

Overview of potential procedural and participant-related confounds for neuroimaging of the resting state

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Studies of intrinsic brain activity in the resting state have become increasingly common. A productive discussion of what analysis methods are appropriate, of the importance of physiologic correction and of the potential interpretations of results has been ongoing. However, less attention has been paid to factors other than physiologic noise that may confound resting-state experiments. These range from straightforward factors, such as ensuring that participants are all instructed in the same manner, to more obscure participant-related factors, such as body weight. We provide an overview of such potentially confounding factors, along with some suggested approaches for minimizing their impact. A particular theme that emerges from the overview is the range of systematic differences between types of study groups (e.g., between patients and controls) that may influence resting-state study results.

Introduction

Studies of the resting brain have proliferated in recent years. These studies of the brain's intrinsic activity have involved a range of imaging modalities, including magnetic resonance imaging (MRI), electroencephalography (EEG) and positron emission tomography (PET), and have been undertaken in a wide range of participant groups. The results have shown great promise in advancing our understanding of the functioning of healthy and diseased brains.^{1,2}

Although there has been a detailed debate over the appropriate methodologies for analyzing resting-state data³⁻⁵ and the interpretation of any findings,^{6,7} and although there have been individual studies describing specific sources of noise,⁸⁻¹⁰ to our knowledge, there has been no literature collating and considering some of the factors that may be relevant to the methodology and interpretation of such experiments in a more general sense. We thus provide an overview of some of the confounding factors that have been shown to alter the properties of resting-state activity and that may be relevant to the approaches to data acquisition and analysis in such experiments. These experimental confounds can be distinguished from issues relating to particular analysis techniques or targets (e.g., negative blood oxygen level-dependent [BOLD] responses,¹¹ functional connectivity¹²) as well as from

questions regarding the purpose or content of resting-state activity.^{6,13}

It must be noted from the outset that the experimental resting state is necessarily only an operationalization of the concept of a "true" resting state (i.e., one composed solely of intrinsic activity).^{7,10} This is because a situation in which there is no activity owing to extrinsic stimuli is impossible, as there is always some input from the environment and body. For our purposes, we considered resting-state studies to be those that involved measures of some aspect of brain function, such as intraregional neuronal changes or inter-regional synchrony, in conditions that did not include any explicit external stimuli. In addition, as many resting-state studies to date have focused on the default mode network (DMN),¹⁴ we considered factors that have been shown to influence this network specifically. We assumed that those factors that affect resting-state measures in the DMN may also have similar effects in other regions and so should be taken into consideration until it has been shown otherwise.

The range of techniques being used to analyze resting-state data is rapidly expanding, as is the variety of regions studied. The techniques and regions discussed in the present review are necessarily limited by the literature available at the time of writing; further research specific to particular novel techniques may be necessary. The range of scanning modalities of studies

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covered for each factor is also necessarily dictated by the range of work that has been published to date. This has meant that the context of the factors covered is skewed somewhat toward functional MRI (fMRI), as this is the modality in which most work has been undertaken. We included other modalities whenever information was available. Given the likelihood that effects seen in one modality will have equivalent effects in others, it has been suggested that attempting to link findings across modalities may be a fruitful overall research direction.

The factors identified for our review are divided into 3 general groups (although in some cases there is some overlap among them). First, we consider those factors that we class as procedure-related. By this we mean those factors that are related to the setup of the experiment itself, as well as those

that are relevant to the manner in which data are analyzed. Second, we consider those factors that can be described as participant-related. These factors are those that are inherent to the participants themselves and to their state at the time of the experiment. Finally, factors that may be relevant but for which there is currently no direct experimental evidence are also presented with a view to suggesting possible research directions. Where relevant, we suggest ways of avoiding the particular confounding factor being discussed. An overview of the confounds discussed can be found in Table 1.

Procedure-related factors

The validity of an experiment depends on its procedural

Table 1: Overview of confounds*

Confounding factor	Effect	Comments/suggestions
Instructions to participant	Differing activity and FC with different instructions to participant. Particular effect in dmPFC.	Consistent instructions should be given to all participants. Care should be taken with groups that may respond differently to instructions.
Time of day	Spatial extent of RSNs generally consistent throughout day (greater variability is seen in some networks, e.g., hippocampal and occipital). Substantial variation in regional usage strength exists, except in midline regions. Global small-world properties of RSNs vary throughout the day.	Care should be taken when scheduling experiment times to reduce time of day effects.
Visual noise	Opening or closing eyes changes resting-state measures significantly, including EEG measures, ALFF and resting activity. Rhythmic visual noise may produce spurious patterns in resting-state measures.	Consistent instructions should be given and compliance may be monitored with a simple camera setup. Eyes closed may be the optimal condition.
Auditory noise	MRI noise reduces measured spatial extent of the DMN; MRI noise alters activity in the auditory cortex and other regions (including cingulate and insula); MRI noise may interfere with EEG data acquired using simultaneous MRI-EEG.	Noise attenuation should be used. Comparisons between modalities may consider noise differences. Systematic differences among groups is important.
Age	Reduced FC in the DMN and increased FC in the somatosensory cortex with age. Long range connectivity reduced with relatively small increases in age. EEG frequency changes throughout life.	Include participant age in analysis.
Body weight	Resting EEG measures vary with BMI, including reduced α activity in overweight. MEG Δ and β synchronicity increased in obese participants. Reduced FC in overweight participants.	BMI can be easily recorded and included in the analysis.
Sex	Women show higher resting-state measures, including FC, network efficiency, ReHo, in the left hemisphere; men in right. Greater α , β , γ power in women; greater EEG entropy in men. Greater frontal NIRS fluctuations in men.	Sex should be controlled for in resting-state studies. Particular care should be taken to match among groups.
Emotional state	Anxiety may alter FC patterns (e.g., increased coupling between insula and thalamus).	Where possible, participants should be familiarized with experimental environment.
Sleep	Transition between wakefulness and sleep alters FC and "anti-correlated" networks. Sleep deprivation reduces coherence and FC in DMN; also reduces "anti-correlations."	Care should be taken with experiment scheduling and participants' state of wakefulness noted. Sleeping during the experiment may be monitored by camera.
Movement	Greater mean movement reduces long-range inter-regional FC, increases short-range FC. Affects ICA and ALFF. Movement may produce neural activity in itself.	Particular care in analysis must be taken when comparing groups with systematic differences in movement.
Caffeine	Alters BOLD response; reduces measures of functional connectivity and ALFF; reduces global α power and increases mean α frequency.	Instruct participants not to consume caffeine in hours before experimental session.
Nicotine	Acute nicotine consumption alters activity and FC in DMN and other networks; increases EEG $\alpha 2$ power; reduces cerebral glucose metabolism. Craving leads to activations in frontal midline regions and deactivations in posterior ones, with EEG α band effects. Chronic use reduces gCBF and has effects on vasculature.	Smokers should abstain for 2–3 hours preceding experimental session. Smoking status and history should be taken for all participants.

