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Role of functional magnetic resonance imaging in drug discovery

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Abstract

In this review, we survey the state of the field of functional magnetic resonance imaging (fMRI) as it relates to drug discovery and drug development. We highlight the advantages and limitations of fMRI for this purpose and suggest ways to improve the use of fMRI for developing new therapeutics, with emphasis on treatments for anxiety disorders. Fundamentally, pharmacological studies with standard psychiatric treatments using standardized behavioral probes during fMRI will need to be carried out to determine characteristic brain signatures that could be used to predict whether novel compounds are likely to have specific therapeutic effects.

Drug discovery for central nervous system drugs in general and for drugs with indications for psychiatric disorders in particular is complicated by a number of factors. Specifically, after almost five decades of a strong focus of research into the biological basis of psychiatric disorders, a basic identifiable biological mechanism for a single psychiatric disorder has yet to be identified. Thus, although numerous biological abnormalities have been described in various psychiatric disorders, there is no clear molecular, biochemical, or neural substrate etiology and pathology. As a consequence, treatments for these disorders are fundamentally based on altering the symptomatology of the disorder without a clear understanding how these symptoms emerge. This basic problem leads to a set of other issues that complicate drug discovery for psychiatric disorders.

Drug Development Phases

The development of a new drug begins with the discovery or synthesis of a novel compound and moves through preclinical and four clinical stages. Initial clues about the potential value of a new therapeutic agent usually come from animal or cell models of a disease, from inferences about the role of an agent in altering disease pathophysiology, or by chemical or physiological analogies with drugs known to have therapeutic value (US Department of Health & Human Services 2004). After the rationale for a drug as a potential therapeutic agent has been validated, a reliable assay of the drug is developed. In the preclinical phase, which typically involves testing in animal models, initial data on the safety of the drug is obtained. At this point the drug developer submits an Investigational New Drug (IND) application to the Federal Drug Administration (FDA) to gain approval for clinical trials, which can involve four phases (National Institutes of Health). Phase I trials establish the pharmacological actions of drugs in humans, evaluate side effects with increasing doses, and preliminarily assess effectiveness. Phase I might involve healthy individuals (this is most common) or patients with a disorder of potential therapeutic interest. Phase II trials are controlled patient studies that

assess the drug's effectiveness in a particular clinical condition. Phase II studies also assess common short-term side effects. Phase III trials are intended to evaluate the benefit-risk relationship of a drug in an expanded sample. These data are used to generalize to a population of individuals with the disorder. The drug developer then submits a new drug application (NDA) to the FDA. If approved, the drug developer might do post-marketing studies to obtain additional information about optimal use.

Fundamental problems of drug discovery in psychiatry

Although we will provide converging evidence for altered neural circuits functioning in anxiety, it must be acknowledged that few psychiatric disorders presently have clear neuroanatomical substrates or circuits as targets. Although abnormalities of structural and functional neural substrates are increasingly being identified for various psychiatric disorders, it is unclear whether altering these abnormalities is necessary or sufficient for successful treatment. Second, the current definition of psychiatric disorders is based on commonly agreed upon diagnostic criteria describing syndromes on a phenomenological level. Although this approach has improved reliability of diagnosis, it is unclear whether these syndromes correspond to specific biological disease entities. Instead, it is more than likely that currently defined psychiatric disorders comprise a mixture of different biological entities with variable genetic, molecular, and other biological pathologies. Third, although the current diagnostic criteria allow clinicians to group individuals into different diagnostic groups, there is considerable heterogeneity in the clinical manifestation of these disorders across individuals. Thus, due to the fact that treatment success is generally identified as symptom reduction and, given the variable expression of symptoms across individuals within a diagnostic category, it is not surprising that treatments effects are inherently variable. Fourth, due to a lack of understanding of the basic etiology of different psychiatric disorders, animal models for these conditions are based primarily on hypothesized abnormal pathophysiology or models of behavioral states that are thought to mimic those states in human mental illness. These animal models range from genetic mutant mice to specific behavioral procedures that have a certain predictive validity for some types of psychotropic drug development. Nevertheless, there is a substantial and model-specific false positive and, possibly more significant, false negative read-out for potential psychiatric therapeutics.

For example, anxiety disorders in humans are characterized by a complex set of cognitive and affective features. Anxious individuals focus on the likelihood (or, as they sometimes perceive it, inevitability) of a future aversive bodily state in certain contexts. The cognitive-behavioral conceptualization of anxiety disorders is based on behavioral theories of fear conditioning and cognitive theories that highlight the role of anxious thinking (Clark, 1986). In the cognitive-behavior literature, "anxiety sensitivity" is the construct used to describe the tendency of certain individuals to view interoceptive sensations as dangerous or threatening (Reiss, Peterson, Gursky, & McNally, 1986). Irrespective of the conceptualization, the anxious anticipation and worry characterizing anxiety disorders is fundamentally a subjective and introspective state that cannot be queried using animal models. Instead, altered learning process models, such as fear-potentiated startle or extinction learning as well as behavioral conflict models such as the Geller Seifter or Vogel conflict procedure are used to probe potential novel anxiolytics in animal paradigms (Crawley, 1985).

Given these difficulties, it is not surprising that drugs aimed at treating psychiatric conditions have some of the highest failure rates in the pharmaceutical industry (Frank & Hargreaves, 2003). This may be due to a variety of different factors. First, psychiatric treatment studies are often characterized by a high placebo response rate, which makes it difficult to identify true positives. Second, psychiatric rating scales are used to identify treatment response. These scales depend either on the accurate completion by the subject or a well-trained clinician observer.

Substantial variability in training, symptom identification, thoroughness of assessment, and other factors contribute to the limited signal to noise ratio of these instruments. Third, treatment outcomes in psychiatric disorders typically occur after days or weeks, which make it difficult to quickly examine the efficacy of these compounds. Instead, costly and long treatment trials are necessary to establish whether a novel drug significantly improves the condition. The frequent reassurance and empathic caring that is provided by clinical trialists during these protracted drug testing periods may well contribute to the high placebo response rate noted above. Taken together, the field of discovery for central nervous system drugs is riddled by difficulties and complex problems, which are not easy to address. Therefore, technological advances in probing brain functioning are being actively considered as novel candidates to help develop new psychiatric treatments. It is within this context that the use of functional magnetic resonance imaging (fMRI) for use in drug discovery has to be discussed.

