From methods to meaning in functional neuroimaging

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9

Discussion & Perspectives

In this final chapter some critical issues are discussed that might shape future research in which neuroimaging is used as a tool for psychiatric research. Several theoretical and mathematical models are described, which in the future hopefully will enhance our insights into mental functioning in psychiatric disorders. It is this basic conviction that has shaped this thesis and has been its main focus: that in order to increase our understanding of normal and abnormal mental functioning, carefully designed experiments have to be chosen, combined with a constant need for methodological and mathematical refinement. Furthermore, this chapter includes a short review on theoretical connectivity literature.

In chapter 1 data collection and analysis in functional neuroimaging was introduced. Chapters 3 and 4 of this thesis reported the methodological refinement of PET data reconstruction. In particular, chapter 3 described an alternative data reconstruction method which has the potential to increase the statistical power in $H_2{}^{15}O$-PET activation studies, while in chapter 4 an alternative attenuation correction was presented. The latter approach showed a reduction in motion artifacts which is beneficial for data interpretation and signal to noise ratio. This method was applied to the data in chapters 5 and 6. Chapter 5 described a philosophical approach (den Boer, 2003) of conscious awareness in dissociative identity disorder (DID) patients. Chapter 6 fully described the results of the study within a two-by-two factorial design setting. Chapters 7 and 8 used the high temporal resolution of fMRI to study the robustness of perception and the moment of conscious awareness in an event-related manner.

The functional studies in this thesis (chapters 5, 6, 7 and 8) test hypothesized differences in regional cerebral activity by creating statistical parametric maps

(SPMs). This approach is based on the concept of functional localization, which was briefly introduced in chapter 2. Functional localization implies that a function can be localized in one segregated brain area. However, one function can also be distributed over several segregated brain areas.

By studying the interactions between these segregated brain areas the functional integration of the brain can be assessed, in other words, how and which brain areas work together to accomplish a particular function. The concept of functional integration acknowledges that the brain needs to integrate (neural) information locally and globally to enable human behavior. For example, during higher cognitive functions a fast integration of information is required between different segregated sensory, motor and cognitive domains. These converging interactions from distributed neural networks are the basis for connectivity analysis in neuroimaging data.

The focus of most neuroimaging research is on the aspects of functional localization (Petersson et al., 1999b) due to using statistical parametric mapping (SPM) (Penny et al., 2001). However, a more interesting approach is to extend this research to determine which brain areas are activated during a task and explore the functional neural network and their relative connection strengths. In other words: which brain areas are activated and how do these brain areas interact to constitute a functional network. In the remainder of this chapter several options in the context of functional and effective connectivity data analysis are offered, moving away from the standard 'hot spot' approach and addressing functional neuroimaging data analysis in terms of functional networks (Petersson et al., 1999a; Sporns et al., 2000; Friston et al., 2003). Throughout this chapter, future perspectives in the context of functional network data analysis methods are given, incorporating the studies of chapters 5 to 8, to allow for an approach which assigns functional meaning to biological neural networks (Tononi et al., 1999; Laughlini and Sejnowski, 2003).

From local to global human brain functioning

Brain functions can be arranged in a hierarchy of complexity. The more vital a function is for survival the higher the degree of localization, i.e. lower in the hierarchy. Basic functions like respiration, blood pressure and heart rate regulation are vital functions, which are localized and mediated in nuclei within the brainstem. Further up in the hierarchy, the cerebral cortex becomes involved. At this level the brain functions are localized in specialized brain areas. Most sensory brain functions are functionally specialized, for example vision in the visual cortex or hearing in the auditory cortex. Higher up in the hierarchy of brain functions, multiple brain areas usually become involved. Emotion regulation can

involve multiple brain areas, e.g. prefrontal cortex and the limbic system (Dolan, 2002). However, in case of the basic vital defense mechanisms an emotional response is mediated by, that is localized in, the amygdala (LeDoux, 2000). On the other hand, higher cognitive functions are never localized. Mental functions, like abstract reasoning or decision making, involve integration of information through a connected network of a large number of segregated brain areas (Cabeza and Nyberg, 2000; Loeb and Poggio, 2002). The most complex function of the human brain is conscious experience, which depends on recurrent neural activity in the thalamic-cortical system, the so-called 'dynamic core' (Tononi and Edelman, 1998). More specifically, the number of brain areas involved in the execution of a brain function is related to the complexity of this brain function. The complexity of the mathematical model describing the relation among brain areas increases with increasing brain function complexity.

