Chapter 8

Genetic Marker Mice and Their Use in Understanding Learning and Memory

Mark Murphy, Yvette M. Wilson and Christopher Butler

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/56498

1. Introduction

The advent of genetically encoded marker proteins to follow functional and structural change in neurons has been a major technical advance in neuroscience. These proteins have been used to image cellular changes *in vitro* and *in vivo* and have enabled the identification of activated neurons which are involved in a diverse array of functions in the brain. Particular marker proteins have also been employed to trace the changes in neuronal activation following different functional stimuli both *in vitro* and *in vivo*. Another major advance in utilising genetically encoded marker proteins has been the development of techniques which allow the specific stimulation or inhibition of neuronal function of specified subsets of neurons which express these proteins. This has allowed the precise targeting of subpopulations of neurons within sub-nuclei within the brain to determine their function. In this article, we will summarise the major types of genetically encoded marker proteins and their uses in studies of neuronal function, predominantly in the mouse. We will give examples where they have been used in behavioural studies, with a particular emphasis on learning and memory.

2. Transgenic marker mice

In contrast to traditional staining or dye-injection techniques, labelling cells using a genetic approach enables the identification of specific cell types, sub-types, as well as the temporal and spatial aspects of genetic expression [1]. One of the most widely used reporter proteins is the bacterial β-galactosidase (βgal) enzyme, encoded by the *E. coli* gene *LacZ* [2]. Inserting the *LacZ* gene into a cell under the control of a given set of transcriptional

elements enables the biochemical labelling of cells in which expression of the gene of interest has taken place. One of the first such studies in learning and memory utilized a transgenic mouse containing the *LacZ* gene regulated by six cAMP response elements (CREs) upstream of a minimal promoter [3, 4]. The CRE system and CRE binding protein (CREB) are important transcription elements involved in learning and memory. CRE-mediated *LacZ* expression was induced by long term potentiation (LTP) in area CA1 of the hippocampus and was also induced in CA1 and CA3 and amygdala following different forms of fear conditioning [3, 4], which is consistent with neurons in these areas of the brain being involved in contextual and fear memory.

This *CRE-LacZ* mouse was also used to examine neuronal activation in the barrel fields of the somatosensory cortex, which receive and map sensory information from the facial whiskers. Removal of all but one facial whisker resulted in highly specific *LacZ* expression in layer IV of the spared whisker barrel, and was accompanied by an increase in responsiveness of neurons in layer II/III of the same barrel. These findings suggested that CRE expression in layer IV was in neurons presynaptic to the altered neurons in layer II/III [5].

Given that βgal diffusion into the processes of a *LacZ* expressing neuron is minimal, this traditional method of *LacZ* reporting can only provide limited information in studies of the nervous system, where data on the morphology, structure, and connections between neurons is often required [1]. Neurons and their processes can thus be visualised by the use of *LacZ*-fusion genes, whereby the *LacZ* gene is fused to a gene encoding a separate, neuronal protein that is known to be trafficked throughout the cell [2]. For example in *Drosophila*, the entire neuron has been labelled by fusing *LacZ* with the gene for the microtubule-binding protein tau [2].

The *Fos-Tau-LacZ* (*FTL*) transgenic mouse was created to enable the identification of functionally activated neurons in the mouse brain following a given behavioural task [6]. The mouse expresses a transgene that encodes the tau-βgal fusion protein, driven by the promoter for the immediate early gene c-*fos.* This immediate-early gene is expressed in neurons following functional stimulation, with expression shown to occur following a range of stimuli including stress, ischemia, sensory stimulation, endocrine hormones and various pharmacological agents [6]. The *FTL* transgene is thus rapidly expressed in neurons following functional stimulation, and trafficked throughout the cell body, dendrites and axon. This labelling enables the localisation of functionally activated neurons, the identification of their cellular morphologies and the connections they make with other neurons in the brain [6]. Labelling experiments that utilise the *FTL* mouse include the identification of the neuronal nuclei involved in osmoregulation [6], as well as the identification of light activated pathways in the visual system [7]. However, the findings most pertinent to this review occur from the analysis of *FTL* mouse brains following context fear conditioning.

Context fear conditioning is a model for learning, and training *FTL* mice to associate the context of a shock chamber with the aversive stimulus of a foot-shock enables the identification of the neurons that are functionally activated by this association. Initially, a discrete population of glutamatergic neurons were identified along the lateral edge of the lateral amygdala [8]. These *FTL* $^{+}$ neurons only appeared in those *FTL* mice that had learned to associate the context with

http://dx.doi.org/10.5772/56498

shock, indicating that these cells may be involved in the circuits responsible for this learning process [8]. A further study was able to identify other nuclei of labelled neurons, in the medial amygdala, the amygdalo-striatal transition region, and the ventromedial hypothalamus [9]. It was also shown that these regions were not activated following the recall of memory or following fear expression, but specifically by the association of context to shock, suggesting that these neurons were involved in this learning event [9]. These anatomically restricted populations were hypothesised to be nodes within a circuit for fear conditioning [9]. In this way, a modified version of the βgal marker protein has contributed to our understanding of the circuitry that underlies the formation of fear memories in mammals.

Perhaps the most widely studied cellular marker protein is the Green Fluorescent Protein (GFP). First isolated from *Aequorea* jellyfish, GFP was found to produce bright green fluorescence in the presence of ultraviolet light [10]. The GFP gene was first used as a marker of genetic expression [11], with the finding that both prokaryotic and eukaryotic cells were capable of expressing the protein and that this expression was non-toxic to the cell. In a similar fashion to βgal, GFP is thus capable of acting as a marker of genetic expression as well as enabling the labelling of specific populations of cells.

Fos-GFP mice have been generated and used to study plasticity in the barrel cortex in a series of studies following on from those using the *CRE-LacZ* mice [5, 12-15]. Similar to the results from the *CRE-LacZ* mice, expression of GFP in the *fos-GFP* mice was specific to the barrel field of the spared whisker in mice where all whiskers bar one were removed. The use of GFP as a marker also permitted electrophysiological recordings of these neurons, and it was found that both the GFP^+ and GFP^- neurons within the same region of the spared barrel had altered action potentials and spike frequencies compared to neurons in non-spared barrels. Subsequent experiments with *fos-GFP* mice identified increased amplitudes of the AMPA glutamate receptor in the spared barrel column [13]. This was due to the specific delivery of AMPA receptors at the inputs to the spared, but not deprived, barrels. These findings suggested that delivery of AMPA receptors is a normal feature of synaptic strengthening underlying experience dependent plasticity [13]. Further experiments studied the effects of ongoing stimulation of the spared whisker [12]. *N*-methyl-D-aspartate (NMDA) receptors were required to initiate synaptic strengthening at the layer IV-II/III synapse. However with additional sensory activity, strengthening was dependent on activation of metabotropic glutamate receptors, which suggests a mechanism whereby continued experience can result in synaptic strengthening over time [12].