Mechanics and applicability of fMRI

The human brain comprises only about 2% of the body mass yet accounts for approximately 20% of its total oxygen consumption (Shulman, Hyder, & Rothman, 2002). Deoxyhemoglobin has paramagnetic effects in the blood upon the nuclear magnetic resonance transverse relaxation times of nearby water protons in the tissue (Ogawa & Lee, 1990). The fact that changes in the oxygen level in the blood can affect the fraction of hemoglobin in the deoxygenated state can be utilized as an image contrast and was termed as blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI) (Ogawa, Lee, Nayak, & Glynn, 1990). Recent BOLD fMRI experiments in the awake human visual cortex have shown that the ratio between BOLD-fMRI signal change and baseline signal is linearly proportional to the change in blood flow relative to the baseline blood flow (Hyder et al., 2001). Moreover, increased baseline blood flow is thought to be proportional to total deoxyhemoglobin within a voxel (Buxton, Uludag, Dubowitz, & Liu, 2004). For example, increased baseline cerebral blood flow by breathing CO₂ reduces the BOLD response to the same task substantially (Kastrup, Kruger, Glover, Neumann-Haefelin, & Moseley, 1999). Therefore, the BOLD signal reflects the effect of neural activity on dynamic changes in cerebral blood flow (CBF), cerebral blood volume, and the cerebral rate of oxygen metabolism through a process generally referred to as neurovascular coupling.

In order to adequately assess the role of BOLD-fMRI within the context of drug development, one has to critically examine some of the main problems with this technique. First, the signal obtained from BOLD-fMRI is fundamentally relative, i.e., although the readout primarily measures in changes blood flow; it cannot be converted easily to a physical quantity such as volume of blood per unit time per volume of tissue. Therefore, the units are only meaningful when considered relative to some reference process. Consequently, all BOLD-fMRI experiments examine changes of the signal between some state of interest and a reference state. Second, the signal change observed in typical BOLD-fMRI experiments is of the order of 0.3–3.0% of the total signal readout from the MRI scanner. Therefore, task-induced signal changes are minute relative to the readout and are subject to other, sometimes significantly larger, noisy fluctuations. Third, the sources of noise within the signal are complex and comprise mechanical or system-level, thermal, and physiological noise (Cohen & DuBois, 1999). For example, both heart rate and breathing can profoundly affect the BOLD-fMRI signal in a complex manner (Abbott, Opdam, Briellmann, & Jackson, 2005). Fourth, signal sensitivity and reliability varies across different brain regions and is strongly influenced by the magnetic susceptibility of surrounding tissues. For example, surrounding air-filled osseous structures reduce signal readout and affect the ability to measure BOLD-fMRI changes particularly in the orbitofrontal cortex (Deichmann, Josephs, Hutton, Corfield, & Turner, 2002). Fifth, the biochemical basis of the BOLD-fMRI signal is complex and incompletely understood (Buxton et al., 2004). Thus, as opposed to positron emission tomography (PET), which enables one to specifically examine

the concentration of a chemical in different brain areas, BOLD-fMRI is fundamentally unspecific and a resultant of possibly many different chemical processes. Lastly, because of the complex nature of the signal, complicated and multi-step statistical signal post-processing methods have been used to improve the signal quality. However, there is no agreed upon standard post-processing pathway within the neuroimaging community. Thus, reports on brain activation changes have to be carefully examined with respect to the post-processing techniques applied.

It may appear from the limitations and problems listed above that BOLD-fMRI is, at best, a questionable technical advance for brain imaging. Nevertheless, there has been an explosion of neuroimaging studies using this tool, which have resulted in a surprisingly consistent systems neuroscience literature ranging from such basic functions as the cortical processing of vision to such complicated cognitive processes as working memory. The increased use of BOLD-fMRI is a consequence of several profound advantages of this neuroimaging technique in comparison to previously prominent neuroimaging tools. First, BOLD-fMRI is fundamentally non-invasive, i.e., imaging the brain does not require the subject to ingest or be injected with a substance or to have blood drawn. Second, this technique is relatively in-expensive. Hourly rates for functional MRI scans range from \$500–\$1000, making per-subject imaging costs a fraction of the cost of PET studies. Third, MRI systems that are capable of measuring BOLD-fMRI signals are almost ubiquitously available. Fourth, no radioactive tracers need to be injected, which allows for repeated scanning of the same subject. Fifth, the combination of clever behavioral probes and BOLD-fMRI enables us to probe the neural substrates underlying specific behavioral processes in unprecedented ways. This is particularly useful for psychiatric disorders because these individuals relative to comparison subjects rarely show global cognitive or affective abnormalities but rather subtle processing differences. Sixth, effect sizes reported from neuroimaging experiments have generally been large even when considering relatively subtle behavioral processes. The presence of a large effect has resulted in many fMRI findings from experiments with relatively few number of subjects ($n = 5-15$). Similar effect sizes have been obtained in pharmacological fMRI studies (Arce, Miller, Feinstein, Stein, & Paulus, 2006; Paulus, Feinstein, Castillo, Simmons, & Stein, 2005), which support the idea that one can conduct relatively small scale (and, therefore, relatively inexpensive) proof of concept studies. For all these reasons, BOLD-fMRI has become the predominant neuroimaging tool to examine brain functioning.

