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Mini-review

Optogenetics to study the circuits of fear- and depression-like behaviors: A critical analysis

Catherine Belzung [a,*], Marc Turiault [b], Guy Griebel [c]

[a] INSERM 930, Université François Rabelais, UFR Sciences, Parc Grandmont, 37200 Tours, France
[b] Le Temps, Geneva, Switzerland
[c] Sanofi R&D, 1 avenue Pierre Brossolette, 91385 Chilly-Mazarin, France

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ABSTRACT

In recent years, the development and extensive use of optogenetics resulted in impressive findings on the neurobiology of anxiety and depression in animals. Indeed, it permitted to depict precisely the role of specific cell populations in various brain areas, including the amygdala nuclei, the auditory cortex, the anterior cingulate, the hypothalamus, the hippocampus and the bed nucleus of stria terminalis in specific aspects of fear and anxiety behaviors. Moreover, these findings emphasized the involvement of projections from the ventral tegmental area to the nucleus accumbens and the medial prefrontal cortex in eliciting depressive-like behaviors in stress-resilient mice or in inhibiting the expression of such behaviors in stress-vulnerable mice. Here we describe the optogenetic toolbox, including recent developments, and then review how the use of this technique contributed to dissect further the circuit underlying anxiety- and depression-like behaviors. We then point to some drawbacks of the current studies, particularly a) the sharp contrast between the sophistication of the optogenetic tools and the rudimentary aspect of the behavioral assays used, b) the fact that the studies were generally undertaken using normal rodents, that is animals that have not been subjected to experimental manipulations shifting them to a state relevant for pathologies and c) that the opportunity to explore the potential of these techniques to develop innovative therapeutics has been fully ignored yet. Finally, we discuss the point that these findings frequently ignore the complexity of the circuitry, as they focus only on a particular subpart of it. We conclude that users of this cutting edge technology could benefit from dialog between behavioral neuroscientists, psychiatrists and pharmacologists to further improve the impact of the findings.

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* Corresponding author.
E-mail address: catherine.belzung@univ-tours.fr (C. Belzung).

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1. Introduction

Much has been said about the disabling effects and the high personal and societal costs of disorders, such as anxiety and mood disorders (for reviews, see Kessler, 2007; Kessler et al., 2011; Ormel et al., 2008; Wittchen et al., 2011). These conditions are by far the most frequently diagnosed psychiatric diseases in Western countries. Although a variety of treatment options are available, mainly pharmacological, there is still a huge ongoing research effort for novel therapies for these disorders (Griebel and Holmes, 2013). This effort is driven by the growing medical need to improve on the effectiveness and the side effects of current treatment options. For example, a significant proportion of patients suffering from anxiety and/or depressive disorders do not achieve sustained symptomatic remission. Current anxiolytics and antidepressants have limited therapeutic efficacy and it has been conservatively estimated that 10 to 30% of patients are treatment-resistant despite multiple treatment attempts (Nierenberg and Amsterdam, 1990; Rush et al., 2006; Trivedi et al., 2006).

An issue that often confounds the discovery of new treatments for pathological anxiety and depression is our limited understanding of the underlying pathogenic mechanisms. The complexity and diversity of anxiety and depressive symptoms along with the heterogeneity of brain regions suggested to play a role in these behaviors have represented major hurdles in the understanding of neural circuits mediating anxiety and mood disorders. Moreover, the lack of adequate tools capable of manipulating distinct populations of neurons with fine spatial and temporal precision in vivo has been a serious drawback in the identification of the precise nature of the dysregulation in the neural circuits in anxiety and depressive disorders (Carter and De Lecea, 2011).

The recent and rapid development of optogenetic tools has provided new opportunities to probe the role of neural circuits mediating anxiety and mood disorders by allowing scientists to manipulate selected neuronal populations (Boyden, 2011; Deisseroth, 2012). Optogenetics combine tissue- and cell type-specific expression of light-sensitive microbial proteins called opsins along with advanced optical methods to reach, and control the activity of specific populations of cell in vitro and in living animals with high temporal precision and rapid reversibility. Since the pioneering work of Nagel et al. (2003) who reported the discovery of channelrhodopsin-2 (ChR2), a light-gated cation channel that could be used to depolarize cultured mammalian cells in responses to light, and the first demonstration that the introduction of the ChR2 gene into mammalian neurons resulted in a sustained reliable control of neural firing (Boyden et al., 2005), the use of opsins spread rapidly throughout neuroscience (Bernstein and Boyden, 2011; Carter and De Lecea, 2011; Lammel et al., 2014; Rein and Deussing, 2011; Tye and Deisseroth, 2012; Yizhar et al., 2011a). Much of this research effort focused on our understanding of the neural circuits governing the pathophysiology of anxiety and depression.

This review highlights the use of optogenetic probes in the investigation of the neural substrates of anxiety and depression. Prior to that, in the first part of the paper, we briefly trace back the conceptual roots and history of the field and highlight recent development of the optogenetic toolbox.

2. A method to establish causal relationships

Before the recent emergence of optogenetics, determining the function of a neuronal type was possible through correlations of information from electrical stimulations, pharmacology, electrophysiology or molecular genetics. However, these tools have serious limitations. For example, electrical stimulations lack specificity as they simultaneously alter surrounding cells and passing axonal fibers. Moreover, the kinetics and reversibility of pharmacology and gene inactivation experiments are slow, while the integration of sensory cues is mediated through electrical and synaptic signals within a millisecond timescale. Optogenetics offer fast light-controlled tools that are able to manipulate the network through reversible neuronal excitations and inhibitions, at key moments during behavior with a millisecond precision triggering action potentials or hyperpolarization (Stamatakis and Stuber, 2012; Tan et al., 2012), providing new means for establishing causal relationships between neural activity and complex behaviors. This dissection of neuronal network is critical to construct models of psychiatric diseases through loss- and gain-of-function experiments especially by combining pharmacological treatment with optogenetics in vivo and in vitro. At present, the optogenetic toolbox contains light-sensitive proteins able to control electrical and biochemical signaling.

3. Overview of strategies

Despite the sustained development of new optogenetic tools, ChR2 is still the most commonly used opsin because it offers large and reliable currents across a wide range of frequencies. Its ease of use has been confirmed by many different labs. It is often expressed through the adeno-associated virus (AAV) DNA, maintained extra-chromosomally, or through the lentivirus vector DNA, integrated into the genome of target cells. The benefits of using viruses are a stable long-term expression and strong promoters inserted in front of the opsin gene to provide a high level of opsin expression (Davidson and Breakefield, 2003) without cell specificity. This technique is of interest when the goal is to identify the function of projections from brain nuclei to another. The virus is then injected at the level of the cell bodies, while the optical fiber is inserted in the nuclei targeted by the axons in order to stimulate the terminals. This method can also be used in vivo or in vitro to act upon synaptic plasticity by applying long term potentiation or depression protocols through aspecific light frequency stimulation (Pascoli et al., 2012).

By fusing opsin genes to a cell-type specific promoter it is possible to achieve protein expression in a specific cell population but viruses have a limited packaging DNA size: AAV can be filled with transgene constructs up to 5 kb and lentiviruses are limited to 10 kb. It is far too limited to encompass entirely most promoters and regulatory elements and often preclude the cellular specificity. In addition, most cell-type specific promoters do not drive strong expression of the downstream gene. To circumvent the packaging limit of viruses and the weak expression of specific promoters (many of the opsins have low conductance or pumping properties) (Yizhar et al., 2011a), transgenic animal lines are used as a powerful complementary tool. Cell type-specificity is given by Cre recombinase expression, while spatial accuracy and strong expression is achieved with stereotaxic injection of a virus containing a conditional allele (i.e. flanked by two loxP sequences) of an opsin gene behind a strong promoter. In the transgenic animals, the Cre recombinase is inserted in bacterial artificial chromosomes (BAC) encompassing the complete sequence of the regulatory elements of a gene of interest and injected into the zygote (Turiault et al., 2007). Numerous Cre mouse and a few Cre rat lines are available from the Jackson Laboratory, Allen Brain Institute for Brain Science or GENSAT, with a specific expression of the recombinase in many specific neuronal population. Once the viral DNA is transfected into each cell of a brain nuclei, the Cre produced in specific cells will recombine the double floxed opsin construct and enable opsin gene transcription (Fig. 1) (Tsai et al., 2009). The strong ubiquitous promoter driving the opsin expression provides enough opsin protein to achieve reliable neural activation or silencing. It is of interest to notice that some cell specificity can be reached by choosing a specific virus serotype with the genetic material of one virus and the capsid of another, but it requires a robust characterization to avoid any undesired cell transfection (Wang et al., 2011).