The neural correlates of consciousness are highly integrated and highly differentiated. High integration of a conscious experience can be measured with for example functional clustering (see page 148, included in the section in which functional connectivity methods are reviewed). Highly differentiated neural processes are defined within a functional connected system (see page 146), for example a functional cluster (see page 148), involving neural complexity (Tononi et al., 1994; Sporns et al., 2000; den Boer, 2003). Neuronal complexity measures to what degree the process is differentiated, i.e. subdivided into different subsets or neural states (Tononi and Edelman, 1998). Interaction strengths and directions between the connected areas in a neural state can be explored with effective connectivity methods (see page 149).

The data in chapter 5 and chapter 6 are of interest for studying the neural correlates of consciousness. In chapter 5 two states of conscious awareness were under investigation. In the first conscious state trauma related information was not integrated into conscious awareness. The second conscious state allowed trauma related information to be integrated in conscious awareness. In addition, the data in chapters 7 and 8 can also be analyzed in this context. This study also includes two conscious states, namely the period prior to pop-out and the period post pop-out.

Functional organization of the brain

The brain exhibits two types of functional organization: functional segregation and functional integration (Friston, 1994; Tononi et al., 1994; Friston, 1997a; Sporns et al., 2000). The distinction between functional segregation and functional integration involves the distinction between localization and (dis-)connections (Friston, 1994, 1997a). Functional segregation does not inform about the presence or

absence of connections between activated brain areas, whereas functional integration concerns the connections between functionally segregated areas and is defined by functional or effective connectivity (see for definition page 145). Figure 9.1 displays the hierarchy of the functional organization of the brain.

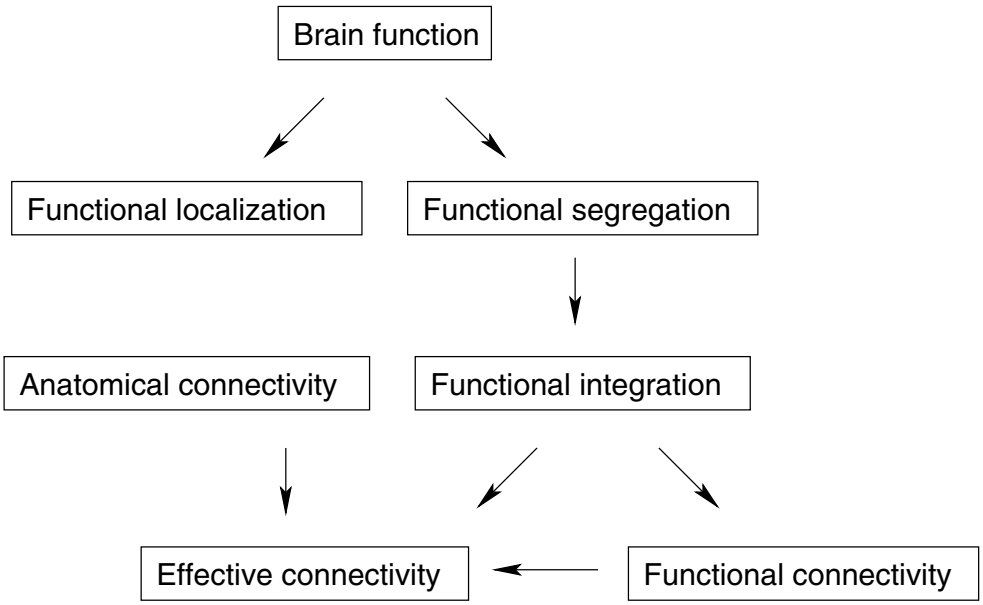

Figure 9.1: *The brain function under investigation can be localized in a single cortical area (functional localization) or be localized in several segregated brain areas (functional segregation). Several functionally localized brain areas, or functional segregated brain areas, might need to cooperate to display behavior (functional integration). Therefore, brain areas need to be anatomically connected directly or indirectly (anatomical connectivity). Functional connectivity determines the correlation between the activity in different brain areas. However, this method does not inform whether brain areas are (directly or indirectly) anatomically connected. Nor does it give any insight into the nature of the connections and their strength. Functional integration analyses using effective connectivity methods inform about the time-independent and causal connections on the basis of a mathematical and a neuroanatomical model (see page 149). Effective connectivity strengths provide information about the functionally interacting networks. Note that functional connectivity may assist in the selection of areas of interest for effective connectivity analysis.*