Recently, the Barth group studied the properties of GFP^+ neurons in the cortex of unstimulated *fos-GFP* mice as a method to study neurons which were recently active in cortex [16]. GFP^+ neurons had higher firing rates compared to GFP^- neurons, which was due to increased excitatory and decreased inhibitory drive of the GFP^+ neurons. Paired cell recordings indicated that the GFP^+ neurons had a greater likelihood of being connected to each other. These results suggested that the GFP^+ neurons represented interconnected neuronal ensembles in neocortex, possibly involved in coding of sensory information [16].

Since its initial discovery, a number of GFP variants have been created, improving the efficiency and stability of the protein as well as altering its spectral properties [17]. In this way,

different colours of emitted light can be produced. Red, yellow and cyan fluorescent proteins (RFP, YFP, and CFP, or XFPs collectively) have each been created, enabling experiments that label multiple cell types or expression profiles within the same biological sample [18]. The use of multiple XFPs is perhaps exemplified by the creation of the 'Brainbow' mouse, whereby individual neurons in the mouse brain express different ratios of the XFPs, enabling the distinctive tagging of individual neurons with at least 90 different fluorescent colours [19]. The Brainbow mouse enables the visualisation of the precise morphology of closely juxtaposed neurons, and has had major contributions to the study of neural connections in the brain [20].

Specifically targeting the expression of XFPs to neurons is typically achieved by driving expression of the XFPs by the promoter for the thymocyte antigen protein Thy-1, a cell surface protein. Thy-1 is a known marker of axonal processes in mature neurons, thus Thy1-XFP transgenes specifically label neurons in the brain [21]. A large number of learning and memory studies that utilise the various XFPs have also used the Thy-1-XFP fusion transgene to fluorescently tag neurons and their processes. These neurons can then be visualised *in vitro* using fluorescence confocal microscopy, as well as visualising *in vivo* using two-photon microscopy.

2.1. Conditional transgenic marker mice

In addition to the marker mice described above, it is also possible to conditionally regulate the gene which controls marker expression. Some of the conditional expression systems include Cre recombinase/lox site insertions, excisions and other modifications, and the tetracycline (tet) systems based on the tet-controlled transactivator (tTa) and reverse tet-on transactivator (rtTA) that allow downregulation or induction of gene expression [22]. A conditional transgenic mouse employing the tet system has been developed and used in studies of learning and memory [23]. This *TetTag* mouse has two transgenes: 1. containing the c-*fos* promoter regulating expression of tTa, which will bind to 2. the *TetO* promoter regulating expression of *tau-LacZ.* Binding to the *TetO* promoter is inhibited by doxycycline, and thus by maintaining the mice on doxycycline, this system is blocked. However, the second transgene also contains a doxycycline insensitive tTa, and once this transgene is activated, a feedback loop is established which will maintain expression of the doxycycline insensitive tTa and consequently also *tau-LacZ.* This allows for the long term tagging of neurons which express c-*fos* during the window when the mice were taken off doxycycline [23]. Using this *TetTag* mouse, a small number of neurons in the basolateral amygdala, which were activated and tagged during fear conditioning learning, were found to be subsequently reactivated during recall of fear conditioning [23]. It was thus suggested that these neurons were a stable neural correlate of fear memory.

A variant of this experiment involved the c-*fos*-tTa transgene in combination with a *TetO*-GFP-GluR1 transgene [24]. GluR1 is a major subunit of the AMPA glutamate receptor and using this conditional marker mouse, the location of newly synthesised AMPA receptors could be followed using GFP fluorescence. Following fear conditioning, newly synthesised GluR1 receptors were found to be selectively associated with mushroom-type dendritic spines on hippocampal CA1 neurons [24]. These results were argued to be consistent with a synaptic

tagging model whereby activated synapses capture new AMPA receptors as part the learning and memory process.

2.2. Viral-mediated gene delivery

Genetic manipulation of the neurons involved in learning and memory has also been achieved using viral methods of transgene delivery, enabling targeting of specific brain regions. In one series of studies, the question of how neurons become involved in memory was addressed and if the transcriptional status of the neuron at the time of learning was important in this process. For this, the function of CREB was manipulated via delivery of a series of different CREB containing viruses to the lateral amygdala [25, 26]. Increasing CREB function in any lateral amygdala neuron appeared to increase the probability that this neuron was recruited into the fear memory trace, suggesting that CREB status is important in determining which neurons are involved in memory [25]. Further, ablation of these overexpressing CREB neurons after learning blocked the expression of the specific fear memory in which they were involved, establishing that these neurons were functionally required for that specific fear memory [26]. Broadly consistent results were found when the CREB viruses were targeted to the auditory thalamus [27].

3. Two-photon imaging using transgenic marker mice

Transgenic marker mice have been used very successfully to follow changes in neuron structure over time. In initial studies of this kind, individual neurons were imaged in developing hippocampus of rat brains expressing enhanced GFP, via infection with GFP encoding Sindbis virus [28]. Imaging of the neurons was done using two-photon laser scanning microscopy, which has the advantage of detecting the fluorescence signal with very low levels of photobleaching and phototoxicity. This allows for repeated high resolution imaging deep into living neural tissue with little effect on the imaged neurons. These studies demonstrated change in dendritic structure driven by high frequency synaptic stimulation, suggesting that synaptic activation during development could contribute to development of neural circuitry [28].

Subsequent studies have undertaken imaging of dendritic spines over time. Dendritic spines are protrusions from dendrites and are the postsynaptic sites of excitatory synapses. Thus imaging changes in dendritic spines over time is a very good approach to studying structural synaptic plasticity. Synaptic plasticity is thought to be a prime candidate mechanism underlying the processes involved in learning and memory. Two-photon imaging of dendritic spines was undertaken in hippocampal slices [29] using one of the lines of thy1-GFP expressing mice (line M) generated by Feng et al. [18]. Induction of LTP in these slices resulted in a transient increase in spine area of a small fraction of spines. Similar to LTP, this increase was dependent on NMDA receptor activation which is hypothesised to contribute to the synapse remodelling that occurs in LTP [29]. Similar results were obtained in experiments using hippocampal slices from non-transgenic rats [30].

