

# Some recent advances in basic neuroscience research in China

Mu-ming Poo<sup>1,2,\*</sup> and Aike Guo<sup>1,3</sup>

<sup>1</sup>*Institute of Neuroscience and Key Laboratory of Neurobiology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, Peoples's Republic of China*

<sup>2</sup>*Division of Neurobiology, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3200, USA*

<sup>3</sup>*State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, Peoples's Republic of China*

Neuroscience as a distinct discipline or research programme has been a rather recent event in most Chinese universities and in the Chinese Academy of Sciences. However, the last few years have witnessed increased funding and an improved research environment for neuroscience, both of which facilitated an influx of Chinese neuroscientists trained abroad. In this review, we have highlighted some recent research advances made by neuroscientists in China. Based on our own expertise, this review is focused mainly on findings that have contributed to our understanding of the mechanisms underlying brain development, neural plasticity and cognitive processes, and neural degeneration.

**Keywords:** neural development; neural plasticity; learning and memory; neural injury; neurodegenerative diseases

## 1. INTRODUCTION

The distinct features of the nervous system are the diversity of cell types that emerge during development, the complexity of neuronal structures and their interconnections, and the highly plastic nature of its structure and function. Active research in various Chinese research institutions in recent years has aimed to understand the mechanisms responsible for the differentiation of neuronal phenotypes, the formation of specific synaptic connections, and the plasticity in the properties of neurons and neural networks during normal brain function and in disease states, as well as the degeneration of neurons and their connections in the ageing brain. While many of these studies have addressed problems and themes in common with ongoing research of their international peers, research resources and societal needs unique to China are likely to shape the future development of Chinese neuroscience.

## 2. GUIDANCE OF NEURONAL MIGRATION AND AXON EXTENSION

The assembly of the nervous system during development depends on the migration of newborn neurons from their birthplace to proper locations in the brain. After arriving at their destination, outgrowth of dendritic and axonal processes must be guided by extracellular signals in the developing tissue in order for them to reach their target neurons and make appropriate synaptic connections. How migrating neurons

and growing axons are guided has been an outstanding question for developmental neurobiologists since Ramon y Cajal first described these phenomena more than a century ago. There is now substantial evidence that guidance of both neuronal migration and axon extension is mediated by attractive or repulsive protein factors, which are secreted by specific cells or bound to cell surfaces and extracellular matrix in the developing tissue. These guidance factors activate neuronal surface receptors and trigger a cascade of cytoplasmic events that lead to directed motility of the entire neuron or the tip of the growing axon, the 'growth cone'. Using an *in vitro* assay for growth cone guidance, Xiaobing Yuan and colleagues at the Institute of Neuroscience in Shanghai have examined the guidance of growth cones of cerebellar granule cells by SDF-1, a chemokine that attracts migrating leukocytes and granule cells via a G protein-coupled receptor (GPCR). They found that a gradient of SDF-1 causes either attractive or repulsive turning of the growth cone, depending on the activity of two different signalling pathways in the neuron, both of which are downstream from heterotrimeric protein Gi and phospholipase C activation. Protein kinase C (PKC) activation leads to repulsion of growth cones, whereas inositol 1,4,5-triphosphate receptor activation leads to attraction (Xiang *et al.* 2002). These findings are of interest to the field of axon guidance because the chemokine family of factors can now be included in the growing families of secreted factors that may serve for axon guidance. Furthermore, GPCR signalling is clearly shown to be capable of mediating directional signals in the cytoplasm of the growth cone, although most guidance factor receptors identified so far are not GPCRs. Since SDF-1 and GPCRs are known to be involved in the guidance of neuronal migration, these results add further support to the notion that

\* Author for correspondence (mpoo@uclink.berkeley.edu).

One contribution of 14 to a Theme Issue 'Biological science in China'.

guidance of neuronal migration and axon extension may share similar guidance factors and cellular transduction mechanisms.

To initiate directed neuronal migration and axon extension, the rearrangement of cytoskeletal structures underlying cellular motility is required. The Rho family GTPases have emerged as the key regulators for cytoskeletal rearrangements in a variety of cells, including neurons. Yuan *et al.* (2003) have examined whether these GTPases are involved in mediating the guidance of growth cones by an attractive factor, brain-derived neurotrophic factor (BDNF), and a repulsive factor, lysophosphatidic acid (LPA). They found that the expression of either dominant-negative or constitutively active Cdc42 in cultured *Xenopus* spinal neurons, at a concentration that did not substantially affect filopodial formation and neurite extension, abolished BDNF-induced attractive turning, while the expression of dominant-negative RhoA abolished LPA-induced repulsion. Besides being necessary for turning, they also found that an asymmetry in Rho kinase across the growth cone, created by a gradient of a specific (membrane permeable) inhibitor Y-27632, is sufficient to trigger the turning response. Furthermore, BDNF can indeed activate Cdc42 and Rac in cultured neurons. Together, these findings provide the best evidence so far that RhoGTPases serve to mediate axon guidance, rather than simply providing permissive conditions for the guidance process.

It is well known that cell motility depends critically on the level of cytosolic  $\text{Ca}^{2+}$ . Recent evidence indicates that several guidance factors, including netrin-1, BDNF and myelin-associated glycoprotein, trigger a gradient of  $\text{Ca}^{2+}$  across the growth cone, which can further trigger the attractive or repulsive turning of the growth cone, depending on the magnitude of  $\text{Ca}^{2+}$  elevation. How does the  $\text{Ca}^{2+}$  signal regulate the cytoskeletal rearrangements? Jin *et al.* (2005) recently demonstrated that  $\text{Ca}^{2+}$  elevation triggered directly by a gradient of ryanodine, at a concentration that caused  $\text{Ca}^{2+}$  release from internal stores and attractive growth-cone turning, directly activated Cdc42 and Rac in cerebellar granule cells. Thus, RhoGTPases act downstream from  $\text{Ca}^{2+}$  signalling. That the  $\text{Ca}^{2+}$  signal may also play a critical role in neuronal migration has long been recognized. Interestingly, Xu *et al.* (2004a) found that there is a gradient of cytosolic  $\text{Ca}^{2+}$  in the soma of cerebellar granule cells migrating on the surface of co-cultured radial glia, with a lower concentration at the front side of soma; this  $\text{Ca}^{2+}$  gradient reverses its polarity in response to the frontal application of a repulsive factor, slit-2, which causes reversal in the direction of neuronal migration. Thus, a  $\text{Ca}^{2+}$  gradient may also be responsible for triggering directed migration of the entire neuron in response to extracellular guidance factors.