Functional localization

Functional localization and functional segregation refer to different concepts. Functional localization implies that a function can be specifically localized in one segregated cortical area. Functional neuroimaging data are three dimensional data consisting of voxels that contain the activation values. Standard SPMs are used to characterize brain function in terms of regionally specific responses. Therefore, SPMs may reveal functionally localized brain areas. SPMs are based on univariate statistical tests, which implies testing each voxel separately against a null hypothesis. SPM performs voxel-wise statistical analyses in parallel and creates an image of statistics. Several voxels surviving a statistical threshold, which are spatially connected with each other in terms of edge, face or vertex, define a cluster of activated voxels, that is an activated brain area. If SPM reveals only one activated brain area, it indicates that the brain function under investigation is localized in a specific brain area, that is functional localization.

Functional segregation

Functional segregation implies that executing a function incorporates more than one brain area. Each participating brain area is incorporating a certain aspect of a specific function. Consequently, the execution of such a function is anatomically segregated in the cortex. If the standard SPM analysis reveals several activated brain areas, it indicates functional segregation. Functional segregation comprises anatomically distributed but functionally integrated neural networks. Functional segregation is specifically meaningful in the context of functional integration and vice versa.

Functional integration

The infrastructure supporting a functionally integrated neural network may involve many brain areas. The cooperation of these areas can be mediated by one central brain area, for example the brain stem, or by the functional integration among the brain areas involved. Functional integration is only meaningful in the context of functional segregation, in other words, multiple functionally localized areas subserve the execution of the function under investigation.

Functional integration implies that segregated brain areas are functionally integrated, i.e., a cortical infrastructure is needed to support a brain function that involves many segregated areas. Functional integration is the process of achieving a specific function by integrating functionally interacting segregated areas. Func-

tional integration is mediated by the interactions between directly or indirectly connected functionally segregated areas.

Functional integration comprises two definitions: functional connectivity and effective connectivity (Gerstein et al., 1989; Friston, 1994; Bullmore et al., 2004). Functional connectivity is defined as the temporal correlations between spatially remote neurophysiological events. Effective connectivity is defined as the influence one neural system exerts over another. The concepts of functional and effective connectivity have been elaborated in the analysis of electrophysiological neuronal spike trains (Gerstein et al., 1989). This terminology was introduced in functional neuroimaging using PET data and voxel-based PCA analysis (Friston et al., 1993b) and thoroughly discussed in Friston (1994) and Petersson et al. (1999b).

Anatomical connectivity

Functional integration depends on direct or indirect anatomical connectivity. Functional, effective and anatomical connectivity are interdependent but all refer to different concepts. Anatomical connections, consisting of axons and dendrites, are the basis for functional connections between brain areas. Anatomical connectivity is a structural definition and is dependent on the presence of direct or indirect neural connections. Large fiber bundles in the white matter of the brain can be made visible using diffusion tensor imaging (DTI) (Lim and Helpern, 2002; Koch et al., 2002; Hagmann et al., 2003). Furthermore, theoretical neuroanatomy can be described on the basis of functional connectivity measures (Sporns et al., 2000, 2002).

Functional connectivity

Functional connectivity does not rely on direct anatomical or functional connections. There can be one mediating area which activates a range of functionally segregated areas. More specifically, areas can display activity independent of each other. Functional connectivity is therefore not necessarily due to effective connectivity, meaning that influences may be indirect. Functional connectivity is an operational definition. It is simply a statement about the observed correlations and characterizes functionally distributed neural networks (Friston, 1994). By definition, the correlation matrix is the functional connectivity matrix. Functional connectivity between two areas implies that their activation fluctuates together. Functional connectivity does not provide any direct insight into the causal mediation of the observed correlations (see for recent examples in psychiatry: Shaw et al., 2002; Lanius et al., 2004).