ALFF = amplitude of low frequency fluctuations; BMI = body mass index; BOLD = blood oxygen-level dependent; DMN = default-mode network; dmPFC = dorsomedial prefrontal cortex; EEG = electroencephalography; FC = functional connectivity; gCBF = global cerebral blood flow; ICA = independent component analysis; MEG = magnetoencephalography; MRI = magnetic resonance imaging; NIRS = near infrared spectroscopy; ReHo = regional homogeneity; RSN = resting-state network.

*Confounding factors identified are listed along with their observed effects on resting state experiments and brief comments on how their effects can be minimized. References can be found in the relevant section of the main text.

consistency across all of the samples and measurements that are to be compared. Similarly, the conclusions that can be drawn from a particular study depend on knowledge of the variables that are altered, regardless of whether they are the direct target of the experiment or whether they are extraneous confounds. In addition, if results from one experiment are to be compared with those of a similar one, then the possibility that differences in results may stem from slight differences in the methods employed must be considered.

In this section, we detail a range of procedural aspects of resting-state experiments that alter the properties of the measurements obtained from participants and that may thus be confounding factors to be considered when making inferences from the data. These factors are those that are inherent to the experimental design and scan-day procedure and, as such, should be consistently relevant across all participants (although there is scope for differences in individual responses to the procedures). Such experimental factors may also be relevant to comparisons among studies owing to any potential interstudy differences. Particular strategies for the analysis of acquired data are beyond the scope of this review and have been discussed elsewhere.³⁻⁵

Participant instructions

Most resting-state experiments involve the participant remaining within the particular scanning equipment for a period of minutes, at rest and with no specific external stimuli.

Although such a setup is simple, there is still a need for the researcher to provide some instruction to the participant before starting the experiment. Thus there is scope for a variability in neural responses that is related to differences in the exact content of instructions across participants or among studies rather than to "true" differences in resting-state activity.

The importance of this factor is demonstrated by the finding of an effect of instruction content on acquired data in a study by Benjamin and colleagues.¹⁵ They showed in this MRI study that instructing the participants to relax and be still during the scan produced different results from those obtained when participants were instructed to either attend to or ignore the scanner noise. More specifically, the activity and connectivity of the dorsomedial prefrontal cortex (dmPFC) was altered, with increased intraregional activity and differential connectivity patterns with other brain regions, such as the posterior cingulate cortex (PCC), when instructions other than just to relax were given.

The different instructions given in the study by Benjamin and colleagues mimic the possible differences between individual participants' approaches to resting-state scans in the absence of specific instructions. Some participants may respond to the scanner noise in a specific way (e.g., actively trying to block it out rather than relaxing), and this difference may then result in differences in the measures obtained for each participant. The authors further pointed out that the situation is of particular interest when comparing 2 participant groups, each with a systematic inclination toward one approach (e.g., patients with schizophrenia v. healthy controls). Such an inclination could result in apparent differences be-

tween the groups related to how they act during the scan rather than to genuine neural differences. Support for this possibility is given by the authors' finding that regions, such as the dmPFC, that have been identified in past studies as regions that differ between patients and controls are also ones that show an effect of instruction content.

The confounding effects of instruction content can be mitigated by using a standardized script for all participants and ensuring that they all understand the instructions. Unfortunately, a complete attenuation of this factor is not possible because participants do not always follow the instructions they have been given to the same degree. Similarly, differences in behaviour between groups that are in some way related to the nature of 1 or more groups (e.g., patients with schizophrenia v. healthy controls) are hard to remove entirely, but can be taken into consideration when interpreting results.

Time of day

Circadian rhythms are a powerful feature of the human body and have wide-ranging effects.^{16,17} Time of day has been shown to affect a number of psychological processes, including memory, executive function and attention.¹⁸⁻²⁰ In addition, glucose metabolism, a common target of PET resting-state studies, has been found to be altered throughout the day in a number of cortical and subcortical regions.^{21,22} In addition, circadian rhythms differ, sometimes quite substantially, among individuals in terms of the peak and nonpeak stages of the daily cycle (i.e., the difference between so-called "owls" and "larks").^{23,24} In conjunction, these observations question the relevance of the time of day at which resting-state experiments are carried out to the results obtained.

