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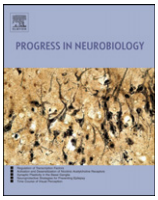

The brain and its resting state activity—Experimental and methodological implications

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ABSTRACT

Despite all the recent progress in neuroscience, we still do not understand the basic principles according to which the brain functions. This may be due, at least in part, to our lack of knowledge how the brain's intrinsic activity, the brain's input, impacts stimulus-induced changes in the brain. We here discuss the neuronal, experimental and methodological relevance of the brain's resting state activity for future studies. Furthermore, we make several suggestions how to best define and include the brain's resting state into our experimental designs. We conclude that experimental consideration of the brain's resting state has major implications for setting up experimental designs and methodological strategies. This may also shed new light on some hitherto unresolved questions like the neuroscientific mechanisms underlying consciousness and psychiatric disorders.

Contents

1. Introduction

Recent developments in neuroscience, such as brain imaging, allow a hitherto unknown insight into brain function and human behaviour (Northoff, 2010). Mental phenomena like consciousness, self and free will that were formerly attributed to the mind are now associated with the brain (Churchland, 2002; Dennett, 1991; Frith, 2007; Gallagher, 2005; Koch, 2004; Northoff, 2004; Searle, 2004). However, the exact role of the brain, especially with regard to its own intrinsic activity (also often referred to as resting state activity), remains largely unclear. A few studies in humans and other animals have focused on the impact of the brain's intrinsic activity on subsequent stimulus-induced activity and the associ-

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Abbreviations: fMRI, functional magnetic resonance imaging; PET, positron emission tomography; DMN, default-mode network; PACC, perigenual anterior cingulate cortex; NBR, negative BOLD responses; BOLD, blood oxygen level dependent; MDD, major depressive disorder.

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ated mental states (Arieli et al., 1996; Boly et al., 2007; Busch et al., 2009; Christoff et al., 2009; Fiser et al., 2004; Fox et al., 2006; Greicius and Menon, 2004; Kenet et al., 2003; Maandag et al., 2007; Muthukumaraswamy et al., 2009; Northoff et al., 2007; Schneider et al., 2008; Shulman et al., 2009b). This has led Raichle (2009) most recently to speak of what he called "paradigm shift". This 'paradigm shift' refers primarily to how we view the brain, which can be broadly described in two ways. In the first, the brain is viewed as a primarily reflexive organ whose neural activity is completely determined by the incoming stimuli and thus the momentary demands of the environment. In the second, the brain is assumed instead to be an active organ which imposes its intrinsic activity upon the stimuli that are to be processed. While the recent findings clearly demonstrate intrinsic activity in the brain, its exact implications for the brain's neural processing of stimuli from the outside world and the associated behavioural and mental states remain far from clear. Moreover, how to properly account for the brain's intrinsic activity in our experimental designs when investigating stimulus-induced activity is presently unknown. If stimulus-induced activity is indeed, at least in part, predetermined by the brain's intrinsic activity (e.g., resting state activity level) we may need to include the latter as a key variable when investigating the former.

How can we methodologically disentangle the effects of the brain's intrinsic activity from the stimuli and tasks which are employed by us as scientists and hence as observers? While we observe changes in neuronal activity when employing our stimuli and tasks, as for instance in neuroimaging, we do not know whether the observed activity changes are related to changes in the brain's intrinsic activity as merely triggered by the task/stimulus or, alternatively, to the causal effects associated with the stimulus itself. We thus need to devise methodological, e.g., experimental and analytical, approaches that allow us to at least partially parse the two inputs—the brain's intrinsic activity (i.e. brain's input) and the task or stimuli as employed by the observer (i.e. the observers input). This is important, since if we do not disentangle the two inputs in our experimental designs and subsequent analyses, we may falsely attribute neural activity to our stimuli or tasks rather than to the brain itself.

The question arises whether such a distinction between the brain's intrinsic activity and stimulus-induced activity is possible at all. This is especially so if intrinsic activity interacts with stimulus-induced activity. Such a mutual entanglement between intrinsic and stimulus-induced activity may make it rather difficult to segregate the two in experimental protocols from a methodological point of view. Even if segregable, one may argue that the resting state may remain irrelevant in our quest to understand the neural underpinnings of specific psychological functions (see, for instance, Buckner and Vincent, 2007; Morcom and Fletcher, 2007; Raichle and Snyder, 2007 for a reply). However, in order to determine the potential irrelevance of the brain's input as distinguished from the observer's input, it follows that one needs to demonstrate it experimentally. This is possible by at least attempting to differentiate both the brain's and the observer's input in our methodological approaches and experimental designs, aiming towards what could be described as the idealised conceptual separation between the two. Only then will we be able to decide whether the brain's intrinsic activity is indeed irrelevant or, alternatively, necessary to give rise to mental states.