The importance of task development

An often under-appreciated aspect of BOLD-fMRI is that the interpretation of the results critically depends on the details of the task conducted during the imaging procedure. As pointed out above, only signal changes can be meaningfully interpreted. Whenever a neural substrate is being described as “activated” in a study or whenever two populations are described as differing in their activation patterns, one has to keep in mind that these differences are described in the context of the task conducted. For example, the amygdala plays a critical role in normal fear conditioning and is implicated in the pathophysiology of anxiety disorders (Rauch, Shin, & Wright, 2003b; Charney, 2003). This structure is also important for other emotional information processing and behavior (LeDoux, 1992). Functional neuroimaging studies have shown amygdala activation in fear conditioning (Buchel, Morris, Dolan, & Friston, 1998), reward related processing (Breiter & Rosen, 1999), encoding of emotionally salient information (Canli, Zhao, Brewer, Gabrieli, & Cahill, 2000), risk-taking (Ernst et al., 2002), processing positively valenced stimuli (Garavan, Pendergrass, Ross, Stein, & Risinger, 2001), and appetitive or aversive olfactory learning (Gottfried, O’Doherty, & Dolan, 2002). Individuals with social anxiety disorder (Stein, Goldin, Sareen, Zorrilla, & Brown, 2002b) or posttraumatic stress disorder (Rauch et al., 2000a) show amygdala hyperresponsivity to fearful or angry faces. However, amygdala functioning as assessed by BOLD-fMRI depends critically

on the reference condition, i.e., what the investigator uses as a task that is thought to induce less neuronal activation in this structure. Therefore, it is not surprising that altered amygdala functioning in individuals with specific anxiety disorders has been reported in some studies (Shin et al., 2005; Bryant et al., 2005) but not others (Shin et al., 2001). Taken together, although there are significant limitations with BOLD-fMRI, this technique has substantial advantages over other neuroimaging tools, which make it an attractive candidate for its use in drug development.

Facial emotion processing and PharmacofMRI

We and other investigators have made extensive use of facial emotion processing tasks as neuroimaging probes. To identify, recognize, and respond to facial emotional stimuli is a complex process. This involves a well-studied neural circuitry, which is altered in individuals with anxiety disorders (REFS). Adjacent to extrastriate cortex are cortical areas that are highly specialized for face processing (Haxby et al., 1994). In particular, bilateral lingual/fusiform gyri and the right parahippocampal gyrus are almost always involved in facial processing (Kapur, Friston, Young, Frith, & Frackowiak, 1995). Processing of faces in this area takes place within 165 ms (Halgren, Raij, Marinkovic, Jousmaki, & Hari, 2000) and the amygdala is required to link visual representations of facial expressions with affective representations such as fear (Adolphs, Tranel, Damasio, & Damasio, 1995). Some groups have suggested that the amygdala is more sensitive to fear relative to other emotional expressions (Morris et al., 1996a), and is involved even in the absence of awareness (Whalen et al., 1998a), which may be mediated via subcortical pathway to the right amygdala, via midbrain and thalamus (Morris, Ohman, & Dolan, 1999). Moreover, an extended circuitry comprising the amygdala, pulvinar, anterior insula and anterior cingulate activates during the processing of fearful faces (Morris et al., 1998), which also appears to be engaged whenever an explicit emotion face judgment is required (Gorno-Tempini et al., 2001). Some investigators have argued that left and right amygdala and extended limbic areas are differentially involved in negative versus positive emotion processing, respectively. For example, left amygdala activity was associated with stronger activation during negative valenced face presentation. In comparison, right amygdaloid activity was stronger when positive facial expressions were evaluated (Iidaka et al., 2001). Others have found emotional expressions of happiness, fear, and sadness but not anger are recognized more efficiently in the right versus the left hemiface (Indersmitten & Gur, 2003). In summary, there is good affective neuroscience support for the role of the amygdala and associated limbic structures for the affective processing of faces.

Several pharmacological studies have used face emotion paradigms during fMRI to begin to delineate the modulation of different neurotransmitter systems. For example, cholinergic stimulation using physostigmine increased activation in anterior fusiform gyrus (Bentley, Vuilleumier, Thiel, Driver, & Dolan, 2003). In contrast, NMDA modulation using ketamine decreased activation in fusiform gyrus and other cortical face processing areas (Abel et al., 2003). Others have found during behavioral studies that antidepressants also affect the processing of emotional faces. Specifically, acute administration of citalopram resulted in a higher correct detection rate and reduced response latency of facial expressions of fear and happiness (Harmer et al., 2003). In contrast, reduction of serotonergic modulation by tryptophan depletion impaired the recognition of fearful facial expressions (Harmer, Rogers, Tunbridge, Cowen, & Goodwin, 2003). The degree to which neural systems involved in this process are changed by serotonergic modulation, however, may be mediated by baseline characteristics of the subjects such as threat sensitivity (Cools et al., 2005). Finally, low doses of alcohol improve emotional discrimination of happy faces (Kano et al., 2003).

Applications of BOLD-fMRI to drug development

Given this background, what are the fundamental questions that fMRI can address in drug development? There are several different application of this technique, however, we will limit ourselves in this review to human BOLD-fMRI and refer to other sources discussing its utility for preclinical development. First, BOLD-fMRI can be used to examine how a drug affects neural substrates that are important for a particular cognitive, affective, or experiential process. For example, we have recently used a simple emotional face processing task to probe how an anxiolytic affects amygdala functioning in healthy volunteers (Paulus et al., 2005). In this study, we were able to show that the benzodiazepine, lorazepam, attenuated activation in the amygdala in a dose dependent manner when visually processing emotional faces relative to processing circles and ovals. Moreover, an anxiolytic dose but not a non-anxiolytic dose of this drug significantly reduced the activation in this structure relative to the placebo condition. Finally, lorazepam did not alter brain activation in visual cortex, which argues against a non-specific drug effect. In combination, this study showed that an anxiety-relevant brain structure could be affected by an anxiolytic at doses that are known to reduce anxiety. In this study, there was no change in levels of anxiety in these healthy volunteers after the higher (typically anxiolytic) dose of lorazepam, which was possibly due to the relatively low level of anxiety among the participants, which may have yielded a “floor effect”. Second, BOLD-fMRI can be used to establish whether a class of drugs, e.g., benzodiazepines, has a “common brain signature”. Specifically, by conducting a series of dose-response experiments with different benzodiazepines one can begin to determine whether the reduction of amygdala activation during emotional face processing is a common feature of all benzodiazepines and possibly relates to the potency of the drug as an anxiolytic. Third, BOLD-fMRI dose-response experiments with anxiolytic and non-anxiolytic drugs can be used to delineate the specificity and sensitivity of this technique in identifying an even broader anxiolytic “brain signature”. This would be particularly important for identifying novel drugs or existing therapeutics with novel indications. Fourth, by extending these experiments to populations of psychiatric patients one can begin to determine whether individuals with anxiety disorders have a qualitatively or quantitatively different brain signature in response to administration of anxiolytic agents. Moreover, one can begin to use this technique to delineate these brain signatures for different anxiety disorders in order to differentiate these disorders from one another. Fifth, one can begin to delineate similarities and differences in brain signatures for classes of therapeutics in response to acute versus chronic administration. This is particularly relevant for the future application of this tool in the drug discovery process. For example, it would be important to determine whether the acute brain signature in response to antidepressants such as serotonin specific reuptake inhibitors (SSRIs) differs from that of the chronic brain signature. The answer to this question is important for the predictive validity of this technique because chronic but not acute administration of this class of drugs is frequently used to treat anxiety.