An fMRI resting-state experiment by Park and colleagues²⁵ that sought to specifically test the consistency of resting-state measures throughout the day showed that most resting-state networks (RSNs) display a generally consistent spatial delineation at different time points. For example, the spatial properties of the DMN were highly conserved throughout the day. However, not all resting-state networks displayed such spatial consistency; some, such as the hippocampal and occipital networks, displayed a greater degree of variability. The finding that the DMN shows consistent spatial properties throughout the day is supported by a study of attention using a Stroop-like task that found no changes in the pattern of task-induced deactivation in DMN regions throughout the day.²⁶ This was in contrast to activity alterations in regions within attention networks, such as the supplementary motor area, parietal lobe and frontal eye fields.

In contrast to the general spatial consistency of RSNs throughout the day, Park and colleagues²⁵ found that within RSNs there was significant variation in regional usage strength — a measure of the degree to which a particular network contributes to intrinsic fluctuations — across time. An exception to this was those network constituents around the central and posterior midline, such as the PCC and superior-parietal region, which displayed consistent usage strength throughout the day. In addition, Park and colleagues²⁵ found that the global small-world properties^{27,28} of RSNs differed

significantly throughout the day, contrasting with consistent local efficiency within regions over time.

With some measures of resting-state activity being consistent throughout the day and others differing considerably, a case can be made for considering time of day when scheduling and analyzing resting-state data, although more research is warranted. Comparisons among groups should, where possible, be based on data acquired at comparable hours.

Visual noise

With the experimental resting state being an operationalization of an ideal, entirely input-free resting state, the degree of extraneous external stimulus present is of key importance.^{7,10} In this context, the amount of visual stimulation present during the experiment must be taken into account.

First to be considered is whether the participant should be instructed to remain at rest with their eyes open or their eyes closed. Significant differences in the properties of the brain's resting activity have been reported between these 2 states.²⁹⁻³⁴ These differences include alterations in multiple EEG bands,^{35,36} changes in the amplitude of low-frequency fluctuations in the visual cortex and paracentral lobule (note that this study acquired data from the posterior portion of the brain only, and so changes in other regions may also occur³⁴) and differences in activity levels in the sensory cortices.³¹

Interestingly, it should be noted that the changes in brain activity seen between the eyes open and eyes closed conditions do not appear to be simply owing to a difference in the amount of light seen. The opening and closing of the eyes in darkness has been found to produce alterations in sensory cortices and cortical midline structures in both blind and sighted individuals,^{30,33,37} suggesting that there is an effect of opening or closing the eyes beyond that relating to visual stimuli per se.

Also to be considered are any sources of visual input that may not be obvious but that may produce responses in the brain that could be mistaken for intrinsic activity. For example, Logothetis and colleagues¹⁰ demonstrated that an almost imperceptible flicker from a visual stimulator produced rhythmic responses in the brain that could be mistaken for organized and physiologically relevant intrinsic activity.

To minimize the potential confounding effects of visual noise, consistent instructions should be given to all participants. Compliance with instructions can be monitored using a simple camera setup or in person by the experimenter. Care should be taken to eliminate sources of noise, such as visual stimulators or lights that operate at a particular frequency. For most resting-state experiments, where the aim is to obtain measures as close to a true baseline as possible, eyes closed in a minimally lit room appears to be the optimal condition.³⁶ This state minimizes the chance of extraneous visual inputs¹⁰ and the issue of external input confounding intrinsic activity,^{31,38} while minimizing the anxiety-inducing effects of complete darkness.³⁹

Although the consideration of eyes open versus eyes closed is not always relevant within studies (given that all participants should be in the same state), it becomes of key

importance when comparisons are made among studies. Apparent differences between 2 groups taken from the literature (e.g., patient group v. healthy controls) may be due to this simple factor rather than a genuine difference between the groups. Thus it is important that this information be clearly reported in resting-state studies.

Auditory noise

Different scanning techniques have different levels of background noise, ranging from none (e.g., EEG) to more than 100 dB (e.g., MRI). Such levels of noise and their differences among techniques may be a source of confounding effects on measurements obtained during resting-state experiments and on the validity of comparisons among studies.

In MRI studies, the presence of scanner noise has been shown to reduce the extent of DMN activity, a common target of resting-state studies, as determined by a contrast between rest and task.⁴⁰ Although this study by Gaab and colleagues⁴⁰ indirectly measured resting-state activity through contrast with an auditory task (raising potential questions as to the nature of the effect measured), the result was suggestive of a general effect of noise on the resting state. This would arguably be analogous to the more robust findings on the effects of visual noise on the resting state described above.