The aim of this paper is to discuss, methodologically, the relevance of the brain's intrinsic activity in mental and behavioural states, thereby pointing out the need to develop appropriate experimental tools for disentangling the brain's intrinsic activity (i.e. the brain's input) from the tasks or stimuli we as observers employ (i.e. the observer's input). Such a methodological approach will allow us to at least in part segregate the brain's input in our experimental designs from the stimuli and tasks we as observers employ. We will discuss possible experimental strategies in this direction and will also attempt to shed some light on broader methodological issues. We will conclude that the methodological issues pertaining to the recent highlighting of the brain's intrinsic activity might provide novel and alternative ways of acquiring insight into the neural mechanisms of the brain.

2. Operational definition of the brain's resting state activity

Recent observation of the brain's high intrinsic resting state activity, apparently independent of any kind of extrinsic stimuli or tasks, may provide some insight into the nature of the brain's input (see below for further definition of the concept of the brain's input). There is indeed empirical evidence for intrinsic activity in the brain. Using electrophysiological recordings such as EEG, Llinas (1988) and others (Arieli et al., 1996; Buzsáki, 2006; Buzsaki and Draguhn, 2004) have observed intrinsic brain activity in the gestalt of auto-rhythmic electrical oscillations (or synchronizations) across different brain regions, for instance the thalamic nuclei and cortical regions. Recent fMRI and PET studies have also revealed high resting state and metabolic activity in a particular network of regions, the so-called default-mode network (DMN), that includes predominantly subcortical and cortical midline regions in both humans (Buckner et al., 2008; Fransson, 2005; Northoff and Bermpohl, 2004; Northoff et al., 2006; Raichle and Gusnard, 2005; Raichle et al., 2001) and non-human animals such as monkeys (Northoff and Panksepp, 2008; Rilling et al., 2007; Vincent et al., 2007) and rodents (Pawela et al., 2009; Shulman et al., 2009a) (but see Morcom and Fletcher, 2007 for an opposing view). This default-mode network shows strong activity, especially in the resting state with the absence of stimulus-induced activity (Fox et al., 2005; Greicius et al., 2003; Greicius et al., 2009).

While these (and other) data clearly indicate intrinsic activity in the brain, a strict definition of the brain's resting state may be rather difficult. One of the issues arising here is that the brain is never really at rest, but is continuously active even in the absence of specific stimuli. This implies that even if the observer does not employ specific stimuli, the brain nevertheless encounters a continuous barrage of sensory input through, for instance, the visual or auditory senses. For instance, a recent EEG study demonstrated clear electrophysiological differences between a resting state with eyes closed and one with eyes open (Barry et al., 2007). The authors assume a true resting state activity, which they call the 'baseline arousal level' and relate experimentally to an eyes closed condition. This baseline level of arousal must be distinguished from what they call 'baseline activation level', investigated with the open eyes condition, which reflects the reception of passive visual input without any active stimulus or task processing. Hence, depending on the sensory context, be it with open or closed eyes, different levels of baseline or resting state activity may be distinguished.

These difficulties have forced researchers to define the brain's resting state activity in a strictly operational fashion. Using closed eyes is considered to be one valid experimental way to tap into the brain's resting state activity (see Logothetis et al., 2009; Raichle, 2010). While this might be regarded as experimentally valid and sufficient, it may, however, prove insufficient when considering the input from the remaining senses, such as audition, that cannot be shut down completely. The input from the remaining senses may still impact the brain's resting state activity, which might make it difficult if not impossible to experimentally isolate the latter completely from the former. Even if we could succeed in shutting down the sensory and thus exteroceptive input completely, we are still confronted with the continuous interoceptive input from the body that is also processed in the brain.

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Nonetheless, given the potential importance of determining the resting state's impact on global functioning, it is essential to use the best methods currently available to approximate the brain's intrinsic activity in our experimental designs. However, exact experimental strategies and designs for doing this remain unclear.

