

Functional network analysis of human brain systems under pharmacological modulation

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"In man is a three pound brain which, as far as we know, is the most complex and orderly arrangement of matter in the universe"

- Isaac Asimov, In the Game of Energy and Thermodynamics You Can't Even Break Even, *Smithsonian* (June 1970), p. 10.

Declaration of originality

I confirm that all unreferenced work described herein is my own unless stated otherwise.

Abstract

Complex alterations in brain function and neurochemistry underlie pathology and treatment in multiple neuropsychiatric disorders, yet remain incompletely characterised. This thesis outlines possibilities for human neuroimaging techniques sensitive to spontaneous fluctuations in large-scale neurobiological signalling, or ‘resting-state network (RSN) functional connectivity’, to address such knowledge gaps.

Novel RSN-sensitive analysis approaches to functional magnetic resonance imaging data are introduced. These techniques are then evaluated experimentally, in contexts relevant for maladaptive cognitive and motivational processing, for their utility to identify and characterise systems-level signatures of individual differences in neurochemistry and psychopharmacological responsiveness.

Firstly, RSN functional connectivity measures are investigated in the context of pharmacological intervention with nicotine replacement therapy in habitual smokers. Results identify connectivity between executive control and ‘default mode’ RSNs as a neural signature of pharmacotherapeutic efficacy in treating cognitive symptoms of nicotine withdrawal. Secondly, RSN connectivity is investigated alongside specific neuroreceptor-sensitive measures to investigate the extent to which network connectivity patterns reflect fundamental neurobiology in healthy subjects. Individual differences in dopamine D3 receptor availability – a possible marker for reward-related behaviours – are associated with topographic connectivity signatures within RSNs implicated in cognitive and motivational control. Thirdly, the ability of RSN metrics to characterise distinct neurochemical manipulations is tested in healthy subjects. Dopamine agonistic and antagonistic neuromodulations display differential effects on signalling in cortico-subcortical and cortico-cortical reward circuitry and interact selectively with subject impulsivity. Finally, RSN cortico-subcortical connectivity metrics are investigated for their sensitivity to clinical-pharmacological effects in Parkinson’s disease. Results reveal evidence for both compensatory large-scale network mechanisms and ‘non-normalising’ dopaminergic medication effects in patients.

Overall, findings indicate novel systems-level neuroimaging methodology probing interactions within and between RSNs to provide sensitive, biologically plausible markers for behavioural and neuropharmacological characteristics of neuropsychiatric disorders. Continued development of functional network analysis approaches may facilitate their direct application to clinical and drug development domains.

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List of abbreviations

BET	Brain Extraction Tool
BGLN	basal ganglia/limbic network
BIS-11	Barratt Impulsiveness Scale (11 th version)
BOLD	blood-oxygenation level-dependent
BP _{ND}	binding potential relative to the nondisplaceable compartment
CNC	change in network coupling
CSF	cerebrospinal fluid
D2R/D3R	dopamine D2/D3 receptor
DA	dopamine agonist
DMN	default mode network
DPU	dorsal putamen
EEG	electroencephalography
EPI	echo planar imaging
(f)ALFF	(fractional) amplitude of low frequency fluctuations
FAST	FMRIB's Automated Segmentation Tool
FDR	false discovery rate
FIRST	FMRIB's Integrated Registration and Segmentation Tool
FLIRT	FMRIB's Linear Image Registration Tool
(F)MRI	(functional) magnetic resonance imaging
FMRIB	Centre for Functional Magnetic Resonance Imaging of the Brain
FNIRT	FMRIB's Nonlinear Image Registration Tool
FPN	frontoparietal network
FSL	FMRIB Software Library
FWE	family-wise error
FWHM	full-width half-maximum
GLM	general linear model
HCS	healthy control subjects
ICA	independent component analysis
ITC	intertemporal choice
MEG	magnetoencephalography
MELODIC	Multivariate Exploratory Linear Optimized Decomposition into Independent Components
MMWS	Modified Minnesota Withdrawal Symptom Scale
MNI	Montreal Neurological Institute
MPRAGE	magnetization-prepared rapid acquisition gradient echo
NRT	nicotine replacement therapy
OFC	orbitofrontal cortex
PCA	principal component analysis
PD	Parkinson's disease
PET	positron emission tomography
ReHo	regional homogeneity
ROI	region of interest
RSN	resting-state network
SBCA	seed-based (partial) correlation analysis
SCA	seed-based correlation analysis
SD	standard deviation
SEN	salience/executive control network
SN/VTA	substantia nigra/ventral tegmental area
TE	echo time
TR	repetition time

List of publications arising from work presented in this thesis

Peer-reviewed articles

DM Cole, SM Smith and CF Beckmann. Advances and pitfalls in the analysis and interpretation of resting-state fMRI data. *Frontiers in Systems Neuroscience*, 4:8, 2010.

DM Cole, CF Beckmann, CJ Long, PM Matthews, MJ Durcan and JD Beaver. Nicotine replacement in abstinent smokers improves cognitive withdrawal symptoms with modulation of resting brain network dynamics. *Neuroimage*, 52(2):590-599, 2010.

DM Cole, CF Beckmann, GE Searle, C Plisson, AC Tziortzi, TE Nichols, RN Gunn, PM Matthews, EA Rabiner and JD Beaver. Orbitofrontal connectivity with resting-state networks is associated with midbrain dopamine D3 receptor availability. *Cerebral Cortex*, 22(12):2784-2793, 2012.

DM Cole, NYL Oei, RP Soeter, S Both, JMA van Gerven, SARB Rombouts and CF Beckmann. Dopamine-dependent architecture of cortico-subcortical network connectivity. *Cerebral Cortex*, in press.

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DM Cole, B Mohammadi, M Milenkova, K Kollwe, C Schrader, A Samii, R Dengler, TF Münte and CF Beckmann. Effects of dopamine agonist treatment on resting-state network connectivity in Parkinson's disease. In revision.

Book chapter

RK Niazy, **DM Cole**, CF Beckmann, SM Smith. "Resting state networks", in *fMRI: from nuclear spins to brain function*, Biological Magnetic Resonance v.30, eds Ugurbil K, Uludag K, Berliner LJ. New York: Springer, in press.

Conference abstracts

- DM Cole**, CF Beckmann, CJ Long, PM Matthews, MJ Durcan and JD Beaver. Nicotine remediates smoking withdrawal by modulating resting-state functional connectivity. *15th Annual Meeting of the Organization for Human Brain Mapping*, San Francisco CA, June 2009. (Proceedings in *Neuroimage*, 47 Supplement 1, p. S67).
- DM Cole**, CF Beckmann, GE Searle, C Plisson, AC Tziortzi, TE Nichols, RN Gunn, PM Matthews, EA Rabiner and JD Beaver. Dopamine D3 receptor availability explains orbitofrontal connectivity with cognitive networks. *16th Annual Meeting of the Organization for Human Brain Mapping*, Barcelona, Spain, June 2010.
- DM Cole**, B Mohammadi, M Milenkova, K Kollewe, C Schrader, A Samii, R Dengler, TF Münte and CF Beckmann. Effects of Parkinson's disease and treatment on cortico-subcortical network functional connectivity. *17th Annual Meeting of the Organization for Human Brain Mapping*, Quebec City, Canada, June 2011.
- DM Cole**, CF Beckmann, AC Andorn, M Bani, PJ Nathan, ET Bullmore, PM Matthews, EA Rabiner and JD Beaver. Coupling between cognitive networks associated with symptoms of compulsive binge eating. *17th Annual Meeting of the Organization for Human Brain Mapping*, Quebec City, Canada, June 2011.
- DM Cole**, NYL Oei, RP Soeter, S Both, JMA van Gerven, SARB Rombouts and CF Beckmann. Dopamine-dependent cortico-subcortical network functional connectivity: association with impulsivity. *18th Annual Meeting of the Organization for Human Brain Mapping*, Beijing, China, June 2012.
- DM Cole**, CF Beckmann, NYL Oei, RP Soeter, S Both, JMA van Gerven, and SARB Rombouts. Linear and nonlinear effects of dopamine neuromodulation on resting-state network connectivity. *18th Annual Meeting of the Organization for Human Brain Mapping*, Beijing, China, June 2012.

Further publications

- JD Beaver, CJ Long, **DM Cole**, MJ Durcan, LC Bannon, RG Mishra and PM Matthews. The effects of nicotine replacement on cognitive brain activity during smoking withdrawal studied with simultaneous fMRI/EEG. *Neuropsychopharmacology*, 36(9):1792-1800, 2011.
- NL Voets, CF Beckmann, **DM Cole**, S Hong, A Bernasconi and N Bernasconi. Structural substrates for resting network disruption in temporal lobe epilepsy. *Brain*, 135(Pt 8):2350-2357, 2012.
- LE Klumpers, **DM Cole**, N Khalili-Mahani, RP Soeter, ET te Beek, SARB Rombouts and JMA van Gerven. Manipulating brain connectivity with δ^9 -tetrahydrocannabinol: a pharmacological resting state FMRI study. *Neuroimage*, 63(3):1701-1711, 2012.
- JD Beaver, G Searle, T Nichols, **DM Cole**, C Plisson, A Andorn, M Bani, G Ghibellini, L Iavarone, A Tziortzi, P Shotbolt, Y Lee, A Bifone, E Merlo-Pich, M Laruelle, R Gunn, PM Matthews and EA Rabiner. Dopamine D3 receptor availability is associated with amygdala reactivity to reward. Under review.

Invited talks

- Modulation of resting-state functional connectivity associated with nicotine withdrawal and treatment. *18th Annual Symposium of the International Society for Magnetic Resonance in Medicine (British Chapter) for PhD Students & Post-Docs*, Imperial College London, United Kingdom, March 2009.
- Dopamine D3 receptor availability explains orbitofrontal connectivity with cognitive networks. *16th Annual Meeting of the Organization for Human Brain Mapping*, Barcelona, Spain, June 2010.
- Network-level neuroimaging of addiction and reward pathways. *Professor David Nutt's Neuropsychopharmacology Unit Meeting*, Imperial College London, United Kingdom, October 2010.

- Brain network functional connectivity in clinical imaging studies: promises and pitfalls. *Annual Summer Meeting of the British Association for Psychopharmacology*, Harrogate, United Kingdom, July 2011.
- Large-scale brain network connectivity, individual differences and addiction. *Special Seminar, Neuroimaging Research Branch of the National Institute for Drug Abuse, National Institute of Health*, Baltimore MD, March 2012.
- Dopamine-dependent cortico-subcortical network functional connectivity: association with impulsivity. *18th Annual Meeting of the Organization for Human Brain Mapping*, Beijing, China, June 2012.
- Systems-level connectivity, neurochemistry & individual differences: neuropsychiatric applications. *Special Seminar, Translational Neuromodeling Unit, University of Zurich and Swiss Federal Institute of Technology*, Zurich, Switzerland, July 2012.
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Chapter 1 – General introduction

1.1 – Theoretical background

The human brain possesses seemingly unrivalled biological and computational complexity. This inherent quality is manifest across multiple, intricately organised and interacting scales: from the genetic level, via molecular and cellular levels, up to microscopic and macroscopic levels of neuronal arrays and large-scale networks of neural systems. Yet, at each level of biological complexity the brain is prone to dysfunction, for example, in various forms of neurological and psychiatric disease. Thus, despite the difficulties facing comprehensive study of this highly evolved organ, the importance of such knowledge for answering key questions of cognition and behaviour, clinical diagnosis, treatment efficacy and prognosis in neuropsychiatric diseases continues to motivate the achievements of researchers in multiple neuroscientific fields, addressing different scales of brain complexity. The result of this is that, in steady strides over the past decades, significant advances have been made in our understanding of the function and dysfunction of distributed regions and networks within this tightly packed, but highly organised, bundle of matter. Arguably, this task has begun to appear a great deal more achievable in recent years due to the advent and continuous refinement of functional neuroimaging techniques.

1.1.1 – Systems-level imaging of the brain: a valuable neuroscientific approach

Conceivably, one of the most promising and, currently, most widely used technique for understanding how the human brain operates is functional magnetic resonance imaging (fMRI) with acquisition sequences sensitive to blood-oxygenation level-dependent (BOLD) contrast. First developed by Ogawa and colleagues (Ogawa et al., 1990), the BOLD fMRI technique uses gradient-echo planar imaging (EPI) sensitive to the deoxygenation of haemoglobin and, by association, the oxygen consumption and energy demands of neuronal populations. It thus provides a comparative, though indirect, measure of the ensemble activity of these populations. Although insufficient to inform *direct* neuronal interpretations, the effective convergence of high spatial and reasonably high temporal resolutions of measurements achievable with fMRI fuels the popularity of this method as a means of testing hypotheses about specialisation of function in the brain (as summarised in a number of excellent texts, e.g., Jezzard et al., 2001; Huettel et al., 2004). To date, this has been achieved chiefly through model-based analyses and statistical contrasts testing the proportion of BOLD signal change across various task and ‘baseline’ conditions. Historically, this approach has been used largely to inform theories of ‘functional localisation’, the spatial segregation across distinct brain regions of specific psychological functions, often with the aim of teasing apart the differential coding of mechanistically related concepts such as face and object perception (Kanwisher et al., 1997), visual and auditory linguistic perception (Dehaene et al., 2002) or comparatively high-level cognitive functions related, for example, to the processing of reward and affective information (e.g., O’Doherty et al., 2003; Behrens et al., 2008).

Within this classical BOLD fMRI approach, brain activity evoked during cognitive or behavioural task paradigms typically has been assumed, for analysis purposes, to conform to a strict temporal model (i.e., an experimenter-derived notion of how functional brain circuitry should *activate* selectively – above a given statistical threshold – to a given experimental manipulation, rather than a data-driven observation of how different aspects of *ongoing* activity within this neurocircuitry are perturbed during environmental or state changes). More recently, however, the ability to identify and quantify the temporal synchronicity of activity between BOLD signalling in distributed brain regions has also been recognised. Correlated dynamic signals across spatially distinct neuroanatomical areas are important because they mirror an inherent phenomenon of complex neural systems, known as “functional connectivity” (Gerstein and Perkel, 1969; Dickson and Gerstein, 1974; Varela et al., 2001; Friston, 2011). In line with principles of neuroplasticity (e.g., those advocated by Hebbian learning theory; Hebb, 1949), patterns of functional connectivity operating across multiple time scales observed using different measures of brain activity, including BOLD fMRI, are thought to reflect distinct neuronal populations activating with a common functional purpose and, potentially, the variability in their inter-regional communication strength (Dickson and Gerstein, 1974; Gevins et al., 1985; Ahissar et al., 1992; Horwitz et al., 1992; Friston et al., 1993; Biswal et al., 1995; Cordes et al., 2000; Buzsaki and Draguhn, 2004).

Functional connectivity analysis techniques have been applied successfully to fMRI data from studies incorporating cognitive-behavioural task models, for which they provide additional information to ‘traditional’ activation-based approaches about network-level processes altering in line with such

models (e.g., Friston et al., 1997; Bokde et al., 2001; Hampson et al., 2002). Despite this, it is arguable that their greatest practical utility may be found in the investigation of task-independent, or “resting-state” BOLD fluctuations (Fox and Raichle, 2007; Fox and Greicius, 2010; Murphy and Mackay, 2011). Since the original discovery of Biswal and colleagues (Biswal et al., 1995) that systems-level functional connectivity phenomena can be identified from BOLD data spontaneously (i.e., without overt experimental stimulation), it has become increasingly clear that resting-state investigations can provide useful complementary conceptual information about the human neural functional architecture to that made available by traditional task-based approaches and, further, that they may be more directly ‘translatable’ to numerous ‘real-world’ applications in clinical and drug development domains (e.g., Greicius et al., 2004; Fox and Raichle, 2007; Castellanos et al., 2008; Fleisher et al., 2009; Varoquaux et al., 2010; Murphy and Mackay, 2011; Khalili-Mahani et al., 2012).

While task-based approaches to fMRI require the assumption that the underlying neural activity of interest conforms to a strict temporal model, many studies assessing functional connectivity (during both tasks *and* the resting state) restrict their hypotheses to strict *spatial* models (e.g., “seed-based correlation analysis”; SCA; see section 2.1.2). However, these temporal and spatial signatures may not reflect fully the patterns of brain activity and connectivity that characterise clinically relevant functional and dysfunctional neural modifications, for example those initiated pharmacologically (Greicius, 2008; Schleim and Roiser, 2009; Murphy and Mackay, 2011). In contrast, multivariate, model-free analysis methods (e.g., “independent component analysis”; ICA; see section 2.1.3) applied to resting-state fMRI data allow the

simultaneous measurement and assessment of multiple, large-scale, functional dynamic systems in the human brain, without the need to impose overly restrictive prior assumptions on the analysis regarding the anatomy or dynamics of pathological or pharmacological effects on said systems (Beckmann et al., 2005; Smith et al., 2009, 2012a). In the context of neuropsychiatric or psychopharmacological applications, it is particularly difficult to impose prior specifications about expected changes in the spatio-temporal patterns of brain activity accompanying, for example, symptom improvements following acute or longitudinal medical or experimental interventions. Therefore, combined resting-state network and model-free analysis approaches may be more appropriate than task- and model-based alternatives for probing brain activity in these unrestricted domains. In this thesis, therefore, these approaches are applied in a number of related contexts and evaluated in terms of both their benefits and limitations.

1.1.2 – Validation of systems-level functional connectivity metrics

With the benefits such as those introduced in the previous section in mind, an increasing number of researchers have begun to include resting-state functional connectivity techniques as standard parts of their testing batteries. However, these methods are not without their criticisms. The lack of *a priori* models describing the temporal characteristics of resting-state fMRI data renders them fundamentally uncontrolled and, in the absence of any experimental intervention, difficult to interpret in terms relevant for cognition and behaviour. One initial and influential critique focussed heavily on debating the value of affording a “special status” to brain activity measured in the resting state, in

particular contesting the idea that such measures could reflect a ‘baseline’ of brain function (Morcom and Fletcher, 2007; although for responses highlighting how this critique is, in part, misdirected, see Buckner and Vincent, 2007; Raichle and Snyder, 2007). Fortunately, consistent observations that the spatial organisation of spontaneous activity strongly mirrors that of task-evoked activity (as measured using BOLD fMRI) somewhat negates these criticisms and allows the neuroimaging community to refocus their attention on the potential (e.g., clinical and pharmacological) applications of these novel, systems-level techniques (e.g., Fox and Raichle, 2007; Poldrack et al., 2009; Smith et al., 2009; Northoff et al., 2010a; Van Dijk et al., 2010; see also section **1.2**).

The burgeoning interest and increased confidence in resting-state functional connectivity techniques is, in part, exemplified by the recent creation of a “Brain Connectivity” journal (Pawela and Biswal, 2011). In the opening issue of this publication, it was highlighted by Friston that functional connectivity fMRI measures historically have been (and still are) interpreted widely and inaccurately as reflecting ‘coupling’ between neuronal populations (Friston, 2011). Although the correlations between measures extracted from fMRI are likely to reflect multiple sources of signal variation, separate from the ‘true’ coupling association between the activity time series of two (or more) neuronal populations, a number of statistical techniques put forward recently show promise in teasing these factors apart (Christova et al., 2011; Friston et al., 2011; Smith et al., 2011). Furthermore, there is compelling evidence from rodent optogenetic fMRI that changes in the correlation between BOLD signals in spatially distinct brain regions do indeed approximate measures of altered functional coupling, within thalamocortical pathways that are known from

neurophysiological studies to be key pathways of oscillatory coupling interactions influencing (in this case motor) function (Lee et al., 2010). In fact, this optogenetic study by Lee and colleagues has provided a crucial validation of functional connectivity metrics as measured using BOLD fMRI, indicating that the mechanisms they represent are fundamentally neuronal in nature.

1.2 – Resting-state networks (RSNs)

Through the application of resting-state fMRI methods¹, a characteristic set of co-activating functional networks (see, e.g., Fig. 1.1) is found consistently across subjects (Beckmann et al., 2005; Damoiseaux et al., 2006; De Luca et al., 2006; Fox and Raichle, 2007; Smith et al., 2009), imaging centres and hardware (Biswal et al., 2010), stages of cognitive development (Fair et al., 2007; Fransson et al., 2007; Doria et al., 2010) and, to some extent, across degrees of consciousness (Boly et al., 2008; Greicius et al., 2008) and across species (Vincent et al., 2007; Hutchison et al., 2010, 2012; Mantini et al., 2011; Lu et al., 2012). Moreover, connectivity within individual networks has been shown to be heritable (Glahn et al., 2010) and altered resting (and stimulus-guided) functioning of large-scale systems has been found in correlation with individual differences in behavioural performance (Fox et al., 2007; Kelly et al., 2008), as well as in neuropsychiatric disease (e.g., Greicius et al., 2004; Castellanos et al., 2008; Di Martino et al., 2009; Seeley et al., 2009) and under pharmacological manipulation (Anand et al., 2005; Hong et al., 2009; Kelly et al., 2009; Khalili-Mahani et al., 2012). As with other functional connectivity phenomena and in line with Hebbian learning principles

¹Portions of this section are adapted from a related publication: Cole DM, Smith SM, Beckmann CF (2010a) Advances and pitfalls in the analysis and interpretation of resting-state fMRI data. *Front Syst Neurosci* 4:8.

(Hebb, 1949), co-activation within these networks is purported to reflect the spontaneous energy demands of activity within neuron populations that, via ‘firing’ together with a common functional purpose, have subsequently ‘wired’ together through synaptic plasticity (e.g., Bi and Poo, 1999, 2001; Saini et al., 2004; Raichle and Mintun, 2006; Lewis et al., 2009). There is, therefore, compelling evidence for these systems, widely referred to as “resting-state” networks (RSNs; Greicius et al., 2003; Beckmann et al., 2005; De Luca et al., 2006), or in some cases “intrinsic connectivity networks” (e.g., Seeley et al., 2007), as core functional networks in the mammalian brain. Within this thesis these large-scale functional brain networks, where appropriate, are referred to as “RSNs”. This nomenclature choice is not intended to imply that the patterns of activity and connectivity observed in this state are fundamentally ‘special’, or even functionally distinct from those modulated during traditional task-based paradigms (thus circumventing some of the criticisms levelled by Morcom and Fletcher, 2007). The intention is merely to imply that the resting state may be a useful context in which to observe and investigate these systems. Indeed, the co-activating networks identified consistently in analyses of resting-state fMRI data are compatible spatially with each of the major distinct brain systems that have been identified using functional imaging methods across a huge number of cognitive and behavioural tasks (Smith et al., 2009; see also section 1.4.1 and Fig. 1.2).

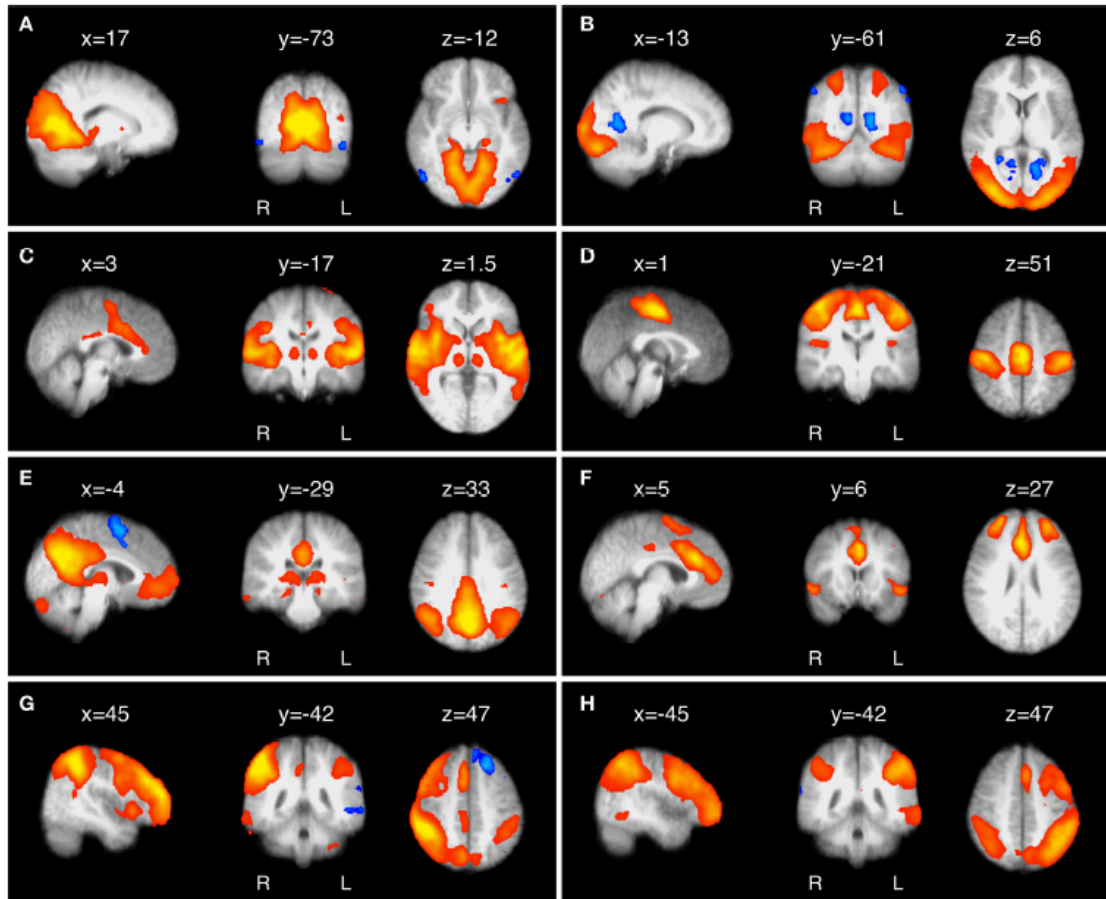


Figure 1.1. Eight of the most common and consistent RSNs identified by ICA: A) RSN located in primary visual cortex; B) extrastriate visual cortex; C) auditory and other sensory association cortices, including portions of bilateral insula and anterior/mid-cingulate; D) the somatomotor cortex; E) the ‘default mode’ network (DMN), involved in episodic memory processes and self-referential mental representations and, conversely, deactivated during demanding cognitive tasks requiring the externalisation of attentional focus; F) a prefrontal network implicated in executive control and salience processing; and G and H) two right- and left-lateralised frontoparietal RSNs spatially similar to the bilateral dorsal attention network and implicated in working memory and cognitive attentional processes (for further details, see Beckmann et al., 2005). Three-dimensional slice co-ordinates relative to the anterior commissure are provided in MNI152 stereotactic space (Montreal Neurological Institute, Canada). Regions showing positive association with the characteristic RSN time course are shown in red-yellow colouring; negative temporal RSN associations in blue-cyan. R = right hemisphere, L = left; in all Figures, brain images are presented in line with radiological convention.

In accordance with the rapidly increasing interest in resting-state FMRI research, a number of analysis techniques have been developed with the aim of probing RSNs in BOLD data (reviewed in Chapter 2). The majority of analysis approaches used thus far have been spatially model-driven, with strong *a priori* hypotheses regarding the functional connectivity of specific regions of interest (ROIs), or

individual voxel locations of interest, within the imaged brain. Recently, however, a great deal of attention has been focused on the patterns of connectivity between multiple ROIs within spatially distributed, large-scale networks – RSNs. RSNs can be identified via both model-driven (e.g., seed-based correlation analysis; SCA; Biswal et al., 1995; Greicius et al., 2003; Fox et al., 2005; see also section 2.1.2) and data-driven analyses (e.g., independent component analysis; ICA; McKeown et al., 1998; Kiviniemi et al., 2003; Beckmann et al., 2005; see also section 2.1.3). There is evidence that they can be reliably and reproducibly detected at individual subject and group levels across a range of analysis techniques (Greicius et al., 2004; Damoiseaux et al., 2006; Shehzad et al., 2009; Zuo et al., 2010a, 2010b). However, different approaches do often provide varying, though complementary, information about the spatio-temporal characteristics of these networks (see, e.g., Chapter 2). Moreover, the ‘ideal’ approach for characterising RSNs tends to depend on the specific questions researchers wish to ask of the data.

This thesis probes the general hypothesis that methods sensitive to large-scale network functional connectivity, in the resting state, may be useful for assessing the systems-level neural implications of underlying neurochemical and psychopharmacological mechanisms. Such contexts and their extensions to combined investigation with clinically relevant questions are relatively under-researched application areas for these methods. Of particular novel interest here is the question of whether or not RSNs can be modulated in ways relevant for neuropsychiatric diseases associated with aberrant reward, motivation and cognitive control processes, such as addiction, depression, Parkinson’s disease and schizophrenia (e.g., Braver et al., 1999; Schultz, 2002; Wise, 2004; Anand et

al., 2005; Mansvelder et al., 2006; Dagher and Robbins, 2009; Koob and Volkow, 2010; Roiser et al., 2012). Localised brain structural or functional deficits associated with many neuropsychiatric disorders are either poorly replicated or non-existent, with successful efforts reported to date focussed almost exclusively on structural classification in Alzheimer's disease and related neurological aging disorders (as discussed by, e.g., Linden, 2012). Therefore, RSN metrics may prove ideal, less reductionist, candidates for tapping into more subtle, systems-level deficits that presumably underlie such clinical problems (see e.g., Filippini et al., 2009; Menon, 2011; Sutherland et al., 2012), as well as the systems-level differences that underlie variability in related behaviours and personality factors in the general, 'healthy' population (Lewis et al., 2009; Mennes et al., 2010; Adelstein et al., 2011).

1.3 – Novel applications of advanced network-sensitive analysis techniques

Despite a huge volume of pre-clinical research into the neural mechanisms and treatment of maladaptive cognition and motivational processing characteristic of multiple neuropsychiatric disorders, clinically effective pharmacotherapies have remained an elusive (and often costly) problem for the scientific community, the pharmaceutical industry and, further, for society at large (World Health Organization, 2008; Insel and Sahakian, 2012; Linden, 2012). The work described in this thesis proceeds on the assertion that, relative to more 'traditional' models and approaches, resting-state fMRI warrants further exploration for its potential to benefit medical research in domains of neuropsychiatric drug development, due to a favourable combination of repeatability, spatio-temporal specificity, statistical sensitivity and

translatability (e.g., Greicius, 2008; Cole et al., 2010a; Fox and Greicius, 2010; Murphy and Mackay, 2011).

As a consequence of the complex systems biology of the brain, it is often difficult both to *identify* variability in brain signals (e.g., BOLD) that may be induced when functional processing is challenged by neuropsychiatric pathology or pharmacological intervention (especially when brain structure overall remains intact) and, further, to *characterise* these spatiotemporal observations in terms of their neural underpinnings and/or behavioural sequelae. Disorders such as addiction, schizophrenia and their treatment, for example, symptomatically affect diverse cognitive and motivational processes, which are known to be associated with multiple and distributed large-scale neuronal and neurochemical systems (Bressler, 1995; Nieoullon, 2002; Mansvelder et al., 2006; Koob and Volkow, 2010; Sesack and Grace, 2010; Goldstein and Volkow, 2011; Newhouse et al., 2011). These systems, in turn, are difficult to probe comprehensively in a controlled experimental setting, such as with any given cognitive task (Greicius, 2008; Fox and Greicius, 2010; Menon, 2011; Murphy and Mackay, 2011). Thus, the distributed neural circuitry underlying cognitive and behavioural aspects of, for instance, addiction (as described in e.g., Koob and Volkow, 2010) may provide an ideal model in which to explore biological and mathematical hypotheses pertaining to RSN functionality, through the use of novel methods in an applied psychopharmacological context (for review see Sutherland et al., 2012). It is argued in the following chapters that one can assess and summarise complex neural dynamics in a manner relevant for clinical and pharmacological applications by quantifying and comparing functional connectivity at the level of (resting-state) networks in BOLD fMRI data,

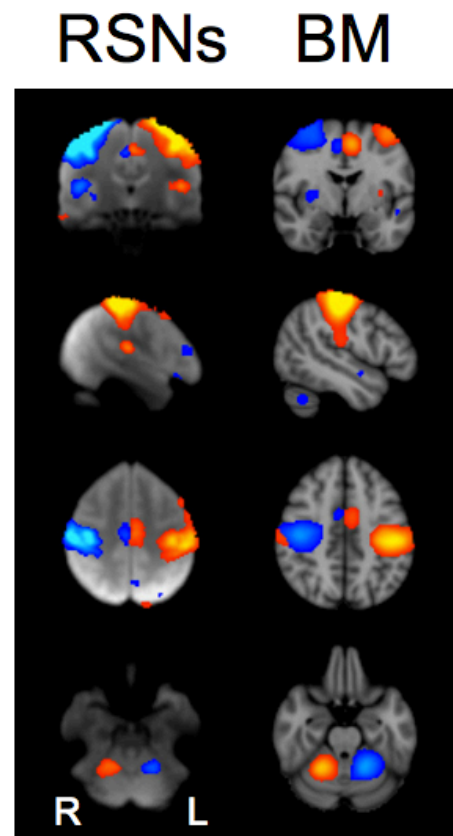
particularly those spanning brain regions involved in higher-level cognitive, emotional and reward processes.

To date, differences from healthy comparison subjects in RSN functional connectivity have been identified in multiple neurological and psychiatric populations, some of which show a high degree of consistency across studies and techniques. Most notable, perhaps, are the findings of altered “default mode network” (DMN; see Fig. 1.1E and section 1.4.1) function in relation to symptoms and markers of Alzheimer’s disease (Greicius et al., 2004; Buckner et al., 2005; Rombouts et al., 2005; Wang et al., 2006; Wang et al., 2007a; Filippini et al., 2009; Rombouts et al., 2009). However, other neuropsychiatric disorders, associated with symptoms of aberrant reward and motivation and their underlying neurochemistry (e.g., addiction, depression, Parkinson’s disease and schizophrenia), by and large, do not enjoy this level of replication. Furthermore, while average differences in connectivity patterns between population groups are reported regularly, validations of their characteristics as useful for diagnostics at the level of individual subjects (Greicius, 2008), or indeed for standardised measures and predictors of how brain signalling may be modulated experimentally over time, for example via disease pharmacological intervention (Matthews et al., 2011), are currently relatively remote prospects (although see, e.g., Varoquaux et al., 2010; Klumpers et al., 2012).

In conjunction with novel functional connectivity analytical approaches, the resting-state BOLD fMRI technique has certainly proven valuable in delineating some previously unclear aspects of the human neural functional architecture relevant for psychological functions and dysfunctions (e.g., Filippini et al., 2009; O’Reilly et al., 2010; limitations to mechanistic neuronal

interpretations notwithstanding). However, the very existence of multiple, though often complementary (see section 2.1), analytical options for investigating large-scale neural signals may actually foster the difficulties of probing these (already complex) systems-level phenomena. This issue is particularly prominent in resting-state fMRI studies, where the aforementioned lack of *a priori* activity models has resulted in the development of a wide range of innovative techniques attempting to characterise these unconstrained data. Prior to expanding further on such issues in Chapter 2, in the remainder of the current chapter these resting-state networks are introduced and described in terms of their specific characteristics.

Figure 1.2. Two distinct right- (blue-cyan) and left-lateralised (red-yellow) large-scale somato-motor cortical co-activation networks, complete with contralateral cerebellar clusters, identified consistently by two different methods. Left: RSNs identified by ICA of a group of healthy subjects ($N = 36$). Right: The same networks identified by meta-analytic ICA methods applied to functional activation data from the BrainMap (BM) database ($N = 29671$). These results are supported by and extend prior knowledge about cortical somato-motor systems, in terms of their function, spatial localisation, lateralisation and connectivity (for more details see Smith et al., 2009).



1.4 – RSN characteristics

1.4.1 – Spatio-temporal characteristics

RSNs are localised to grey matter regions (Beckmann et al., 2005; De Luca et al., 2006) and it is becoming increasingly evident that they reflect functional systems supporting core perceptual and cognitive processes² (e.g., Seeley et al., 2009; Menon, 2011). Fig. 1.1 (reproduced from Beckmann et al., 2005) displays eight RSN maps commonly identified in resting-state FMRI data, in this case using independent component analysis (ICA; see Chapter 2 for details). These patterns of spontaneous functional connectivity are consistent with stimulus-evoked co-activation patterns in, for example, sensory and motor cortices, language and memory systems and higher cognitive control networks (Biswal et al., 1995; Lowe et al., 1998; Cordes et al., 2000; Hampson et al., 2002; Beckmann et al., 2005; Seeley et al., 2007; Smith et al., 2009). Indeed, in Fig. 1.2 (reproduced from Smith et al., 2009) functionally relevant, hemisphere-lateralised distinctions can be seen between somato/sensory-motor systems involving cortical and contralateral cerebellar nodes, identified as separate RSNs using a meta-analysis of the ‘BrainMap’ functional activations database (<http://brainmap.org>) and, in an independent sample, a group ICA approach applied to resting-state BOLD FMRI data. The same meta-analytic work also ascribes sensory and higher-level ‘cognitive’ functional relevance to a number of other RSNs, such as those displayed in Fig. 1.1 (Smith et al., 2009). Furthermore, in some instances, certain RSNs appear to display either up-regulation or down-regulation of activity during specific cognitive tasks. Thus they may be described

²Portions of this section are adapted from a related publication: Cole DM, Smith SM, Beckmann CF (2010a) Advances and pitfalls in the analysis and interpretation of resting-state FMRI data. *Front Syst Neurosci* 4:8.

as either “task-positive” or (in the case of the commonly-studied ‘default mode’ network; DMN; see e.g., Fig. 1.1E) “task-negative”, in terms of the direction of correlation between the mean network activity and the event timings during the task (Shulman et al., 1997; Gusnard and Raichle, 2001; Greicius et al., 2003; Fox et al., 2005; Kelly et al., 2008).

The DMN, a notable example in terms of the regularity of its investigation, consists chiefly of bilateral precuneus/posterior cingulate, medial prefrontal and (posterior) inferior parietal cortical ‘nodes’ and first rose to prominence due to being formed of regions consistently *deactivated* during experimental paradigms that required task-relevant, or ‘on-line’ allocation of attentional resources (Shulman et al., 1997). Thus, it was initially considered to represent a “baseline” system of brain activity that was taken ‘off-line’ when such resources were required for (e.g.) external stimulus monitoring (Gusnard and Raichle, 2001; Raichle et al., 2001). More recently, however, the DMN has been found to display increased activity during tasks that have components requiring attention to be directed introspectively, or “self-referential” cognition (Gusnard et al., 2001; Harrison et al., 2008a; Leech et al., 2011). Thus, it is no longer regarded as simply a ‘task-negative’ or ‘task-independent’ network (e.g., Buckner et al., 2008; Leech et al., 2011). Furthermore, increased interest has been developing in the functional relevance of the interplay *between* the DMN and RSNs regarded as ‘task-positive’. These latter frontoparietal cognitive RSNs (e.g., those depicted in Fig. 1.1F-H) are usually described as consisting of three or more bilateral nodes in anterior cingulate/pre-supplementary motor area, anterior insula, dorsolateral prefrontal and (anterior) inferior parietal cortices and are variously implicated in functions of externalised attention, executive control and

emotional/motivational salience processing (Fox et al., 2005; Dosenbach et al., 2007; Seeley et al., 2007; Vincent et al., 2008). Such ‘higher-level’ cognitive functions are precisely those implicated in the symptomatology and treatment of multiple neuropsychiatric disorders (Greicius, 2008; Menon, 2011; some of the functions often ascribed to other RSNs are outlined briefly in the legend of Fig. 1.1).

In addition to the proposed functional “specialisations” of the DMN and other, ‘task-positive’, cognitive networks, their unconstrained nature (i.e., the fact that variability in the behaviour of their measured signals is not confined to specific, experimenter-derived paradigmatic scenarios) makes them ideal functional neuroimaging candidates for characterising systems-level differences, changes or modulations that cannot easily be assumed to conform to a strict *a priori* temporal or spatial model (e.g., those reflecting large-scale brain network reorganisation in neuropsychiatric disease or following pharmacological challenge). Indeed, the activity and connectivity patterns of the DMN and other ‘cognitive’ RSNs are thought to be relevant for a number of clinical and pharmacological neuroscientific applications (Fox and Raichle, 2007; Buckner et al., 2008; Broyd et al., 2009; Fox and Greicius, 2010; Murphy and Mackay, 2011; Sutherland et al., 2012).

Importantly, the occurrence and characteristics of the various spatio-temporal observations labelled as ‘RSNs’ depend on the nature of neural processes being evoked or induced by a given paradigm, or even the surrounding context of the resting-state scan (also see sections 2.1.1 and 7.3.1). Furthermore, subtle changes in the analytic approach to resting data, for example using slightly different spatial seeds in SCA (see Fig. 2.1 and also Buckner et al., 2008;

Hayasaka and Laurienti, 2010), or altering the model order dimensionality estimation in ICA (Kiviniemi et al., 2009; Smith et al., 2009), can have a significant impact on the spatial characteristics of the RSNs identified. In the latter cases, Kiviniemi, Smith and colleagues independently found that raising or lowering the ICA model order caused some networks to, respectively, ‘split’ into smaller sub-systems or ‘fuse’ into larger-scale brain systems (see also Abou-Elseoud et al., 2010). Convincingly, these dissociative and integrative characteristics seem to reflect the functional hierarchy of brain systems across various levels of neurobiological precision (compare, for example, the single bilateral somatomotor RSN in Fig. 1.1D and the two lateralised RSNs from a more detailed data decomposition in Fig. 1.2), although the extent to which each of these observations reflect actual neural distinctions or are artificially induced by the analyses remains unclear (Cole et al., 2010a; see also section 2.1.3). Moreover, for both biological and statistical reasons, sub-regions or ‘nodes’ of a given RSN may share “nonstationary” (i.e., time-varying) connectivity relationships within that network or with other identified nodes or RSNs (see section 2.1.3, Chapter 3 and section 7.3.3; also see Chang and Glover, 2010). Inferred characteristic RSN patterns can therefore be affected by multiple factors, in terms of the resultant connectivity relationships within and between networks. Such caveats are discussed in more detail in Chapter 2.