While the importance of  $\text{Ca}^{2+}$  signalling in axon guidance is well recognized, it has been unclear how the guidance cue triggers the  $\text{Ca}^{2+}$  signal in the growth cone. The dependence of growth-cone guidance on extracellular  $\text{Ca}^{2+}$  for many guidance factors suggests that  $\text{Ca}^{2+}$  influx through the plasma membrane may provide the initial trigger for cytoplasmic  $\text{Ca}^{2+}$  elevation, which may also be further enhanced by

$\text{Ca}^{2+}$  release from internal stores. For example, BDNF-induced chemoattraction of growth cones requires  $\text{Ca}^{2+}$  signalling, but how  $\text{Ca}^{2+}$  at the growth cone is regulated by BDNF remains largely unclear. In a collaborative work between X. Yuan's and Y. Wang's laboratories at the Institution of Neuroscience, Li *et al.* (2005) recently showed that in cultured cerebellar granule cells, transient receptor-potential canonical (TRPC) channels contribute to the BDNF-induced  $\text{Ca}^{2+}$  elevation at the growth cone and are required for BDNF-induced chemoattraction. Several members of the TRPC family are highly expressed in these neurons, and both  $\text{Ca}^{2+}$  elevation and growth-cone turning induced by BDNF were abolished by pharmacological inhibition of TRPC channels, by overexpressing a dominant negative form of TRPC3 or TRPC6, or by downregulating the expression of TRPC3 with small interference RNA. Thus, TRPC channel activity is essential for nerve growth-cone guidance by BDNF. It is now well established that ion channels formed by the TRP superfamily of proteins act as sensors for temperature, osmolarity, mechanical stress and taste. The finding of Li *et al.* (2005) has now expanded the function of TRP channels in sensory processes to include the chemosensory response of the nerve growth cone towards extracellular guidance factors.

### 3. PLASTICITY OF SYNAPSE AND NEURAL CIRCUITS

One of the most striking properties of the nervous system is the use-dependent plasticity of neural circuits. Changes in the structure and function of neural circuits triggered by prior neuronal activity underlie many higher cognitive functions, from learning and memory to perception. The most plastic region of the neural circuit is the synapse, where presynaptic molecular machineries for transmitter secretion, the property and distribution of postsynaptic transmitter receptors and their subsynaptic-associated proteins, are all highly susceptible to modulation by synaptic activities. Bliss & Lomo (1973) first discovered that a brief period of high-frequency activity can induce persistent enhancement of synaptic transmission in the hippocampus, a phenomenon now known as long-term potentiation (LTP). Later studies also demonstrated that prolonged, low-frequency activity leads to long-term depression (LTD) at many synapses. Synaptic modifications associated with LTP/LTD are generally considered to be the cellular basis for learning and memory (Martin *et al.* 2000) and for activity-dependent refinement of developing neural circuits (Zhang & Poo 2001). Markram *et al.* (1997) showed that the timing of postsynaptic backpropagating action potentials relative to the onset of excitatory postsynaptic potentials (EPSPs) plays a critical role in determining the polarity of synaptic modifications; postsynaptic spiking 10 ms after EPSP onset leads to LTP, whereas the reverse order leads to LTD. Such spike timing-dependent plasticity (STDP) of synapses has attracted the attention of many experimental and computational neuroscientists in recent years since it endows a sensitivity of the neural circuit to the temporal pattern of neuronal activity (Dan & Poo 2004),

a feature important for associative learning and memory in the brain.

Although most studies on STDP were focused on synaptic plasticity, other neuronal functions 'beyond synapse' are also susceptible to modification by neuronal activity. There is now strong evidence that, in addition to persistent changes in synaptic efficacy, correlated activity can result in persistent changes of the intrinsic excitability of pre- and postsynaptic neurons. In cultures of hippocampal neurons, induction of LTP by correlated pre- and postsynaptic activation is accompanied by an immediate and persistent enhancement of the intrinsic neuronal excitability of the presynaptic neuron, a modification caused by enhanced activation kinetics of  $\text{Na}^+$  channels (Ganguly *et al.* 2000). Li *et al.* (2004) at the Institute of Neuroscience in Shanghai recently showed that there is a reduction of intrinsic neuronal excitability following the induction of LTD by correlated pre- and postsynaptic activation in hippocampal cultures and in somatosensory cortical slices. This presynaptic effect can be attributed to an enhanced activation of voltage-dependent slow-inactivating  $\text{K}^+$  channels, a process that requires postsynaptic  $\text{Ca}^{2+}$  elevation and presynaptic protein kinase A (PKA) and PKC activities. Together, these results implicate an immediate retrograde signalling associated with the induction of LTP/LTD and a rapid spread of cytosolic signals throughout the presynaptic neuron, leading to global modifications of ion channels.

In addition to global changes of intrinsic excitability in the presynaptic neuron, local morphological changes of dendrites and alteration in the property or distribution of ion channels are also likely to modify dendritic summation of synaptic potentials. Wang *et al.* (2003b) of the Institute of Neuroscience in Shanghai found that in CA1 pyramidal neurons of the hippocampus, the induction of LTP/LTD is accompanied by corresponding bi-directional changes in the linearity of spatial summation of EPSPs (Wang *et al.* 2003b). Local modifications of  $\text{I}_h$  channels and N-methyl-D-aspartate (NMDA) receptors, which are known to influence dendritic summation of EPSPs, may account for the observed changes in dendritic integration. Thus, correlated pre- and postsynaptic activities can induce persistent modification of dendritic integration, providing additional activity-dependent plasticity beyond synaptic potentiation or depression. These new findings are likely to be highly relevant to the function of neural circuits in information processing and storage.

#### 4. NEURON–GLIA INTERACTIONS

There is increasing evidence that glial cells, the most abundant cell type in the brain, play important regulatory roles in neurogenesis, synaptogenesis and synaptic function and plasticity. Of particular interest is the role of astrocytes, which are found to associate closely with neuronal synapses and may serve to modulate synaptic transmission and plasticity. A series of recent studies in Shumin Duan's laboratory at the Institute of Neuroscience in Shanghai have shown that factors secreted by astrocytes can exert both short- and

long-term effects on the efficacy of synaptic transmission. In cultures of hippocampal neurons, Zhang *et al.* (2003) showed that endogenously released ATP tonically suppresses glutamatergic synapses via pre-synaptic P2Y receptors, an effect that depends on the presence of co-cultured astrocytes. Glutamate release accompanying neuronal activity also activates non-NMDA receptors of nearby astrocytes and triggers ATP release from these cells, which in turn causes homo- and heterosynaptic suppression of neuronal synaptic transmission. In CA1 pyramidal neurons of hippocampal slices, a similar synaptic suppression was also produced by adenosine, an immediate degradation product of ATP released by glial cells. These studies demonstrated a dynamic neuron–glia crosstalk that participates in activity-dependent synaptic modulation with a duration of the order of seconds.

