

Chapter 10

Carving Out the Path to Computational Biomarkers for Mental Disorders

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Abstract

For the last 20 years, there has been impressive progress in cognitive neuroscience and neuroimaging. Unfortunately, the translation from basic research to real world clinical applications in the domain of mental disorders has been limited. A major contribution of functional MRI would be to provide computational biomarkers for precision medicine and treatment optimization. Before this is possible, a number of open conceptual and methodological challenges need to be addressed, and some of them will be outlined in this chapter. For illustration purposes, we focus on the amygdala and its relevance in anxiety disorders. We cover a few central aspects on how certain pitfalls can preclude reliable empirical work on the amygdala and, thus, robust computational biomarkers. Overcoming them requires multidisciplinary collaboration on the level of psychiatric and neuroscientific theories on the one hand, and on fMRI acquisition, signal processing, and analysis on the other. While we are optimistic that neuroimaging will provide a substantial contribution for the discovery of computational biomarkers in psychiatry, psychopathologies and their neuropathophysiology are complex so we cannot propose an easy solution. Instead, we focus on some challenges that emerge in the overlap of theory and application.

Key words Emotions, Anxiety, Mental disorders, Amygdala, Functional MRI

1 Introduction

1.1 The Complexity of Understanding Brain Function and Dysfunction

In his lectures in 1890, the Viennese psychiatry professor Theodor Meynert deplored that, in contrast to other fields in medicine, psychiatry has not made the transition from a descriptive discipline toward an *explaining science*. In fact, over the last 130 years, psychiatry has made tremendous progress in the diagnostics and treatment of mental illnesses. However, compared to other fields in medicine, a precise understanding of the etiology, pathophysiology, and progression is insufficient for many diseases. Despite the availability and success of a wide range of treatment options, core brain mechanisms and neural principles that indicate or explain efficacious treatments are unclear. Admittedly, this is not surprising considering that the brain is the most complex organ in the

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human body and constantly shaped by biological, psychological, and social factors in a complex, nonlinear fashion. Appreciating this complexity, Theodor Meynert already proposed that psychopathologies and their treatment should be addressed by an interdisciplinary and mechanistic perspective on the brain, just like anatomy has evolved toward an explanatory science by relating its observations from histology, developmental, and comparative biology.

While none of the factors relevant for development and progression of psychopathologies appear monocausal, it is only reasonable to assume that if mental illnesses are (at least in part) brain illnesses there have to be some observable biomarkers in the brain for the presence or absence of these illnesses based on the computational level of pathophysiological mechanisms. Functional neuroimaging has been very successful in mapping brain functions to brain areas, unveiled quantifiable differences between study populations, and in so doing informed more and more refined theories about brain mechanisms. Proper theorizing and application of these theories are important. The history of neuroscience has taught us that these mechanistic concepts have always been inspired or explained by referring to metaphors and narratives that reflected the cultural and technological state of the art. With varying degree of seriousness, the brain has been illustrated as operating using cogwheels, hydraulic pumps, steam engines, electric cables, or, most recently, computer hardware that performs computations, implements the mind as a software, or instantiates some form of virtual reality. Is it organized like a manufacturing line in a factory, an office building with several different departments, a homunculus sitting in a Cartesian theater, like a democratic parliament, or a social network?

Thinking about these questions is not an intellectual pastime activity. They provide the conceptual framework for how hypotheses are formed and experiments are designed. However, that is not the only motivation as pragmatic considerations also play a role in experimental design and in doing so influence the way brain functions are operationalized. The empirical results are then used to update theories, yet they are not independent of hidden assumptions, methodological (quasi) standards, and narratives taken for granted. This means, methodological choices—may they be well-founded and necessary or naïve and arbitrary—can change the direction of how theories evolve over time. On the other hand, not paying attention to pragmatic methodological constraints is not fruitful, either. An effect cannot be studied if it is not operationalized for the empirical methods at hand. In sum, theoretical background, methodological choices, empirical results, and their interpretation conditionally depend on each other.

We will discuss how these considerations apply to the identification of computational biomarkers in psychiatry. In doing so, this chapter will focus mainly on the amygdala and anxiety disorders.

Yet, many of the principles described herein generalize to other psychopathologies and brain areas and illustrate general principles of empirical neuroscientific research. First, we will investigate the justification of using the amygdala as a potential target of interest by discussing its function and operationalization in experiments. Second, we address potential challenges in measuring neural activation in the amygdala both because of its complex anatomy and methodological challenges specific to functional MRI. We argue that finding optimal solutions for these two issues are of central importance for the identification and practical application of amygdala-based biomarkers.