Currently, the position of BOLD-fMRI in the drug development process is unclear. Ideally, one would like to identify an acute brain signature in healthy volunteers that has predictive validity for anxiolytic action in clinical populations. For example, if attenuation of amygdala activation during emotional face processing in healthy volunteers predicts anxiolytic action, one would be able to conduct small scale proof of concept trials in non-disorder populations to screen out potential anxiolytic agents. The use of acute versus chronic administration of drug and the ability to recruit healthy volunteers as opposed to clinical populations would significantly reduce cost and shorten the duration of the study.

In this case, BOLD-fMRI would become an invaluable tool during early phase I development of compounds. Nevertheless, even if one would need to conduct chronic administration in disorder populations to obtain predictive validity, one may be able to conduct multi-center

trials, which include BOLD-fMRI as a component in relatively few subjects to obtain a critical brain signature.

Hurdles to anxiolytic drug development

There is no shortage of new drug development candidates for anxiety (Gilligan & Li, 2004; Bergink, van Megen, & Westenberg, 2004; Spooen & Gasparini, 2004; Korpi & Sinkkonen, 2005; Nemeroff & Vale, 2005). A plethora of preclinical research, including major advances in molecular neuroscience (especially gene expression) (Gordon & Hen, 2004), point to alterations in a variety of key neurotransmitter and neuromodulatory systems in the pathogenesis of anxiety states (Holmes, Yang, Lesch, Crawley, & Murphy, 2003; Lesch, Zeng, Reif, & Gutknecht, 2003; Gross et al., 2002; Santarelli et al., 2001; Merikangas & Pine, 2004; Charney & Bremner, 2004). Despite these remarkable advances in understanding, no new class of anxiolytic drug has reached the market in the past two decades! This speaks to the difficulty in moving compounds from Phase I to Phase II and Phase III human studies. Current state-of-the-art is to pick a lead candidate – based almost entirely on Phase I safety and pharmacokinetic considerations – and to move it into large-scale clinical trials in patients with particular anxiety disorders, often with little or no understanding of optimal dosing for the anxiety target. PharmacofMRI techniques would be applied to relatively small numbers of subjects as a preemptory step in the earliest part of Phase II, using compounds that have shown promise and safety in Phase I. The availability of human biomarkers of anxiolytic efficacy would substantially facilitate the movement of anxiolytic drugs from pipeline to clinic by enhancing the likelihood that a compound chosen for registration trials would be efficacious. This would reduce much of the cost (and hence, much of the risk) of bringing new compounds beyond Phase I and would enable drug developers to consider a wider range of candidates as potential anti-anxiety therapies.

A biomarker (Frank et al., 2003) is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention. Biomarkers are proposed to measure the delivery of drugs to their intended targets and to understand and predict the pathophysiology and how it is altered by therapy through monitoring variables known to have clinical relevance. Biomarkers are especially valuable, as they can help to prioritize drug discovery resources by enabling early proof-of-concept studies for novel therapeutic targets. A useful biomarker should meet the following requirements: (1) a consistent response across studies and drugs; (2) a clear response of the biomarker to a therapeutic dose; (3) a dose-response relationship; (4) and a plausible relationship between biomarker, pharmacology or pathogenesis. Though we are aware that the use of biomarkers to predict anti-anxiety therapeutics has been much bandied about in a drug development context, we are aware of precious few cases where such approaches have been implemented and even fewer where they have been successful. The use of a variety of biomarkers for benzodiazepine efficacy – including slowing in saccadic peak velocity, visual analogue scales showing reduced alertness, and changes in spectral electroencephalographic (EEG) – was the subjects of a recent review (de Visser et al., 2003) where the authors concluded that sensitivity and reproducibility of the tests was low. Another potential biomarker, the reduction in fear-potentiated startle (FPS) with a particular drug, has been used to make inferences about the drug's anxiolytic potential (Grillon, Cordova, Levine, & Morgan, III, 2003). Although promising and deserving of further study in this regard, the fact that benzodiazepines – a well-established class of anxiolytic drugs – have no effect on FPS in humans (Baas et al., 2002) suggests that this particular approach has serious limitations as a human bioassay for anxiety efficacy. Carbon dioxide inhalation, thought to be a susceptibility marker for panic disorder (Stein & Rapee, 1998), has had some limited testing in this context (Gorman, Martinez, Coplan, Kent, & Kleber, 2004), but will require further development and may be restricted to identifying compounds with promise for panic – but not other anxiety

disorders. Other approaches are clearly needed, particularly those that reflect biological processes even more proximate to the neural circuitry underlying anxiety and its disorders.