Brief correlated pre- and postsynaptic activities are known to cause persistent LTP of synaptic transmission between neurons. Interestingly, Yang *et al.* (2003) found that repetitive correlated activation of pre- and postsynaptic neurons induced LTP of synaptic transmission among hippocampal neurons grown on a layer of astrocytes, but not among neurons cultured in glial-conditioned medium (GCM). A supplement of D-serine, an agonist previously known to serve as a co-agonist of the glycine-binding site of the NMDA subtype of glutamate receptors (NMDARs), enhanced NMDAR activation and enabled the LTP induction in GCM cultures. The induction of LTP in both mixed neuron–astrocyte cultures and hippocampal slices was suppressed by NMDAR antagonists, glycine-binding site blockers of NMDARs or an enzyme that degrades endogenous D-serine. By providing extracellular D-serine that facilitates activation of NMDARs, astrocytes thus play a key role in activity-dependent long-term synaptic plasticity. Together, these results underscore the importance of dynamic bi-directional communication between neurons and glia in setting the synaptic efficacy and the function of neural circuits. Glial cells thus not only serve for long-term trophic support of neurons, but also actively participate in the undergoing signal processing of neural circuits by receiving activity-dependent neuronal factors and providing feedback signals that further modulate signal processing.

#### 5. OPIATE ADDICTION AND HIPPOCAMPAL PLASTICITY

Opiate addiction has become an increasingly serious social problem in China, especially in southwest provinces. An understanding of the underlying neural adaptive mechanisms may lead to useful clues to the treatment of opiate addiction. An emerging view is that drug addiction is an aberrant form of learning, mediated by adaptive changes in the brain's memory and reward systems. Hippocampus is known to be important for the formation of episodic memory, and long-term activity-induced LTP and LTD in various regions of the hippocampus have been widely recognized as the cellular basis for hippocampus-related learning and memory. Gang Pei and colleagues at the Institute of Biochemistry and Cell Biology in Shanghai

have examined the effect of chronic morphine or heroin treatment on LTP induction in the CA1 region of the rat hippocampus. They found that chronic exposure to opiates, which induced severe drug tolerance and dependence, markedly reduced the capacity for LTP induction during the period of drug withdrawal (Pu *et al.* 2002). Interestingly, the capacity for LTP could be restored to the normal level by re-exposure of the animals to opiates, suggesting that the mechanism for synaptic plasticity is adapted to a state of dependence on opiates. Upregulation of the cAMP pathway may be responsible for the adaptive changes in hippocampal plasticity, since opiate-reduced LTP was restored by inhibitors of PKA. Further studies (Xu *et al.* 2004b) showed that the expression of SNAP-25, a t-SNARE protein essential for synaptic vesicle exocytosis, was regulated in the hippocampus after the chronic morphine treatment, with a reduction of phosphorylation of Ser<sup>187</sup>, a PKC phosphorylation site of SNAP-25, which leads to a reduction in the formation of a ternary complex of SNARE proteins in hippocampal synaptosomes.

Adaptive changes in the hippocampus were also examined by Xu *et al.* (2003) during the period of withdrawal after chronic morphine treatment in rats. The glutamate uptake in hippocampal synaptosomes was significantly increased, apparently due to an increase in the number of functional glutamate transporters. The expression of glutamate transporter subtype 1 (GLT1) was indeed shown to be upregulated specifically in hippocampal synaptosomes. Ultrastructural evidence revealed that GLT1 expression was markedly increased in the nerve terminals of the hippocampus and associated with the plasma membrane *in vivo*. This selective translocation of GLT1 to hippocampal nerve terminals during morphine withdrawal may cause increased uptake of released glutamate and a modulation of excitatory neurotransmission in the hippocampus during opiate withdrawal.

An interesting aspect of drug addiction is that the propensity of an individual for drug abuse, drug seeking and relapse is influenced by stress or glucocorticoids. There is evidence that stress may interact with drug addiction through a common mechanism of synaptic plasticity in the ventral tegmental area and hippocampus. Xu Lin and colleagues at Kunming Institute of Zoology have made a concerted effort to examine the role of the hippocampus in the interaction between stress and drug addiction, based on the fact that the hippocampus is sensitive to stress and glucocorticoids and may play a significant role in learning and memory associated with drug addiction. They found that acute stress enables LTD induction by low-frequency stimulation (LFS; Xiong *et al.* 2003), but acute morphine treatment causes synaptic potentiation. Interestingly, exposure to an acute stressor reverses the effect of morphine from synaptic potentiation to depression, and precluded further LTD induction by LFS (Yang *et al.* 2004). The synaptic depression caused by stress with morphine is blocked either by the glucocorticoid receptor or by the NMDA receptor antagonist. Application of corticosterone with morphine during the initial phase of drug use promotes

later delayed-escape behaviour (using the Morris water maze test), leading to persistent morphine seeking after withdrawal. These results support the notion that hippocampal synaptic plasticity may play a significant role in the effects of stress or glucocorticoids on opiate addiction. This work has shed new light on the interesting relationship between stress, synaptic plasticity and drug addiction.

## 6. LEARNING, MEMORY AND COGNITIVE PROCESSES

Modern primate facilities have been established recently in the Institute of Zoology of the Chinese Academy of Sciences in the southwestern city of Kunming, providing an excellent environment for experimental primate research in all areas. The abundant resources for primate research have also attracted international collaborations in neuroscience. An interesting example is the recent collaboration between A.G. Levinthal's group from the University of Utah with Y. F. Zhou of the Chinese University of Science and Technology at Hefei and Y. Ma of the Kunming Institute of Zoology. Senescence in monkeys is known to be accompanied by a degradation of receptive field properties and by prolonged hyperactivity of visual cortical neurons. Leventhal *et al.* (2003) discovered that administration of  $\gamma$ -aminobutyric acid (GABA) and its agonist muscimol in the visual cortex of senescent monkeys improved orientation and direction detection capability of neurons in the striate cortex, suggesting that GABA agonists may be useful for the treatment of decaying sensory functions in the ageing brain. Further studies showed that in ageing monkeys exhibiting hyperactivity, there is a marked delay in visual information transfer both intracortically and from the striate to extra-striate cortex (Wang *et al.* 2005). Future studies of functional alterations in the ageing visual cortex at the circuit and synapse levels, using *in vivo* or brain slice recordings, may yield useful clues to the mechanisms underlying the ageing process and potential approaches for impediment or prevention.

The prefrontal cortex of the primate brain serves an important role in higher cognitive functions, guiding the thought, feeling and action of the animal. By processing and withholding information from motor and sensory cortices in the form of 'working memory', the prefrontal cortex regulates attention, inhibits inappropriate behaviours and coordinates goal-directed actions. In collaboration with F. A. Wilson of the University of Arizona, Y. Ma and his colleagues have focused their attention on the function of the dorsolateral prefrontal cortex (dlPFC) in the planning and execution of spatially directed movements in freely moving monkeys. They found that lesions in the dlPFC resulted in no impairment of allocentric spatial memory in which the geometric relationships between environmental cues are used to identify spatial location. However, the egocentric spatial memory guided by visual or tactile cues that depends on the location of objects relative to the monkey during testing was impaired (Ma *et al.* 2003). Further physiological recording of neuronal activities showed that dlPFC neurons are involved in

monitoring the spatial nature of behavioural sequences in an egocentric memory task, while hippocampal neurons are active during spatially directed locomotion that depends on allocentric memory (Ma *et al.* 2004). Ma's group has also developed a reliable physiological recording method for freely moving rhesus monkeys (Tian *et al.* 2002). This is potentially an important technical advance in the field, since it would allow studies of the operation of the primate nervous system responsible for initiating behaviours that are relevant to the animal in a natural environment.