1.4.2 – Spectral characteristics

A degree of controversy surrounds the importance of time-frequency spectral information expressed by resting-state networks measured using BOLD fMRI. RSNs are often referred to in the literature as “low-frequency”, in terms of their

spectral power distributions, although the neurobiological significance of such observations cannot easily be assumed. Early frequency characterisation work (Cordes et al., 2000; Cordes et al., 2001) localised functionally relevant, spontaneous BOLD oscillations in the lower frequency ranges (0.01-0.08 Hz), separable from respiratory (0.1-0.5 Hz) and cardiovascular (0.6-1.2 Hz) signal frequencies. However, more recent fMRI evidence suggests that, while it is true that the predominant spectral power of RSNs appears in practice at low frequencies, the signal contributions that extend into higher frequencies do so with equal consistency (Niazy et al., 2011). Specifically, it has been shown that filtering RSN signals to account for the frequency content of their haemodynamic response function ‘flattens’ their power distribution from 0.01 Hz up to 0.15 Hz, instead of being biased towards the lower-frequency end of the spectrum (Niazy et al., 2011). This suggests that the low peak power characteristics of BOLD fMRI-derived RSNs are largely induced by the haemodynamics and that underlying RSN ‘neural’ dynamics may be more “broadband” than previously thought (see also section 7.3.4). Additionally, it should be noted that many artefactual signals also have spectral peaks that are either truly within similar low frequency ranges attributed to RSN activity, or are aliased by the fMRI temporal sampling into these ranges (e.g., De Luca et al., 2006; Birn et al., 2008). However, it has been shown that certain corrective methods, for example involving physiological monitoring during scanning (Glover et al., 2000) or post-acquisition denoising with session-level ICA (Starck et al., 2010) can be used to significantly reduce or even remove these confounds (see section 2.1.1). For these reasons, the work presented in this thesis does not employ preprocessing techniques that specifically restrict the measured BOLD signals of interest within

'low-frequency' spectra often described as stereotypic of RSN fluctuations. In Chapter 2, a detailed discussion is provided of additional technical and methodological choices pertaining to this work, complete with discussion of the scientific rationale influencing these decisions.

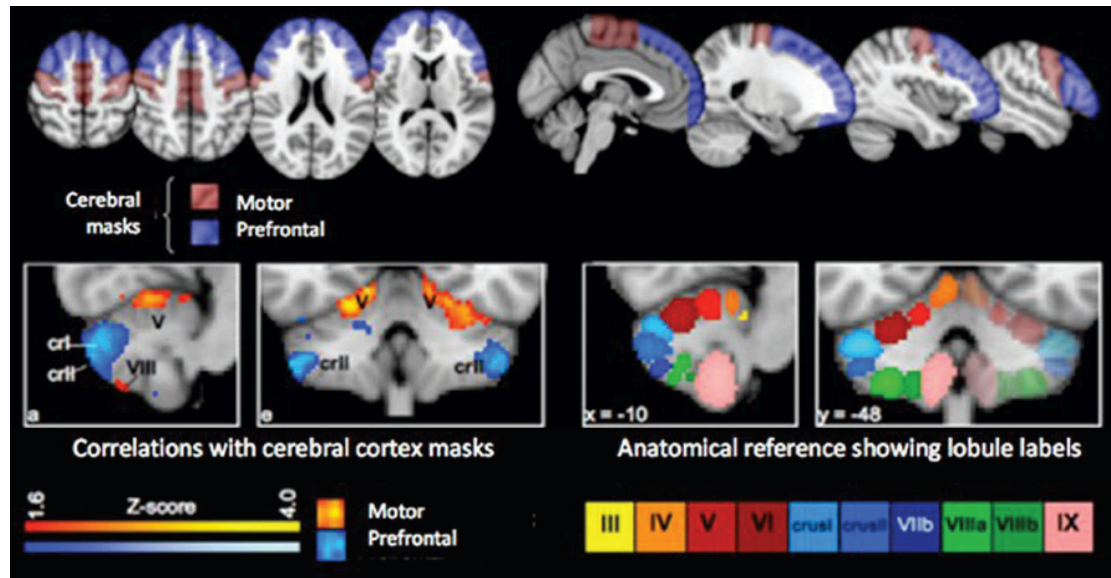


Figure 1.3. Functional connectivity parcellation of the cerebellum into (centre left) distinct regions of preferential functional connectivity with (top) multiple large-scale cortical areas (in this example two: motor, red; prefrontal, blue). Some correspondence of these functional connectivity findings with anatomically defined cerebellar subdivisions is apparent (centre right). Details of cerebellar connectivity profiles with additional large-scale cerebral systems (O'Reilly et al., 2010), including RSNs (Habas et al., 2009), have also been reported.

1.4.3 – Characteristic relationships between network connectivity and anatomical measures

RSNs display reliable and consistent functional connectivity patterns with specific thalamic (Zhang et al., 2008) and cerebellar sub-regions (Habas et al., 2009; Krienen and Buckner, 2009; O'Reilly et al., 2010). Studies of RSNs may therefore enable functionally relevant investigations of both cortico-subcortical and cerebro-cerebellar connectivity associations, potentially in greater detail than previously achieved with structural connectivity measures. In particular,

due to extant constraints on our ability to image micro-structure in certain axonal white matter pathways with sufficient resolution, connectivity relationships between sub-nuclei of the cerebellum and the rest of the brain are currently measurable in more detail with functional connectivity parcellation approaches than with structural connectivity measures from diffusion tensor imaging. For example, Fig. 1.3 (reproduced from O'Reilly et al., 2010) shows how cerebro-cerebellar connectivity pathways, not normally traceable *in vivo*, may be imaged and characterised in terms of their spontaneous functional interactions. Critically, other than providing additional information about *direct* connections, by helping to circumvent the resolution limitations of current anatomical imaging techniques functional connectivity metrics are also sensitive to indirect, polysynaptic neuronal connections (e.g., Fig. 1.3). These indirect connections cannot easily be tested for their *functional* relevance with other imaging measures, yet they are an inherent property of the large-scale neural networks thought to support higher-level cognitive processes (e.g., Bressler, 1995; Honey et al., 2009; Van Dijk et al., 2010; Lu et al., 2011; Mantini et al., 2011). They can seemingly exist in the absence of measurable *structural* connections, which (assuming current state-of-the-art diffusion-weighted imaging) require mono-synaptic connectivity pathways mediated by myelinated fibres and are not sensitive, for example, to spreading oscillatory activity and synchronisation patterns within cortico-cortical or cortico-subcortical feedback loops sustaining cognition (e.g., Alexander et al., 1986; Llinas et al., 1998; Varela et al., 2001; Buzsaki and Draguhn, 2004). Similarly, functional connectivity BOLD fMRI measures also provide complementary information to that gained from other functional, metabolic and molecular imaging modalities (using, for example,

positron emission tomography; PET), as well as structural connectivity/covariance MRI metrics (e.g., Buckner et al., 2005; De Luca et al., 2006; Greicius et al., 2009; Honey et al., 2009; Seeley et al., 2009). This convergence with other data types increases confidence in the utility of these measures to help comprehensively map and quantify the neural substrates of systems-level function and dysfunction.

1.5 – Overview of chapters

In Chapter 2 a more detailed review of technical and methodological issues affecting multiple RSN analysis techniques is provided. Subsequent chapters describe studies in which novel methods are applied, along with evidence-based interpretation of the resultant findings, discussion of their utility and how they may inform the optimisation of future research. Chapter 3 presents an example, in contexts of nicotine addiction, of how these metrics can show sensitivity to clinically relevant, but biologically non-specific, neuropsychopharmacological effects. In Chapter 4, a specific dopaminergic candidate for more fundamental neurobiological mediation of large-scale network connectivity relationships and topographies is investigated. This is followed by descriptions of research, again in contexts of dopamine modulation, establishing the sensitivity of RSN measures to patterns of functional connectivity associated with different pharmacological manipulations or neuropsychiatric disease states, in Chapters 5 and 6 respectively.

Chapter 2 – Methodology

2.1 – Methodological background

Mirroring the increasing interest in resting-state FMRI research, over the past 10-15 years a rich array of signal processing techniques has been developed specifically to evaluate these data. This section of the chapter is a summary and review of the most widely applied of these methods³, focussing primarily, but not exclusively, on seed-based correlation analysis (SCA) and independent component analysis (ICA). The following is a discussion of the commonalities, differences and potential interpretative pitfalls of these and other techniques, beginning with an account of some of the specific pre-statistical requirements of the data.

2.1.1 – Acquisition and preprocessing of resting-state BOLD FMRI data

It has been shown that a comparatively wide range of sampling rates and a small number of data points can be used to measure sufficient BOLD activity for identifying RSNs, relative to the rate and number of samples acquired during the majority of task FMRI studies (e.g., De Luca et al., 2006; Van Dijk et al., 2010). Typical resting experiments therefore are of the order of 5-10 min, though the identification of an optimal duration of a resting FMRI session (and the possible need for multiple sessions) is an open issue. Van Dijk and colleagues suggest that 5 min of recording time is near-asymptotic with regard to correlation map

³Portions of this section are adapted from a related publication: Cole DM, Smith SM, Beckmann CF (2010a) Advances and pitfalls in the analysis and interpretation of resting-state FMRI data. *Front Syst Neurosci* 4:8.

stability (Van Dijk et al., 2010). However, the networks defined by this group occur at what is generally recognised to be the macroscopic end of the brain network spectrum. It is unlikely that this generalises to cases where a more detailed parcellation of functional connectivity patterns is sought, for example, by means of a higher-dimensional ICA decomposition (Kiviniemi et al., 2009; Smith et al., 2009; see also Fig. 1.2), as in these cases the degree of partial temporal correlation between sub-systems increases, reducing the ability to delineate them easily. Additionally, no consensus exists as to whether there is a significant impact of the precise experimental setting, for example, whether data are acquired while subjects are asleep or awake (Horovitz et al., 2008) and with eyes closed or open, with or without fixation (Marx et al., 2004; McAvoy et al., 2008; Bianciardi et al., 2009a; Yan et al., 2009; Van Dijk et al., 2010). However, several recent studies of the stability of RSN patterns through various sleep states (e.g., Fukunaga et al., 2006; Horovitz et al., 2009) indicate that the correlation patterns are relatively stable, except for weakening in deep sleep.

Resting BOLD data benefit from the majority of preprocessing steps routinely applied to ‘traditional’ task-related BOLD FMRI data (Beckmann et al., 2005; Birn et al., 2006). However, there are a number of subtle differences worth noting. For example, high-pass temporal filtering thresholds applied to task FMRI data may be overly aggressive with respect to removing some of the relevant RSN frequency information (as discussed in section 1.4.2) and a more conservative approach is required in order to preserve powers at low frequency.

Importantly, a substantial portion of the FMRI signal obtainable during rest can be attributed to spontaneous BOLD activity, relative to that attributable to scanner and physiological artefacts, even at high field strengths (Bianciardi et

al., 2009b). However, it has been shown that non-neuronal physiological signals may interfere with network functional connectivity patterns in BOLD data and the resultant interpretations thereof (Birn et al., 2006). Birn and colleagues showed that monitoring and controlling for (regressing out) ‘low-frequency’ respiratory-related fluctuations from the data could strengthen certain functional connectivity patterns plausibly neuronal in origin, particularly within the DMN, in both task-related and resting-state contexts. Indeed, removal of a range of confounding signals, such as respiratory, cardiovascular or cerebrospinal fluid (CSF) pulsatile noise is shown to improve the quality of data attributed to neural activity (Birn et al., 2006; Birn et al., 2008; Van Dijk et al., 2010). It has therefore become common practice in fMRI research (particularly resting-state) to monitor such signals during scanning, with specific software packages developed to correct retrospectively for their confounding effects post-acquisition (e.g., Glover et al., 2000). It can be argued that such noise removal is of particular importance for functional connectivity studies, given the data-driven nature of the analysis, where spurious correlations induced by the presence of structured noise effects may (in the worst case) severely increase the number of false-positive detections (Power et al., 2012; Van Dijk et al., 2012). Similarly, other sources of regionally-specific noise, such as white matter and CSF signals, should be accounted for in the analysis (e.g., Fox et al., 2005), as optimal BOLD signal to noise ratio in these regions is far more susceptible to artefact than in cortical grey matter (Tohka et al., 2008). A range of approaches can be employed here, either by (i) restricting the functional data analysis with binary grey matter masks thresholded at an arbitrary level, (ii) by including time series from these tissues as nuisance covariates (see section 2.1.2 and Fig. 2.1,

below), or (iii) by employing probabilistic, volumetric grey matter covariates in inferential analysis stages; i.e., by using additional, three-dimensional confound regressors at the between-subject analysis stage which, for any given voxel, encode the relative proportion of grey matter for each subject (see, e.g., Voets et al., 2012). A number of the various techniques designed to minimise the effects of signal confounds and thus increase signal-to-noise ratio in functional connectivity analyses are employed in the following research chapters.

Although concerns about the confounding influence of physiological noise and other structured artefacts in fMRI datasets are clearly warranted, in most cases it has been shown that session-level ICA methods can reliably identify and account for the artefactual influence of non-grey matter, respiratory and cardiovascular signal fluctuation on RSNs (Kiviniemi et al., 2003; Beckmann et al., 2005; De Luca et al., 2006; Birn et al., 2008; Kiviniemi et al., 2009). Note, however, that potential difficulties arise when attempting to separate physiological noise components from ‘true’ neural components using ICA (see section 2.1.3). Attempts to create automated artefact classification algorithms for components identified by ICA have generated mixed results, often with relatively high levels of misclassification (e.g., rates of between 0.2 and 0.3; Tohka et al., 2008). At the group level (see section 2.2.1), ICA methods can potentially lose some of the power of single-session data cleanup, so group-ICA approaches may benefit from further (ICA-based or other) cleanup in preprocessing stages (Biswal et al., 2010). Additionally, it is apparent that some artefactual components share a large degree of spatial and spectral overlap with RSNs, and at low dimensionalities even ‘mix’ and form parts of the same component in the end decomposition (Birn et al., 2008). However, the spatial overlap of, for

example, the ‘default mode network’ (DMN; e.g., Fig. 1.1E and Fig. 2.1) and artefactual respiratory components identified by Birn and colleagues is relatively minimal, both in the majority of single-subject cases and at the group-ICA level, with peak DMN parietal nodes being markedly distant from occipital regions strongly affected by respiratory fluctuation (Birn et al., 2008). Moreover, it has recently been demonstrated that separating these signals post-acquisition by manually increasing the dimensionality of the ICA model order, rather than having to collect and utilise additional physiological data, may more easily account for these confounding effects (Starck et al., 2010). Finally, of topical importance and discussed in detail elsewhere, recent evidence suggests that one specific preprocessing procedure commonly applied in (SCA) connectivity analyses, that of subtracting or regressing out the global mean signal from the model, may induce spurious negative correlations between RSNs and thereby artificially bias end interpretations of connectivity relationships (Murphy et al., 2009; Cole et al., 2010a). These criticisms are not applicable to the analytical techniques employed within this thesis, which do not include global mean signal regression at any point.

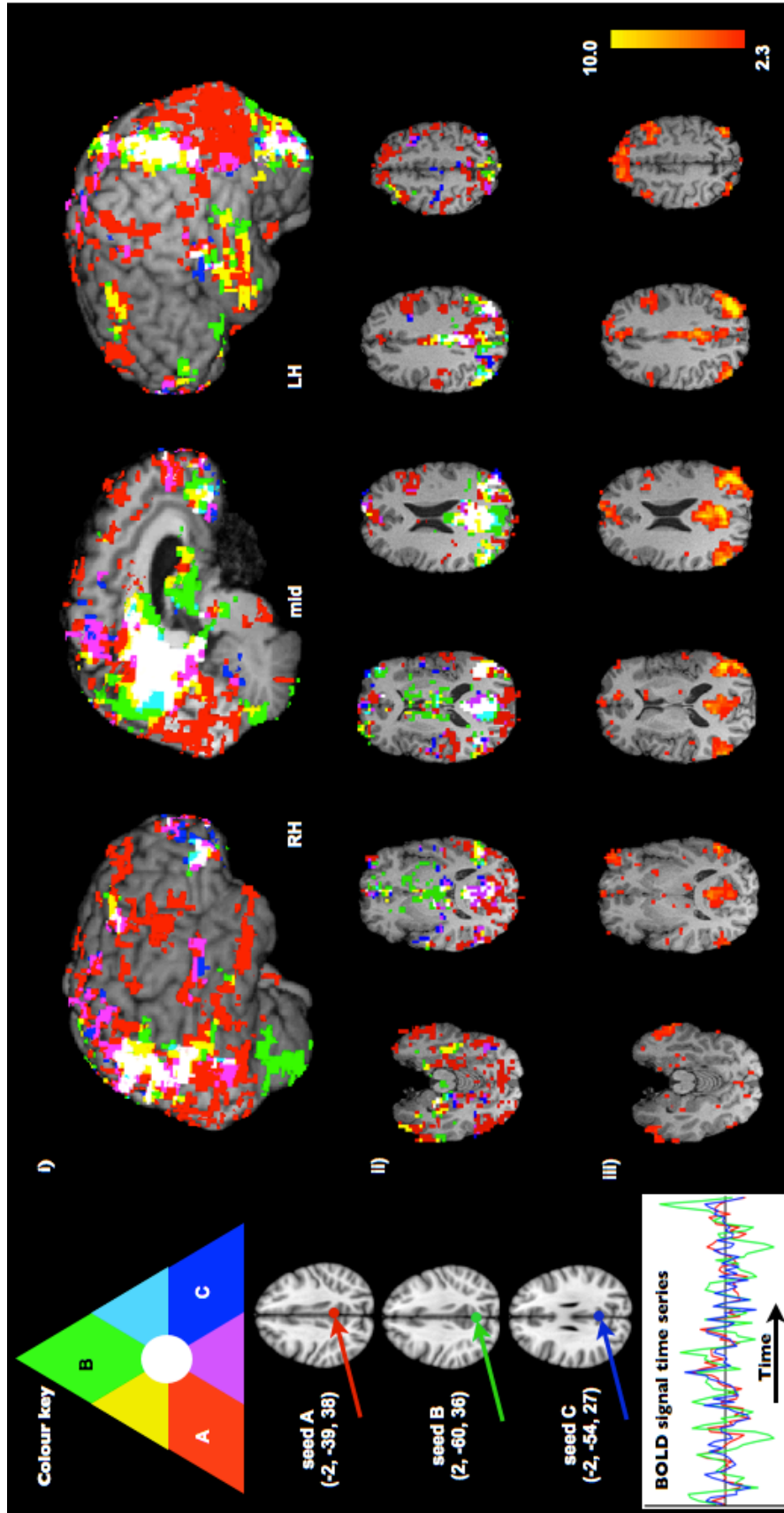


Figure 2.1. Single-subject comparison of SCA-derived versions of the DMN using three different seed voxel locations proposed in the literature (A: Fox et al., 2005 in red; B: Singh and Fawcett, 2008 in green; C: Greicius et al., 2003, in dark blue; x, y, z co-ordinates provided in MNI152 space). The results of SCA analysis using these seed time series are displayed (i) as maximum intensity projections (searching up to 12 voxels below the surface or slice on 3-D renderings of a single subject's high-resolution MRI, RH = right hemisphere, mid = midline, LH = left hemisphere); and (ii) as binarised thresholded z -statistic images overlaid on selected anatomical slices, but at the resolution of the subject's functional scan (cluster-corrected $z = 2.3, p < 0.05$). (iii) ICA-derived DMN map (colour bar shows z -statistic values). Figure reproduced from Cole et al. (2010a).

2.1.2 – Methods of RSN identification (i): seed-based correlation analysis (SCA)

Biswal and colleagues first identified coherent, spontaneous BOLD fluctuations bilaterally in the somatomotor cortical regions using a seed-based approach to derive time course models of functional connectivity (Biswal et al., 1995). This method requires the *a priori* selection of a voxel, cluster or atlas ‘seed’ ROI – perhaps based on previous literature or functional activation maps from a localiser experiment – from which to extract time series data (see, e.g., Fig 2.1). This time series vector is then used as a regressor in a linear correlation analysis or – when augmenting the model with other regressors (of interest or confounds) – a general linear model (GLM) analysis, in order to calculate voxel-wise (potentially whole-brain) maps of correlation or covariance with the seed region BOLD activity, which are interpreted as measures of functional connectivity (Fig. 2.1). The SCA approach is termed “univariate” because the time series ‘model’ is regressed against the time series data in each voxel separately from that in every other voxel. SCA has proven useful in revealing the functional connectivity properties of many brain ROIs, and has been applied in the literature by numerous groups (e.g., Greicius et al., 2003; Fox et al., 2005; Margulies et al., 2007). The primary advantage of SCA over other methods is that the approach provides a direct answer to a direct question – it shows the network of regions most strongly functionally connected with the seed voxel or ROI (Fig. 2.1). This straightforward interpretability, relative to other methods, makes SCA an attractive approach for many researchers investigating large-scale signalling relationships. Recent assessment of the test-retest reliability of these methods has indicated that RSN connectivity relationships can be identified by SCA with moderate to high reliability (Shehzad et al., 2009).

One potential weakness of SCA methods concerns the influence on the signal of structured spatial confounds, such as other RSNs (than the one under consideration) or structured noise, for example, residual head motion effects or scanner-induced artefacts. Some of these effects may be partially removed by incorporating specific preprocessing steps such as temporal filtering, but the presence of residual confounds in the SCA reference time course can have a detrimental influence on SCA correlation maps, in that estimated ‘networks’ also include voxels that describe the spatial extent of the artefact. More generally, the univariate approach of correlating the time series of a single voxel with those of each other voxel in a brain image disregards the richness of information available within the statistical relationships between multiple data points. Prior selection of the time series of *one* sub-region to correlate with and inform the activity of the network *as a whole* imposes anatomical restrictions on the measurement of network connectivity and, consequently, on interpretations of systems-level hypotheses. Fundamentally there are as many possible ‘networks’ to be derived as there are possible seeds, so discussing and interpreting one resulting spatial map as a distinct and meaningful neurobiological system is an under-representation of the data, as all but one possible ‘network’ in the data are being ignored. Biologically, the choice of seed may bias connectivity findings towards specific, smaller or overlapping sub-systems, rather than larger, distinct networks (e.g., Buckner et al., 2008; Mennes et al., 2010; Leech et al., 2011). Finally, these issues are all contingent on investigator-specific (seed size or location) and subject-specific (spatial normalisation or functional localisation) biases potentially resulting from the method of *a priori* seed selection employed (see Fig. 2.1). As a caveat, however, one must not underplay the importance to

the current knowledge-base in the field of subjective expertise in carefully selecting seed regions, as well as in identifying and classifying RSNs, both of which have played a major role in shaping our current understanding of these effects.

To illustrate the issue of potential biases attached to seed selection in SCA, Fig. 2.1 presents a number of SCA-derived versions of the DMN, alongside the same RSN estimated by ICA (for a related approach see (Buckner et al., 2008)). The DMN seed locations were selected from three important papers in the resting-state fMRI literature (Greicius et al., 2003; Fox et al., 2005; Singh and Fawcett, 2008). These and all other spatial co-ordinates in this thesis are presented in the space of the MNI152 standard template (Montreal Neurological Institute, QC, Canada). Results of SCA using these seeds were calculated using white matter, CSF and motion confounds and are displayed as: maximum intensity projections (Fig. 2.1i) and as binarised statistical maps on selected slices in the subject's structural space (Fig. 2.1ii, cluster-corrected $z > 2.3$, $p < 0.05$). Though there is significant overlap in the extent of the inferred networks, independent of the seed voxel location (white), it is clear from the extent of primary (non-overlapping) colours visible (largely red and green), particularly in prefrontal and occipital cortices and subcortical regions, that biases inherent in the seed-selection process can result in a large amount of variability carried into the results and subsequent interpretations. In order to validly discuss SCA results in terms of "networks", some form of consensus mapping is required, where the different versions of a network are combined in order to generate a single consistent representation (e.g., using information theoretic principles such as clustering or principal component analysis (PCA) across the different maps).

Fig. 2.1iii, for comparison, shows the ICA-derived DMN map from the same data (where the model order, i.e., the number of components, was estimated from the data; for details see Beckmann and Smith, 2004). Amongst the set of four spatial maps this component map has the highest mean spatial correlation with the three SCA estimates of the DMN (Cole et al., 2010a). The ICA approach, discussed in the next section, therefore can be viewed as one possible way of generating such consensus maps, eliminating the need to specify explicit seed locations, though at the expense of losing specificity in relation to a single well-defined seed of interest. Note, however, that other aspects of the analysis (such as the choice of the model order in ICA, see section 2.1.3; or the number and nature of confound regressors in SCA) are likely to introduce other types of variability into the final outputs.

It is important to note here that ‘validating’ network connectivity maps by simply highlighting visual similarities with a network identified by ICA, a practice adopted increasingly frequently in SCA studies, is not necessarily optimal for comparator selection or useful in terms of inference, without detailed quantification of this similarity. Importantly, for the above-demonstrated reasons, a large number of researchers are beginning additionally to adopt multivariate methods such as ICA in their standard approaches to analysing spontaneous BOLD fluctuations. Such approaches avoid many of these problems and thereby have complementary advantages to those of SCA methods, enabling a more comprehensive mapping of network-level functional phenomena and their response to experimental manipulation.

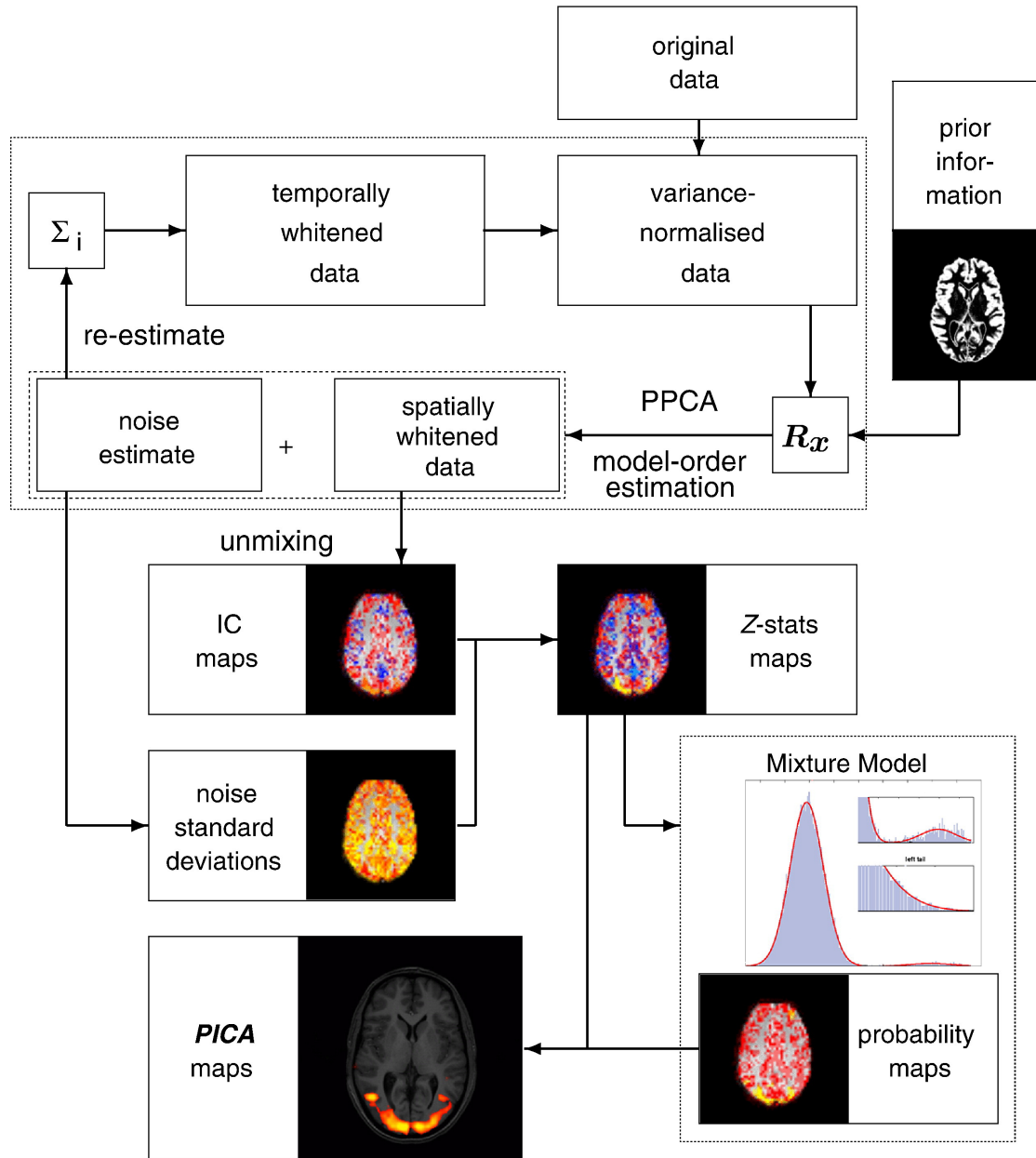


Figure 2.2. Schematic for probabilistic independent component analysis, reproduced from (Beckmann and Smith, 2004; Beckmann, 2012).

2.1.3 – Methods of RSN identification (ii): independent component analysis (ICA)

Initially recognised within neuroscience as a valuable method of separating uncorrelated signal waveforms in electroencephalography (EEG) data, ICA was first applied to fMRI data collected during an experimental task (McKeown et al., 1998). The same techniques were later applied to resting-state fMRI data (Kiviniemi et al., 2003). ICA works by decomposing a two-dimensional data

matrix into the time courses and associated spatial maps of the underlying 'hidden' signal sources. Although a number of differing approaches to ICA are used widely in neuroimaging (implemented as separate software packages), common concepts and core methods underlie their application (for review see Beckmann, 2012). One popular, probabilistic approach is to estimate maximally statistically and spatially independent, non-Gaussian components from FMRI data, by optimising a measure of non-Gaussianity in the estimated maps (see Fig. 2.2). Although probabilistic ICA estimates component maps of maximal spatial independence (from each other), this does not preclude spatial overlap between components (for details see Beckmann et al., 2005). The ICA method of exploratory FMRI analysis is regarded as preferable to that of PCA, as the spatial independence enforced upon components by (spatial) ICA dictates only that their time courses not be highly co-linear, resulting in a more biologically plausible systems model than that obtained from a PCA decomposition, where the analysis enforces orthogonality between time courses, precluding the detection of signals that are partially correlated in the temporal domain. Note that while temporal ICA can be carried out as an alternative to spatial ICA (component time courses are orthogonalised but spatial maps are not), it suffers from the same orthogonality issue as PCA and is more susceptible to noise due to the typically smaller number of observations available to drive the estimation. For these reasons, the preferred method of RSN identification employed in this work is that of spatial ICA, which will thus be referred to as "ICA" for the remainder, unless stated otherwise.

Importantly, as with SCA, use of the ICA approach has identified networks of spontaneous coherence comparable to known sensory and cognitive

processing systems (e.g., Fig. 1.1). Persuasively, these include the somatomotor cortical connectivity network found in the first resting connectivity experiments (Biswal et al., 1995), sensory systems in visual and auditory cortices and, of particular interest to those applying imaging to neuropsychiatric populations and psychopharmacological experiments, networks apparently reflecting higher-level cognitive processes (e.g., the DMN). In this comparatively unrestricted way, ICA has been used to generate a ‘complete’ (if simple) picture of the functional hierarchy of dissociative and integrative relationships making up the spontaneous and evoked activity of the human brain, particularly with respect to the potential for network ‘splitting’ and ‘fusing’ across levels of neurobiological complexity (Kiviniemi et al., 2009; Smith et al., 2009; Laird et al., 2011). RSNs identified by ICA can also be less prone to artefactual interference effects from noise (including fluctuations in the mean global signal) than those from SCA (see section 2.1.1; also Birn et al., 2008; Murphy et al., 2009), due to the ability of the method to account for the existence of such structured noise effects within additional (non-RSN) components.

The primary advantages of ICA over SCA approaches are the ability to avoid prior spatial assumptions and noise attached to the seed, along with the ability to simultaneously compare the coherence of activity in multiple distributed voxels. However, employing ICA is not without its methodological and interpretative challenges. First, unlike PCA, data decomposition with ICA is obtained by means of iterative optimisation. The stochastic nature of this approach induces a degree of run-to-run variability, so the results obtained can differ between analysis runs on even the same data. This type of variability can be reduced when selecting more stringent convergence criteria and software

now exists that enables ICA repeatability testing (e.g., Himberg et al., 2004), which can be used to investigate the degree of variability and estimate ‘average’ decompositions across multiple ICA repetitions.

Second, the processes of dimensionality reduction and model order selection in ICA can be somewhat arbitrary. Specifically, one has to either specify in advance how many components to estimate from the data (see, e.g., Smith et al., 2009) or opt to allow automatic dimensionality estimation based on the internal signal and noise characteristics of the data (e.g., Beckmann and Smith, 2004; Seeley et al., 2007). While such approaches do exist to optimally select the number of independent components for a given dataset according to *statistical* criteria (for recent reliability testing of multiple models see Zuo et al., 2010a), it must be recognised that there can be no single, ‘best’ dimensionality or model order for the underlying neurophysiology of multiple distributed systems. There will always be multiple valid solutions for characterising the hierarchical complexity of RSN functional neurobiology. This level of ambiguity simply mirrors the general ambiguity in characterising the brain’s functional organisation; while we may validly conceptualise the existence of a visual, auditory, somatomotor or language system, a more fine-grained characterisation might separate this into specific areas such as the “hand knob”, “visual word form area”, “fusiform face area” (e.g., Kanwisher et al., 1997; Dehaene et al., 2002) and so forth. Each of these (and other) different types of characterisation is valid at a particular level of complexity. In the case of ICA decompositions, higher dimensionalities of the model, giving more fine-grained network parcellations (see also Fig. 1.2), have recently been advocated to provide a more ‘complete’ insight into the complex functional hierarchy of neural systems and

sub-systems spontaneously active in health (Kiviniemi et al., 2009; Smith et al., 2009) and disease (Abou Elseoud et al., 2011). However, the robustness of a given level of decomposition relies on being supported by data quality (e.g., one cannot expect a *robust* 100-dimensional ICA decomposition from a typical 5-minute single fMRI scan in which only 100 EPI volumes might be acquired). Furthermore, it is important to distinguish between the value of varying the model order to prove a technical methodological point (i.e., to demonstrate that systems *can* be made to split into sub-systems mathematically, potentially unrelated to the ability to assign biologically meaningful interpretations to such splits) and the value of concentrating on interpreting the results of a testable systems-level hypothesis in larger networks identified at lower (and data-driven) model orders.

Third, whereas SCA guarantees a result in terms of identifying the brain regions most associated, or functionally connected, with the selected seed (presumed to closely correspond to the associated RSN), ICA results may be ‘split’ into a number of sub-networks, depending on the parameters of the analysis (i.e., more RSN ‘splitting’ at high model order dimensionalities) and the internal characteristics of the data. This can result in the estimation of a large number of components, which may be difficult to identify and classify (Tohka et al., 2008). On the other hand, in some instances this splitting appears functionally relevant. For example, using session-level ICA followed by a template matching procedure, Seeley and colleagues identified such a split in a network suggested to be involved in higher-level cognitive function, revealing separate purported ‘executive control’ and ‘salience’ networks (Seeley et al., 2007). Related dissociations have since been reported using a variety of single-

session and group-ICA post-processing methods (Sridharan et al., 2008; Kiviniemi et al., 2009; Cole et al., 2012a) and further bolstered by functional and structural evidence from separate neurological disorders (Seeley et al., 2009), increasing confidence in the functional relevance of the observation.

Finally, one ICA decomposition of a given dataset may hide the fact that any given brain region may, over time, share varying connectivity patterns with multiple networks. This variability, or ambiguity, of regional co-activations between network nodes can be referred to as the “nonstationarity” of a given brain area in terms of its connectivity with one or more RSNs, which equally affects multiple analysis approaches that do not account for it (for specific investigations of this, see Chang and Glover, 2010; Kiviniemi et al., 2011; Smith et al., 2012a; and, with respect to nonstationarity at the neuronal level, see Popa et al., 2009). However, there are early indications that, despite its above-mentioned shortcomings for fMRI data decomposition and RSN identification, novel applications of *temporal* ICA can be used to better address issues of connectivity nonstationarity within and between network nodes (Smith et al., 2012a). Such approaches may prove valuable in future clinical-pharmacological applied work focussing on measuring connectivity modulations over time.

2.1.4 – Methods of RSN identification (iii): other

Since Cordes and colleagues originally characterised a number of functional networks of interest as low-frequency BOLD fluctuations (Cordes et al., 2000), analysis techniques probing the frequency-specific characteristics of RSNs have developed in parallel to correlation-based methods. Indeed, it is often tempting when investigating novel phenomena to assume that a property that appears to

be prominent is also of functional importance. Techniques that have emerged to probe frequency-specific aspects of RSN phenomena include ‘amplitude of low frequency fluctuations’ (ALFF) indices (Zang et al., 2007). The ALFF index is calculated by averaging the square root of the power spectrum of a given (spatially constrained) BOLD time course across the frequencies filtered, then standardising the value relative to the global mean ALFF value. The resulting value represents the degree to which the signal measured within the region/voxel of interest differs from all other regions of BOLD signal included in the global average, in terms of the proportion of its fluctuations that fall within the pre-defined frequency band. However, the assumption that all relevant neuronal information contributing to resting-state BOLD fluctuations can be represented by a single quantity, calculated only from information inherent in the frequency domain, is problematic. Firstly, this measure does not account for the existence of multiple, independent and interacting brain systems and therefore interpretations relating to specific ‘networks’ are unlikely to be borne out. Secondly, when considering the argument for a greater level of broadband content in neural RSN oscillations than previously thought (see section 1.4.2; Niazy et al., 2011), a frequency-dependent metric seems overly restrictive. This raises the possibility that potentially interesting information is being removed from the analysis by these mathematical procedures. Additionally, prior work has suggested that low-frequency measures of resting data may be rather susceptible to cerebral vascular and respiratory artefacts (Zuo et al., 2010b). Indeed, some aspects of the spatial maps derived from these techniques can appear, at least under qualitative examination, to resemble patterns queried as

artefactual by experimenters using other techniques, particularly in midline brain regions (e.g., Birn et al., 2006; Birn et al., 2008).

With these issues in mind the ALFF approach has been more recently refined to account for ‘fractional’ inclusion of information in frequencies outside of the normal range (fALFF; Zou et al., 2008). Zou and colleagues accomplish this by calculating “the ratio of the power at each frequency to the integrate power of the entire frequency range” (i.e., summing the oscillatory amplitudes across the ‘typical’ 0.01-0.08 Hz range, then dividing by the amplitude sum across a more inclusive range of 0-0.25 Hz). Additionally and optimally (see section 1.4.2), this amended approach involves no bandpass filtering. Although questions may remain over the susceptibility of these techniques to physiological noise, recent independent testing reveals both ALFF and fALFF to have moderate to high levels of reliability and consistency in terms of the (primarily midline) spatial patterns generated (Zuo et al., 2010b). Furthermore, some useful information about individual or group variability in neural processes may be present in BOLD oscillatory amplitude envelopes (e.g., Zang et al., 2007; Zuo et al., 2010b). Such techniques may thereby provide a useful complement to approaches using SCA or ICA to investigate inter-regional coherencies between BOLD signals (e.g., as in that applied to a clinical-pharmacological question by Anand et al., 2005).

A number of other frequency-dependent and time series statistical approaches exist that can be applied to the analysis of spontaneous oscillatory activity in BOLD data. These include linear or nonlinear comparison of fractal dynamics (Wink et al., 2008), measures of frequency-specific mutual information (Salvador et al., 2007), and graph theoretic investigation of such networks in the context of their ‘small-world’ characteristics by multivariate partial correlation

of spectral information from pre-defined ROIs (Salvador et al., 2005; Achard et al., 2006; Stam and Reijneveld, 2007). As discussed in section 2.1.1, all ROI-based approaches are subject to the primary criticisms of subjective node/seed definitions affecting SCA methods. Studies employing graph theory usually fall into this category (Smith, 2012). Further, graph theoretic approaches, which typically carry out a large number of comparisons of node pairings across the brain, are particularly affected by uncertainties regarding the selection of optimal threshold criteria for statistical significance of inter-regional connectivity effects measured and alterations therein (Mumford et al., 2010). Moreover, this approach is increasingly associated with extremely abstract interpretations that reduce these rich connectivity data to simple, often singular, numeric information describing theoretical network quantities and, therefore, remove results from *the* brain network level and the biological translatability associated with it (Smith, 2012). Similar criticisms can be levelled at recent work combining ICA identification of RSNs with graph theoretic evaluation of their properties (Ding et al., 2011), although not to the same extent. This amalgamation may therefore hold promise in terms of future *applied* work. Another novel approach to frequency-based assessment of RSN activity has benefited from combination with ICA methodology (Damoiseaux et al., 2006), although such alternative techniques have been replicated sparsely (see e.g., Damoiseaux et al., 2008), partly due to uncertainties surrounding the frequency-specificity of RSN activity as identified by BOLD FMRI (as outlined in section 1.4.2).

Another method regularly applied to resting-state FMRI data assesses regional homogeneity (ReHo; Zang et al., 2004), based on “Kendall’s coefficient of

concordance". This technique is sensitive to the 'purity' of clusters identified as expressing high functional connectivity with a model time series within a given cluster. By virtue of the assumption that neighbouring voxels are temporally similar, clusters identified as strongly connected during task or rest can be tested for their inner homogeneity and the degree to which this is modulated by a given paradigm or differs between groups (e.g., Liu et al., 2006; Paakki et al., 2010). The temporal variability within a cluster is reflected in the assigned homogeneity score. Advantages of the ReHo technique over, for example, SCA, include its relative insensitivity to possible region-to-region and/or trial-to-trial variability of the haemodynamic response function. Also, unlike with ICA, no assumptions are made regarding the spatial independence of identified ReHo maps and extensions to group analysis are relatively straightforward (Zang et al., 2004). However, this approach is fundamentally local in nature and therefore exhibits a high degree of sensitivity to different levels of spatial smoothing. Similarly to ALFF measures, the insensitivity to functional boundaries precludes drawing inferences on the degree of correspondence between spatially remote regions, making it difficult to use ReHo to characterise the distributed nature of RSNs (Zang et al., 2004).

Finally, a class of methodologies that may be particularly powerful for characterising distributed, functionally relevant connectivity patterns (including in the resting state) is that of 'machine learning'. Machine learning techniques employ classifier training and pattern classification algorithms (e.g., '*k*-nearest neighbour', 'leave-one-out cross-validation', 'support vector machine'), which in some cases have been shown to enable accurate and predictive decoding of different cognitive states or group/population stratification, potentially in both

task and resting states (Poldrack et al., 2009; Shirer et al., 2011; Duff et al., 2012). However, the utility of these techniques depends to a large extent on being able to test and validate their accuracy on a large number of subjects from many different populations undergoing a variety of state modulations. In the longer term, the continuous updating of large imaging databases with novel and replication datasets will doubtless aid this. Thus, in the short to medium term, applications of machine learning techniques may be more interpretable in combination with other data-driven analytical techniques (e.g., those that are the primary focus of this thesis and presented in subsequent sections), but they will add little to the mechanistic interpretability of findings resulting from the latter approaches within any individual study.