Another possibility to get a cell specific opsin expression is the use of knock-in transgenic animals, where the opsin gene is integrated into the host's germ line. It has the advantage of avoiding variations in the expression level across animals and guarantees greater comparability between experiments in different brain structures. In this case spatial

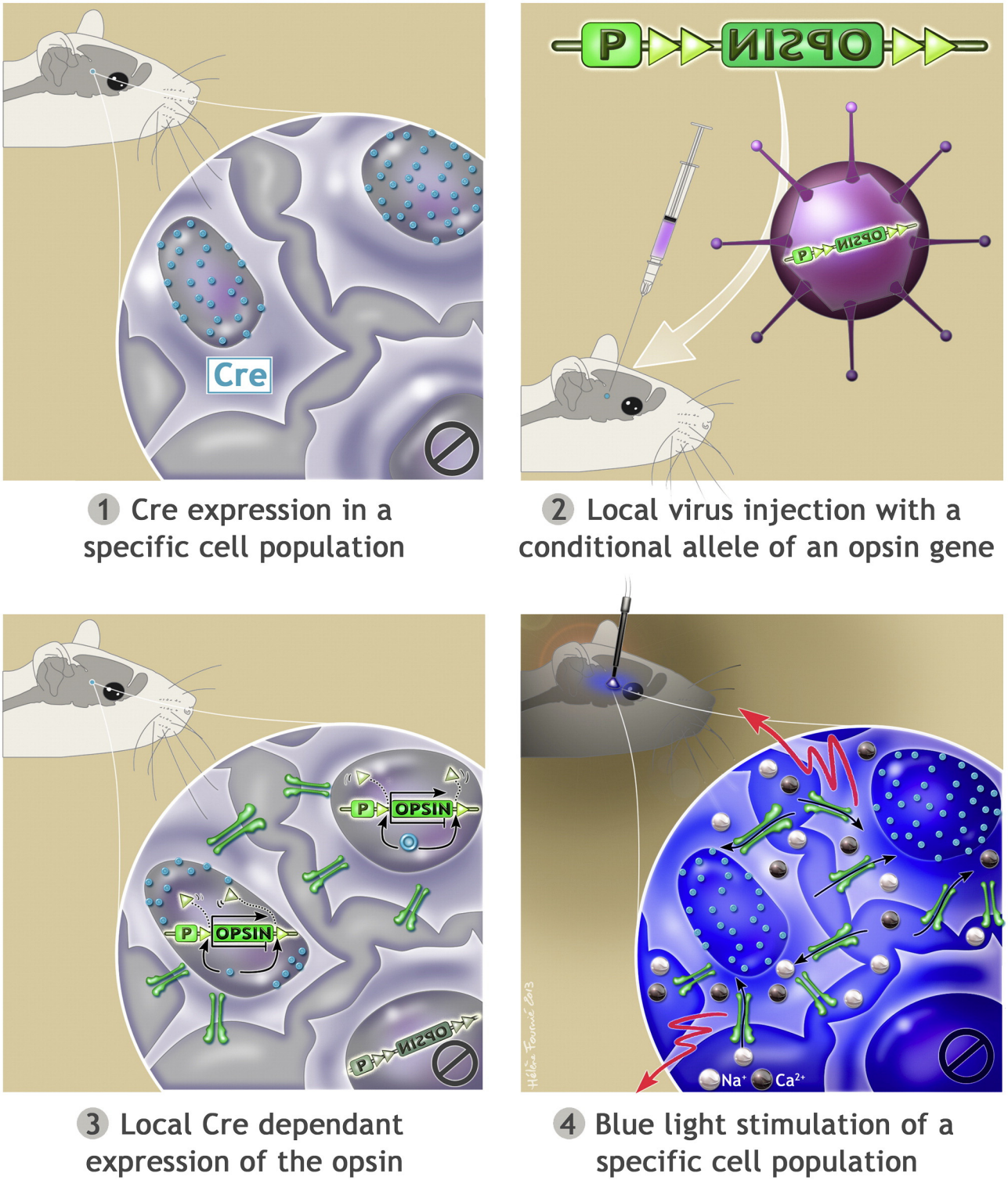


Fig. 1. Method for a cell specific strong opsin expression in a targeted brain nucleus. 1. Rodent transgenic line expressing the Cre recombinase under a specific cell type promoter. 2. The adeno-associated virus or the lentivirus vector is filled with a conditional allele of an opsin gene and stereotaxically injected into the brain nuclei of interest of the transgenic animal. 3. The viral DNA infects each cell of the brain structure with an inactive opsin gene. In the Cre expressing cells the recombination of the viral DNA lead to the reversal of the opsin gene and set the open reading frame behind the strong promoter. The opsin gene is strongly expressed and efficient trafficking results in a high protein membrane insertion throughout the soma and axon terminals. 4. An optical fiber is inserted above the opsin expressing nuclei to allow an in vivo illumination of the targeted cells. Light induce a conformational change of the opsin, open its ion channel and induce a cell specific activation (ChR2) or inhibition (NpHR3.0/Arch).

specificity can be reached by the local injection of a Cre recombinase expressing virus, while the opsin gene integrated into the host genome is flanked by two lox P sites. Stable integrated transgenes are more suitable for comparisons between different developmental times.

4. Circuit-specific targeting

It is widely documented that cell populations identified by a common molecular signature, such as dopamine neurons are with dopamine

transporter expression, can still be classified into different subtypes. For instance, dopamine transporter-positive dopamine neurons from the ventral tegmental area (VTA) project to several downstream brain structures. Dopamine neurons projecting to the dorsal striatum play a major role in locomotor function, while dopamine neurons projecting to the nucleus accumbens (NAcc) are involved in reward conditioning. In order to assess the function of specific cell population in a projection specific manner, the optogenetic toolbox contains trans-synaptic proteins and viral vectors with unique anterograde- or retrograde-transporting properties (Maskos et al., 2002; Wickersham et al., 2007). If the trans-synaptic tracer WGA–Cre, a fusion protein of Cre and wheat germ agglutinin (WGA) is inserted into a virus and injected in a region targeted by dopamine neurons such as the NAcc, it will be transferred into the pre-synaptic neurons, and be retrogradely transported into the cell body of dopamine neurons. If another virus containing a conditional allele of ChR2 is injected in the VTA, the WGA–Cre would recombine the lox-P sites and induce the expression of ChR2. In this case illumination of the VTA with blue light would stimulate specifically dopamine neurons projecting to the NAcc. In the same way, retrograde- and anterograde-transporting viral vectors (Beier et al., 2011; Wall et al., 2010) like the herpes simplex virus 1 (HSV-1) or the rabies virus (RV) can also be used to deliver recombinase in a projection specific manner.

5. Opsins

The number of new light sensitive molecules with improved features continues to grow, as do the methods for transgene delivery. The diversity in the opsins spectral properties makes them useful to selectively inhibit or excite neurons. After ChR2, the chloride-pumping halorhodopsin from *Natronomonas pharaonis* (NpHR3.0) has been shown to hyperpolarize neurons with a yellow light (Zhang et al., 2007). Because ChR2 and NpHR, response to equivalent light power and have almost separated action spectra (~470 nm for ChR2, ~590 nm for NpHR3.0), it should be possible to express both opsins simultaneously in the same cells, thus enabling a bidirectional control of neurons activity (Zhang et al., 2008), taking into consideration that a residual undesired opsin activation might occur.

In order to alternatively activate two different cell populations, the same type of combination could be used with ChR2 and channelrhodopsin-1 from *Volvox carteri* (VChR1) that is still responding at 589 nm (Zhang et al., 2008), a wavelength where ChR2 should be no longer responsive. Other inhibitory opsins can also drive large inhibitory currents, including archaerhodopsin-3 (Arch), a green light (~550 nm) activated proton pump isolated from an archaebacteria (Chow et al., 2010; Idnurm and Howlett, 2001) and bacteriorhopsin (BR) with maximum currents at ~560 nm.

Using mutagenesis of the ChR2 gene, the spectral properties, conductance and kinetics have been modified. The mutant ChETAs has shorter steady state photocurrent and responds efficiently to very high frequency of light stimulation. Such accelerated channel closure kinetics allows long term potentiation protocols since it can be stimulated at up to 200 Hz, meaning recovery from desensitized state is not limiting. A limitation is that faster deactivation goes together with reduced light sensitivity.