Information processing in the dlPFC is known to be influenced by neuromodulatory inputs, including the noradrenergic projections from the locus coeruleus. Attention-deficit/hyperactivity disorder patients showed evidence of genetic alterations in genes related to catecholamine transmission. B. M. Li and colleagues at Fudan University in Shanghai have shown that  $\alpha_2$ -adrenergic modulation of prefrontal cortical neuronal activity affects spatial working memory in monkeys (Li *et al.* 1999). They have also shown recently that chronic and bilateral infusion of the  $\alpha_2$ -adrenergic antagonist yohimbine into the dlPFC of monkeys resulted in marked locomotor hyperactivity, suggesting involvement of  $\alpha_2$ -adrenergic receptors in the inhibitory control of locomotor activity in dlPFC (Ma *et al.* 2005). This adrenergic influence appears to be widespread in the PFC and responsible for regulating diverse PFC functions. In the ventral PFC, an area known to be important for the monkey to learn new visuomotor associations (Wang *et al.* 2000), infusion of the  $\alpha_{2A}$ -adrenergic agonist guanfacine improved the monkey's ability to learn new associations (Wang *et al.* 2004). Noradrenergic modulation of memory acquisition and storage has been shown for various forms of learning and in many different brain regions besides PFC, including the amygdala and the hippocampus, but different adrenergic receptors appear to be involved. Infusion of the  $\alpha$ -adrenergic receptor antagonist DL propranolol into the CA1 region of the hippocampus results in a deficit in context fear conditioning in rats (Ji *et al.* 2003a) and interferes with the consolidation of spatial memory, as indicated by the poor performance of the rat in the Morris water maze test (Ji *et al.* 2003b). Given the extensive information that now exists for the circuitry and synaptic plasticity mechanisms of hippocampus and amygdala, we may soon arrive at a complete understanding of the cellular mechanisms underlying the neuromodulatory actions of catecholamine in the cognitive functions of the nervous system.

Despite rapid progress, mechanistic understanding of higher cognitive functions beyond learning and memory remains at a rather primitive stage. Perception, consciousness, decision making and language, for example, all require information processing involving a large number of brain areas. The complexity of underlying neural circuits imposes a formidable task for neuroscientists. One approach to this problem is to examine the circuitry underlying analogous cognitive functions of simpler organisms such as *Drosophila* and honeybee, with the hope that elementary circuit properties underlying cognitive functions may be revealed. A significant step in the use of simple organisms for studying cognitive function was taken

by Aike Guo and colleagues at the Institute of Neuroscience in Shanghai. By an ingenious design of behavioural studies of *Drosophila* using a flight simulator, a paradigm pioneered by Heisenberg and colleagues in Germany, Guo & Guo (2005) showed that associative conditioning with either visual cue or olfactory cue can be performed in the flight simulator, and that there is synergism in learning when the fly is using both visual and olfactory cues simultaneously for association with punishment. They found that the threshold for memory retrieval using the single cue is significantly reduced after dual-cue conditioning. Interestingly, following preconditioning of the fly simultaneously to coincident visual and olfactory stimuli in the absence of punishment, the memory induced by subsequent associative conditioning of the visual cue with punishment can be transferred to the olfactory system, allowing the fly to associate the olfactory cue with the punishment.

The *Drosophila* flight behaviour has also been used to examine a cognitive function analogous to decision making in higher organisms. Tang & Guo (2001) show that one can assay the fly's ability in decision making by imposing conflicting visual cues (either the colour or the shape of an object in the fly's visual field) to the fly after prior associative conditioning of these cues with punishment. Wild-type flies were found to make 'decisive' choice in their flight behaviour, based on the relative saliency of the conflicting cues, but this ability was severely impaired when the mushroom body of the fly is impaired by mutation or ablated by chemical treatment. Another higher cognitive function known as translational invariance in visual perception can also be demonstrated in flies. Using the same flight simulator, S. M. Tang at the Institute of Biophysics in Beijing, in collaboration with M. Heisenberg's group in Wurzburg, Germany, has shown that the fly can recognize visual pattern independent of the region of their visual field where it was trained to recognize the pattern (Tang *et al.* 2004). Future studies using molecular genetic tools that interfere with the activity of selective populations of neurons may allow further dissection of the underlying neural circuitry in the fly brain for cognitive functions.

## 7. BRAIN IMAGING STUDIES

For understanding human cognitive functions, advances in functional brain imaging have resulted in significant progress in delineating the spatial and temporal characteristics of neural activities in various brain regions, especially when imaging studies can be added by the use of electroencephalograms of high spatial resolution and new non-invasive stimulation techniques, e.g. transcranial magnetic stimulation.

Using functional magnetic-resonance imaging (fMRI), D. Zhang and colleagues at the University of Science and Technology of China in Hefei, in collaboration with Xiaoping Hu's group, now at Emory University, have examined the difference in brain regions activated during playing the Chinese game of 'Go', in comparison with chess playing. Although many cortical areas are similarly activated in both games, the Go playing involves a stronger

activation of the right parietal area than the left, whereas the left parietal area was activated more during chess playing (Chen *et al.* 2003b). Using fMRI, these investigators also examined the involvement of various brain areas associated with forward and backward digit recall in young and old subjects. They found that there is an age dependence in the area of activation and that different neural mechanisms may be involved in forward and backward digit recall; brain functions associated with these two types of recall are differentially affected by ageing (Sun *et al.* 2005).