Functional connectivity analyses include eigenimage analysis and multidimensional scaling, e.g. principal coordinate analysis and three-way multidimensional scaling, which are used only descriptively (see below). These multivariate methods are closely related to each other but have different interpretations in relation to functional imaging data. Multidimensional scaling maps anatomy into functional space and eigenimage analysis maps function into anatomical space. Neither eigenimage analysis nor principal coordinate analysis is suitable for making statistical inferences about what is observed. Both cannot estimate the significance of results obtained in terms of a p value.

Eigenimage analysis

Functional connectivity on the basis of eigenimage analysis (Friston, 1997b) characterizes distributed neural networks by subjecting the functional imaging data to principal component analysis (PCA) (Friston et al., 1993b; Bullmore et al., 1996) or singular value decomposition (SVD) (Friston, 1994; Bullmore et al., 1996). Eigenimage analysis is a simple characterization (in terms of principal components, eigenvectors or spatial modes) of the most important features of the data. Usually, only a few principal components (eigenvectors) are needed to explain a majority of variance in the data (Bullmore et al., 1996). These eigenvectors can be displayed as eigenimages. The eigenimages are mutually independent, because the principal components are orthogonal, each representing an independent pattern of activation.

Multidimensional scaling

A complementary approach, called multidimensional scaling, is (in its simplest form) equivalent to eigenimage analysis and constitutes mapping anatomy into a functional space (Friston, 1997b). This method involves constructing a functional space where functional connectivity is used to define the proximity of two anatomical areas, for example by using principal coordinate analysis which is the simplest variant of multidimensional scaling (Friston et al., 1996a). More specifically, the higher the functional connectivity the closer the anatomical regions are in this functional space (Friston, 1994). This method was recently extended to a method of three-way multidimensional scaling for group analysis and includes the possibility to perform statistical testing (Welchew et al., 2002).

ManCova

Neither eigenimage analysis nor principal coordinate analysis are statistical inference techniques for testing significance of distributed neural networks. In contrast, the technique of multivariate analyses of (co-)variance (ManCova)

includes statistical inferences on brain activation patterns. ManCova requires the number of observations, i.e. scans, to be greater than the number of components of the multivariate observations, i.e. voxels. Therefore, in PET the number of observations must first be reduced by using eigenimage analysis. In addition, the ManCova multivariate analysis requires a ManCova appropriate general linear model and canonical variates analysis (CVA) (Friston et al., 1995a, 1996b; Fletcher et al., 1996; Worsley et al., 1997).

Partial least squares

Partial least squares (PLS) aims to identify spatiotemporal interactions within or between neuron populations (McIntosh et al., 1996; Friston, 1997b; Lin et al., 2003) and is related to subscale profiles (Moeller et al., 1987) and eigenimages (Friston et al., 1993b). PLS provides an opportunity for identifying the key regions that form the nodes of a functional network, which are differentially engaged due to task parameters, across the experiment (McIntosh et al., 1996, 1997).

Functional clustering

A different approach to functional connectivity is functional clustering (Tononi et al., 1998). A functional cluster is a set of neural elements (the brain areas) which interact strongly among themselves but interact only weakly with the rest of the brain, in other words internal cohesion and external isolation. Functional clustering does not incorporate causal interactions and the underlying anatomical connectivity need not be continuous. A cluster, consisting of the set of brain regions of interest, needs to be defined on the basis of functional localization analysis within SPM or on basis of *a priori* hypotheses. Functional clustering can then be assessed by calculating a cluster index for each subset of brain regions.

The cluster index (CI) is obtained by dividing the statistical dependence within the cluster by the statistical dependence of the cluster and the rest of the brain. A CI value near 1 indicates a homogeneous system, meaning that the interactions within the cluster are similar to the interactions between the cluster and the rest of the brain. A CI larger than 1 describes a functional cluster with strong internal interactions and weak interactions with the rest of the brain. More specifically, a high CI indicates a cluster having functional boundaries with the rest of the brain. A CI smaller than 1 describes a cluster with weaker internal interactions than with the rest of the brain.