Without reviewing the voluminous literature on neuroimaging and anxiety (see (Cannistraro & Rauch, 2003) for an excellent review in this regard), we do wish to highlight several studies that indicate the likely involvement of candidate brain regions for the projects proposed in this application. The amygdala is thought to play an important role in the response to fear, as evidenced by amygdala activation to emotional (usually fearful or angry) human faces (possibly to the eyes themselves (Whalen et al., 2004)) in numerous positron emission tomography (PET) and fMRI studies (see (Rauch, Shin, & Wright, 2003a) for review; (Davidson & Irwin, 1999; Morris et al., 1996b; Whalen et al., 1998b)). Interestingly, exaggerated amygdala activation to emotional human faces has been noted in several of the anxiety disorders – namely generalized social phobia (GSP) (Stein, Goldin, Sareen, Zorrilla, & Brown, 2002a) and posttraumatic stress disorder (PTSD) (Rauch et al., 2000b) and also probably in panic disorder (PD) and generalized anxiety disorder (GAD) (Thomas et al., 2001), whereas it has *not* been observed in either specific phobia (Wright, Martis, McMullin, Shin, & Rauch, 2003) or obsessive compulsive disorder (OCD) (Cannistraro et al., 2004). These differential task effects across anxiety disorders serve as a reminder that the neural circuitry of all anxiety disorders is not uniform, and indicate that a bioassay for “anti-anxiety” activity based on changes in activity in neural structures will almost certainly be constrained in its predictive validity to those disorders that share this circuit dysfunction. For example, in the case of an emotional face task designed to elicit amygdala activation that could be modified by drug, the a priori expectation might be that it would be predictive of efficacy in GSP, PTSD, PD, and GAD, but not necessarily in specific phobia or OCD. Although it would be premature at this stage to test differential effects across subjects with different anxiety disorders, we will take a dimensional approach and measure distinct symptom domains (e.g., social anxiety – a cardinal symptom of GSP; worry – a cardinal symptom of GAD; anxiety sensitivity – a cardinal symptom of PD) to determine if these differentially influence response. This approach has proven fruitful in recent fMRI research where social anxiety symptoms were found to best predict amygdala activation in adolescents viewing fearful faces (Killgore & Yurgelun-Todd, 2005).

As detailed above, there is a strong rationale for hypothesizing that the use of cognitive and/or behavioral paradigms in conjunction with fMRI can activate neural circuits that are important for the experience of anxiety and in the pathophysiology of certain anxiety disorders. There is also preliminary evidence that activity within key elements (e.g., amygdala) of these circuits can be modified with known anti-anxiety treatments, and that the extent of change may correlate with therapeutic response. Taken together, this information would suggest that measurement of change in BOLD activity within key neural structures following the administration of a drug would be indicative of the anxiolytic potential of that drug. This model, if validated, would provide a human *in vivo* bioassay of anxiolytic activity, and could be used to predict therapeutic efficacy of new anti-anxiety compounds or even new classes of anti-anxiety agents. A caveat, as noted above, is that the phenomenon of “anxiety” is itself heterogeneous, and we must be alert to the possibility (indeed, to the likelihood) that some circuits will mediate global aspects of anxiety whereas others may be more symptom specific. Once the particulars of these dimensional phenomena are better understood at a brain systems level, this caveat could actually become a strength of this approach by enabling the more targeted prediction of response (e.g., prediction of whether a particular compound is likely to be effective for obsessive compulsive disorder as opposed to panic disorder).

Following this presumptive new development pathway, knowledge of a drug’s safety and tolerability profile would emerge from Phase I, and several doses of drug could be administered to a relatively small number of subjects (dependent on the sensitivity of the bioassay, but it

would need to be considerably better than the gold standard which, at present, is self-report of anxiety symptoms) who participate in pharmacofMRI testing in a new, earlier stage of Phase II. This bioassay would then, in theory, provide information not only about the likely efficacy of the drug as an anxiolytic, but also information about its probable anti-anxiety dose-response relationship. These data would be used, in conjunction with other complementary findings (e.g., toxicity) that emerges in Phase I, to inform a “go, no-go” decision for further, larger-scale Phase II RCT testing, and would enlighten selection of the dose(s) of drug to be tested in such trials.

The future of pharmacofMRI and anxiolytic drug development

There are many gaps in current knowledge that must be bridged before this technique can be successfully and routinely applied to the drug development process. In order to function as a test of predictive validity of new anti-anxiety compounds, pharmacofMRI in this context must meet certain stringent requirements for human bioassay validation. There are basically five steps that need to be considered. First, one has to identify a brain area that is important for anxiety and has been shown to be functionally altered in anxiety disorders. Second, one has to identify an experimental paradigm that probes this brain area. This experimental paradigm should be sensitive to the behavioral effects of anxiety, show no ceiling or floor effects, be repeatable with negligible learning effects (i.e., good test-retest reliability), should be simple and relatively independent of “volitional” effects, sensitive to basic pharmacological manipulations, activate areas in the brain that are of relevance for anxiety, show behavioral effects and/or brain imaging effects that correlate with ratings of anxiety. Third, one has to determine whether there is a correlation between reduction in anxiety and BOLD change in the predicted direction with standard anxiolytics. Fourth, one has to demonstrate that standard anxiolytic drugs affect the brain area in the hypothesized direction. Moreover, this effect should occur in a dose-response relationship. Finally, for BOLD-fMRI to be useful in drug discovery one has to demonstrate advantages over the “gold standard” (i.e., standardized rating scales) in one or more of: effect size for drug-placebo differences, reduced exposure to risk, or cost. Though several of these criteria have already been fully or partially met others have not yet been tested. Importantly, it must also be demonstrated that non-specific drug effects on cerebral vasculature can be differentiated from specific effects of drug on activity within key neuronal structures (Brown, Jernigan, & Cato, 2005). Of direct relevance to our application, recent research suggests that with respect to test-retest reliability (i.e., “stability”) of the BOLD response to affective faces, certain contrasts are more stable than others (e.g., emotional faces vs. baseline cf. emotional vs. neutral faces) (Johnstone et al., 2005).

Pharmacological studies using BOLD-fMRI represent an emerging discipline with potential to address a variety of neural systems and drug development questions (Salmeron & Stein, 2002) aside from its role in drug development. Among the most exciting new advances over the past 10 years in the field of drug development has been the implementation of biomarkers to speed the development cycle. Several biomarkers have been developed for examining treatment effects in Alzheimers disease, however, few biomarkers have yet been developed for psychiatric disorders. BOLD-fMRI provides a unique technique to inquire on a systems neuroscience level about the functioning of brain areas as they relate to many cognitive and affective processes. Moreover, this relatively inexpensive, non-invasive technique can be repeated many times to obtain brain activation patterns. The precise role of BOLD-fMRI in the drug development pipeline is yet to be identified. Nevertheless, initial results are promising and support cautious optimism that this technique may be one of the major technological advances in decades to improve the development of therapeutics for brain diseases.