2.2 – Novel approaches to RSN analysis

This section provides a summary of the advanced methods used for higher-level data analysis in this thesis, while reviewing in parallel some of the more general issues with analysing resting-state fMRI data at the level of groups consisting of multiple subjects.

The majority of techniques for multi-subject analysis of resting-state functional connectivity are not yet as well developed as at the single-subject level. The research chapters of this thesis use novel multivariate and mass univariate analysis approaches aimed at surmounting existing challenges in combining resting-state fMRI data from multiple subjects with group-level inference. The techniques are applied to assess systems-level signalling variability that: (i) characterises individual differences in the susceptibility or reactivity to neurochemical modulation; and (ii) distinguishes clinical subjects

(e.g., Parkinson's disease patients) from healthy controls. In this context, the scope of review in this section considers only the fundamental principles and recent advances relating to the two methodologies extended most widely to multi-subject data: SCA and ICA. Most of the pros and cons of these two approaches, discussed at the single-subject level above, still apply at the group level. Moreover, they are compounded by caveats common to all attempts to combine functional neuroimaging datasets in this way, for example, issues related to co-registration of data into a common space and identification of networks or nodes (see e.g., sections 2.2.1-3 and Fig. 2.1). Gross variability in cortical thickness and folding across individuals or subject populations may instil a registration bias in the location of group analysis inputs (seed-ROIs) or outputs (one or more functionally connected nodes) towards one group or other, or towards a specific subjective characteristic. Thus, such variability can cause problems for group level inferences about functional-anatomical localisation (at regional or network levels). Similarly, the potential for mis-registration of individual session fMRI data following spatial normalisation may result in functionally segregated, but proximal, regions being assigned the same functional/neuroanatomical label across subjects, similarly marring valid inference. These sources of variability are local in nature and therefore their impact on inferred connectivity patterns is more prominent in voxel-based SCA. In cases of ICA or a *region*-based SCA such variability typically results in blurring of the estimated spatial patterns (see e.g., Fig. 1.1). One recent study suggests that the network properties of systems, in terms of 'small-world' characteristics inherent in connectivity relationships between nodes, are better approximated by using single voxel seeds rather than larger ROI seeds (Hayasaka and

Laurienti, 2010). However, it seems probable that this may be true of *a priori* ROIs (such as derived from a standard space template), but that data-derived ROIs (in analogy to ICA spatial maps) would result in seed regions with better performance.

In short, the more macroscopic approaches to measuring functional connectivity should be preferable for characterising signalling patterns at the level of networks corresponding to large-scale neural systems. However, the caveats outlined previously in this chapter must inform any assumptions made when extending these findings to robust, efficient and unambiguous group-level interpretations of alterations in biologically meaningful networks and their relevance to behaviour. Moreover, a central philosophy of this thesis is that meaningful interpretations are best applied to RSN observations through careful consideration in combination with individual difference metrics. For example, via measuring the correlation or covariance of RSN functional connectivity measures with behavioural or personality metrics, relevant neural systems may be identified whose influences span (one or more) functional scales.

2.2.1 – Group-ICA

Although interest in RSN analysis has grown exponentially (e.g., Pawela and Biswal, 2011) over the past few years, coherent methods for comparing such distributed, systems-level activity patterns across subjects and/or sessions within an ICA-based framework have only been proposed and validated fairly recently. One immediate problem when running separate ICA decompositions is that of having to select which components to carry forward to a between-subject analysis. Considering the possible existence of multiple different decomposition

solutions, even within the same subject's data, there might not be any consistent one-to-one mapping between estimated sets of component maps when compared across different subjects.

Early efforts advocated running single-session ICA, separately for each subject, then attempting to find the 'best-fit' component to an *a priori* RSN template, to carry forward to group comparison stages (e.g., Greicius et al., 2004; Esposito et al., 2005; De Luca et al., 2006). However, template-matching approaches are susceptible to the effects of multiple sources of gross variability, particularly between-subject variability, inherent to unconstrained resting-state fMRI data (Beckmann et al., 2005). Although we know RSNs to be largely consistent across healthy individuals (Damoiseaux et al., 2006), there are no guarantees of one-to-one correspondence of identified component maps, including RSNs, across subjects, groups or studies. As mentioned above, at a given ICA dimensionality, one RSN could potentially be split into two or more sub-networks in some subjects, and appear as a single component in others. Differences in the amount of structured noise between subjects or groups, such as might be induced by head motion, can also influence characteristics of measured 'networks' and lead to misinterpretation of apparent subject differences (Power et al., 2012). Furthermore, numerous parameters, including the number of subjects, group-ICA model-order and statistical thresholds used to derive and define network maps can vary across studies attempting to identify such 'templates'. In an effort to avoid such sources of variability, some researchers have advocated the use of separate "group-ICA" runs per group or experimental condition to be compared prior to further GLM comparison (e.g., Harrison et al., 2008a, 2008b). However, this approach may also be sub-optimal,

as it biases towards false-positive findings of group or between-session differences (Calhoun et al., 2001; Beckmann and Smith, 2005).

Finally, while single-subject ICA has greater power to model/ignore session-level structured noise than group-level ICA approaches, extending the former approach to group-level matching of components across subjects fails to take advantage of the increased effective signal-to-noise ratio provided by the latter wherein all subjects are analysed simultaneously (Calhoun et al., 2001). In this way, group-level ICA can generally support a much higher-dimensional (and therefore more finely-detailed) decomposition than single-session ICA (e.g., Kiviniemi et al., 2009; Smith et al., 2009). Furthermore, working from the ‘top down’, by starting with a group-level ICA and *then* generating subject-specific versions of the resulting group maps, solves the problem of between-subject RSN correspondence inherent in the process of combining single-session ICA data. Thus, in subsequent research chapters the group-ICA approach is employed as the method of choice for identifying RSN spatiotemporal information to hypothesis-test in a number of applied contexts.

The first group-ICA model to emerge for fMRI was applied to task data (Calhoun et al., 2001). In the first step of this procedure, data are spatially normalised and dimensionality-reduced via PCA (separately for each subject). These reduced datasets are then assumed to contain the most important source signals that have been ‘mixed’ into the measurements. All reduced datasets are temporally concatenated prior to the application of group-ICA. This identifies voxels that share common temporal patterns of response within and between subjects. By means of temporal concatenation of multiple datasets (Fig. 2.3A; also see Calhoun et al., 2001), group-ICA can thereby estimate group-level

independent components, including RSNs (Beckmann et al., 2005). Due to the relatively unconstrained spatiotemporal characteristics of BOLD signals in resting data across sessions and subjects, such a concatenation approach is more suitable for analysing these data than an alternative ‘tensor’ ICA method that assumes similar temporal fluctuations in each dataset (such as during performance of a cognitive task with a fixed model; Beckmann and Smith, 2005). Tensor ICA, nonetheless, can be used as a model-free probe of network-level brain activity across multiple subjects during task conditions with a fixed temporal model, reportedly with increased sensitivity to group differences relative to standard GLM-based approaches (Rombouts et al., 2009; Voets et al., 2009).

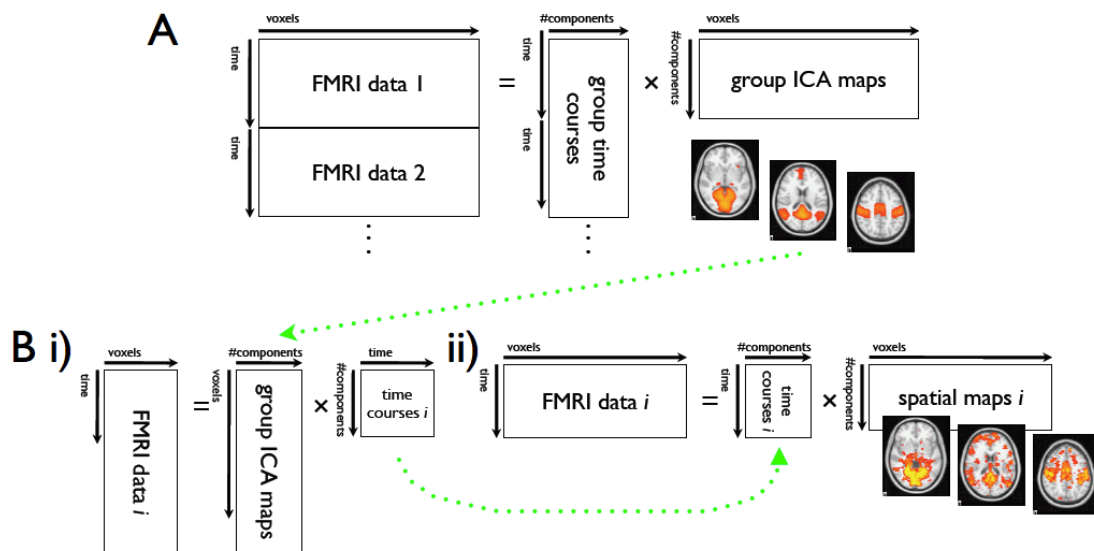


Figure 2.3. Schematics for (A) temporal concatenation group-ICA and (B) first (i) and second (ii) stages of the ‘dual regression’ technique of cross-subject RSN comparison; adapted from Beckmann et al. (2009). Green arrows indicate which pieces of information are carried forward to subsequent stages of the analysis.

2.2.2 – Optimal statistical inference following group-ICA

The ideal method for computing subject-level components from spatial maps identified via initial group-ICA remains contentious. Indeed, a number of such models have been suggested for this, predictably each with their particular pros and cons. In order to enable voxel-wise, between-subject comparisons of ICA-derived components, Calhoun and colleagues proposed to create individual subject components from the group decomposition via PCA backprojection/reconstruction (Calhoun et al., 2001). Further extensions of this approach have enabled the testing of within-network and between-network connectivity relationships across different task conditions or subject groups (Calhoun et al., 2004a; Jafri et al., 2008). The backprojection method estimates, at the subject level, temporal and spatial information associated with each group component, by projecting the original single-subject data onto projection matrices that combine the group-level unmixing matrix and the subject-level PCA-derived matrices used for dimensionality reduction. Because these PCA matrices are calculated separately for each subject there is no guarantee that, in the reduced data space, consistent information (across subjects) is retained. Hence this approach faces comparable problems to those attempting to combine or template-match single-session ICA datasets prior to group analysis. Moreover, the dependence on subject-specific PCA reduction raises the probability of session-specific noise contributions sub-optimally influencing further analyses, thereby confounding any final (usually within a GLM framework) cross-subject RSN comparisons. Thus the backprojection approach is susceptible to both false-positive and false-negative detection of effects at the group level. In response to these challenges, a more recently developed approach (Filippini et al., 2009;

Beckmann et al., 2009) estimates subject-specific RSNs from information contained within the original functional data via a 'dual regression' technique (Fig. 2.3B). This approach is applied extensively in the following research chapters.

The dual regression technique differs from back-reconstruction through performing spatiotemporal regression of the components from group-ICA against the original, individual session, functional datasets. The spatial maps output by a (temporal concatenation) group-ICA decomposition are first used as a set of GLM (spatial) regressors in a fixed-effects multiple regression analysis (Fig. 2.3Bi). This process generates individualised, session-specific time courses for each independent component in each subject's functional dataset (also see Calhoun et al., 2004b). These time courses, rather than matrices calculated as part of back-projection, are then normalised and used as GLM (temporal) regressors in a second multiple linear regression against the functional datasets (Fig. 2.3Bii). This generates individualised spatial maps for each original group-level component. The analysis is carried out in a standard coordinate space, so that cross-subject, mixed-/random-effects voxel-wise (ideally non-parametric, e.g., Nichols and Holmes, 2002) statistical testing of RSNs can be carried out. The statistical demeaning of data and regression variables is 'standard' across both stages of the dual regression. However, some debate still surrounds the choice of whether, or not, to variance normalise the temporal variables entered into the second regression. The former approach is widely preferred, as it is sensitive to additional variability across subjects in terms of (potentially meaningful) RSN/component amplitudes, not just the synchronisation of voxel-wise BOLD signal fluctuations with the time course of the (RSN) BOLD signal (e.g.,

Beckmann et al., 2009; Roosendaal et al., 2010). This distinction is very similar to that between linear regression and correlation techniques, respectively, in that ‘connectivity’ associations identified are informed by similarities in both the *size* and the *shape* of the signals in the former case, but by *shape* similarities alone in the latter. The dual regression approach has been validated in terms of its ability to estimate session-level RSNs from group-level ICA spatial maps, consistently and more reliably than single-session template-matching approaches (Zuo et al., 2010a). For higher-level analyses of RSN data, performed within a GLM framework (the ‘norm’), a non-parametric statistical approach is recommended, due to the inherently non-Gaussian nature of signals contributing to components identified by ICA. For the reasons explicated above, standard procedures followed in this thesis include dual regression with variance normalisation of second-level time course regressors, followed by non-parametric permutation testing at the higher level. Correction of the statistical maps produced by conducting null hypothesis tests across multiple voxels is also appropriate.

More recently, it has been proposed that the dual regression approach may lack sensitivity to estimate ‘true’ subject-specific component time courses and spatial maps, due to assumptions made when estimating subject-level characteristics from ‘standardised’ group maps (Erhardt et al., 2011). Erhardt and colleagues advocate an alternative approach using “noise-free” group-ICA followed by an updated version of the back-reconstruction method to derive subject-specific component information, which is found to more accurately identify component spatial maps inserted within simulated data. However, at its most basic level this critique and its resultant recommendations fail to take into account that the goal of most researchers looking to apply such techniques is to

be able to provide interpretations, backed up by statistics, of how a group or individual characteristic differs from some *standard* (or normal) ‘ideal’ of network-level connectivity behaviour (e.g., the work presented in subsequent chapters). These claims also fail to recall the inherent variability and complexity in structured neural network activity playing out within BOLD FMRI data, particularly that collected without any concurrent task paradigm and in the absence of an associated model. Outside of studies using simulations, data-derived evidence remains lacking that the noise-free group-ICA and augmented backprojection approach is superior to the dual regression method for performing valid group-level comparisons of ICA-derived networks, especially in the resting-state. Furthermore, the methods proposed in lieu of dual regression are still prone to the same issues affecting PCA backprojection mentioned previously, in that important variability in the data at the group level that is not represented in the PCA reduction space for each subject is being ignored. Thus, in the following research chapters, the dual regression methodology is a mainstay to the multi-session/cross-subject comparison of RSNs.

Pragmatically, the RSN characterisations resulting from the network-sensitive methods employed in this work are regarded as *in most cases* more complex than univariate seed-based techniques widely applied across the history of the field (e.g., Biswal et al., 1995; Lowe et al., 1998; Fox et al., 2005), but *in most cases* less complex than untargeted, whole-brain connectivity approaches exemplified by recent applications of graph theoretical models (for reviews see e.g., Bullmore and Sporns, 2009; Smith, 2012). It is proposed that the balanced level of complexity in this approach provides a more intuitive ‘middle ground’: 1) to better approximate the apparently insurmountable (although see

Laird et al., 2011) ‘ideal’ of comprehensively characterising a systems-level neurocognitive functional ontology; and 2) to explain how modulations of large-scale network connectivity processes are important for brain function (and dysfunction) with sufficient detail and necessary clarity to inform clinical-pharmacological applications.

In Chapters 3 to 5 the utility of a combined multi-subject (temporal concatenation) ICA and dual regression approach is explored. These ‘unsupervised’ analysis methods are adapted here to characterise large-scale within- and between-network connectivity relationships across the whole brain. In addition, this work investigates how such an approach may be usefully applied to questions of cognitive-behavioural functioning relevant for clinical and psychopharmacological applications related, primarily, to aberrant cognition, reward and motivational processing (such as in addiction) and treatment thereof.

2.2.3 – Group-level SCA

It is important to attempt to ground discovery methods of brain functional network connectivity within the realms of hypothesis-driven science and appropriately selected statistics (e.g., Biswal et al., 2010; Fox and Greicius, 2010; Wig et al., 2011). Thus, in addition to assessing benefits to novel applications of the (comparatively ‘exploratory’) network-sensitive multivariate techniques discussed above, later research chapters introduce an advanced approach to seed-based connectivity analysis applied to group-level questions.

As with group-ICA, higher-level approaches to SCA, while widely applied and sharing some basic principles, are not universally standardised. Specifically,

group-level SCA methodologies can vary in terms of the precise information brought forward from single-subject analyses to the higher level. In practice, most SCA group studies carry forward voxel-wise regression coefficients (e.g., Greicius et al., 2003) or bivariate correlation coefficients (e.g., Fox et al., 2005). However, a number of more recent approaches use partial correlation coefficients (e.g., Fransson and Marrelec, 2008; O'Reilly et al., 2010). All of these values are identified from an initial, whole-brain analysis of the functional connectivity with the time series extracted from a given seed region. At the higher level, these values are then converted to z-statistics and averaged across subjects in a standard GLM (for technical outlines of GLM methods applied to FMRI data see e.g., Worsley and Friston, 1995; Beckmann et al., 2003), prior to some form of hypothesis testing. The latter may or may not take into account between-subject variability (i.e., be a mixed-effects or fixed-effects cross-subject analysis). It has been highlighted that SCA methods display variability and disparity in identifying meaningful neurobiological 'networks' and consensus mapping (see section 2.1.2). Therefore, the analytical approaches applied to test systems-level hypotheses in this thesis focus on multivariate techniques that more completely capture the characteristics of these phenomena.

Although this work primarily presents applications of the ICA-based methods outlined in the previous section to clinical-pharmacological questions, Chapter 5 also introduces the application of group-level statistics to a recently-developed 'seed-based (partial) correlation analysis' (SBCA) technique. At the level of single subjects, SBCA is a useful method of parcellating specified anatomical 'seed' regions in terms of their voxel-wise functional connectivity relationship 'preferences' (and strengths of said correlations) with multiple

‘target’ areas in the brain. One of the first uses of this method, for example, involved parcellating the cerebellum in terms of its preferential functional connectivity relationships with large-scale, contiguous cortical areas (O'Reilly et al., 2010). As implemented in Chapters 5 and 6, these target maps can also assume the definition of more distributed neural systems, such as particular RSNs of interest, among other possible instantiations. Furthermore, data resulting from subject-specific SBCA can be probed for across-subject or group differences (see Chapter 5). As applied here, the approach shares some practical similarities with dual regression, but is more hypothesis-driven in terms of the *a priori* spatial information entered. Therefore, SCBA may provide more focussed measures of how connectivity interactions between networks and specific anatomical (e.g., subcortical) regions are altered in pathological processes of cognition, reward, emotion or motivation and, further, how these patterns can be modulated pharmacologically.

Chapter 3 – Network sensitivity to pharmacotherapeutic modulation

Nicotine withdrawal remediation reflected in RSN dynamics

In nicotine addiction, the neural mediators of treatment effects and efficacy and, further, their interactions with individual differences in disease severity are incompletely characterised. In this context, resting-state fMRI metrics may provide more applicable, 'global' characterisations of drug modulations and relevant functions. This first experimental study tested for network-level functional connectivity patterns associated with symptoms of cigarette smoking withdrawal and subsequent improvements following nicotine replacement therapy (NRT) in 17 heavy smokers, in a double-blind, placebo-controlled, crossover design. Individual differences in cognitive withdrawal symptom improvements after NRT were associated with increased inverse coupling between salience/executive control (SEN) and default mode (DMN) networks. Furthermore, symptom improvements were negatively correlated with altered functional connectivity within the DMN and with connectivity between the SEN and regions implicated in reward processing. Findings reveal that, in abstinent smokers, NRT modulates dynamic interactions between large-scale cognitive RSNs in line with withdrawal remediation. Individual differences in RSN functional connectivity may predict therapeutic outcomes in nicotine addiction and other conditions associated with cognitive impairments.

3.1 – Introduction

In addition to promoting commonly recognised symptoms such as craving and impaired mood, smoking cessation is known to cause cognitive withdrawal symptoms including difficulty concentrating⁴ (Parrott et al., 1996). Such cognitive impairments are thought to contribute to smoking relapse and may be relieved by nicotine replacement therapy (NRT; Parrott and Roberts, 1991; Heishman et al., 1994; Bell et al., 1999). Perfusion and BOLD fMRI studies in abstinent smokers associate specific symptoms with increased activity, relative to states of smoking satiety, in localised brain regions; including abstinence-induced craving represented by orbitofrontal, anterior cingulate and insular cortices (Wang et al., 2007b) and activity increasing in line with impaired cognitive processing in dorsolateral prefrontal cortex (Xu et al., 2005). Administering NRT prior to withdrawal symptom onset has been shown to modulate neural activity in many such regions, even though these effects are observed independently of symptoms of cognitive impairment or improvement therein (Lawrence et al., 2002; Hahn et al., 2007).

Imaging studies of smoking and nicotine neuropsychopharmacology typically employ behavioural tasks to evoke brain activity (Newhouse et al., 2011). This approach provides valuable insight into localised brain mechanisms underlying task-specific manifestations of behaviour. However, studies of resting-state activity may provide complementary information about spontaneous neural processes occurring at a more distributed, systems level (Fox and Raichle, 2007; Buckner et al., 2008; Newhouse et al., 2011). The current

⁴Portions of this chapter are reproduced from a related publication: Cole DM, Beckmann CF, Long CJ, Matthews PM, Durcan MJ, Beaver JD (2010b) Nicotine replacement in abstinent smokers improves cognitive withdrawal symptoms with modulation of resting brain network dynamics. *Neuroimage* 52:590-599.

study used resting-state ‘pharmacological fMRI’ to establish neural signatures associated with NRT remediation of withdrawal-induced lapses in concentration, as well as the gross effects of the drug treatment itself. Of particular interest were withdrawal symptom relationships with connectivity fluctuations between two RSNs implicated in mediating cognitive efficiency.

In this study it was hypothesised that nicotine withdrawal would reduce negative temporal correlations between the DMN and one or more ‘task-positive’ frontoparietal networks in line with cognitive impairment, with nicotine replacement ameliorating this effect in line with symptom improvements. Previous task-related and resting-state fMRI research indicates that activity is often negatively coupled, or “anti-correlated”, between the DMN and task-positive networks, such as ‘salience processing’ and ‘executive control’ networks (SENs), such that BOLD activity reductions in DMN foci occur concomitantly with *increased* activity (and *vice versa*) in SEN regions including anterior cingulate, anterior insular, dorsolateral prefrontal and inferior parietal cortices (Greicius et al., 2003; Fox et al., 2005; Fransson, 2005; Castellanos et al., 2008; Kelly et al., 2008; Zuo et al., 2010a). It has been proposed further that the strength of this inverse relationship mediates the efficient allocation of cognitive resources (Castellanos et al., 2008). Here this hypothesis was tested in the context of cognitive impairment during nicotine withdrawal.

3.2 – Materials and methods

3.2.1 – Subjects

Participants were 17 right-handed smokers (4 female; mean age = 30.3 years $\pm$ 1.78 standard error, range = 19-49 years), studied as part of a larger cohort

undergoing simultaneous EEG-fMRI during a cognitive task. These data have been reported elsewhere and provide complementary findings to those presented in this chapter (Beaver et al., 2011; see also section 3.4.2). To ensure that subjects with a history of neurological or psychiatric illness were excluded from taking part in the study, written confirmation was obtained directly from each participant's general practitioner prior to enrolment. All subjects smoked regularly for at least one year (mean = 11.2 ± 6.6 standard deviation (SD), range = 2-27 years), smoked approximately ten or more commercially manufactured cigarettes on average per day (mean = 15.7 ± 5.4 SD, range = 7-30 per day), and their first daily cigarette was usually consumed within 30 min of waking. Subjects completed the Fagerström Test for Nicotine Dependence (Heatherton et al., 1991) during initial screening (mean score = 5.6 ± 1.7 SD, range = 3-9). Procedures on the day of the study included breath and urine testing to avoid scanning subjects with recent alcohol consumption or other recreational drug use. All subjects were deemed free of structural brain abnormalities following review of anatomical MRI scans by a qualified neuroradiologist.

3.2.2 – Study Design

The study was randomised and placebo-controlled in a crossover design. Each subject underwent two resting-state fMRI scanning sessions, with at least 48 hr and a maximum of 3 weeks between sessions. Drug administration was investigator- and subject-blind. Subjects smoked a cigarette on arrival at the study centre and then abstained from smoking, under observation, for 8 hr before the first dosing and thereafter until the end of the session. This time frame is sufficient to induce withdrawal symptoms in most habitual smokers

(Hughes et al., 1994). Participants received two identical doses on each visit, either with a 4mg nicotine lozenge (NiQuitin®, GlaxoSmithKline) or a taste-matched placebo lozenge containing capsicum. The first and second doses were respectively administered 3.5 and 1.5 hr prior to the resting-state scan. This dosing regimen has previously been shown to significantly reduce cognitive withdrawal symptoms (Atzori et al., 2008). Immediately prior to the 5 min resting-state scan, subjects performed an attentionally demanding rapid visual information processing task (Lawrence et al., 2002) for 28 min (for detailed discussion of these data see Beaver et al., 2011) and for a brief summary see section 3.4.2). For the resting-state scan, subjects were instructed to lie quietly still with their eyes closed, and to stay awake. All subjects gave written, informed consent in which confidentiality, anonymity, and the opportunity to withdraw without penalty were assured. The study was carried out in accordance with the standards of the Declaration of Helsinki and the protocol was approved by the Charing Cross Hospital Research Ethics Committee.

3.2.3 – Withdrawal symptom measures

Nicotine withdrawal was assessed using the Modified Minnesota Withdrawal Symptom (MMWS) scale (Hughes and Hatsukami, 1986; Hughes, 1992) immediately after the resting-state scan, approximately corresponding to 1.75 hr after the second dose and 11.75 hr after a subject's last cigarette. The MMWS is a self-report measure of the intensity of cognitive, affective and interoceptive dimensions of nicotine withdrawal. Subjects provided a rating between 0 (no withdrawal) and 4 (severe withdrawal) on items such as difficulty concentrating, anxiety, craving and increased appetite (see Table 3.1 for complete list), giving a

possible total score between 0 and 28 for broad-spectrum, clinical withdrawal. Paired one-tailed t-tests were carried out on the MMWS scores in order to confirm which withdrawal symptoms were significantly reduced ($p < 0.05$, uncorrected) by NRT administration relative to placebo.

3.2.4 – fMRI data acquisition and standard preprocessing

Single-shot, T2*-weighted, gradient-EPI data sensitive to BOLD contrast were acquired continuously on a 3-Tesla MAGNETOM Tim Trio whole-body MRI scanner (Siemens, Erlangen, Germany) with a 12-channel birdcage radio frequency head coil. Each scan session consisted of 98 volumes of whole-brain, 41-slice acquisition (TR = 3000 ms, TE = 30 ms, flip angle = 90°, voxel size = 3.5 mm³). The first four volumes were discarded from further analyses to allow for magnetic equilibration, resulting in a 94-point time series at each voxel per scan. Additional high-resolution T1-weighted anatomical MRI scans (MPRAGE) were acquired for each subject to aid with EPI co-registration (TR = 3000 ms, TE = 3.66 ms, flip angle = 9°, voxel size = 1 mm³, 208 slices).

Table 3.1. Results of MMWS self-report testing for nicotine and placebo treatment sessions, including mean score, standard deviation (SD) and range for each category scale. Reported t-statistics and *p*-values of significance for differences between nicotine and placebo treatment conditions, based on paired t-tests (*N* = 17).

MMWS Item	Nicotine		Placebo		Paired t-test outcome
	Mean (SD)	Range	Mean (SD)	Range	
Desire or craving to smoke	1.94 (0.97)	0-3	2.76 (0.66)	2-4	<i>t</i> = 3.00, <i>p</i> = 0.0042**
Difficulty concentrating	1.41 (1.28)	0-4	2.00 (1.27)	0-4	<i>t</i> = 1.98, <i>p</i> = 0.0329*
Restless, impatient	1.06 (1.19)	0-4	1.88 (0.99)	0-3	<i>t</i> = 2.64, <i>p</i> = 0.0089**
Increased appetite, hungry	1.41 (1.28)	0-4	2.18 (1.33)	0-4	<i>t</i> = 3.49, <i>p</i> = 0.0015**
Depressed mood, sad	0.18 (0.39)	0-1	0.53 (0.87)	0-3	<i>t</i> = 1.46, <i>p</i> = 0.0817
Angry, irritable, frustrated	0.76 (1.15)	0-4	1.18 (0.88)	0-3	<i>t</i> = 1.24, <i>p</i> = 0.1169
Anxious, nervous	0.47 (0.72)	0-2	0.76 (0.97)	0-3	<i>t</i> = 0.89, <i>p</i> = 0.1926
Total MMWS Score	7.24 (4.85)	0-18	11.29 (4.66)	2-21	<i>t</i> = 2.52, <i>p</i> = 0.0113*

***p* < 0.01; **p* < 0.05; all uncorrected (one-tailed)

Initial preprocessing⁵ was carried out using tools from the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL Version 4.1.2; Oxford, United Kingdom; <http://www.fmrib.ox.ac.uk/fsl>; Smith et al., 2004). EPI data were motion-corrected with Motion Correction FLIRT (Jenkinson et al., 2002), stripped of non-brain information using BET (Smith, 2002), spatially smoothed with a Gaussian kernel of 5 mm full-width half-maximum (FWHM) and high-pass temporally filtered at 100 s. EPI volumes were registered to the individual subject's high-resolution anatomical volume and the MNI152 2 mm³ stereotactic standard space template using FNIRT, the FMRIB non-linear image registration tool (Andersson et al., 2007).

3.2.5 – Resting-state network functional connectivity analyses (i): Summary

The following is a brief summary of statistical analysis procedures. Step 1: probabilistic ICA with temporal concatenation was used to identify group independent component spatial maps including RSNs. Step 2: dual regression (see section 2.2.2) was used to convert these into scan-specific component time courses and spatial maps for each individual. These data were the focus of subsequent analyses. Step 3: a selective partial correlation analysis was conducted of the temporal dynamics between the identified SEN and DMN, to examine variability in RSN coupling behaviour related to individual differences in improved concentration following NRT (relative to placebo). Step 4: a more inclusive, voxel-wise multiple regression analysis was conducted, separately for both the SEN and DMN, testing: (i) the relationship between RSN functional

⁵Preprocessing steps implemented in this chapter are identical to those described in subsequent chapters, unless stated otherwise.

connectivity across the whole brain and individual differences in clinical withdrawal symptom improvements by NRT (relative to placebo); and (ii) a between-session comparison of treatment effects (nicotine vs. placebo) on RSN connectivity across the whole brain, independent of clinical improvements. These steps are described in greater detail below.

3.2.6 – RSN functional connectivity analyses (ii): ICA and dual regression

Group probabilistic ICA with temporal concatenation was carried out using FSL MELODIC (Beckmann and Smith, 2004; Beckmann et al., 2005; Beckmann and Smith, 2005), to identify independent components across all 34 datasets (17 subjects $\times$ 2 treatment sessions). This identifies voxels that: (i) share correlated temporal patterns of BOLD signal variation, or functional connectivity, within individual scan sessions; and (ii) explain spatially-normalised components of resting-state functional connectivity across the group, including group-level RSNs. The total number of components generated by ICA was optimally selected for the dataset via dimensionality estimation using the Laplace approximation to the full Bayesian evidence of the model order (see section 2.1.3). The resulting maps were thresholded using an alternative hypothesis test fitting a Gaussian/Gamma mixture model to a histogram of intensity values, with a significance level of $p > 0.5$ (for details see Beckmann and Smith, 2004). Components corresponding to the DMN and SEN were visually identified from the final set of group-ICA maps through comparison with previous literature, based on their spatial configurations and the power spectral density of the associated time courses (e.g., Beckmann et al., 2005; Damoiseaux et al., 2006; Mantini et al., 2007; Damoiseaux et al., 2008).

Dual regression was used to derive individual scan session time courses and spatial maps for the SEN and DMN (Beckmann et al., 2009; Filippini et al., 2009). These data were then tested for RSN connectivity changes across treatment sessions, as well as for relationships between such changes and individual differences in symptom reductions; both in terms of: (i) dynamic changes in temporal coupling between the two networks; and (ii) exploration of whole-brain, within-subject changes in RSN connectivity patterns. In dual regression, for each IC, voxel-wise maps of functional connectivity strength (regression coefficients) at the subject/scan level, representing ‘individualised’ versions of the group-level components, were calculated as follows. Step 1: all unthresholded group maps from the ICA output were entered into linear model fits (spatial regression) against the preprocessed fMRI data from each individual resting-state acquisition, to produce subject- and scan-specific time courses of signal fluctuation corresponding to each group-level component (one per column in the resulting matrix; 14 components $\times$ 94 time points). Step 2: the within-session time courses were variance-normalised and then used in linear model fits (temporal regression) against the corresponding fMRI data, converting each time series into scan-specific spatial maps of the corresponding component; two per subject (one for each treatment session). This method reliably produces scan session-specific approximations to all the unthresholded spatial maps in the group-ICA output (Zuo et al., 2010a). These data constitute separate single 4-D files for each functional scan, with one scan-specific version of each group component spatial map per 3-D volume, each in turn containing whole-brain voxel-wise regression coefficient maps of network functional connectivity with the respective IC. These individualised, subject/scan-specific instantiations of

equivalent group-level components were subsequently split into individual 3-D files to isolate the SEN and DMN maps for each subject and session. In order to conduct statistical comparisons in the next step, 3-D maps were then merged across all subjects to create a single 4-D file per RSN. Step 3: the resulting scan-specific spatial maps were probed voxel-wise via mixed-effects linear model contrasts in a multiple regression framework. This entailed non-parametric permutation testing as implemented within FSL randomise (Nichols and Holmes, 2002) to compare SEN and DMN maps separately across the whole brain for correlations between within-subject functional connectivity changes and withdrawal symptom improvements, as well as mean network functional connectivity differences between treatment sessions (5000 permutations).

3.2.7 – RSN functional connectivity analyses (iii): Changes in between-network coupling

The hypothesis that cognitive symptom improvements by NRT relate to *between-network* connectivity was tested by examining the relationship between the change in MMWS scores (nicotine session – placebo session) and variation in the coupling between the SEN and DMN time course dynamics. Session-specific network time courses derived from Step 1 of the dual regression (see section 3.2.6) were subject to a partial correlation analysis⁶ to examine the temporal association between the two RSNs of interest, controlling for the influence of variance shared with other independent components present in the functional data. In this analysis the time courses of the DMN and SEN were correlated,

⁶As implemented in the Statistical Package for the Social Sciences version 12.0 (SPSS Inc., Chicago, IL, USA).

separately for each individual scanning session, controlling for the session-specific time courses of all other components identified by ICA and subsequent dual regression. The resulting partial correlation coefficients from each subject's placebo session were first Fisher-transformed to a normal (z-score) distribution, then subtracted from those in the nicotine session, producing 'change in network coupling' (CNC) measures, or difference scores, of the relationship between the DMN and SEN for each subject, between treatment conditions. Of particular interest was the relationship between CNC scores and individual variability in improvements of self-reported difficulty concentrating. CNC values were therefore correlated (Pearson's r) with nicotine-placebo session difference scores of 'difficulty concentrating,' an item measured by the MMWS scale, as well as total MMWS difference scores. For completeness, CNC scores were also compared with difference scores on the remaining MMWS items, correcting for multiple comparisons. As the MMWS items predictably displayed a degree of statistical non-independence, a Bonferroni correction was applied for (the significance $p < 0.05$ was divided by) six eigenvectors explaining $> 99\%$ of the variance in the MMWS dataset calculated using PCA⁷ (thus significance $p < 0.0083$, uncorrected). Paired permutation testing (10000 permutations) of the single-session SEN-DMN partial correlation coefficients was carried out in order to test for main effects of treatment type on RSN coupling between sessions.

⁷Principal component analysis (PCA) of behavioural data was carried out using the "eig" function as implemented in MATLAB 7 (The MathWorks Inc., Natick, MA, USA).

3.2.8 – RSN functional connectivity analyses (iv): Whole-brain effects of individual differences in NRT-induced withdrawal symptom reduction on RSN connectivity

Further to testing the hypothesis of between-network coupling reflecting improvements in concentration, additional analyses explored whether individual differences in treatment-induced clinical withdrawal symptom improvements were associated with variation in RSN functional connectivity. Total MMWS scores were therefore demeaned across the group and entered as positive and negative covariates of interest in Step 3 of the resting-state dual regression data analysis (see section 3.2.6). Unlike the comparison of between-network dynamics, this approach probed the effects of individual differences on functional network connectivity across the entire brain. This spatially sensitive method potentially provides a more global spatial characterisation of regions in which changes in connectivity with RSNs are positively or negatively correlated with psychological withdrawal symptom improvements. To control for potential differences in RSN functional connectivity affected by prior task performance (see e.g., Albert et al., 2009), separately from the cognitive and clinical withdrawal symptoms of interest, accuracy scores in the most difficult condition of the information processing task (Beaver et al., 2011) were similarly included as confound regressors. Normalised 2 mm³ voxels displaying zero signal in at least one individual scan session, or falling below a 30% threshold of containing grey matter based on a study population-specific probabilistic atlas calculated using FAST (part of FSL; Zhang et al., 2001), were excluded from this analysis. In conjunction with the above procedures, this produced group inference spatial maps of voxel-wise t-statistics showing the difference in functional connectivity between the treatment conditions, plus separate connectivity profiles for both

the SEN and the DMN, indicating regions displaying altered connectivity with the RSN, positively or negatively correlated with MMWS withdrawal symptom changes.

Clusters resulting from the treatment effect contrasts, and those from mean effect maps correlating with individual differences in symptoms, were defined as significant if there were at least 15 contiguous voxels that survived the correction for multiple comparisons at the $p < 0.05$ level, controlling for the local false discovery rate (FDR; Efron, 2004). The 15-voxel extent threshold was employed to exclude regional effects that survived the voxel-wise local FDR correction, but were minimally spatially extensive and, therefore, likely to be of reduced functional significance and/or liable to an increased probability of expressing diminished signal-to-noise ratio. To characterise statistically significant positive or negative correlations between connectivity and behaviour, clusters identified from the multiple regression incorporating MMWS covariates (Tables 3.2 and 3.3) were further used as post-hoc ROI masks. These ROI masks were applied to the associated individual subject/scan-specific RSNs of interest as identified by Step 2 of the dual regression (see section 3.2.6). In this way, parameter estimates of RSN-ROI functional connectivity for each scan session were extracted from all combined and individual significant regions identified from separate statistical behavioural mean effect images, so that connectivity changes, or difference scores, could be calculated between the nicotine and placebo sessions for each subject. These values were in turn correlated with individual total and itemised MMWS difference scores between treatments, to provide a preliminary, post-hoc illustration of the functional relevance of the inferred significant effects (see Tables 3.2 and 3.3). The significance p-values ($p <$

0.05, corrected) of the resulting correlation coefficients were Bonferroni corrected for multiple comparisons as in the comparison of changes in between-network coupling (section 3.2.7).

Table 3.2. Complete cluster information for ROI functional connections with the SEN expressing group level (N = 17) main effects of treatment type and altering in correlation with total MMWS score withdrawal symptom reductions (t-statistics, all $p < 0.05$, local FDR corrected), plus preliminary post-hoc correlation coefficients of the MMWS item most strongly associated with the latter effects.

Statistical contrast or mean effect	Anatomical location (R = right; L = left)	Cluster extent (2mm ³ voxels in MNI space)	Primary associated (post-hoc) clinical endpoint (and Pearson's r coefficient)	x, y, z co- ordinates of peak voxel	t-value of peak voxel association with total MMWS reductions
1. Nicotine > Placebo SEN connectivity	R (inferior) precentral gyrus/premotor cortex	38	-	58, -2, 26	6.07
	R (superior) precentral gyrus/premotor cortex	17	-	56, -6, 36	6.60
2. Nicotine < Placebo SEN connectivity	L posterior insula/planum polare	21	-	-42, -10, -6	5.99
	R temporal pole/orbitofrontal cortex	17	-	36, 16, -26	5.90
	L temporal pole/orbitofrontal cortex	16	-	-44, 14, -18	5.55
	L dorsal anterior cerebellum	19	-	-24, -32, -30	4.70

3. SEN connectivity negatively correlated with MMWS difference scores (positively related to withdrawal reductions)	L orbitofrontal cortex	53	Difficulty concentrating (-0.80)***	-28, 12, -20	6.51
	L dorsomedial prefrontal cortex	46	Depressed/sad (-0.81)***	-18, 44, 30	6.80
	L thalamus	34	Depressed/sad (-0.82)***	-4, -26, 2	6.00
	R dorsal medial cerebellum	17	Anxious (-0.83)***	12, -56, -14	6.67
4. SEN connectivity positively correlated with MMWS difference scores (negatively related to withdrawal reductions)	R posterior insula	26	Anxious (0.79)***	38, -6, 6	8.98
	L post-central gyrus/premotor cortex	75	Depressed/sad (0.79)***	-60, -12, 30	5.03
	R pre-central gyrus	27	Depressed/sad (0.84)***	58, -6, 36	5.47
	R central operculum/sylvian fissure	17	Angry (0.86)***	62, -10, 12	4.73
	R posterior middle/inferior temporal gyri	16	Anxious (0.70)*	50, -56, -6	5.36
	L temporo-occipital fusiform cortex	16	Restless/impatient (0.78)**	-36, -52, -22	4.78
	L posterior dorsal cerebellum	83	Anxious (0.82)***	-24, -76, -22	7.60
	***p < 0.001; **p < 0.01; *p < 0.05; all Bonferroni corrected (two-tailed)				

Table 3.3. Complete cluster information for ROI functional connections with the DMN expressing group level (N = 17) main effects of treatment type and altering in correlation with total MMWS score withdrawal symptom reductions (t-statistics, all $p < 0.05$, local FDR corrected), plus preliminary post-hoc correlation coefficients of the MMWS item most strongly associated with the latter effects.