Lin Chen and colleagues at the Institute of Biophysics in Beijing have used fMRI to examine whether form perception plays a role in the human visual perception of apparent (illusory) motion (AM), created by sequential presentation of a pair of adjacent motionless images. The current view assumes that AM is processed by the same brain pathway used for detection of real motion, the dorsal 'where' pathway. Zhuo *et al.* (2003) found that for long-range AM (with larger separation of the two images), but not for short-range AM, there is significant activation of the anterior temporal lobe in the ventral 'what' pathway, suggesting involvement of form perception. Furthermore, the degree of activation is directly proportional to the geometric differences between the pair of images, with highest activation when the topological property of the image is changed. The dependence of anterior temporal lobe activation on geometry strongly supports the notion that form perception is important in the human perception of long-range AM. These results are consistent with the view that the topological property of the visual image represents the primary element and is the first to be perceived in form perception (Chen 1982, 2001). Chen's 'holistic' view of visual perception differs sharply from the popular 'bottom-up' view based on the binding of local features of the visual image into a more global perception (Chen 2001). Evidence for topological perception has long been supported by human visual psychophysical studies of visual sensitivity, AM, illusory conjunctions and relative salience of different geometric invariants (see review Chen 2001) as well as by studies of honeybee learning of pattern discrimination (Chen *et al.* 2003a). Chen's study on topological perception nicely illustrates the challenge facing many cognitive neuroscientists. There is little doubt that topological perception constitutes an important element in visual perception and further brain-imaging studies may delineate various brain regions involved in such perception. However, true neurobiological understanding of cognitive functions must entail not only a spatio-temporal map of the active brain regions responsible for the cognitive function, but also the underlying neural circuits and their operational principles that give rise to the cognitive function. Thus, the real challenge resides in the formulation of experimental paradigms that can effectively address the underlying neural mechanism at the level of neural circuits.

## 8. NEURAL INJURY, DEGENERATION AND REGENERATION

Traumatic and ischaemic injury of the nervous system often leads to serious dysfunction. Understanding the

cellular and molecular changes associated with the injury may offer insights into potential therapeutic approaches. For example, a peripherally axotomized animal represents one model for studying the mechanisms underlying neuropathic pain. To determine the molecular alterations in dorsal root ganglia (DRGs), Xu Zhang's group at the Institute of Neuroscience in Shanghai have performed microarray analysis of genes from the cDNA libraries of lumbar DRGs of normal rats and of rats 14 days after peripheral axotomy (Xiao *et al.* 2002). They have identified a large number of molecular alterations, including neuropeptides, receptors, ion channels, signal transduction molecules and synaptic vesicle proteins, many of which have not previously been associated with axotomy. These findings provide useful clues to the origin of neuropathic pain and the design of therapeutic agents.

An interesting issue associated with peripheral axotomy is the sprouting of thick myelinated afferents (A-fibres) from laminae III–IV into laminae I–II of the spinal cord, a process that may underlie pathogenesis of neuropathic pain. Bao *et al.* (2002) examined this issue using fluorescence tracing with the cholera toxin-B subunit. They found that only a small number of A-fibres sprouted and made synaptic contacts in inner lamina II, a region normally innervated by C-fibres, but not in outer lamina II or lamina I. Neuropeptide Y was found in these sprouts in inner lamina II, an area very rich in Y1 receptor-positive processes. These results suggest that axotomy-induced sprouting from deeper to superficial layers is much less pronounced than previously assumed. It would be of interest to know whether this limited sprouting may constitute functional circuitry involved in neuropathic pain. Tienle Xu's group at the Institute of Neuroscience in Shanghai has discovered recently that acid-sensing ion channels (ASICs), a group of ligand-gated cation channels activated by extracellular protons, are expressed at a high level in dorsal horn neurons (Wu *et al.* 2004). Peripheral complete Freund's adjuvant-induced inflammation resulted in increased expression of two subunits, ASIC1a and ASIC2a, in the dorsal horn, consistent with the idea that ASICs of dorsal horn neurons participate in central sensory transmission/modulation and contribute to inflammation-related persistent pain.

One group of important players in nociception in the spinal cord is the  $\delta$ - and  $\mu$ -opioid receptors (DORs and MORs), which are involved in the presynaptic inhibition of afferent sensory inputs. The regulation of membrane insertion of these receptors is likely to be of critical importance in normal and pathological nociception. Bao *et al.* (2003) discovered a novel mechanism for plasma-membrane insertion of DORs. Unlike most membrane receptors destined for the plasma membrane, DORs appear to reside in the secretory granules of small dorsal root ganglion neurons; activation of surface DORs causes  $\text{Ca}^{2+}$  release from  $\text{IP}_3$ -sensitive stores and  $\text{Ca}^{2+}$  entry, resulting in a long-lasting exocytosis of DOR-containing granules and DOR insertion into the plasma membrane, together with other excitatory neuropeptides. This suggests that treatment of neuropathic pain should include a blockade of DORs.

Opioid and tachykinin systems are involved in modulation of pain transmission in the spinal cord. Regulation of surface opioid receptors on nociceptive afferents is critical for opioid analgesia. The finding by Bao *et al.* (2003) that plasma-membrane insertion of DORs is induced by stimulus-triggered exocytosis of DOR-containing large dense-core vesicles (LDCVs) led to the immediate question of how DORs become sorted into the regulated secretory pathway. Guan *et al.* (2005) recently made the surprising discovery that direct interaction between protachykinin and DORs is responsible for sorting DORs into LDCVs. This interaction is mediated by the substance-P domain of protachykinin and the third luminal domain of DORs. Furthermore, deletion of the preprotachykinin-A gene reduced stimulus-induced surface insertion of DORs, and abolished DOR-mediated spinal analgesia and morphine tolerance. Thus, protachykinin is essential for modulation of the sensitivity of nociceptive afferents to opioids, and the opioid and tachykinin systems are directly linked by protachykinin/DOR interaction. This series of studies by X. Zhang's group nicely illustrates the fact that much remains to be learned about the cell biology of neurons in order to understand the cellular basis of neuropathic pain and to formulate therapeutic strategies in the treatment of this disease.

The hippocampus has been a useful system for studying not only cellular mechanisms underlying learning and memory, but also neural plasticity following injury of the adult nervous system. Following transection of the perforant path input from the entorhinal cortex to the hippocampal dentate gyrus, reorganization of connectivity in the deafferented area occurs as a result of terminal sprouting, proliferation and synaptogenesis of other uninjured afferent axons in the deafferented areas. This model system has been fruitfully exploited by C. F. Zhou and his colleagues at the Shanghai Institute of Physiology for studying cellular changes following neural injury. Using cDNA microarrays for differential screening, Ying *et al.* (2001) have identified a set of genes whose expression is altered 10 days after perforant path transection. One of these is cystatin C, a member of the endogenous type-II cysteine protease inhibitors that can modulate the turnover of extra- and intracellular proteins. Ying *et al.* (2002) further showed that this protein is selectively upregulated in the deafferented zones within the hippocampus. Interestingly, most of the cystatin C appears to reside in the astrocytes of these areas, suggesting that secretion of cystatin C from astrocytes may play an important role in the sprouting and remodelling of connectivity in the deafferented areas. Besides cystatin C, increased expression was also found for several other proteins, including major histocompatibility complex (MHC)I, MHCII,  $\beta$ 2-microglobulin and interferon- $\gamma$  receptor, suggesting potential immune system involvement in neural plasticity following injury. This is of particular interest in view of the finding that in MHCII-deficient mice, activity-induced LTP is enhanced and LTD is eliminated (Huh *et al.* 2000).