In further experiments using the thy1-GFP-M mice [18], two-photon microscopy was used to study relationships between spines following LTP [31]. Following induction of LTP at individual synapses of hippocampal pyramidal neurons, the response thresholds at closely neighbouring synapses on the same dendrite were found to be altered [31]. Thus, presentation of low level stimuli, which were normally too weak to induce LTP, resulted in robust LTP and spine enlargement at these neighbouring synapses. The reduction in this threshold for LTP was short lived (~10 minutes) and extended over 10 micron of dendrite length. It was proposed that these interactions between neighbouring synapses were consistent with clustered models of plasticity in memory storage as well as providing a mechanism for binding of behaviourally linked information within a small region of a dendrite [31].

3.1. Two-photon imaging *in vivo*

The two-photon imaging approach has been extended to studies of living animals to great effect. This is done by removing a small area of skull from the mice, which allows for repeated imaging of the exposed cortex using two-photon microscopy. The major advance in this approach is that single neurons can be studied in living mice over extended periods of time, up to many months. This allows for the mapping of spines on a particular dendrite and the tracking of the changes in spine number, morphology and lifetime of individual spines over this time. Thus, one can examine the effects of learning on spines, and accompanying studies can ask if the observed spine changes result in synaptic changes.

The first studies to use this approach undertook imaging of spines in individual pyramidal neurons in visual cortex and barrel cortex over periods of a month to over a year [32, 33]. Using thy1-GFP-line H mice [18], they found that dendritic structure was essentially stable, and that spines appear and disappear. In barrel cortex, 50% of spines were stable for at least a month, with the other spines present for days or less [32]. These spine changes were shown to correlate with synaptic change. Further, sensory experience of the facial whiskers (the principle input for the barrel cortex) resulted in increased spine turnover [32]. In adult visual cortex, the great majority of spines were stable for at least one month [33]. However, in visual cortex of young mice during the critical period of visual cortical development, about 70% of spines were stable for at least one month, with most changes due to spine elimination [33]. These findings thus demonstrated spine turnover in cortex, and that developmental stage and sensory experience can alter that turnover. Further studies in different regions of the mouse cortex also confirmed that spine turnover varies across the cortex [34].

Most synapses which occur on dendritic spines are excitatory, and most of the changes described above probably represent changes in excitatory synapses. There is no obvious morphological hallmark for inhibitory synapses. Recently, genetic markers have been developed to allow the visualisation of both inhibitory synapses and dendritic spines on pyramidal neuron dendrites. The markers were a) teal fluorescent protein fused to gephyrin, a postsynaptic protein only expressed in inhibitory synapses, and b) YFP to label neuronal morphology [35]. Plasmids expressing these markers were inserted into the embryonic cortices of mice via electroporation. Using this combination of markers, it was found that inhibitory synapses and dendritic spines (as proxy for excitatory synapses) differed in their distribution pattern across

http://dx.doi.org/10.5772/56498

the dendritic arbor [35]. However, remodelling of both inhibitory synapses and dendritic spines occurred within the same spatially clustered regions on the dendritic arbor and this clustering was influenced by sensory input. These findings suggested that both excitatory and inhibitory synapse rearrangement occurs and may be coordinated at the dendritic level [35].

Whereas dendritic structure is stable in pyramidal neurons, other classes of neurons in the cortex show dynamic changes in dendritic structure over time. Imaging of thy1-GFP-S mice [18] showed that GABA+ inhibitory interneurons extend and retract dendritic branches over periods of months and in a small proportion of neurons, new branch tips emerge [36]. In the visual cortex, visual deprivation stimulates this structural remodelling, affecting up to 16% of branch tips [37]. Visual deprivation induces branch retractions, which is accompanied by loss of inhibitory inputs to neighbouring pyramidal neurons and results in a decrease in inhibitory tone [37]. Further studies show that interneuron remodelling occurs across the major primary sensory cortex regions, but may differ in degree between primary and higher order sensory cortical areas [38]. These studies show that the dendritic arbor of inhibitory neurons changes over time, is influenced by sensory input, and that these changes correlate with functional changes in sensory cortex.

3.2. Two-photon imaging in learning and memory

The effects of learning have been directly studied using *in vivo* imaging of dendritic spines. In two such studies, young (1 month) and adult thy1-GFP-H mice [18] were trained specific motor skills and the effects of that training on motor cortex were followed [39, 40]. Training in a forelimb reaching task resulted in formation of dendritic spines within one hour in the pyramidal neurons in contralateral motor cortex [39]. Training on a rotarod also increased production of new spines in motor cortex [40]. These new spines were stabilised by subsequent training and persisted long after training stopped and into adulthood [39, 40]. However, spines present before training were selectively eliminated and thus overall spine density returned to its original level. Other motor skills resulted in production of different sets of spines [39]. These findings suggested that specific motor skills are encoded by particular sets of newly generated and long lasting synaptic connections [39, 40].

Subsequent studies using the motor learning model showed that a third of the new spines formed during learning emerged as clusters, generally as pairs of spines [41]. These clustered spines were more likely to persist than newly formed single spines. The clusters were formed in succession, with later spines in the cluster formed during repetition of the motor task [41]. Thus, these new clusters are formed by repetitive activation of particular cortical circuits and correspond to the strength of the motor memory.

Other studies in learning and memory using two-photon imaging of YFP+ dendritic spines have provided somewhat counter-intuitive findings. Studies of fear conditioning by pairing an auditory cue with a foot-shock provide evidence that this results in an increase in the rate of spine elimination in frontal association cortex [42]. In contrast, extinguishing the fear memory by presenting the auditory cue without foot-shock, increased the rate of spine formation. Both of these changes in spine number were observed on the same dendrites and within the same region of the dendrite. Further reconditioning of the mice tended to result in

elimination of the spines which were formed by extinction [42]. These findings suggest both that the fear memory trace is partly generated through reduction of particular synaptic contacts and that this is eliminated through opposing actions of extinction on these synapses.