The problems associated with isolating the brain's resting state have led some to presuppose a purely operational definition of the brain's resting state, namely the state of the brain before it is perturbed by any kind of stimuli from outside of the brain itself (Buzsaki and Draguhn, 2004; Shulman et al., 2009b). However, as we have seen, even this seemingly simple operational definition may be put into doubt by the fact that it only concerns those stimuli specifically employed by the observer, without accounting for either the continuous unspecific exteroceptive inputs or the interoceptive inputs from the body. How can we nevertheless approach the issue of the brain's resting state activity in our experimental designs? One indirect way would be to first show the empirical relevance of the brain's intrinsic activity in the gestalt of its interacting effects with stimulus-induced activity amounting to what can be called rest–stimulus and stimulus–rest interaction. This approach will be the focus of the next sections which will serve as the basis for subsequent discussion on some potential experimental strategies and methodological issues for future studies.

Before proceeding to the empirical data, one brief conceptual remark shall be made. As described, it may be difficult to empirically and experimentally disentangle the brain's resting state activity from stimulus-induced activity be it intero- or exteroceptively induced. This empirical and experimental fuzziness may contrast with our concepts and descriptions that, at least on a purely conceptual level, seem to clearly segregate the brain's input from the observer's input. Hence there may be discrepancy between the brain itself and the concepts we use to describe it. While the boundaries between resting state and stimulus-induced activity seem to be rather fuzzy in both empirical and experimental regards, their clear-cut conceptual segregation seems to suggest otherwise. Such an empirical/experimental-conceptual discrepancy should be kept in mind in the following description. More specifically, we should be aware that our concepts may suggest a more clear-cut segregation between resting state and stimulus-induced activity, as well as between the brain's and observer's input, than actually exists.

3. Empirical relevance of the brain's input I: rest–stimulus interaction

In order for the brain's input to be functionally relevant it must interact with the neural activity as induced by the stimuli and tasks employed by the observer; this is so because we are able to access and experience these stimuli and tasks in a conscious way, and thus in the gestalt of mental states. Hence, we must search for how the brain's input (i.e. its intrinsic activity) modulates, predisposes, and possibly even determines stimulus-induced activity. We must thus investigate what we call the 'brain–stimulus interaction' or 'rest–stimulus interaction' (see also Northoff et al., 2010).

A few studies have indeed demonstrated that the brain's intrinsic activity impacts subsequent stimulus-induced activity. Q2 Greicius and Menon (2004) investigated how the default-mode network (DMN) impacts subsequent stimulus-induced activity in visual and auditory tasks during passive sensory tasks. They observed that the level of activity in the DMN during stimulation predicted the neuronal activity in both visual and auditory cortices during the auditory and visual tasks. The lower the activity in the task-negative networks of the DMN during auditory/visual stimulation, the higher the stimulus-induced neuronal activity in auditory and visual cortex. This strongly suggests that the level of resting state in the DMN impacts the stimulus-induced neuronal activity in other stimulus-related regions.

In an animal study, experimental manipulation of the brain's resting state has been reported by Maandag et al. (2007). They created pharmacologically induced (using halothane and chloralose) high and low resting state activity in rats and subsequently measured neural activity during forepaw stimulation using fMRI. High resting state activity was associated with widespread activity across the cortex and rather weak activity in the sensorimotor cortex. This activity pattern was reversed in animals with low resting state activity, where neural activity was stronger in the sensorimotor cortex and virtually absent in other cortical regions. These results demonstrate that the level of resting state activity may modulate the distribution and intensity of stimulus-induced activity in regions like the sensorimotor cortex and cannot simply be explained by increased anesthesia-induced inhibition (Shulman et al., 2009b; van Eijsden et al., 2009). In addition, this resting state–stimulus interaction may, in part, help to explain variations in output seen between studies of awake vs. anesthetized animals under similar experimental conditions (for recent examples see Chen et al., 2009; Huetz et al., 2009; Kiyatkin and Brown, 2007). Other groups have also used similar approaches to investigating the brain's resting state properties in rodents (Biswal and Kannurpatti, 2009; Zhao et al., 2008), and newer developments in methodology which allow for a mapping of the resting state in conscious non-human animals will contribute greatly to future studies (Zhang et al., 2010).