Statistical contrast or mean effect	Anatomical location (R = right; L = left)	Cluster extent (2mm ³ voxels in MNI space)	Primary associated (post-hoc) clinical endpoint (and Pearson's r coefficient)	x, y, z co-ordinates of peak voxel	t-value of peak voxel association with total MMWS reductions
1. Nicotine > Placebo DMN connectivity	L posterior precuneus	32	-	-6, -74, 46	5.77
	R posterior cuneus	22	-	10, -86, 34	5.30
2. Nicotine < Placebo DMN connectivity	Occipital striate /Primary visual cortex	24	-	2, -92, 6	4.87
3. DMN connectivity negatively correlated with MMWS difference scores (positively related to withdrawal reductions)	L mid-cingulate	21	Restless/impatient (-0.77)**	-2, -8, 32	9.20
	R occipito-parietal cortex	30	Difficulty concentrating (-0.81)***	24, -62, 44	6.33
	L medial orbitofrontal cortex	15	Angry (-0.74)**	-16, 34, -22	5.68
	R temporo-parietal junction	16	Anxious (-0.70)*	66, -26, 26	4.33
	L inferior cerebellum	22	Anxious (-0.71)**	-28, -48, -52	4.81

4. DMN connectivity positively correlated with MMWS difference scores (negatively related to withdrawal reductions)	L dorsal hippocampus	29	Restless/impatient (0.79)***	-28, -38, -6	6.09
	R medial temporal fusiform/parahippocampal gyrus	31	Restless/impatient (0.77)**	36, -26, -24	5.77
	L medial temporal fusiform/parahippocampal gyrus	17	Difficulty concentrating (0.67)*	-28, -40, -16	5.00
	L precuneus	24	Craving (0.75)**	-12, -74, 10	4.58
	R precuneus	21	Angry (0.63)*	10, -52, 6	4.45
	R angular gyrus	19	Craving (0.82)***	44, -48, 50	4.96
	R lingual gyrus	17	Depressed/sad (0.70)*	6, -68, -6	4.76
	L superior parietal/motor cortex	101	Craving (0.83)***	-26, -26, 56	6.76
	R superior parietal/motor cortex	24	Difficulty concentrating (0.84)***	6, -26, 66	5.41
	L visual striate cortex	41	Depressed/sad (0.74)**	-6, -88, 0	5.61
	Bilateral medial inferior cerebellum	37	Depressed/sad (0.77)**	-6, -66, -42	4.60
	L posterior cerebellum	35	Angry (0.82)***	-36, -80, -28	5.76
	L dorsal cerebellum	29	Depressed/sad (0.75)**	-18, -72, -20	5.81
***p < 0.001; **p < 0.01; *p < 0.05; all Bonferroni corrected (two-tailed)					5.35
L dorsal anterior cerebellum					

3.3 – Results

3.3.1 – Behavioural withdrawal symptom measures

As expected, subjects varied widely both in cognitive withdrawal symptom severity and in treatment-related symptom improvements (see Table 3.1). Overall, paired t-tests showed that NRT (relative to placebo) significantly reduced total MMWS ($t = 2.52$, $p < 0.05$, uncorrected) and difficulty concentrating scores ($t = 1.98$, $p < 0.05$, uncorrected), as well as other MMWS single item sub-scores (Table 3.1).

3.3.2 – RSN functional connectivity analyses (i): Group-ICA

Group-ICA identified fourteen independent components in the resting-state FMRI datasets. Eight of these corresponded closely to previously described RSNs (e.g., Beckmann et al., 2005; Damoiseaux et al., 2008; Sridharan et al., 2008): 1) a representative SEN map, including the anterior cingulate, anterior insula/frontal operculum, supramarginal gyrus and dorsolateral prefrontal cortex (Fig. 3.1A); 2) the DMN, including the posterior cingulate, precuneus, angular gyrus and ventromedial prefrontal cortex (Fig. 3.1B); 3) a somatomotor network; 4) the dorsal visual processing stream; 5) the ventral visual processing stream; 6) the primary visual cortex; and separate 7) left- and 8) right-lateralised frontoparietal networks (for details on the spatial characteristics of these additional networks, see e.g., Fig. 1.1; Beckmann et al., 2005). The remaining six components represented artefacts from magnetic susceptibility changes, head motion and respiratory and cardiovascular motion effects (see e.g., Kiviniemi et al., 2009). Subsequent analyses focussed on the SEN and DMN maps in line with the *a priori* hypothesis.

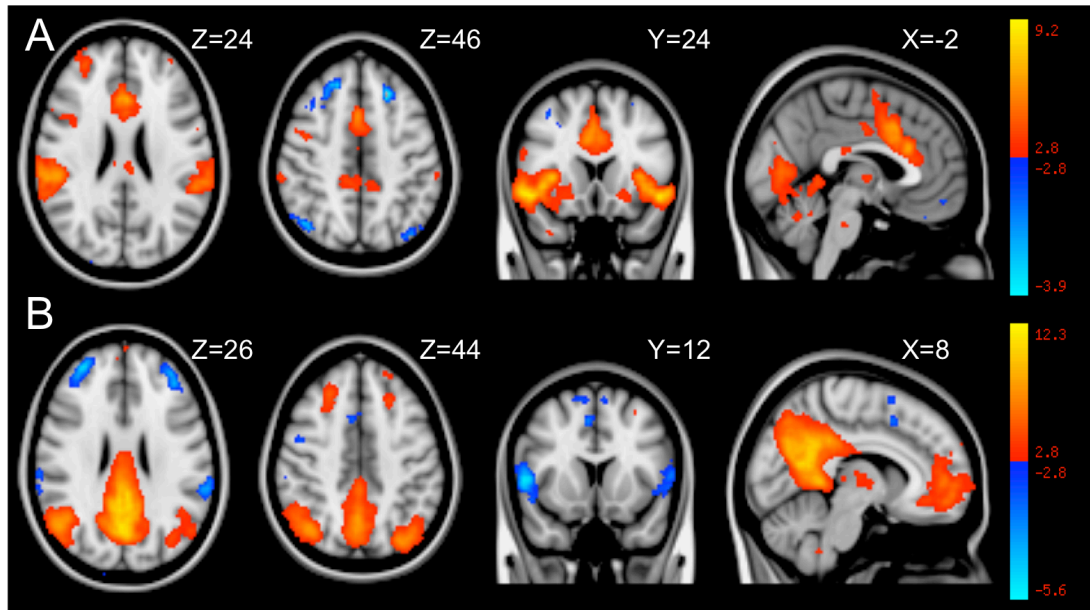


Figure 3.1. Spatial characteristics of the SEN (A) and the DMN (B) as estimated by group-ICA of both nicotine and placebo treatment conditions (N = 17). Colour intensities are voxel-wise z-statistics of the mean strength of regional functional connectivity with the mean temporal activity of the larger network. Red-yellow indicates a positive connectivity association with the characteristic network time course, while blue indicates a negative network connectivity association.

3.3.3 – RSN functional connectivity analyses (ii): DMN-SEN coupling

In line with the hypothesis, there was a wide variation in the SEN-DMN partial correlation coefficients across subjects (nicotine: mean $r = -0.15 \pm 0.31$ SD, range = -0.73 to 0.33; placebo: mean $r = -0.17 \pm 0.21$ SD, range = -0.47 to 0.22; see Fig. 3.2 for individual subject examples). To test whether this variation related to individual differences in the reduction of withdrawal symptoms it was necessary to calculate (i) the normalised ‘change in network coupling’ (CNC) between the SEN and DMN across treatment conditions and (ii) MMWS difference scores between NRT and placebo treatments for each subject. Correlations between the CNC and MMWS difference scores were then carried out. Consistent with the hypothesis that coupling between the SEN and DMN is related to the cognitive symptoms of smoking abstinence, CNC values were positively correlated with

MMWS ‘difficulty concentrating’ difference scores ($r = 0.62$; $p < 0.05$, corrected; uncorrected $p = 0.0080$; Fig. 3.3). There were no other strong correlations between CNC measures and difference sub-scores for other MMWS items or the total scores (all r values < 0.45 , all uncorrected $p > 0.10$). At a group level, without accounting for individual differences in MMWS symptom improvements between sessions, permutation testing for a main effect of treatment revealed no significant effect of NRT (relative to placebo) on changes in coupling between the SEN and DMN networks defined in the resting-state data ($p = 0.47$).

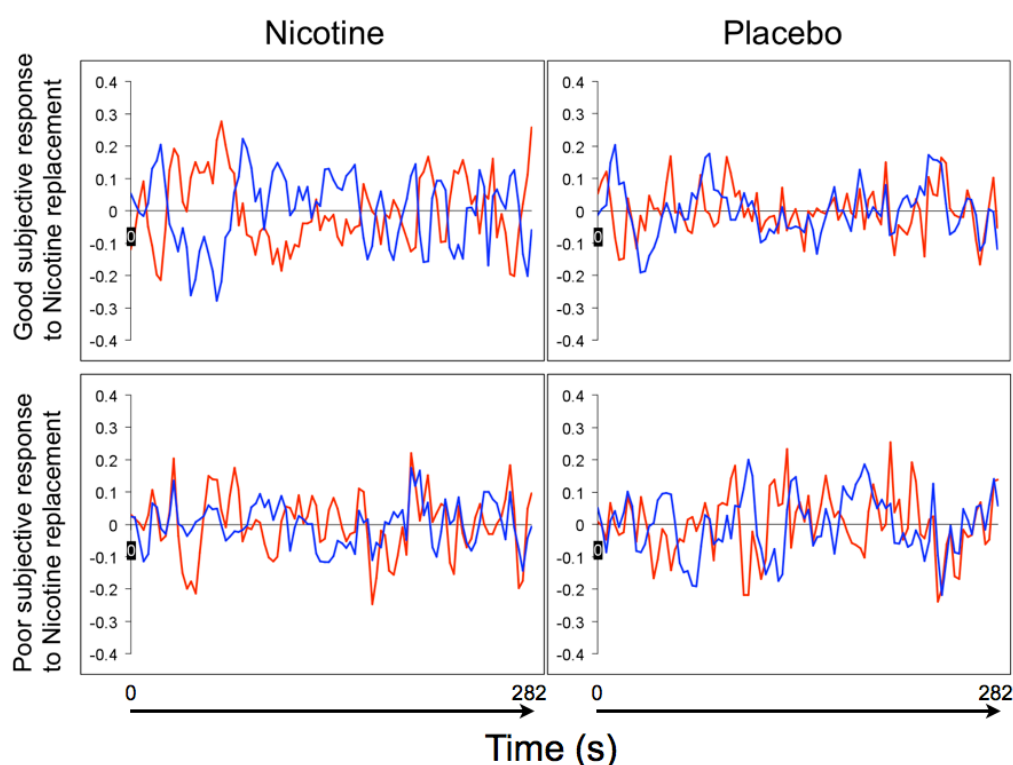


Figure 3.2. Dynamic signal fluctuations of the SEN (red) and DMN (blue) from nicotine and placebo sessions of two individual subjects. The first example subject (top row) responded well to nicotine replacement in terms of a large reduction in withdrawal symptoms in the nicotine compared to the placebo session. The concurrent increase in coupling between the characteristic network time courses of the SEN and DMN is clear from the associated plots. The second example subject (bottom row) displayed no overall reduction in withdrawal symptoms between the nicotine and placebo sessions. Accordingly, this subject displays no overt change in SEN-DMN coupling between treatment conditions. Y-axis values represent normalised mean signal fluctuation in the RSN; the x-axis denotes time, ranging from the start of the resting-state scan (0 s) to the end of acquisition (282 s).

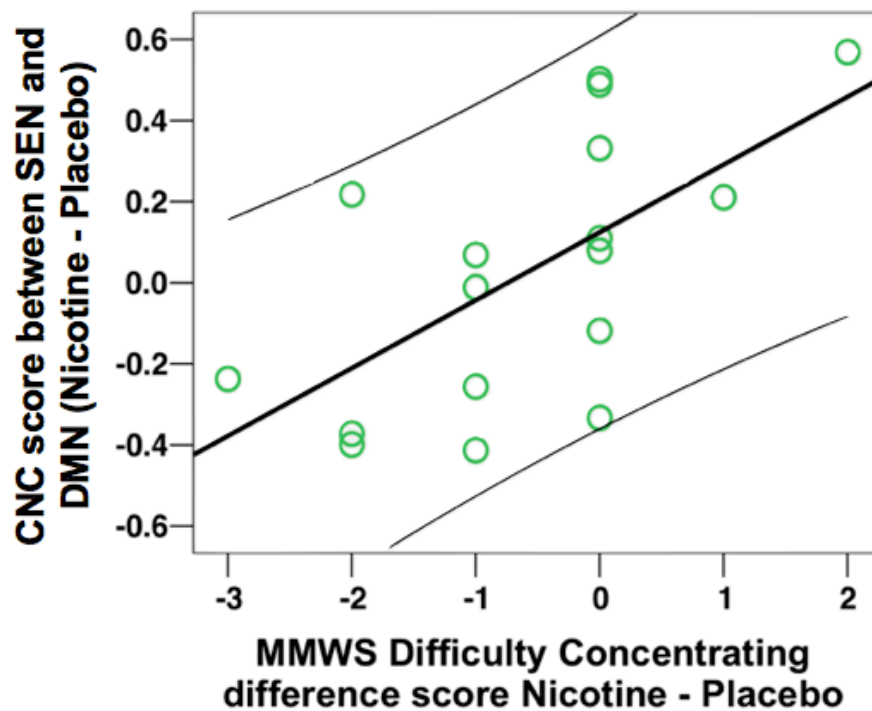


Figure 3.3. (A) Positive correlation ($r = 0.62$, $p < 0.05$, corrected) between the change in network coupling (CNC) of the SEN and DMN between treatment conditions and self-reported difference scores of nicotine-induced improvements in difficulty concentrating ($N = 17$). Centre line denotes the mean group effect; outer lines indicate 95% confidence intervals for individual cases.

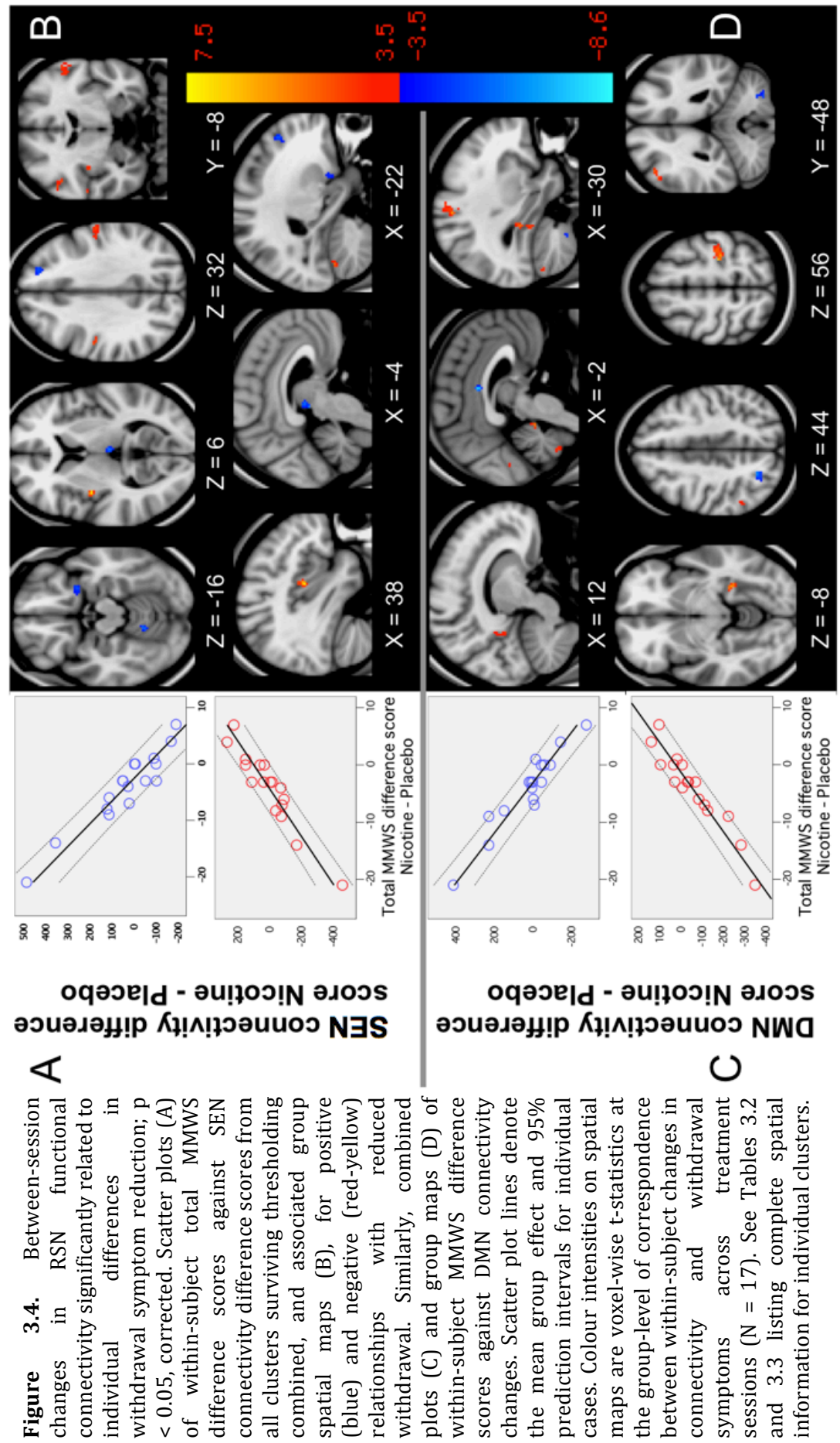
3.3.4 – RSN functional connectivity analyses (iii): connectivity changes correlated with individual treatment response variability

To explore effects of NRT on brain functional changes related to clinical symptoms of tobacco withdrawal across the whole brain, subject- and session-specific maps of RSN functional connectivity were defined via dual regression. The effects of individual differences in total MMWS symptom improvements⁸ by NRT (relative to placebo) on: (i) SEN whole-brain network functional connectivity and (ii) DMN whole-brain connectivity were separately assessed by

⁸Total MMWS scores were found to explain more variance in the self-report data than any other behavioural variables, as calculated by PCA (see section 3.2.7) and are thus likely to provide the most relevant covariate for elucidating individual differences in related brain metrics. For this reason these values for each subject were used in this higher-level analysis, rather than scores on the “difficulty concentrating” item alone, which was most strongly associated with the change in DMN-SEN coupling dynamics (reported in section 3.3.3).

a within-subject analysis, using the GLM to perform voxel-wise paired comparisons.

Significant correlations ($p < 0.05$, local FDR corrected) were identified between MMWS difference scores and treatment-related changes in the functional connectivity of both RSNs of interest (the SEN and the DMN) with resting activity fluctuations in other brain regions (Fig. 3.4; see Tables 3.2 and 3.3 for full results). These included negative correlations between MMWS difference scores (and thus *positive* correlations between symptom *reductions*) and the connectivity of the SEN with activity in regions including the left orbitofrontal cortex, left dorsomedial prefrontal cortex and left thalamus (Fig. 3.4A,B; blue). Conversely, positive correlations between MMWS difference scores and functional connectivity changes with the SEN (*negatively* related to symptom reductions) were found in regions including the right posterior insula, right posterior middle/inferior temporal gyri, left post-central gyrus and right precentral gyrus/central sulcus (Fig. 3.4A,B; red-yellow). MMWS difference scores were negatively correlated with functional connectivity changes between the *DMN* and clusters including the left mid-cingulate and left lateral orbitofrontal cortices (Fig. 3.4C,D; blue). Positive correlations were found between MMWS difference scores and functional connectivity changes of the DMN with activity in regions including the left dorsal hippocampus, bilateral precuneus and bilateral parahippocampal gyri (Fig. 3.4C,D; red-yellow).



3.3.5 – RSN functional connectivity analyses (iv): Drug effects of nicotine on RSN functional connectivity

The dual regression and subsequent voxel-wise analysis also identified brain regions showing significant functional connectivity changes with either the SEN or DMN between treatment sessions, independent of individual differences in symptom improvements ($p < 0.05$, local FDR corrected). Relative to placebo, NRT significantly reduced functional connectivity between the SEN and the time courses of activity in clusters located in the bilateral temporal poles extending into frontal opercular/orbitofrontal cortex, left posterior insula and left dorsal anterior cerebellum. NRT significantly increased functional connectivity between the SEN and activity in superior and inferior regions of right precentral gyrus. NRT administration significantly reduced functional connectivity between the DMN and the time course of activity in one region of occipital striate cortex, and increased connectivity between the DMN and activity in left posterior precuneus and right posterior cuneus (Tables 3.2 and 3.3).

3.4 – Discussion

3.4.1 – Summary

This study investigated the utility of RSN functional connectivity measures in providing sensitive systems-level markers of the clinical effects of NRT on cognition supported by large-scale neural networks. The primary hypothesis was tested that NRT-related symptom improvements in abstinent smokers are associated with modulations in the functional connectivity between salience/executive control and default mode RSNs. Therapeutic effects of nicotine to reduce cognitive impairments during smoking abstinence correlated

significantly with the changes in coupling between SEN and DMN network dynamics. This provides evidence that acute therapeutic effects are related to modulation of brain processes implicated in salience processing, executive control, attention and motivated behaviour. Exploratory analysis of the connectivity of these RSNs with voxel-wise activity across the whole brain identified additional pharmacological effects. Functional connectivity changes between the SEN and several regions contributing to the DMN, and *vice versa*, were positively correlated with beneficial clinical effects of nicotine replacement. Conversely, negative correlations with therapeutic effects were identified by changes in the *within-network* connectivity of both the SEN and DMN, suggesting distinct functional consequences for alterations in connectivity within and between core cognitive networks in nicotine addiction. These results emphasise that NRT not only reduces nicotine craving, but also alters brain signalling and functional connectivity (as measured using BOLD FMRI) in ways that contribute to subjective improvements in cognition.

3.4.2 – SEN-DMN coupling and subjective measures of concentration

Improved concentration following nicotine administration was associated with increased negative coupling between the SEN and DMN. This finding provides direct support for theories relating increased SEN-DMN negative coupling to improvements in cognitive control. Previous research in healthy populations has shown that activity patterns in these two RSNs are negatively correlated in many cognitive task models (Shulman et al., 1997; Greicius et al., 2003; Sridharan et al., 2008). Castellanos and colleagues have suggested that the degree of coupling between these networks is sensitive to individual variability in cognitive

performance, and that a greater negative correlation, as demonstrated here with NRT relative to placebo, is associated with improved cognition or more 'efficient' cognitive control (Castellanos et al., 2008; Kelly et al., 2008). Seed-based (SCA) studies of connectivity during both task performance (Baliki et al., 2008; Kelly et al., 2008) and rest (Wang et al., 2007a; Castellanos et al., 2008) provide support for these differences mediating cognitive abilities in healthy subjects and multiple neuropsychiatric populations. Because activity in the SEN is linked to cognitive control (Bush et al., 2000; Dosenbach et al., 2007; Laird et al., 2011), as is its negative relationship with the DMN (Kelly et al., 2008; Leech et al., 2011), one would expect, as the current results demonstrate, to see a stronger correlation between SEN-DMN coupling and cognitive symptoms such as difficulty concentrating, relative to other symptoms of smoking withdrawal. Further work is needed to distinguish potential elements of SEN-DMN coupling (and related intra- and inter-RSN functional connectivity patterns) mediating specific processes adversely affected during impaired cognitive states (e.g., withdrawal), including attention (Castellanos et al., 2008), novelty/salience monitoring (Seeley et al., 2007; Froeliger et al., 2009) and working memory (Vincent et al., 2006; Dosenbach et al., 2007). Indeed, in a related experiment recently reported, promising initial findings have revealed novel insights into the neural underpinnings of nicotine's effects on cognition, by showing that NRT simultaneously increases and decreases model-related activation during a demanding cognitive task in large-scale 'task-positive' and default mode neural networks, respectively (Beaver et al., 2011). Of chief importance in terms of neuronal interpretations, this study also found this NRT-related enhancement to be strongly coupled with alpha band activity as measured by co-acquired EEG.

This study focussed on investigating functional connectivity in the context of nicotine withdrawal and subsequent pharmacotherapy. However, some of the results are consistent with prior findings of task-related fMRI activation (Lawrence et al., 2002; Hahn et al., 2007) and SCA resting connectivity studies (Hong et al., 2009) in smokers not experiencing withdrawal symptoms. Hahn and colleagues previously demonstrated reduced BOLD signal in regions corresponding to the DMN during a visuo-spatial attention task in smokers given nicotine, which was interpreted as acute nicotinic “down-regulation” of DMN activity during task performance (Hahn et al., 2007). Combined with reported nicotine-related *increases* in cingulate resting functional connectivity (Hong et al., 2009), this is consistent with the current finding of between-network coupling during withdrawal altered by pharmacotherapy. Differences between these results and those of preceding studies, in terms of nicotine affecting *within-network* connectivity between the SEN and fronto-temporal/insular cortex, may be related to the longer abstinence period in this study, designed to induce withdrawal symptoms prior to NRT administration.

The multivariate analysis employed here complements prior approaches to understanding the neural mechanisms underlying beneficial effects of NRT on cognition, affording ‘bidirectional’ descriptions of dynamic, temporally correlated BOLD oscillations within and between networks. Unidirectional, single-network interpretations, positing a nicotine-induced down-regulation of the DMN during tasks or “up-regulation” at rest (Hahn et al., 2007; Beaver et al., 2011), have been based on stationary (i.e., time-invariant) approaches to analysing dynamic BOLD data, necessitated by both the task-related activation paradigms and seed-based connectivity models employed (see sections 1.3 &

2.1). Unidirectional approaches may be anatomically restrictive to systems-level hypotheses, especially those addressing interactions between distinct networks (Chang and Glover, 2010). Here it is demonstrated (i) that the co-activation signal *within* the DMN is often accompanied by an inversely correlated signal fluctuation across the SEN at the systems-level, and (ii) that the dynamics of these two RSNs couple inversely in a nonstationary, state-specific manner correlated with individual differences in acute cognitive symptom improvements.

3.4.3 – RSN connectivity and improvements in withdrawal symptoms

Results showed that improvements in clinical symptoms of smoking withdrawal were positively correlated with SEN connectivity changes, both in brain regions contributing to the DMN (e.g., medial prefrontal cortex and thalamus) and in regions involved in reward processing (e.g., orbitofrontal cortex; OFC; Kringelbach and Rolls, 2004; O'Doherty, 2004). The DMN also displayed functional connectivity with *SEN* mid-cingulate cortex regions and OFC that correlated positively with improvements in withdrawal symptoms after nicotine replacement. One could speculate that the analysis identifies key integrative nodes (e.g., prefrontal cortex and thalamus) that mediate interactions between the two major cognitive processing networks. Future studies could also address whether altered connectivity of the DMN *and* SEN with regions of OFC may further link their coupling dynamics to changes in reward or salience processing relevant for addiction and other maladaptive motivated behaviours (O'Doherty, 2004; Volkow et al., 2011; Meunier et al., 2012; Tomasi and Volkow, 2012; see also Chapter 4).

Results also highlighted that reduced withdrawal symptoms were negatively correlated with changes in within-network functional connectivity in the DMN, including with the hippocampus and parahippocampal gyrus. Networks involving these regions have been proposed to play a critical role in aspects of episodic memory (Greicius et al., 2004; Vincent et al., 2006), but also have been widely implicated in drug-dependence (e.g., Kenney and Gould, 2008; Koob and Volkow, 2010). Links between hippocampal integration with associative cortex in the precuneus (identified here in the same regression contrast) and cognitive deficits are apparent from findings of reduced default mode connectivity with the hippocampus in Alzheimer's disease (Greicius et al., 2004), along with abnormally *increased* DMN-hippocampal connectivity in populations genetically 'at-risk' for the disease (Filippini et al., 2009). These neural changes could contribute to memory deficits associated with smoking abstinence, subjectively alleviated by NRT (Atzori et al., 2008; Froeliger et al., 2009). Further, the *SEN* displayed functional connectivity changes with the insula negatively correlated with clinical symptom improvements after nicotine replacement. Therefore, pharmacotherapy may also ameliorate smoking withdrawal symptoms by modulating executive functional integration of activity in a region thought to be involved in processing unpleasant salient physical and emotional sensations, and found to be critical to the desire to continue cigarette smoking in addicts following acquired brain injury (Naqvi et al., 2007).

3.4.4 – Methodological considerations

A number of methodological points warrant discussion. First, as detailed in section 2.1.1 (see also Cole et al., 2010a), controversy surrounds the use of global

mean signal regression (used widely in univariate SCA connectivity models to correct for non-neuronal, physiological noise) as a preprocessing step in resting-state FMRI studies. Removal of this information could induce artefactual negative correlations between time series (Murphy et al., 2009), although counter-arguments exist (Fox et al., 2009). Nevertheless, in the current study modulations were inferred in the correlation between the SEN and DMN by multivariate analysis of within-subject data obtained in two controlled conditions *without* performing global signal regression. It remains possible that the partial correlation technique used to measure the within-session relationship between the SEN and DMN may increase the probability of finding negative correlations (Marrelec et al., 2009; Smith et al., 2011). However, such a bias cannot account for the observed correlation between withdrawal symptom reductions and increased negative coupling between cognitive RSNs (i.e., associations between the changes in within-subject variables) across two controlled experimental conditions.

Second, because BOLD FMRI is sensitive to changes in the relative ratio of oxygenated to deoxygenated haemoglobin (Ogawa et al., 1990), it is possible that functional connectivity measurements could be influenced by direct effects of nicotine on cortical vasodilation altering neurovascular coupling (Mathew and Wilson, 1991; Iida et al., 1998). Similar considerations are necessary for the majority of pharmacological FMRI studies (Matthews et al., 2006; Iannetti and Wise, 2007). However, global changes in cortical vasodilation cannot account for the significant correlations found here between the connectivity dynamics of FMRI signals and individual differences in self-reported clinical improvements. Additionally, it has been shown with perfusion FMRI methods (allowing absolute

measurement of cerebral blood flow) that NRT does not alter global cerebral blood flow or neurovascular coupling in a regionally non-specific manner (Hahn et al., 2007; Hahn et al., 2009).

Finally, the resting-state data presented in this study were acquired immediately following performance of a demanding cognitive processing task. Performance of cognitive tasks may influence subsequent observations of RSNs (Albert et al., 2009; Lewis et al., 2009; Pyka et al., 2009). Although it is possible that prior task performance could have influenced the primary findings of nicotine influencing RSN connectivity during smoking withdrawal, this is unlikely because (i) effects of pharmacotherapy were compared across treatment sessions with testing in each session conducted in an identical order, and (ii) differences in performance on the cognitive task were controlled for via the inclusion of a confound regressor in the within-subject analysis.

3.4.5 – Conclusions

This study investigated functional connectivity dynamics of brain resting-state networks in habitual smokers to characterise the beneficial effects of nicotine replacement therapy on cognitive processing during smoking abstinence. Effects of NRT on functional connectivity were identified both *within and between* default mode and executive control networks. Moreover, individual variations in functional connectivity were shown to be associated with established psychological measures of withdrawal response to NRT. Results highlight widespread effects of NRT on brain functional networks in habitual smokers during cigarette abstinence associated with subjective cognitive and clinical improvements.

These findings imply that RSN functional connectivity measures can provide sensitive markers of the clinical effects of pharmacological compounds on distributed neurochemical and neurobiological systems and, thus, are relevant for mechanisms of aberrant cognitive and reward processing in neuropsychiatric disorders such as drug dependence. Results particularly highlight the sensitivity of the techniques in detecting individual differences in symptom severity and/or treatment response.

It must be noted that nicotine is a drug with a comparatively high degree of neurochemical ambiguity in terms of its central nervous system action. For example, exogenous nicotine administration is known to have a broad profile of modulatory and 'knock-on' effects on multiple neurotransmitter systems (e.g., Wonnacott et al., 2006). Therefore, in order to practicably apply the same imaging and analysis techniques to drug development settings, where pharmacotherapies are often more selective for specific neuroreceptor families or sub-types (and clinical benefits are perhaps less immediately evaluable), it is important to establish whether or not these measures can be grounded in fundamental neurobiology. Indeed, while opportunities to apply similar approaches in clinical practice may be apparent, it is important for the medicines development community to prove that novel techniques can be of use in targeting and teasing apart specific, neurobiological phenotypes. With this in mind, the following chapter describes a multimodal neuroimaging study investigating relationships between RSN activity (providing 'intermediate phenotypic' markers of individual differences relevant for neuropsychiatric disease) and discrete neuroreceptor systems (molecular phenotypes), which are

implicated in disease and of current interest in pharmacotherapy development settings.

Chapter 4 – Linking network dynamics to molecular neurobiology

Dopamine D3 receptors modulate orbitofrontal RSN integration

Evidence highlights a key role for brain dopamine D3 receptor (D3R) function in multiple neuropsychiatric disorders, including addiction. Disrupted frontal cortical connectivity with distributed brain networks has also been implicated in processes relevant for such diseases. This potential conjunction between molecular and functional imaging markers has not, however, been tested directly. This second study, in 12 healthy male subjects, used a novel within-subject combination of [^{11}C]-(+)-PHNO positron emission tomography (PET) and resting-state fMRI to investigate associations between specific dopamine neuroreceptor sub-types and large-scale RSN functional connectivity patterns. High midbrain D3R availability was associated with reduced functional connectivity between orbitofrontal cortex (OFC) and RSNs implicated in cognitive control and salience processing. The opposite pattern was observed in subcortical reward circuitry and the DMN, which showed greater connectivity with OFC in individuals with high D3R availability. Findings demonstrate that differential interactions between OFC and RSNs implicated in cognitive control and reward are associated with variability in specific molecular neuroreceptor profiles.

4.1 – Introduction

It has been established here (in Chapter 3) that methods sensitive to large-scale network functional connectivity in the resting state display some utility in identifying gross drug effects in the central nervous system, as well as neuropsychopharmacological variability across individuals. However, the comparative lack of neurobiological specificity, in this case of NRT, was also noted. Therefore, in order to validate systems-level connectivity metrics as relevant markers for the treatment of neuropsychiatric disorders, it may be beneficial to investigate how they interact with specific neurochemical systems of topical interest in medicines development.

Dopamine has been widely implicated in motivated behaviour, reward processing and related neuropsychiatric disorders, particularly substance dependence⁹ (see e.g., Schultz, 2002; Wise, 2004). Pre-clinical studies suggest that the D2-like family of dopamine receptors (D2, D3 and D4) mediates reward-related behaviour (Dalley et al., 2007; Heidbreder and Newman, 2010). D3 receptor (D3R) function specifically has been related to sensitivity to positive reinforcement, contributing to reward-seeking and motivated behaviours characteristic of relapse to addiction (Caine and Koob, 1993; Guillin et al., 2001; Sokoloff et al., 2006). D3Rs in the substantia nigra/ventral tegmental area (SN/VTA) of the midbrain are thought to act as inhibitory modulators of post-synaptic dopamine release in regions implicated in reward processing, including orbitofrontal cortex (OFC) and striatum (Levant, 1997; Buckholtz et al., 2010; Heidbreder and Newman, 2010; Koob and Volkow, 2010; Sesack and Grace,

⁹Portions of this chapter are reproduced from a related publication: Cole DM, Beckmann CF, Searle GE, Plisson C, Tziortzi AC, Nichols TE, Gunn RN, Matthews PM, Rabiner EA, Beaver JD (2012a) Orbitofrontal connectivity with resting-state networks is associated with midbrain dopamine D3 receptor availability. *Cereb Cortex* 22:2784-2793.

2010). However, while the D2 receptor (D2R) system has been explored extensively, little has been discovered about the role of the D3 system in human brain function and dysfunction due to the lack, until recently, of D3R-selective imaging probes (Rabiner and Laruelle, 2010; Boileau et al., 2012).

Studies in primates and patients with frontal lobe lesions have shown that the OFC is crucial for decision-making processes subserving inhibitory control and goal-directed behaviour (Damasio et al., 1990; Bechara et al., 2000; Izquierdo et al., 2004, 2005). fMRI studies have expanded on these findings, identifying task-specific BOLD activity in functionally heterogeneous regions of human OFC that are associated with distinct aspects of reward processing and goal-directed behaviour (O'Doherty et al., 2001; O'Doherty et al., 2003). Thus, OFC functions strongly parallel those often attributed to the D3R system. The OFC also has extensive reciprocal anatomical connections with cortical, sub-cortical and midbrain regions, consistent with the notion that it regulates the functioning of multiple neuronal networks underlying behavioural decision-making (Kringelbach and Rolls, 2004; Frank and Claus, 2006). Despite these functional similarities, and although large-scale brain networks including frontal circuitry are known to underlie many of the complex behaviours contributing to reward and addiction (as described in Chapter 3; also see e.g., Robbins, 2000; Frank and Claus, 2006; Cole et al., 2010b; Koob and Volkow, 2010; Sesack and Grace, 2010; Goldstein and Volkow, 2011), little is currently known about the potential role of midbrain D3R in modulating interactions between orbital prefrontal regions and networks relevant to cognitive control, motivational salience and reward processing.

This study used PET with the D3-preferring radioligand [^{11}C]-(+)-PHNO and resting-state FMRI in the same individuals, to examine midbrain D3R availability in conjunction with measures of OFC functional connectivity with distinct ‘cognitive’ RSNs. It was hypothesised that RSNs spanning frontoparietal cortical regions, previously shown to support cognitive control and motivational salience processing (Gusnard et al., 2001; Greicius et al., 2004; Seeley et al., 2007, 2009), would show variation in OFC functional connectivity between subjects, associated with individual differences in midbrain D3R availability. In line with reported dissociations between cognitive control and reward-related brain activity in addiction and other neuropsychiatric disorders (Baler and Volkow, 2006; Cools, 2006), it was further hypothesised that an opposing relationship would exist between D3R availability and OFC connectivity with RSNs comprising reward-processing structures in the limbic system and basal ganglia.

Recent findings have shown that [^{11}C]-(+)-PHNO can be used to quantify the *in vivo* availability of both D3R and D2R sub-types (Rabiner et al., 2009; Searle et al., 2010). [^{11}C]-(+)-PHNO binding in the midbrain SN/VTA is attributable specifically to D3R availability (> 90% of the total signal), while the majority of binding (> 90%) in the dorsal putamen (DPU) is attributable to D2R (Tziortzi et al., 2011). Based on this and other emerging evidence of anatomical and functional dissociations between D2R and D3R (van Gaalen et al., 2009; Heidbreder and Newman, 2010; Watson et al., 2012), a secondary objective of the study was to investigate possible differential effects on RSN connectivity patterns associated with D2R, relative to D3R availability.

4.2 – Materials and methods

4.2.1 – Participants

Participants were twelve right-handed healthy male volunteers (mean age = 37.3 years, range = 31.2 - 52.4 years). No subjects had a history of medical, neurological or psychiatric illness, nor were any taking concomitant medications or non-prescription drugs. Suitability for inclusion in the study was assessed by complete medical history as confirmed by general practitioner and a qualified study physician, review of systems, physical examination, psychiatric interview, routine blood tests, urine toxicology and electrocardiogram. Subjects were excluded from the study if any history of neuropsychiatric illness was established, including substance use disorder. All subjects were deemed free of structural brain abnormalities following review of anatomical MRI scans by a qualified neuroradiologist. Screening prior to scanning included urine testing to exclude subjects positive for recent alcohol or nicotine use and other drugs of abuse. Subjects were excluded if presenting with a history of regular alcohol consumption within six months of the study, defined as an average weekly intake of greater than 21 units or an average daily intake of greater than 3 units. Subjects with urinary cotinine levels indicative of smoking or history or regular use of tobacco- or nicotine-containing products within six months prior to study enrolment were also excluded. Subjects abstained from ingesting caffeine- or xanthine-containing products (e.g., coffee, tea, cola drinks and chocolate) for 24 hr prior to their scans.

4.2.2 – Study design

Each subject was PET scanned with [^{11}C]-(+)-PHNO. This was followed on the same day by resting-state BOLD fMRI acquired twice in a single session. PET scanning was carried out for 90 min, and participants were transferred for fMRI scanning within 60 min of PET scan completion. During fMRI each subject underwent two 5.4 min resting-state scans separated by 20 min. Participants completed a cognitive task between the two resting scans (to be reported on in Beaver et al., under review). All participants provided written, informed consent in which confidentiality, anonymity, and the opportunity to withdraw without penalty were assured. Imaging data were collected during baseline sessions of a larger drug study¹⁰. The study protocol was approved by the UK Health Protection Agency Administration of Radioactive Substances Advisory Committee and the Welwyn Clinical Pharmacology Ethics Committee, University of Hertfordshire and procedures were carried out in accordance with the standards of the Declaration of Helsinki.

4.2.3 – [^{11}C]-(+)-PHNO production

[^{11}C]-(+)-PHNO was prepared from the N-despropyl PHNO, (+)-3,4,4a,5,6,10b-hexahydro-2H-naphtho[1,2-b][1,4]oxazin-9-ol, hydrochloride, by [^{11}C]propionyl chloride acylation followed by reduction of the [^{11}C]amide intermediate (Wilson

¹⁰Results from analyses of additional [^{11}C]-(+)-PHNO PET and cognitive task-related fMRI data, collected in conjunction with a pharmacological challenge, are in preparation for reporting elsewhere (Beaver et al., under review). As part of that extended study, a team of researchers conducted the procedures described in sections 4.2.3-5 of this thesis. In brief, results identified that high midbrain D3R availability ([^{11}C]-(+)-PHNO binding) was associated with greater BOLD signal in the amygdala in response to the receipt of monetary reward and that this activation was attenuated by administration of a selective D3R antagonist. This association between molecular and functional quantities is novel. However, as highlighted in Chapter 1, interpretations resulting from such experiments are restricted to those of regional brain function altering in line with a strict task model; they may be complemented by investigation of comparatively unconstrained systems-level phenomena, such as RSN functional connectivity.

et al., 2005). This reaction was fully automated to reliably produce high purity [^{11}C]-(+)-PHNO in a radiochemical decay-corrected yield of $16 \pm 5\%$ after a synthesis time of 35 min (Plisson et al., 2012). Injected activity of [^{11}C]-(+)-PHNO ranged from 114.5-404.7 MBq across subjects (mean = 222.8 ± 96.5 SD) and injected mass ranged from 1.04-4.24 μg (mean = 2.01 ± 0.91 SD).

4.2.4 – PET image acquisition

Dynamic PET scans were acquired in 3-D mode using a Siemens Biograph 16 HI-REZ PET-CT scanner. A CT scan was acquired for attenuation correction of PET data prior to administration of [^{11}C]-(+)-PHNO. The radioligand was administered as an intravenous bolus over 30 s. Dynamic PET data were acquired for 90 min, binned into 26 frames (durations: 8×15 s, 3×60 s, 5×2 min, 5×5 min, 5×10 min), then reconstructed using Fourier rebinning and 2-D filtered back projection with a ramp filter at Nyquist cut-off frequency. Image data were smoothed with a Gaussian kernel of 5 mm FWHM.