4. Genetically engineered calcium indicators

Ca^{2+} is one of the master second messengers for the cell, being involved in a vast array of cellular processes. Many studies have employed various chemical Ca^{2+} indicators to study Ca^{2+} flux in the cell. These chemical Ca^{2+} indicators are generally based on the Ca^{2+} chelator BAPTA (1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid). A particular advantage of the Ca^{2+} indicators is the very high temporal resolution (millisecond scale) as these indicators are changing their fluorescence essentially in time with Ca^{2+} flux in the cell. In recent years, a new class of Ca^{2+} indicators has been developed; the genetically engineered Ca^{2+} indicators (GECI; 43-45). These indicators are formed by the fusion of genetically engineered fluorescent proteins with proteins which bind Ca^{2+}. Upon binding of Ca^{2+} the confirmation of the GECI changes, which results in a change in its fluorescence properties. The principal advantages of the GECIs over the chemical Ca^{2+} indicators is that they can be targeted to specific functional subpopulations of neurons by the use of cell specific gene promoters to control their expression, they can be delivered to particular brain regions using viral injection, and expression is relatively stable for several months.

The first GECIs were the Cameleons, which were fusions of blue- or cyan- variants of GFP with calmodulin, the calmodulin-binding peptide M13, and an enhanced green- or yellow-emitting GFP [45]. Binding of Ca^{2+} results in consequent binding of M13 with calmodulin and an increase in fluorescent resonance energy transfer between the two GFPs in the protein [45]. Another form of GECI is the GCaMP (GFP–Calmodulin–M13 Protein), which uses a circularly permuted GFP where the N- and C- termini of GFP are fused [46, 47]. Calmodulin and M13 are fused to this circularly permuted GFP, and on binding of Ca^{2+}, the conformation of the fusion protein is altered which results in increased fluorescence of GFP [46, 47]. Other forms of GECIs use Troponin C instead of Calmodulin and M13 to induce binding of Ca^{2+} and conformational change in the fusion protein [48]. The different types of GECIs have different properties and particular advantages in Ca^{2+} imaging studies [43].

Ca^{2+} influx and regulation of signalling plays a fundamental role in the molecular mechanisms underlying learning and memory. For example, the NMDA glutamate receptor is regarded as one of the most important neurotransmitter receptors in the initial acquisition process of learning and memory [49-51]. The NMDA receptors are highly permeable to Ca^{2+} ions, but this permeability only occurs during both membrane depolarisation and glutamate binding [52]. Such conditions are regarded as a requirement for memory acquisition. Inside the neuron, Ca^{2+} regulates many intracellular signalling processes involved in memory formation [50, 51, 53]. Thus the use of GECIs may be useful in learning and memory studies; for example in identifying neuronal populations undergoing changes in Ca^{2+} concentrations during learning and memory and in studying the temporal progression of such changes. However, there have been few studies to date which have used this approach in learning and memory research.

Recent studies have developed methods for the cellular imaging of neural activity in awake behaving mice and which can be suitable for analysis of cellular responses during learning and memory. For example, one study describes a method to visualise cellular imaging of neural activity in the visual cortex of awake head restrained mice during visual discrimination learning as well as passive viewing of visual stimuli [54]. Neural activity was measured using the yellow Cameleon 3.6 GECI, virally transfected into visual cortex. Another approach has been developed which enables imaging the activity of neurons in head restrained mice which can still perform spatial behaviours within a virtual reality system [55]. In the example given, the activity of neurons in the CA1 region of the hippocampus was imaged through the expression of the GCaMP3 GECI. Populations of place cells were thus identified based on their place specific activity within the virtual environment and correlated with their location within the local hippocampal circuit [55].

An extension of the use of GECIs is the development of indicators which detect the Ca^{2+} activation of Ca^{2+}/calmodulin-dependent protein kinase (CaMKII; 56). These are GECIs using CaMKII as the Ca^{2+} binding protein and thus are specific for CaMKII activation. This indicator has been used to detect changes in CaMKII activity in individual spines of particular regions of cortex before and after visual deprivation [56]. Visual deprivation is a model of experience dependent plasticity and thus this approach could be used in the analysis of spine changes occurring during learning and memory formation.

5. Optogenetics and learning and memory

Optogenetics is a technology currently sweeping through many areas of neuroscience. It relies on the targeted expression of light activated ion channels within any neuronal population one wishes to study [57-59]. The light activated channels belong to the family of microbial opsins. Two classes of these opsins are currently used: 1. Positive ion channels which upon light activation result in depolarisation and activation of the neuron (such as channelrhodopsins ChR1, ChR2, and VChR1), and 2. negative ion channels which upon light stimulation result in hyperpolarisation and inhibition of the neuron (such as *Natronomonas pharaonis* halorhodopsins, NpHR, enhanced halorhodopsins, eNpHR2 and eNpHR3, *Archaerhodopsin, Leptosphaeria maculans* fungal opsin, and enhanced bacteriorhodopsin). The channels are activated very quickly by light, allowing for the precise temporal control of neuronal activation. The light can be delivered by optic fibres to a small volume of brain tissue allowing for good spatial definition of activation and expression of the opsins can be genetically targeted to subpopulations of neurons within the brain region of interest. This combination thus permits the examination of the consequences of either activation or inhibition of neuronal function at a fine temporal, spatial and neuron-type level [58, 59].

5.1. Optogenetics in reward learning

Optogenetic technology has been used to study a number of different types of learning and memory. These include classical conditioning to both rewarding and aversive stimuli, and

spatial learning and the role of the hippocampus. Most of these studies have been done in mice, with one study undertaken in *Drosophila* to date [60]. In studies of conditioning to a reward, one of the most important classes of neuron studied is the dopaminergic neuron in the ventral tegmental area (VTA), postulated to be involved in mediating the reward stimulus [61, 62]. However, it was unclear if firing of these neurons alone could result in reward conditioning. To test the role of these neurons, the Cre-inducible adeno-associated virus vector carrying the ChR2 gene fused to enhanced YFP (EYFP) was used [63]. Injection of this vector into the VTA of *Tyrosine hydroxylase – Cre* transgenic mice results in specific expression of ChR2-EYFP in the dopamine neurons. They then tested the effects of optogenetic stimulation of these dopamine neurons on conditioned place preference. The mice received phasic (50 Hz) optical stimulation in one chamber and 1 Hz stimulation in the other chamber of the place preference apparatus. The mice developed a clear place preference to the chamber in which they received the phasic stimulation [63]. These findings demonstrate that phasic firing of the dopamine neurons alone (in the absence of reward) is sufficient for reward conditioning.

These experiments involved conditioned place preference, which is passive behavioural conditioning. To look at the role of the dopaminergic neurons in operant conditioning, these neurons were optogenetically stimulated during an active food seeking operant task [64]. Phasic activation of the dopaminergic neurons enhanced the positive reinforcing actions (pressing a specific lever for a food reward) in this task. This enhancing effect was dependent on the presence of the food reward, in contrast to that seen in the passive conditioning task [64]. However, activation of the dopaminergic neurons alone was sufficient to reactivate a previously extinguished food seeking behaviour. These findings together suggested that activation of the dopaminergic neurons facilitates development of positive reinforcement during active reward seeking [64].