Support for an effect of scanner noise on resting-state data comes from studies that have been carried out using methods, such as sparse sampling, that present stimuli during the silent periods between spaced MRI acquisitions. In addition to altering the response of the auditory cortex,⁴¹ such studies have found that scanner noise can interact with stimuli to produce differential activity in, for example, the insula, cingulate gyrus and occipital cortex.^{40,42,43} We can thus infer that scanner noise has an effect on these regions in the absence of stimuli, although further research on this front is required. Also of note is the possibility that MRI noise will corrupt EEG data acquired simultaneously during rest using combined fMRI-EEG. Such a confounding effect has been reported in the context of studies of memory (in both the auditory and nonauditory domain), where MRI scanner noise influenced the EEG responses observed.⁴⁴

The probable effect of noise on resting-state properties points to the need for quality noise attenuation when using MRI and for robust control of background noise when using other modalities. Of equal importance is consistency in the level of noise attenuation across participants. The difference in noise levels between imaging modalities (e.g., between MRI and PET) may also be worth considering when comparing resting-state results, as subtle but spurious differences may be introduced. A final consideration may be the possibility that there are systematic differences in responses to background noises between healthy controls and groups with altered auditory attention, such as people with schizophrenia.⁴⁵

Participant-related factors

Efforts are generally taken to ensure that study participants are as comparable as possible. This can be through selection

for only those who are, for example, right handed or within a certain age range, or by screening for certain psychiatric conditions. However, despite a general assumption of uniformity within such screened samples, there is potentially a great amount of variability among participants. With such variation in physical and psychological traits, there is the risk that experimental results are confounded by these traits. This may be particularly true in comparisons among groups that have systematic differences.

Age

The brain and its functioning are known to change over an individual's lifetime.^{46,47} The factor of age is an explicit variable in studies that compare functional properties among groups at different stages of development (e.g., adolescents v. adults) where there is a *prima facie* reason to believe that this factor will have an effect. The question arises, however, as to whether the variances in the ages of participants that make up a single resting-state study group also have the potential to be a confounding factor for the properties of interest in such experiments.

Age has been shown to affect resting-state functional connectivity between regions. For example, a reduction in functional connectivity within the DMN and within somatosensory networks has been seen in older compared with younger participants.^{48,49} Specifically, Tomasi and Volkow⁴⁹ demonstrated that long-range connectivity (connectivity between different regions) was reduced, but that connectivity within regions (the coherence of regional activity) was not affected. They described a 6% decrease in long-range functional connectivity within the DMN with each decade of life along with a corresponding 3.4% decrease in the dorsal attentional network. The magnitude of these changes over such a relatively small age difference suggests that aging effects may be relevant even within participant groups that are quite closely matched for age (e.g., a healthy group of participants with ages ranging from 18 to 35 years).

It should also be noted that there is some evidence that the effect of aging on the results gained through MRI resting-state analyses observed may to some degree depend on the data analysis methods used. For example, Koch and colleagues⁵⁰ found that independent component analysis (ICA) proved to be more sensitive than cross-correlation methods in detecting age-related alterations in their participant population. Also of note in the context of MRI resting-state studies is evidence that the brain's metabolic properties alter with advancing age. Specifically, the link between BOLD response and neural activity may be altered due to age-related changes in the cerebral oxygen metabolic rate, meaning that changes in BOLD properties in older participants may be of physiological rather than neuronal origin.⁵¹

In the context of EEG, the absolute spectral power has been seen to be sensitive to age differences in young participants aged 3–17 years.⁵² Relative spectral power does not appear to be sensitive to age in the same way,^{53,54} but it should be noted that changes in relative power in a particular band do not necessarily reflect power changes in that band alone and can

give results that are skewed toward the dominant frequency band.⁵⁵ Using a database of 4651 patients, Aurlien and colleagues⁵⁶ found that resting α rhythm frequency increased through childhood until about 20 years of age, at which point it stabilized until about 45 years of age. The amplitude of the resting α rhythm declines from youth until around 35–40 years of age, at which point the amplitude stabilizes. In the case of both the α rhythm frequency and amplitude, female participants have higher measures than male participants, with the differences increasing with age.

Body weight

Perhaps a less obvious factor than age, the body weight of participants has been found to have some effect on resting-state measures. Babiloni and colleagues⁵⁷ measured resting EEG rhythms in participants who were either of normal weight, underweight or obese (no participants had eating disorders). They found differences in $\alpha 1$ and $\alpha 2$ low resolution brain electromagnetic tomography current densities in the occipital, parietal and temporal lobes among the groups. Obese participants showed the lowest values in all regions, with normal-weight participants generally showing the highest and underweight participants being between these 2. It must be noted, however, that the effects seen may have been due to altered EEG properties with differing body composition rather than an underlying physiologic change. However, the effect on resting-state measures would still need to be considered if this were the case.

The finding that body weight in nonpathological participants is related to differences in resting-state measures and that there appears to be a continuum (although not an obviously linear one for some measures) from underweight through to overweight extends previous findings of alterations in resting-state measures in obese compared with lean individuals. In obese participants, a left-sided asymmetry in prefrontal resting α -activation, as measured using EEG, has been observed,⁵⁸ as has been an increase in synchronization likelihood in the Δ and β bands, as measured using magnetoencephalography (MEG).⁵⁹

Magnetic resonance imaging functional connectivity changes have also been observed in obese individuals. Using an ICA approach, Kullmann and colleagues⁶⁰ found a reduction of functional connectivity strength in the right anterior cingulate and left insula, along with an increase in strength in the bilateral precuneus. The functional connectivity strength in these regions correlated with participant body mass index (BMI). In addition, functional connectivity strength in the left orbitofrontal cortex and the right putamen were found to correlate with insulin levels, as measured after fasting. This latter finding is of interest in the context of prior findings that nasally administered insulin produces a change in MEG θ band path length that is correlated to BMI,⁶¹ suggesting an interaction between resting-state, hormonal properties and BMI. However, more research in this area is required.