Minimum spanning tree and clustering

Other exploratory data analysis techniques include minimum spanning trees (Baumgartner et al., 2001; Baumgartner and Somorjai, 2001) and data-driven ap-

proaches like cluster analysis (Baumgartner et al., 1998; Golay et al., 1998; Baune et al., 1999; Fadili et al., 2001; Dimitriadou et al., 2004). These approaches may provide an alternative and/or complementary approach to the functional connectivity methods mentioned above.

Effective connectivity

The functional integration of a distributed function can be examined with effective connectivity. Effective connectivity is defined as the influence one neural system exerts over another and specifically models causal interactions between brain areas (Gerstein et al., 1989; Friston et al., 1993b). Effective connectivity is closer to the intuitive interpretation of connectivity analysis than functional connectivity (Friston, 1994).

Effective connectivity consists of two models: a neuroanatomical model and a mathematical model. The neuroanatomical model describes which areas are connected. The mathematical model describes the nature of these connections (Büchel and Friston, 1997a). By combining these models, the influence of one brain area on another is calculated in terms of connection strengths in a least square sense. One fascinating option of connectivity analysis would be to investigate neural plasticity by assessing changes in connection strength (Büchel and Friston, 1997a; Büchel et al., 1999).

The validity of effective connectivity depends in turn on the validity of the neuroanatomical model as well as the mathematical model that are utilized. Validity issues (Friston, 1994) include: (i) construct validity and (ii) predictive validity. (i) Construct validity can be considered in terms of neuroanatomical connectivity and accuracy of the modeled biological neural network. This neuroanatomical model defines the effective connections of a particular area and must conform to the (direct or indirect) anatomical efferent and afferent connections of that area. Information on human anatomical connectivity can be assessed by DTI (Lim and Helpern, 2002; Koch et al., 2002; Hagmann et al., 2003). More detailed information of anatomical connectivity can be obtained from nonhuman primates (see for example: Stephan et al., 2001). (ii) Predictive validity addresses the accuracy of the model's prediction of the behavior of brain networks. This mathematical model must describe the biological neural network accurately. More specifically, the model accurately predicts changes in a brain area of interest using connection strengths which are estimated from independent data (Friston, 1994; Friston et al., 1995b).

Due to the model dependency of effective connectivity the results are also dependent on the mathematical and neuroanatomical model applied (Sporns et al., 2000). Therefore, results that are found might depend on a misspecified

model. More specifically, the number or kind of areas involved, or the nature of the connections may be misspecified. In these cases, the inclusion of an explorative method may be considered. Another option is to test the validity of the model under consideration. More specifically, an automated search for the best path model may be considered (Bullmore et al., 2000).

Linear mathematical models

The simplest model for effective connectivity expresses the activity in one area as a weighted sum of activity in other areas, which is regarded as simple linear regression (Friston, 1994). In this case the response of the areas in the network and the connection weights, i.e. the model parameters, are linear. Linear models have been applied on separate subsets of a PET scanning sessions to investigate time-dependent changes in effective connectivity (Friston et al., 1993a).

Structural equation modeling (SEM) (McIntosh and Gonzalez-Lima, 1994; McIntosh et al., 1994) is a linear regression model for effective connectivity, which includes a statistical comparison of different models. SEM models the causal relation between selected brain areas. Thus, brain functioning is described by differences in the strength of connections between selected brain areas (McIntosh and Gonzalez-Lima, 1994). In SEM connections between brain areas are based on neuroanatomy, therefore construct validity must be taken into account, and the interregional activity covariance matrix is used to calculate path strengths, which represent the effective connectivity values. The statistical inference in SEM addresses two points: the goodness of the overall fit of the model and the difference between models. Free models, allowing changes in effective connections, and constrained models, with fixed connection strengths, can be defined (Büchel and Friston, 1997a; Büchel et al., 1999).

Nonlinear mathematical models

Nonlinear mathematical models describe the nonlinear response of a biological neural network. SEM is usually restricted to linear models. However, additional terms can be included containing a nonlinear function of the original variables. Interactions between variables or modulation of effective connections can be incorporated in a similar manner (Büchel and Friston, 1997a,b).