4.2.5 – PET analysis

Dynamic PET images were registered to the subject's T1-weighted anatomical volume (see section 4.2.6), and corrected for motion using a frame-to-frame registration process with a mutual information cost function (as implemented in SPM5; Wellcome Trust Centre for Neuroimaging, University College London, United Kingdom; <http://www.fil.ion.ucl.ac.uk/spm>). Bilateral SN/VTA ROIs were defined manually on each subject's PET integral image, using a fixed size template calculated via a previously published method (Searle et al., 2010). DPU ROIs were bilaterally and manually defined on each subject's T1 image, using a

technique shown to reliably distinguish the region from others in the striatum (Tziortzi et al., 2011). ROIs were applied to the dynamic PET data to derive regional time-activity curves. Nondisplaceable binding potential (BP_{ND}) values were calculated for both bilateral ROIs via the basis function implementation of the simplified reference tissue model (Lammertsma and Hume, 1996; Gunn et al., 1997; see Equation 1) with the cerebellum as the reference region defined using an in-house atlas (Tziortzi et al., 2011).

$$BP_{ND} = \frac{f_{ND}B_{\max}}{K_D}$$

Equation 1. The $[^{11}\text{C}]\text{-(+)-PHNO}$ binding potential relative to the nondisplaceable compartment (BP_{ND}) is equal to the product of receptor density ($B_{\max}$), affinity of ligand for the target ($1/K_D$) and the free fraction of the ligand in the brain (f_{ND}).

This produced values of $[^{11}\text{C}]\text{-(+)-PHNO}$ BP_{ND} in SN/VTA and DPU regions for each subject. BP_{ND} values were demeaned prior to inclusion as covariates in the fMRI analyses (see section 4.2.8).

4.2.6 – fMRI acquisition

BOLD contrast-sensitive, T2*-weighted, echo-planar images were acquired continuously on a 3-Tesla Siemens Trio scanner with a 32-channel head coil. Each scan consisted of 154 volumes of 38-slice acquisition (TR = 2100 ms; dual TE = 13/31 ms, 31 ms data *only* reported; flip angle = 78°; 3.5 × 3.5 × 3.0 mm voxels). It is well established that OFC regions can exhibit signal dropout with echo-planar imaging (see e.g., Kringelbach and Rolls, 2004). Therefore, the following acquisition techniques were used to recover signal in these regions: (i) slice acquisition was angled 30° coronally upwards from the anterior-posterior

commissural plane; (ii) slice thickness was minimised to 3 mm. These measures minimised OFC signal dropout but led to loss of coverage over a small posterior portion of the superior parietal lobe in some subjects, which was therefore excluded from whole-brain group analyses. The first four volumes were removed from each fMRI dataset to allow for magnetic equilibration, resulting in a 150-datapoint BOLD time series at each voxel per acquisition. Additional high-resolution T1-weighted anatomical scans (MPRAGE) were acquired with whole-brain coverage for each subject to aid with both fMRI and PET image co-registration and PET ROI definition (TR = 3000 ms, TE = 3.66 ms, flip angle = 9°, voxel size = 1 mm³, 208 slices). For the resting-state scans, subjects were instructed to lie still with their eyes closed, but to stay awake.

4.2.7 – fMRI analysis

Image preprocessing and analyses were performed with FSL (version 4.1.4). Preprocessing, group-ICA with temporal concatenation and dual regression techniques applied were equivalent to those described in Chapter 3. Both resting-state acquisitions from each subject were included in the analyses (150 volumes per scan × 2 resting-state scans per subject × 12 subjects = 3600 image volumes).

Group-level ICA identified 33 components in the resting-state fMRI data (24 scans in total, two per subject), all of which underwent subsequent dual regression. These included RSNs commonly found in previous studies (e.g., Beckmann et al., 2005; Kiviniemi et al., 2009). Six RSNs of interest were selected from the group-ICA results for further analyses based on their neuroanatomical configurations, in comparison with previous literature (e.g., Beckmann et al.,

2005; Seeley et al., 2007; Kiviniemi et al., 2009; Smith et al., 2009). Those chosen for higher-level analyses are thought to be relevant for cognitive control and goal-directed behaviour (Table 4.1). These were (i) three bilateral frontoparietal RSNs thought to support high-level cognitive and motivational processes (e.g., Seeley et al., 2007), with nodes located within three or more of the dorsal anterior cingulate, dorsolateral prefrontal, anterior insular and inferior parietal cortices (typically referred to as “salience” and/or “executive control” networks; they are referred to here as SEN1, SEN2 and SEN3; Fig. 4.1A-C), (ii) the DMN (Fig. 4.1D) and (iii) a network comprising basal ganglia/limbic regions (the BGLN; Fig. 4.1E), with potential relevance to dopamine function (Kelly et al., 2009; Sesack and Grace, 2010). Although they were here identified as maximally spatially independent components, the three SENs each shared some spatial features with systems reported previously using similar nomenclature, for example the single SEN studied in Chapter 3. The implications of such spatiotemporal variability that can occur across different studies and/or populations are discussed elsewhere in this thesis (see sections 2.1.3 and 7.3.3). The DMN was examined because of its putative role in spontaneous cognition (Gusnard et al., 2001; Raichle et al., 2001; Buckner et al., 2008) and the functional relevance of its “anti-correlated” relationship with SENs identified previously (Fox et al., 2005; Cole et al., 2010b; see also Chapter 3). A basal ganglia/limbic network (BGLN) including the striatum, amygdala, thalamus and ventral anterior cingulate (Fig. 4.1E) was also examined, as many of these regions have high D3R concentrations (Rabiner et al., 2009; Searle et al., 2010; Tziortzi et al., 2011) and this network has been strongly implicated in reward processes and dopamine function (Kelly et al., 2009; Sesack and Grace, 2010). A somatomotor RSN spanning bilateral superior

parietal cortices, thought to relate to primary sensory and motor processing (Biswal et al., 1995), served as a ‘control’ RSN (i.e., not expected to be influenced by D3R availability) in higher-level analyses. The spatial patterns of functional connectivity for these RSNs were consistent with prior observations (Greicius et al., 2004; Beckmann et al., 2005; Seeley et al., 2007, 2009; Smith et al., 2009; Cole et al., 2010b; see Table 4.1).

4.2.8 – PET-FMRI comparison

The dual regression produced subject-wise whole-brain maps of voxel-wise functional connectivity with each RSN. These were arranged into 4-D files comprising, for each RSN of interest, the associated maps from every functional session and then analysed to find regions where individual differences in RSN connectivity were significantly positively or negatively associated with PET BP_{ND} measures. Voxel-wise, mixed-effects nonparametric permutation testing was used (as implemented in FSL randomise), with 5000 permutations and 5 mm variance smoothing, the latter being a recommendation for studies with comparatively modest sample sizes (Nichols and Holmes, 2002). PET studies typically use the smallest possible sample size required to detect an effect to minimise potential risks associated with exposing healthy volunteers to ionising radiation (see section 4.4.4 for additional discussion). Here the primary interest was in relating D3R availability to OFC-RSN connectivity patterns, so [¹¹C]-(+)-PHNO BP_{ND} values from SN/VTA were entered as a covariate of interest in voxel-wise analyses of separate RSN maps. Of secondary interest were effects associated with D2R availability, so DPU [¹¹C]-(+)-PHNO BP_{ND} scores were also included as a covariate in these analyses. To control for the effects of a task

carried out between the two resting FMRI scans in each session, an additional order covariate was included, differentially modelling and contrasting for potential pre- and post-task resting session effects across subjects. Normalised voxels displaying zero signal in at least one individual acquisition, or falling below a 20% threshold of containing grey matter based on a study population-specific probabilistic atlas calculated using FSL FAST, were exempt from all mixed-effects regression analyses. Significant regional effects were defined using cluster-mass thresholding ($t > 2.3$, $p < 0.05$) correcting for family-wise error (FWE).

Additionally, correlations were explored between SN/VTA (D3) BP_{ND}, DPU (D2) BP_{ND} and subject age to test whether these factors were significantly associated (e.g., Volkow et al., 1998). For completeness, age was also correlated (post hoc) with individual scores from any BP_{ND}-related OFC-RSN connectivity effects identified by the PET-FMRI comparison (see section 4.3).

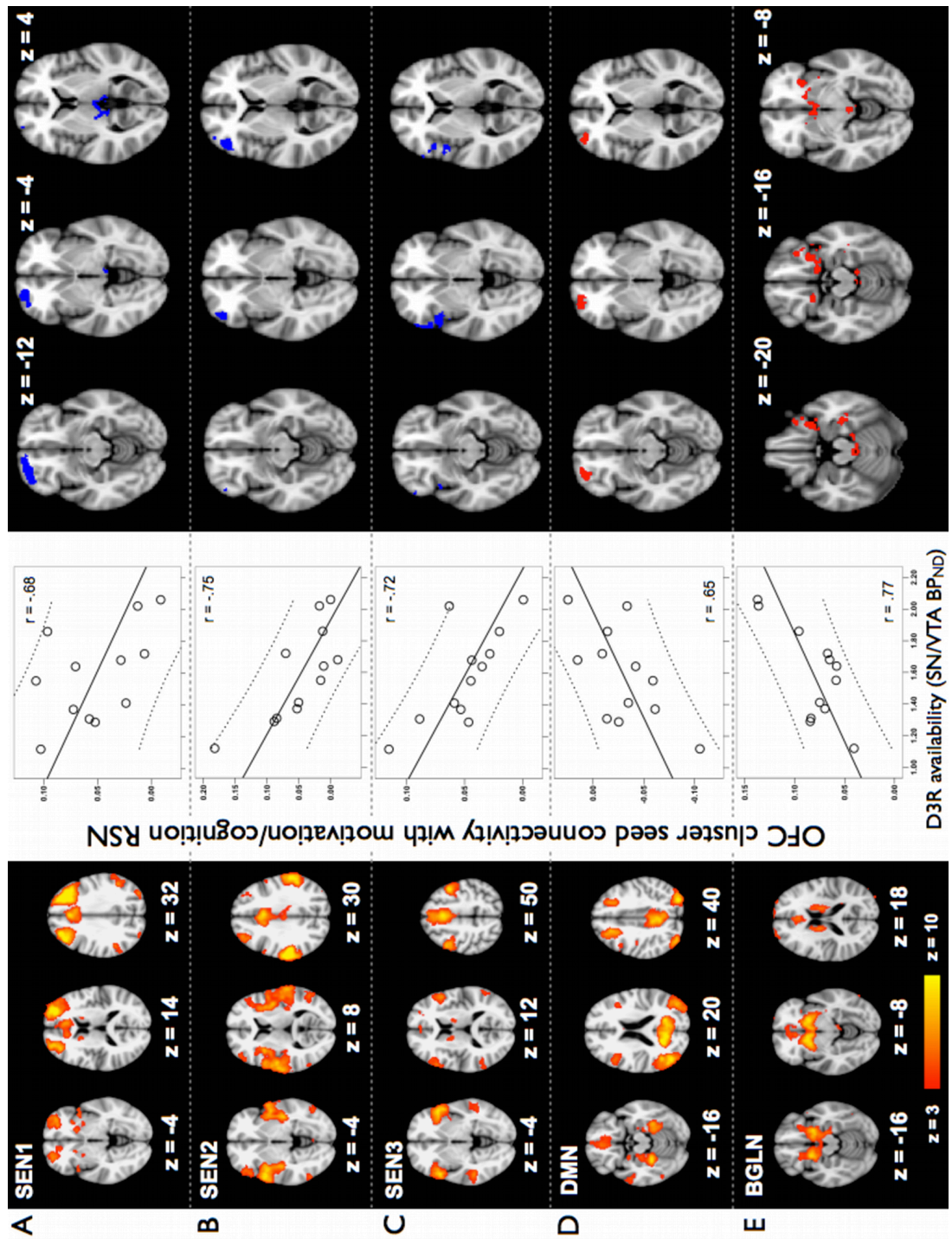
Based on the results of the single-network voxel-wise analyses (Fig. 4.1, see also Tables 4.2 and 4.3), an additional mixed-effects multiple regression analysis was carried out, with identical parameters and covariates to those already described, but entering the 4-D concatenated, subject-specific 3-D spatial maps of two RSNs (the DMN and SEN1) displaying inverse D3-dependent relationships, heavily overlapping the same OFC region (Fig. 4.2). Whole-brain, differential contrasts were carried out between the two networks, in order to fully examine the D3-related dependencies of this, and other, patterns of regional connectivity with one RSN relative to the other (reported in section 4.3.5).

Table 4.1. Spatial information for nodes in RSNs of interest identified in FMRI data by group-ICA (N = 12).

RSN	Node location (R = right hemisphere; L = left)	MNI x, y, z co-ordinates (peak z-statistic)
SEN1	1. L middle frontal gyrus/dmPFC/vlPFC/OFC	-26, 38, 36 (15.0)
	2. R middle frontal gyrus/dmPFC/vlPFC/OFC	38, 36, 32 (12.5)
	3. Bilateral dorsal anterior cingulate cortex	0, 20, 40 (10.4)
	4. Bilateral dorsal precuneus	-4, -60, 50 (9.7)
	5. R lateral cerebellum	34, -58, -34 (8.1)
	6. L supramarginal/angular gyri	-52, -50, 38 (7.8)
	7. L lateral cerebellum	-48, -56, -38 (6.3)
	8. L inferior parietal/superior occipital cortex	-34, -82, 34 (6.1)
	9. R supramarginal/angular gyri	50, -46, 30 (5.9)
	10. L dorsal and ventral striatum	-16, 16, -2 (5.2)
	11. R dorsal and ventral striatum	16, 14, 10 (5.2)
	12. L anterior insula	-40, 12, -2 (4.8)
	13. R anterior insula	34, 16, 2 (4.6)
SEN2	1. R supramarginal gyrus/parietal operculum	60, -28, 32 (17.1)
	2. L supramarginal gyrus/parietal operculum	-62, -30, 18 (15.1)
	3. Bilateral dorsal anterior-mid cingulate	6, 6, 38 (12.4)
	4. R insula/frontal operculum/temporal pole	40, -6, -14 (9.0)
	5. L insula/frontal operculum/temporal pole	-38, 4, 2 (8.9)
	6. R middle frontal gyrus/dlPFC/vlPFC/OFC	26, 44, 20 (6.3)
	7. L temporo-occipital cortex	-54, -62, 6 (6.0)
	8. L posterior inferior cerebellum	-16, -72, -52 (5.6)
	9. R temporo-occipital cortex	56, -62, -6 (5.2)
	10. L medial OFC	-24, 34, -14 (4.2)
	11. L middle frontal gyrus/dlPFC	-30, 34, 34 (3.8)
	12. R posterior thalamus	10, -20, 4 (3.2)
SEN3	1. Bilateral superior frontal gyrus/pre-supplementary motor area/dorsal anterior cingulate	-2, 12, 56 (11.6)
	2. L inferior frontal gyrus/frontal operculum/anterior insula	-50, 18, -6 (10.0)
	3. R inferior frontal gyrus/frontal operculum/anterior insula	52, 18, -4 (9.9)
	4. L middle frontal gyrus/dlPFC	-44, 2, 50 (9.6)
	5. R middle frontal gyrus/dlPFC	50, 6, 46 (8.2)
	6. R middle temporal gyrus	54, -38, -2 (6.8)
	7. L supramarginal/angular gyri	-60, -46, 22 (6.1)
	8. L middle frontal gyrus/dmPFC	-24, 52, 20 (5.2)
	9. R supramarginal/angular gyri	62, -52, 18 (5.2)
	10. L middle temporal gyrus	-56, -32, -8 (4.9)
	11. R middle frontal gyrus/dmPFC	26, 50, 26 (4.5)
	12. R globus pallidus/anterior thalamus	14, -2, 12 (4.3)
	13. L globus pallidus/anterior thalamus	-14, 4, 6 (4.2)

DMN	1. Bilateral ventral precuneus	8, -54, 6 (15.3)
	2. R inferior parietal/superior occipital cortex	42, -76, 28 (12.8)
	3. Bilateral dorsal precuneus	2, -52, 46 (12.3)
	4. L inferior parietal/superior occipital cortex	-34, -86, 34 (12.0)
	5. R middle frontal gyrus/dmPFC	22, 26, 36 (8.5)
	6. L temporal fusiform/parahippocampal gyrus/hippocampus	-26, -46, -10 (8.2)
	7. R temporal fusiform/parahippocampal gyrus/hippocampus	28, -40, -14 (9.8)
	8. L middle frontal gyrus/dmPFC	-24, 8, 50 (7.8)
	9. R inferior frontal gyrus/dlPFC	48, 20, 26 (6.4)
	10. Bilateral vmPFC	2, 48, -12 (6.2)
	11. R anterior middle temporal gyrus	56, -4, -20 (5.6)
	12. L posterior inferior cerebellum	-10, -82, -42 (5.4)
	13. L inferior frontal gyrus/dlPFC	-40, 16, 22 (4.8)
BGLN	1. L ventral/dorsal striatum extending into amygdala/OFC	-18, 10, -12 (11.2)
	2. R ventral/dorsal striatum extending into amygdala/OFC	18, 8, -14 (10.5)
	3. R anterior-medial thalamus	10, 0, 8 (10.5)
	4. L anterior-medial thalamus, extending into midbrain	-8, -2, 6 (10.0)
	5. Bilateral rostral/ventral anterior cingulate/vmPFC	-4, 44, 0 (5.8)
Somatomotor	1. L post-central/pre-central gyri (somatomotor 'strip')	-56, -8, 22 (16.5)
	2. R post-central/pre-central gyri (somatomotor 'strip')	60, 04, 20 (16.1)
	3. Bilateral superior parietal cortex	-2, -10, 52 (12.4)
	4. L dorsal medial cerebellum	-14, -64, -22 (4.7)
	5. R inferior parietal/superior occipital cortex	46, -68, 32 (4.6)
	6. R dorsal medial cerebellum	18, -60, -20 (4.5)

OFC = orbitofrontal cortex; dmPFC = dorsomedial prefrontal cortex; dlPFC = dorsolateral prefrontal cortex; vmPFC = ventromedial prefrontal cortex; vlPFC = ventrolateral prefrontal cortex



4.3 – Results

4.3.1 – PET measures of D3 and D2 receptor availability are unrelated

Midbrain D3R availability was estimated for twelve healthy male subjects by calculating the specific binding potential (BP_{ND}) of $[^{11}C]$ -(+)-PHNO in the SN/VTA (mean = 1.59 ± 0.30 SD, range = 1.12-2.06). $[^{11}C]$ -(+)-PHNO BP_{ND} values from the putamen region of the dorsal striatum (DPU) were calculated to provide measures of D2R availability (mean = 2.47 ± 0.21 SD, range = 2.09-2.78). No correlation was found between D3R and D2R availabilities ($r = -0.03$, $p = 0.93$). There was also no correlation between either BP_{ND} measure and age (D3: $r = 0.01$, $p = 0.98$; D2: $r = 0.29$, $p = 0.36$) in the current sample. Similarly, there were no significant correlations between subject age and any of the BP_{ND} -related OFC-RSN connectivity measures identified in sections 4.3.3-4.3.4 below (D3: all p -values > 0.37 ; D2: all $p > 0.22$).

4.3.2 – Distinct effects of midbrain D3R availability on orbitofrontal connectivity with salience/executive RSNs relative to limbic networks

Brain regions in which subject-specific functional connectivity differences with separate RSNs of interest were significantly associated with midbrain D3R availability are displayed in Fig. 4.1A-E and Table 4.2 (cluster $t > 2.3$, $p < 0.05$, FWE-corrected). In support of the primary hypothesis, negative associations were found between SN/VTA $[^{11}C]$ -(+)-PHNO BP_{ND} and the connectivity of each SEN with distinct anterior and lateral OFC or ventrolateral prefrontal cortex regions (Fig. 4.1A-C). Functional connectivity negatively associated with midbrain D3 BP_{ND} was also observed between the characteristic activity of the SEN1 system and some of its other primary ‘nodes’ (see Table 4.1), including

bilateral dorsolateral and dorsomedial prefrontal cortices (Table 4.2). These results identified regions where higher midbrain D3R availability explained reduced positive connectivity with the RSN.

In further support of the primary hypothesis, a positive association was found between midbrain [^{11}C]-(+)-PHNO BP_{ND} and bilateral posterior OFC connectivity with the BGLN, indicating regions of greater positive connectivity associated with higher D3R availability (Fig. 4.1E; Table 4.2). A positive association was also found between D3R availability and connectivity of the DMN with a specific OFC region (Fig. 4.1D; reflecting reduced negative connectivity associated with higher D3R availability). This OFC cluster overlapped with the region exhibiting a negative association between functional connectivity with SEN1 and midbrain D3R availability (overlap = 438 mm^3 , Fig. 4.2).

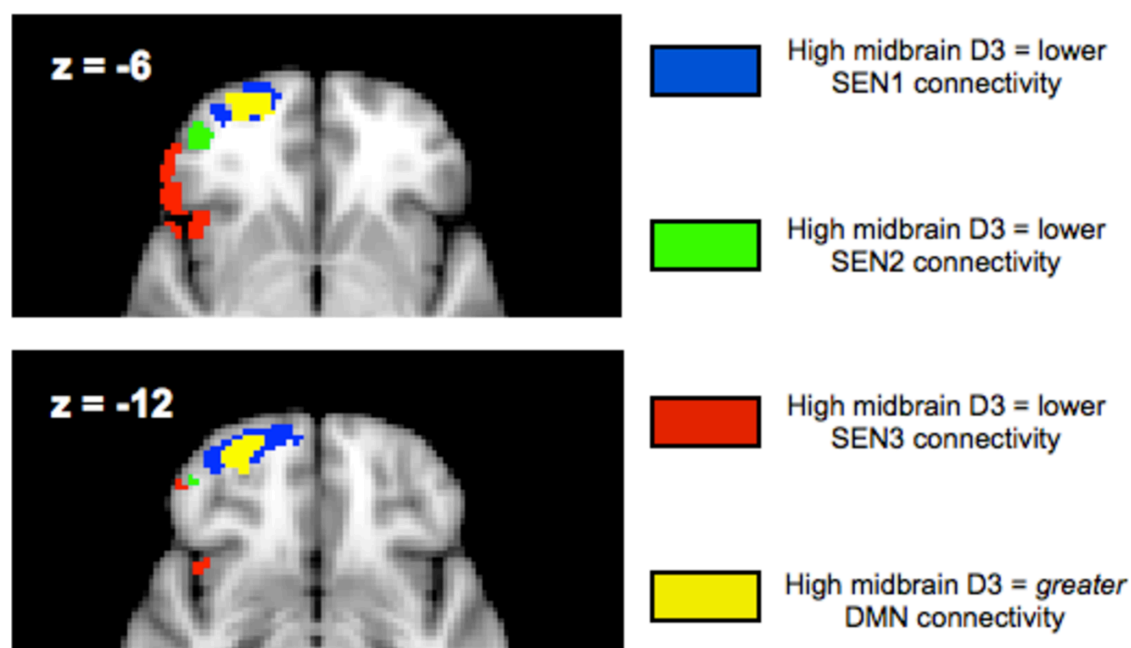


Figure 4.2. Overlap and topography of D3 effects on OFC-RSN connectivity. OFC functional connectivity with SENs and the DMN displays significant negative and positive associations, respectively, with midbrain D3R availability ($N = 12$).

Variation in somatomotor RSN ('control' network) connectivity with the OFC showed no relationship to individual differences in midbrain D3 BP_{ND} (Table 4.2). Neither was any evidence found of significant order effects on the functional connectivity of any of the RSNs studied, by testing for differences between the two resting fMRI scans acquired in each session.

Table 4.2. D3 receptor availability explains functional connectivity relationships between multiple brain regions and RSNs of interest.

RSN	Association between SN/VTA (D3) BP _{ND} and RSN-ROI functional connectivity	Cluster anatomical location	Cluster extent (2mm ³ voxels in MNI152 space)	MNI x, y, z coordinates (peak t-statistic)
SEN1	Negative	R anterior lateral OFC	462	24, 60, -8 (5.92)
		R dmPFC/dlPFC	521	20, 62, 22 (6.59)
		L dmPFC/dlPFC	786	-22, 62, 12 (6.19)
		Bilateral precuneus	695	0, -50, 46 (5.49)
		Bilateral thalamus/L caudate	464	-14, -18, 20 (6.29)
SEN2	Negative	L inferior cerebellum	470	-16, -54, -54 (6.05)
		R vlPFC/lateral OFC	346	44, 46, 6 (6.37)
		L superior parietal cortex/central sulcus	457	-34, -14, 68 (5.38)
SEN3	Negative	R dlPFC/vlPFC/lateral OFC/anterior insula/temporal pole	683	50, 34, 16 (7.05)
		R supramarginal/angular gyri	412	64, -44, 26 (5.77)
		Bilateral superior parietal/L premotor cortex	401	-44, 4, 48 (5.54)
DMN	Positive	R anterior lateral OFC	386	28, 48, -16 (5.40)
	Negative	Bilateral superior lateral occipital cortex/precuneus	1169	54, -66, 24 (6.95)
		L fusiform cortex	414	-22, -64, -14 (5.42)
BGLN	Positive	L posterior OFC/anterior insula extending into bilateral ventral striatum/caudate	731	14, 20, 4 (4.03)
		Midbrain extending into L temporal fusiform/parahippocampal gyrus	347	0, -36, -20 (5.97)
		L inferior cerebellum	323	-16, -54, -56 (5.41)
Somato-motor	Negative	L superior parietal cortex/pre-central/post-central gyri	499	-40, -2, 54 (5.16)

All results: cluster $t > 2.3$, $p < 0.05$, FWE-corrected; R = right hemisphere, L = left; OFC = orbitofrontal cortex; dmPFC = dorsomedial prefrontal cortex; dlPFC = dorsolateral prefrontal cortex; vlPFC = ventrolateral prefrontal cortex; SN/VTA = substantia nigra/ventral tegmental area

Table 4.3. Putamen D2 receptor availability explains functional connectivity relationships between select brain regions and RSNs of interest.

RSN	Association between putamen (D2) BP _{ND} and RSN-ROI functional connectivity	Cluster anatomical location	Cluster extent (2mm ³ voxels in MNI152 space)	MNI x, y, z co-ordinates (peak t-statistic)
SEN1	Positive	L superior parietal/supramarginal/central opercular cortex	706	-38, -18, 22 (4.27)
SEN2	Negative	R dlPFC/vlPFC	373	46, 46, 16 (4.91)
DMN	Negative	R dlPFC/vlPFC/lateral OFC	526	50, 40, -10 (5.14)
		R superior lateral occipital cortex/angular gyrus	527	48, -66, 42 (4.76)
		Bilateral lingual gyrus/ventral precuneus	477	-12, -58, 4 (5.91)
BGLN	Negative	R anterior lateral/bilateral medial OFC	479	4, 56, -12 (5.28)

All results: cluster $t > 2.3$, $p < 0.05$, FWE-corrected; R = right hemisphere, L = left; OFC = orbitofrontal cortex; dlPFC = dorsolateral prefrontal cortex; vlPFC = ventrolateral prefrontal cortex

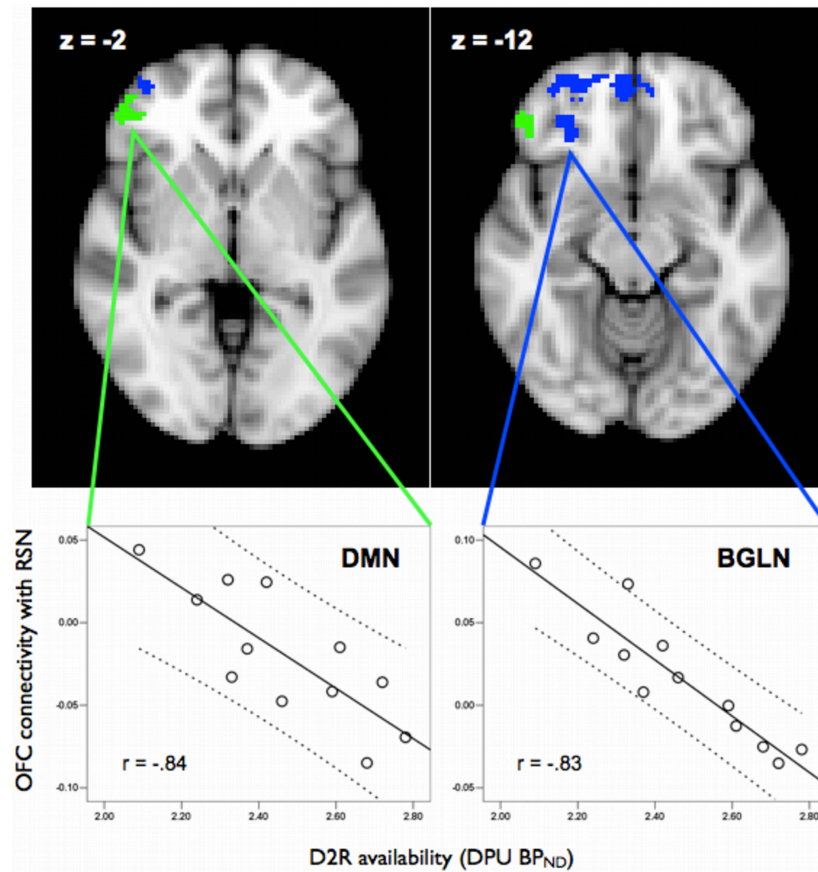


Figure 4.3. OFC-RSN functional connectivity patterns associated with putamen D2R availability are sparse relative to D3-related effects and opposite in direction. In contrast to relationships between midbrain D3R availability and OFC connectivity with all frontoparietal and limbic RSNs, individual differences in DPU D2R availability ($N = 12$) only significantly explain OFC functional connectivity with the DMN (green) and BGLN (blue), but with no SEN systems (cluster $t > 2.3$, $p < 0.05$, FWE-corrected). These negative relationships are in the opposite direction to those associated with D3R availability found between OFC regions and these two RSNs (see Fig. 4.1; plots as described therein).

4.3.4 – Effects of putamen D2R availability on RSN connectivity differ from those of midbrain D3R availability

Brain regions in which subject-specific RSN functional connectivity differences were associated with DPU [^{11}C]-(+)-PHNO BP_{ND} variation (reflecting D2R availability) were much less abundant than those associated with variation in D3R availability and also showed different anatomical patterns (Table 4.3). No D2-related connectivity associations were found between SENs and clusters in distinct or overlapping OFC sub-regions. However, two RSNs (the BGLN and the

DMN) showed effects of D2R availability on connectivity with clusters located in (or extending to) OFC (Fig. 4.3), but these had opposite relationships (negative) to the (positive) associations found between D3R availability and OFC functional connectivity (see Fig. 4.1D-E).

4.3.5 – Midbrain D3R availability associated with inverse connectivity between OFC and RSNs

Anatomically overlapping, but inverse, associations between antero-lateral OFC functional connectivity with the DMN relative to SEN1, which were explained by midbrain [¹¹C]-(+)-PHNO BP_{ND} (Fig. 4.2), were identified by separate analyses of *single*, functionally distinct RSNs. To confirm that these results were indicative of D3-dependent differences in connectivity between the OFC region and one RSN relative to another, a further whole-brain multiple regression analysis was conducted, this time incorporating the subject-specific connectivity measures from *both* RSNs (see section 4.2.8). Inverse functional connectivity with the DMN relative to SEN1 in regions including right antero-lateral OFC was found to be associated with variation in midbrain D3R availability across subjects (Fig. 4.4). High D3R availability was associated with greater (less negative) right antero-lateral OFC connectivity with the DMN and lower positive functional connectivity with SEN1 (peak $t = 6.81$; MNI co-ordinates: $x = 24, y = 60, z = -8$). This contrast also revealed inverse RSN connectivity effects dependent on midbrain D3R availability (Fig. 4.4) in the left dorsomedial prefrontal cortex ($t = 6.67$; $-22, 62, 12$), right inferior parietal cortex ($t = 6.03$; $42, -58, 52$) and the precuneus ($t = 5.33$; $2, -46, 40$), as well as the cerebellum ($t = 6.99$; $-14, -54, -52$). Therefore, higher midbrain D3R availability across individuals was associated with a

significant reduction in negative functional connectivity between the DMN and SEN1, as measured within a number of their key cortical regions (see Fig. 4.1). Based on the combined and single-network analyses, the OFC in particular appears to influence interactions of large-scale cognitive RSNs to a greater degree in individuals with *low* midbrain D3R availability.

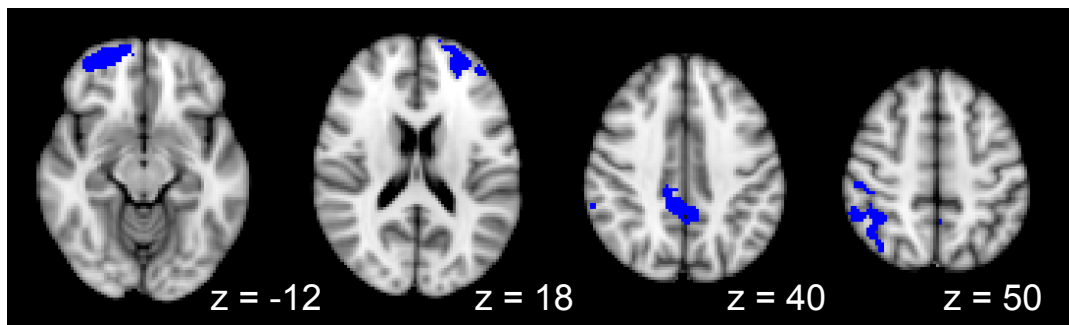


Figure 4.4. High midbrain D3 receptor availability explains reduced inverse functional connectivity with the DMN relative to SEN1, in right antero-lateral OFC and key regions of cognitive RSN overlap. Higher midbrain D3 receptor availability is associated, across individuals ($N = 12$), with a significant reduction of the inverse functional connectivity patterns with the DMN relative to the SEN1 system displayed by (reading left to right) (i) the right antero-lateral OFC, (ii) left dorsomedial prefrontal cortex, (iii) bilateral precuneus and (iv) right inferior parietal cortex (cluster $t > 2.3$, $p < 0.05$, FWE-corrected).

4.4 – Discussion

4.4.1 – Summary

This study has shown, in healthy human subjects, that individual differences in midbrain dopamine D3 receptor availability relate to distinct variations in functional connectivity between the orbitofrontal cortex and multiple, large-scale brain networks (RSNs). High midbrain D3R availability was associated with reduced connectivity between the OFC and frontoparietal RSNs involving brain regions implicated in executive control and salience processing (Seeley et al., 2007). Conversely, OFC connectivity with the basal ganglia and with the DMN was greater in individuals with high D3R availability. The results further

emphasised that sub-regions of the antero-lateral OFC could be distinguished on the basis of their relative (D3-dependent) connectivity with one or more RSNs. Previous work has indicated that dopaminergic mechanisms may cause frontal cortical and basal ganglia activity patterns to dissociate during aberrant reward processing (Baler and Volkow, 2006; Cools, 2006), while the DMN is often 'down-regulated' during cognitive processes associated with increased salience or executive network activity (Greicius et al., 2003; Fox et al., 2005). The current results therefore provide novel evidence of connectivity dissociations in humans, in line with these findings, relating the functional modulation of neocortical pathways relevant to cognition, salience and reward processing specifically to D3R-mediated dopaminergic signalling. In this way, these functional effects are further found to have substantial relationships with meaningful, quantitative measures of fundamental molecular neurobiology (see also Footnote [10], section 4.2.2).

4.4.2 – Molecular control of systems-level functioning

The results presented here relate molecular signalling through a specific dopamine neuroreceptor system to activity modulations in brain resting-state networks. Together with recent studies of pharmacological or genetic effects (see e.g., Achard and Bullmore, 2007; Cole et al., 2010b; Liu et al., 2010; Gordon et al., 2012; Kunisato et al., 2011; Wiggins et al., 2012), these findings provide important insights into the molecular bases of large-scale functional connectivity patterns. Parallels between resting-state BOLD fMRI activity fluctuations and those evoked by specific cognitive tasks are becoming increasingly apparent (Fox and Raichle, 2007; Buckner et al., 2008; Smith et al., 2009; Cole et al., 2010a;

Northoff et al., 2010a; Shirer et al., 2012), reinforcing the notion that these phenomena reflect core aspects of neural processing relevant for cognitive and behavioural functioning. Furthermore, the same large-scale networks show altered activity in certain neuropsychiatric disease states with partially- or clearly-established molecular underpinnings (e.g., Greicius et al., 2004; Seeley et al., 2009; Cole et al., 2010b; Koob and Volkow, 2010).

The results demonstrate that functional interactions between OFC and networks implicated in cognitive control and reward are associated with midbrain D3R signalling. Pre-clinical, clinical and post-mortem evidence implicates dysfunction of both the D3R system and salience/executive RSNs in a number of neuropsychiatric disorders (Staley and Mash, 1996; Gurevich et al., 1997; Allman et al., 2005; Sokoloff et al., 2006; Seeley et al., 2009; Kim et al., 2012) and in aspects of emotional and social behaviours in healthy subjects (Seeley et al., 2007; Allman et al., 2010). Primary 'nodes' of SEN systems in fronto-insular and anterior cingulate cortices contain large quantities of long-range, bipolar projection neurons found only in humans and a minority of other mammals with highly evolved neocortices (Nimchinsky et al., 1999; Allman et al., 2010). Moreover, regions rich in these cells exhibit strong anatomical connections with anterior orbitofrontal cortices (Allman et al., 2010) and immunocytochemical evidence shows these neurons to be labelled abundantly with D3R antibodies (Allman et al., 2005). Together with the current findings, the collective evidence therefore is suggestive of an association between D3R function, specialised frontal lobe cytoarchitecture and large-scale network connectivity patterns underlying complex behaviour in humans.

An earlier study reported a positive association between D2/D3 receptor availability in the striatum and metabolism in OFC, measured using [^{11}C]raclopride PET and [^{18}F]fluorodeoxyglucose PET, respectively (Volkow et al., 2001). However, radioligands such as [^{11}C]raclopride do not distinguish between D2 and D3 receptor sub-types (Graff-Guerrero et al., 2008). The current study was able to make use of the relative selectivity of [^{11}C]-(+)-PHNO binding in midbrain SN/VTA to delineate specific measures of D3R availability (Rabiner et al., 2009; Searle et al., 2010). Combining this information with fMRI data in the same subjects enabled further characterisation of the influence of D3R availability on large-scale networks (RSNs), in terms of their relative functional connectivity with anatomically distinct OFC sub-regions. Future work needs to characterise clinical and behavioural changes associated with this systems-level connectivity modulation.

4.4.3 – Functional implications of OFC-RSN connectivity under molecular control

OFC regions are thought to be crucial for regulating impulsive and adaptive behaviours related to reward sensitivity (Bechara et al., 2000; Izquierdo et al., 2004; Kringelbach and Rolls, 2004) and goal-directed decision-making (Frank and Claus, 2006; Noonan et al., 2010; Walton et al., 2010). A previous meta-analytic review of functional imaging studies relates activity in anterior and lateral OFC regions analogous to those described in the current study to “impending changes in behaviour” in response, for example, to ‘punisher’ stimuli signalling negative emotional, social, or reward-related connotations for maintaining a behavioural strategy (Kringelbach and Rolls, 2004). Similar types of adaptive behaviour across multiple facets of complex cognition and emotional

processing are found to form functional correlates of activity in SENs (Seeley et al., 2007). The current results expand upon these interpretations by showing that antero-lateral OFC integration in reward and cognitive control systems is explained to some extent by midbrain D3R signalling.

These findings additionally highlight, in the living human brain, functional distinctions between the influences of dopaminergic signalling through D3 relative to D2 receptor sub-types, in terms of how individual differences in these neurobiological quantities impact upon BOLD signal dynamics of large-scale networks during undirected rest. There is currently much debate on precisely how post-synaptic dopamine inhibition relates to D2/D3 function in midbrain relative to striatal regions (Levant, 1997; Volkow et al., 2001; Dalley et al., 2007; Buckholtz et al., 2010; Heidbreder and Newman, 2010; Koob and Volkow, 2010; Sesack and Grace, 2010). High midbrain D3R availability may reflect increased autoreceptor inhibition of dopamine release in frontal and striatal projection areas (Levant, 1997; Buckholtz et al., 2010; Heidbreder and Newman, 2010; Koob and Volkow, 2010; Sesack and Grace, 2010), which the current data indicate may result in: (i) reduced antero-lateral OFC modulation of interactions within and between inversely-related frontoparietal cognitive RSNs, while (ii) increasing the posterior OFC modulation of striatal (BGLN) reward pathways (also associated here with *low* DPU D2R availability). Such neurochemically mediated connectivity modulations could feasibly lead to increased compulsive or reward-seeking behaviours (e.g., Volkow et al., 2001; Boileau et al., 2012). Example evidence of such a relationship between these behaviours and the defined brain processes comes from associations between *increased* drug cue responsiveness and *reduced* cognitive control in addictive disorders (see e.g.,

Baler and Volkow, 2006). Further support for this interpretation stems from recent evidence in addiction and obsessive compulsive disorder showing disorder-dependent alterations in resting-state functional connectivity between OFC and equivalent networks to those studied here (Cole et al., 2010b; Wilcox et al., 2011; Meunier et al., 2012).

Other PET studies in humans with non-selective D2/D3 ligands support relationships between reward-related personality traits and dopamine receptor availability (Zald et al., 2008; Lee et al., 2009; Buckholtz et al., 2010; Gjedde et al., 2010). Although there are no prior published data in humans specifically linking D3R with behavioural risk factors for addiction, such as impulsivity or reward sensitivity (Zald et al., 2008), non-human studies with selective antagonists show that D3-blocking specifically abolishes motivated reward-seeking behaviours (Heidbreder and Newman, 2010). Conversely, one rodent study used preferential and selective antagonists for D2R and D3R sub-types, respectively, to demonstrate specific *D2R*-mediated increases in impulsive behaviour following amphetamine challenge (van Gaalen et al., 2009). The current observation that OFC functional connectivity with both the DMN and BGLN is *positively* associated with midbrain D3R but *negatively* associated with DPU D2R availability extends this evidence of functional dissociations between dopamine neuroreceptor sub-types in humans. Moreover, these results identify the OFC as a potentially key region for modulating interactions between SENs implicated in cognition and motivated behaviour, in conjunction with D3R (but not D2R) availability. Such dissociations may relate to existing theories of the ‘tonic-phasic’ hypothesis of dopaminergic influence on cognitive control (e.g., Cools, 2006; Sokoloff et al., 2006; Sesack and Grace, 2010). For example, D3

autoreceptors in the midbrain are found to control phasic, but not tonic, dopaminergic activity (Sokoloff et al., 2006), whereas such distinctions are less clear for distributed D2Rs. Future investigations may seek to link these effects more directly to specific behavioural factors related to addiction or affective disorders, such as salience-dependent motivation, anxiety or reward-sensitivity.