Obtaining a metric for a person's body weight, such as the BMI, requires no measures beyond those that are routinely taken (i.e., height and weight) when setting up the MRI scanner

for each participant and would be easily obtained in the context of other scanning techniques, requiring no additional measurements. This should make body weight a simple measure to include in group-level analyses. Participant body weight may be of particular relevance in comparisons between healthy controls and patient groups, such as depressed patients, in which there are tendencies toward greater BMI,⁶² leading to systematic differences between groups. Potential hormonal effects (i.e., insulin levels) are harder to account for, and would involve measurements that are impractical in most circumstances. Studies that focus on resting-state measures in individuals with eating disorders may, however, wish to take this variable into account.

Sex

Differences in neuroanatomy between male and female participants have been suggested by a number of studies. For example, at a population level men have larger brains than women, with a lower proportion of grey matter.^{63,64} Some differences in anatomic lateralization between men and women have also been reported, specifically greater hemispheric asymmetries in both white and grey matter in men^{65,66} (although other studies have found no difference^{67,68}). The existence of gross anatomic differences has been shown to be reflected in differences in anatomic network properties, with, for example, local and global network efficiency suggested to be slightly higher in women than men.^{69,70}

Such anatomic differences are mirrored by differences in resting-state measures between men and women. Using a combination of fMRI and ICA, Filippi and colleagues⁷¹ found that functional connectivity within the canonical RSNs⁷² differed between male and female participants (although another, smaller, fMRI ICA study found no difference⁷³). For example, within the DMN, men showed greater connectivity than women within the right cuneus cortex, whereas in the executive network, women showed greater functional connectivity than men in the left inferior temporal gyrus. In addition, connectivity between specific networks (e.g., between the DMN and auditory networks) has been shown to be greater in men than women. Using a data set of more than 1400 participants, Biswal and colleagues⁷⁴ also found differences in functional connectivity between men and women and showed differences in the amplitude of low-frequency fluctuations (ALFF). Small-world functional network properties also appear to differ between men and women.⁷⁵ More specifically, men had somewhat greater local efficiency in the right hemispheric networks, whereas women had greater local efficiency in the left hemispheric networks. Finally, in the context of fMRI, differences in regional homogeneity have been observed, with men showing greater regional homogeneity in the right hemisphere and women showing greater regional homogeneity in the left hemisphere.^{76,77} This difference is linked to some degree with differences in grey matter density.

Although there is some inconsistency in the literature, reasonably robust sex differences in EEG resting-state measures have been reported. In the Δ and θ bands, no clear pattern emerges;⁷⁸ however, in the α , β and γ bands, multiple studies have reported higher power in women.^{56,78–81} In addition to sex

differences in frequency band power, studies have reported differences in resting-state approximate entropy, a measure reflecting the complexity of neural generators,⁸² within separate bands. Specifically, approximate entropy was found to be greater in men in the β and γ bands, and greater in women in the α band.^{78,80} Greater entropy in the α band in women was also shown using a spectral entropy approach.⁷⁹ In addition to EEG, Jausovec and Jausovec⁷⁸ acquired resting-state near infrared spectroscopy data from the same participants, showing that men had greater frontal hemoglobin saturation than women, coupled with faster and more irregular spontaneous fluctuations in oxo- and deoxyhemoglobin. This result appears to tie in well with the concurrent greater entropy measures.

The described range of sex effects on resting measures highlights the importance of controlling for this factor in analyses. Where groups are to be compared, it also underscores the need to match participants for sex. Finally, it should be noted that it is somewhat unclear to what extent the differences described are due to basic anatomic features (e.g., skull thickness, cortical volume) and to what extent they are due to genuine functional differences between the sexes. This, however, does not detract from the need to control for this variable.

Emotional state

Participating in any experiment is an unusual experience for most people. This is particularly true with MRI and PET experiments owing to the unusual environment or to the need for an injection, respectively. As such, the experience can have quite a profound emotional effect on participants — most often an anxious response⁸³ — that has been shown to produce subjective and hormonal changes that correlate with brain activity.⁸⁴

Whereas such emotional responses are of consideration for all experiments, they have, in the form of induced transient emotional responses, been shown to have direct effects on resting-state measures. Eryilmaz and colleagues⁸⁵ presented emotional videos (fearful, joyful and neutral) to participants directly before resting measures were obtained. When compared with neutral videos, joyful and fearful videos produced a number of different changes in resting properties. First, inter-regional coupling was altered, with, for example, greater coupling between the insula and thalamus following fearful videos. In addition, the pattern of network hubs was altered, with the strongest hub shifting from the precuneus to the PCC following emotional videos. The effects observed were particularly pronounced following fearful videos, relating directly to the degrees of anxiety that different participants may experience during scanning.

With the experimental experience being necessarily somewhat unusual, it seems difficult to eliminate the issue of anxiety completely. However, some steps, such as giving participants sufficient information in a form that they can easily understand about what will be happening and why, can be taken. With MRI scans, dummy scanners allow the participants to experience a simulation of the scanner environment before scanning. If such a facility is not available, then prior

exposure to scanner sounds may be beneficial. Also of note in this context is the potential difference between individuals who have experienced 1 or more scanning sessions before the resting-state study (e.g., patients, “professional” study participants), compared with those who have never been in a similar situation. This difference in familiarity with the experimental environment may lead to differences in resting-state measures and should be at least noted during the study.

