whether the detection of environmental uncertainty might also prime processing of positivity.

The transient hyper-vigilance induced by exposure to unpredictable tones suggests that healthy subjects can end up looking a lot like anxious subjects when exposed to the right situations. Perhaps this basic vigilance function in response to uncertainty is quite similar across anxious and healthy individuals, at least initially. What might differ between these individuals is how the prefrontal cortex handles the calculation of actual danger when environmental uncertainty is encountered. Those without a disorder might not cross a diagnosable line because, when appropriate, they are able to counter with a prefrontal cortical response that overrides, regulates and ultimately quells this initial amygdala hyper-responsiveness [8,10–13]. Indeed, this regulatory function appears compromised in pathological anxiety [14]. In short, at least a portion of the healthy amygdala acts as if it has an anxiety disorder – searching for threat in response to uncertainty. This design enables the amygdala to operate based on principles that are more primal and rigid [2,15] while the more educated and flexible prefrontal cortex possesses the ability to bend these rules. Overt behavior ends up being the balance struck between these processes and therein lies a basis for individual differences.

For those specifically interested in amygdala function, the study by Herry, Bach and colleagues reminds us that although amygdala output has an important role in emotional responding, the associative functions of the amygdala are primary and ubiquitous in nature. That is, the influence of the amygdala is constant as it monitors the environment for events that have predicted clear outcomes in the past. If clear predictive signals are lacking, the amygdala can boost vigilance (e.g. lower sensory thresholds throughout sensory cortex [5]) in response to uncertain events, in an attempt to help determine any causal relationships between such events [7]. When appropriate, the amygdala (via extensive efferent circuits) can then give rise to an emotional-state change [2,5,6]. In the human subjects studied by Herry, Bach and colleagues, amygdala response to unpredictable tones did not evoke a measured change in emotional state in its own right, but it did modulate emotional behavior during subsequent biologically-relevant situations. Thus, higher amygdala activity can precipitate, but might not necessarily dictate, a change in your emotional state. This should be comforting news. You are not a prisoner of your emotions. In the face of uncertainty, the amygdala just gives you a jump-start. What you do with it is what makes you. . . 'you.'

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Letters

Combining electrophysiology and functional imaging – different methods for different questions

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The integration of functional magnetic resonance imaging (fMRI) and electrophysiological methods, such as electroencephalography (EEG) or magnetoencephalography (MEG), is highly attractive to the cognitive neuroscientist, because it promises a temporospatial resolution that cannot be obtained with either technique alone. In fMRI-constrained source analysis, the task-related spatial information from the fMRI is used to weight or constrain

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the EEG source analysis [1] and to elucidate the activation sequence of cognitive processes [2–4].

In their discussion of fMRI-constrained source analysis, Debener *et al.* [5] correctly state that the assumption of a correspondence between neural generators of haemodynamic signals and the EEG might not always be correct. They use this point to argue that the analysis of single-trial covariation between EEG and blood oxygen level dependent (BOLD) signals is preferable. Their proposed single-trial EEG–fMRI analysis uses trial-to-trial variability of statistically independent components (IC) derived from the EEG signal to derive predictors for the local fMRI signal [5].

However, this single-trial approach does not use any constraints on physically plausible locations of the sources of the scalp signal. It could, therefore, identify brain areas whose metabolic activity, as measured by fMRI, also coarsely correlates with the IC-derived predictor, although it is not the generator. This can occur when an area is tightly coupled with the generator but with a different time course or frequency profile of electrical activity. In principle, it would also be necessary to quantify the mutual correlation of all IC-derived predictors.

The single-trial EEG–fMRI analysis assumes linear correlation of the BOLD signal with the single-trial IC feature of interest. It might thus miss neurophysiological processes in which both the IC feature of the EEG and the BOLD signal deviate with the same sign from the baseline but are modulated with the opposite sign by changing task demands, such as memory load [3]. Finally, the single-trial approach faces the inherent problems of independent components analysis (ICA), such as the pre-existence of knowledge about the number of sources required by current algorithms [6].