1.2 The Role of Emotions in Anxiety and Other Mental Disorders

On a symptomatic level, the experience of negative emotions and impairments in emotion regulation are well-known phenotypes of many psychiatric disorders [1–3]. In anxiety disorders, in particular, excessive fear and anxiety are hallmark symptoms (ICD-11, https://icd.who.int). Modifying the experience of these negative emotions or regulating their intensity are targets or outcomes of successful therapeutic interventions [4]. Experiencing negative emotions biases the perception of the world [5] and executive functions [6, 7], making anxiety disorders a high-risk factor for major depressive disorders [8] and disability [9]. There is also a well-established relationship between negative emotional experience and chronic stress [10], which in turn leads to negative somatic and psychological downstream effects [11].

While symptoms and consequences of anxiety and other mental disorders are multifaceted, we can assume that there is a relationship to disordered brain functions that might be observable using neuroimaging. In fact, applications of fMRI have shown that deficits in motor areas can be informative to evaluate motor impairments [12], language areas for language impairments [13], and visual areas for vision impairments [14]. The same should hold true for brain areas that are involved in perceiving and experiencing emotions given that emotions play a key role in anxiety and other mental disorders.

1.3 The Role of the Amygdala in Emotions

Historically, the amygdala has been considered one of the key regions for experiencing and perceiving emotions [15]. Since then, robust empirical evidence has led to the popular notion that the amygdala is the emotion center of the brain. Amygdala's role in fear and anxiety is likely the most well-studied topic due to its high relevance for clinical and social neuroscience as well as because of the comparably simple experimental implementations and strong behavioral and neural effects. After all, there is a high ecological significance and value to efficient and effective responses toward danger [16], which are understood as a set of stereotypical and automatic threat responses [17, 18]. Beyond animal research and human neuroimaging studies, there is also strong evidence from

humans with amygdala lesions that exhibit strongly reduced threat responses and subjective feelings of fear [19] and, e.g., marked impairments in detecting deceit in social decision making [20, 21]. Later on, we will discuss some challenges to this simplified notion of the amygdala's involvement in emotions and highlight a more nuanced role in how emotions are processed in the brain.

On a neural level, amygdala reactivity during threat perception is assumed to be implemented by input from sensory cortices and the thalamus to the amygdala in partly independent processing streams [22]. This traditional dual road model has been extended to a more complex multi-road model [23] strengthening the notion that emotion perception is not implemented in the amygdala alone but in a more distributed brain network. Likewise, the experience of emotions is also considered to rely on distributed brain networks [24]. While the amygdala appears to be central to emotional experience, it is neither sufficient nor necessary. This is most convincingly evidenced in a person with amygdala lesions, who can experience strong fear in response to CO_2 inhalation, and was unaffected in other tested fear-induction settings, such as fear-inducing emotional images and videos, a visit to a house of horrors, and life-threatening situations in real life [25]. On the other hand, there is strong evidence from a controlled amygdala stimulation study in epilepsy patients using acute monopolar electrical stimulation that did not affect the subjective experience of emotions despite robustly observable physiological changes [26].

In sum, the present evidence shows clearly that the amygdala is involved in the perception and experience of emotions. Yet, there is neither a specialization for fear alone nor is it the only brain area relevant for emotion and fear processing. Its undisputed central role in a variety of emotional processes makes it a highly valued research target in clinical neuroscience and an ideal biomarker candidate.

1.4 Amygdala Activation and Connectivity in Anxiety and Other Mental Disorders

A typical neuroimaging finding in anxiety disorders is an increased amygdala response to fear-inducing stimuli [27, 28]. This hyperactivation is in contrast to an amygdala hypoactivation toward rewarding stimuli, which is observed in the context of depression-related anhedonia symptoms [29, 30] or initial hyperactivation followed by hypoactivation in schizophrenia [31]. This means, in these and other conditions, amygdala activation can be an informative biomarker taking into account the difference in BOLD response [32].

Amygdala hyperactivation in anxiety disorders can be explained in part by an increased reactivity toward threat and uncertainty in anxiety disorders [33]. On the other hand, there is also a reduction in prefrontal regulation that leads to hypervigilance (i.e., increased attentional focus toward threat), impaired safety learning, and dysfunctional cognitive control [34]. In particular, the orbitofrontal

cortex plays a relevant role in amygdala regulation [35, 36], and reduced resting state and task connectivity has been shown, e.g., in social anxiety disorder [37–39]. In contrast, e.g., selective serotonin reuptake inhibitors, a pharmacological intervention with demonstrated applicability in anxiety and affective disorders, were shown to increase amygdala downregulation in healthy volunteers [40] consistent with connectivity changes following successful psychotherapeutic interventions [41] and theories of reappraisal success [42]. Additionally, amygdala activation and connectivity changes during emotion processing have been observed for the NMDA receptor antagonist ketamine [43] and the mixed serotonin receptor agonist psilocybin [44], which have both demonstrated the potential of therapeutic efficacy in anxiety and other mental disorders [45, 46].