The mutants ChR2 (C128A) and ChR2 (C128S) are useful when photocurrents are needed for a long period of time. These "step function opsins" dramatically increase the time constant for channel closing. They respond to a brief pulse of blue light (470 nm) by a stable current influx that can last up to 30 min and can be terminated by a pulse of green light (542 nm) (Berndt et al., 2009). Theses mutants should be of great interest for a physiological intervention since they can allow a depolarization state that could intensify the spontaneous activity by an increase of release probability without imposing an artificial pattern.

The optogenetic toolbox also contains recombinant or chimeric photo-inducible G protein-coupled receptors (GPCR), allowing in vivo and in vitro optical modulation of metabotropic receptors signaling pathways (Airan et al., 2009). These opsins, called OptoXRs, provide temporal and cellular accuracy that were not possible to achieve with pharmacological and genetic tools and make them suitable for controlling behavior in behaving animals.

6. Limitations

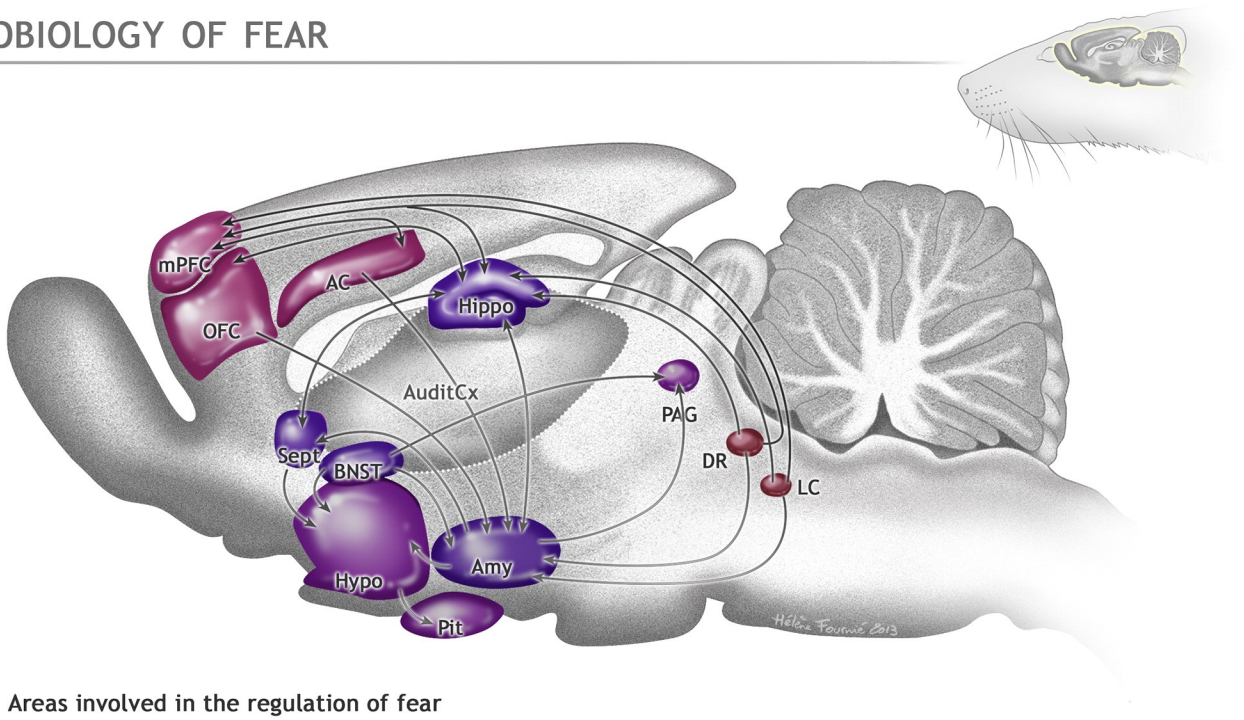
Despite the fact that optogenetics have already reshaped neuroscience, there are inherent artifacts to this new technique that may impact the interpretation of the results and should be considered with care. A potential caveat for optical stimulation of both ChR2 family and hyperpolarizing opsin, is their respective permeability for $Ca2+$ or $Cl-/H+$ that could modify the neurotransmitter release probability. For example, $Cl-$ intracellular influx through NpHR3.0 alters the GABAA receptor reversal potential and subsequently the post-inhibition reactivity to spontaneous GABA release (Raimondo et al., 2012). Therefore, reversibility might not be complete immediately after turning off the light. Another caveat to consider concerns projection targeting techniques because opsin-expressing axons that pass through the illuminated targeted structure but end somewhere else may also be stimulated. Trans-synaptic viruses used to express opsins in anatomically and genetically controlled cell populations will likely circumvent this issue. But it is not yet possible to avoid light-induced depolarization that may backpropagate from the illuminated brain structure, along the axons, to the cell body thus inducing numerous indirect side effects such as somato-dendritic release that could locally modulate the activity of other neurons.

Results from optogenetic studies provide information about the targeted cell type function but not directly about the neurotransmitter released. For instance some dopamine neurons also release glutamate, precluding specific dopamine effects interpretation. Furthermore, electrical activity is not always correlated with neurotransmitter release (Garris et al., 1999; Gonon, 1988) meaning that techniques like voltammetry or amperometry should be used to measure neurotransmitter release. Voltammetry can be employed to directly measure changes in DA release caused by optical manipulations.

Optically stimulating a large structure like the striatum may be difficult particularly in primates. First, because optical fibers delivering light can effectively illuminate a volume of less than 1 mm^3 due to scattering and absorption of photons in tissue. Solutions have been described such as using fibers with wide numerical apertures (Yizhar et al., 2011a), or using multiple fibers (Bernstein and Boyden, 2011) may mitigate this problem in some cases. Second, because the restricted area of viral infection may not be sufficient when targeting extended structures and thus need multiple injections. On the opposite, an additional caveat is the spatial targeting of viral transfection by stereotaxic injection in small brain nuclei. It always needs post-hoc confirmation, particularly when targeting small brain nuclei.

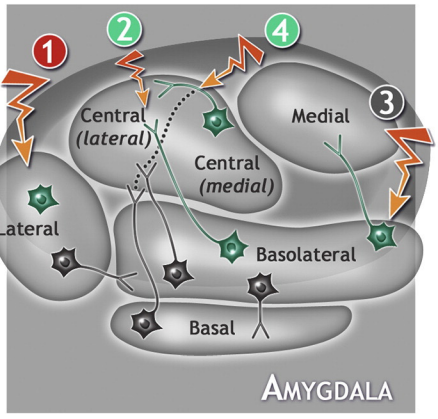
Another concern is about the frequency of the stimulation which is in some cases not compatible with the physiological conditions so that the relevance of the findings can be questioned. Indeed, when studying the neurobiology of fear or depression, not only the cell type but also the pattern of stimulation of the cells matters. For example, in their paper, Chaudhury et al. (2013) used optogenetic stimulation of the ventral tegmental area (VTA) to induce depressive-like behaviors: while a tonic 0.5 Hz stimulation did not elicit any behavioral change, the phasic 20 Hz stimulation induces the susceptible phenotype. Further, in their paper, Tye et al. (2013) used a 30 Hz stimulation of the same cells to elicit antidepressant-like effects. This has to be compared to the firing rate of VTA dopaminergic neurons observed earlier on in the literature. For example Krishnan et al. (2007) showed that after 25 days of social defeat, the firing rate of these cells was 2 Hz in unstressed controls, 3 Hz in resilient mice and 2.3 Hz in susceptible mice, indicating that the frequency of the VTA stimulation used in optogenetic experiments was not within physiological range.