Part of the immune function in the nervous system may be performed by glial cells. Glial activation and associated release of cytokines and growth factors may constitute an important cellular reaction to neural

injuries. Entorhinal deafferentation leads to microglial activation and an upregulation of profilin transcript, which appears to be involved in morphological change, migration and phagocytic behaviour of microglial cells (Dong *et al.* 2004). Wang & Zhou (2005) recently also discovered an axotomy-induced upregulation of interferon- $\gamma$  (IFN- $\gamma$ ) receptor mRNA in the mouse hippocampus following transections of the entorhinal afferents. The upregulation of both IFN- $\gamma$  and its receptor expression coincided spatio-temporally with astroglial activation, suggesting the potential involvement of IFN- $\gamma$  and its receptor in the activation process of astrocytes in the hippocampus following entorhinal deafferentation.

Plasticity associated with injury-induced circuit rearrangements may involve reactivation and enhancement of cellular mechanisms that are used during early development and in the plastic changes associated with the normal adult brain. A potential link between regeneration and development is the ephrin-Eph system, two ligand/receptor families of membrane proteins that are known to contribute to the guidance and formation of retinotopic projections in the developing visual system. There is also increasing evidence that ephrin-Eph interactions also participate in the function and plasticity of mature synapses in the adult nervous system. Using semi-quantitative RT-PCR, Wang *et al.* (2003a) showed that the expression of mRNAs for ephrin A1, A2, A3, A5 and B1 are markedly upregulated in rat hippocampus in the first two weeks following transection of entorhinal afferents, during which rearrangement of connections in the deafferented areas occurs. The expression of these surface proteins may account for the receptiveness of the deafferented neurons to further innervation by sprouting axons.

Sprouting of nerve terminals of intact afferents triggered by partial afferentation, as in the case of perforant path transection described above, represents a limited regeneration and reorganization of the nervous system following injury. Regeneration of injured axons in the central nervous system has been rather limited. This is generally attributed to the presence of inhibitory factors generated near the injured tissue in the central nervous system rather than a lack of growth capacity of injured axons. Thus, an understanding of the identity of these inhibitory factors, the mechanisms that regulate their expression and the cellular actions they exert upon growing axons may all contribute to the design of therapeutic approaches that may promote neuronal regeneration after injury.

With an ageing population, older people now constitute an increasingly large percentage of Chinese society and neurodegenerative diseases have become a pressing health-care issue. Research directed to an understanding of the biological basis of these diseases and development of effective diagnostic and therapeutic approaches is now well supported by the government. In the case of Alzheimer's disease (AD), the most common cause of dementia, Jianzhi Wang and co-workers in Tongji Medical School in Wuhan have focused on a microtubule-associated protein, tau, in the ageing brain. AD is characterized by the presence of neurofibrillary

tangles (NFTs) and senile plaques, and a selective loss of cholinergic neurons in the brain. The NFTs consist mostly of insoluble fibrils of hyperphosphorylated forms of tau. Downregulation of protein phosphatases 2A and 1 *in vivo* may cause tau hyperphosphorylation; specific inhibition of these two enzymes by injection of calyculin A into rat hippocampus resulted in tau hyperphosphorylation and impairment of spatial memory retention, as shown by using the standard test of Morris' water maze (Sun *et al.* 2003). An interesting spin-off of these studies by the Wang group was the discovery of hyperphosphorylated tau in the cerebrospinal fluid of AD patients (Hu *et al.* 2002a), which led to the development of an ultrasensitive bioassay of these proteins in cerebro-spinal fluid as a method for early AD diagnosis (Hu *et al.* 2002b). The latter is a significant advance beyond the traditional neurological diagnosis of AD and pathological identification of the presence of excessive NFTs and senile plaques in autopsied brain tissues.

One of the most vulnerable brain regions in the AD brain is the hippocampus. Based on cumulated epidemiological and clinical evidence that oestrogen use may delay the onset or progression of AD, J. N. Zhou and colleagues at the University of Science and Technology in Hefei have examined the distribution of the oestrogen receptor- $\alpha$  (ER $\alpha$ ) in the hippocampus of control and AD patients. Immunocytochemical staining showed that the number and density of GFAP- and ER $\alpha$ -positive astrocytes were increased in the CA1 region of the AD hippocampus (Lu *et al.* 2003). The general increase in astrocyte density represents pathological changes in the AD hippocampus, whereas the presence of ER $\alpha$  in many of these astrocytes suggests that the neuroprotective effects of oestrogen may be mediated through neurotrophic astrocyte–neuron interactions.

## 9. CONCLUDING REMARKS

Like many other countries, China is facing an increasingly higher proportion of ageing population. Research relevant to the prevention and cure of neurodegenerative diseases will have to be given higher priority in the biomedical research agenda. Significant advances in both basic and clinical neuroscience are likely to be made in coming years, through increased government funding together with the appreciation that high-quality laboratory research holds the key to the definitive solution of neurodegenerative and other neurological diseases.