Fox et al. (2006) investigated how intrinsic resting state neuronal oscillations in a stimulus-related region impacts subsequent behaviour in humans. They showed that the ongoing intrinsic neuronal oscillations in the somatomotor cortex, which persisted during stimulus-induced activity, predicted a high percentage of the trial-to-trial variability in somatomotor cortical task-related activity and reaction time in a subsequent button press task. Spontaneous BOLD fluctuations and task-related responses in the somatomotor cortex were superimposed onto one another and appeared to have a near linear relationship. Hence, the spontaneous BOLD fluctuations seem to determine, or perhaps predispose, the subsequent behaviour, i.e. the task-related responses. This clearly indicates the functional and behavioural significance of intrinsic resting state activity for stimulus-induced neural activity in the respective stimulus-related regions (see also Boly et al., 2008; Busch et al., 2009; Fiser et al., 2004; Fox and Raichle, 2007, for similar approaches in humans, as well as Kenet et al., 2003, for similar approaches in animal visual cortex).

Finally, even the resting state level of biochemicals like GABA may impact subsequent stimulus-induced activity. Using combined MRS and fMRI, Northoff et al. (2007) investigated the level of GABA in a typical DMN region, the perigenual anterior cingulate cortex (PACC), which shows predominantly negative BOLD responses (NBR). The resting state level of GABA in the PACC correlated with the degree of NBR as induced by an emotional judgment task in the very same region. Higher resting state concentrations of GABA in the PACC correlated with higher NBR in the very same region during stimulus-induced activity. This study demonstrated that the resting state concentration of GABA in the PACC may indeed impact stimulus-induced activity changes in the PACC (see Muthukumaraswamy et al., 2009 for analogous results with regard to the visual cortex).

Taken together, these studies indicate the empirical relevance of the brain's intrinsic activity for stimulus-induced activity in both animals and humans. The brain's input may consequently be considered a variable by itself that researchers should attempt to keep as independent and distinct of other variables as is currently possible, like the stimulus as the observer's input. This, however,

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has major implications for both methodological approaches and experimental designs.

4. Empirical relevance of the brain's input II: stimulus–rest interaction

The brain's input may itself be modified by the stimulus-induced activity. A recent study by Lewis et al. (2009) investigated the effects of visual perceptual learning on resting state connectivity. The subjects underwent training of a shape-identification task constrained to one visual quadrant. After several days of training, subjects underwent fMRI during a visual training task. This revealed an effect of training of the respective side, i.e. quadrant, in the visual cortical activation when compared to the untrained side. In addition, subjects underwent two sets of fMRI resting state scans with visual fixation before and after behavioural training. These comparisons yielded a difference in the resting state connectivity between the visual cortex, fronto-parietal regions involved in spatial attention, and regions of the default-mode network.

Another study investigated the effects of motor learning on resting state activity (Albert et al., 2009). Resting state activity was investigated in fMRI before and after an 11 min visuomotor training session. Neural activity in the fronto-parietal resting state network (i.e. lateral frontal and parietal regions) and the cerebellum was significantly increased after the visuomotor training session when compared to before the session. Interestingly, the same network was not recruited during mere motor performance, thus being specific for motor learning. This suggests that resting state activity in this network may be closely related to visuomotor learning rather than mere visuomotor performance.

These examples of stimulus–rest interaction (see also Pyka et al., 2009; Schneider et al., 2008, for further examples) indicate that the resting state activity level is not fixed but that it is modulated by the incoming stimuli. This suggests that the brain's input is flexible rather than static. What does this imply for the definition of the brain's resting state? It means that the brain's resting state does not reflect a pure resting state but is always already integrated with stimulus-induced activity. Hence, a complete isolation of the brain's resting state activity from stimulus-induced activity may remain impossible. The brain's neural activity seems to be rather a *mixtum compositum* of what we, on a conceptual level, distinguish as the brain's resting state and stimulus-induced activity. This may have important consequences for our experimental designs raising methodological issues that should be discussed in more detail.

5. Experimental relevance of the brain's resting state activity

We demonstrated empirical support for mutual interaction between resting state and stimulus-induced activity, e.g., rest–stimulus and stimulus–rest interaction. This implies that resting state and stimulus-induced activity may empirically, i.e. neuronally, not be as clearly segregated as suggested by our concepts. What does this imply for our experimental designs and methodological strategies?

Considering the results especially from rest–stimulus interaction experiments, we saw that the resting state activity level is a variable that needs to be accounted for when investigating stimulus-induced activity. While stimulus-induced activity is clearly the dependent variable, these results suggest a different role for the resting state activity in our experimental designs. Either the resting state activity level is entered as an independent variable in the experimental design or it is accounted for as modulatory (or confounding) variable. Both options shall be discussed in the following.