By and large, the present literature suggests some involvement of the amygdala in anxiety and other mental disorders [47]. Connectivity studies and changes after therapeutic interventions suggest that it plays a role in the pathophysiological mechanisms providing further justification for its usefulness as a biomarker.

1.5 Structural and Functional Alterations in the Amygdala as a Possible Differential Diagnostic Biomarker for Mental Disorders

Considering its role in emotion processing and the impairment of the emotional response being a consistent phenomenological state across the majority of mental disorders, the amygdala could also be a morphological substrate with differential diagnostic value in psychiatry. For instance, a reduction of the bilateral amygdala volume has been established in major depressive disorder, bipolar disorder, and also first-episode schizophrenia [48]. In addition, the volume reduction was significantly more pronounced in the latter two than in major depressive disorder.

A meta-analysis by McTeague et al. demonstrated convergent amygdala hyperactivation during emotion-processing tasks in non-psychotic (e.g., anxiety, unipolar depression, bipolar depression, obsessive-compulsive disorder, etc.) versus psychotic disorders (e.g., schizophrenia, schizoaffective disorder, etc.) [2]. Furthermore, in the same meta-analysis an overlapping increased activation of the left amygdala and the hippocampus was observed in anxiety and major depressive disorders whereas aberrant activity of the right amygdala and right ventrolateral prefrontal cortex was associated with bipolar disorder.

Differences in amygdala connectivity have also been implicated in various mental disorders. Resting state functional connectivity of the amygdala and the right middle cingulate has been shown to be reduced in patients with first episode schizophrenia as compared to individuals at high-risk for schizophrenia and healthy controls [49]. Decreased resting state functional connectivity between the right amygdala and the left anterior hippocampus has been noted as a possible discriminating alteration in unipolar major depressive disorder compared to bipolar disorder and healthy individuals [50].

Moreover, bipolar disorder type II has been differentiated from borderline personality disorder via a lower resting state functional connectivity between the amygdala and the middle frontal gyrus, which was further associated with affective shifts, higher impulsivity, functional impairment, etc. [51]. All of the hitherto described findings suggest the possibility of the amygdala being a possible differentiating biomarker for mental illnesses.

1.6 Real World Challenges for Amygdala-Based Biomarkers

So far, we have discussed a number of reasons why the amygdala could be an excellent biomarker for psychiatric conditions such as anxiety disorders. However, despite the robust theoretical basis, there are a number of challenges regarding the practical applications. In the following, we will focus on a few key issues that influence the outcome of this endeavor. Admittedly, there is a strong interaction of these issues, which creates the impression that any research in this domain is hopelessly stuck in a vicious circle. To overcome this deadlock, we aim at making these challenges explicit and unmask some of the mutual dependencies. We argue that there is not one overall grand solution that magically dissolves all challenges. There is not the one ideal scanner or sequence parameter that provides the optimal data at the press of a button. There is not the one ideal analysis method that will provide answers to complex research problems given just some arbitrary data. Instead, the challenges are multi-faceted and follow the Anna Karenina principle named after a quote from Leo Tolstoy's novel: *All happy families are alike; each unhappy family is unhappy in its own way.*

Emphasizing the complexities as well as the various sources of limitations is not meant to frustrate the reader. Instead, we want to raise awareness on the reasons for these complexities and that there is not a single monolithic solution or investment that can solve all issues at the same time. Instead, we encourage to investigate the challenges from an engineering perspective and applying the successful paradigm that goes back to Julius Caesar: *I divide and conquer*. This means to appreciate the complexity of an unsurmountable task and then to break it apart into independent subtasks as far as needed to make them easy to handle. Having smaller tasks also allows for feasible outcome evaluation, i.e., to verify that a specific goal has been met, that the protocol is working as expected, and that a device is actually functioning. In practice this can mean, e.g., the following: Is my task valid for my target brain region, do I have a problem with the timing and synchronization of my scripts, and is it actually possible to measure the brain response with my imaging sequence settings? This kind of verification and troubleshooting is usually only possible if the subtasks and preliminary goals have been operationalized properly before setting course for novel scientific discoveries.

2 Materials

2.1 Magnetic Resonance Imaging Hardware

To date, magnetic resonance imaging (MRI) provides the best way for in vivo neuroimaging of the amygdala and other subcortical brain regions. Not only does it allow for high resolution anatomical imaging, functional MRI (fMRI) can be used to acquire time-resolved images that are sensitive to local changes in blood oxygenation, which is a well-established proxy for clustered neural activity. MRI scanners are becoming more available and affordable as scanner manufacturers are able to optimize their hardware and software designs to reduce construction, material, and consumable costs. However, while the versatility of the fMRI method allows for many compromises, there is no such thing as a free lunch. In fact, some of the methodological challenges that will be discussed below can be mitigated by investing in research grade MRI scanners with field strengths of 3 Tesla and more, a sensitive multi-channel head coil for high signal to noise ratio (SNR), and an optimal gradient system (i.e., fast rise time and high maximum gradient strength) to allow for the use of fast echo planar imaging (EPI) methods. In detail, compromises in field strengths entail a linear reduction of overall MRI signal intensity impacting factors such as specificity (e.g., the ability to distinguish between tissue types). High sensitivity of the receiver coil, on the other hand, can improve the amount of detected original signals despite higher levels of noise.