Specific interactions between brain areas can be explored by extending a linear model to include a modulatory interaction, which influences the activity of the areas involved (Friston et al., 1995b; Büchel and Friston, 1997a). In this type of analysis the modulatory interaction term models the nonlinear response of the biological neural network. Furthermore, the nonlinear response of a biological neural network can also be modeled with dynamic changes in effective connectivity as

characterized by variable parameter regression (Büchel and Friston, 1998). Another option to describe a nonlinear biological neural network is to take a generic model, e.g. involving Volterra series, and obtain the specific parameters that describe the biological neural network under consideration (Friston and Büchel, 2000).

A method including psychophysiological and modulatory interactions (Friston et al., 1997) is based on experimental factors. A psychophysiological interaction investigates whether the contribution, that is effective connectivity, of an area to another area changes significantly due to the experimental context.

Dynamical causal modeling (DCM) (Friston et al., 2003) also focuses on experimentally induced changes in effective connectivity. DCM is used to test specific hypotheses, as motivated by the experimental design, and allows for statistical inference about the connections. DCM treats the brain as a nonlinear dynamic system that receives inputs and produces outputs (Friston et al., 2003). Inputs can affect the system in two manners. (i) An input directly changes neural activity, e.g., a visual stimulus causes a direct response in primary visual cortex. (ii) An input may indirectly change the effective connections or interactions, for example modulation by attention (Büchel and Friston, 1997b). DCM differs from other effective connectivity procedures in that it approaches connectivity on a neural or synaptic activity level. The neural activities in the regions involved cause changes in cerebral blood flow, i.e. the BOLD response, representing the output. Therefore, DCM models neural activity causing the region-specific BOLD response. DCM is included in the SPM2 software (www.fil.ion.ucl.ac.uk/spm/spm2.html).

Future perspective: dissociative identity disorder

The discussion about model validity and accuracy of the mathematical connectivity models is a necessary one and it basically boils down to the question: how does this serve a meaningful interpretation for functional imaging data? A fascinating area of research is to apply functional or effective connectivity analysis on the data described in chapter 6. Different patterns of brain areas were found to be involved in functioning as two different parts of the personality. The term neural networks is already used in the interpretation of that data. However, it is unknown if these areas are included in several distributed functional networks or if these areas are functioning as one functional network. The first question may be addressed by applying functional connectivity analysis like PCA or functional clustering. In addition, an explorative approach might inform about effective connection between the areas as defined by functional connectivity or functional clustering. This would then also open the possibility to investigate differences in these effective connections between the two brain states.

As mentioned above on page 142, the data in chapter 5 and 6 are of interest for studying the neural correlates of consciousness. In chapter 5 two conscious scenes (Tononi and Edelman, 1998), i.e. two states of conscious awareness, were under investigation. In the first conscious scene trauma related information was not integrated into conscious awareness. The second conscious scene allowed trauma related information to be integrated in conscious awareness. Therefore, in the case of dissociative identity disorder two different patterns of activation exist for the two different states of conscious awareness, which are found in the main effect analysis on page 75 and supported by the 'within identity' conjunction analysis (also on page 75). These two patterns of activation could represent two distinct functional clusters, or two functional connectivity patterns, each with a separate dynamic core and therefore two separate states of conscious experience, comprising two separate conscious scenes (Tononi et al., 1994; den Boer, 2003).

An additional extension in this field of research involves incorporating fMRI instead of PET scanning, perhaps even to scan a personality switch and analyzing the event-related data in terms of changes over time in effective connectivity. Several connectivity methods can be applied to specifically investigate the biological neural networks and the mediating connections between the areas in this network to gain more knowledge about the neural correlates sustaining the different parts of the personality in dissociative identity disorder.

Future perspective: fear perception

In a typical functional neuroimaging experiment the investigator may want to know which brain areas are involved during the performance of a task, as well as the interactions between these areas, for example in the experiment as described in chapter 7. Sometimes it is possible to specify a detailed model on the basis of prior research. In that case, DCM can perfectly be used to determine the effective connectivity for the brain areas. This then opens the possibility to investigate the changes in effective connections, and consequently, the dynamics of interacting brain areas. The design presented in chapter 7 is suitable for addressing questions about effective connectivity changes around the moment of pop-out (see also chapter 8) in healthy subjects as well as in psychiatric patients. The DCM which may be employed for this research is depicted in figure 9.2.