Few studies, using either humans or animals as subjects, have considered the resting state, i.e. the brain's input, as an independent variable. The study by Maandag et al. (2007), described above, was perhaps the first to have considered the brain's input as an independent variable within the animal literature. Forepaw stimulation (i.e. the observer's input) was subsequently used as an additional independent variable and activity from the primary somatosensory cortex was considered as the dependent variable (i.e. brain's output). Subsequent studies by Kannurpatti et al. (2008), Pawela et al. (2009) and Zhao et al. (2008) have continued to investigate the properties of resting state activity using related approaches in non-human animals. Studies involving humans have been subject to similar issues. One approach, which considers the brain's input as an independent variable, takes advantage of recent technical and analytical advances in combining simultaneous functional brain imaging with electroencephalography (Laufs et al., 2008). With this approach, the authors suggest, it is possible to measure the brain's activity from two angles—with one signal interpreted as the independent variable, and the other as the dependent variable.

One hallmark of approaches using the brain's resting state activity as an independent variable is the experimental variation. Using anaesthetics, Maandag et al. (2007) induced two different resting state activity levels whose impact on the dependent variable, i.e. the neural activity during motor stimulation, was measured. One difficulty in human studies is to develop strategies to manipulate the resting state activity level without impairing the cognitive and sensory facilities required for subsequent stimulus-induced processing. While this may prove to be impossible in an absolute way, we may at least approximate it in our experimental designs. One approach would be to consider the issue in an anatomical sense. One may, for instance, investigate the impact of the default-mode network's resting state activity level (e.g., in its predominantly medial cortical regions) on cognitive activity associated with lateral prefrontal regions (e.g., working memory). Manipulation of the DMN resting state activity level may be possible in animals by intraregional pharmacological manipulation or electrical stimulation, while in humans intra- or postoperative deep brain stimulation in midline regions may achieve the same purpose. Another instance of quasi-manipulation of the resting state activity level is psychiatric disorders like depression, where the resting state activity has been shown to be abnormally elevated in anterior midline regions (see below for details).

Notwithstanding these suggestions, it will remain rather difficult to experimentally manipulate the level of resting state activity and to enter it as an independent variable in human designs. One alternative is to use the resting state activity level as a modulatory rather than an independent variable. This has been done recently in humans in the above mentioned fMRI-MRS studies where the resting state level of GABA was entered as a modulatory variable into the design and analysis of the fMRI data. The same could be done regarding resting state functional connectivity between, for instance, regions of the DMN that may bias and predispose the functional connectivity during stimulus-induced activity. An analogous strategy has been pursued when combining anatomical and functional resting state connectivity measures showing that the latter strongly overlap, though incompletely, with the former (Greicius et al., 2009). Finally, the resting state level of biochemicals like GABA and glutamate in one particular region may also be entered as a modulatory variable into the analysis of stimulus-induced functional connectivity of that region with another one. For instance, a recent study demonstrated that the level of resting state glutamate in the perigenual anterior cingulate cortex predicts the degree of functional connectivity of that region with the supragenual anterior cingulate cortex during emotional stimulation (Duncan et al., submitted for publication). Q3

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While these suggestions for possible experimental designs concern humans, it should be mentioned that in non-human animal studies inclusion of the resting state activity as a modulatory variable is common practice with some neuroscientific methods. There are many experimental methods in the animal literature that consider the brain's resting state prior to the observer's input, though not typically as an independent variable. Instead, baseline recordings are generally used to normalize otherwise variable data sets. For instance, virtually all experiments employing electrophysiological techniques, or pharmacological techniques investigating in vivo changes in neurochemical concentrations (such as microdialysis or cyclic voltammetry), rely on initial baseline measurements in order to compare subsequent stimulus-induced changes.

What is done in animals is also possible in human imaging studies. One way to account for the resting state activity level prior to the stimulus presentation is to include the baseline condition as a regular condition. Usually the baseline condition, as it is called in imaging, consists of a fixation cross which is considered as an intertrial interval. One could now design and model the baseline condition as a regular condition randomized in between the conditions related to stimulus presentation. That makes it possible to compare those stimulus-related conditions that follow a prior baseline condition to those without preceding baseline conditions. The difference between the two stimulus-related conditions may then be due to the prior resting state activity level and its interaction with the stimulus. While this provides one possible experimental option to account for the impact of the prior resting state activity level, we should be careful though in making clear that what the imagers call baseline does not reflect a proper measure of resting state activity. This is so because the often used fixation cross requires the eyes to be open and an additional effort of fixation which therefore cannot be considered an appropriate measure of resting state activity (Logothetis et al., 2009).