Within the dopamine system, the firing rate of the dopamine neurons is increased for only a very short time following reward events (200 milliseconds) and it was unclear if this was sufficient to be involved in reward learning. To test for this, mice with expression of ChR2-EYFP targeted to the dopamine neurons of the VTA were placed in testing chambers with a port, which when investigated with a nose-poke, triggered a 200 millisecond optogenetic stimulation. This resulted in the mice rapidly learning to nose-poke the port and receive the brief optical stimulations [65]. This demonstrated that the brief time of dopamine neuron firing was sufficient to drive reward learning. Optogenetics has also been used to study the role of GABA neurons in the VTA and shown that these neurons negatively regulate consummatory behaviour and dopamine release from the VTA [66].

Further experiments have looked at the role of other neurons in the putative reward circuit. The nucleus accumbens is strongly implicated in the reward pathway and its input from the basolateral amygdala (BLA) is thought to be involved in cue-triggered motivated behaviours. In order to investigate the function of the BLA to nucleus accumbens pathway during behaviour, the ChR2-EYFP virus was injected into the BLA, and the pathway to the accumbens was targeted for optogenetic stimulation [67]. Mice were then placed in the testing chambers which triggered optogenetic stimulation with a nose-poke. The mice rapidly learnt to receive optical stimulations [67]. To inhibit this pathway, the BLA was injected with a NpHR-EYFP virus,

http://dx.doi.org/10.5772/56498

which results in hyperpolarisation upon light stimulation. Optically induced inhibition of the pathway reduced co-evoked intake of sucrose, demonstrating that this pathway controls naturally occurring reward related behaviour. These findings together show that the pathway from BLA to the nucleus accumbens promotes motivated behavioural responding in conjunction with the dopamine pathway from VTA [67].

The striatum is another part of the reward circuit and has been implicated both in positive learning reinforcement as well as negative reinforcement. The striatum contains two populations of projection neurons, characterised by their expression of either dopamine receptor 1 (D1) or 2 (D2). To determine possible roles of these two populations of neurons, they were selectively targeted for optogenetic stimulation [68]. Optical stimulation of the D1 receptor–expressing neurons induced persistent reinforcement, whereas stimulating D2 receptor–expressing neurons induced transient negative reinforcement, indicating that activation of these different populations of neurons has opposite behavioural effects and can result in distinctly different learning outcomes.

5.2. Optogenetics in classical fear conditioning

The amygdala is heavily implicated not only in reward but also in classical conditioning to aversive stimuli that occurs in fear conditioning paradigms. In particular the lateral amygdala is considered to be a site of plasticity underlying fear memory. In order to determine if stimulation of the principle neurons of the lateral amygdala could directly contribute to fear conditioning, mice were infected with the ChR2-EYFP virus to target these neurons [69]. The mice then received an auditory stimulus paired with optical stimulation of the LA neurons instead of being paired with a conventional aversive stimulus. It was found that pairing resulted in successful fear conditioning of the mice. These findings provided direct evidence that fear learning can be a consequence of a stimulus induced activation of the principle neurons of the lateral amygdala [69].

The central amygdala is thought to be involved in transmitting the behavioural response signal to other parts of the brain. Recent information also implicates the central amygdala in fear learning. To investigate this possibility, a series of different approaches, including optogenetically targeted activation of subpopulations of neurons in central amygdala were employed [70]. Neuronal activity in the lateral division of the central amygdala was found to be required for fear memory formation, whereas optogenetic stimulation of neurons in the medial division of the central amygdala indicated that these neurons were involved in fear related (freezing) behavioural expression [70]. These findings suggested that a part of the fear memory is acquired in inhibitory neurons of the medial division, which project to the lateral division of the central amygdala to control their output fear signalling.

Contextual fear conditioning is a form of fear conditioning which is dependent on the hippocampus. It was unclear if the hippocampal neurons which are activated during context fear learning contain enough information to drive fear behaviour when they are specifically re-activated. To test this, neurons which were activated during fear learning in the dentate gyrus of mice were targeted to express ChR2 [71], using a modified *TetTag* mouse described above [23]. Optical stimulation of dentate gyrus alone resulted in freez-

ing, indicating light induced fear memory recall. Further, activation of cells targeted in a context not associated with fear did not result in freezing, suggesting that light-induced fear memory recall is context specific [71]. Essentially similar findings were obtained using non-optogenetic techniques [72]. Together these findings indicate that activation of a sparse and specific population of neurons in dentate gyrus, which were activated during learning, is sufficient for recall of that memory.

Another important issue on the role of the hippocampus in learning and memory is the observation that contextual and explicit memories are first dependent on hippocampus but loss of hippocampus some period of time after acquisition of these memories does not result in loss of these memories [73]. Based on these observations, it has been thought that memories somehow transfer from hippocampus to the cortex over time. Optogenetic approaches were employed to examine the contribution of the hippocampus to long term memories in real-time [74]. Excitatory neurons in dorsal CA1 hippocampus were virally targeted to express the chloride channel, eNpHR3.1. Rapid optical stimulation to inhibit these neurons resulted in reversible abolition of short and long term context fear memory (up to 9 weeks old), indicating hippocampal involvement throughout the period of memory retention [74]. However, when inhibition was extended significantly, the context fear memory became hippocampal independent; suggesting long term memory normally involves hippocampus but can shift to alternate structures. The anterior cingulate cortex had previously been implicated in storage of long term memories, and optogenetically induced inhibition of this region of the cortex resulted in inhibition of long term but not recent context fear memories [74]. These findings thus indicate a permanent role for hippocampus in context memory, with additional roles for anterior cingulate cortex in long term memory.

Another form of fear conditioning involves pairing the aversive stimulus to an auditory stimulus. This auditory fear conditioning is independent of hippocampus and probably involves auditory regions of the brain. Recent experiments indicate that auditory fear conditioning depends on recruitment of a disinhibitory microcircuit in the auditory cortex [75]. Disinhibition in auditory cortex is driven by foot-shock-mediated cholinergic activation of layer I interneurons, which generates inhibition of layer II/III parvalbumin-positive interneurons and subsequently leads to disinhibition of the layer II/III cortical pyramidal neurons. Importantly, optogenetic block of pyramidal neuron disinhibition abolishes fear learning [75]. These findings thus show the involvement of auditory cortex in associative fear learning, but also suggest that layer 1 disinhibition may be an important mechanism underlying different types of learning throughout the cortex.