Sleep

With resting-state experiments requiring participants to sit or lie still and at rest for a period of some minutes, the possibility that they may drift into sleep becomes a real concern. This is particularly true for elderly individuals, in whom the supine position used in most MRI and PET scanners has been shown to induce drowsiness,⁸³ and in students, in whom there is a propensity for lack of sleep.⁸⁶

Functional connectivity in the DMN and anticorrelated networks (often referred to as “task-positive” networks) have been shown to alter between sleep and wakefulness,^{87,88} meaning that apparent differences among participants could be severely confounded by this factor should some stay awake while others sleep. Similarly, EEG measures differ between sleep and wakefulness, making this a consideration in studies using this method.⁸⁹

Also relevant is the finding that partial sleep deprivation alters functional connectivity, as measured using MRI. Sämann and colleagues⁹⁰ found that a single night of disrupted sleep (participants sleeping only from 10 pm until 1:30 am) reduced the strength of coherence within the DMN and task-positive network compared with a full night’s sleep. De Havas and colleagues⁹¹ similarly found that 24 hours of sleep deprivation compared with a normal night’s sleep reduced functional connectivity strength within the DMN and reduced anticorrelations between the DMN and the task-positive network (including the insula, temporoparietal junction and intraparietal sulcus). At the biochemical level, sleep disruption (through shift work patterns) has also been shown to alter frontal lobe γ -aminobutyric acid (GABA) concentrations.⁹² As GABA has been implicated in resting-state related processes¹¹ and is the major inhibitory transmitter in the brain, this raises further questions as to the effects due to sleep deprivation.

Scheduling scans at times of the day when sleepiness is less likely to be an issue (e.g., avoiding scanning very early in the morning, late in the evening and during the “post-lunch dip”) would be a prudent precaution.⁹³ Participants can also be questioned about their levels of tiredness and quality of sleep the preceding evening to gain some information regarding the potential presence of sleep deprivation effects.

The issue of participants sleeping during the experiment is one that is in most circumstances amenable to direct monitoring through a simple video camera setup. Observing the participant’s behaviour during the scan (where an eyes open resting state is used) or recording it for later analysis will allow those who had problems with staying awake to be identified. Such an approach would be a desirable addition to a

simple participant self-report about their wakefulness during the session.

Participant movement

Any participant movement, particularly head movement, is a serious issue for most scanning modalities; thus efforts are generally made to minimize movement. Similarly, a range of methods has been developed to account as much as possible for any movement when analyzing acquired data.^{94,95} The issue of movement is particularly relevant in studies that compare very young or very old participants with healthy adults, as systematic differences in movement can be expected.

In the context of MRI resting-state measures, Van Dijk and colleagues⁹⁶ analyzed the effect of head movement on inter-regional functional connectivity, as determined using a cross-correlation method and local functional coupling.⁹⁷ Participants who displayed a greater mean amount of head motion were found to have reduced inter-regional functional connectivity in the DMN and the frontoparietal control network. In contrast to reductions in functional connectivity in these networks, greater mean head movement was found to increase functional connectivity between the left and right motor cortices, suggesting that head movement can affect the measures obtained from different regions differently. Similar to the increase in functional connectivity seen between the motor cortices, a greater mean motion was associated with an increase in local, intraregional, functional coupling. These findings were replicated in a large juvenile sample⁹⁸ in a study that also looked at the effect of head movement on different resting-state analysis methods, specifically dual-regression ICA and ALFF.³⁴ As with the correlation-based approach, movement was found to substantially affect the other methods studied. Importantly, Power and colleagues,⁹⁹ having found that head movement introduced spurious correlation structures into resting-state results (specifically that long-range inter-regional correlations were reduced and short-range ones were increased), observed that standard preprocessing steps did not eliminate these effects. Even following the typical regressing out of head movement parameters, the spurious correlations remained.

The effects of head movement on functional connectivity observed in these studies were relatively small, but were of a sufficient magnitude to be plausibly mistaken for “true” neuronal effects of interest. Van Dijk and colleagues⁹⁶ also observed that individual participants displayed a similar degree of head movement across scanning sessions, increasing the likelihood that systematic differences resulting from head movement could be mistaken for neuronal differences in comparisons between different groups. Similarly, Satterthwaite and colleagues⁹⁸ observed that the amount of participant movement was correlated with age in their juvenile sample, meaning that systematic differences among age groups are likely.

In addition to head movement influencing the data acquisition methods by altering, for example, the position of the brain in the MRI field,¹⁰⁰ the motion itself can also induce neural activity. Jansen and colleagues¹⁰¹ investigated the effect of movement when EEG signals are used as predictors for

BOLD signals. Participants were cued to move their feet a small amount at marked times during the scan to induce small movements of the head that mimicked those that occur normally during scanning. When convolved with a canonical hemodynamic response function, the artifacts in the EEG signal were found to correlate with BOLD responses in multiple brain regions (including the mid-cingulate cortex, insula and thalamus, all areas of interest in resting-state studies). However, when the artifacts were not correlated with the hemodynamic response function, there were far fewer correlating regions, suggesting that the observed effect was due to motion-related neural activity rather than the effect of motion on the MRI acquisition properties alone.

Caffeine

Commonly taken into consideration in task-related fMRI is the consumption of caffeine. This is based on findings that caffeine alters the properties of the BOLD response.¹⁰²⁻¹⁰⁴ With such physiologic effects in the context of MRI, it seems likely that caffeine also has effects on data acquired using other modalities.