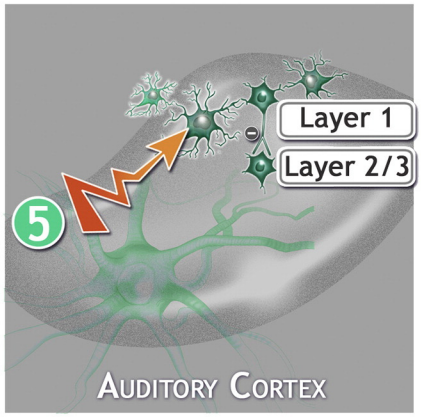
Neurobiology of fear

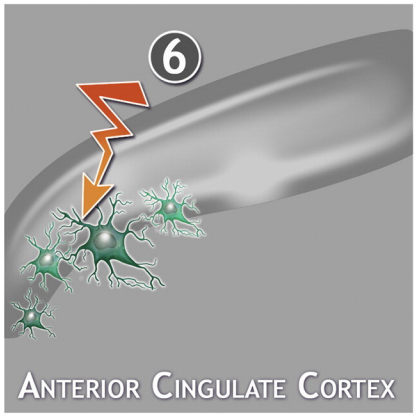


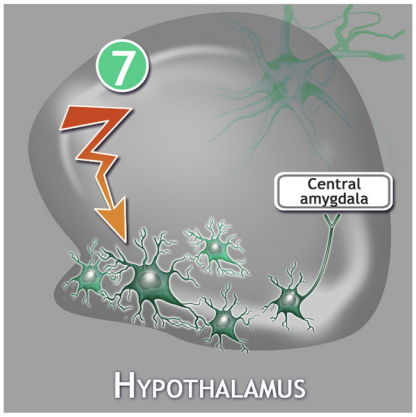
- Areas involved in the regulation of fear
- Areas involved in the generation of fear
- Areas involved in the expression of aspects of the fear response
- Monoaminergic nuclei

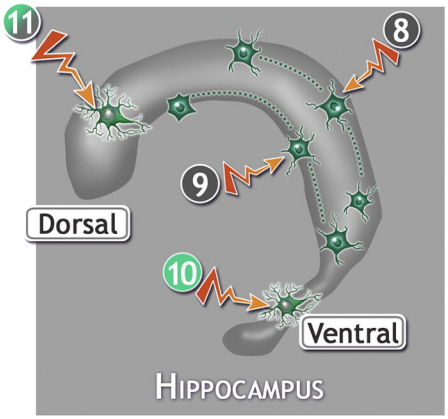
- Induction of anxiogenesis
- Induction of anxiolysis
- Other findings

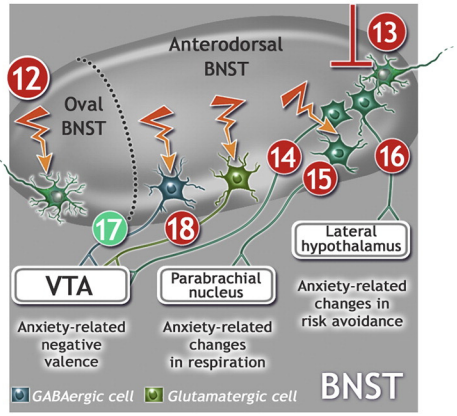












Finally, the promoter strength should be adapted to the experiment since a long term high level of ChR2 expression can induce abnormal axonal morphology and may impair cellular function (Miyashita et al., 2013). These caveats are linked to the basic use of ChR2 and NpHR3.0 and need further investigation since most of these unwanted effects are not yet well characterized. For trans-synaptic techniques or bidirectional control (opsins with separated action spectra), since they have not yet been largely diffused and published, their limitations are quite unknown.

6.1. Tools for anxiety and depression study

The elementary model of the brain structures implied in anxiety and depression were established using local lesions, pharmacological treatment, and electrophysiological studies (Ehrlich et al., 2009; LeDoux, 2000). However, the technical limitations of these tools prevented the establishment of a causal link between behavior and specific circuit elements. Recently, optogenetics has begun to fill this gap, mostly through the conditional expression of ChR2 and NpHR3.0 with an illumination of the cell body or the projections areas. As it will be detailed below, these experiments have increased our understanding of the functional link between anxiety-related behaviors and brain nuclei such as intra-amygdala microcircuitry or its connections with distal regions (Nieh et al., 2013). Interestingly fast light control tools has achieved surprising results by showing, for instance, the fundamental role of dopamine neurons in depression and fear related behaviors (Chaudhury et al., 2013; Tan et al., 2012) or the rewarding properties of the glutamatergic projection from Basolateral Amygdala (BLA) to the NAcc (Stuber et al., 2011). Other opsins have been occasionally used such as the step function opsin (Yizhar et al., 2011b). In this study, applying a pulse of light induced a long depolarization of mPFC pyramidal neurons and increased the spontaneous activity. By changing the excitation/inhibition balance, they were able to confirm the importance of mPFC in fear learning.

7. Summary of the results from optogenetic experiments, which aimed at investigating the neurobiology of fear and depression

7.1. Neurobiology of fear and/or anxiety

In the last decades, the neural mechanisms that subserve fear and/or anxiety have been characterized in a large number of experimental studies both in humans, mainly using functional neuroimaging, and in animals, using immunohistochemistry and focal brain lesions. These findings led to the description of a largely conserved circuit, including highly interconnected limbic cortical and subcortical areas. Fig. 2 shows a simplified view of this network highlighting a hierarchically-organized system with brain areas involved in the expression of the fear/anxiety response that are controlled by structures generating and coordinating these responses. These latter are innervated by areas involved in the regulation of emotions. More precisely, a pivotal role is exerted by brain areas involved in the generation of the fear and/or anxiogenic experience, such as the amygdala (Amano et al., 2011; Davis, 1992; Duvarci et al., 2011; Ehrlich et al., 2009; Herry et al., 2008; Killcross et al., 1997; LaBar et al., 1998; Pape and Pare, 2010), the hippocampus (mainly its ventral part) (Kjelstrup et al., 2002), the bed nucleus of stria terminalis (BNST) (Davis and Shi, 1999; Davis et al., 2010; Sink et al., 2011) and the septum (Sheehan et al., 2004). These brain areas control efferent structures (Veening et al., 1984), which translate the anxiogenic experience into its physiological (hypothalamus), hormonal (hypothalamus and pituitary) and behavioral correlates (periaqueductal gray (PAG)) (Davis, 1992), while being controlled by regions involved in the regulation of emotions such as the cingulate cortex and subparts of the frontal cortex (Sotres-Bayon and Quirk, 2010). Finally, this network is strongly innervated by monoaminergic fibers originating from mesencephalic nuclei such as the raphe nucleus (serotonin) (Azmitia and Segal, 1978) or the locus coeruleus (noradrenaline) (Sara, 2009).

Logically, the initial optogenetic experiments that aimed to investigate the cellular substrate of fear and/or anxiety, focused on subparts of this network (Fig. 2 and Table 1). As is evidenced in Table 1, these studies predominantly investigated the role of the amygdala, the hippocampus and the BNST (respectively 22%, 22% and 33% of the experiments). The remaining optogenetic studies investigated the contributions of the hypothalamus, the auditory cortex and the anterior cingulate cortex in these behaviors, again because they have been suggested in earlier studies to play a role in the regulation of fear/anxiety responses.

While the use of optogenetic tools in these studies did not enable the identification of brain areas previously not reported to play a role in anxiety, they nevertheless led to deeper understanding of the fear network, highlighting the neural substrates underlying the temporal dynamic of the generation of fear (mainly in the context of fear memory) and region-specific cell types. Since optogenetic stimulation can activate or inhibit neurons at a very precise moment, it is possible to distinguish more precisely the temporal windows during fear learning, thus segregating the involvement of different cells during acquisition, expression, long term storage and extinction of fear memories. Several of these studies showed that the acquisition of fear memory in mice relies upon interneurons within the auditory cortex (in case of fear conditioning to complex tones), along with dorsal, but not ventral, dentate gyrus granule cells, CA1 pyramidal neurons and several cell types within the amygdala (glutamatergic neurons of the basolateral nucleus, GABAergic neurons from the lateral and central amygdala). The expression of already stored fear memory was related in these experiments to hypothalamic oxytocin neurons projecting to the central amygdala, dentate granule neurons in the dorsal hippocampus and CA1 neurons, and GABAergic neurons in the central amygdala. However, glutamatergic cells in the basolateral amygdala were not involved. Finally, remote memory of conditioned fear was proposed to be under the control of glutamatergic neurons within the anterior cingulate, while extinction relied upon glutamatergic neurons in the basolateral amygdala.