6. Methodological relevance of the brain's resting state activity

We demonstrated the empirical and experimental relevance of the brain's resting state activity as the brain's input to its own neural processing of stimuli. Different ways of how to account for the brain's resting state activity as the brain's input into our experimental designs were suggested. If we need to include the brain's resting state activity as an additional input into our experimental designs as either an independent or modulatory variable, the question arises how this affects the other variables. This concerns the stimuli the observer employs, the observer's input, the measured and observed neural activity, i.e. the brain's output, and the investigated subject's role or input, the subject's input (see Fig. 1).

The above described results of rest–stimulus and stimulus–rest interaction as well as their experimental implications clearly show that the neural activity we observe may not be completely related to and determined by the stimuli we as observers employ. Instead, what we observe as neural activity, the brain's output, may rather reflect a *mixtum compositum* of both the brain's resting state activity level and the stimulus-induced activity. This however means that the effects of the stimuli we employ, the observer's input, cannot be completely traced back to the observer himself. In other words, what we observe and measure as neural activity, i.e. the brain's output, may not be completely and exclusively related to our stimuli, the observer's input, but rather to the interaction between stimuli and the brain's resting state activity. This however means methodologically that the observer's input cannot be regarded as a completely independent variable in our experimental designs. Instead, it may also be conceived, at least in part, as a dependent variable in that its effects are very much dependent upon the resting state activity level (then considered the independent variable).

This approach may have serious implications for our designs. For instance, if one wants to investigate the impact of stimulus-induced activity on the resting state activity level, one may enter the latter as a modulatory or even a dependent variable in the experimental designs. Rather than modelling the events in the design matrix in imaging experiments according to the stimulus-related conditions, one may enter the latter as a main regressor of interest when taking the baseline or resting state periods as main event. One may then compare the regional activity changes from