If these hardware investments are not an option, increasing the number of data points provides a solution to improve data quality by suppressing the effect of noise through averaging, following the law of large numbers. One way to achieve this is by generating more data points by increasing the sampling rate, i.e., reducing the repetition time of the image acquisition. While this option also achieves a more accurate sampling of the BOLD signal, its possibilities are limited by the sluggishness of the BOLD effect.

Another solution for noise suppression is to increase the repetition of task trials, which entails either longer experiments and measurements or fewer individual task conditions. Increasing the repetitions of task trials is in many cases undesired as patients in particular will struggle with long experimental runs leading to less task compliance and excessive head motion. Beyond that, prolonged exposure to the task stimulus will create learning effects resulting in neuronal adaptation processes, such as habituation, a phenomenon particularly relevant in the amygdala. A reduction of individual task conditions, on the other hand, can be achieved by formulating precise hypotheses and experimental interventions. This requires a precise understanding of the effects of interest and their neural mechanisms. We will discuss these issues in detail in the Subheading 3.

Overall, these explanations demonstrate the consequences of several investment and experimental choices that require careful deliberation, setup, and ongoing empirical evaluation of the setup, experimental protocol, and data. The complexity of the imaging part additionally highlights the need for dedicated research support by personnel with experience in neuroimaging and MR physics—and that hardware investments alone are not sufficient.

2.2 Computing Hardware

For data analysis and modeling dedicated computing hardware is required. In comparison to an MRI scanner, however, the costs will be negligible. Based on previous experience, modern prosumer and small enterprise hardware allow for successful data processing, as long as fast data access and long-term data security demands can be met. This means, e.g., that a state-of-the-art desktop workstation with sufficiently large internal disk space and RAM attached to an external redundant backup system could be a cost-efficient solution. While multi-core servers and high-performance computing clusters allow for parallel data processing and high data throughput, they also require experienced research support personnel and end-users with high levels of technical expertise. With this in mind, the computing infrastructure should be designed to address the end-users' skill level and demands. Investing time and resources in usability and ongoing domain-general training are central requirements for planning new computing infrastructure. Overlooking or ignoring these human factors inevitably degrades the usefulness and cost-effectiveness of the infrastructure provided.

3 Methods

3.1 Experimental Design: Amygdala Function

Typical tasks to investigate amygdala reactivity involve presentation of emotional stimuli. Amygdala function is not restricted to only one sensory domain, in fact it is known to receive multimodal input and perform multisensory integration [52]. Consequently, a wide range of experimental paradigms are used, e.g., *visual*, such as negatively valanced (e.g., shocking or disgusting) [53, 54] or positively valanced (e.g., humorous or erotic) emotional images [55] and faces [56], *auditory*, such as dissonant audio cues [57], *olfactory*, such as pleasant or unpleasant odors [58], and *somatic*, such as painful stimulation [59].

Detailed work in rodent models has demonstrated that amygdala output [60] is used to regulate and initiate defensive behaviors [61–63] and plays a central role in fear conditioning [64, 65]. A direct translation from rodent to primate research is challenging due to phylogenetic changes specifically in the amygdala region [66]. While there is good cross-species evidence for similar defensive behavior initiation and regulation [67], there is less consensus if mere anticipation of negative stimuli robustly increases amygdala

activation [68]. Yet, it was observable for highly anxious participants [69–71] as a consequence of negative expectations and biases during anticipation, e.g., in anxiety disorders [34] and depression [72]. On the other hand, there is also some risk that elevated feelings of anxiety and stress when undergoing fMRI experiments could be an important confound [73], in particular at the beginning of the scanning [74]. This means, an increase in amygdala activation in people with anxious predispositions could be an artifact of the experimental setup and less specific for psychopathologies per se [75]. This demonstrates that translational neuroscience can be complex and task demands do not necessary translate across different species, i.e., what might be ecologically relevant for rodents does not necessarily entail the same relevance for human participants.