The consumption of caffeine has been found to alter the properties measured in the resting state. For example, using fMRI, Rack-Gomer and Liu¹⁰⁵ demonstrated that the consumption of 200 mg of caffeine 45 minutes before the scanning session reduced both the measured functional connectivity within the motor cortex and the power of low frequency fluctuations (< 0.1 Hz) in this region. Although this study acquired data from the motor cortex alone, the prior results referred to previously that showed an effect of caffeine across the brain in the context of fMRI tasks suggest that its results can be generalized to all regions.

An effect of caffeine on the resting state has also been observed in studies using EEG. Barry and colleagues^{35,36} found that 250 mg of caffeine administered 30 minutes prior to testing produced a global reduction in α power along with an increase in mean α frequency. The reduction in power was particularly pronounced in the lower portion of the α band, a frequency range of particular interest in the context of resting-state studies. These findings suggest that participants' caffeine consumption must be considered in EEG studies as well as MRI studies.

To minimize the effects of caffeine on resting-state experiments, participants should be instructed to not ingest caffeine in the hours before the scanning session (including a reminder that caffeine increasingly appears in products and foods other than the obvious tea and coffee).

Nicotine

As with caffeine, nicotine is a stimulant that is widely used. Importantly, the proportion of individuals using nicotine is much greater among those who have mental disorders than among those who do not.¹⁰⁶ This is of key importance when comparing patient and control groups, as there may be a systematic difference in nicotine use between the groups and thus scope for spurious differential imaging results.

The potential role of nicotine as a confounding variable affecting resting-state experiments is multifactorial, as both its acute administration and craving due to abstinence in addicts can influence the brain, as can the effects of chronic use. Nicotine administration has been observed to have cognitive effects, particularly on attention.¹⁰⁷ In comparisons between nicotine and placebo, these cognitive effects have been linked to deactivation of the DMN in response to tasks¹⁰⁸ in conjunction with increases in activity in other attention-related regions.¹⁰⁹ In addition to such task-related changes in the DMN, a similar pattern of changes in response to nicotine administration (i.e., a reduction in DMN activity and an increase in activity in attention networks) has been observed in an fMRI resting-state study,¹¹⁰ along with changes in fMRI functional connectivity between subregions of the DMN and other brain regions.¹¹¹ Similarly, a recent EEG study found that resting EEG measures in nonsmokers were altered by nicotine administration; specifically an increase in left frontal $\alpha 2$ power was reported.¹¹²

Task-induced nicotine craving has been seen to alter activity within regions, including the anterior cingulate cortex (ACC), dmPFC, PCC and precuneus, that are of typical interest in resting-state studies.^{113,114} The changes observed in these studies were found to be even more extensive when the participants were instructed to consciously resist their cravings, with additional activations in prefrontal regions. Cued nicotine craving has also been suggested to alter α band properties in EEG studies.¹¹⁵ Finally, arterial spin-labelling resting-state scans acquired after a period of nicotine abstinence (≥ 12 h) have identified increases in blood flow in the ACC/orbitofrontal cortex.¹¹⁶

In addition to regional effects within the brain, nicotine use and nicotine withdrawal have more global cerebral effects that may be relevant to resting-state measures. Nicotine administration has been shown to reduce global cerebral glucose metabolism,^{117,118} a factor that may be of relevance in fluorodeoxyglucose (FDG)-PET resting-state studies. Reductions in global cerebral blood flow are also seen after nicotine administration and, importantly, after chronic nicotine use.^{106,119,120} These findings are of particular relevance to studies using methods that depend on blood flow properties, such as fMRI. Also of relevance in this context is the acute effect that nicotine has on the vasculature through nitric oxide-induced vasodilatation, with chronic smoking also leading to dysfunction of the nitric oxide system in the cerebral vasculature.¹²¹

With a range of chronic and acute effects, nicotine use represents a potential confound for resting-state studies, particularly in comparisons among groups with systemic differences in nicotine use. To minimize short-term effects of nicotine administration and craving, smokers (and those on nicotine replacement therapy) may be instructed to abstain in the 2-3 hours preceding the scanning session; this time period fits between the nicotine elimination half-life of around 2 hours¹²² and the onset of withdrawal at 6-12 hours after abstinence.¹²³ For chronic effects and between-group differences, the only solution seems to be awareness of the possibility of a confounding effect and noting all participants' current and historical smoking status.

Potential factors of interest

Menstrual cycle

The menstrual cycle is a powerful modulator of the female body as a whole and has been found to have a range of effects on the brain that may be relevant to resting-state studies. First, at a functional level the menstrual cycle has been found to alter neural responses (as measured using MRI) to different stimuli. These include negative emotional^{124,125} and food-related^{126,127} stimuli. Such effects on stimulus-induced responses suggest that the menstrual cycle may produce a similar modulation of resting-state measures.

At the neurotransmitter level, the concentration of GABA in different parts of the brain, including the occipital and motor cortices, changes in the follicular, as compared with the luteal, phase.^{128,129} An alteration in dopaminergic function across the menstrual cycle has also been observed.^{130,131} It seems reasonable to suppose that such alterations in transmitter function will have concurrent effects on resting-state measures.

Taken together, the described changes that can be brought about by the menstrual cycle at both a functional and physiological level suggest that the question of whether it can also lead to alterations in resting-state activity is a valid one. Future research in this area is thus required.

Posture

Different imaging modalities require participants to be placed in different positions. For example, most MRI scanners require that the participant lie down, whereas most EEG experiments are carried out with the participant in a seated position. Could such a difference in posture lead to differences in the properties of resting-state measures acquired through the different methods?