Fig. 2. The neurobiology of fear and/or anxiety: recent advances thanks to the use of optogenetics. Upper panel: the brain circuit of fear and/or anxiety as characterized before the use optogenetic tools. mPFC: medial Prefrontal Cortex, OFC: Orbitofrontal Cortex, AC: Anterior Cingulate, Audit CX: Auditory Cortex, Hippo: Hippocampus, Sept: Septum, Amy: Amygdala, BNST: Bed Nucleus of Stria Terminalis, Hypo: Hypothalamus, Pit: Pituitary, PAG: Periaqueducal Gray, DR: Dorsal Raphe, LC: Locus Coeruleus. Lower panels: new findings obtained using optogenetics. 1. Optical stimulation of pyramidal neurons within the lateral amygdala elicits anxiogenic behavior. 2. The central amygdala can be subdivided into two functional subparts (lateral and medial). Stimulation of BLA terminals in the lateral subdivision of the CeA produces anxiolytic effects. 3. Stimulation of glutamatergic pyramidal neurons from basolateral amygdala inhibits the medial amygdala and impairs fear memory. 4. Stimulation of basolateral terminals in the central amygdala produces anxiolytic-like effects while inhibition elicits opposite effects. 5. Interneurons from Layer 1 of auditory cortex, controlled by acethylcholine, generate inhibition of layer 2/3 parvalbumin-positive interneurons thus reducing fear. 6. Inhibition of anterior cingulate inhibits remote but not recent contextual fear memory. 7. Stimulation of oxytocin neurons in the hypothalamus decreases conditioned fear. 8. Stimulation of dentate gyrus granule cells (ventral or dorsal extension not known) activated during fear learning induce freezing in the absence of shock in a different context. 9. Inhibition of glutamatergic CA1 neurons (ventral or dorsal extension not indicated) inhibition blocks contextual fear acquisition and retrieval. 10. Stimulation of granule cells in the ventral dentate gyrus produces anxiolytic-like effects, but does not affect fear learning. Inhibition has no effect. 11. Stimulation of granule cells in the dorsal dentate gyrus decreases conditioned fear, but does not affect spontaneous anxiety-related behaviors. Inhibition of these cells decreases encoding of contextual fear learning, without affecting anxiety-like behavior. 12. Oval BNST activity promotes anxiety. 13. Inhibition of anterodorsal BNST activity is anxiogenic. 14. Activation of the anterodorsal BNST-VTA projections produces anxiety related changes in valence. 15. Activation of the anterodorsal BNST-parabrachial projections produces anxiety-related changes in respiration. 16. Activation of the anterodorsal BNST-lateral hypothalamus projections produces anxiety-related risk avoidance. 17. Activation of BNST GABAergic projections elicits rewarding and anxiolytic phenotypes. 18. Photostimulation of BNST glutamatergic projections results in aversion and anxiogenic behavioral phenotypes. VTA: ventral tegmental area.

Table 1
Summary of findings using optogenetic tools to study the neurobiology of anxiety and/or fear.

Phenotype	Region	Cell type	Strain	Construct	Test	Finding	Reference
Spontaneous anxiety	Basolateral and central amygdala	Glutamatergic	C57BL/6 mice (4–6-week-old)	AAV5-CamKIIα-hChR2-eYFP	Elevated plus-maze, Open-field, Locomotion	Stimulation of BLA terminals in the CeA produced anxiolytic-like effects	Tye et al. (2011)
Spontaneous anxiety	Basolateral and central amygdala	Glutamatergic	C57BL/6 mice (4–6-week-old)	AAV5-CamKIIα-eNpHR3.0-eYFP	Elevated plus-maze, Open-field, Locomotion	Inhibition of BLA terminals in the CeA produced anxiogenic-like effects	Tye et al. (2011)
Learned fear	Basolateral amygdala	Glutamatergic	Thy1::ChR2 transgenic mice		Conditioned fear stress	Pyramidal glutamatergic cells from BLA inhibit cells located in medial amygdala impairing fear memory	Jasnow et al. (2013)
Learned fear	Central amygdala	GABAergic	C57BL/6 mice (2–3-month-old)	AAV2/7-Syn-ChR22A-tdimer	Fear conditioning	Subpopulations of CeA (CE lateral) are required for fear acquisition, Fear response are driven by output neurons from CeM to CeL	Ciocchi et al. (2010)
Learned fear	Lateral amygdala	GABAergic	Sprague–Dawley rats (275–300 g)	AAV-CamkIIα-ChR2-eYFP	Conditioned learning	The pairing of auditory sensory cue with optical stimulation of LA neurons produced anxiogenic-like behaviors	Johansen et al. (2010)
Learned fear	Auditory cortex	Cortical pyramidal	Parvalbumin (PV):: Cre C57BL/6 J mice (1.5–6-month-old)	AAV2/75-EF1α-DIO-ChR2	Conditioned learning	Stimulation of parvalbumin interneurons during and for after the shock reduced 24 h later fear responses to the conditioned stimulus, Cholinergic activation of Layer 1 interneurons generate inhibition of layer 2/3 parvalbumin interneurons	Letzkus et al. (2011)
Learned fear	Hypothalamus	Oxytocin	Female Wistar rats	AAV-OT-ChR2-eYFP	Conditioned learning	Oxytocin release from axon terminals from the hypothalamus within the central amygdala results in attenuation of conditioned fear	Knobloch et al. (2012)
Spontaneous anxiety	BNST	Somata	C57BL/6 mice	AAV-hSyn-eNpHR3.0-eYFP	Elevated plus-maze, open-field	Inhibition of anterodorsal BNST somata produced anxiolytic-like activity	Kim et al. (2013)
Spontaneous anxiety	BNST oval nucleus	Dopaminergic—D1	Drd1a::Cre mice	AAV-hSyn-eNpHR3.0-eYFP	Elevated plus-maze, open-field	Inhibition of BNST oval nucleus produced anxiogenic-like activity	Kim et al. (2013)
Spontaneous anxiety	BNST oval nucleus	Dopaminergic—D1	Drd1a::Cre mice	AAV-EF1α-DIO-ChR2(H134R)-eYFP	Elevated plus-maze, open-field	Activation of BNST oval nucleus produced anxiolytic-like activity	Kim et al. (2013)
Spontaneous anxiety	Basolateral amygdala	Glutamatergic	C57BL/6 mice	AAV-CamKIIα-eNpHR3.0-eYFP	Elevated plus-maze, open-field	Inhibition of the basolateral–anterodorsal BNST projections produced anxiolytic-like effects	Kim et al. (2013)
Spontaneous anxiety	Basolateral amygdala	Glutamatergic	C57BL/6 mice	AAV-CamKIIα-ChR2-eYFP	Elevated plus-maze, open-field	Activation of the basolateral–anterodorsal BNST projections produced anxiogenic-like effects	Kim et al. (2013)
Spontaneous anxiety	BNST anterodorsal	Glutamatergic	C57BL/6 mice	AAV-CamKIIα-ChR2-eYFP	Elevated plus-maze, open-field	Activation of the anterodorsal BNST–lateral hypothalamus projections produced anxiogenic-like effects	Kim et al. (2013)
Spontaneous anxiety	BNST anterodorsal	Glutamatergic	C57BL/6 mice	AAV-CamKIIα-ChR2-eYFP	Elevated plus-maze, open-field, place preference	Activation of the anterodorsal BNST-VTA projections produced place preference, without affecting anxiety-like behaviors	Kim et al. (2013)
Spontaneous anxiety	BNST, VTA	Glutamatergic	Vglut2::IRES-Cre mice	AAV-ChR2-eYFP	Open-field	Stimulation of the glutamatergic BNST-VTA pathway resulted in anxiogenic-like behaviors	Jennings et al. (2013)

Spontaneous anxiety	BNST, VTA	GABAergic	Vgat2::IRES-Cre mice	AAV-ChR2-eYFP	Elevated plus-maze	Stimulation of the GABAergic BNST-VTA pathway produced anxiolytic-like effects	Jennings et al. (2013)
Spontaneous anxiety	VTA	GABAergic	Vgat2::IRES-Cre mice	AAV-eNpHR3.0-eYFP	Elevated plus-maze	Stimulation of GABAergic neurons in the VTA produced anxiolytic-like effects	Jennings et al. (2013)
Spontaneous anxiety	BNST, VTA	GABAergic	Vgat2::IRES-Cre mice	AAV-ChR2-eYFP	Elevated plus-maze 3 h after foot-shock application	Stimulation of the GABAergic BNST-VTA pathway produced anxiolytic-like effects	Jennings et al. (2013)
Learned fear	Dentate gyrus	Granule cells	c-fos-tTA mice	AAV-TRE-ChR2-eYFP	Conditioned fear stress	Stimulation of cells activated during fear learning induced freezing in the absence of shock in a different context	Liu et al. (2012)
Spontaneous anxiety and Learned fear	Ventral dentate gyrus	Granule cells	ROSA26-CAG-stop^{flox}-ChR2(H134R)-tdTomato (Ai27) mice backcrossed to POMPC-BAC Cre mice		Active avoidance, conditioned fear, elevated plus-maze, open-field	Stimulation of granule cells in the ventral dentate gyrus produced anxiolytic-like effects, but did not affect fear learning	Kheirbek et al. (2013)
Spontaneous anxiety and Learned fear	Ventral dentate gyrus	Granule cells	ROSA26-CAG-stop^{flox}-eNpHR3.0-eYFP mice backcrossed to POMPC-BAC Cre mice		Active avoidance, conditioned fear, elevated plus-maze, open-field	Inhibition of granule cells in the ventral dentate gyrus had no effect	Kheirbek et al. (2013)
Spontaneous anxiety and Learned fear	Dorsal dentate gyrus	Granule cells	ROSA26-CAG-stop^{flox}-ChR2(H134R)-tdTomato (Ai27) mice backcrossed to POMPC-BAC Cre mice		Active avoidance, conditioned fear, elevated plus-maze, open-field	Stimulation of granule cells in the dorsal dentate gyrus decreased conditioned fear, but did not affect anxiety-related behaviors	Kheirbek et al. (2013)
Spontaneous anxiety and Learned fear	Dorsal dentate gyrus	Granule cells	ROSA26-CAG-stop^{flox}-eNpHR3.0-eYFP mice backcrossed to POMPC-BAC Cre mice		Active avoidance, conditioned fear, elevated plus-maze, open-field	Inhibition of granule cells in the dorsal dentate gyrus decreased the encoding contextual learning, without affecting anxiety-like responses	Kheirbek et al. (2013)
Learned fear	Hippocampus CA1	Glutamatergic	C57BL/6	LV-CamKIIα-eNpHR3.0-eYFP	Conditioned fear stress	CA1 inhibition blocks contextual fear acquisition and retrieval	Goshen et al. (2011)
Learned fear	Anterior cingulate cortex	Glutamatergic	C57BL/6	LV-CamKIIα-eNpHR3.0-eYFP	Conditioned fear stress	Inhibition of AC inhibits remote but not recent contextual memory	Goshen et al. (2011)