5.3. Hippocampus and spatial learning

Where many studies have looked at the role of excitatory granule cells of the dentate gyrus in spatial learning, the function of the GABA-ergic inhibitory interneurons, which control the granule neuron activity, is unclear. To investigate the role of these neurons, their activity was inhibited via expression of targeted expression of eNpHR3.0 [76]. Optogenetic inhibition of these GABA-ergic interneurons impaired spatial learning and memory retrieval, without

affecting memory retention, as determined in the Morris water maze, thus establishing a role for these neurons in spatial learning and retention [76].

5.4. Other studies in learning and memory

Sleep has been implicated in memory consolidation for many years. Sleep disruption results in memory deficits, which raises the question of whether the continuity of sleep is important for memory consolidation. However, it is difficult to disrupt one feature of sleep (i.e. sleep continuity) without disrupting other sleep features (such as duration and intensity). To introduce a precise way of disrupting sleep continuity, optogenetics was used to target hypocretin/orexin neurons, which play a key role in arousal [77]. Optogenetic activation of these neurons could fragment sleep without affecting total amount or intensity of sleep [77]. Fragmenting sleep this way disrupted performance of the mice in an object recognition task once the duration of sleep episodes decreased below 66% normal. These findings indicated that a minimum of uninterrupted sleep is required for memory consolidation [77].

6. Conclusion

The employment of genetically encoded markers both in transgenic mice and in viral constructs has been a major technical advance for neuroscience and for whole animal biology generally. In studies of learning and memory, the use of this technology is leading to improved understanding in many aspects of this large and varied field of knowledge. The use of this approach is aiding in the identification of the neurons which are involved in learning and memory, in identifying the changes within those neurons which may underlie different parts of the learning process, in understanding potential mechanisms which specify which neurons are involved in learning and memory, and in describing ensembles of neurons which together code the contextual memory in the hippocampus. Two photon imaging using genetic markers in living animals is producing remarkable findings of what synaptic changes occur in learning and memory and how synaptic homeostasis is achieved. The use of Genetically engineered Calcium indicators is at an early stage in learning and memory, but it promises to inform us of real time changes in neuronal activation during learning and memory events.

Optogenetics, which relies on the ability to specifically activate or inhibit specific markers, is rapidly becoming a critical technique throughout neuroscience. Overall, optogenetics is delivering in its promise to enhance our understanding of learning and memory, through its ability to target specific populations of neurons and activate or inhibit them very rapidly and reversibly. This has helped to define the role of these neurons in behaviours associated with the learning and memory process, to ask if these neurons are involved in learning or memory *per se*, and to determine directly the role of these neurons - without the complexity of relatively slow lesioning studies and attendant compensation which the brain undertakes to circumvent the lesion.

Author details

Mark Murphy*, Yvette M. Wilson and Christopher Butler

*Address all correspondence to: m.murphy@unimelb.edu.au

Department of Anatomy and Neuroscience, University of Melbourne, Melbourne, Victoria, Australia

References

[1] Callahan CA, Yoshikawa S, Thomas JB. Tracing axons. Curr Opin Neurobiol. 1998;8(5):582-6. Epub 1998/11/13.

[2] Callahan CA, Thomas JB. Tau-beta-galactosidase, an axon-targeted fusion protein. Proc Natl Acad Sci U S A. 1994;91(13):5972-6.

[3] Impey S, Mark M, Villacres EC, Poser S, Chavkin C, Storm DR. Induction of CRE-mediated gene expression by stimuli that generate long-lasting LTP in area CA1 of the hippocampus. Neuron. 1996;16(5):973-82. Epub 1996/05/01.

[4] Impey S, Smith DM, Obrietan K, Donahue R, Wade C, Storm DR. Stimulation of cAMP response element (CRE)-mediated transcription during contextual learning. Nat Neurosci. 1998;1(7):595-601.

[5] Barth AL, McKenna M, Glazewski S, Hill P, Impey S, Storm D, et al. Upregulation of cAMP response element-mediated gene expression during experience-dependent plasticity in adult neocortex. J Neurosci. 2000;20(11):4206-16.

[6] Wilson Y, Nag N, Davern P, Oldfield BJ, McKinley MJ, Greferath U, et al. Visualization of functionally activated circuitry in the brain. Proc Natl Acad Sci U S A. 2002;99(5):3252-7.

[7] Greferath U, Nag N, Zele AJ, Bui BV, Wilson Y, Vingrys AJ, et al. Fos-tau-LacZ mice expose light-activated pathways in the visual system. Neuroimage. 2004;23(3): 1027-38.

[8] Wilson YM, Murphy M. A discrete population of neurons in the lateral amygdala is specifically activated by contextual fear conditioning. Learn Mem. 2009;16(6):357-61.

[9] Trogrlic L, Wilson YM, Newman AG, Murphy M. Context fear learning specifically activates distinct populations of neurons in amygdala and hypothalamus. Learn Mem. 2011;18(10):678-87. Epub 2011/10/05.

[10] Shimomura O, Johnson FH, Saiga Y. Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, Aequorea. Journal of cellular and comparative physiology. 1962;59:223-39. Epub 1962/06/01.

[11] Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC. Green fluorescent protein as a marker for gene expression. Science. 1994;263(5148):802-5. Epub 1994/02/11.

[12] Clem RL, Celikel T, Barth AL. Ongoing in vivo experience triggers synaptic metaplasticity in the neocortex. Science. 2008;319(5859):101-4.

[13] Clem RL, Barth A. Pathway-specific trafficking of native AMPARs by in vivo experience. Neuron. 2006;49(5):663-70.

[14] Barth AL, Gerkin RC, Dean KL. Alteration of neuronal firing properties after in vivo experience in a FosGFP transgenic mouse. J Neurosci. 2004;24(29):6466-75.

[15] Benedetti BL, Glazewski S, Barth AL. Reliable and precise neuronal firing during sensory plasticity in superficial layers of primary somatosensory cortex. J Neurosci. 2009;29(38):11817-27. Epub 2009/09/25.

[16] Yassin L, Benedetti BL, Jouhanneau JS, Wen JA, Poulet JF, Barth AL. An embedded subnetwork of highly active neurons in the neocortex. Neuron. 2010;68(6):1043-50. Epub 2010/12/22.

[17] Tsien RY. The green fluorescent protein. Annual review of biochemistry. 1998;67:509-44. Epub 1998/10/06.

