis of differential cell localization (nuclear versus cytoplasmic) of the RNA signals induced by the stimuli [45]. This procedure does not, however, represent repeated measures in the strictest sense and remains technically challenging and less versatile than fMRI.

[f]Multiple ME-MRI experiments can be carried out on the same subjects provided a minimum of two weeks is allowed between them so that the Mn^{2+} has disappeared from the brain before the second injection.

Table 2. Examples of the application of available MRI techniques for the study of various neuronal functions and circuits in very small animals such as mice and songbirds[a]

MRI method	Animal condition	Neuronal circuit or function	Mice	Songbirds
BOLD fMRI	Healthy brain	Auditory		[24–27,29,32,36]
		Visual	[46]	
		Somatosensory	[47]	
		Olfactory	[48,49]	
		Cognitive function		[36]
	Pathologic or transgenic brain	Entire brain	[50]	
		Somatosensory	[51]	
ME-MRI	Healthy brain	Auditory	[39,52]	
		Song control system		[8–10,13,53,54]
		Olfactory	[55,56]	
		Neuroplasticity	[39]	[9,10,53]
		Hypothalamus	[57]	
	Pathologic or transgenic brain	Olfactory	[58,59]	
		Visual	[60] (more available)	
DTI	Healthy brain	Entire brain, white matter	[61,62] (more available)	[17]
		Neuroplasticity		[18]
	Pathologic or transgenic brain	Entire brain, white matter	[63]	
		Corpus callosum	[64]	
		Nigrostriatal	[65]	
		Entire brain	[66] (more available)	

[a]The table focuses on studies published after year 2000 and presents representative recent examples for all topics that were investigated by these techniques. For some topics, a large number of articles are available and this is then indicated in the table. Note that most studies on neuroplasticity and the only study on cognition were performed in birds. This is why we focused the present review on these animal models.

promote a new generation of studies on neuroplasticity and cognition in small animals such as rodents. Cognitive studies are still missing in rodents (Table 2) but recent experiments have exploited the potential of ME-MRI to study, for example, experience-driven plasticity in the mice auditory midbrain [39] and axonal sprouting in a rat model of epilepsy [40]. These techniques open new windows on brain activity and structure that will now permit, in conjunction with more traditional approaches, further analyses of multiple questions in neurosciences in general. Because the same imaging approaches can be used in humans and in animals that can be submitted to invasive manipulations (e.g. lesions, implantation and tract-tracing), MRI represents a unique tool that should promote translational research by enabling parallel analyses of physiological and pathological processes in the human and animal brain.