In fact, the amygdala has been proposed to play an important role for relevance and novelty detection to guide attention and learning [76]. Given that understanding the feelings and intentions of conspecifics has a high ecological relevance for human beings, it is not surprising that recognizing [77, 78], distinguishing [79], passively viewing [80], and even subliminal presentation of emotional faces increase amygdala activity [81, 82]. In this context, amygdala hyperactivation has been observed in anxiety disorders [27, 28] and increased amygdala reactivity toward negatively contextualized neutral face images is a risk factor for impaired mental well-being [83]. Besides different forms of hypo- or hyperactivation [29–31], another relevant biomarker could be delayed amygdala recovery from an emotional stimulus, which has been linked with trait neuroticism [84] and inhibited temperament [85]. This means, other than peak amygdala activation, which is the focus of conventional fMRI analysis, there are informative signal properties that could be identified using a time course analysis, such as time to peak, slope, recovery to baseline time, area under the curve, and features observable using frequency domain analyses [86, 87].

Besides inter-trial temporal dynamics, there is also an ongoing debate about the temporal reliability and stability of the amygdala response to emotional faces across trials, runs, and sessions. While some studies report high stability across task sessions [88] and trials [89–91], other studies demonstrate amygdala habituation in healthy participants [92–94]. In the context of social anxiety disorders, however, amygdala habituation could also be seen as a distinctive reaction to the exposure of an inherently aversive stimulus [91]. This means, in addition to amygdala reactivity, signal changes across task trials and within an individual task trial could be informative.

Another lesson from animal models is the central role of the amygdala in various forms of learning, especially in volatile or vicarious contexts [95]. In support of this notion, it has been shown in humans that participants with (basolateral) amygdala

lesions perform worse in a social learning task [20, 21], where they had to repeatedly decide whether to trust another player in an economic decision-making game. This finding has been replicated in neurotypical participants [96], highlighting the amygdala's important role in prolonged instrumental learning. In this task, in contrast to face stimuli, no habituation was observed, indicating a longer-lasting involvement to maintain successful task performance. This means that ecologically valid, social, and possibly non-social probabilistic learning tasks could have particular potential for identifying pathology-specific amygdala and behavioral biomarkers.

In sum, we conclude that the amygdala, in theory, would be a good biomarker candidate given its central role in a variety of neuronal systems, cognitive processes, and experiential phenomena. However, conceptual flaws in the study and task design lead to suboptimal amygdala responses or even a complete absence of task-related activation. Careful planning and validation of the experimental setup and model will be essential.

3.2 Experimental Design: Amygdala Anatomy

In addition to the amygdala's functional intricacies, another level of complexity is added by its anatomical and regional functional heterogeneity. In human neuroimaging, the amygdala is typically treated as a singular structure [97], which might be an invalid oversimplification [98]. Two important subnuclei are the basolateral (BLA) and the central part of the amygdala (CeA), which possibly extends to the bed nucleus of the stria terminalis (BST) [99], which is heavily connected to the CeA but anatomically separated by a white matter structure known as the internal capsule in primates [52]. On a functional level, the BLA is considered a primary input region for multimodal sensory data, while the CeA, on the other hand, projects strongly to brainstem and hypothalamus to facilitate automatic responses to threat [52]. Previous studies have found highly relevant fear- and anxiety-related CeA responses [65, 69], which highlight that this region, despite being small and some of its effects overshadowed by the larger BLA, should be addressed in a differentiated way. While the CeA could be related to fast threat responses, the BST appears to play a role in more prolonged states of anxiety [100]. Taken together, the BLA, CeA, and BST could provide the necessary architecture to form a computational network that hierarchically encodes associations between states of the world and their affective value on different time scales and levels of abstractness. It can be assumed that there are functional dissociations between the amygdaloid subregions that are reflected by a difference in the temporal profiles of the task response. This can lead to potentially destructive signal mixing, as time delayed or anticorrelated signals that are sampled and averaged together can lead to attenuation of the overall measured BOLD response.

Besides the BLA, CeA, and BST, there are more subnuclei with different cytoarchitecture, suggesting an even more complex involvement in the neural computation. A recent review suggests that the optimal strategy for amygdala parcellation is not even clear on an anatomic level (Ufer et al., in preparation). It can be expected that the missing consensus might lead to future revisions of how subregions should be defined anatomically. The debate on amygdala segmentation might appear only of theoretical value, yet there are important pragmatic implications. Many amygdala interneurons are GABAergic [101] leading to a strong inhibitory effect. This means that, e.g., amygdala regulation by prefrontal regions is achieved by long range excitatory glutaminergic projections, which target amygdala GABAergic neural populations that implement local inhibition [102]. Amygdala output, in turn, is frequently implemented by a neural mechanism known as disinhibition, i.e., neural activation that is facilitated by suppressing an ongoing inhibition. Output suppression and regulation plays a major role in amygdala's function leading to the notion that it is a *silent region* as local spontaneous neural activation does not easily propagate to other brain areas [52, 103], suppressing behavioral downstream consequences. As a consequence, uncovering the actual neural mechanisms that caused an observable effect on the fMRI BOLD signal is not straightforward as relevant amygdala output could have been suppressed as a consequence of neurotypical or pathological physiology. Likewise, the absence of an observable difference in the fMRI BOLD signal alone does not necessarily mean that there were no differences in neural computation between different study populations or conditions. The reason for this is that local metabolic demands that cause BOLD signal fluctuations can either be caused by the activation of excitatory or inhibitory neural subpopulations. To some extent, apparent amygdala hyperactivation observed in fMRI could reflect the opposite of the actual neural activation. In this case, the relatively increased BOLD signal could be caused by more local inhibition, leading to less amygdala neural activation but more metabolic demand.