[18] Feng G, Mellor RH, Bernstein M, Keller-Peck C, Nguyen QT, Wallace M, et al. Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. Neuron. 2000;28(1):41-51.

[19] Livet J, Weissman TA, Kang H, Draft RW, Lu J, Bennis RA, et al. Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. Nature. 2007;450(7166):56-62. Epub 2007/11/02.

[20] Lichtman JW, Livet J, Sanes JR. A technicolour approach to the connectome. Nat Rev Neurosci. 2008;9(6):417-22. Epub 2008/05/01.

[21] Andra K, Abramowski D, Duke M, Probst A, Wiederhold KH, Burki K, et al. Expression of APP in transgenic mice: a comparison of neuron-specific promoters. Neurobiology of aging. 1996;17(2):183-90. Epub 1996/03/01.

[22] Bockamp E, Sprengel R, Eshkind L, Lehmann T, Braun JM, Emmrich F, et al. Conditional transgenic mouse models: from the basics to genome-wide sets of knockouts and current studies of tissue regeneration. Regenerative medicine. 2008;3(2):217-35. Epub 2008/03/01.

[23] Reijmers LG, Perkins BL, Matsuo N, Mayford M. Localization of a stable neural correlate of associative memory. Science. 2007;317(5842):1230-3.

[24] Matsuo N, Reijmers L, Mayford M. Spine-type-specific recruitment of newly synthesized AMPA receptors with learning. Science. 2008;319(5866):1104-7.

[25] Han JH, Kushner SA, Yiu AP, Cole CJ, Matynia A, Brown RA, et al. Neuronal competition and selection during memory formation. Science. 2007;316(5823):457-60.

[26] Han JH, Kushner SA, Yiu AP, Hsiang HL, Buch T, Waisman A, et al. Selective erasure of a fear memory. Science. 2009;323(5920):1492-6.

[27] Han JH, Yiu AP, Cole CJ, Hsiang HL, Neve RL, Josselyn SA. Increasing CREB in the auditory thalamus enhances memory and generalization of auditory conditioned fear. Learn Mem. 2008;15(6):443-53.

[28] Maletic-Savatic M, Malinow R, Svoboda K. Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. Science. 1999;283(5409):1923-7. Epub 1999/03/19.

[29] Lang C, Barco A, Zablow L, Kandel ER, Siegelbaum SA, Zakharenko SS. Transient expansion of synaptically connected dendritic spines upon induction of hippocampal long-term potentiation. Proc Natl Acad Sci U S A. 2004;101(47):16665-70. Epub 2004/11/16.

[30] Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H. Structural basis of long-term potentiation in single dendritic spines. Nature. 2004;429(6993):761-6. Epub 2004/06/11.

[31] Harvey CD, Svoboda K. Locally dynamic synaptic learning rules in pyramidal neuron dendrites. Nature. 2007;450(7173):1195-200. Epub 2007/12/22.

[32] Trachtenberg JT, Chen BE, Knott GW, Feng G, Sanes JR, Welker E, et al. Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. Nature. 2002;420(6917):788-94. Epub 2002/12/20.

[33] Grutzendler J, Kasthuri N, Gan WB. Long-term dendritic spine stability in the adult cortex. Nature. 2002;420(6917):812-6.

[34] Majewska AK, Newton JR, Sur M. Remodeling of synaptic structure in sensory cortical areas in vivo. J Neurosci. 2006;26(11):3021-9. Epub 2006/03/17.

[35] Chen JL, Villa KL, Cha JW, So PT, Kubota Y, Nedivi E. Clustered dynamics of inhibitory synapses and dendritic spines in the adult neocortex. Neuron. 2012;74(2):361-73. Epub 2012/05/01.

[36] Lee WC, Huang H, Feng G, Sanes JR, Brown EN, So PT, et al. Dynamic remodeling of dendritic arbors in GABAergic interneurons of adult visual cortex. PLoS biology. 2006;4(2):e29. Epub 2005/12/22.

[37] Chen JL, Lin WC, Cha JW, So PT, Kubota Y, Nedivi E. Structural basis for the role of inhibition in facilitating adult brain plasticity. Nat Neurosci. 2011;14(5):587-94. Epub 2011/04/12.

[38] Chen JL, Flanders GH, Lee WC, Lin WC, Nedivi E. Inhibitory dendrite dynamics as a general feature of the adult cortical microcircuit. J Neurosci. 2011;31(35):12437-43. Epub 2011/09/02.

[39] Xu T, Yu X, Perlik AJ, Tobin WF, Zweig JA, Tennant K, et al. Rapid formation and selective stabilization of synapses for enduring motor memories. Nature. 2009;462(7275):915-9. Epub 2009/12/01.

[40] Yang G, Pan F, Gan WB. Stably maintained dendritic spines are associated with lifelong memories. Nature. 2009;462(7275):920-4. Epub 2009/12/01.

[41] Fu M, Yu X, Lu J, Zuo Y. Repetitive motor learning induces coordinated formation of clustered dendritic spines in vivo. Nature. 2012;483(7387):92-5. Epub 2012/02/22.

[42] Lai CS, Franke TF, Gan WB. Opposite effects of fear conditioning and extinction on dendritic spine remodelling. Nature. 2012;483(7387):87-91. Epub 2012/02/22.

[43] Tian L, Akerboom J, Schreiter ER, Looger LL. Neural activity imaging with genetically encoded calcium indicators. Prog Brain Res. 2012;196:79-94. Epub 2012/02/22.

[44] Tian L, Hires SA, Looger LL. Imaging neuronal activity with genetically encoded calcium indicators. Cold Spring Harbor protocols. 2012;2012 Jun(6):647-56. Epub 2012/06/05.

[45] Miyawaki A, Llopis J, Heim R, McCaffery JM, Adams JA, Ikura M, et al. Fluorescent indicators for Ca2+ based on green fluorescent proteins and calmodulin. Nature. 1997;388(6645):882-7. Epub 1997/08/28.

[46] Nakai J, Ohkura M, Imoto K. A high signal-to-noise Ca(2+) probe composed of a single green fluorescent protein. Nature biotechnology. 2001;19(2):137-41. Epub 2001/02/15.

[47] Tian L, Hires SA, Mao T, Huber D, Chiappe ME, Chalasani SH, et al. Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. Nature methods. 2009;6(12):875-81. Epub 2009/11/10.

[48] Mank M, Santos AF, Direnberger S, Mrsic-Flogel TD, Hofer SB, Stein V, et al. A genetically encoded calcium indicator for chronic in vivo two-photon imaging. Nature methods. 2008;5(9):805-11. Epub 2009/01/23.