References

1 Jarvis, E.D. (2004) Learned birdsong and the neurobiology of human language. *Ann. N. Y. Acad. Sci.* 1016, 749–777
2 Jarvis, E.D. *et al.* (2005) Avian brains and a new understanding of vertebrate brain evolution. *Nat. Rev. Neurosci.* 6, 151–159
3 Doupe, A.J. and Kuhl, P.K. (1999) Birdsong and human speech: common themes and mechanisms. *Annu. Rev. Neurosci.* 22, 567–631
4 Nottebohm, F. *et al.* (1982) Connections of vocal control nuclei in the canary telencephalon. *J. Comp. Neurol.* 207, 344–357
5 Brenowitz, E.A. (2004) Plasticity of the adult avian song control system. *Ann. N. Y. Acad. Sci.* 1016, 560–585
6 Ball, G.F. *et al.* (2004) Seasonal Plasticity in the song control system. Multiple brain sites of steroid hormone action and the importance of variation in song behavior. *Ann. N. Y. Acad. Sci.* 1016, 586–610
7 Van der Linden, A. *et al.* (1998) Non invasive *in vivo* anatomical studies of the oscine brain by high resolution MRI microscopy. *J. Neurosci. Methods* 81, 45–52
8 Van der Linden, A. *et al.* (2002) *In vivo* manganese-enhanced magnetic resonance imaging reveals connections and functional properties of the songbird vocal control system. *Neuroscience* 112, 467–474
9 Van Meir, V. *et al.* (2004) Differential effects of testosterone on neuronal populations and their connections in a sensorimotor brain nucleus controlling song production in songbirds: a manganese enhanced-magnetic resonance imaging study. *Neuroimage* 21, 914–923
10 Van Meir, V. *et al.* (2006) *In vivo* MR imaging of the seasonal volumetric and functional plasticity of song control nuclei in relation to song output in a female songbird. *Neuroimage* 31, 981–992
11 Margoliash, D. (1986) Preference for autogenous song by auditory neurons in a song system nucleus of the white-crowned sparrow. *J. Neurosci.* 6, 1643–1661
12 Mooney, R. (2000) Different subthreshold mechanisms underlie song selectivity in identified HVc neurons of the zebra finch. *J. Neurosci.* 20, 5420–5436
13 Tindemans, I. *et al.* (2003) *In vivo* dynamic ME-MRI reveals differential functional responses of RA- and area X-projecting neurons in the HVC of canaries exposed to conspecific song. *Eur. J. Neurosci.* 18, 3352–3360
14 Taber, K.H. *et al.* (2002) The future for diffusion tensor imaging in neuropsychiatry. *J. Neuropsychiatry Clin. Neurosci.* 14, 1–5
15 Cascio, C.J. *et al.* (2007) Diffusion tensor imaging: application to the study of the developing brain. *J. Am. Acad. Child Adolesc. Psychiatry* 46, 213–223
16 Lebel, C. *et al.* (2008) Microstructural maturation of the human brain from childhood to adulthood. *Neuroimage* 40, 1044–1055
17 De Groof, G. *et al.* (2006) *In vivo* diffusion tensor imaging (DTI) of brain subdivisions and vocal pathways in songbirds. *Neuroimage* 29, 754–763
18 De Groof, G. *et al.* (2008) Seasonal rewiring of the songbird brain: an *in vivo* MRI Study. *Eur. J. Neurosci.* 28, 2475–2485
19 Mello, C.V. *et al.* (2004) Song-induced gene expression: a window on song auditory processing and perception. *Ann. N. Y. Acad. Sci.* 1016, 263–281
20 Grace, J.A. *et al.* (2003) Selectivity for conspecific song in the zebra finch auditory forebrain. *J. Neurophysiol.* 89, 472–487
21 Petkov, C.I. *et al.* (2006) Functional imaging reveals numerous fields in the monkey auditory cortex. *PLoS Biol.* 4, e215
22 Kayser, C. *et al.* (2007) Functional imaging reveals visual modulation of specific fields in auditory cortex. *J. Neurosci.* 27, 1824–1835
23 Petkov, C.I. *et al.* (2008) A voice region in the monkey brain. *Nat. Neurosci.* 11, 367–374
24 Van Meir, V. *et al.* (2005) Spatiotemporal properties of the BOLD response in the songbirds' auditory circuit during a variety of listening tasks. *Neuroimage* 25, 1242–1255
25 Boumans, T. *et al.* (2007) Neural representation of spectral and temporal features of song in the auditory forebrain of zebra finches as revealed by functional MRI. *Eur. J. Neurosci.* 26, 2613–2626
26 Voss, H.U. *et al.* (2007) Functional MRI of the zebra finch brain during song stimulation suggests a lateralized response topography. *Proc. Natl. Acad. Sci. U. S. A.* 104, 10667–10672
27 Vignal, C. *et al.* (2008) Measuring brain hemodynamic changes in a songbird: responses to hypercapnia measured with functional MRI and near-infrared spectroscopy. *Phys. Med. Biol.* 53, 2457–2470
28 Vignal, C. *et al.* (2004) Background noise does not modify song-induced genic activation in the bird brain. *Behav Brain Res.* 153, 241–248
29 Boumans, T. *et al.* (2008) Functional magnetic resonance imaging in zebra finch discerns the neural substrate involved in segregation of conspecific song from background noise. *J. Neurophysiol.* 99, 931–938
30 Appeltants, D. *et al.* (2005) The effect of auditory distractors on song discrimination in male canaries (*Serinus canaria*). *Behav. Processes* 69, 331–341
31 Poirier, C. *et al.* (2008) A three-dimensional MRI atlas of the zebra finch brain in stereotaxic coordinates. *Neuroimage* 41, 1–6
32 Boumans, T. *et al.* (2008) Functional MRI of auditory responses in the zebra finch forebrain reveals a hierarchical organisation based on signal strength but not selectivity. *PLoS One* 3, e3184
33 Prather, J.F. and Mooney, R. (2004) Neural correlates of learned song in the avian forebrain: simultaneous representation of self and others. *Curr. Opin. Neurobiol.* 14, 496–502
34 Theunissen, F.E. *et al.* (2004) Song selectivity in the song system and in the auditory forebrain. *Ann. N. Y. Acad. Sci.* 1016, 222–245
35 Bauer, E.E. *et al.* (2008) A synaptic basis for auditory-vocal integration in the songbird. *J. Neurosci.* 28, 1509–1522
36 Poirier, C. *et al.* (2009) Own-song recognition in the songbird auditory pathway: selectivity and lateralization. *J. Neurosci.* 29, 2252–2258
37 Keenan, J.P. *et al.* (2001) Self-recognition and the right hemisphere. *Nature* 409, 305
38 Nakamura, K. *et al.* (2001) Neural substrates for recognition of familiar voices: a PET study. *Neuropsychologia* 39, 1047–1054
39 Yu, X. *et al.* (2007) Large-scale reorganization of the tonotopic map in mouse auditory midbrain revealed by MRI. *Proc. Natl. Acad. Sci. U. S. A.* 104, 12193–12198
40 Immonen, R.J. *et al.* (2008) Manganese enhanced MRI detects mossy fiber sprouting rather than neurodegeneration, gliosis or seizure-activity in the epileptic rat hippocampus. *Neuroimage* 40, 1718–1730
41 Febo, M. *et al.* (2005) The neural consequences of repeated cocaine exposure revealed by functional MRI in awake rats. *Neuropsychopharmacology* 30, 936–943
42 Febo, M. *et al.* (2008) Nursing stimulation is more than tactile sensation: it is a multisensory experience. *Horm. Behav.* 54, 330–339