4.4.4 – Methodological considerations

A number of methodological considerations warrant discussion. Firstly, [^{11}C]-(+)-PHNO binding potential has been shown to be sensitive to extracellular dopamine in the living human brain (e.g., Willeit et al., 2008; Shotbolt et al., 2012). Thus, extracellular dopamine levels may be a factor in the relationship between midbrain [^{11}C]-(+)-PHNO binding potentials and OFC-RSN functional connectivity. Additional research is therefore necessary to determine the precise contributions of midbrain D3R *density* and extracellular dopamine levels to these effects.

Secondly, this study employed a modest sample size, thus results should be generalised judiciously. For example, an association between the availability of different dopamine receptor sub-types (D2 and D3) was entirely absent in the current study, but could in theory emerge through testing in a different population. Nevertheless, the primary concern with small sample sizes is that they may suffer from an increased incidence of false negative results ('Type II' error), *not* false positive results ('Type I' error). Indeed, here highly robust associations were found between PET measurements of D3R (and D2R) availability and fMRI measurements of functional connectivity across a number of networks involving the OFC, demonstrating that the current sample is

sufficient to detect significant effects in accordance with the *a priori* hypotheses. Moreover, although Type I error is of minimal risk in some studies with modest sample sizes, its risk was controlled for within the multiple regression framework of the current analysis, through a conservative combination of nonparametric permutation testing, cluster-mass thresholding and FWE-correction across the whole brain for multiple comparisons.

4.4.5 – Conclusions

In summary, using a novel approach combining [^{11}C]-(+)-PHNO PET and resting-state fMRI, it has been shown directly in healthy humans that variation in midbrain dopamine D3 receptor availability is associated with individual differences in functional connectivity between OFC regions and multiple neural networks underlying cognitive control, goal-directed behaviour and reward. To date, dopaminergic pharmacotherapies have not proven clinically effective in treating most addictions. This work contributes to the validation of D3R as a target for novel treatments for addiction or other motivational disorders. Evidence has already indirectly associated dysfunction of both D3R and frontal cortical networks with a number of neuropsychiatric disorders (Staley and Mash, 1996; Gurevich et al., 1997; Allman et al., 2005; Sokoloff et al., 2006; Seeley et al., 2009; Kim et al., 2012). Future studies can extend this to test directly in these disorders for dysfunctional connectivity of the regions of OFC defined here.

This study provides valuable evidence for the molecular basis of large-scale network functional connectivity patterns. A role is highlighted for the dopamine system specifically, although this does not rule out a likely role for other neurochemical systems or molecular pathways in modulating such

phenomena (see e.g., Cole et al., 2010b; Liu et al., 2010; Kunisato et al., 2011; Hahn et al., 2012; Wiggins et al., 2012). In any case, it will be important and reassuring to be able to relate such findings to individual differences in behavioural or personality measures relevant for cognition, reward, motivational processing and neuropsychiatric disorders.

Finally, it will be of chief importance – particularly with respect to contexts of medicines development and clinical nosological applications – to confirm that RSN connectivity patterns under specific neurochemical control can be modulated pharmacologically, in a biologically plausible manner in line with existing pharmacological literature. In Chapter 5 some of these factors are addressed in a study reporting the effects of broad-spectrum dopamine neuromodulation on network functional connectivity, as well as interactions with individual differences in self-report measures of impulsivity.

Chapter 5 – Towards stratifying drug modulations via large-scale network connectivity differences

Differential dopamine drug effects on RSN connectivity

Dopaminergic medications, used to treat neurochemical pathology and associated symptoms in multiple neuropsychiatric disorders, are often of mixed efficacy and are regularly associated with unwelcome side effects. This outcome variability, which may be attributable to the existence of both linear and ‘inverted U-shaped’ effects of dopamine modulation on cognitive functions, clouds the majority of attempts to characterise such drug effects using functional neuroimaging. Due to the proposed ability to map biologically plausible effects on systems-level signalling throughout the brain, RSN functional connectivity measures may provide a sensitive alternative. In this placebo-controlled, parallel-design study in healthy volunteers, broad-spectrum (agonistic and antagonistic) pharmacological manipulations were used to assess the effects of dopamine neuromodulation on large-scale cortico-subcortical and cortico-cortical RSN functional connectivity relationships. Both linear and nonlinear neuromodulatory effects were identified on distinct large-scale functional connectivity patterns of RSNs known to be involved in aspects of cognition and reward processing. Further, drug-specific associations were found between RSN reactivity to dopamine modulation and individual differences in trait impulsivity. Findings reveal differential RSN sensitivity to biologically plausible drug effects.

5.1 – Introduction

Dopaminergic regulation of neural processing is seemingly critical for core functions of cognition, motivated behaviour and reward response, as established by decades of animal research¹¹ (e.g., Brozoski et al., 1979; Nieoullon, 2002; Schultz, 2002; Wise, 2004). Dopamine neurotransmission is also linked with reward-seeking and impulsive behaviours in humans (Pessiglione et al., 2006; Buckholtz et al., 2010). Specific neuroreceptor proteins regulating dopaminergic signalling, particularly D2-like receptors (a category that includes D3R; see section 4.1), are thought to mediate individual differences in sensitivity to neurochemical or pharmacological manipulation and, accordingly, the probability of developing and sustaining symptoms of pathological reward, behavioural inhibition or salience processing in disorders such as addiction, Parkinson's disease and schizophrenia (Schafer et al., 2001; Dalley et al., 2007; Dagher and Robbins, 2009; Buckholtz et al., 2010).

There is, therefore, wide appreciation of the potential for interventions modulating dopamine neurotransmission to treat cognitive symptoms across a range of neuropsychiatric disorders (Goldberg et al., 1993; Robbins, 2000; Volkow et al., 2004; Cools, 2006), or even in experimental enhancement of 'normal' cognitive processes (Robbins, 2000; Volkow et al., 2009; Cools and D'Esposito, 2011). However, the efficacy of dopamine-targeting therapies has proven extremely variable, depending largely on the disease or cognitive process in question (see e.g., Crow, 1980; Davis et al., 1991; Laruelle et al., 2003; Cools,

¹¹Portions of this chapter are reproduced from two related publications: Cole DM, Oei NY, Soeter RP, Both S, van Gerven JM, Rombouts SA, Beckmann CF (2012) Dopamine-dependent architecture of cortico-subcortical network connectivity. *Cereb Cortex* [Epub ahead of print] doi:10.1093/cercor/bhs136; Cole DM, Beckmann CF, Oei NY, Both S, van Gerven JM, Rombouts SA (under review) Differential and distributed effects of dopamine neuromodulations on resting-state network connectivity.

2006; Dagher and Robbins, 2009; Heidbreder and Newman, 2010; Martinez et al., 2011). In particular, the use of psychopharmacological medications to ‘correct’ hypo- or hyper-dopaminergic states in associated neuropsychiatric disorders (e.g., schizophrenia and Parkinson’s, respectively) is, itself, thought to be (at least partly) responsible for adverse sensory-motor and cognitive side effects or comorbid presentations (e.g., Goldberg et al., 1993; Cools, 2006; Dagher and Robbins, 2009; Martinez et al., 2011).

Importantly, recent insights into understanding how brain dopamine regulates core psychological functions, particularly those related to cognitive control and attention, emphasise a key role for differences in baseline levels of the molecule in determining variable characteristics of psychological processing, both across populations and within individual subjects. In particular, it is becoming increasingly clear that simple ‘linear’ relationships – those positing (e.g.) that increasing dopamine neurotransmission improves functionality while attenuating it is detrimental – although extant (e.g., Pessiglione et al., 2006; Diaconescu et al., 2010), do not describe fully the complex associations between levels of dopamine and cognitive qualities (Cools and D’Esposito, 2011). It is an increasingly common observation that both hypo- and hyper-dopaminergic states can (in certain instances) have deleterious effects on performance in tasks of cognition and attention, indicative of an ‘inverted U-shaped’ (i.e., nonlinear) association between dopamine neuromodulation and cognitive processing (Cools and D’Esposito, 2011). This could imply the existence of an ‘optimum’ dopamine level required to balance the interplay between competing psychological processes and thus promote function. Moreover, this may provide an explanation for instances where pharmacological intervention to treat one

psychological faculty (e.g., elevating dopaminergic activity to minimise the motor symptoms of Parkinson's disease) proves detrimental to another (i.e., produces an adverse side effect, for example a behavioural addiction, that would not occur if dopamine neurotransmission had remained at the previous lower levels). However, somewhat paradoxically this supposedly optimum level appears to vary, not just across different individuals and dopamine-dependent behaviours, but also across the brain regions involved (Cools and D'Esposito, 2011). The apparent unpredictability of this ability of dopamine to, for example, improve one faculty while impairing another has significant ramifications for the pharmacological management of multiple neuropsychiatric disorders. Indeed, it is likely that dopamine's intimate and distributed entanglement with a range of cognitive faculties has directly contributed to the fact that, in the living human brain, therapeutic psychopharmacological effects of dopamine neuromodulation remain incompletely characterised (Cools and D'Esposito, 2011).

In Chapter 4, evidence was provided that differential configurations in molecular dopaminergic systems contribute to variability, across individuals, in large-scale functional patterns of network-level connectivity measurable by fMRI. Indeed, dopaminergic influences in the brain are shown, here and previously, to be investigable through fMRI measures of functional connectivity within large-scale networks (Honey et al., 2003; Cole et al., 2012a). It has been proposed further that the complex cognitive processes and personality factors relevant for reward-related behaviour and impulsivity are mediated by analogous large-scale neuronal systems, communicating via cortico-subcortical and cortico-cortical pathways (Bressler, 1995; Everitt and Robbins, 2005; Koob and Volkow, 2010). However, broad-spectrum dopaminergic manipulations of

connections within multiple large-scale networks and, moreover, interactions with individual difference measures relevant for psychopharmacological modulation of pathological processing have not yet been investigated.

This chapter describes a novel study combining agonistic (levodopa; L-dopa) and antagonistic (haloperidol) pharmacological manipulations in healthy subjects with multivariate and mass univariate fMRI data analysis techniques (see Chapter 2) sensitive to RSN functional connectivity. This pharmacological fMRI study focussed specifically on RSNs relevant for cognitive control, impulsivity and reward processing (Seeley et al., 2007; Smith et al., 2009; Cole et al., 2010b; Koob and Volkow, 2010; Shannon et al., 2011), which have also been implicated as dysfunctional in neuropsychiatric disorders regularly treated with dopamine-targeting medications (e.g., Castellanos et al., 2008; Kelly et al., 2009; Wolf et al., 2011). The primary aim was to establish differential roles for the dopamine neurotransmitter system in modulating resting-state functional interactions within distinct large-scale cortico-subcortical and cortico-cortical pathways, as well as to further characterise these modulatory effects as either linear or nonlinear. Currently, only limited and ill-reconcilable indications for nonlinear effects of dopamine neuromodulation on functional connectivity have been provided (Cohen et al., 2007; Wallace et al., 2011). A secondary aim was to identify potential ‘drug-personality’ interactions, by exploring associations between individual differences in dopamine-dependent RSN connectivity patterns and trait impulsivity.

Inverted U-shaped associations between dopamine and cognition are most commonly reported during prescribed tasks engaging higher-level cognitive processes (Cools and D'Esposito, 2011). However, accumulating

evidence indicates that the influence of the dopamine system on neuronal signalling at the level of several large-scale cognitive control networks can also be probed outside of specific tasks with resting fMRI (Achard and Bullmore, 2007; Kelly et al., 2009; Gordon et al., 2012; see also Chapter 4). Indeed (and as discussed briefly in Chapter 3 and in more detail in Chapter 7), a growing body of literature emphasises fundamental, predictive associations between activity or connectivity patterns evoked during cognitive tasks and those spontaneously emerging during the resting state (e.g., Fox et al., 2007; Pyka et al., 2009; Smith et al., 2009; Sala-Lluch et al., 2012). Furthermore, the translational value of resting-state measurements for addressing clinically relevant questions of diagnostics and prognostics is becoming increasingly apparent (also see, e.g., Greicius et al., 2004; Filippini et al., 2009; Cole et al., 2010b; Fox and Greicius, 2010; Varoquaux et al., 2010; Murphy and Mackay, 2011). Consequently, it was hypothesised that, in the healthy human brain, differential (linear and nonlinear) effects of pharmacological modulations on the dopamine system would be detectable in distinct RSN connectivity patterns and, further, that a minority of these interactive relationships (primarily involving subcortical pathways) would be associated selectively with individual differences in impulsive personality.

5.2 – Materials and methods

5.2.1 – Participants and study design

55 healthy male volunteers naïve to the experimental drugs were recruited and assigned randomly to three groups¹² (L-dopa, haloperidol or placebo). Data are

¹²Non-analytical study procedures described in sections 5.2.1-3 were conducted by a team of researchers at the Department of Radiology, Leiden University Medical Center, Leiden, The Netherlands.

reported from 49 participants who completed the study in full (mean age = 22.4 years $\pm$ 4.1 SD; see Table 5.1). Eligibility criteria were: no current (or history of) psychiatric problems as determined by the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998); no medical history indicating a risk in using L-dopa or haloperidol (e.g., cardiac illness, depressive disorders, thyroid disorders, glaucoma); no current or recent use (less than 12 weeks before participation) of psychopharmacological medication and other medications or psychotropic drugs that might interfere with the central nervous system action of L-dopa or haloperidol (e.g., cannabis or cocaine).

Drug administration was double-blind and followed a previously published, 'placebo-counterbalanced' protocol (Pessiglione et al., 2006), ensuring resting-state fMRI data were acquired at projected peak plasma concentrations for both drugs. Briefly, participants received either a fixed dose of 3 mg haloperidol (Haldol®; N = 18) four hours prior to scanning ($T_{\max}$ = 3-6 hrs, half-time = 14-36 hrs) followed by a placebo one hour prior, a placebo four hours prior to scanning followed by 100 mg levodopa combined with 25 mg of carbidopa (Sinemet®; N = 16) one hour prior ($T_{\max}$ = 45 min, half-time = 1-2 hrs), or a placebo at both time points (N = 15). Pharmaceuticals and 'clinical' doses were selected based on their regular use in treating symptoms of neuropsychiatric disorders such as schizophrenia and Parkinson's disease. All tablets were over-encapsulated to ensure that participants and experimenters were blind to the dosages and could not compare or identify the drugs. The study was approved by the Medical Ethics Committee of the Leiden University Medical Center and carried out in accordance with the standards of the Declaration of Helsinki. Each participant gave signed, informed consent in which

confidentiality, anonymity, and the opportunity to withdraw without penalty were assured.

5.2.2 – Questionnaires

Participants completed questionnaires immediately after ingestion of the first pill. To assess individual differences in impulsivity, the Barratt Impulsiveness Scale (BIS-11; Patton et al., 1995) was administered to subjects (see Table 5.1; data absent for a single subject in the L-dopa group).

Table 5.1. Descriptive statistics of subject variables for each drug group and associated one-way ANOVA results.

	Haloperidol (N = 18)	Placebo (N = 15)	L-Dopa (N = 16; 15 for BIS-11)	F (p)
Age (mean ± SD)	22.25 ± 3.53	21.47 ± 3.05	23.38 ± 5.30	0.86 (0.43)
BIS-11 total (mean ± SD)	66.06 ± 6.46	63.53 ± 9.01	66.67 ± 11.58	0.51 (0.61)

5.2.3 – Image acquisition

Imaging was carried out on a 3-Tesla Achieva MRI scanner (Philips, Best, The Netherlands) using an 8-channel sensitivity encoding head coil. A T1-weighted structural volume was acquired for registration purposes. For the resting-state fMRI scan, 220 whole-brain volumes of T2*-weighted gradient EPI sensitive to BOLD contrast were obtained in the axial direction (TR = 2.2 s, TE = 30 ms, flip angle = 80°, isotropic voxels of 2.75 mm, slice gap = 0.25 mm, 38 slices). Participants were instructed to remain awake with their eyes closed throughout.

5.2.4 – Image preprocessing

Resting-state fMRI data were preprocessed and initially analysed in individual subject/session-level EPI space. As described in previous chapters, image preprocessing was performed with FSL (Version 4.1.8; Smith et al., 2004). The first four volumes were removed from each fMRI dataset to allow for magnetic equilibration, resulting in a 216-datapoint BOLD time series at each voxel per session.

Seed-based ‘partial’ correlation analysis (SBCA; as introduced in section 2.2.3; see O'Reilly et al., 2010) was carried out for each subject within an anatomically-derived subcortical seed mask incorporating regions with established dopamine-dependent functionality or connectivity (Honey et al., 2003; Kelly et al., 2009; Buckholz et al., 2010; Koob and Volkow, 2010). Every voxel within each ‘individualised’ mask was quantitatively tested in terms of its connectivity with each of a number of RSN ‘target’ maps, which collectively covered the majority of neo-cortex.

To construct subject-specific subcortical seed masks, T1 structural images were segmented using FSL FIRST (Patenaude et al., 2011). Bilateral regions included in these masks were the entire striatum (comprising regions of caudate, putamen and ventral striatum), globus pallidus, amygdala, hippocampus and thalamus (and midbrain, see below). The unthresholded versions of these segmented structures (i.e., without boundary correction) were combined into a single, ‘liberal’ mask image for each subject. Absolute/fine-detailed anatomical accuracy at the single subject level was not required, as ultimately information from all subject masks was combined to form the space for higher-level group analyses. To include midbrain voxels within these masks, nonlinear warp

transformation (as implemented in FSL FNIRT; Andersson et al., 2007) of six binary, bilateral volumes from the Talairach Daemon atlas (Lancaster et al., 2000; labels = midbrain, substantia nigra, subthalamic nucleus, red nucleus, mammillary body and medial geniculus body) to the high-resolution space of each subject was carried out. This midbrain information was then added to the mask containing subjects' other subcortical regions. These subject-specific combined masks were then affine transformed to EPI space using FSL FLIRT (Jenkinson et al., 2002) and used in subsequent subject-wise SBCA.

Twenty binary RSN spatial maps (seven of interest and 13 'nuisance', see section 5.2.6) from a group probabilistic ICA of the subjects given placebo were used to construct RSN masks for use as target neural functional connectivity networks in SBCA. Placebo data only (216 volumes per scan $\times$ 15 subjects = 3240 image volumes) were entered into multi-session ICA with temporal concatenation (as implemented in FSL MELODIC), to avoid biasing this spatial target selection towards the larger haloperidol group (e.g., Langers, 2010). Automatic dimensionality estimation using the Laplace approximation identified 43 independent components in total in the placebo group FMRI data. Twenty of these were selected for further analyses based on their neuroanatomical configurations and neurophysiological interpretability as RSNs, in comparison with previous literature (Beckmann et al., 2005; Kiviniemi et al., 2009; Smith et al., 2009; Cole et al., 2010b). The remaining 23 components were deemed artefacts of motion, non-neuronal physiology or magnetic susceptibility (see e.g., Kiviniemi et al., 2009) and thus not included in further analyses. Networks identified by group-ICA and entered into further analyses have been described previously by multiple groups (and in this thesis) and comprise core systems

and sub-systems implicated in multiple sensory, motor and cognitive functions (Biswal et al., 1995; Vincent et al., 2006, 2008; Seeley et al., 2007; Smith et al., 2009; Andrews-Hanna et al., 2010; Cole et al., 2010b). All 20 template RSN maps were thresholded (at $z > 3$), then binarised and affine transformed from MNI space to the functional space of each EPI session using the FSL FLIRT tool; first to the space of the associated high-resolution T1 structural scan, then to functional EPI space. While in high-resolution space (prior to transformation to EPI space), voxels with $< 20\%$ probability of containing grey matter in the equivalent T1 structural (as calculated using FSL FAST) were removed from all 'subject-specific' RSN spatial maps.

5.2.5 – Neocortical RSN connectivity with subcortical regions (i): SBCA

In SBCA the subcortical seed masks from each session were individually examined for their voxel-wise spatial distributions of functional connectivity strength with the characteristic activity of each of the 20 RSNs (seven subjected to higher-level analysis, 13 nuisance). All 20 non-artefactual components were included in this first-level analysis to ensure that potential extraneous interactions, or temporally overlapping relationships, between any of the seven RSNs of interest (see section 5.2.6; Fig. 5.1A-G) and any of the 13 nuisance RSNs (e.g., visual, auditory or somatomotor networks) could be factored out of the analysis, in effect treating the latter as confound regressors. Voxel-wise connectivity strengths were quantified by calculating partial correlation coefficients between the BOLD signal time series at each mask voxel and that of the weighted principal eigenvariate associated with each RSN (the latter calculated via subject-wise singular value decomposition). Voxel-wise

coefficients are termed ‘partial’ because the analysis associated with a given target RSN controlled, in turn, for the seed voxel’s activity relationship with each of the other 19 RSNs examined as targets in separate correlation analyses¹³. These analyses also controlled for the confounding influences of structured noise from white matter and CSF tissue types and residual motion artefact. To this end, binary T1-segmented maps of white matter and CSF (calculated using FSL FAST) were transformed to EPI space using FLIRT and, for each session, used as masks against the associated, preprocessed functional datasets, enabling the extraction of confound time series that were calculated as the mean BOLD signal within these tissue masks¹⁴. In addition to white matter and CSF confounds, six time series resulting from the motion correction procedure describing individual subject motion parameters were also regressed out of the SBCA.

¹³For a detailed evaluation of the use of partial, relative to full, correlation calculations in network modelling based on resting-state functional connectivity data, see Smith SM, Miller KL, Salimi-Khorshidi G, Webster M, Beckmann CF, Nichols TE, Ramsey JD, Woolrich MW (2011) Network modelling methods for FMRI. *Neuroimage* 54:875-891.

¹⁴Higher-level analyses reported in subsequent sections of this chapter were repeated with the additional inclusion of voxel-wise grey matter density covariates for each subject, which served to factor out potential additional effects of structural variability unaccounted for by the above-described tissue-specific preprocessing procedures on BOLD signal-dependent functional connectivity relationships. However, the results of these separate analyses were almost identical to those reported in section 5.3, thus they are not reported in detail. This lack of difference implies that the dopamine-dependent network functional connectivity relationships examined in this study are relatively independent from structural/anatomical variability, at least in terms of grey matter density.

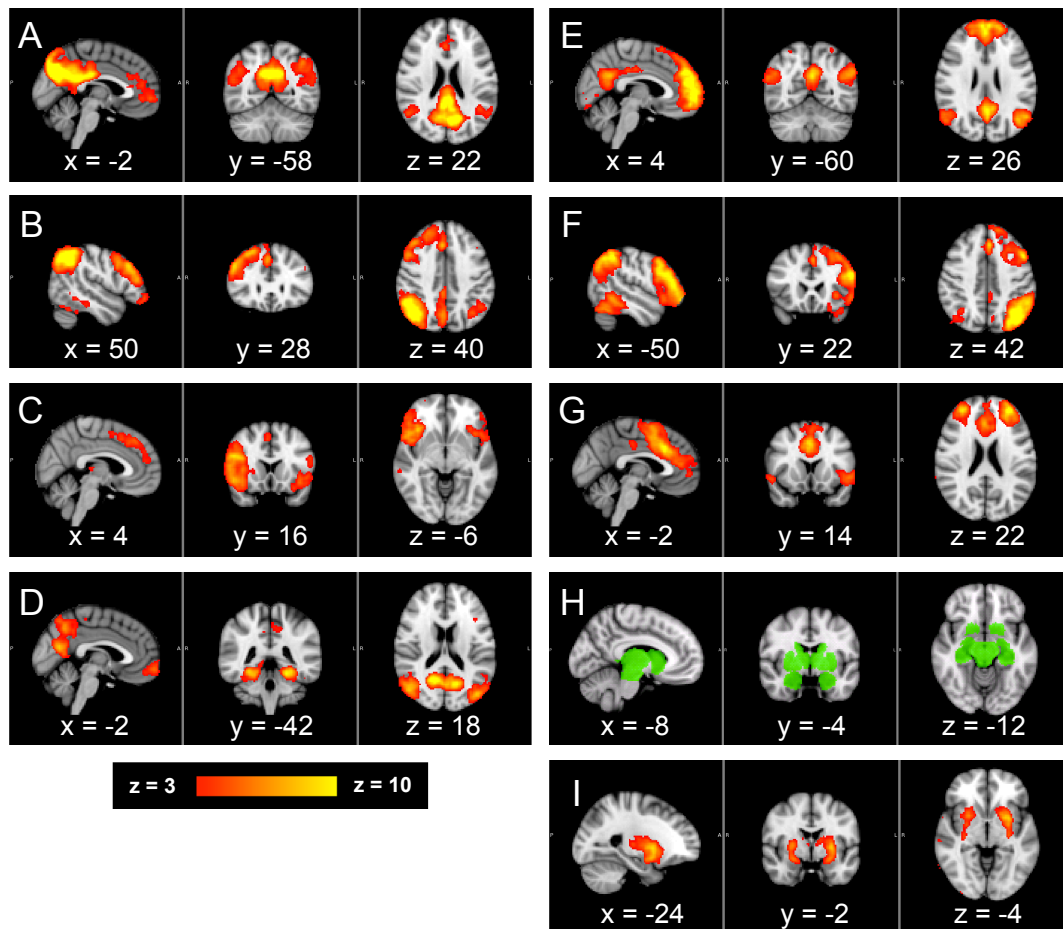


Figure 5.1. RSNs of interest from ICA of placebo group data (N = 15) and group subcortical seed mask. (A-G) Seven RSNs subjected to higher-level post-SBCA analysis of dopamine-dependent subcortical functional connectivity and post-dual regression whole-brain connectivity. (A) Postero-centric default mode network (DMN); (B) Right-lateralised frontoparietal network (FPN) (C) inferior fronto-insular salience/executive network (SEN); (D) hippocampal-parietal/ventral DMN; (E) Antero-centric DMN; (F) Left-lateralised FPN; (G) dorsal medial-lateral frontal SEN. (H) Subcortical mask used in higher-level analyses. (I) Basal ganglia/limbic RSN (BGLN) including bilateral striatum, pallidum and amygdala subjected only to post-dual regression analyses of whole-brain connectivity.

5.2.6 – Neocortical RSN connectivity with subcortical regions (ii): Higher-level analysis

Further analyses examining drug effects on RSN-subcortical functional connectivity focussed on a subset of seven RSNs (Fig. 5.1A-G), of interest due to their reported involvement in complex cognitive control and motivational processes potentially relevant for impulse control, reward processing or

dopamine function (Greicius et al., 2004; Vincent et al., 2006, 2008; Seeley et al., 2007; Kelly et al., 2009; Smith et al., 2009; Andrews-Hanna et al., 2010; Cole et al., 2010b, 2012a; Koob and Volkow, 2010; Shannon et al., 2011; Wolf et al., 2011). In line with this amassed literature, these RSNs are here referred to as the (i) postero-centric (Fig. 5.1A; peak MNI co-ordinates in the dorsal precuneus: $x = 0$, $y = -76$, $z = 34$), (ii) hippocampal-parietal/ventral (Fig. 5.1D; left ventral precuneus peak: $x = -10$, $y = -62$, $z = 12$) and (iii) antero-centric (Fig. 5.1E; right dorsomedial prefrontal cortex peak: 2, 54, 20) sub-systems of the DMN, the (iv) right- (Fig. 5.1B; right inferior parietal/superior occipital cortex peak: 44, -66, 48) and (v) left-lateralised (Fig. 5.1F; left inferior parietal/superior occipital cortex peak: -38, -72, 46) frontoparietal networks (FPNs), the (vi) fronto-insular (Fig. 5.1C; right inferior frontal gyrus/dorsolateral prefrontal cortex peak: 50, 18, 24) and (vii) dorsal medial-lateral frontal (Fig. 5.1G; left frontopolar/dorsolateral prefrontal cortex peak: -30, 48, 24) salience/executive networks (SENs).

To test the correlation maps resulting from SBCA for between-group differences (seven RSNs $\times$ 49 subjects = 343 maps), they were transformed to a common stereotactic space, firstly by applying previously calculated affine transformations to high-resolution structural space, then by FNIRT nonlinear warp transform to MNI template space. Correlation maps were then concatenated in a single 4-D file per RSN, containing, for each subject, a subcortical map of connectivity with said RSN (thus 49 per RSN). These RSN-specific subcortical connectivity maps were then analysed within the GLM framework, using non-parametric permutation testing (5000 permutations; as implemented in FSL randomise) to identify subcortical regions in which

functional connectivity with a given RSN of interest differed between dopamine drug treatment groups, in terms of being more strongly or weakly positive or negative. Explicitly, contrasts tested for both linear (L-dopa > placebo > haloperidol; and the inverse contrast) and quadratic/nonlinear (placebo > L-dopa + haloperidol; and the inverse) drug effects on RSN-subcortical connectivity. Significant effects were defined by cluster-mass thresholding ($t > 2.3$, $p < 0.05$, FWE-corrected) and are presented in Table 5.2. For the group subcortical mask used in these analyses, to be inclusive and allow for small inter-subject structural variations, the subcortical masks from each subject were nonlinearly warped to MNI space (using FSL FNIRT), summed and the resulting image was then binarised (Fig. 5.1H).

5.2.7 – Whole-brain analysis of RSN connectivity using dual regression

The analysis described in section 5.2.6 tested specific hypotheses about the integration of pre-defined subcortical structures within macroscopic brain networks. However, the human brain systems influenced by dopamine neurotransmission are distributed in nature throughout the cortex and subcortex and the precise mechanisms of functional integration across the regions involved in dopaminergic processing are not clear (Koob and Volkow, 2010). Therefore, to avoid overlooking more widespread linear and nonlinear effects of dopamine modulation on network connectivity, particularly within neocortical regions, a second higher-level analysis was performed using dual regression methods described in previous chapters, followed by GLM permutation testing. Thus, this approach was sensitive to systems-level RSN

connectivity relationships across the whole brain, rather than restricting all *a priori* hypotheses to cortico-subcortical functional connections.

The full set of 20 unthresholded, weighted RSN maps identified by group-ICA of the placebo data was entered into dual regression to produce subject-specific RSN time courses and spatial maps. Artefactual signals from white matter, CSF and six motion parameters (defined using FSL tools as described in section 5.2.5) were regressed out of this analysis for each subject. Group comparisons examining both linear and nonlinear effects of dopamine drug modulation were carried out within an identical framework to the post-SBCA higher-level analyses (section 5.2.6), but with the additional inclusion of an eighth RSN of interest spanning a (largely striatal) basal ganglia/limbic network (BGLN) of regions (Fig. 5.11; peak co-ordinates in the left dorsal putamen: $x = -22$, $y = 4$, $z = 2$), with potential relevance for dopaminergic function, reward processing and related neuropsychiatric disorders (Kelly et al., 2009; Robinson et al., 2009; Cole et al., 2012a). In preparation for the group GLM comparison, the individualised maps corresponding to the eight RSNs of interest were normalised to z-statistics and concatenated across subjects, creating a 4-D file containing the map corresponding to each RSN for one subject per volume (thus 49 per RSN).

5.2.8 – Measuring associations between connectivity and impulsivity

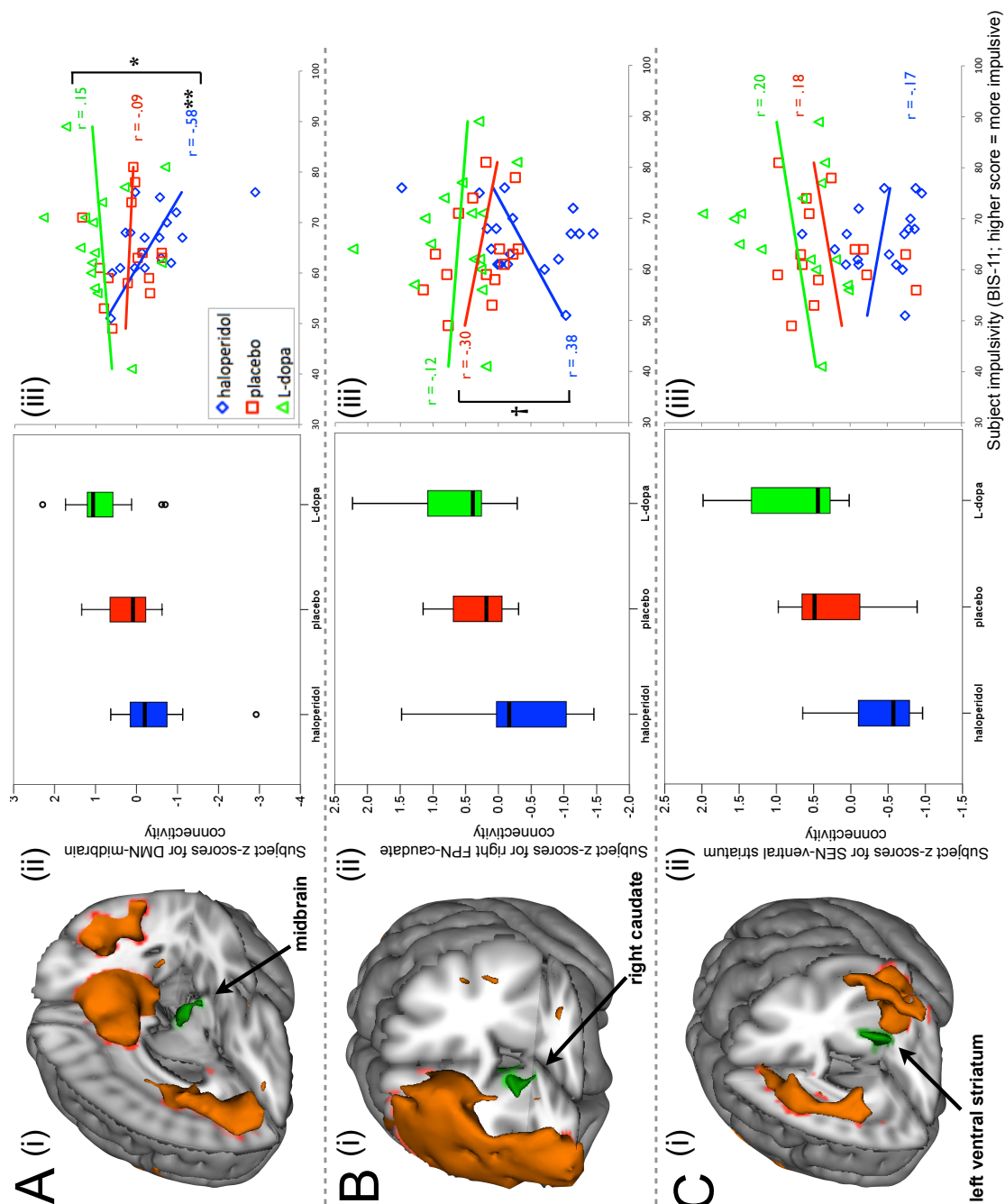
To examine drug-specific network functional connectivity associations with impulsivity, fMRI results were correlated, within-group, with total BIS-11 scores. Significant clusters identified from the post-SBCA and post-dual regression higher-level analyses were thus used, respectively, as masks to

extract mean connectivity scores from the RSN-specific correlation and connectivity maps used initially as inputs to these analyses (in the case of the post-SBCA correlation maps, Fisher z-transformed normalised versions). The resulting values were then grouped by drug condition and correlated (Pearson's r) with BIS-11 total scores to find significant within-group associations ($p < 0.05$, two-tailed). These correlations were then compared across groups to investigate drug-personality interactions, using a z-test to identify significant differences between the resulting (Fisher z-transformed) correlation coefficients. All post-hoc correlations with impulsivity scores are regarded as exploratory and are uncorrected for comparisons across multiple RSNs.

Table 5.2. Clusters displaying significant linear effects of dopamine modulation on RSN functional connectivity: spatial information and associations with impulsivity.

RSN (see Fig. 1)	Subcortical cluster anatomical location	Cluster MNI x, y, z co-ordinates (peak t-statistic) and volume	Association between drug and RSN-subcortical connectivity	Association with BIS-11 scores
A. Postero-centric DMN	Midbrain (bilateral posterior)	-6, -28, -6 (5.08), 1680 mm ³	Linear: L-dopa (> placebo) > haloperidol	Significant negative correlation in haloperidol group; significantly different from L-dopa group
B. Right FPN	Right dorsal caudate	12, 8, 6 (4.08), 1656 mm ³	L-dopa (> placebo) > haloperidol	Positive trend in haloperidol group; close-to-significantly different from placebo group
C. Fronto-insular SEN	Left ventral striatum	-16, 12, -10 (4.21), 1160 mm ³	L-dopa (> placebo) > haloperidol	None significant (n.s.)
F. Left FPN	Bilateral ventro-medial thalamus	6, -14, 2 (4.00), 1392 mm ³	L-dopa (> placebo) > haloperidol	n.s.

Figure 5.2. Significant linear effects of antagonistic (haloperidol) and agonistic (L-dopa) dopaminergic neuromodulation on cortico-subcortical resting-state network functional connectivity ($N = 49$) and correlations with subject BIS-11 impulsivity scores. (A) (i) Default mode network (DMN)-midbrain connectivity shows (ii) a linear effect ($t > 2.3$, $p < 0.05$, FWE-corrected) of treatment (L-dopa > placebo > haloperidol), which (iii) is negatively correlated with impulsivity within the haloperidol group, differentially to within the L-dopa group. (B) (i) Right frontoparietal control network (FPN)-caudate connectivity shows (ii) a similar linear effect and (iii) a trend towards an opposite relationship with impulsivity to the DMN-midbrain result. (C) (i) Salience/executive network (SEN)-ventral striatum connectivity shows (ii) the same linear drug effect but (iii) no significant interaction with impulsivity. Left panels: RSNs presented in orange, subcortical regions in green. Centre panels: Boxplots represent mean connectivity scores ($\pm 95\%$ confidence intervals) for each drug group. Right panels: **Denotes significant within-group correlation with impulsivity ($p < 0.05$); *Significant difference between two correlation coefficients ($p < 0.05$); †Near-significant trend towards difference between coefficients ($p < 0.07$).



5.3 – Results

5.3.1 – Dopamine-dependent linear modulations of cortico-subcortical connectivity

From analyses of fMRI data collected in conjunction with broad-spectrum pharmacological challenges, this study first revealed multiple significant linear associations (L-dopa > placebo > haloperidol) between dopaminergic drug group and cortico-subcortical functional connectivity (cluster $t > 2.3$, $p < 0.05$, FWE-corrected). These effects on connectivity, greater in the L-dopa group and reduced in the haloperidol group, were apparent between: (i) the postero-centric DMN and the midbrain (peak $t = 5.08$; co-ordinates: $x = -6$, $y = -28$, $z = -6$; Fig. 5.2A; see Table 5.2 for more details); (ii) the right-lateralised FPN and the right dorsal caudate ($t = 4.08$; $x = 12$, $y = 8$, $z = 6$; Fig. 5.2B); and (iii) the inferior fronto-insular SEN and the left ventral striatum ($t = 4.21$; $x = -16$, $y = 12$, $z = -10$; Fig. 5.2C). A significant effect of drug group was also found on the connectivity between (iv) the left-lateralised FPN and bilateral thalamic regions (see Table 5.2). This post-SBCA analysis revealed no significant ‘inverse linear’ (haloperidol > placebo > L-dopa) or nonlinear effects of dopamine modulation on cortico-subcortical connectivity.

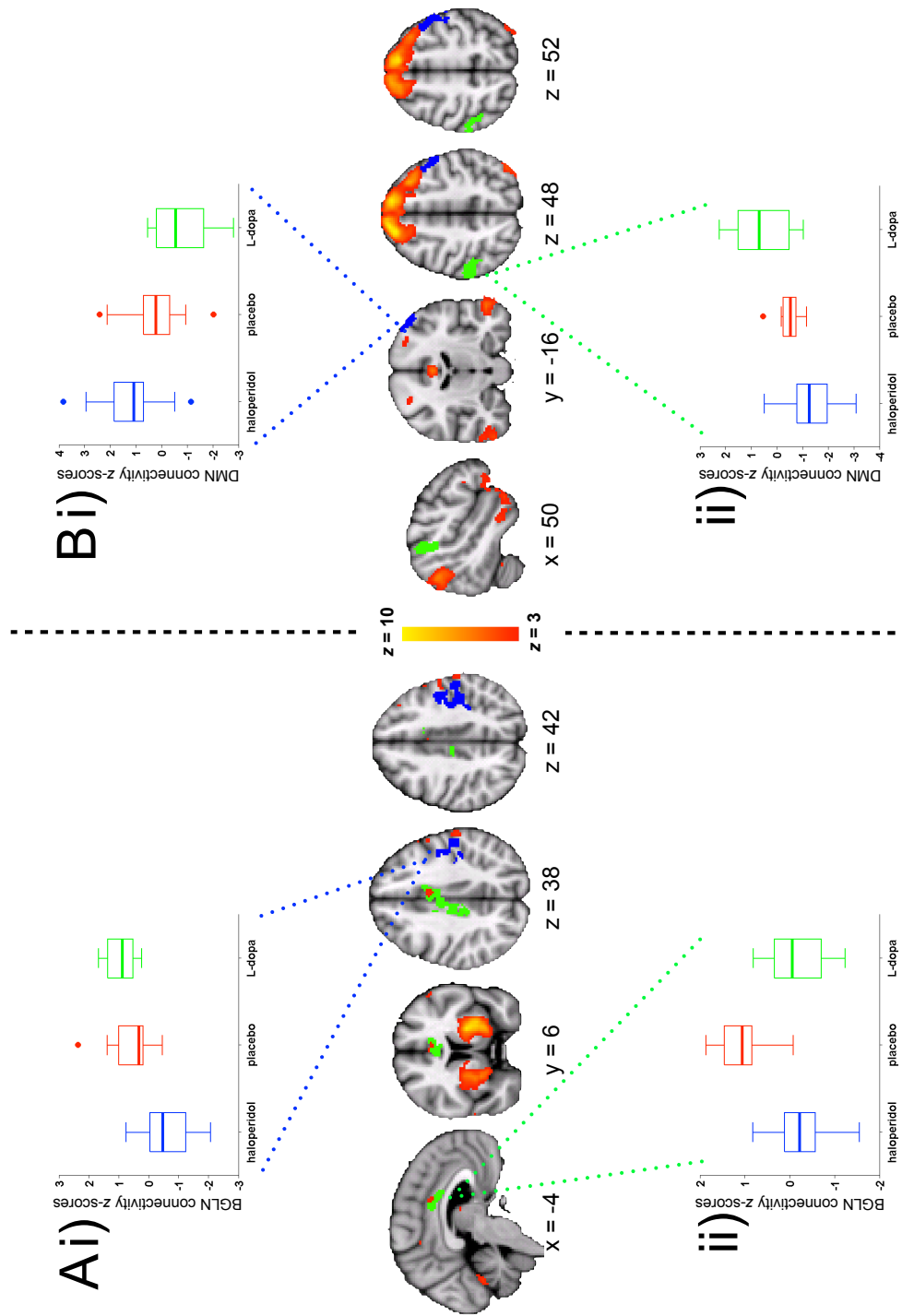
5.3.2 – Dopamine modulates distinct RSN connectivity patterns differentially

A second, whole-brain analysis examined how the distributed signalling of macroscopic brain networks could be altered in a dopamine-dependent manner. Multiple significant effects were identified, both linear and ‘inverted U-shaped’, of dopaminergic agonistic and antagonistic drug modulations on the functional connectivity patterns of behaviourally relevant resting-state networks identified by group-ICA. The predominantly subcortical BGLN (Fig. 5.1I), which covered the

majority of the bilateral striatum and portions of the pallidum and amygdala, showed a significant linear effect of drug group (cluster $t > 2.3$, $p < 0.05$, FWE-corrected). Specifically, BGLN functional connectivity with regions of left pre- and post-central gyri/motor cortex (peak $t = 6.18$; $x = -38$, $y = -26$, $z = 42$; Fig. 5.3Ai) was greater in the L-dopa group and lower in the haloperidol group, relative to the placebo group. In addition, an inverted U-shaped (i.e., quadratic) effect of dopamine drug group was found on the connectivity association between the BGLN and a region of dorsal anterior/mid-cingulate cortex ($t = 5.27$; $x = 4$, $y = -8$, $z = 36$), where this connectivity was significantly higher in the placebo group than in both drug groups (on average lowest in the haloperidol group; Fig. 5.3Aii).