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Reference List

- Abbott DF, Opdam HI, Briellmann RS, Jackson GD. Brief breath holding may confound functional magnetic resonance imaging studies. *Hum Brain Mapp* 2005;24:284–290. [PubMed: 15678482]
- Abel KM, Allin MP, Kucharska-Pietura K, David A, Andrew C, Williams S, et al. Ketamine alters neural processing of facial emotion recognition in healthy men: an fMRI study. *Neuroreport* 2003;14:387–391. [PubMed: 12634489]
- Adolphs R, Tranel D, Damasio H, Damasio AR. Fear and the human amygdala. *J Neurosci* 1995;15:5879–5891. [PubMed: 7666173]
- Arce E, Miller DA, Feinstein JS, Stein MB, Paulus MP. Lorazepam dose-dependently decreases risk-taking related activation in limbic areas. *Psychopharmacology (Berl)*. 2006
- Baas JM, Grillon C, Bocker KB, Brack AA, Morgan CA III, Kenemans JL, et al. Benzodiazepines have no effect on fear-potentiated startle in humans. *Psychopharmacology (Berl)* 2002;161:233–247. [PubMed: 12021826]
- Bentley P, Vuilleumier P, Thiel CM, Driver J, Dolan RJ. Cholinergic enhancement modulates neural correlates of selective attention and emotional processing. *Neuroimage* 2003;20:58–70. [PubMed: 14527570]
- Bergink V, van Megen HJ, Westenberg HG. Glutamate and anxiety. *Eur Neuropsychopharmacol* 2004;14:175–183. [PubMed: 15056476]
- Breiter HC, Rosen BR. Functional magnetic resonance imaging of brain reward circuitry in the human. *Ann N Y Acad Sci* 1999;877:523–547. [PubMed: 10415669]
- Brown, GG.; Jernigan, T.; Allison Cato, M. FMRI in neuropsychiatric disorders. In: Edelman, RR.; Hesselink, JR.; Zlatkin, MB.; Crues, JV., III, editors. *Clinical Magnetic Resonance imaging*. 3. London: Elsevier; 2005.
- Bryant RA, Felmingham KL, Kemp AH, Barton M, Peduto AS, Rennie C, et al. Neural networks of information processing in posttraumatic stress disorder: a functional magnetic resonance imaging study. *Biol Psychiatry* 2005;58:111–118. [PubMed: 16038681]
- Buchel C, Morris J, Dolan RJ, Friston KJ. Brain systems mediating aversive conditioning: an event-related fMRI study. *Neuron* 1998;20:947–957. [PubMed: 9620699]
- Buxton RB, Uludag K, Dubowitz DJ, Liu TT. Modeling the hemodynamic response to brain activation. *Neuroimage* 2004;23(Suppl 1):S220–233. [PubMed: 15501093]
- Canli T, Zhao Z, Brewer J, Gabrieli JD, Cahill L. Event-related activation in the human amygdala associates with later memory for individual emotional experience. *J Neurosci* 2000;20:RC99. [PubMed: 11000199]
- Cannistraro PA, Rauch SL. Neural circuitry of anxiety: evidence from structural and functional neuroimaging studies. *Psychopharmacol Bull* 2003;37:8–25. [PubMed: 15131515]
- Cannistraro PA, Wright CI, Wedig MM, Martis B, Shin LM, Wilhelm S, et al. Amygdala responses to human faces in obsessive-compulsive disorder. *Biol Psychiatry* 2004;56:916–920. [PubMed: 15601600]
- Charney DS. Neuroanatomical circuits modulating fear and anxiety behaviors. *Acta Psychiatr Scand Suppl* 2003;38–50. [PubMed: 12950435]
- Charney, DS.; Bremner, JD. The neurobiology of anxiety disorders. In: Charney, DS.; Nestler, EJ., editors. *Neurobiology of Mental Illness*. 2. New York: Oxford University Press; 2004. p. 605–627.
- Clark DM. A cognitive approach to panic. *Behav Res Ther* 1986;24:461–470. [PubMed: 3741311]
- Cohen MS, DuBois RM. Stability, repeatability, and the expression of signal magnitude in functional magnetic resonance imaging. *J Magn Reson Imaging* 1999;10:33–40. [PubMed: 10398975]
- Cools R, Calder AJ, Lawrence AD, Clark L, Bullmore E, Robbins TW. Individual differences in threat sensitivity predict serotonergic modulation of amygdala response to fearful faces. *Psychopharmacology (Berl)*. 2005