We list a number of challenges for amygdala fMRI originating from the anatomical, cytoarchitectonic, and functional heterogeneity of the amygdaloid cluster. As countermeasure, we propose (a) the implementation of validated experimental protocols that avoid destructive signal mixing resulting from the temporal differences in the neural activation of the individual subpopulations, (b) the use of a high temporal and spatial resolution in fMRI to distinguish the different functional contributions during task performance (discussed in more detail in [1, 2]), and (c) taking into account amygdala connectivity and regulation by other brain regions (discussed in more detail in Subheading 3.3).

3.3 Experimental Design: Connectivity and Amygdala Regulation

The amygdala is extensively connected to a wide range of subcortical and cortical regions that are involved in a diverse set of different brain functions. On one hand, this is the reason for the importance of the amygdala and functions as an important hub in many domains. On the other hand, the amygdala might only be one aspect of a compound biomarker that involves a network of functionally connected brain areas. When considering the brain as a self-organized dynamic system, the causal interaction of these brain areas could be pivotal for understanding the mechanisms leading to regional activation as well as for behavioral, computational, and experiential phenotypes and how they relate to psychopathologies. For example, fast amygdala reactivity to an aversive stimulus might not necessarily lead to automatic threat responses or subjectively experienced distress, if efficient downregulation by other brain regions occurs or if attentional resources are utilized elsewhere [104]. In fact, the context-dependency of prefrontal-amygdala regulation has been shown [38] and successful reappraisal alters prefrontal network connectivity [42], which could be an explanation for amygdala connectivity changes after successful psychotherapy [41]. In this context, with fMRI-based neurofeedback of the amygdala gaining popularity, regulation success could be a promising tool as it reflects the participants' ability to voluntarily control their amygdala activation on demand.

3.4 Functional MRI of the Amygdala

So far, we have addressed issues pertaining mainly to the experimental design and already mentioned that amygdala fMRI also requires imaging with high spatial and temporal resolution. Even though fMRI can be an excellent method for subcortical functional imaging, it faces a number of challenges that need to be addressed.

Historically, there were serious concerns in the neuroimaging community that the amygdala and ventral prefrontal regions might be inaccessible for reliable BOLD fMRI [105], due to the air cavities formed by the paranasal sinuses that cause magnetic field inhomogeneities leading to signal dropouts in the ventral brain. As BOLD fMRI relies on detecting susceptibility-related signal differences (i.e., the difference between oxygenated and deoxygenated hemoglobin), the imaging sequences used are particularly affected by these susceptibility artefacts, e.g., gradient echo planar imaging sequence with relatively long echo times. Noteworthy, these issues are a lesser concern for other imaging modalities (e.g., anatomical MRI) or for fMRI in other species with different cranial anatomy. For example, in dogs the most profound signal dropouts are observed in medial dorsofrontal and not ventral brain areas such as the amygdala or hypothalamus [106].

The effects of signal loss due to spin dephasing can be compensated by using a high spatial resolution with small voxel sizes [107], which is also indicated to distinguish the functional contributions of the individual amygdala subregions to efficiently avoid signal mixing. Two factors limit the use of arbitrarily high resolutions.

First, decreasing the slice thickness reduces the covered acquisition volume. To compensate, more slices need to be acquired, which leads to longer repetition times reducing the temporal resolution. A whole-brain coverage may not be essential when using optimized experimental protocols that only target the amygdala and other more ventral areas such as the ventral striatum, midbrain regions, anterior insula, ventral temporal lobe (e.g., the fusiform face), as well as ventrolateral and ventromedial prefrontal areas. All these regions have been implicated in a wide range of mental disorders before, which makes them an ideal focus for an optimized imaging protocol. The disadvantage of this approach is that the experimental hypotheses need to be clear and explicit and there are less possibilities for post-hoc troubleshooting. For example, to verify the accuracy of the experimental timing and synchronization of the stimulus computer and MRI scanner clocks, the motor response in the hand area of the primary motor cortex provides a robust signal that can be related to button presses. This means that careful validation of the experiment is key before applying it to the actual research context. Finally, if support by the MRI scanner is available, novel optimized acquisition methods such as multiband echo planar imaging [108] can provide a good solution to achieve both high temporal and spatial resolution.