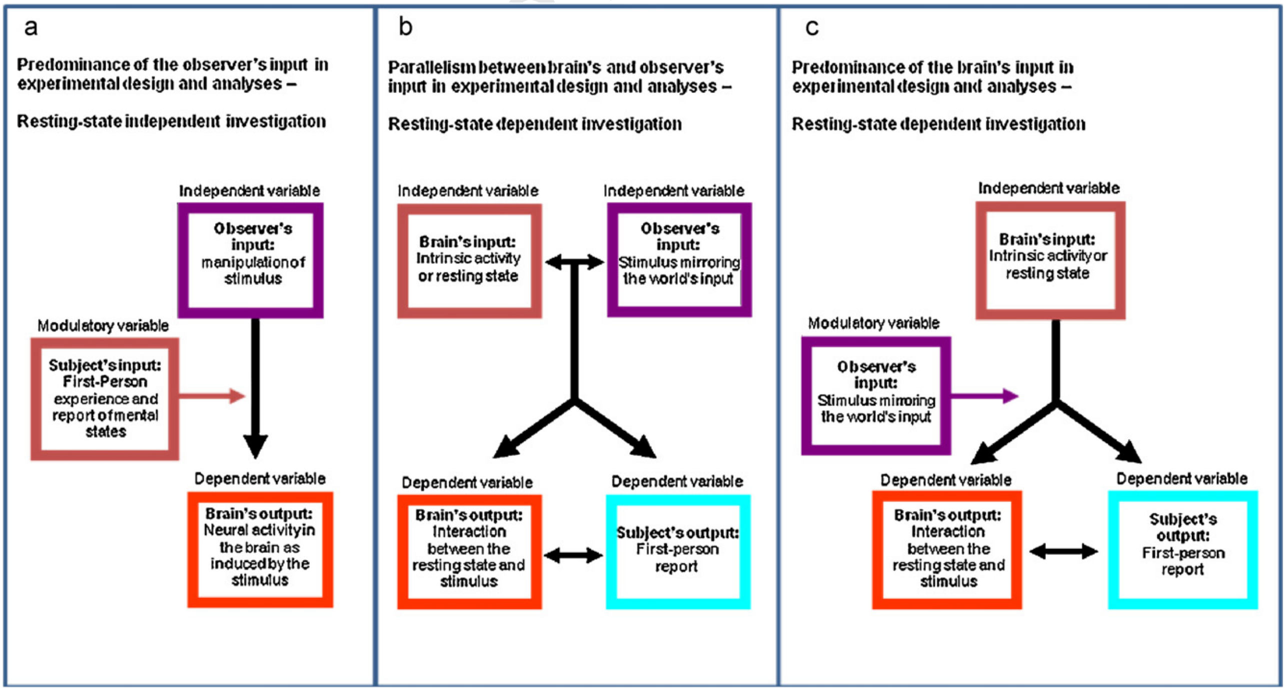

Fig. 1. Methodological approaches to experimental design and analyses in the neurosciences and particularly brain imaging.

the design matrix that included the stimulus-related conditions with those that did not. While this may be one way to circumvent the difficulties of the subtraction method in imaging designs, it may fail in others. This is especially the case when subtracting two stimulus-related conditions, main and control, from each other. Possible differential interaction of the resting state with the main and the control condition are not accounted for here. One way to do that is to include the independently measured resting state activity level for either the whole brain or the respective region of interest as a co-variate into the design matrix. Hence, the inclusion of the brain's resting state activity in the experimental designs may pose some serious challenges to the typical subtraction designs presupposed in imaging. We may thus need to extend the subtraction approach to be more inclusive such that it can take the resting state activity level as either an independent or a modulatory variable into account.

Let us briefly summarize on a more general level. These considerations make it clear that the inclusion of the brain's resting state activity into the experimental designs entails shifts in the relationship between the measured neural activity, the brain's output, and the stimuli employed by the observer, the observer's input. While often neglected in many current designs (see Fig. 1a), the brain's input may be included in the experimental set up as a parallel (Fig. 1b) or non-parallel (see Fig. 1c) independent variable. The inclusion of the brain's input also changes the definition of the brain's output. The brain's output can thus no longer be defined merely as stimulus-induced activity but, instead, must be considered as a rest–stimulus interaction while still occupying the position as a dependent variable. The observer's input, the stimuli the neuroscientist employs, may then either be a second independent variable alongside the brain's input (Fig. 1b) or, even more radically, merely a modulatory variable (Fig. 1c; for instance, see Freeman, 2003, as an advocate of such a solution).

What about the subject's input? The subject's input describes the subjective experience and thus the behavioural or self-reported manifestation of the mental state in question which is taken as the subject's own input into the experimental design. In other words, the subject's input may describe the experimental target variable if one wants to understand the neural mechanisms underlying mental states that can be accessed only in subjective experience. If the subject's input is to be modulated or manipulated by the observer's input, the subject's input may also function as a dependent variable, thereby making a distinction between the subject's input and output necessary. This however changes the whole scenario. The subject's input is then no longer merely a modulatory variable but is instead an independent variable that stands alongside the brain's input as independent variable. While the subject's output may function as a dependent variable alongside the brain's output.

Taken together, it is clear that inclusion of the brain's input as an independent variable may make changes in the methodological approach necessary, although it remains unclear how far these changes would need to go. It is particularly unclear, for instance, whether it would be sufficient to include the brain's input as an additional independent variable alongside the observer's input, or whether we would have to go one step further and treat the observer's input as a modulatory variable. Due to lack of data, we are currently unable to decide which kind of methodological approach would be most appropriate—a decision which, ultimately, may also vary according to the neuronal mechanisms and psychological function in question.

7. Implications of brain's resting state activity

We demonstrated empirical evidence for the interaction between resting state and stimulus-induced activity. This was followed by a discussion of the experimental and broader methodological implications of the brain's resting state activity in our experimental designs. As such, specific strategies for approaching and investigating the brain and its resting state activity were suggested. Besides the purely methodological aspect, one may now raise the question whether all this will help us in getting a better grip on some currently unresolved issues in neuroscience.

One question regards how a stimulus-specific neural response can be elicited on the basis of a stimulus-unspecific response in, for instance, the DMN. As shown in many studies (Raichle et al., 2001; Shulman et al., 1999), the neural activity elicited in the DMN is unspecific to the stimuli with different types of stimuli inducing the same kind and degree of neural activity (as for instance predominant negative BOLD responses in especially anterior midline regions). The question is how such stimulus-unspecific responses in for instance the DMN translates into the rather stimulus-specific responses we observe when employing our specific stimuli. While no stimulus-specific activity may be observed at all in the absence of the resting state activity, the latter is not sufficient to account for the observed neural activity. Hence, it seems that both resting state activity and stimuli are necessary and are needed to interact in a complementary way to generate the kind of stimulus-specific activities we observe as the brain's output. However, neither the functional principles underlying the transition from stimulus-unspecific to -specific responses, nor those guiding rest–stimulus interaction, are currently known yet.