Additionally, two significant linear, but opposing, effects of dopamine neuromodulation were found on the – predominantly neocortical – antero-centric DMN (Fig. 5.1E). These were: (i) greater connectivity in the haloperidol, relative to placebo and L-dopa groups, with a cluster in the left precentral and middle frontal gyri ($t = 4.51$; $x = -40$, $y = 6$, $z = 56$; Fig. 5.3Bi); and (ii) reduced (or *increased negative*) connectivity in the haloperidol group, relative to placebo and L-dopa groups, between this RSN and the right supramarginal gyrus/intraparietal sulcus ($t = 6.40$; $x = 52$, $y = -34$, $z = 46$; Fig. 5.3Bii). No quadratic (inverted U-shaped or ‘non-inverted’ U-shaped) dopaminergic effects were identified across the whole brain in terms of the connectivity with this (or any other) cortical RSN of interest.

Figure 5.3. Significant linear and nonlinear effects of dopamine neuromodulation on whole-brain RSN functional connectivity (N = 49). (A) Centre: BGLN connectivity with left pre- and post-central gyri/motor cortex (blue) shows (i) a linear effect (t > 2.3, $p < 0.05$, FWE-corrected) of dopamine (L-dopa > placebo > haloperidol), while BGLN connectivity with dorsal anterior-mid cingulate (green) displays (ii) a *nonlinear* effect of dopamine (placebo > haloperidol + L-dopa). (B) Centre: antero-centric DMN connectivity with left precentral and middle frontal gyri (blue) shows (i) an *inverse* linear drug effect (haloperidol > placebo > L-dopa), while anterior DMN-right supramarginal gyrus connectivity displays (ii) the opposite relationship with dopamine modulation. Red-yellow overlays depict regions of high functional connectivity within the RSNS themselves, as defined by placebo group-ICA.



5.3.3 – Connectivity-impulsivity associations

Further exploratory analyses of the significant post-SBCA results revealed that dopamine-dependent connectivity between the posterior DMN and the midbrain was significantly negatively correlated with subject BIS-11 total scores in the haloperidol group ($r = -0.58$, $p = 0.012$ two-tailed). Further, this effect size was significantly different ($z = 2.1$, $p = 0.038$ two-tailed) from an equivalent *positive* (non-significant) correlation in the L-dopa group, thereby indicating an interaction effect between impulsivity scores and drug type on network functional connectivity (Fig. 5.2Aiii). Although both haloperidol and L-dopa groups visibly contain outliers¹⁵ for the contrast in question, by removing the scores of these subjects from the respective connectivity-impulsivity correlations this interaction effect was further amplified ($z = 2.6$, $p = 0.010$ two-tailed). Of potential interest, the drug effects on connectivity between the right FPN and caudate showed an opposing relationship with BIS-11 scores to the midbrain-DMN pattern identified (Fig. 5.2Biii). No within-group correlation reached significance for this FPN-caudate relationship, but the haloperidol group correlation was again opposite in direction to the other two groups and in this case showed a trend towards a significant difference from the BIS-11 correlation with the placebo group ($z = -1.8$, $p = 0.067$ two-tailed). The dopamine-dependent SEN-ventral striatum connectivity pattern showed no within- or between-group associations with BIS-11 scores (Fig. 5.2Ciii).

In terms of results identified by whole-brain, post-dual regression analyses, the dopamine-dependent connectivity (L-dopa > placebo > haloperidol) between a right supramarginal gyrus cluster and the DMN (Fig.

¹⁵As determined by the boxplot algorithm in SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

5.3Bii) was significantly negatively correlated with subject BIS-11 scores in the group given haloperidol ($r = -0.51$, $p = 0.031$ two-tailed; Fig. 5.4). Non-significant equivalent correlations in the placebo and L-dopa groups were both directionally opposite (positive) to that in the haloperidol group, to a significantly different degree ($z = 2.43$, $p = 0.015$ two-tailed) and at trend levels ($z = 1.69$, $p = 0.091$), respectively. No other connectivity patterns showed within-group correlations or between-group interactions with BIS-11 scores.

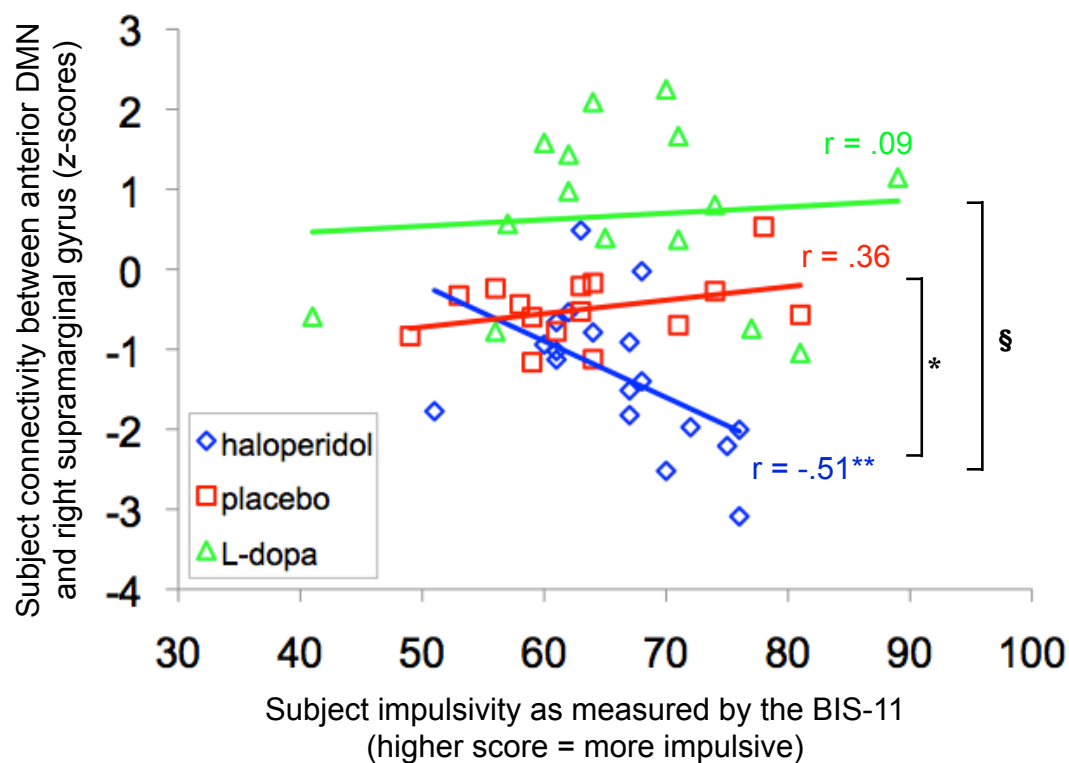


Figure 5.4. Drug-specific association between anterior DMN-parietal connectivity and impulsivity. Antero-centric DMN connectivity in right supramarginal gyrus (see Fig. 5.3Bii) is significantly negatively correlated with subject BIS-11 scores in the group given haloperidol ($r = -0.51$, $p < 0.05$ two-tailed, denoted by '**'), but not in the placebo or L-dopa group. The connectivity-impulsivity correlation in the haloperidol group is significantly different to that in the placebo group ($p < 0.02$, denoted by '*') and shows trend levels of difference to that in the L-dopa group ($p = 0.09$, denoted by '\$').

5.4 – Discussion

5.4.1 – Summary

This study identified novel and important links between dopamine neuromodulation and systems-level signalling within the human brain. Results provide evidence that, relative to a placebo, dopamine agonistic and antagonistic manipulations at ‘clinical’ doses (see section 5.2.1) affect large-scale resting-state network connectivity relationships in the healthy human brain in both opposing (linear) and inverted U-shaped (nonlinear) fashions. Linear effects of dopamine drug modulation were widespread, occurring within cortico-subcortical and cortico-cortical networks. Only a single nonlinear dopaminergic effect was identified, occurring within cortico-subcortical RSN circuitry. Further, drug-specific associations were pinpointed between RSN reactivity to dopamine modulation and individual differences in trait impulsivity, revealing dissociable drug-personality interaction effects across distinct dopamine-dependent cortico-subcortical and cortico-cortical circuitry.

5.4.2 – *Linear and nonlinear dopamine modulation of cortico-subcortical network connectivity*

Firstly, opposing effects were demonstrated of agonistic and antagonistic dopaminergic challenges on functional connectivity relationships between dopamine-rich subcortical regions (specifically midbrain and striatum) and corresponding neocortical cognitive RSNs, as well as between the large-scale subcortical BGLN and regions of left somatomotor and premotor cortex. Similar neural circuits have been implicated in aspects of cognition, personality and reward processing relevant for neuropsychiatric disorders commonly treated

with dopamine-targeting medications (e.g., Castellanos et al., 2008; Cole et al., 2010b, 2012a; Koob and Volkow, 2010; Tost et al., 2010; Shannon et al., 2011; Wolf et al., 2011). The observed linear, dopamine-dependent BGLN-motor/premotor connectivity relationship is, in part, a resting-state corroboration of task-related fMRI functional connectivity results reported by Tost and colleagues, which showed a reduction of connectivity between comparable (left hemisphere) striatal and cortical regions in healthy subjects under haloperidol (dopamine antagonism) during a motor activation paradigm (Tost et al., 2010). Moreover, the current results provide intuitive confirmation that an opposing (agonistic) pharmacological manipulation with L-dopa has the opposite effect on this same motor network and other cortico-subcortical circuitry. Indeed, L-dopa generally *increases* cortico-subcortical network connectivity in this study sample, whereas haloperidol tends to *decrease* it (although see below and section 5.4.3). These findings suggest that RSN functional connectivity can, in some cases, provide an indirect measure of dopamine neurotransmission. Of note, the latter observation is also in line with prior electrophysiological evidence of L-dopa increasing cortico-subcortical neuronal coupling in Parkinson's disease (Williams et al., 2002), indicating further that multimodal functional connectivity measures can provide converging evidence of neurotransmitter effects on macroscopic brain signalling.

Secondly and conversely, whole-brain analyses revealed an inverted U-shaped association between dopamine modulation and resting-state connectivity between the BGLN and regions of dorsal anterior/mid-cingulate cortex. In contrast to the *linear* effects of dopamine drug group on cortical-BGLN connectivity subserving motor functioning (Tost et al., 2010), this observed

connectivity nonlinearity might reflect the involvement of cingulate circuitry implicated in the dopamine-dependent regulation, or *optimisation*, of higher-level cognitive, emotional or motivational processes (Bush et al., 2000; Botvinick, 2007; Cools and D'Esposito, 2011). The variability of action that dopaminergic drugs seem able to exert, even on two distinct, distributed interaction patterns of a single subcortical RSN, may go some way towards explaining the variability in symptom severity, treatment response, and motor and cognitive side effects observed consistently in the neuropsychiatric clinic (Goldberg et al., 1993; Dagher and Robbins, 2009; Heidbreder and Newman, 2010). For example, this variability may be responsible in some way for the fact that a number of Parkinson's disease patients treated with dopamine agonist drugs develop cognitive-behavioural complaints and/or addictive disorders, whereas a majority do not (Cools, 2006; Dagher and Robbins, 2009; Weintraub et al., 2010; Cilia and van Eimeren, 2011; see also Chapter 6). Future investigation of the relevance of large-scale network functional connectivity signal variability for dopamine-mediated neuroplastic processes might also account for potential interactions with other, for instance glutamatergic and related, neurotransmitter or neurochemical systems (e.g., Reynolds and Wickens, 2002; Laruelle et al., 2003).

5.4.3 – Distinct linear dopamine modulations of cortico-cortical RSN connectivity

In addition to revealing differential effects of dopamine on the functional connectivity between subcortical circuitry and distinct cortical regions, analyses identified two separate and opposing linear dopaminergic effects on signalling between the predominantly anterior neocortical DMN and separate regions of

cortex. Firstly, results demonstrated an inverse linear association between the pharmacological modulation of dopamine neurotransmission and DMN functional connectivity with regions of the left premotor cortex. Unlike all other current and the majority of previous findings (Achard and Bullmore, 2007; Tost et al., 2010; although see Diaconescu et al., 2010), this result is indicative of dopamine antagonism actually *increasing*, rather than *decreasing*, functional connectivity. Thus, while it has been hypothesised that haloperidol given acutely, via a suppressive effect on synaptic plasticity, is more likely to decrease than increase neuronal connectivity (Tost et al., 2010), the current findings suggest conversely that the mechanisms by which dopamine mediates network functional connectivity *as measured by BOLD FMRI* may not be explained with such parsimony¹⁶. Nevertheless, a second linear group effect of dopamine neuromodulation was found on DMN connectivity with the right supramarginal gyrus/intraparietal sulcus regions of the inferior parietal cortex. This may be relevant for the widely described “anti-correlation”, or negative temporal coupling, between the DMN and frontoparietal cognitive RSNs involving supramarginal and intraparietal regions, the strength of which is thought to reflect the efficiency of cognitive processing (Fox et al., 2005; Baliki et al., 2008; Castellanos et al., 2008; Kelly et al., 2008; Cole et al., 2010b; see also Chapter 3 and section 7.3.3). Indeed, the linear effect of dopamine in these data shows haloperidol to *increase negative functional connectivity* between the DMN and anterior inferior parietal regions.

¹⁶It should be noted that the approach of variance normalisation applied here (as described in section 2.2.2), which is sensitive to ‘amplitude’ as well as ‘shape’ contributions to functional connectivity measures, may provide differential sensitivity, for example, to effects that would not be detectable with purely correlative approaches.

The DMN is often labelled as a ‘task negative’ RSN, of which the angular gyrus portion of the inferior parietal cortex is an integral part (Raichle et al., 2001). Conversely, the frontoparietal RSNs, of which more anterior supramarginal and intraparietal regions form a key node, are deemed to be ‘task positive’ (Fox et al., 2005; Dosenbach et al., 2007; Vincent et al., 2008). This anatomical contiguity has led some researchers to put forward the inferior parietal cortex as a “transition zone” between default mode and frontoparietal RSNs (Cohen et al., 2008; Mennes et al., 2010). In line with the common finding that these spatially distinct networks are temporally negatively coupled, it could therefore be inferred that this parietal transition region is ideally spatially situated for, or plays an important part in, promoting the dynamic interplay between functionally dissociable cognitive networks. Although their primary reported finding centred on the role of anterior insula in ‘driving’ the switching between major cognitive RSNs, such a possibility has been suggested previously by Sridharan and colleagues (as part of supplementary discussion; Sridharan et al., 2008), based on directional, ‘effective’ connectivity analyses of resting-state FMRI data (although note that their study incorporated Granger causality methods, which face important caveats regarding their applicability to FMRI data; see e.g., Smith et al., 2011; see also section 7.3.5). Indeed, one of the major hypothesised mechanisms by which brain dopamine acts is in mediating the dynamic balance between processes of “flexible updating and cognitive stabilization” (Cools and D’Esposito, 2011). Thus, the current finding of dopamine-dependent parietal transition region connectivity with the DMN could feasibly reflect the purported role of the neurotransmitter in network switching relevant for efficient cognitive functioning, although further studies are required

to confirm this interpretation. Initial support for the relevance of this finding for neurochemical processes underlying cognitive efficiency, flexibility or optimisation stems, perhaps, from the drug-dependent association found here between this specific connectivity pattern and self-reported impulsivity measures (noting, however, that similar impulsivity associations are found with dopamine-dependent cortico-subcortical connectivity patterns identified in separate analyses).

5.4.4 – Drug-specific associations between dopamine-dependent RSN functional connectivity and trait impulsivity

Aside from the gross linear and nonlinear effects of dopaminergic pharmacological challenges on systems-level functioning, selective associations were also identified between self-reported impulsive personality traits and individual responses to dopamine modulation of cortico-subcortical and cortico-cortical network connectivity patterns. The predictive strength and direction of these patterns differ depending on the RSN involved, perhaps reflecting distinct network functions (Beckmann et al., 2005; Seeley et al., 2007; Smith et al., 2009). For example, the DMN and frontoparietal networks are thought to subserve opposing aspects of cognition, but are seldom related directly to impulsivity, although meta-analytic work strongly implicates the right-lateralised FPN in inhibitory control (Smith et al., 2009). Findings further expand on previous evidence by highlighting that dopaminergic network differences in impulsivity extend beyond the midbrain and basal ganglia (Dalley et al., 2007; Buckholz et al., 2010) to large-scale, functionally dissociable neocortical systems.

Moreover, it seems that subject impulsivity correlates preferentially and differentially with the effects of haloperidol, relative to L-dopa, on connectivity. The neurobiological reasons for this are unclear, but may relate to pharmacological differences between the drugs in specificity or potency. Administering exogenous L-dopa indirectly increases neurotransmission by raising existing levels of the dopamine precursor, whereas acute haloperidol challenge preferentially blocks neurotransmission at dopamine D2 receptors. The latter is thought to suppress mechanisms governing synaptic plasticity, an effect likely to be reflected in functional connectivity measures (Bi and Poo, 2001; Saini et al., 2004; Lewis et al., 2009; Tost et al., 2010). The current findings raise the possibility that markers of impulsive personality may also influence these mechanisms. Alternatively, in a healthy, neuroleptic-naïve population, genetic, metabolic or neurophysiological differences in susceptible individuals may increase sensitivity to novel dopamine antagonism with clinical doses of haloperidol more dramatically than sensitivity to indirect agonism with the naturally present L-dopa molecule. Such propensity may be only indirectly related to high trait impulsivity, although equivalent interactions have previously been identified between impulsivity differences and the functional effects of *direct* dopamine agonists (Cools et al., 2007; Dalley et al., 2007).

5.4.5 – Methodological considerations

It is important to highlight that correlations with impulsivity measures were exploratory and, therefore, significant results limited to the group given haloperidol should be interpreted cautiously, as should the fact that such an association is only apparent with network connectivity relationships displaying

linear, and not nonlinear, effects of dopamine modulation. It should also be noted that the objectivity of the BIS-11 self-report scale in assessing multi-factorial impulsivity is a subject of some debate in the personality psychological literature (for review see, e.g., Evenden, 1999). However, as a general construct it displays robust inverse associations with molecular imaging measures of subcortical D2/D3 receptor availability pertinent to dopamine neurotransmission (Buckholtz et al., 2010); a result that provides reassurance in the context of the current study.

Secondly, similar to the study described in Chapter 3 using a nicotine lozenge intervention (and as is a concern in the majority of fMRI studies involving pharmacological manipulations), direct vasoactive effects of the dopamine drugs used here are difficult to rule out (see also section 3.4.4). Nonetheless, there is no precedent to expect that any such non-neuronal drug effects would be spatially specific or directionally non-specific, rather than globally pervasive and unidirectional. Thus, the fact that the current study identified multiple different types of drug effects, occurring in different directions in distinct, distributed neural networks, serves to minimise any such concerns.

Finally, it should be noted that, by virtue of the study design, the conceptualisation of the 'inverted U' presented here is not precisely analogous to that put forward previously (Cools and D'Esposito, 2011). This study specifically tested, in three independent groups, the linear and nonlinear effects on average of increasing and decreasing dopamine neurotransmission with neuromodulatory drugs. The framework posited by Cools and D'Esposito, however, emphasises the contribution of individual differences in baseline

dopamine concentrations to dopamine's inverted U 'continuum'. To interpret nonlinear observations resulting from the current study as evidence of inverted U-shaped effects of dopamine levels on functional processing requires the assumption that, prior to drug administration, subjects in the three experimental groups did not differ, on average, in their basal brain dopamine levels. Although this assumption is well founded, it cannot be confirmed unequivocally from the current data. Indeed, future functional connectivity studies investigating the 'inverted U' hypothesis may benefit from administering repeated or varying doses of dopaminergic drugs, or through combining resting-state fMRI and PET imaging methodologies (as in Chapter 4), to provide within-subject measures to elucidate how baseline dopamine levels or receptor availabilities influence the reactivity of brain functional networks to pharmacological challenge. Furthermore, such multimodal imaging investigations would only benefit from the additional inclusion of genotyping procedures, to cross-examine the genetic, neurobiological and neurochemical factors influencing intermediate phenotypic, large-scale network functional connectivity measures of individual differences in the response to experimental interventions such as dopamine manipulation (see e.g., Liu et al., 2010; Gordon et al., 2012; see also sections 7.3.2 and 7.3.6).

5.4.6 – Conclusions

Despite intensifying neuropsychiatric interest in the potential diagnostic and prognostic value of resting-state brain activity (Greicius et al., 2004; Filippini et al., 2009; Cole et al., 2010b; Fox and Greicius, 2010; Matthews et al., 2011; Murphy and Mackay, 2011), functional neuroimaging measures sensitive to these distributed, systems-level connectivity phenomena have, thus far, revealed

little evidence that linear or ‘inverted U-shaped’ effects of dopaminergic processing, similar to those observable during prescribed cognitive tasks, are measurable in spontaneous neural signalling patterns. Nonetheless, if such patterns exist, RSN functional connectivity measures should, in theory, be able to extricate them, due to the proposed ability to map biologically plausible effects on systems-level signalling throughout the entire brain (Beckmann et al., 2005; Smith et al., 2009).

In line with these assertions, this study found distinct cortico-subcortical and cortico-cortical network functional connectivity patterns to be affected differentially by dopamine agonist (L-dopa) and antagonist (haloperidol) drugs at clinical doses used regularly to treat neuropsychiatric disorders, relative to a placebo. While linear effects of dopamine neuromodulation were apparent across multiple cortico-cortical and cortico-subcortical functional connections, an inverted U-shaped connectivity response to pharmacological challenge was found only within basal ganglia-cingulate circuitry. Further, findings reveal dissociable drug-personality interaction effects across distinct dopamine-dependent cortico-subcortical and cortico-cortical circuitry. Results indicate that the systems-level brain response to targeted pharmacological D2 receptor blockade with a selective antagonist may be a more sensitive intermediate phenotype for certain neuropsychiatric indications, such as trait impulsivity, than the response to indirectly increasing dopamine neurotransmission by raising precursor levels. This study identifies possible systems underlying pathogenesis and treatment efficacy in disorders of dopamine deficiency and provides support for the objective utility of RSN connectivity metrics in classifying the effects and efficacy of psychopharmacological medications.

It can be concluded that, despite the incomplete standardisation of RSN connectivity measures for assessing clinical diagnosis, prognosis and treatment efficacy, their ability to characterise biologically plausible linear and nonlinear effects of broad-spectrum pharmacological (dopamine) modulations, at the systems level, supports their continued application and development as useful markers for these purposes in neuropsychiatry. Future studies should extend systems-level investigation of RSN connectivity associations with personality, behavioural or genetic factors to patient populations regularly medicated with selective dopamine receptor agonists or antagonists (Schafer et al., 2001; Cools, 2006; Dagher and Robbins, 2009; Heidbreder and Newman, 2010), revealing the possible impact of brain network functional interactions dependent on these factors on treatment and prognosis. With this in mind, Chapter 6 reports a study applying SBCA techniques sensitive to cortico-subcortical network connectivity patterns and modulations therein, equivalent to those described in this chapter, to patients with Parkinson's disease undergoing dopamine agonist treatment withdrawal and age-matched healthy controls, along with further extensions to investigating the association between dopamine-dependent connectivity modulations and measures of impulsivity.

Chapter 6 – Investigating systems-level functional connectivity in a disorder of neurochemistry

Effects of Parkinson's disease and treatment on RSN signalling

Dopamine agonist (DA) medications are commonly used to treat motor symptoms of Parkinson's disease (PD), but may also facilitate cognitive changes such as increased impulsivity in decision-making. Variability in the neural response to dopamine modulation within cortico-basal ganglia circuitry is thought to play a deterministic role in these 'non-normalising' treatment effects, although such phenomena are incompletely characterised. This study combined resting-state fMRI with DA medication modification techniques in PD patients, to establish whether RSN functional connectivity metrics could identify disease-relevant effects of DA on systems-level neural processing. Via comparison with age-matched healthy control subjects (HCs), multiple treatment-independent and non-normalising DA effects on RSN cortico-subcortical circuitry were found in PD, with a relative lack of evidence for 'normalising' connectivity effects. Differential associations were also found between connectivity within basal ganglia systems and individual impulsivity measures, which were positively correlated in HCs but not in PD. Thus, this study provides systems-level evidence for anomalous DA effects in PD on large-scale networks supporting cognition and motivated behaviour. Moreover, findings support the theory that dysfunctional striatal and basal ganglia signalling patterns in PD are compensated for by increased recruitment of other large-scale cortico-subcortical systems.

6.1 – Introduction

Classically, Parkinson's disease (PD) is understood to be a disorder of hypodopaminergic neurotransmission (for review see, e.g., Fahn, 2008). This aberrancy of neurochemical systems primarily afflicts PD patients with pronounced motor deficits. However, some patients reportedly experience a number of other debilitating symptoms. These include higher-order cognitive and neuropsychiatric symptoms such as impairments in working memory, decision-making and motivation (Owen et al., 1992; Aarsland et al., 1999; Pluck and Brown, 2002; Dagher and Robbins, 2009). Importantly, growing evidence implies that, in a notable minority of PD patients, some such higher-order symptoms may be unaffected, exacerbated, or perhaps even caused, by the same dopamine agonist (DA) medications used widely and successfully to treat the more common motor complaints (for reviews see Cools, 2006; Dagher and Robbins, 2009; Cilia and van Eimeren, 2011). Of particular neuropsychiatric pertinence, undesirable side effects of DA treatment are often found to occur in the form of increases in impulsive and compulsive behaviours and, in severe cases, addictions (Dagher and Robbins, 2009; Weintraub et al., 2010).

It has been suggested that: "Persistent pharmacological stimulation of dopamine receptors, as occurs in medicated PD patients, could [... result in...] increased engagement in reward-seeking behaviours and reduced ability to disengage in the face of negative consequences" (Dagher and Robbins, 2009, p. 506). This statement applies primarily to dopamine receptors within the mesolimbic pathway, which are not primarily deficient in PD, but may be hyperstimulated by the administration of DA drugs (Dagher and Robbins, 2009). Thus, by altering signalling between spatially distinct – but functionally/anatomically

connected – neuronal populations, DA medications, although serving to ‘normalise’ motor deficits caused by degeneration of the nigrostriatal dopamine pathway, may also effectively promote impulsive/compulsive behaviours in PD via incidental neuroplastic mechanisms. The neural bases of these latter, ‘non-normalising’ DA effects are not well characterised, although they are thought to be represented prominently in striatal regions and connected areas of the mesolimbic and cortico-striatal dopamine pathways – circuitry known to be involved in many higher-order cognitive processes supported by dopamine, relevant for impulsivity and disturbed in PD – with susceptibility to such effects varying across patients in line with neurochemical and cognitive/neuropsychiatric phenotypes (e.g., Everitt and Robbins, 2005; Evans et al., 2006; Dagher and Robbins, 2009; Buckholz et al., 2010; Housden et al., 2010; Tost et al., 2010, O’Sullivan et al., 2011). Indeed, differences in neuropsychiatric side effects of DA medication exist across individuals with PD, just as differences in the behavioural response to dopamine manipulation are apparent between healthy individuals (Dagher and Robbins, 2009; Cilia and van Eimeren, 2011; Cools and D’Esposito, 2011). This cognitive and neuropsychiatric variability could reflect individual differences in baseline (mesolimbic and cortico-subcortical) dopaminergic functioning, leading to differential neuroanatomical sensitivity to subsequent drug modulation; particularly within distinct striatal and other subcortical regions (Cools, 2006), but also in higher-level cortical areas (Cools and D’Esposito, 2011). Furthermore, a growing body of fMRI research has suggested that some of these individual differences may be explained by systems-level variability measurable in cortico-subcortical network functional connectivity patterns relevant for both dopamine function (e.g., in

healthy subjects, see Nagano-Saito et al., 2008; Cole et al., 2012b; see also Chapter 5) and dysfunction, for example in PD (Helmich et al., 2010; Kwak et al., 2010).

From the existing neuroimaging literature, considerable variability and uncertainty is apparent regarding the ability to characterise clinically relevant, systems-level dopaminergic effects, such as DA medication effects in PD, using fMRI. As with analogous investigations into other neuropsychiatric populations and associated treatments, this may result from the broad and non-standardised array of experimenter-derived behavioural paradigms employed by different research groups during scanning (Greicius, 2008; Murphy and Mackay, 2011). Here, previous chapters have demonstrated evidence of specific large-scale, higher-order neural systems possessing functionality relevant for dopaminergic and other neurochemical modulations, as measured by resting-state network connectivity.

In Chapter 5, it was established that analytical techniques sensitive to RSN functional connectivity, including SBCA, could be used to measure systems-level differences between (dopamine) drug manipulations. This approach may thus help to expound some of the aforementioned ambiguity in the literature regarding anomalous effects of DA therapeutics in PD. Therefore, this final research chapter extends the use of these methods to study a patient population undergoing relevant neurochemical modulations, by examining the effects of PD and its treatment with DA pharmacotherapies on RSN functional connectivity patterns within cortico-subcortical circuitry thought to be dysfunctional in the disease. A repeated measures approach was applied, using DA withdrawal techniques in PD patients (see section 6.2.1), which enabled a ‘closer-to-causal’

estimation of dopaminergic influences on systems-level processing within large-scale RSNs (in terms of measuring an experimentally controlled, temporally directed influence of DA medication modification on connectivity changes). To test the hypothesis that DA treatments exert, at the level of large-scale systems, both beneficial (normalising) and detrimental (non-normalising) influences on distinct psychological processes (sensory-motor and cognitive/motivational, respectively), connectivity differences from healthy control subjects (HCs) in PD were investigated in circuitry thought to support such functions. Due to their potential relevance for neuropsychiatric dysfunction in PD, particular interest was placed in the functional connectivity of cortico-subcortical networks involving striatal and other basal ganglia regions. To relate systems-level observations more directly to a psychological construct relevant for PD, associations were also explored between functional connections involving striatal regions showing significant effects of disease or DA withdrawal and a behavioural measure of impulsivity in decision-making; the latter defined using an intertemporal choice (ITC) questionnaire.

6.2 – Materials and methods

6.2.1 – Participants and study design

Fifteen adults diagnosed with PD (mean age = 60 ± 7.4 SD, range = 42-74 years) and 19 HCs matched for age (mean = 61 ± 7.0 , range = 44-69) and years of education (PD mean = 14 ± 2.6 SD, range = 11-19; HCs mean = 15 ± 3.0 SD, range = 11.5-22) were included in the study. All subjects were drawn from a sample described, along with other non-imaging procedures, in a previously published

article¹⁷ (Milenkova et al., 2011). Patients had idiopathic PD, as diagnosed by an experienced neurologist, without comorbid depression, dementia or impulse control disorders (i.e., no history of gambling problems, compulsive shopping, hypersexuality, punning, or abuse of their medication) and were judged to be able to function to a reasonable degree (e.g., they did not express pronounced motor symptoms) when their medication was withdrawn for approximately 12 hrs. HCs were all free from neuropsychiatric disorders. All patients were being treated with DA medication primarily targeting D2/D3 receptors (12 treated with 1.05-3.50 mg pramipexole, two with 4-15 mg ropinirole and one with 300 mg piribedil). Eight of the 15 patients received DA monotherapy, while seven also required concomitant L-dopa medication (150-850 mg) that was *not* withdrawn during the study. The implications of this variability are considered in more detail in section 6.4.4.

Patients were scanned using resting-state fMRI on two separate visits, the first prior to ('PD On') and the second 12 hrs after ('PD Off') DA withdrawal. HCs were scanned only once, *without* any pharmacological challenge. All study procedures were approved by the University of Magdeburg ethical committee.

6.2.2 – Intertemporal choice (ITC) questionnaire

The ITC questionnaire provides a measure of 'delay discounting' of monetary rewards for individual subjects, which refers to a reduction in the subjective value of a larger later reward, relative to that of a smaller earlier one, as the temporal delay to the former increases (Kirby et al., 1999). Delay discounting in

¹⁷Non-analytical study procedures described in sections 6.2.1-3 were conducted by a team of researchers at the Department of Neurology, Medical School Hannover, Hannover, Germany.

ITC has been shown to occur with increased frequency in disorders associated with maladaptive reward processing, including addiction, pathological gambling and PD (Kirby et al., 1999; Petry, 2001; Housden et al., 2010; Milenkova et al., 2011). However, relationships between this measure of impulsivity, brain dopamine function and individual differences in large-scale cortico-subcortical network functional connectivity remain less clear, as do the specific identities of the key connectivity pathways mediating these associations (see e.g., Peters and Buchel, 2010; Camchong et al., 2011; Christakou et al., 2011; Kayser et al., 2012). Moreover, a recent publication examining behavioural ITC data from subjects also included in the current fMRI study found that, on average, DA withdrawal had no effect on delay discounting in PD, which was nonetheless increased overall in the patients relative to HCs (Milenkova et al., 2011). This absence of a main drug effect on behaviour may have been influenced by the aforementioned variability across individuals in disease severity or sensitivity to treatment.

The ITC questionnaire was administered at each visit as described previously (Milenkova et al., 2011). In brief, the ITC requires subjects to choose in sequence between paired monetary rewards, one smaller and immediate and the other larger and delayed. Subjects were incentivised to make realistic decisions with the information that they would later have the opportunity to actually achieve one of these reward choices through a probabilistic die-throw. A measure k of the delay discount rate is produced for each subject. The k -value computation follows a (quasi-) hyperbolic function such that a higher value for k indicates that the participant in question subjectively devalued delayed rewards at a progressively steeper rate than an individual displaying a lower k -value. A version of the ITC questionnaire sensitive to the effects of different reward

magnitudes on delay discounting was employed, although as the original behavioural study found no evidence of differential magnitude effects between groups and conditions (Milenkova et al., 2011), the mean k -value is the variable of primary interest in the current context.

6.2.3 – Image acquisition

BOLD fMRI data were acquired for six minutes in the resting state on a 3-Tesla Magnetom Allegra MRI scanner (Siemens, Erlangen, Germany). In total, 178 volumes of T2*-weighted EPI data were acquired per session (TR = 2.0 s, TE = 30 ms, flip angle = 80°, isotropic voxel size = 3.0 mm³, 34 slices with 0.75 mm gaps), angled parallel to the anterior-posterior commissural plane. High-resolution (1 mm³) T1-weighted MPRAGE volumes were also acquired for each subject.

6.2.4 – Image preprocessing and first-level analysis/SBCA

Resting-state fMRI data were preprocessed with FSL tools as described in previous chapters. Following this, the data of HCs were entered into group-ICA with temporal concatenation, which resulted in the automatic estimation of 32 independent components. In line with the approach taken in previous chapters, eleven of these components were judged to be viable RSNs based on their spatial characteristics, of which eight were considered to be of higher-level interest due to their purported functional associations and relevance to the current study (see section 6.2.5). Subject/session-specific measures of voxel-wise subcortical connectivity with each of the 11 RSNs were obtained using SBCA as described in sections 5.2.4-5, for all HCs and PD patients in both treatment conditions separately. There were two variations on the analysis approach used in Chapter

5, both of which were incorporated to account for the potential (but relatively unclear) contribution of tissue macro- or micro-structural variations (e.g., atrophy, cortical thinning) to regional neuropathology in non-demented PD patients (Burton et al., 2004; Dalaker et al., 2009; Menke et al., 2010; Jubault et al., 2011; Tinaz et al., 2011). Firstly, to ensure more accurate alignment of neural systems defined at a group level to the brain space of individuals, prior to affine transformation to EPI space, RSN target maps from the HCs group-ICA were *nonlinearly* (as opposed to linearly) transformed from MNI space to the high-resolution space of each subject (as implemented in FSL FNIRT; Andersson et al., 2007); wherein, secondly and prior to registration to EPI space, voxels failing to exceed a comparatively liberal threshold of > 10% probability of containing grey matter (relative to > 20% in Chapter 5) in the equivalent T1 structural were removed from these ‘subject-specific’ RSN spatial maps. These extra procedures were applied to the data of all subjects, whether PD patients or HCs.

6.2.5 – Higher-level analyses

Eight RSNs of interest were subject to higher-level analyses, with their selection based chiefly on criteria and findings outlined in previous chapters (e.g., networks located largely outside of primary sensory cortices, with spatial characteristics reflecting neural systems thought to be relevant for dopamine-dependent processes, impulsivity or motivated behaviour). These were: (i) the BGLN (Fig. 6.1A, peak MNI co-ordinates in the left anterior putamen: $x = -24$, $y = 6$, $z = 2$), (ii) a fronto-temporo-parietal SEN (Fig. 6.1B, left postcentral gyrus/central operculum peak: $x = -64$, $y = -22$, $z = 16$), (iii) right- (Fig. 6.1C, right inferior parietal/superior occipital cortex: 46, -58, 48) and (iv) left-lateralised

(Fig. 6.1D, left lateral orbitofrontal/frontopolar cortex: -46, 44, -12) FPNs, (v) antero-centric (Fig. 6.1E, dorsomedial prefrontal cortex peak: 0, 46, 30) and (vi) postero-centric (Fig. 6.1F, left precuneus/ posterior cingulate cortex: -4, -54, 16) DMNs, (vii) a cerebellar RSN (Fig. 6.1G, medial cerebellum peak: -2, -56, -30) and (viii) a somatomotor RSN (Fig. 6.1H, right central sulcus/superior parietal cortex: 38, -26, 58).

In the study outlined in Chapter 5, the BGLN was not subject to post-SBCA higher-level analysis, as its close spatial consistency with the subcortical seed mask used may have raised questions of interpretability, related to the potential ‘circularity’ of such an analysis (e.g., Kriegeskorte et al., 2009). In the group-ICA results from the current control dataset, however, the BGLN demonstrated large nodes of high functional connectivity within extended portions of ventromedial and orbital prefrontal cortex, outside of the subcortical volume used as the seed (Fig. 6.1A). This system strongly matches circuitry described by Ersche and colleagues as: “large-scale striato-thalamo-orbitofrontal networks implicated in the processing of natural rewards and the regulation of behavior” (Ersche et al., 2010). Furthermore, it comprises regions known to be modulated by dopamine and compromised in addiction and other disorders associated with impulsive/compulsive behaviours (Honey et al., 2003; Everitt and Robbins, 2005; Koob and Volkow, 2010; Sesack and Grace, 2010). It was thus included as a network of interest in the current post-SBCA analysis (the implications of spatiotemporal variability that can occur across different studies and/or populations, such as the differences between the BGLN identified here, relative to in Chapter 5, are discussed elsewhere in this thesis; see sections 2.1.3 and 7.3.3). Similarly, the cerebellar RSN (Fig. 6.1G) was included as emerging

evidence has highlighted a compensatory role for cerebellar functioning and connectivity in PD, which is hypothesised to arise in response to impaired dopaminergic function in striatal regions and their reduced integration with large-scale networks (Appel-Cresswell et al., 2010). Finally, the somatomotor RSN was included because DA medications are used widely to treat motor system dysfunction in PD.

Statistical contrasts in the higher-level analysis specifically examined RSN functional connectivity patterns for: (i) basic paired effects of DA treatment withdrawal in PD patients (i.e., PD On >/< PD Off); (ii) 'normalising' effects of DA in PD patients (measured using quadratic contrasts; i.e., HCs + PD On >/< PD Off); (iii) 'non-normalising' effects of DA in PD patients (i.e., HCs + PD Off >/< PD On), which might provide support for non-beneficial effects of DA on certain cognitive functions in PD (e.g., Dagher and Robbins, 2009); and finally (iv) gross (treatment-independent) differences between HCs and PD patients (i.e., HCs >/< PD On + PD Off). In this study, the subcortical mask used for higher-level analyses in MNI space was formed from the subject-level masks of PD patients *only*. The aim of this was to provide a comparatively conservative masking approach, to better control for potential (although inconclusively established) brain structural differences between groups impacting on observations of functional processes (e.g., Burton et al., 2004; Dalaker et al., 2009; Menke et al., 2010).

Higher-level analyses differed further from those reported in previous chapters, as statistical contrasts applied consecutively to the (subcortical) connectivity measures of each of the eight RSNs of interest were not conducted in separate analyses. Rather, post-SBCA functional connectivity data from all

eight RSNs were concatenated within the same, single design matrix, which was then probed with contrasts testing for differences across groups and DA treatment conditions. This approach was chosen as it afforded an increased level of computational efficiency, which was unavailable during the analyses of data reported in previous chapters due to computing hardware specifications.