## REFERENCES

- Bao, L., Wang, H. F., Cai, H. J., Tong, Y. G., Jin, S. X., Lu, Y. J., Grant, G., Hokfelt, T. & Zhang, X. 2002 Peripheral axotomy induces only very limited sprouting of coarse myelinated afferents into inner lamina II of rat spinal cord. *Eur. J. Neurosci.* **16**, 175–185. (doi:10.1046/j.1460-9568.2002.02080.x)
- Bao, L. *et al.* 2003 Activation of delta opioid receptors induces receptor insertion and neuropeptide secretion. *Neuron* **37**, 121–133. (doi:10.1016/S0896-6273(02)01103-0)
- Bliss, T. V. & Lomo, T. 1973 Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol.* **232**, 331–356.
- Chen, L. 1982 Topological structure in visual perception. *Science* **218**, 699–700. (doi:10.1126/science.7134969)
- Chen, L. 2001 Perceptual organization: to reverse back the inverted (upside-down) question of feature binding. *Vis. Cogn.* **8**, 287–303. (doi:10.1080/13506280143000016)
- Chen, L., Zhang, S. & Srinivasan, M. V. 2003a Global perception in small brains: topological pattern recognition in honey bees. *Proc. Natl Acad. Sci. USA* **100**, 6884–6889. (doi:10.1073/pnas.0732090100)
- Chen, X., Zhang, D., Zhang, X., Li, Z., Meng, X., He, S. & Hu, X. 2003b A functional MRI study of high-level cognition. II. The game of GO. *Cogn. Brain Res.* **16**, 32–37. (doi:10.1016/S0926-6410(02)00206-9)
- Dan, Y. & Poo, M. M. 2004 Spike timing-dependent plasticity of neural circuits. *Neuron* **44**, 23–30. (doi:10.1016/j.neuron.2004.09.007)
- Dong, J. H., Ying, G. X. & Zhou, C. F. 2004 Entorhinal deafferentation induces the expression of profilin mRNA in the reactive microglial cells in the hippocampus. *Glia* **47**, 102–108. (doi:10.1002/glia.10355)
- Ganguly, K., Kiss, L. & Poo, M. 2000 Enhancement of presynaptic neuronal excitability by correlated presynaptic and postsynaptic spiking. *Nat. Neurosci.* **3**, 1018–1026. (doi:10.1038/79838)
- Guan, J. S. *et al.* 2005 Interaction with vesicle luminal protachykinin regulates surface expression of  $\delta$ -opioid receptors and opioid analgesia. *Cell*. **122**, 619–631.
- Guo, J. Z. & Guo, A. K. 2005 Cross-model interreaction and memory transfer in *Drosophila* learning. *Science*. **309**, 307–310.
- Hu, Y. Y., He, S. S., Wang, X. C., Duan, Q. H., Khatoon, S., Iqbal, K., Grundke-Iqbal, I. & Wang, J. Z. 2002a Elevated levels of phosphorylated neurofilament proteins in cerebrospinal fluid of Alzheimer disease patients. *Neurosci. Lett.* **320**, 156–160. (doi:10.1016/S0304-3940(02)00047-2)
- Hu, Y. Y., He, S. S., Wang, X., Duan, Q. H., Grundke-Iqbal, I., Iqbal, K. & Wang, J. 2002b Levels of nonphosphorylated and phosphorylated tau in cerebrospinal fluid of Alzheimer's disease patients: an ultrasensitive bienzyme-substrate-recycle enzyme-linked immunosorbent assay. *Am. J. Pathol.* **160**, 1269–1278.
- Huh, G. S., Boulanger, L. M., Du, H., Riquelme, P. A., Brotz, T. M. & Shatz, C. J. 2000 Functional requirement for class I MHC in CNS development and plasticity. *Science* **290**, 2051–2055. (doi:10.1126/science.290.5499.2155)
- Ji, J. Z., Zhang, X. H. & Li, B. M. 2003a Deficient spatial memory induced by blockade of beta-adrenoceptors in the hippocampal CA1 region. *Behav. Neurosci.* **117**, 1378–1384. (doi:10.1037/0735-7044.117.6.1378)
- Ji, J. Z., Wang, X. M. & Li, B. M. 2003b Deficit in long-term contextual fear memory induced by blockade of beta-adrenoceptors in hippocampal CA1 region. *Eur. J. Neurosci.* **17**, 1947–1952. (doi:10.1046/j.1460-9568.2003.02620.x)
- Jin, M. *et al.* 2005 Ca<sup>2+</sup>-dependent regulation of rho GTPases triggers turning of nerve growth cones. *J. Neurosci.* **25**, 2338–2347. (doi:10.1523/JNEUROSCI.4889-04.2005)
- Leventhal, A. G., Wang, Y., Pu, M., Zhou, Y. & Ma, Y. 2003 GABA and its agonists improved visual cortical function in senescent monkeys. *Science* **300**, 812–815. (doi:10.1126/science.1082874)
- Li, B. M., Mao, Z. M., Wang, M. & Mei, Z. T. 1999 Alpha-2 adrenergic modulation of prefrontal cortical neural activity related to spatial working memory in monkeys. *Neuropsychopharmacology* **21**, 601–610. (doi:10.1016/S0893-133X(99)00070-6)
- Li, C., Lu, J., Wu, J., Duan, S. & Poo, M. 2004 Bidirectional modification of presynaptic neuronal excitability