- Crawley JN. Exploratory behavior models of anxiety in mice. *Neuroscience and Biobehavioral Reviews* 1985;9:37–44. [PubMed: 2858080]
- Davidson RJ, Irwin W. The functional neuroanatomy of emotion and affective style. *Trends Cogn Sci* 1999;3:11–21. [PubMed: 10234222]
- de Visser SJ, van der Post JP, de Waal PP, Cornet F, Cohen AF, van Gerven JM. Biomarkers for the effects of benzodiazepines in healthy volunteers. *Br J Clin Pharmacol* 2003;55:39–50. [PubMed: 12534639]
- Deichmann R, Josephs O, Hutton C, Corfield DR, Turner R. Compensation of susceptibility-induced BOLD sensitivity losses in echo-planar fMRI imaging. *Neuroimage* 2002;15:120–135. [PubMed: 11771980]
- Ernst M, Bolla K, Mouratidis M, Contoreggi C, Matochik JA, Kurian V, et al. Decision-making in a Risk-taking Task. A PET Study. *Neuropsychopharmacology* 2002;26:682–691. [PubMed: 11927193]
- Frank R, Hargreaves R. Clinical biomarkers in drug discovery and development. *Nat Rev Drug Discov* 2003;2:566–580. [PubMed: 12838269]
- Garavan H, Pendergrass JC, Ross TJ, Stein EA, Risinger RC. Amygdala response to both positively and negatively valenced stimuli. *Neuroreport* 2001;12:2779–2783. [PubMed: 11522965]
- Gilligan PJ, Li YW. Corticotropin-releasing factor antagonists: recent advances and exciting prospects for the treatment of human diseases. *Curr Opin Drug Discov Devel* 2004;7:487–497.
- Gordon JA, Hen R. Genetic approaches to the study of anxiety. *Annu Rev Neurosci* 2004;27:193–222. [PubMed: 15217331]
- Gorman JM, Martinez J, Coplan JD, Kent J, Kleber M. The effect of successful treatment on the emotional and physiological response to carbon dioxide inhalation in patients with panic disorder. *Biol Psychiatry* 2004;56:862–867. [PubMed: 15576063]
- Gorno-Tempini ML, Pradelli S, Serafini M, Pagnoni G, Baraldi P, Porro C, et al. Explicit and incidental facial expression processing: an fMRI study. *Neuroimage* 2001;14:465–473. [PubMed: 11467919]
- Gottfried JA, O'Doherty J, Dolan RJ. Appetitive and aversive olfactory learning in humans studied using event-related functional magnetic resonance imaging. *J Neurosci* 2002;22:10829–10837. [PubMed: 12486176]
- Grillon C, Cordova J, Levine LR, Morgan CA III. Anxiolytic effects of a novel group II metabotropic glutamate receptor agonist (LY354740) in the fear-potentiated startle paradigm in humans. *Psychopharmacology (Berl)* 2003;168:446–454. [PubMed: 12709777]
- Gross C, Zhuang X, Stark K, Ramboz S, Oosting R, Kirby L, et al. Serotonin1A receptor acts during development to establish normal anxiety-like behaviour in the adult. *Nature* 2002;416:396–400. [PubMed: 11919622]
- Halgren E, Raji T, Marinkovic K, Jousmaki V, Hari R. Cognitive response profile of the human fusiform face area as determined by MEG. *Cereb Cortex* 2000;10:69–81. [PubMed: 10639397]
- Harmer CJ, Bhagwagar Z, Perrett DI, Vollm BA, Cowen PJ, Goodwin GM. Acute SSRI administration affects the processing of social cues in healthy volunteers. *Neuropsychopharmacology* 2003;28:148–152. [PubMed: 12496951]
- Harmer CJ, Rogers RD, Tunbridge E, Cowen PJ, Goodwin GM. Tryptophan depletion decreases the recognition of fear in female volunteers. *Psychopharmacology (Berl)* 2003;167:411–417. [PubMed: 12677354]
- Haxby JV, Horwitz B, Ungerleider LG, Maisog JM, Pietrini P, Grady CL. The functional organization of human extrastriate cortex: a PET-rCBF study of selective attention to faces and locations. *Journal of Neuroscience* 1994;14:6336–6353. [PubMed: 7965040]
- Holmes A, Yang RJ, Lesch KP, Crawley JN, Murphy DL. Mice Lacking the Serotonin Transporter Exhibit 5-HT(1A) Receptor-Mediated Abnormalities in Tests for Anxiety-like Behavior. *Neuropsychopharmacology* 2003;28:2077–2088. [PubMed: 12968128]
- Hyder F, Kida I, Behar KL, Kennan RP, Maciejewski PK, Rothman DL. Quantitative functional imaging of the brain: towards mapping neuronal activity by BOLD fMRI. *NMR Biomed* 2001;14:413–431. [PubMed: 11746934]
- Iidaka T, Omori M, Murata T, Kosaka H, Yonekura Y, Okada T, et al. Neural interaction of the amygdala with the prefrontal and temporal cortices in the processing of facial expressions as revealed by fMRI. *J Cogn Neurosci* 2001;13:1035–1047. [PubMed: 11784442]

- Indersmitten T, Gur RC. Emotion processing in chimeric faces: hemispheric asymmetries in expression and recognition of emotions. *J Neurosci* 2003;23:3820–3825. [PubMed: 12736352]
- Johnstone T, Somerville LH, Alexander AL, Oakes TR, Davidson RJ, Kalin NH, et al. Stability of amygdala BOLD response to fearful faces over multiple scan sessions. *NeuroImage* 2005;25:1112–1123. [PubMed: 15850729]
- Kano M, Gyoba J, Kamachi M, Mochizuki H, Hongo M, Yanai K. Low doses of alcohol have a selective effect on the recognition of happy facial expressions. *Hum Psychopharmacol* 2003;18:131–139. [PubMed: 12590407]
- Kapur N, Friston KJ, Young A, Frith CD, Frackowiak RS. Activation of human hippocampal formation during memory for faces: a PET study. *Cortex* 1995;31:99–108. [PubMed: 7781323]
- Kastrup A, Kruger G, Glover GH, Neumann-Haefelin T, Moseley ME. Regional variability of cerebral blood oxygenation response to hypercapnia. *Neuroimage* 1999;10:675–681. [PubMed: 10600413]
- Killgore WD, Yurgelun-Todd DA. Social anxiety predicts amygdala activation in adolescents viewing fearful faces. *NeuroReport* 2005;16:1671–1675. [PubMed: 16189475]
- Korpi ER, Sinkkonen ST. GABA(A) receptor subtypes as targets for neuropsychiatric drug development. *Pharmacol Ther*. 2005
- LeDoux JE. Brain mechanisms of emotion and emotional learning. *Curr Opin Neurobiol* 1992;2:191–197. [PubMed: 1638153]
- Lesch KP, Zeng Y, Reif A, Gutknecht L. Anxiety-related traits in mice with modified genes of the serotonergic pathway. *Eur J Pharmacol* 2003;480:185–204. [PubMed: 14623362]
- Merikangas, KR.; Pine, DS. Genetic and other vulnerability factors for anxiety and stress disorders. In: Davis, KL.; Charney, DS.; Coyle, JT.; Nemeroff, CB., editors. *Neuropsychopharmacology: Fifth Generation of Progress*. Philadelphia: Williams & Wilkins; 2004. p. 867-882.
- Morris JS, Friston KJ, Buchel C, Frith CD, Young AW, Calder AJ, et al. A neuromodulatory role for the human amygdala in processing emotional facial expressions. *Brain* 1998;121:47–57. [PubMed: 9549487]
- Morris JS, Frith CD, Perrett DI, Rowland D, Young AW, Calder AJ, et al. A differential neural response in the human amygdala to fearful and happy facial expressions. *Nature* 1996a;383:812–815. [PubMed: 8893004]
- Morris JS, Frith CD, Perrett DI, Rowland D, Young AW, Calder AJ, et al. A differential neural response in the human amygdala to fearful and happy facial expressions. *Nature* 1996b;383:812–815. [PubMed: 8893004]
- Morris JS, Ohman A, Dolan RJ. A subcortical pathway to the right amygdala mediating "unseen" fear. *Proc Natl Acad Sci USA* 1999;96:1680–1685. [PubMed: 9990084]
- National Institutes of Health. <http://clinicaltrials.gov>
- Nemeroff CB, Vale WW. The neurobiology of depression: inroads to treatment and new drug discovery. *J Clin Psychiatry* 2005;66(Suppl 7):5–13. [PubMed: 16124836]
- Ogawa S, Lee TM. Magnetic resonance imaging of blood vessels at high fields: in vivo and in vitro measurements and image simulation. *Magnetic Resonance in Medicine* 1990;16:9–18. [PubMed: 2255240]
- Ogawa S, Lee TM, Nayak AS, Glynn P. Oxygenation-sensitive contrast in magnetic resonance image of rodent brain at high magnetic fields. *Magnetic Resonance in Medicine* 1990;14:68–78. [PubMed: 2161986]
- Paulus MP, Feinstein JS, Castillo G, Simmons AN, Stein MB. Dose-dependent decrease of activation in bilateral amygdala and insula by Lorazepam during emotion processing. *Arch Gen Psychiatry* 2005;62:282–288. [PubMed: 15753241]
- Rauch SL, Shin LM, Wright CI. Neuroimaging studies of amygdala function in anxiety disorders. *Ann N Y Acad Sci* 2003b;985:389–410. [PubMed: 12724173]
- Rauch SL, Shin LM, Wright CI. Neuroimaging studies of amygdala function in anxiety disorders. *Ann N Y Acad Sci* 2003a;985:389–410. [PubMed: 12724173]
- Rauch SL, Whalen PJ, Shin LM, McInerney SC, Macklin ML, Lasko NB, et al. Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: a functional MRI study. *Biol Psychiatry* 2000a;47:769–776. [PubMed: 10812035]