Second, the image signal to noise ratio degrades as voxel sizes decrease. The common way to reduce the signal noise during preprocessing is to apply spatial smoothing. Finding a good trade-off between sensitivity (i.e., detecting the BOLD response despite the noise) and specificity (i.e., avoiding signal mixing) can be an empirical question that is tested with an independent pilot dataset, ideally. Smoothing that respects the tissue boundaries can be a good compromise. This can be achieved, e.g., in a volume of interest analysis, by extracting all the individual time courses within a given tissue boundary and averaging over these time courses. Another solution for increasing the SNR is to conduct longer experiments with more task trials [109]. Besides the longer scan times that can be intolerable for patients, the disadvantage is that there are learning and exposure effects that can alter the consistency of the amygdala signal, as we discussed in Subheading 3.1. Agreeably, the least practical but best solution to increase SNR in the amygdala is to move to higher field strengths such as 7 Tesla, which will be discussed in more detail in Subheading 3.5.

With respect to imaging parameters, besides the temporal and spatial resolution, the echo time (TE) plays an important role. Image quality can be improved by using short echo times, as distortions and other susceptibility-related artifacts are visibly reduced. Unsurprisingly, a reduction in susceptibility-related signal effects also reduces the BOLD-sensitivity, leading to less image contrast between deoxygenated and oxygenated hemoglobin. This means that longer echo times that approximately optimize

for the hemoglobin-specific relaxation time contrasts while preventing the dominance of imaging artifacts is key. Validation of optimal BOLD sensitivity in the amygdala, given the experimental setup, should occur before the actual measurements. A straightforward approximation is to calculate and systematically compare the temporal signal to noise ratio SNR_t for each voxel based on its time course y defined as:

$$SNR_t(y) = \frac{\mu_y}{\sigma_y} = \frac{\text{mean}(y)}{\text{stddev}(y)}$$

In practice, to quantify BOLD sensitivity in the amygdala based on the SNR_t, resting state or task datasets can be used. Robust tasks such as the emotion discrimination task [79] would be a good option as the they can be used both to quantify the size of a contrast of interest and as an independent functional localizer for a more specific task. To quantify BOLD sensitivity on a more fundamental level, a breath holding paradigm can be used as it leads to well-observable whole brain changes in blood oxygen levels [110].

3.5 Ultra-High Field Functional MRI of the Amygdala

Increasing the number of time points by faster or longer measurements increases SNR_t by reducing the impact of noise (σ_y). Another possibility to increase the SNR_t is by increasing the amount of detectable BOLD signal (μ_y). Higher magnetic field strengths lead to an almost linear increase in image SNR [111], which only in part translates to an increase in temporal SNR_t as the reduction of thermal noise is counter acted by the increase of physiological noise [112]. Consequently, there could be concerns that increases in field strengths do not provide benefits for BOLD fMRI in the amygdala and other ventral brain areas, as they are particularly susceptible to physiological artefacts.

Using an emotional face discrimination task [79], we demonstrated that amygdala BOLD sensitivity was indeed increased at 7 T compared to 3 T in the amygdala [113]. A limitation of this study was the use of two different study populations, minor differences in the task implementations, and differences in signal parameters. To overcome this limitation, we conducted an additional study [114], where all these factors were matched between the scanner, except the echo times, to account for the different field strength specific *T2** relaxation effects.

Sixteen healthy volunteers (11f/5 m, 24.56 ± 3.1 years) underwent two sessions of fMRI in a Siemens 7 T MAGNETOM and a Siemens 3 T TIM Trio whole-body MRI scanner using a single-shot echo planar sequence with the following sequence parameters: matrix size = 128 × 128 px × 20 slices, repetition time of TR = 1.8 s, TE_{7T} /TE_{3T} = 23/40 ms, FoV = 190 × 190 mm^2, 3 mm slice thickness. We employed an emotional face (Faces) and an object discriminated task (Objects) as introduced by Hariri et al. [79]. Data were preprocessed (slice timing correction, realignment,