- accompanying spike timing-dependent synaptic plasticity. *Neuron* **41**, 257–268. (doi:10.1016/S0896-6273(03)00847-X)
- Li, Y., Jia, Y. C., Cui, K., Li, N., Zheng, Z. Y., Wang, Y. Z. & Yuan, X. B. 2005 Essential role of TRPC channels in the guidance of nerve growth cones by brain-derived neurotrophic factor. *Nature* **434**, 894–898. (doi:10.1038/nature03477)
- Lu, Y. P., Zeng, M., Hu, X. Y., Xu, H., Swaab, D. F., Ravid, R. & Zhou, J. N. 2003 Estrogen receptor alpha-immunoreactive astrocytes are increased in the hippocampus in Alzheimer's disease. *Exp. Neurol.* **183**, 482–488. (doi:10.1016/S0014-4886(03)00205-X)
- Ma, Y. Y., Tian, B. P. & Wilson, F. A. 2003 Dissociation of egocentric and allocentric spatial processing in prefrontal cortex. *Neuroreport* **14**, 1737–1741. (doi:10.1097/00001756-200309150-00016)
- Ma, Y. Y., Ryou, J. W., Kim, B. H. & Wilson, F. A. 2004 Spatially directed movement and neuronal activity in freely moving monkeys. *Prog. Brain Res.* **143**, 513–520.
- Ma, C. L., Arnsten, A. F. & Li, B. M. 2005 Locomotor hyperactivity induced by blockade of prefrontal cortical alpha2-adrenoceptors in monkeys. *Biol. Psychiatry* **57**, 192–195. (doi:10.1016/j.biopsych.2004.11.004)
- Markram, H., Lubke, J., Frotscher, M. & Sakmann, B. 1997 Regulation of synaptic efficacy by coincidence of post-synaptic APs and EPSPs. *Science* **275**, 213–215. (doi:10.1126/science.275.5297.213)
- Martin, S. J., Grimwood, P. D. & Morris, R. G. 2000 Synaptic plasticity and memory: an evaluation of the hypothesis. *Annu. Rev. Neurosci.* **23**, 649–711. (doi:10.1146/annurev.neuro.23.1.649)
- Pu, L., Bao, G. B., Xu, N. J., Ma, L. & Pei, G. 2002 Hippocampal long-term potentiation is reduced by chronic opiate treatment and can be restored by re-exposure to opiates. *J. Neurosci.* **22**, 1914–1921.
- Sun, L., Liu, S. Y., Zhou, X. W., Wang, X. C., Liu, R., Wang, Q. & Wang, J. Z. 2003 Inhibition of protein phosphatase 2A- and protein phosphatase 1-induced tau hyperphosphorylation and impairment of spatial memory retention in rats. *Neuroscience* **118**, 1175–1182. (doi:10.1016/S0306-4522(02)00697-8)
- Sun, X., Zhang, X., Chen, X., Zhang, P., Bao, M., Zhang, D., Chen, J., He, S. & Hu, X. 2005 Age-dependent brain activation during forward and backward digit recall revealed by fMRI. *Neuroimage* **26**, 36–47. (doi:10.1016/j.neuroimage.2005.01.022)
- Tang, S. & Guo, A. 2001 Choice behavior of *Drosophila* facing contradictory visual cues. *Science* **294**, 1543–1547. (doi:10.1126/science.1058237)
- Tang, S., Wolf, R., Xu, S. & Heisenberg, M. 2004 Visual pattern recognition in *Drosophila* is invariant for retinal position. *Science* **305**, 1020–1022. (doi:10.1126/science.1099839)
- Tian, S., Qi, H., Wang, J., Cai, J. & Ma, Y. 2002 Differential amplitude modulation of auditory evoked cortical potentials associated with brain state in the freely moving rhesus monkey. *Neurosci. Lett.* **331**, 159–162. (doi:10.1016/S0304-3940(02)00886-8)
- Wang, Y. & Zhou, C. F. 2005 Involvement of interferon-gamma and its receptor in the activation of astrocytes in the mouse hippocampus following entorhinal deafferentation. *Glia* **50**, 56–65. (doi:10.1002/glia.20152)
- Wang, M., Zhang, H. & Li, B. M. 2000 Deficit in conditional visuomotor learning by local infusion of bicuculline into the ventral prefrontal cortex in monkeys. *Eur. J. Neurosci.* **12**, 3787–3796. (doi:10.1046/j.1460-9568.2000.00238.x)
- Wang, Y., Ying, G., Liu, X. & Zhou, C. 2003a Semi-quantitative expression analysis of ephrin mRNAs in the deafferented hippocampus. *Mol. Brain Res.* **120**, 79–83. (doi:10.1016/j.molbrainres.2003.10.005)
- Wang, Z., Xu, N. L., Wu, C. P., Duan, S. & Poo, M. M. 2003b Bidirectional changes in spatial dendritic integration accompanying long-term synaptic modifications. *Neuron* **37**, 463–472. (doi:10.1016/S0896-6273(02)01189-3)
- Wang, M., Tang, Z. X. & Li, B. M. 2004 Enhanced visuomotor associative learning following stimulation of alpha 2A-adrenoceptors in the ventral prefrontal cortex in monkeys. *Brain Res.* **1024**, 176–182. (doi:10.1016/j.brainres.2004.07.062)
- Wang, Y., Zhou, Y., Ma, Y. & Leventhal, A. G. 2005 Degradation of signal timing in cortical areas V1 and V2 of senescent monkeys. *Cereb. Cortex.* **15**, 403–408. (doi:10.1093/cercor/bhh143)
- Wu, L. J., Duan, B., Mei, Y. D., Gao, J., Chen, J. G., Zhuo, M., Xu, L., Wu, M. & Xu, T. L. 2004 Characterization of acid-sensing ion channels in dorsal horn neurons of rat spinal cord. *J. Biol. Chem.* **279**, 43 716–43 724. (doi:10.1074/jbc.M403557200)
- Xiang, Y., Li, Y., Zhang, Z., Cui, K., Wang, S., Yuan, X. B., Wu, C. P., Poo, M. M. & Duan, S. 2002 Nerve growth cone guidance mediated by G protein-coupled receptors. *Nat. Neurosci.* **5**, 843–848. (doi:10.1038/nn899)
- Xiao, H. S. *et al.* 2002 Identification of gene expression profile of dorsal root ganglion in the rat peripheral axotomy model of neuropathic pain. *Proc. Natl Acad. Sci. USA* **99**, 8360–8365. (doi:10.1073/pnas.122231899)
- Xiong, W., Yang, Y., Cao, J., Wei, H., Liang, C., Yang, S. & Xu, L. 2003 The stress experience dependent long-term depression disassociated with stress effect on spatial memory task. *Neurosci. Res.* **46**, 415–421. (doi:10.1016/S0168-0102(03)00120-2)
- Xu, N. J., Bao, L., Fan, H. P., Bao, G. B., Pu, L., Lu, Y. J., Wu, C. F., Zhang, X. & Pei, G. 2003 Morphine withdrawal increases glutamate uptake and surface expression of glutamate transporter GLT1 at hippocampal synapses. *J. Neurosci.* **23**, 4775–4784.
- Xu, H. T., Yuan, X. B., Guan, C. B., Duan, S., Wu, C. P. & Feng, L. 2004a Calcium signaling in chemorepellant Slit2-dependent regulation of neuronal migration. *Proc. Natl Acad. Sci. USA* **101**, 4296–4301. (doi:10.1073/pnas.0303893101)
- Xu, N. J., Yu, Y. X., Zhu, J. M., Liu, H., Shen, L., Zeng, R., Zhang, X. & Pei, G. 2004b Inhibition of SNAP-25 phosphorylation at Ser187 is involved in chronic morphine-induced down-regulation of SNARE complex formation. *J. Biol. Chem.* **279**, 40 601–40 608. (doi:10.1074/jbc.M406896200)
- Yang, Y., Ge, W., Chen, Y., Zhang, Z., Shen, W., Wu, C., Poo, M. & Duan, S. 2003 Contribution of astrocytes to hippocampal long-term potentiation through release of D-serine. *Proc. Natl Acad. Sci. USA* **100**, 15 194–15 199. (doi:10.1073/pnas.2431073100)
- Yang, Y., Zheng, X. G., Wang, Y. F., Cao, J., Dong, Z. F., Cai, J. X., Sui, N. & Xu, L. 2004 Stress enables synaptic depression in CA1 synapses by acute and chronic morphine: possible mechanisms for corticosterone on opiate addiction. *J. Neurosci.* **24**, 2412–2420. (doi:10.1523/JNEUROSCI.5544-03.2004)
- Ying, G., Huang, C., Jing, N. & Zhou, C. 2001 Identification of differentially expressed genes in the denervated rat hippocampus by cDNA arrays. *Neurosci. Lett.* **306**, 121–125. (doi:10.1016/S0304-3940(01)01882-1)
- Ying, G. X., Huang, C., Jiang, Z. H., Liu, X., Jing, N. H. & Zhou, C. F. 2002 Up-regulation of cystatin C expression

- in the murine hippocampus following perforant path transections. *Neuroscience* **112**, 289–298. (doi:10.1016/S0306-4522(02)00083-0)
- Yuan, X. B., Jin, M., Xu, X., Song, Y. Q., Wu, C. P., Poo, M. M. & Duan, S. 2003 Signalling and crosstalk of Rho GTPases in mediating axon guidance. *Nat. Cell Biol.* **5**, 38–45. (doi:10.1038/ncb895)
- Zhang, L. I. & Poo, M. M. 2001 Electrical activity and development of neural circuits. *Nat. Neurosci.* **4**(Suppl), 1207–1214. (doi:10.1038/nm753)
- Zhang, J. M., Wang, H. K., Ye, C. Q., Ge, W., Chen, Y., Jiang, Z. L., Wu, C. P., Poo, M. M. & Duan, S. 2003 ATP released by astrocytes mediates glutamatergic activity-dependent heterosynaptic suppression. *Neuron* **40**, 971–982. (doi:10.1016/S0896-6273(03)00717-7)
- Zhuo, Y., Zhou, T. G., Rao, H. Y., Wang, J. J., Meng, M., Chen, M., Zhou, C. & Chen, L. 2003 Contributions of the visual ventral pathway to long-range apparent motion. *Science* **299**, 417–420. (doi:10.1126/science.1077091)