Perspectives: from data to biological neural networks

As described above, effective connectivity requires both a mathematical and a neuroanatomical model. However, one or both may not be available *a priori*. Therefore, four combinations arise that must be considered (see figure 9.3). On the

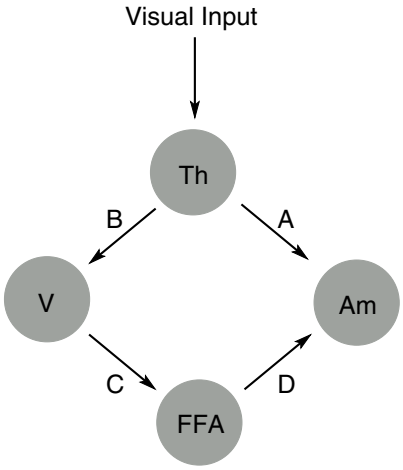

Figure 9.2: *With this dynamic causal model the change in connection strengths around the moment of pop-out (see chapters 7 and 8) may be investigated. A) The direct subcortical route from the thalamus (Th) (after visual input through the superior colliculus) to the amygdala (Am) to allow for a pre-conscious response. B) The indirect cortical route, via the visual cortex (V), passes information through C) to the fusiform face area (FFA) to D) the amygdala. At the moment close to pop-out (see chapter 8) it is hypothesized that the connection strength A) has a high value and the connection strengths B) and C) have low values. For conscious perception (see chapter 7) it is hypothesized that the connection strengths increases for the B-C-D route. In addition, higher connection strengths to the amygdala are expected when presenting fearful faces as compared to neutral faces. More specifically, for neutral faces the route B-C-D may be dominant and for fearful faces route A) may be dominant*

mathematical level, the nature of the connections mediating the activation in the brain areas may either be known or unknown. On the neuroanatomical level no, or only partial, information may be available about the brain areas required for the execution of an experimental task. In addition, the information on the anatomical connections between the activated brain areas may be incomplete (see remarks on construct validity on page 149).

Situation I

This is the desired situation. The investigator has a complete definition of the experimentally induced brain activation in terms of activated brain areas and the anatomical connections between these areas, which is the neuroanatomical model, as well as the nature of the connections (if any connections are present)

		Neuroanatomical model	
		Known	Unknown
Mathematical model	Nature of connections known	I	II
	Nature of connections unknown	III	IV

Figure 9.3: *Effective connectivity requires both a mathematical and a neuroanatomical model. One or both may not be available* a priori*, creating four possible situations.*

between the activated brain areas, which is the mathematical model. As discussed above, the nature of these connections can either be linear or nonlinear. One of the effective connectivity methods (see page 150) may then be employed to determine the effective connectivity.

Situation II

In this case the neuroanatomical model is not known or only partly known, but the nature of connections (linear or nonlinear) in the network under investigation is known. More specifically, the mathematical model is known, to which the data can be fitted. To make the transition from situation II to situation I, brain areas can be obtained by applying the function representing the nature of the connections on the data. For example, in the case of (non-)linear regression, those brain areas will be found that are best fitted by the mathematical model. Networks of brain areas, which do not satisfy this model will not be fitted and therefore excluded.

Another method to approach the neuroanatomical model is to simply apply SPM. The experimental conditions under which the data are obtained are known and the general linear model can be applied to detect a set of activated brain areas. However, not all of these brain areas need to take part in the neural network for which the nature of connections is known. The participation of the brain areas should therefore be additionally tested by applying the mathematical connectivity model. If no or insufficient hypotheses about the neuroanatomical connectivity patterns are present, a more explorative approach may be required. For example, eigenimage analysis or functional clustering can be applied to describe several

distributed neural networks. Each of these distributed neural networks may then be investigated in more detail, taking into account construct validity (see page 149).

Situation III

In this situation, the experimentally activated brain areas are known. However, the nature of the connections are unknown. Generally speaking, this is the situation that is normally the case. Usually this situation exists after data-analysis with SPM, but activated areas can also be known without the application of SPM, for example on the basis of individual anatomy, a simple and straightforward experimental design and accompanied by properly formulated *a priori* hypotheses.