In contrast to the single-trial correlation approach, which uses statistical information to identify sources but disregards the physical validity of the result, fMRI-constrained source analysis assumes that sites with task-correlated fMRI activation are potential sources of the scalp EEG (and MEG) and then tries to reconstruct the electrical source activity based on a physical (overdetermined) model (Box 1). In addition, fMRI-constrained source analysis does not assume a linear coupling. The quality of a discrete inverse model depends on the dipolarity of the sources and a correct estimate of their number. Hence, validation steps are necessary without any guarantee that a source model that successfully passes these steps can always be found.

When the interest is in the haemodynamic correlate of a specific EEG/IC amplitude modulation, single-trial correlation has currently no alternative. Conversely, the fMRI-constrained source analysis identifies the time courses of electrical generators underlying the scalp signal. Both methods share the assumption that generators of scalp

Box 1. FMRI-constrained source analysis

The spatial information from functional magnetic resonance imaging (fMRI) can be used to provide physical constraints for the solution of the electromagnetic inverse problem of determining the brain sources from scalp activity using a model of multiple discrete sources [1]. In this approach, event-related potentials/fields (ERP/ERF) and fMRI data are acquired separately or simultaneously and co-registered into a common coordinate system (Figure Ia–c). The possible locations of the electric/magnetic sources are derived from the task-related fMRI activity (Figure Ie). The source analysis then models the source time courses and reflects activity in the cluster with millisecond resolution (Figure Id).

Validation procedures: A 'crosstalk' analysis excludes the possibility that the model overestimates the number of sources [3,7] (Figure If). Conversely, the risk of underestimating can be reduced by lowering statistical thresholds for the fMRI data and assessing whether any additional sources make a notable contribution in explaining scalp ERP/ERF variance (Figure Ig). Scanning the brain with an additional ('probe') source added to the current solution will detect locations of possible generators not included in the current model; these locations will be indicated by a large fraction of scalp signal variance explained by the probe source (e.g. posterior brain activity in Figure Ih).