43 Cardin, J.A. and Schmidt, M.F. (2004) Noradrenergic inputs mediate state dependence of auditory responses in the avian song system. *J. Neurosci.* 24, 7745–7753

44 Yu, X. *et al.* (2005) *In vivo* auditory brain mapping in mice with Mn-enhanced MRI. *Nat. Neurosci.* 8, 961–968

45 Guzowski, J.F. *et al.* (2001) Imaging neural activity with temporal and cellular resolution using FISH. *Curr. Opin. Neurobiol.* 11, 579–584

46 Nair, G. and Duong, T.Q. (2004) Echo-planar BOLD fMRI of mice on a narrow-bore 9.4 T magnet. *Magn. Reson. Med.* 52, 430–434

47 Ahrens, E.T. and Dubowitz, D.J. (2001) Peripheral somatosensory fMRI in mouse at 11.7 T. *NMR Biomed.* 14, 318–324

48 Xu, F. *et al.* (2003) Odor maps of aldehydes and esters revealed by functional MRI in the glomerular layer of the mouse olfactory bulb. *Proc. Natl. Acad. Sci. U. S. A.* 100, 11029–11034

49 Xu, F. *et al.* (2005) Simultaneous activation of mouse main and accessory olfactory bulbs by odors or pheromones. *J. Comp. Neurol.* 489, 491–500

50 Suarez, S.V. *et al.* (2008) Brain activation by short-term nicotine exposure in anesthetized wild-type and β_2-nicotinic receptors knockout mice: a BOLD fMRI study. *Psychopharmacology (Berl)* 202, 599–610

51 Zhang, L. *et al.* (2007) Changes of brain activity in the aged SAMP mouse. *Biogerontology* 8, 81–88

52 Yu, X. *et al.* (2008) Statistical mapping of sound-evoked activity in the mouse auditory midbrain using Mn-enhanced MRI. *Neuroimage* 39, 223–230

53 Van der Linden, A. *et al.* (2004) Applications of manganese-enhanced magnetic resonance imaging (MEMRI) to image brain plasticity in song birds. *NMR Biomed.* 17, 602–612

54 Tindemans, I. *et al.* (2006) IR-SE and IR-MEMRI allow *in vivo* visualization of oscine neuroarchitecture including the main forebrain regions of the song control system. *NMR Biomed.* 19, 18–29

55 Pautler, R.G. and Koretsky, A.P. (2002) Tracing odor-induced activation in the olfactory bulbs of mice using manganese-enhanced magnetic resonance imaging. *Neuroimage* 16, 441–448

56 Chuang, K.H. *et al.* (2009) Manganese enhanced MRI reveals functional circuitry in response to odorant stimuli. *Neuroimage* 44, 363–372