Hypotheses about the directions of the connections are available, because the neuroanatomical model is known. Therefore, to make the transition from situation III to situation I, an explorative method can be considered, e.g. using Volterra series. Another option is to apply one of the effective connectivity methods as described above, taking the goodness of the overall fit of the model into account.

Situation IV

In this last situation, the brain areas which are involved in the experiment are not known and the mathematical model for the mediating connections between brain areas is also not known. A direct transition from situation IV to situation I appears impossible. At the moment no technique employs the raw data directly to determine the brain areas involved in the experiment as well as connections between these brain areas.

To perform the transition from situation IV to situation II a hypothesis is required about the nature of connections, in the network under investigation, which may even be derived from primate data by using single cell recordings. Alternatively, a set of brain areas may emerge after applying mathematical functions by trial and error.

To make the transition from situation IV to situation III requires the usual approach, by using standard SPM, to define brain areas by applying the GLM that incorporates the experimental effects. Note that a standard SPM analysis requires the definition of the GLM but not of the connections between brain areas. A different option for defining a set of brain areas is to make an estimation on the brain areas involved, for example by using PCA or correlation analysis with a low threshold. A set of brain areas with a low correlation involves a connected network which does not necessarily involve a linear relation. On the basis of this set of brain areas the nature of connections can be explored by, e.g. using a Volterra series or other methods described previously.

To be able to continue to work with unbiased raw data, the data can be reduced by defining brain areas, i.e. volumes of interest, on the basis of Brodmann's map

of cortical areas of the human brain. This map is based on the cytoarchitectonic properties of the cortical areas. These Brodmann areas can be submitted for exploration of the nature of the connections.

Future perspectives: methods

Standard functional localization neuroimaging experiments are, and must be, thoroughly designed by incorporating *a priori* hypotheses about regional specific effects which may even incorporate a definition of the biological neural network under investigation, including the direction of connections (see for example chapter 7). These types of experiments are perfectly suitable for applying SEM or DCM. Biological neural networks which have been extensively studied provide basic information about network functioning.

As previously described, the validity of effective connectivity is dependent on the validity of the defined model. In chapter 6 we could define an *a priori* hypothesis on the basis of a related psychiatric disorder, that is PTSD. This provides a valid hypothesis about the areas which might be involved. However, network analysis might bias results in the direction of PTSD, thereby ignoring DID specific connectivity information. In such cases an investigator prefers to explore the biological neural networks involved, independent of biased *a priori* hypotheses. Many psychiatric disorders lack a validated neuroanatomical model, because the disturbed brain networks are poorly understood.

Therefore, an open area for exploration is area IV of figure 9.3, depicting the situation in which the neuroanatomical nor the mathematical model are known. This situation is interesting from an explorative point of view. In such a case, as much as possible data should be included in the analysis. However, since functional integration is being assessed, the researcher may restrict the analysis to the brain areas of interest, also considering computability. Therefore, instead of approaching all the voxels in the brain, the data might be reduced by defining specific brain areas, such as Brodmann areas (see above), and submitting these areas for exploration of the mathematical nature of the connections. This reduced set of data might also be modeled by a mathematical neural network. The connection strengths between the mathematical neural network nodes represent the connection strengths between the biological neural network nodes. A future improvement for construct validity may involve an increase in spatial resolution of DTI to obtain better fiber tracking results to function as an anatomical basis for effective connectivity.

Nevertheless, a model is always a simplification of reality. Although ever increasing computational capabilities enable the analysis of extremely large and complex models, effective connectivity analysis are limited by the accuracy of the

neuroanatomical and mathematical model, and also by the ability to interpret complex models. Further limitations comprise the temporal resolution of the BOLD response, as well as the spatial resolution and the signal to noise ratio of the data. Connectivity analysis should therefore compromise between complexity, anatomical accuracy and interpretability.

Prospects

Embedded in this chapter several options were offered for future research. Perspectives for future research are mainly methodologically based to serve refinement of methodological and mathematical (connectivity) models in order to increase our understanding of normal and abnormal mental functioning. Therefore, it is a challenge to explore new methods in the field of computational neurobiology to benefit neuroimaging research. This requires multidisciplinary groups in which psychiatrists, neurobiologists, neuropsychologists, information technicians, neural network theoreticians and mathematicians work together. It is only through this concerted research effort that methods can be converted into meaning.

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