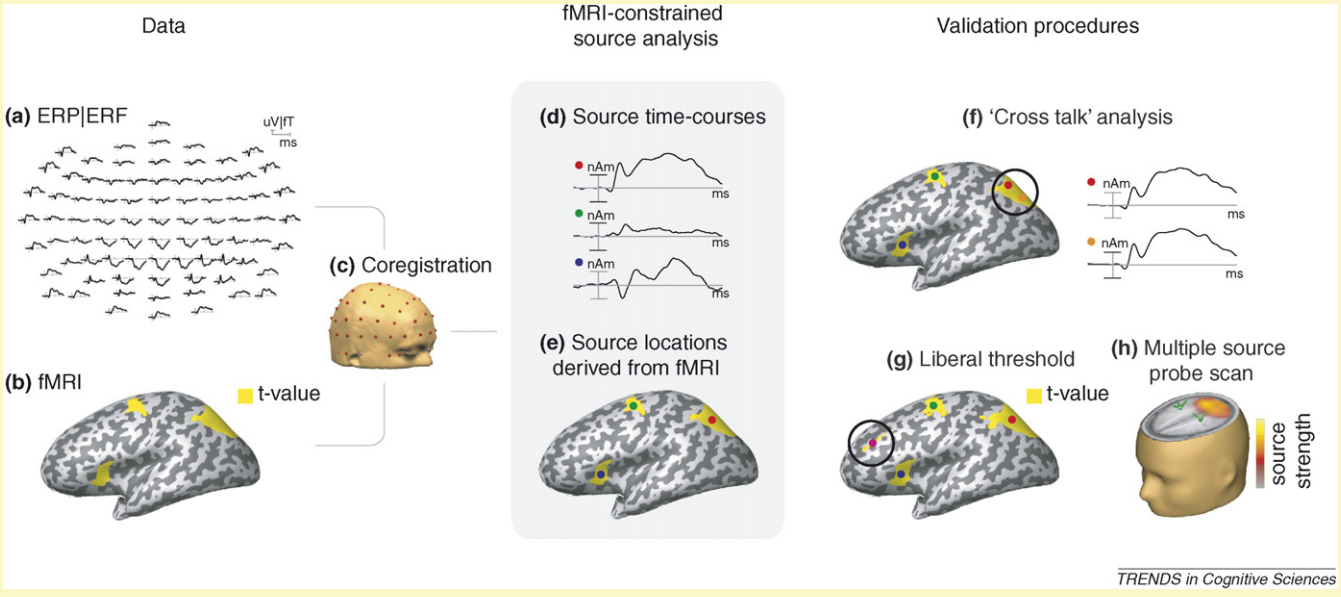

Figure I. FMRI-constrained source analysis.

EEG signals and fMRI activation overlap to some extent. It is the scientific question that determines which method will be appropriate.

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Letters Response

Towards single-trial analysis in cognitive brain research

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Bledowski and coworkers advocate functional magnetic resonance imaging (fMRI)-constrained source analysis of event-related potentials (ERPs), that is, trial-averaged electroencephalogram (EEG) responses, over the single-trial based EEG–fMRI integration technique we recently proposed [1]. The authors focus on three arguments.

First, they argue that our EEG-informed fMRI analysis approach might misidentify cortical generators of EEG activity by not taking into account physically plausible locations of ERP sources. We agree that if the goal is to identify the neural sources of ERPs, spatial constraints are valuable and should be used in the analysis. Independent component analysis (ICA) does not explicitly include this information, but it is readily gleaned by a comparison of the location(s) identified by fMRI with the dipole source analysis of the independent component(s). Indeed, we have previously shown a close correspondence between the dipole source location of the selected independent component and the single-trial EEG–fMRI integration result [1]. Therefore, although the ICA-based trial-by-trial approach can easily incorporate ERP source analysis, the reverse is not feasible. We consider it an advantage of our analysis that it is, in principle, not limited to the identification of common generators of EEG and fMRI. By contrast, the method can deliberately be used to identify functionally defined neural networks that are correlated with temporally well-localized EEG features, using the spatial resolution of fMRI [2].

Second, Bledowski and colleagues argue that the EEG-informed fMRI analysis approach assumes a linear correlation between fMRI and EEG features. Although a linear model is a natural starting point for this analysis scheme, the proposed method is, in fact, not limited to a linear correlation. The method can be generalized to any non-linear relationship simply by constructing corresponding non-linear fMRI regressors from the single-trial EEG features of interest [3].

Third, Bledowski *et al.* argue that the trial-by-trial approach suffers from the assumptions inherent in ICA. In particular, they state that current ICA algorithms require knowledge about the number of sources contributing to the mixed data. The infomax ICA algorithm we use is among the most widely applied algorithms [4] and does not in practice require this knowledge. However, the authors might be referring to the underlying problem, which is the selection of those independent components that can be reliably identified. We have successfully used different strategies to tackle this issue [5,6] and, consistent with others, have found ICA to be of great value – in particular for the direct integration of EEG and fMRI [1,2,7,8]. By contrast, the 'number of sources' problem applies to the fMRI-constrained ERP analysis approach because fMRI does not unambiguously identify the number of possible ERP dipole sources. It is worth recapitulating that fMRI could be blind to some EEG phenomena and vice versa. Hence, none of the currently available EEG–fMRI integration approaches unambiguously tells how many sources are relevant.

To advance EEG–fMRI integration, we need to further our understanding of how these signals relate to each other. However, the fMRI-constrained ERP source-analysis approach does not have much potential in addressing the fundamental question of EEG–fMRI coupling. The sole consideration of trial-averaged data in each modality neglects the amount of information that can be extracted from fluctuations across trials. By contrast, the trial-by-trial approach can help to identify which fractions of EEG

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