Another unresolved issue is consciousness. While abundant neural theories of consciousness have been suggested (see Koch, 2004; Tononi and Koch, 2008, for overviews), the exact neural mechanisms giving rise to consciousness remain unclear. Does the brain's resting state have a role in generating consciousness? A recent suggestion assumes the low-frequency oscillations in the range between 0.01 and 1 Hz to be crucial (He and Raichle, 2009) which however may not be sufficient to account for the conscious contents (Koch, 2009). If however the low-frequency oscillations cannot account for conscious contents, they cannot be considered sufficient conditions and thus neural correlates of consciousness (NCC). They may be relevant, for instance, in that they may be necessary but not sufficient neural conditions of consciousness (see Shulman et al., 2009b for a step in this direction). This means that without a proper resting state activity, consciousness cannot be generated at all while its mere presence is not yet sufficient by itself to generate conscious states. Rather than of neural correlates as sufficient conditions of consciousness, one may then better speak of what we call 'neural predispositions' that describe enabling or necessary but non-sufficient conditions. If so, the brain's resting state activity level, the brain's input, may be a neural predisposition rather than a neural correlate that enables and predisposes us to develop consciousness.

Another issue hitherto unresolved in neuroscience is the exact pathophysiological mechanisms underlying psychiatric disorders like depression and schizophrenia. The behavioural relevance of the brain's resting state activity and its impact on subsequent stimulus-induced activity and behaviour is further underlined by consideration of pathological conditions. Altered resting state activity and connectivity have been implicated in a range of common neuropsychiatric conditions including major depressive disorder (Grimm et al., 2009), schizophrenia (Zhou et al., 2007; Garrity et al., 2007; Kim et al., 2009; Whitfield-Gabrieli et al., Q4 2009), Alzheimer's disease (Liu et al., 2008), and autism (Kennedy and Courchesne, 2008). Considering major depressive disorder (MDD), resting state activity in specifically the sub/pregenual anterior cingulate cortex has been characterized by various changes including abnormal functional connectivity to the

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thalamus (Greicius et al., 2007), abnormal modulation by glutamate rather than GABA (Alcaro et al., 2009; Walter et al., 2009) and decreased activity during external stimulation (Alcaro et al., 2009; Grimm et al., 2009; Sheline et al., 2009), which have all been shown to be related to depressed symptoms. These resting state abnormalities in MDD, along with those in other neuropsychiatric disorders, underline the crucial relevance of the brain's resting state activity, the brains input, for our behavioural and mental states. While at the same time, this may give us a new understanding about the often rather bizarre looking mental states in these patients.

8. Conclusion: do we need to adapt our experimental strategies to the brain?

Despite attempts to dig deeper into the brain itself and decipher its input, we are still left with the question of the principles that guide the brain's function. The current data clearly suggest a central role for the brain's intrinsic activity, e.g., its resting state activity. While conceptually the brain's resting state activity might be clearly segregated from stimulus-induced activity, this looks different when it comes to the brain itself and its way of neuronal processing. As presented here data show interaction between resting state and stimulus-induced activity amounting to rest–stimulus and stimulus–rest interaction. This means that what we observe and measure as neural activity, the brain's output, may be a hybrid of both resting state and stimulus-induced activity. While this makes it impossible to clearly define and segregate both resting state and stimulus-induced activity as distinct variables in our experimental designs, these data show the need to at least approximately account for the brain's resting state activity. We need to design our experiments in relation to the brain's intrinsic activity and its impact on stimulus-induced changes in neural activity including the modifications the latter seems to induce in the former. This will require novel methodological strategies, some of which are discussed here.

Remaining unclear for now, the development of novel methodological strategies may also make possible the investigation of some unresolved issues in neuroscience related to consciousness and psychiatric disorders in a new and original way. The brain's resting state activity may prove a promising arena which will likely have widespread implications for all neuroscience-related fields. In order to take advantage of this, we need to prepare ourselves well by adapting our experimental designs and strategies to the reality of our own brain as suggested by our recent data.

Conflict of interest

The authors have no conflicts of interest to declare.

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