BLA: Basolateral Amygdala, CeA: Central Amygdala, CeM: Medial part of central Amygdala, CeL: Lateral part of central Amygdala, BNST: Bed Nucleus of Stria Terminalis, VTA: Ventral Tegmental Area, AC: Anterior Cingulate Cortex.

Similar to conditioned fear, the use of optogenetic tools enabled a more precise description of the microcircuits and cell types involved in unconditioned anxiety in mice. These studies highlighted notably the contribution of the amygdala in the expression of spontaneous anxiety by showing that the stimulation of basolateral amygdala terminals in the lateral part of the central amygdala produced anxiolytic-like effects, while their inhibition elicited opposite effects as observed in the elevated plus-maze, a prototypical model of unconditioned anxiety. In the same procedure, the optogenetic stimulation of the somata within the basolateral amygdala revealed to be without effect. Other studies focused on the hippocampus. Using again the elevated plus-maze, they found that stimulation of granule cells located in the dorsal part of the dentate gyrus of the hippocampus did not modify the behavior of mice, while stimulation of the same cells but located in the ventral part of the hippocampus caused anxiolytic-like effects.

Although the BNST has often been suggested to play a role in the modulation of unconditioned anxiety (Walker and Davis, 1997; Walker et al., 2003), its precise contribution within the anxiety circuit remained to be clarified. The use of optogenetic methods allowed a better understanding of the role of this structure in anxiety behavior. First, it was found that activation of the oval BNST promoted anxiety, while stimulation of the anterodorsal BNST elicited opposite effects. Second, going a step further, the authors of this study were able to demonstrate a dissociation of anxiety-related changes in valence, in respiration and in avoidance behavior, depending on the projection area from the BNST (respectively related to anterodorsal BNST-Ventral tegmental, anterodorsal BNST-parabrachial and anterodorsal BNST-lateral hypothalamus projections). Finally, these studies revealed in the BNST an unprecedented role of GABA and glutamate neurons in anxiety behaviors. They demonstrated that activation of BNST GABAergic projections elicited anxiolysis, while stimulation of BNST glutamatergic projections resulted in anxiogenesis. These new discoveries would not have been possible using other methods. At best the dissociation of the neural substrates involved in the different phases of the temporal dynamic underlying fear conditioning could be obtained using transient inactivation of these brain areas, for example via local administration of muscimol. However, it would not have permitted to study the cell types involved. Moreover, the use of double labeling immunohistochemistry would permit to characterize and correlate different neural types associated with fear and/or anxiety, but not to study causal participation of a particular type of cells.

7.2. Neurobiology of depression

Current knowledge on the brain network altered in major depression as demonstrated by studies in humans and in animals is depicted in Fig. 3. The circuit elements include areas involved in the regulation of emotions and executive functions (medial prefontal cortex and anterior cingulate cortex), areas involved in depression-related anxiety and increased salience for negative information (hippocampus, lateral habenula and amygdala), areas involved in depression-related anhedonia (VTA and nucleus accumbens), monoaminergic neurotransmission (serotoninergic, noradrenergic and dopaminergic) and depression-related suppression of positive affects (PAG) (for a review, see Tanti and Belzung, 2010; Willner et al., 2013). This brain circuitry has been mainly identified as a result of brain imaging studies while the involvement of monoaminergic neurotransmission is based on the observation that all current available treatment act either specifically to stimulate serotoninergic neurotransmission (selective serotonin reuptake inhibitors), or specifically on noradrenergic neurotransmission (selective noradrenalin reuptake inhibitors) or on both systems (tricyclics, dual noradrenaline and serotonine reuptake inhibitors) (see Willner et al., 2013) for a review.

Strangely enough, experiments that used optogenetic tools to dissect the mechanisms underlying depression (see Table 2) focused on the dopaminergic system, and more particularly on the dopaminergic cells within the VTA (probably because at a methodological level, it would be more complex to target serotoninergic and noradrenergic neurotransmission). Using chronic stress to elicit a depressive-like phenotype, Tye et al. (2013) showed that photostimulation of these cells reduced depressive-like behaviors in mice subjected to chronic stress, while their inhibition produced the opposite effect. In contrast, using social defeat, a more severe model of depression, Chaudhury et al. (2013) showed the opposite pattern: in their study, stimulation of the same VTA dopaminergic neurons elicited a depressive-like phenotype, as mice resilient to this stress became susceptible after the VTA stimulation. The authors of these two studies argued that the differences in the model used (10-week chronic stress versus only 2 sessions of social defeat the same day) might explain these discrepancy, which is probable as 2 sessions of social defeat rather evoke a situation relevant for anxiety disorders such as acute traumatic stress for example. Further on, the Chaudhury et al. (2013) study enabled to precise the function of different VTA projection as the stimulation of the VTA-nucleus accumbens projection was sufficient to produce a depressive-like phenotype, while inhibition elicited again opposite effects. In contrast, in the same study it was shown that inhibition of VTA-medial prefrontal projections elicited depressive-like effects. Finally, another study (Covington et al., 2010) revealed that stimulation of medial prefrontal GABAergic and glutamatergic cells induced antidepressant-like effects.

Though an important role of dopaminergic cells in the VTA in vulnerability and resilience to depressive-like behavior had already been shown (Berton and Nestler, 2006; Krishnan et al., 2007; Nestler and Carlezon, 2006; Willner et al., 2005; Yadid and Friedman, 2008), the novelty of these optogenetic experiments enabled to go beyond simplistic correlative experiments, by revealing the causal role played by these cells in triggering depressive-like effects. However, the contrasting results obtained using the two models of depression was at the same time exciting and disappointing. Further on, these studies enabled to dissect the function of different projections from the VTA.

8. Critical analysis of the optogenetic experiments aimed at investigating the neurobiology of fear and depression

8.1. Behavioral tests used

Optogenetics represent a cutting edge method, but surprisingly enough, the anxiety or depressive-like phenotypes assessed following photostimulation are observed in tests with mostly limited validity as models of anxiety disorder or depression (open-field, elevated plus-maze and fear conditioning for the anxiety-like phenotype; forced swimming, tail suspension, social withdrawal and sucrose preference for depression-like behaviors). From the 25 experiments described in Table 1, 8 used fear conditioning procedures, 13 assessed spontaneous anxiety using forced confrontation to novelty (elevated plus-maze, open-field), while the remaining ones used both fear conditioning and spontaneous anxiety tests. To some extent it is understandable to use well validated behavioral tests, as this also offers the advantage that the results will be comparable with data from the literature obtained using more classical neurobiological methods (lesions, immunohistochemistry, etc.). An extensive literature exists in the field of psychology, distinguishing more precise anxiety notions, such as for example tonic and phasic anxiety (Davis et al., 2010), state and trait anxiety (Belzung and Griebel, 2001; Griebel et al., 1993), spontaneous versus learned fear (Kim et al., 2013). This dissociation is supported by neurobiological data, and it has for example been suggested that while phasic anxiety is sustained by projections from the basolateral amygdala to the medial part of the central amygdala, tonic fear corresponds to activation of projections from the basolateral amygdala to the lateral BNST (Davis et al., 2010). However, this dissociation has not been assessed in the optogenetic experiments.