The relevance of posture as a confounding factor for imaging studies has been discussed previously, with evidence to support the view that there is a general effect.⁸³ Of particular relevance to MRI and PET studies of the resting-state, regional cerebral blood flow (rCBF) has been shown to differ between different postures, with, for example, an increase in rCBF in the frontal and parietal cortices when lying down compared with standing or sitting.¹³² In addition, regional neuronal activity has been found to alter with different postures, as different neural processes are involved in the maintenance of different positions.¹³³

In the context of EEG studies, Harmon-Jones and Peterson¹³⁴ carried out an identical anger-evocation experiment with the participant either upright or supine. A difference in functional lateralization was observed between the 2 positions that corresponded to differences in lateralization that had previously been reported between fMRI and EEG results in a number of studies (i.e., differences between results from upright and supine scanning methods). Although the study by Harmon-Jones and Peterson involved a psychological process that is potentially modulated by position through the difference in capacity to respond to insult be-

tween the 2 states, the observed effect may be transferable to other contexts. Further supporting this suggestion are findings in children that posture affects EEG acquisition, altering the patterns of neural activity observed.⁸³

Finally, evidence points to the influence of posture on brain dynamics being of particular relevance in populations, such as elderly individuals, in whom postural hypo- or hypertension or coronary heart disease are prevalent, as an association between such conditions and postural changes in the brain has been observed. For example, in individuals with the former condition, the posture in which a test is performed has been seen to influence cognitive performance.^{135,136} This would seem likely to mirror a change at the neural level. Similarly, in those with coronary heart disease, rCBF has been seen to differ from reference ranges as a function of posture,¹³⁷ which could affect those resting-state measures, such as MRI and PET, that rely on the physiological properties of the brain.

Taken together, the effects of posture on brain function and physiology described would point to at least a potential relevance of this factor to resting-state studies. However, more research into this question is required.

Conclusion

Although we make no claims of our review being exhaustive, we have presented a range of factors that have been observed to affect resting-state measures across different experimental modalities. These have been observed in both the context of the manner in which experiments are planned and carried out and in the context of natural variability among participants that is unrelated to resting-state measures, *per se*.

The range of factors discussed highlights the need for robust experimental methods when carrying out studies of the resting state. Some comment along these lines has recently been made in the context of cognitive neuroimaging studies,^{138,139} and the findings described here seem to reinforce this position. Indeed, it is worth noting also that most of the issues identified here are likely to be equally relevant to such paradigm-based studies. Although intrasubject test-retest reliability has been shown to be acceptable for some resting-state measures,^{140,141} as the research targets and experimental methods used become more precise and fine-grained, it will be increasingly important to control for the effect of any confounding variables. It should also be noted that the range of techniques now being used to analyze resting-state data is rapidly increasing, as is the variety of regions studied; however, the techniques and regions discussed here are necessarily limited by the literature available at the time of writing, and research specific to novel techniques may be necessary. Along similar lines, it remains to be shown if particular modalities or analysis methods as more robust than others. This information could be beneficial in situations where resting-state measures are used for diagnosis, where the effect of confounds could have major consequences.

As noted previously, those confounding factors where there is potential for a systematic difference among participant groups are of particular importance. Such confounds

include the instructions given to participants and the level of background noise in comparisons between healthy controls and groups that have altered auditory attention, such as people with schizophrenia.⁴⁵ Another example is comparisons between groups that have systematic differences in head movement, such as juvenile or elderly participants in comparison to young adults, where this movement can alter resting measures. If such confounding factors are not taken into consideration, then the strength of claims of differences among groups are greatly diminished. This is a key point as progress is made toward potentially using resting-state measures as biomarkers for particular conditions.

The range of factors we described that can affect resting-state measures also vividly illustrates the difficulty that exists in providing a satisfactory definition of the concept of “rest.” As discussed elsewhere⁷ and shown through empirical methods,¹⁴² the resting-state is an operationalization that is most likely to consist of an amalgamation of true intrinsic activity along with environmental and bodily inputs. Evidence relating to the effect of cognitive load on resting-state measures would also suggest that the individual’s particular attentional state must be included in this “rest” mixture.¹⁴³ Experimentally separating out the different factors that form the resting state thus seems to be one of the key research directions to be followed (where possible), and is an area in which the considerations put forward here will be of particular importance.

Finally, there is potentially a distinction to be drawn between resting-state activity and resting-state measures. The experimental methods used in resting-state studies are generally indirect and so should be seen as providing information about the latter but not necessarily the former. For example, fMRI provides a measure of BOLD response from which information about neuronal responses are inferred,¹³⁹ however, as seen in the current review differences in this value can come about through mechanisms other than neuronal ones, meaning that differences in resting-state measures may not reflect actual differences in resting-state activity. A difference in resting-state measures may thus be seen between 2 groups, but this difference may be due to a non-neuronal feature of one of these groups interacting with the experimental method. Given this, as accurate as possible a knowledge of all the factors that may be influencing resting-state measures in a particular experiment is required to make the inference from these measures to resting-state activity with a reasonable degree of certainty (although, importantly, this still remains an inference).

Research into the brain’s intrinsic activity forms a burgeoning part of the neuroscientific endeavour. However, care must be taken with the experimental procedures adopted to avoid any of the different factors detailed in the present review confounding results or producing spurious differences among participant groups.

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