- Rauch SL, Whalen PJ, Shin LM, McInerney SC, Macklin ML, Lasko NB, et al. Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: a functional MRI study. *Biol Psychiatry* 2000b;47:769–776. [PubMed: 10812035]
- Reiss S, Peterson RA, Gursky DM, McNally RJ. Anxiety sensitivity, anxiety frequency and the prediction of fearfulness. *Behav Res Ther* 1986;24:1–8. [PubMed: 3947307]
- Salmeron BJ, Stein EA. Pharmacological applications of magnetic resonance imaging. *Psychopharmacol Bull* 2002;36:102–129. [PubMed: 12397851]
- Santarelli L, Gobbi G, Debs PC, Sibille ET, Blier P, Hen R, et al. Genetic and pharmacological disruption of neurokinin 1 receptor function decreases anxiety-related behaviors and increases serotonergic function. *Proc Natl Acad Sci USA* 2001;98:1912–1917. [PubMed: 11172050]
- Shin LM, Whalen PJ, Pitman RK, Bush G, Macklin ML, Lasko NB, et al. An fMRI study of anterior cingulate function in posttraumatic stress disorder. *Biol Psychiatry* 2001;50:932–942. [PubMed: 11750889]
- Shin LM, Wright CI, Cannistraro PA, Wedig MM, McMullin K, Martis B, et al. A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. *Arch Gen Psychiatry* 2005;62:273–281. [PubMed: 15753240]
- Shulman RG, Hyder F, Rothman DL. Biophysical basis of brain activity: implications for neuroimaging. *Q Rev Biophys* 2002;35:287–325. [PubMed: 12599751]
- Spooren W, Gasparini F. mGlu5 receptor antagonists: a novel class of anxiolytics. *Drug News Perspect* 2004;17:251–257. [PubMed: 15334174]
- Stein MB, Goldin PR, Sareen J, Zorrilla LT, Brown GG. Increased amygdala activation to angry and contemptuous faces in generalized social phobia. *Arch Gen Psychiatry* 2002b;59:1027–1034. [PubMed: 12418936]
- Stein MB, Goldin PR, Sareen J, Zorrilla LT, Brown GG. Increased amygdala activation to angry and contemptuous faces in generalized social phobia. *Arch Gen Psychiatry* 2002a;59:1027–1034. [PubMed: 12418936]
- Stein, MB.; Rapee, RM. Anxiety sensitivity: Is it all in the head?. In: Taylor, S., editor. *Anxiety Sensitivity: Theory, Research and Treatment of the Fear of Anxiety*. Mahwah, NJ: Lawrence Erlbaum Associates; 1998. p. 199-215.
- Thomas KM, Drevets WC, Dahl RE, Ryan ND, Birmaher B, Eccard CH, et al. Amygdala response to fearful faces in anxious and depressed children. *Arch Gen Psychiatry* 2001;58:1057–1063. [PubMed: 11695953]
- US Department of Health and Human Services and Food & Drug Administration. *Innovation or Stagnation: Challenge and Opportunity on the Critical Path to New Medical Products*. 2004. p. 1-38.
- Whalen PJ, Kagan J, Cook RG, Davis FC, Kim H, Polis S, et al. Human amygdala responsivity to masked fearful eye whites. *Science* 2004;306:2061
- Whalen PJ, Rauch SL, Etcoff NL, McInerney SC, Lee MB, Jenike MA. Masked presentations of emotional facial expressions modulate amygdala activity without explicit knowledge. *J Neurosci* 1998a;18:411–418. [PubMed: 9412517]
- Whalen PJ, Rauch SL, Etcoff NL, McInerney SC, Lee MB, Jenike MA. Masked presentations of emotional facial expressions modulate amygdala activity without explicit knowledge. *J Neurosci* 1998b;18:411–418. [PubMed: 9412517]
- Wright CI, Martis B, McMullin K, Shin LM, Rauch SL. Amygdala and insular responses to emotionally valenced human faces in small animal specific phobia. *Biol Psychiatry* 2003;54:1067–1076. [PubMed: 14625149]