Table 2
Summary of findings using optogenetic tools to study the neurobiology of depression.

Phenotype	Region	Cell type	Strain	Construct	Test	Finding
Medial prefrontal cortex	GABAergic	C57BL/6 mice (10-week-old)	HSV-ChR2-mCherry	Elevated plus-maze, Locomotion, Social interaction, Social recognition, Sucrose preference	Stimulation in stress-susceptible mice following social defeat resulted in antidepressant-like activity, without affecting anxiety-related behaviors	Covington et al. (2010)
VTA	Dopaminergic	TH::IRES-Cre mice	AAV-EF1α-eNpHR3.0-eYFP	Sucrose preference, open-field, Tail-suspension	Inhibition of VTA produced a depressive-like phenotype	Tye et al. (2013)
VTA	Dopaminergic	TH::IRES-Cre mice	AAV-EF1α-ChR2 (H134R)-eYFP	Chronic mild stress: Sucrose preference, open-field, Tail-suspension	Activation of VTA attenuated the deleterious effects of repeated stress exposure	Tye et al. (2013)
Nucleus accumbens, VTA	Dopaminergic	TH::Cre BAC rats	AAV-EF1α-DIO-ChR2(H134R)-eYFP	Forced swimming, open-field	Activation of VTA produced antidepressant-like effects	Tye et al. (2013)
VTA	Dopaminergic	TH::IRES-Cre mice	AAV-DIO-ChR2-eYFP	Subthreshold social defeat: social avoidance, sucrose preference	Phasic stimulation of VTA in stress-susceptible mice increased depressive-like behaviors	Chaudhury et al. (2013)
VTA	Dopaminergic	TH::IRES-Cre mice	HSV-ChR2-eYFP	Social defeat in resilient mice: social avoidance, sucrose preference	Phasic stimulation of VTA converted resilient mice to stress-susceptible mice	Chaudhury et al. (2013)
VTA, nucleus accumbens	Dopaminergic	TH::IRES-Cre mice	AAV-DIO-ChR2-eYFP	Social defeat: social avoidance, sucrose preference	Phasic stimulation of VTA-Nac neurons induced stress susceptible phenotype	Chaudhury et al. (2013)
VTA, nucleus accumbens	Dopaminergic	TH::IRES-Cre mice	AAV-DIO-eNpHR3.0-eYFP	Social defeat: social avoidance	Inhibition induced resilient phenotype	Chaudhury et al. (2013)
VTA, mPFC	Dopaminergic	TH::IRES-Cre mice	AAV-DIO-ChR2-eYFP	Subthreshold social defeat: social avoidance, sucrose preference	Phasic stimulation of VTA-mPFC neurons had no effect	Chaudhury et al. (2013)
VTA, mPFC	Dopaminergic	TH::IRES-Cre mice	AAV-DIO-eNpHR3.0-eYFP	Subthreshold social defeat: social avoidance, sucrose preference	Inhibition of VTA-mPFC neurons induced stress-susceptible phenotype	Chaudhury et al. (2013)

VTA: Ventral Tegmental Area, mPFC: medial Prefrontal Cortex, NAcc: Nucleus Accumbens.s

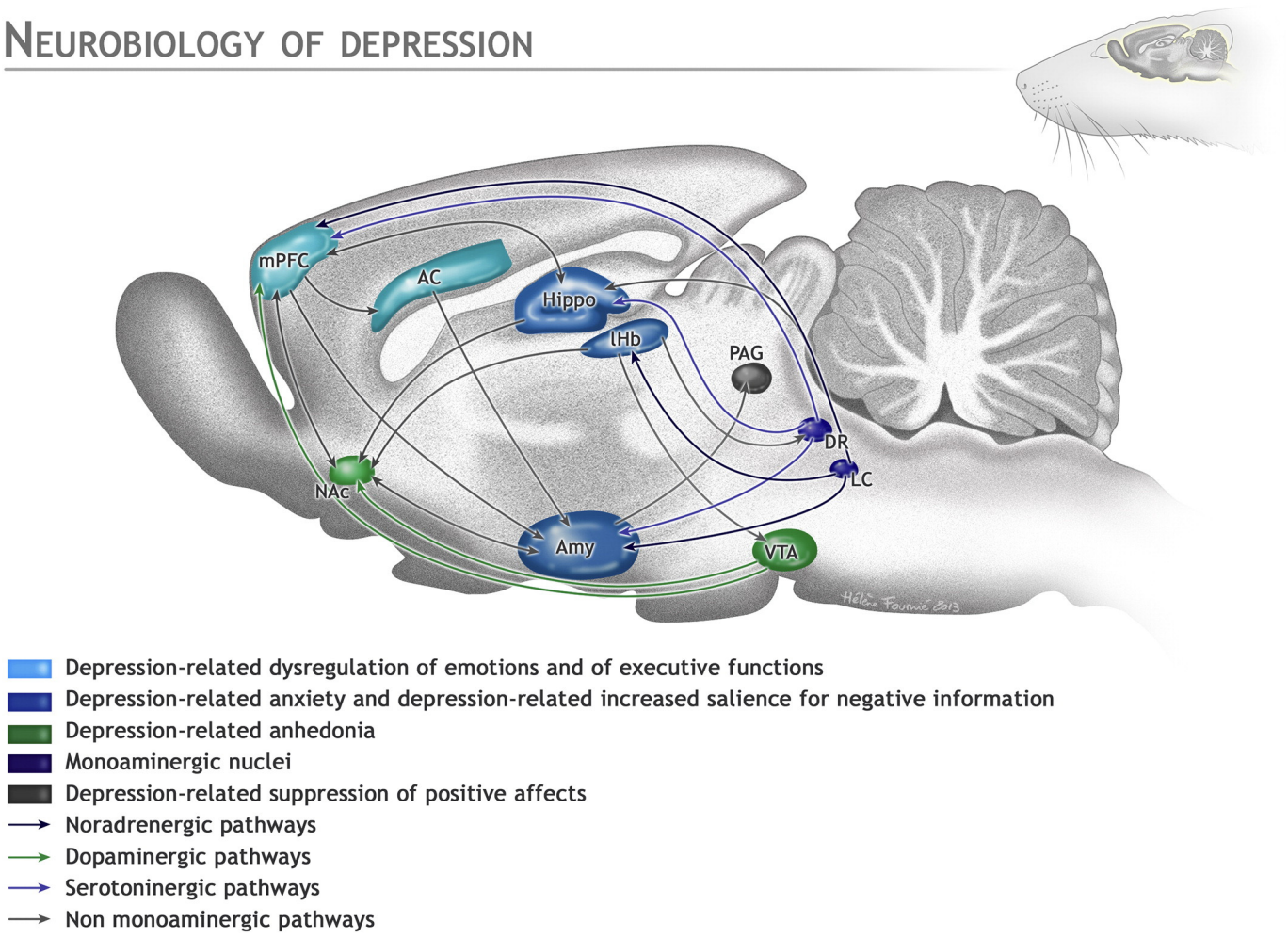
8.2. Normal versus pathological conditions: the case of anxiety

The particularity of anxiety as compared to other behaviors is that it can be both an adaptive, and thus a normal behavior, and a pathological condition. Several types of anxiety disorders have been described in the DSM-5: social phobia, panic attack or generalized anxiety disorder (American Psychiatric Association, 2013). A variety of animal models have been designed to model these disorders or aspects of these nosological entities. For example, excessive state anxiety exhibited by BALB/c mice spontaneously, in the absence of constraints, has been suggested to model aspects of generalized anxiety disorder (Belzung and Griebel, 2001), while certain behaviors exhibited by mice confronted to a natural threat (a rat) in the mouse defense test battery or by rats after dorsal periaqueducal gray electrical stimulation or tonic inhibition of GABAergic activity in the dorsomedial hypothalamus have been suggested to relate to panic-like responses (Griebel et al., 2001, 2003). Other examples are the use of some mutant models (such as mice knockout mice), mice subjected to specific environmental manipulations (for example living in chronic subordinate colony) or selected lines (for example High anxiety behavior (HAB) mice) as models of pathological anxiety, or the use of the 129S1 mouse as a models of impaired fear extinction. Indeed, in these models alterations in the brain circuit underlying anxiety (particularly the amygdala) have been extensively described in these models (Hefner et al., 2008; Sartori et al., 2011; Singewald, 2007; Whittle et al., 2010). These models of anxiety disorders require either to use animals that spontaneously exhibit high levels of anxiety (for example because they have been exposed to poor maternal care, as is the case in BALB/c mice) or to subject animals to experimental manipulations aimed at inducing a pathological state (electrical stimulation or inhibition of a given brain area). These manipulations induce a pathological-like state characterized by both neurobiological and behavioral changes. Surprisingly enough, none of the optogenetic studies which aimed at describing the fear/anxiety circuit used models of pathological state, thus missing a unique opportunity to use a cutting edge method having technical potential to help in dissociating the cerebral substrates underlying different pathological subtypes (for example panic and generalized anxiety disorder) or in designing innovative therapies (see below).