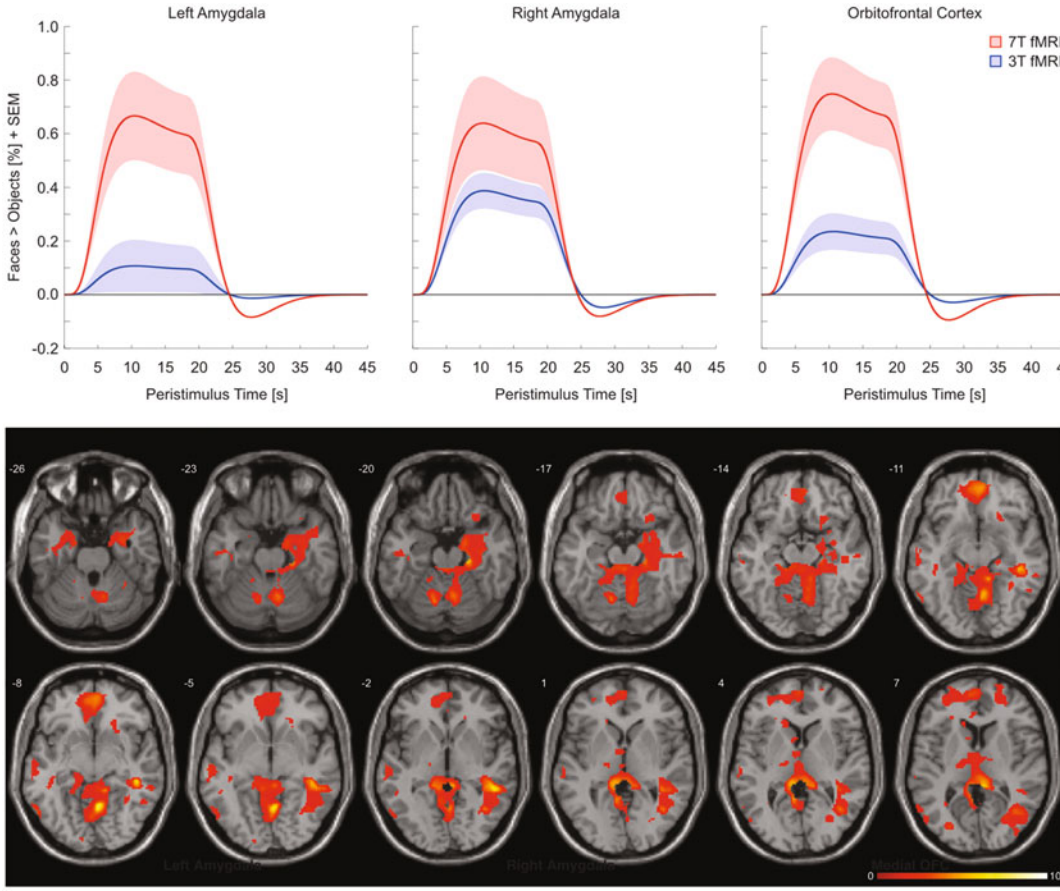

Fig. 1 Comparison of 3T and 7T amygdala fMRI. Amygdala activation was higher at 7 Tesla ($t_{right} = 6.00$, $t_{left} = 4.81$) compared to 3 Tesla ($t_{right} = 2.59$, $t_{left} = 2.71$). Activation was also increased in other ventral areas, such as the medial orbitofrontal cortex, which plays an important role in amygdala regulation. The upper panel depicts the estimated percent signal change differences between the faces and the objects condition at 3T and 7T. The lower panel depicts the paired t-test t-map with the significant increases of 7T vs. 3T

normalization, 8 mm FWHM spatial smoothing) and analyzed in SPM8 (FIL Methods Group, University College London, UK). A paired t-test was used for comparison between the 3 T and 7 T scans and revealed a significant increase in amygdala activation at 7 T (Fig. 1).

These findings are in line with theoretical predictions and support similar comparison studies [111], in particular those that targeted subcortical brain areas [113, 115]. In practice, the most important benefits of amygdala fMRI at 7 T are the possibility to increase image resolution, while maintaining optimal levels of image and temporal SNR, leading to an increased BOLD sensitivity (i.e., increasing the chance of detecting weak signal differences) and localization specificity (i.e., increasing the chance of spatially

discriminating the source of the BOLD signal) [116]. Both of these positive effects are particularly relevant for detecting and discriminating activation in amygdala subnuclei [100].

4 Conclusion

In this chapter we explained multiple crucial aspects that are relevant for discovering and using fMRI-based computational biomarkers. For illustration, we focused on the amygdala's role in anxiety disorders but argue that most of the principles will generalize to other mental disorders and target regions.

Despite recent progresses in big data science that sparked promises of data-driven biomarker discovery, we would like to emphasize the benefits of hypothesis-driven research. It aims for conception of mechanistic models of psychopathologies that are intelligible and applicable for humans, in particular clinical professionals and, ideally, patients and their families as well. Additionally, well-founded theories allow to constrain the search space, given that even successful data-driven discovery of brain–behavioral phenotype associations are complex and require large datasets of more than 50,000 individuals [117]. Finally, as we explained in this chapter, there are many diverse pitfalls on the different levels of functional neuroimaging, from biased implicit theoretical assumptions to inadequate operationalization of brain functions to insufficient data quality. Using adequate theoretical models that provide testable predictions allows for avoiding these pitfalls and uncover their reasons. Ultimately, overcoming the challenges can allow for explanatory computational biomarkers that can transform clinical practice and neuroscientific theories of psychopathologies.

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