57 Kuo, Y.T. *et al.* (2006) Manganese-enhanced magnetic resonance imaging (MEMRI) without compromise of the blood-brain barrier detects hypothalamic neuronal activity *in vivo*. *NMR Biomed.* 19, 1028–1034

58 Smith, K.D. *et al.* (2007) *In vivo* axonal transport rates decrease in a mouse model of Alzheimer's disease. *Neuroimage* 35, 1401–1408

59 Serrano, F. *et al.* (2008) Assessing transneuronal dysfunction utilizing manganese-enhanced MRI (MEMRI). *Magn. Reson. Med.* 60, 169–175

60 Bearer, E.L. *et al.* (2007) Role of neuronal activity and kinesin on tract tracing by manganese-enhanced MRI (MEMRI). *Neuroimage* 37 (Suppl. 1), S37–S46

61 Zhang, J. *et al.* (2002) Three-dimensional diffusion tensor magnetic resonance microimaging of adult mouse brain and hippocampus. *Neuroimage* 15, 892–901

62 Chahboune, H. *et al.* (2007) Neurodevelopment of C57B/L6 mouse brain assessed by *in vivo* diffusion tensor imaging. *NMR Biomed.* 20, 375–382

63 Song, S.K. *et al.* (2004) Diffusion tensor imaging detects age-dependent white matter changes in a transgenic mouse model with amyloid deposition. *Neurobiol. Dis.* 15, 640–647

64 Nair, G. *et al.* (2005) Myelination and long diffusion times alter diffusion-tensor-imaging contrast in myelin-deficient shiverer mice. *Neuroimage* 28, 165–174

65 Boska, M.D. *et al.* (2007) Quantitative diffusion tensor imaging detects dopaminergic neuronal degeneration in a murine model of Parkinson's disease. *Neurobiol. Dis.* 26, 590–596

66 Mac Donald, C.L. *et al.* (2007) Diffusion tensor imaging reliably detects experimental traumatic axonal injury and indicates approximate time of injury. *J. Neurosci.* 27, 11869–11876

67 Brenowitz, E. *et al.* (1997) An introduction to birdsong and the avian song system. *J. Neurobiol.* 33, 495–500

68 Wild, J.M. (2004) Functional neuroanatomy of the sensorimotor control of singing. *Ann. N. Y. Acad. Sci.* 1016, 438–462

69 Reiner, A. *et al.* (2004) Revised nomenclature for avian telencephalon and some related brainstem nuclei. *J. Comp. Neurol.* 473, 377–414

70 Doupe, A.J. *et al.* (2004) Cellular, circuit, and synaptic mechanisms in song learning. *Ann. N. Y. Acad. Sci.* 1016, 495–523

71 Vates, G.E. *et al.* (1996) Auditory pathways of caudal telencephalon and their relation to the song system of adult male zebra finches (*Taenopygia guttata*). *J. Comp. Neurol.* 366, 613–642

72 Wild, J.M. (1993) Descending projections of the songbird nucleus robustus archistriatalis. *J. Comp. Neurol.* 338, 225–241

73 Maul, K.K., *et al.* (2007) How developmental song learning affects auditory responses in the songbird [abstract]. *Society for Neuroscience*, Program No. 430.431/KKK429

74 Sen, K. *et al.* (2001) Feature analysis of natural sounds in the songbird auditory forebrain. *J. Neurophysiol.* 86, 1445–1458

75 Lin, Y.J. and Koretsky, A.P. (1997) Manganese ion enhances T1-weighted MRI during brain activation: an approach to direct imaging of brain function. *Magn Reson Med.* 38, 378–388

76 Pautler, R.G. (2004) *In vivo*, trans-synaptic tract-tracing utilizing manganese-enhanced magnetic resonance imaging (MEMRI). *NMR Biomed.* 17, 595–601

77 Van der Linden, A. *et al.* (2007) Current status of functional MRI on small animals: application to physiology, pathophysiology, and cognition. *NMR Biomed.* 20, 522–545

78 Mori, S. and Zhang, J. (2006) Principles of diffusion tensor imaging and its applications to basic neuroscience research. *Neuron* 51, 527–539

79 Nair, D.G. (2005) About being BOLD. *Brain Res. Brain Res. Rev* 50, 229–243

80 Logothetis, N.K. *et al.* (2001) Neurophysiological investigation of the basis of the fMRI signal. *Nature* 412, 150–157