8.3. Can optogenetics be used to design innovative therapies?

As indicated above, despite extensive preclinical research to develop innovative therapies to treat anxiety and major depressive disorders, very few new treatments have been introduced in clinical practice over the last decades, and they are not considered as first or second-line options, which considerably limits their use. These include repetitive transcranial magnetic stimulation (rTMS) of the prefrontal cortex (see Berlim et al., 2013) for a review) and deep brain stimulation (DBS) of specific brain areas. The general rationale of rTMS consists in interfering directly on the electrical activity of the brain circuits that are disrupted in affective disorders. Indeed, activity in the left dorsolateral prefrontal cortex is decreased in patients with major depressive disorder (for a review, see (Willner et al., 2013)) and rTMS of this region might counteract this dysfunction, thus eliciting remission. The same rationale applies to DBS. In depressed patients, DBS of the subgenual cingulate cortex (Cg_{25}) (Lozano et al., 2008, 2012; Mayberg et al., 2005), of the anterior limb of the capsula interna (Malone et al., 2009), of the nucleus accumbens (Bewernick et al., 2012) or of the lateral habenula (Sartorius et al., 2010) are sufficient to elicit remission. Similar results have been obtained using animal models. Indeed, in rodents, it was found that DBS of the lateral habenula, of the ventromedial prefrontal cortex, of the nucleus accumbens or of the cingulate cortex elicit antidepressant-like effects (Dournes et al., 2013; Falowski et al., 2011; Hamani et al., 2012; Hamani and Nobrega, 2010; Meng et al., 2011; Schmuckermair et al., 2013). However, few studies investigated the network underlying these effects: indeed, with the exception of the study of Schmuckermair et al. (2013) who showed the DBS of the accumbens elicited changes in the hippocampus, the lateral habenula and the prefrontal cortex in HAB mice, most studies did not describe the DBS-induced alteration in the brain circuitry. Further, no study was able to determine the type of cells involved in these effects. Thus, optogenetic stimulation could be used to understand the microcircuit involved in these beneficial effects, identifying particular neuronal populations.

The observation that the beneficial effects of rTMS or DBS require repetitive stimulations suggests that repeated photostimulation would be necessary to obtain sustained effects. Repeated optogenetic stimulation



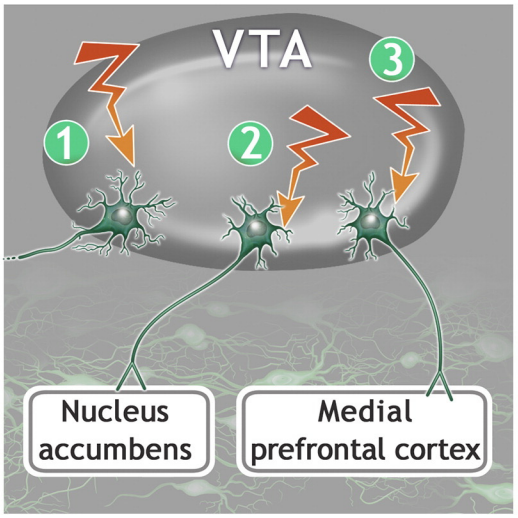


Fig. 3. The neurobiology of depression: recent advances in the optogenetic era. Upper panel: the brain circuit underlying susceptibility/resilience to depression as depicted before the use of optogenetics. The circuit includes areas involved in emotional regulation and executive functions (medial prefontal cortex and anterior cingulate cortex), areas involved in depression-related anxiety and increased salience for negative information (hippocampus, lateral habenula and amygdala), areas involved in the depression-related anhedonia (ventral tegmental area and nucleus accumbens), monoaminergic nuclei (dorsal raphe, locus coeruleus and ventral tegmental area) and depression-related suppression of positive affects (periaqueducal grey). mPFC: medial Prefrontal Cortex, AC; Anterior Cingulate, NAcc: Nucleus Accumbens, Hippo: Hippocampus, Amy: Amygdala, lHb: lateral Habenula, PAG: Periaqueducal Grey, DR: Dorsal Raphe, LC: Locus Coeruleus, VTA: Ventral Tegmental Area. Lower panel: new findings obtained using optogenetics. 1. Stimulation of dopaminergic cells within the VTA elicited antidepressant-like effects in stressed mice while inhibition produced a depressive-like phenotype. If stimulation of VTA neurons is phasic it elicits a depressive-lie phenotype. 2. Phasic stimulation of VTA-Nucleus accumbens neurons produces a depressive-like phenotype while inhibition elicits opposite effects. 3. Inhibition of VTA-medial prefontal projections elicits depressive-like effects.

has already been used to elicit persistent changes in behavior, to mimic for example pathological conditions as described recently for obsessive–compulsive disorder (OCD). Here, repeated orbitofrontal cortex-ventromedial striatum photostimulation elicited persisting long term changes mimicking symptomatology of OCD (Ahmari et al., 2013; Meng et al., 2011). Similar patterns of activation of the fear or of the depression circuit could be used as preclinical models of chronic anxiety and depression-like behavior, or to reverse, in rodents, the effects of chronic manipulations such as for example those of repeated unpredictable stress, thus enabling to further design new therapeutic targets.

8.4. Optogenetics research ignores the complexity of the cerebral network sustaining anxiety and depression

As already mentioned above, clinical as well as preclinical data show that the cerebral network sustaining anxiety and depression is characterized by a high degree of complexity, involving a great number of interconnected brain areas (see Figs. 2 and 3) and cell types (pyramidal cells, interneurons of different types, neurons releasing particular neurotransmitters, etc.). Studies using optogenetic tools have focused either on one particular brain area, on one particular network (for example projections from the VTA to the nucleus accumbens) or on one specific cell type, therefore ignoring the remaining part of the network. Even if findings generally show that photostimulation or inhibition of the specific network that had been investigated is sufficient and/or necessary to elicit behavioral changes, this does not permit to conclude that the fear or the depression circuit can be reduced to that particular circuit. For example, currently all optogenetic research in the depression field focused on VTA projections to the nucleus accumbens or to the medial prefrontal cortex. Yet, this does not mean that the other brain structures shown to be pivotal in depression, such as the cingulate cortex, the amygdala, the lateral habenula or the hippocampus are not involved as well. The same applies to the optogenetic findings on anxiety: even if stimulation of parts of the amygdala or of the BNST is sufficient to elicit sometimes impressive anxiety behavior, this does not mean that these brain areas are not regulated by cortical areas, for example. Therefore, this research enables only to assess a part of the depression and/or of the anxiety circuit, but does not enable to gain knowledge on the complex network underlying these disorders. Further on, when the objective is to target with high specificity a certain cell type, optogenetic requires the availability of engineered rodents. Currently available animals include mice designed to express the light-gated cation channel channelrhodopsin-2 (ChR2) under the regulation of the Thy1.2 promoter or transgenic Cre recombinase driver lines allowing for cell-specific gene manipulations in the central nervous system, such as those enabling to control the firing of GABAergic, glutamatergic, cholinergic, serotonergic, dopaminergic or parvalbumin + neuron populations. Further on, recently transgenic mouse lines expressing functional ChR2 under the control of cell-type specific promoter elements have been designed (Zhao et al., 2011). A complex description of the involvement of a brain area in a given behavior or in a given pathology should use different lines enabling to target different cell types in order to approach the complexity of the system. For example, depression research focused on the participation of dopaminergic cells in depressive-like behavior. Even if the contribution of dopaminergic cells in the depression-like phenotype had already been described, the participation of the serotoninergic, noradrenergic, GABAergic and glutamatergic systems in depression has received strong support since many years (for a review, see Willner et al., 2013) and cannot be ignored.

9. Conclusions

To summarize, there is a sharp contrast between the sophistication of optogenetics on one hand, and the rudimentary behavioral testing and the poor use of animal models of pathological states. Improvement on this could enable to use this technique also to further understand the mechanisms of currently used treatments, and thus to design innovative therapies, which has been poorly addressed up to now. Further improvement would require promoting active dialog between researchers in the field of optogenetic and behavioral neuroscientists, psychiatrists and behavioral pharmacologists.

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