[49] Nicoll RA, Malenka RC. Expression mechanisms underlying NMDA receptor-dependent long-term potentiation. Ann N Y Acad Sci. 1999;868:515-25.

[50] Dunning J, During MJ. Molecular mechanisms of learning and memory. Expert reviews in molecular medicine. 2003;5(25):1-11. Epub 2004/02/28.

[51] Johansen JP, Cain CK, Ostroff LE, LeDoux JE. Molecular mechanisms of fear learning and memory. Cell. 2011;147(3):509-24. Epub 2011/11/01.

[52] Seeburg PH, Burnashev N, Kohr G, Kuner T, Sprengel R, Monyer H. The NMDA receptor channel: molecular design of a coincidence detector. Recent progress in hormone research. 1995;50:19-34. Epub 1995/01/01.

[53] Soderling TR. Calcium-dependent protein kinases in learning and memory. Advances in second messenger and phosphoprotein research. 1995;30:175-89. Epub 1995/01/01.

[54] Andermann ML, Kerlin AM, Reid RC. Chronic cellular imaging of mouse visual cortex during operant behavior and passive viewing. Frontiers in cellular neuroscience. 2010;4:3. Epub 2010/04/22.

[55] Dombeck DA, Harvey CD, Tian L, Looger LL, Tank DW. Functional imaging of hippocampal place cells at cellular resolution during virtual navigation. Nat Neurosci. 2010;13(11):1433-40. Epub 2010/10/05.

[56] Mower AF, Kwok S, Yu H, Majewska AK, Okamoto K, Hayashi Y, et al. Experience-dependent regulation of CaMKII activity within single visual cortex synapses in vivo. Proc Natl Acad Sci U S A. 2011;108(52):21241-6. Epub 2011/12/14.

[57] Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K. Millisecond-timescale, genetically targeted optical control of neural activity. Nat Neurosci. 2005;8(9):1263-8. Epub 2005/08/24.

[58] Tye KM, Deisseroth K. Optogenetic investigation of neural circuits underlying brain disease in animal models. Nat Rev Neurosci. 2012;13(4):251-66. Epub 2012/03/21.

[59] Fenno L, Yizhar O, Deisseroth K. The development and application of optogenetics. Annu Rev Neurosci. 2011;34:389-412. Epub 2011/06/23.

[60] Keene AC, Masek P. Optogenetic induction of aversive taste memory. Neuroscience. 2012;222:173-80. Epub 2012/07/24.

[61] Wise RA. Dopamine, learning and motivation. Nat Rev Neurosci. 2004;5(6):483-94. Epub 2004/05/21.

[62] Everitt BJ, Robbins TW. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci. 2005;8(11):1481-9. Epub 2005/10/28.

[63] Tsai HC, Zhang F, Adamantidis A, Stuber GD, Bonci A, de Lecea L, et al. Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. Science. 2009;324(5930):1080-4. Epub 2009/04/25.

[64] Adamantidis AR, Tsai HC, Boutrel B, Zhang F, Stuber GD, Budygin EA, et al. Optogenetic interrogation of dopaminergic modulation of the multiple phases of reward-seeking behavior. J Neurosci. 2011;31(30):10829-35. Epub 2011/07/29.

[65] Kim KM, Baratta MV, Yang A, Lee D, Boyden ES, Fiorillo CD. Optogenetic mimicry of the transient activation of dopamine neurons by natural reward is sufficient for operant reinforcement. PLoS One. 2012;7(4):e33612. Epub 2012/04/17.

[66] van Zessen R, Phillips JL, Budygin EA, Stuber GD. Activation of VTA GABA neurons disrupts reward consumption. Neuron. 2012;73(6):1184-94. Epub 2012/03/27.

[67] Stuber GD, Sparta DR, Stamatakis AM, van Leeuwen WA, Hardjoprajitno JE, Cho S, et al. Excitatory transmission from the amygdala to nucleus accumbens facilitates reward seeking. Nature. 2011;475(7356):377-80. Epub 2011/07/01.

[68] Kravitz AV, Tye LD, Kreitzer AC. Distinct roles for direct and indirect pathway striatal neurons in reinforcement. Nat Neurosci. 2012;15(6):816-8. Epub 2012/05/01.

[69] Johansen JP, Hamanaka H, Monfils MH, Behnia R, Deisseroth K, Blair HT, et al. Optical activation of lateral amygdala pyramidal cells instructs associative fear learning. Proc Natl Acad Sci U S A. 2010;107(28):12692-7. Epub 2010/07/10.

[70] Ciocchi S, Herry C, Grenier F, Wolff SB, Letzkus JJ, Vlachos I, et al. Encoding of conditioned fear in central amygdala inhibitory circuits. Nature. 2010;468(7321):277-82. Epub 2010/11/12.

[71] Liu X, Ramirez S, Pang PT, Puryear CB, Govindarajan A, Deisseroth K, et al. Optogenetic stimulation of a hippocampal engram activates fear memory recall. Nature. 2012;484(7394):381-5. Epub 2012/03/24.

[72] Garner AR, Rowland DC, Hwang SY, Baumgaertel K, Roth BL, Kentros C, et al. Generation of a synthetic memory trace. Science. 2012;335(6075):1513-6. Epub 2012/03/24.

[73] Milner B, Squire LR, Kandel ER. Cognitive neuroscience and the study of memory. Neuron. 1998;20(3):445-68.

[74] Goshen I, Brodsky M, Prakash R, Wallace J, Gradinaru V, Ramakrishnan C, et al. Dynamics of retrieval strategies for remote memories. Cell. 2011;147(3):678-89. Epub 2011/10/25.

[75] Letzkus JJ, Wolff SB, Meyer EM, Tovote P, Courtin J, Herry C, et al. A disinhibitory microcircuit for associative fear learning in the auditory cortex. Nature. 2011;480(7377):331-5. Epub 2011/12/14.

[76] Andrews-Zwilling Y, Gillespie AK, Kravitz AV, Nelson AB, Devidze N, Lo I, et al. Hilar GABAergic interneuron activity controls spatial learning and memory retrieval. PLoS One. 2012;7(7):e40555. Epub 2012/07/14.

[77] Rolls A, Colas D, Adamantidis A, Carter M, Lanre-Amos T, Heller HC, et al. Optogenetic disruption of sleep continuity impairs memory consolidation. Proc Natl Acad Sci U S A. 2011;108(32):13305-10. Epub 2011/07/27.

IntechOpen