To investigate interactions between disease- or DA treatment-dependent connectivity patterns and impulsive decision-making as measured by the ITC questionnaire, bivariate within-group/condition correlations (Pearson's r , $p < 0.05$, two-tailed) were carried out between individual subject measures of RSN-subcortical connectivity and mean k -values from the ITC scores (the latter were log-transformed to allow for parametric testing). Significant clusters identified from the post-SBCA higher-level analysis were thus used as masks to extract mean connectivity scores from normalised (Fisher z -transformed) versions of RSN-specific correlation maps initially used as inputs to higher-level analyses. As variation in both temporal discounting and impulsivity more generally have been related to functional processes recruiting striatal dopamine (Dalley et al., 2007; Riba et al., 2008; Buckholtz et al., 2010; Cai et al., 2011; Christakou et al., 2011), statistical tests examining associations between impulsivity and functional connectivity were focussed on RSN connectivity relationships involving the striatum that were affected by PD or DA treatment. It was hypothesised that these functional connections would be differentially associated with individual differences in temporal discounting across groups and conditions. Correlations of ITC mean k -values with subject age and duration of treatment in PD were also carried out and revealed no significant associations,

thus confirming a lack of influence of these factors on ITC-related RSN connectivity results.

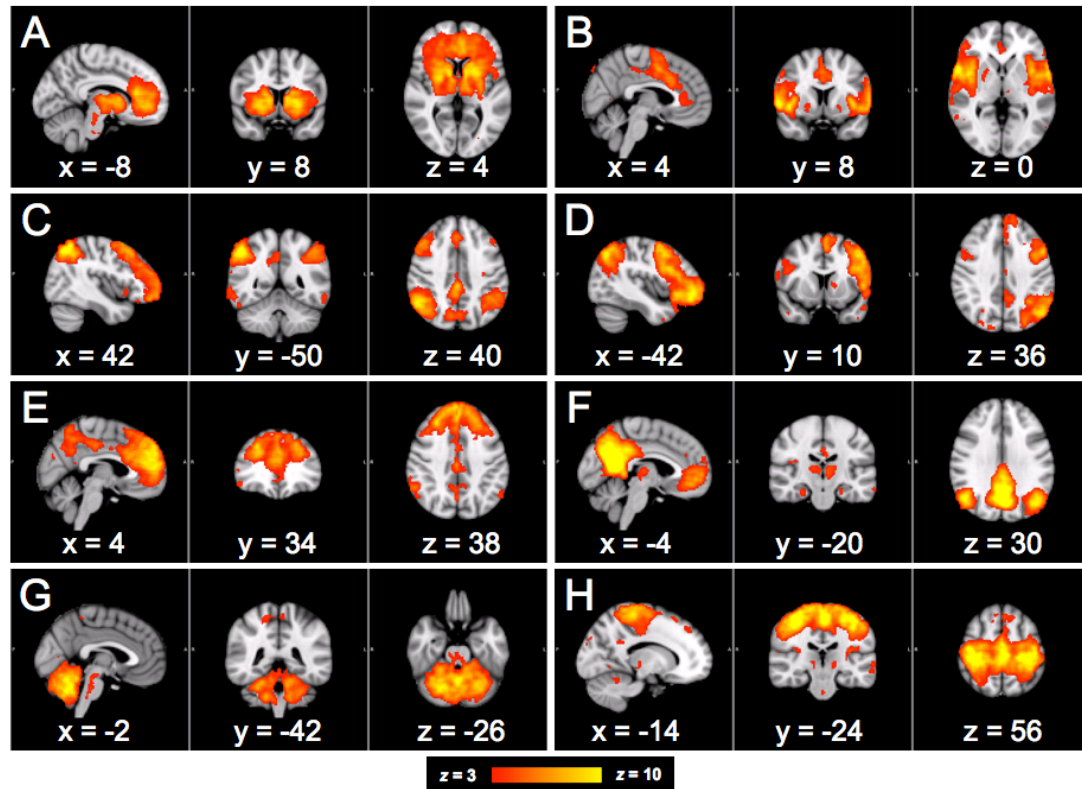


Figure 6.1. RSNs of interest from ICA of healthy control group data (N = 19).

6.3 – Results

6.3.1 – Basic effect of dopamine agonist medication withdrawal on RSN cortico-subcortical functional connectivity in PD

Paired analyses within PD patients revealed a single significant effect (cluster $t > 2.3$, $p < 0.05$, FWE-corrected) of DA medication on their cortico-subcortical RSN functional connectivity. This finding showed an increase in functional connectivity between the BGLN and a cluster located in bilateral antero-medial thalamus in ‘PD Off’ relative to ‘PD On’ treatment conditions (peak $t = 4.98$; coordinates: $x = -6$, $y = -4$, $z = 14$). This result reflects either a *decreasing* effect on network connectivity of DA medication, or an *increasing* effect of DA withdrawal.

The thalamic region identified overlaps with the result of a different contrast examining the same RSN presented in Fig. 6.2Ai (see also next section 6.3.2), thus it is not portrayed independently. Indeed, the finding of minimal basic medication effects in paired analyses of PD patients' FMRI data mirrors that of their ITC behavioural data (Milenkova et al., 2011). It was therefore hypothesised further that comparing PD treatment conditions differentially relative to un-medicated HCs, in terms of 'normalising' or 'non-normalising' (quadratic) effects, might provide increased sensitivity to detecting DA effects on higher-order RSN functional connectivity.

6.3.2 – Non-normalising effects of DA treatment on cortico-subcortical RSN functional connectivity in PD

Additional analyses with quadratic contrasts testing for DA medication-dependent differences between PD patient data (On or Off DA) and that of HCs were employed. In line with expectations, these identified a number of significant non-normalising effects of DA medication on cortico-subcortical RSN functional connectivity. First, bilateral antero-medial thalamic functional connectivity with the BGLN was shown to be reduced significantly in PD On relative to both PD Off and HCs (peak $t = 5.55$; co-ordinates: $x = -6$, $y = -4$, $z = 12$; Fig. 6.2Ai). Second, a similar non-normalising DA connectivity effect was apparent between the BGLN and regions of the right (ventral and anterior mid-) striatum/putamen and anterior pallidum ($t = 4.29$; $x = 20$, $y = 16$, $z = -2$; Fig. 6.2Aii). Third, right posterior hippocampal/thalamic-BGLN connectivity ($t = 4.16$; $x = 8$, $y = -32$, $z = 6$) showed an 'inverse' non-normalising effect of DA *increasing* connectivity in PD On relative to both HCs and PD Off (Fig. 6.2Aiii).

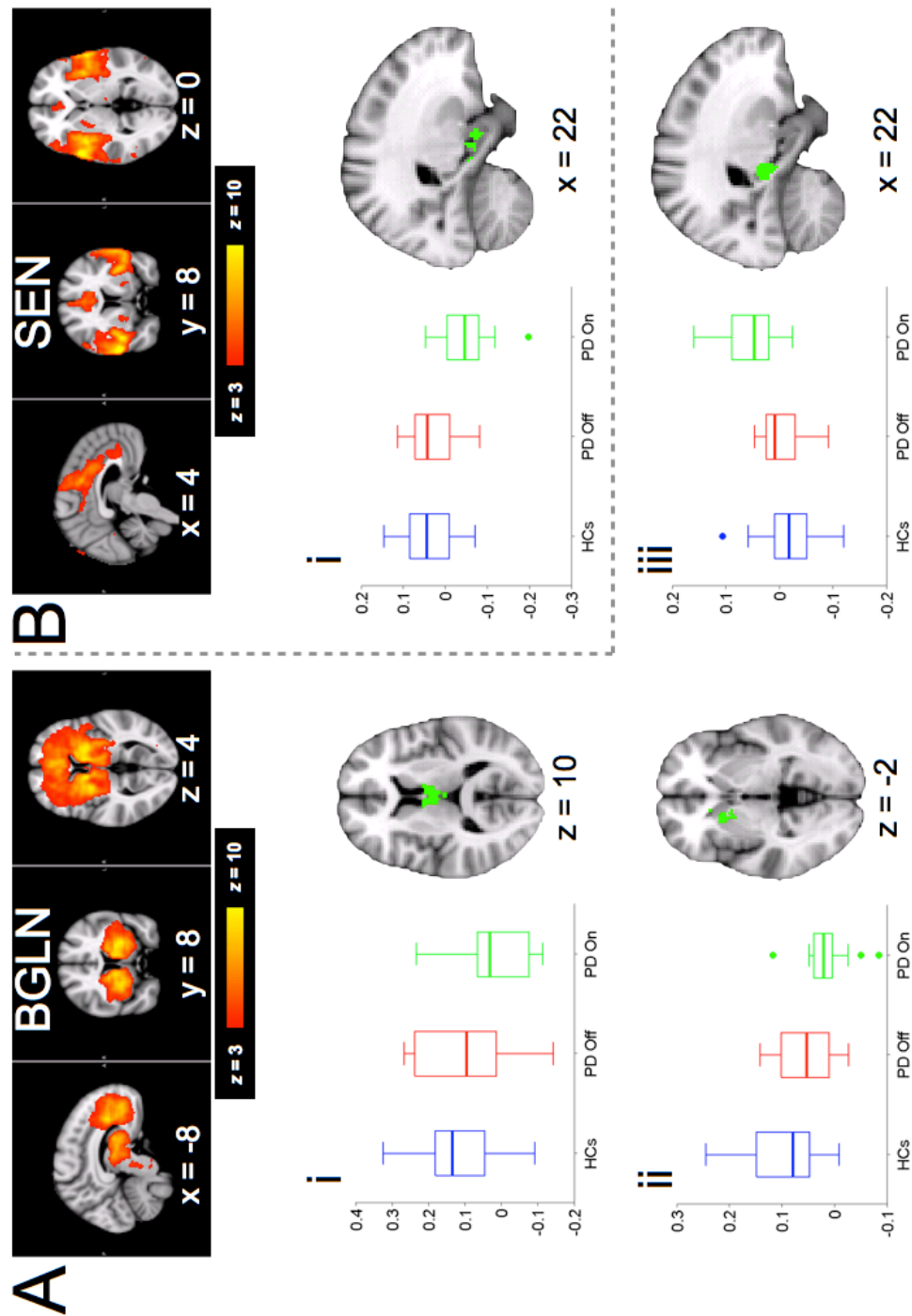
Finally, right hippocampal and amygdalar regions ($t = 4.89$; $x = 16$, $y = -14$, $z = -20$) also showed a non-normalising effect of DA, in terms of reduced connectivity with the fronto-temporo-parietal SEN in the PD On condition (Fig. 6.2B).

6.3.3 – Normalising effect of DA treatment on cerebro-cerebellar RSN functional connectivity in PD

No significant normalising effects of DA medication on RSN *cortico-subcortical* functional connectivity were found¹⁸. However, a single normalising effect of DA medication on connectivity was found within other functional circuitry, occurring between the cerebellar RSN and a cluster located primarily within the right amygdala ($t = 4.90$; $x = 18$, $y = -16$, $z = -14$), extending into midbrain regions (Fig. 6.3). This result identified a region of *increased negative* connectivity in PD Off, relative to PD On and HCs.

¹⁸This and other absences of a detected effect should be interpreted carefully, bearing in mind the often-conservative nature of null hypothesis testing.

Figure 6.2. Significant ‘non-normalising’ effects of DA treatment (i.e., normalising effects of DA *withdrawal*) on RSN connectivity subcortical functional connectivity measures ($t > 2.3$, $p < 0.05$, FWE-corrected), defined by SBCA ($N = 34$). (A) the BGLN displays reduced connectivity in PD On relative to PD Off and HCs with (i) bilateral anterior thalamus and (ii) right ventral-mid striatum/anterior pallidum, while displaying (iii) increased connectivity in PD On relative to PD Off and HCs in right posterior hippocampus. (B) (i) A fronto-temporo-parietal SEN shows reduced connectivity with amygdala/anterior hippocampus in PD On relative to PD Off and HCs. Clusters of significant regional effects are shown in green on representative images. Red-yellow overlays depict regions of high functional connectivity within the RSNs themselves, as defined by HCs group-ICA. Boxplots display RSN-ROI correlation coefficients for each group and condition.



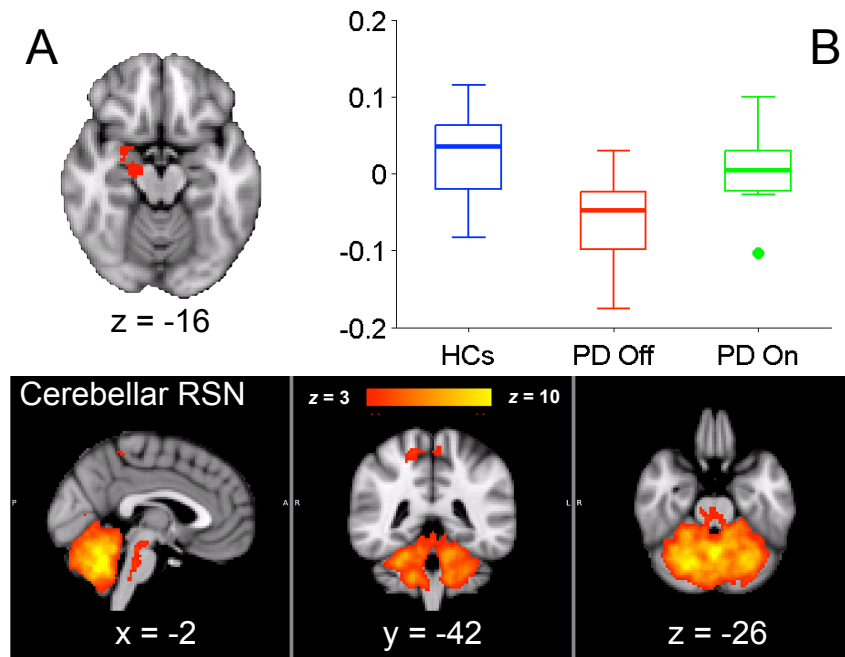


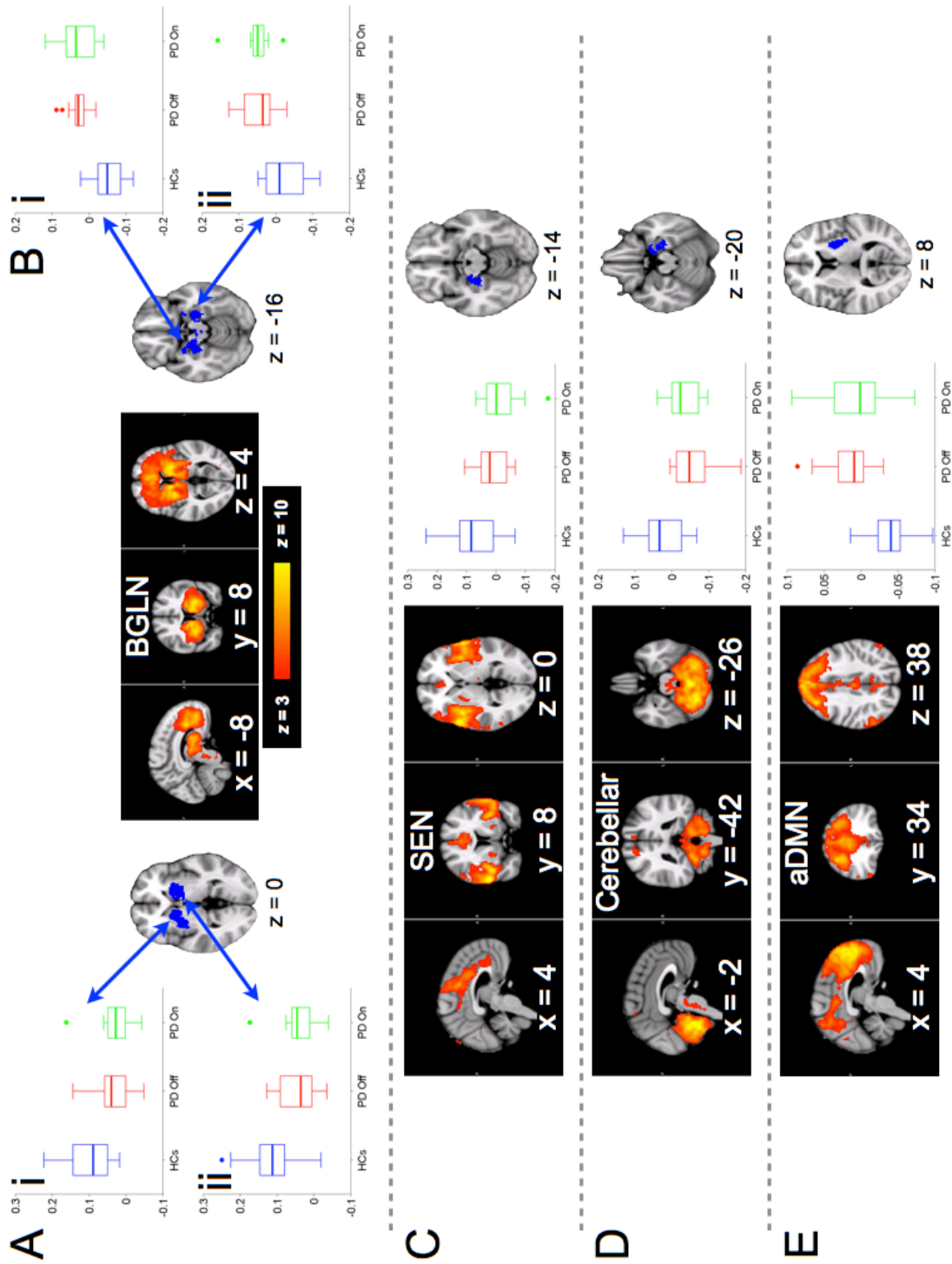
Figure 6.3. A significant ‘normalising’ effect of DA treatment (i.e., a non-normalising effect of DA *withdrawal*) on cerebro-cerebellar RSN functional connectivity measures ($t > 2.3$, $p < 0.05$, FWE-corrected), defined by SBICA ($N = 34$). Connectivity between the cerebellar RSN and (A) right amygdala, extending into midbrain regions is (B) reduced in PD Off relative to PD On and HCs. Formatting as described in Fig. 6.2 legend.

6.3.4 – Treatment-independent cortico-subcortical RSN functional connectivity differences in PD

In addition to the described effects of DA medication and modifications therein, a number of (seemingly treatment-independent) disease-driven effects on cortico-subcortical RSN functional connectivity were identified. A significant reduction in BGLN functional connectivity was found between the bilateral (ventral and extended) striatum/putamen and pallidum (left hemisphere cluster also extending into anterior thalamus: peak $t = 5.17$; $x = -6$, $y = 0$, $z = 2$; right $t = 5.38$; $x = 18$, $y = 14$, $z = 0$) in PD relative to HCs (Fig. 6.4Ai-ii). This widespread basal ganglia regional effect overlapped with the (more spatially constrained) region of right ventral/anterior mid-striatum described above as displaying a non-normalising connectivity effect of DA (as well as the regions showing a similar, but more extensive, thalamic effect; see section 6.3.2 and Fig. 6.2Ai-ii).

Conversely, bilateral hippocampal and amygdalar regions (cluster extending into midbrain and bilateral posterior thalamus; left $t = 4.73$; $x = -20$, $y = -20$, $z = -16$; right $t = 5.87$; $x = 26$, $y = -24$, $z = -10$) showed *increased* treatment-independent BGLN connectivity in PD relative to HCs (Fig. 6.4Bi-ii). In the right hemisphere, there was some overlap between these regions and the posterior hippocampus cluster described as showing an inverse non-normalising effect of DA (Fig. 6.2Aiii). Additional gross effects of disease were found, including reductions of both right hippocampal connectivity with the fronto-temporo-parietal SEN ($t = 5.06$; $x = 24$, $y = -26$, $z = -16$; Fig. 6.4C; although this region was also spatially overlapping with regions showing a seemingly treatment-dependent effect; Fig. 6.2B) and left hippocampal-amygdalar connectivity with the cerebellar RSN ($t = 4.30$; $x = -24$, $y = -26$, $z = -16$; Fig. 6.4D). Finally, a large cluster located within the left dorsal putamen showed increased (i.e., reduced negative) connectivity with the anterior DMN in PD relative to HCs ($t = 4.41$; $x = -32$, $y = -8$, $z = 8$; Fig. 6.4E).

Figure 6.4. Significant, 'treatment-independent', effects of PD on RSN cortico-subcortical functional connectivity measures ($t > 2.3$, $p < 0.05$, FWE-corrected), defined by SBICA ($N = 34$). (A) The BGLN displays reduced 'within-network' connectivity in bilateral (Ai, right; Aii, left) regions of striatum, pallidum and thalamus in PD patients relative to HCs. (B) BGLN connectivity with bilateral (Bi, right; Bii, left) amygdalo-hippocampal regions is *increased* in PD relative to HCs. (C) Right posterior hippocampus shows reduced connectivity with the fronto-temporo-parietal SEN in PD relative to HCs. (D) Reduced left amygdalo-hippocampal connectivity with the cerebellar RSN in PD. (E) Increased connectivity between left dorsal putamen and the antero-centric DMN in PD. Formatting as described in Fig. 6.2 legend.



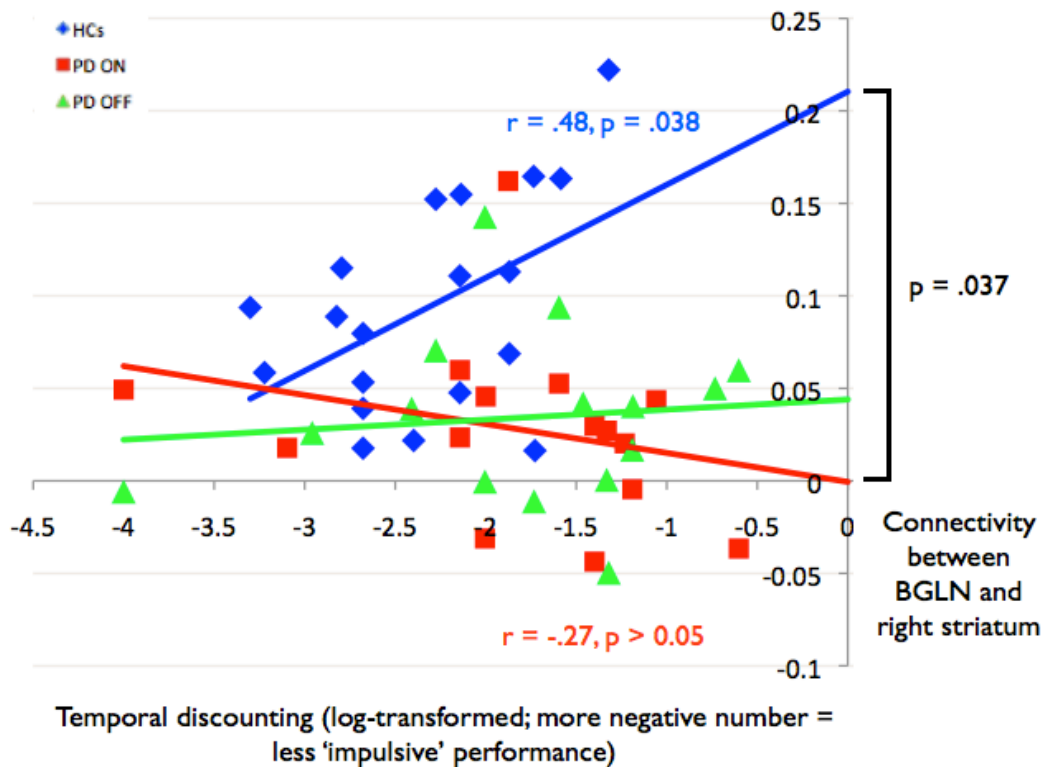


Figure 6.5. Association between BGLN-striatal functional connectivity and impulsivity in decision-making. A positive correlation (blue) was found between delay discounting behaviour (ITC questionnaire mean k -values, log-transformed to allow for parametric testing) and BGLN-striatal connectivity in HCs ($r = 0.48$, $p < 0.05$, two-tailed; $N = 19$), but not in PD patients (red = PD On; green = PD Off; $N = 15$). This significant impulsivity-connectivity association was significantly different ($p < 0.05$, two-tailed) from the equivalent negative (but non-significant) correlation in PD On DA medications (but not during DA withdrawal).

6.3.5 – Association between PD-related, but not DA-related, connectivity modulations and impulsivity

Post-hoc correlations revealed a significant association in HCs ($r = 0.48$, $p = 0.038$), but in neither of the PD treatment conditions ($p > 0.05$), between ITC questionnaire measures of impulsive decision-making (log-transformed mean k -values) and BGLN connectivity within an extended striatal cluster identified in the image analysis (Fig. 6.5). This association was found specifically in the right hemisphere region (Fig. 6.4Ai) displaying significantly greater BGLN connectivity in HCs relative to PD (independent of DA condition). Furthermore, this positive

correlation was significantly different from an equivalent negative (but non-significant; $r = -0.27$) correlation in PD On ($p = 0.037$).

6.4 – Discussion

6.4.1 – Summary

By using an approach combining resting-state FMRI with multivariate (ICA) and mass univariate (SBCA) functional connectivity analysis techniques, this study identified cortico-subcortical RSN functional connectivity patterns suggestive of aberrant systems-level processing in Parkinson's disease – a disorder of dopamine neurochemistry – relative to age-matched healthy control subjects. By studying the effects of dopamine agonist medication withdrawal in PD patients without clear neuropsychiatric comorbidities, considerable evidence was also revealed for 'non-normalising' effects of DA on a range of cortico-subcortical functional connections, echoing neuropsychological observations reported widely in the literature (for reviews see Cools, 2006; Dagher and Robbins, 2009; Cilia and van Eimeren, 2011). Notably, in this study sample, individual differences in impulsive decision-making were found to be associated with connectivity within striatal/basal ganglia circuitry in healthy subjects, but not in the PD patients in whom this same circuitry displayed aberrant functional connectivity. Findings (i) support the existence of compensatory neural connectivity mechanisms in PD, via increased recruitment of cerebellar and other large-scale resting-state networks in response to striatal dysfunction (e.g., Appel-Cresswell et al., 2010), while (ii) extending neurobiological evidence for the prior reported exacerbating effects on cognitive and motivational symptoms of modulating (increasing) dopamine neurotransmission pharmacologically,

with commonly-prescribed DA drugs (e.g., Dagher and Robbins, 2009; Weintraub et al., 2010).

6.4.2 – Functional relevance of DA withdrawal effects on connectivity in PD

It is perhaps surprising, given the known beneficial effects of DA medication on (largely motor) symptoms of PD, that this study found no normalising, but a number of non-normalising, DA effects on cortico-subcortical RSN connectivity patterns in the disease. In particular, there is a notable lack of any observable effect of DA medication withdrawal, or indeed of PD itself, on somatomotor cortical RSN connectivity with subcortical regions. This result differs somewhat from related, prior reported findings (Williams et al., 2002; Wu et al., 2009; Kwak et al., 2010) and, to a certain extent, those reported here in healthy subjects (Chapter 5). Divergence from other studies in PD patients could result from methodological differences, for example, in the systems-level group-ICA approach used here for detecting and selecting RSNs, or from the novel SBCA methodology used to characterise their cortico-subcortical network functional connectivity patterns (subtleties and caveats of distinct analytical approaches are detailed here in Chapter 2). Alternatively, across-study variability may relate to residual neuropharmacological effects of L-dopa medication in some PD patients (see section 6.4.4 and Chapter 5). Either way, this apparent lack of an effect does not rule out the possibility of DA medication exerting a normalising (or non-normalising) influence, over and above that of L-dopa, on RSN cortico-cortical functional connectivity patterns in motor and other neural circuitry. Furthermore, here cerebellar RSN connectivity with the right amygdala (and midbrain) was found to be normalised by DA drugs in patients. Future combined

exploration of a broader range of whole-brain systems-level and neurochemical processes in PD may therefore be warranted. Having said this, the BGLN system identified in this study displayed the most sensitivity to effects of PD and DA on connectivity (in terms of the largest number of significant results identified) and comprises striato-thalamo-orbitofrontal circuitry implicated in dopamine function, reward processing, motivated behaviour and related neuropsychiatric disorders (Honey et al., 2003; Everitt and Robbins, 2005; Ersche et al., 2010; Koob and Volkow, 2010; Sesack and Grace, 2010). It is, therefore, likely to be one of the most relevant RSNs for studying brain functions and dysfunctions underpinned by dopamine and dopaminergic pathology in PD.

Incidences of addictive behaviours and impulse control disorders, such as compulsive gambling and hypersexuality, are reported with increasing regularity in PD patients, having been observed in up to 10% of those treated with DA drugs (Dagher and Robbins, 2009; Weintraub et al., 2010; Cilia and van Eimeren, 2011). It is therefore conceivable that cortico-subcortical networks subserving some of the cognitive and motivational processes relevant for these behaviours would be altered differentially in PD On conditions, relative to PD Off and HCs (i.e., non-normalised). This assertion is supported by the current finding of differential associations, across groups and conditions, between large-scale cortico-subcortical network connectivity and impulsivity in decision-making. The inclusion criteria for this study dictated that no subjects, including PD patients, had comorbid addictions or other impulse control disorders. The absence of a direct correlation between ITC scores and connectivity in either treatment condition may, therefore, reflect this lack of overt behavioural pathology (although increased mean *k*-values in the PD patients in the current

study allude to changes in impulse control at the sub-syndromal level). Nonetheless, even in the absence of diagnosed comorbid disorders, RSN connectivity metrics do appear to differentiate patient and control groups in terms of their within-group associations (or lack thereof, in the case of PD) with individual variability in this behavioural measure of impulsivity.

The findings related to impulsive decision-making are, it must be noted, lateralised to the right hemisphere and restricted to the regions of striatum, putamen and pallidum identified by the contrast testing for disease-related, rather than DA-related, connectivity differences (Fig. 6.4Ai). Although this result does overlap partially with the cluster showing a non-normalising effect of DA medications on connectivity (Fig. 6.2Aii), this latter BGLN-striatal connectivity relationship shows no significant association with impulsive behaviour in any group or condition (although there is a comparable trend, both in this case and that of the treatment-*independent*/disease-related left hemisphere result; all $p > 0.05$). At a glance, this differential association with behaviour in similar, apparently overlapping, subcortical circuitry might engender interpretative difficulties. Equally, however, the very fact that these regions were differentiable in the original image analysis, based on their within-network functional connectivity patterns varying by association with PD or DA, may be functionally important. Fundamentally, the behavioural and RSN connectivity data do seem to corroborate each other, in terms of different (if spatially contiguous/overlapping) basal ganglia sub-regions expressing a distinct BGLN functional connectivity profile. Specifically, in the extended right hemisphere regions of striatum, putamen and pallidum, which exhibit gross disease-related connectivity reductions in PD, this profile is also associated with individual

differences in delay discounting in HCs. Conversely, the profile of the more focal striatopallidal cluster, which displays no such association with behaviour, nonetheless shows pathologically reduced connectivity *only* in the PD On DA medication state. These findings imply a functional dissociation between nearby populations of basal ganglia neuronal assemblies, in terms of whether their large-scale communication patterns are sensitive primarily to chronic deficiencies in dopamine neurotransmission or the (comparatively acute) neuropharmacological effects of attempting to ‘reverse’ this pathology via the exogenous administration of agonist drugs. Further, this dissociation may be reflective of the prior reported functional heterogeneity in primate ventral and dorsal striatal regions (the latter appear better represented by the more extensive PD-dependent result in the current analysis), which encode different information relevant for distinct aspects of reward processing in ITC (Cai et al., 2011). However, any additional interpretation would require such findings to be extended to human populations, in order to circumvent structural differences precluding a concrete cross-species comparison of functional neuroanatomy.

Finally, it is important to highlight that a number of other clusters described in the results share a degree of spatial overlap, despite being identified in separate contrasts sensitive to non-normalising DA and treatment-independent effects on connectivity *with a common RSN*. As well as the intra-BGLN connectivity focussed on above, this caveat specifically affects anterior thalamic-BGLN and right hippocampal-SEN functional connections. In this circuitry, therefore, a more graded, linear effect (e.g., HCs > PD Off > PD On) of dopamine modulation is implied, where DA withdrawal has only a *partial* normalising effect on RSN functional connectivity. This lack of complete

normalisation in certain signalling patterns could be present for a number of reasons; for example, the reasonably short duration DA withdrawal period (up to 12 hrs) used in this study, the fact that variability in drug treatment regimens was not straightforward to account for in the analysis, or possible limiting factors related to underlying neurological damage in PD patients.

6.4.3 – Compensatory neural mechanisms in PD

Both increases and decreases in RSN cortico-subcortical functional connectivity were found in PD patients, relative to age-matched HCs. Many (but not all) of these aberrant functional connections also appear to be spatially similar to those that are normalised by withdrawal of DA medications (i.e., non-normalised by DA) in PD. As noted in the previous section, the connectivity of relatively broad, bilateral regions of ventral and extended striatum (and pallidum) within the BGLN is reduced in PD regardless of medication conditions, while connectivity within the same RSN of a much smaller (although slightly overlapping) region of the right mid-striatum/anterior pallidum displays a normalising increase during DA withdrawal. It has been suggested previously that both increases in impulsive decision-making and neuroplastic reductions in striatal connectivity, which may be promoted by DA medications in PD, result from impaired dopaminergic functioning in basal ganglia (including striatum; e.g., Riba et al., 2008; Appel-Cresswell et al., 2010; Cai et al., 2011; Milenkova et al., 2011). Impulsive decision-making could therefore be a behavioural indication reflecting underlying neural mechanistic attempts to compensate for reduced striatal functional signalling/connectivity in PD. This is corroborated by the current differential connectivity associations with individual differences in delay

discounting (as measured by the ITC questionnaire), which show more impulsive behavioural decision-making (i.e., a higher k -value) to be associated with significantly greater intra-BGLN connectivity in HCs, but with *reduced* (although not significantly so) intra-BGLN connectivity in PD On. In addition, it has been proposed that striatal dysfunctions in PD may be actively compensated for by increased functional recruitment of the cerebellum (Appel-Cresswell et al., 2010). Support for this theory is provided by the current observation of DA-normalised (reduced negative) cerebellar RSN connectivity with the right amygdala, although no association was discovered between the functional connectivity of this circuitry and ITC scores. Moreover, similar (but bilateral) amygdalar and hippocampal regions were found to display greater connectivity with the BGLN in PD relative to HCs; an effect of disease opposite in direction to the reduced striatal connectivity found within the BGLN. The current results thus imply that compensatory functional circuitry in PD may extend beyond the cerebellum to more distributed cortical and subcortical networks.

6.4.4 – Methodological considerations

In this study, the repeated measures design within PD patients was not counterbalanced, as measurements were always taken firstly before, then during, DA medication withdrawal. This sequence was necessary in order to ensure the testing of clinically relevant treatment hypotheses related to ‘normalisation’ and ‘non-normalisation’ of functional connectivity by DA in PD, by maintaining experimental control of the temporally directed, ‘closer-to-causal’ influence of short-term medication withdrawal. While such a design feature could conceivably induce order effects that were difficult to account for

in this study, results in previous chapters revealed RSN functional connectivity metrics to be quite impervious to such effects that are incidental to the experimental manipulation in question. Nonetheless, future studies might explore this possibility, for example, by employing a placebo-controlled, crossover design in early stage, pre-medicated PD patients.

A second important methodological issue for the current study design was the variability in treatment type and regimen across PD patients (see section 6.2.1). Specifically, some patients were on DA monotherapy, while others were taking concomitant L-dopa medication. The latter was not withdrawn from those patients. Furthermore, doses of DA and L-dopa, as well as the exact DA medications being taken, varied across patients. No controlling measures could easily be applied here for these potential sources of variability, as it was important to limit interference with patients' ongoing medical care to the minimum required to test the study hypotheses. This constraint, along with the procedures limiting the DA withdrawal period to approximately 12 hrs, could provide an explanation for why only a single normalising effect of DA on connectivity was found in the current sample. However, the fact that many non-normalising DA effects *were* identified, even in PD patients with no comorbid behavioural disorders of inhibition or addiction, implies that such confounds do not, comparatively, influence sensitivity of the systems-level metrics applied here to the primary experimental pharmacological manipulation. Nonetheless, future investigations might benefit from alternative (e.g., cross-sectional) designs enabling direct assessment of the influences of these multiple factors on large-scale network connectivity patterns relevant for PD and other disorders of neurochemistry.

Finally, it is necessary to highlight the potential for functional connectivity differences between subjects or groups to be influenced by structural anatomical variability within neuronal networks (see also section 1.4.3). This is particularly pertinent to research conducted in neurological populations, who may exhibit varying degrees of macro- or micro-structural pathology in the underlying grey or white matter that account for some of the variability in BOLD signal fluctuation synchronicity (e.g., Seeley et al., 2009; Voets et al., 2012). The current study controlled for the possible (if unclear) impact of this in PD, by employing an ‘inclusive’ method of subcortical seed definition in subject-level SBCA and restricting group-level analyses to subcortical space defined by PD patients (see sections 6.2.4-5). These steps were taken in order to avoid biasing results towards being influenced excessively by gross structural differences between groups or sub-groups of individuals. It is also feasible to employ more advanced methods of, for example, regressing out the effect of voxel-wise grey matter density on functional connectivity metrics (see, e.g., Voets et al., 2012). In future investigations this approach might provide increased sensitivity to delineating neuroimaging differences driven by structural or functional variability and, moreover, may even increase sensitivity to the functional effects of acute or protracted neurochemical modulations via removal of variance associated with unrelated structural differences (although see Footnote [14], section 5.2.5). Nonetheless, as noted, little agreement and clarity exist currently regarding the contribution of structural neuropathology to functional deficits in non-demented PD patients (Burton et al., 2004; Dalaker et al., 2009; Menke et al., 2010; Jubault et al., 2011; Tinaz et al., 2011), such as were sampled in the current study.

6.4.5 – Conclusions

Using methods shown to be sensitive to differential dopamine neuropharmacological manipulations of large-scale brain networks (Chapter 5), this study characterised diverse systems-level functional connectivity modulations associated with Parkinson's disease – a disorder of dopamine neurochemistry – and dopamine agonist treatments in PD. In line with emerging evidence of potentially adverse behavioural effects of DA in PD, analyses showed DA to have largely 'non-normalising' effects on functional connectivity patterns of multiple large-scale cortico-subcortical networks corresponding to known dopaminergic pathways relevant for cognitive and motivational functional processes. In addition, a number of treatment-independent connectivity differences between patients and age-matched healthy control subjects were revealed. Finally, differential associations between connectivity within basal ganglia RSN circuitry and questionnaire scores of impulsivity in decision-making were also apparent in PD patients, relative to controls. The findings provide brain systems-level evidence to support current theories that: (i) while DA drugs are known to improve motor symptoms of nigrostriatal dopamine degeneration, they may also promote unwelcome cognitive changes in neurochemically predisposed individuals through hyperstimulation of the mesolimbic dopamine pathway; and (ii) neural mechanisms compensating for dysfunctional striatal and basal ganglia signalling patterns in PD proceed via increased recruitment of large-scale cerebro-cerebellar and cortico-subcortical systems. Overall, the findings identify functional connectivity pathways that may prove useful targets for future neuromodulatory intervention studies testing, for example, treatment

efficacy or side effects in PD and other disorders in which individual neurochemical predispositions influence therapeutic and behavioural outcomes.

Chapter 7 – Towards network functional connectivity patterns as imaging biomarkers

7.1 – Summary of research

The primary aim of this thesis has been to test the value and sensitivity of large-scale functional network analysis approaches to multi-subject fMRI data for characterising distributed neural interaction patterns in human brain cognitive and reward systems, in a manner both clinically relevant and biologically plausible.

Firstly, theoretical and methodological reasoning was outlined for favouring multivariate analytical techniques, sensitive to the activity fluctuations within and between resting-state network phenomena, in this applied approach to understanding fMRI data (Chapters 1 and 2). This was followed by a description of research applying measures of inter-RSN coupling and whole-brain RSN connectivity in an addiction context, to characterise associations between large-scale network connectivity patterns and withdrawal symptom improvements in abstinent smokers administered nicotine replacement therapy (Chapter 3). The findings provide support for RSN functional connectivity measures as useful imaging markers of clinical-psychopharmacological efficacy. However, exogenous nicotine (as in NRT) targets multiple neuropharmacological and neurotransmitter systems (Wonnacott et al., 2006) and thus (as is not the case with every other pharmacotherapy) has knock-on effects on a broad and potentially non-specific array of neural circuitry (Mansvelder et al., 2006;

Newhouse et al., 2011). Therefore, a second study addressed, in healthy subjects, the effects of individual differences in fundamental neurobiology and neurochemistry, specifically of dopamine D2 and D3 receptor sub-systems (for which a selective PET radioligand was available; Rabiner and Laruelle, 2010), on RSN functional connectivity (Chapter 4). It was found that the specific anatomical availability of distinct dopamine neuroreceptor subtypes (particularly D3R), as defined by [^{11}C]-(+)-PHNO PET, has distinct and topographic effects on connectivity between orbitofrontal cortices and multiple higher-order RSNs. This is the first established link between such novel, task-independent functional and molecular neuroimaging measures. Chapters 3 and 4 thus established methodological sensitivity, respectively, to both broad and specific neurochemical processes. Nonetheless, additional work was required to confirm that RSN connectivity patterns under specific neurochemical control could be modulated pharmacologically, in a manner both biologically plausible and relevant for individual subject stratification (Matthews et al., 2011). Following this, therefore, further pharmacological fMRI investigation of the dopamine system was performed in healthy subjects (Chapter 5). This cross-sectional study combined resting-state fMRI measures during broad-spectrum (agonistic and antagonistic) dopamine drug manipulations with whole-brain multivariate and anatomically targeted mass univariate analytical techniques. Results showed that linear effects of dopamine neuromodulation could be observed within both cortico-cortical and cortico-subcortical RSN cognitive and motor circuitry, while ‘inverted U-shaped’ dopaminergic effects were restricted to cingulate-striatal RSN functional interactions. These findings identified possible systems underlying pathogenesis and treatment efficacy in disorders of

dopamine deficiency (Cools, 2006; Cools and D'Esposito, 2011). Associations found between individual differences in trait impulsivity and sensitivity to dopamine-dependent (particularly cortico-subcortical) connectivity modulation further implied a role for these techniques in pinpointing or stratifying maladaptive processes relevant for such neuropsychiatric disorders (Cools et al., 2007). Finally, in order to establish whether or not the anatomically informed mass univariate analytical techniques introduced here could reveal clinically relevant effects in a direct context of disordered dopaminergic processing, they were applied in a study assessing patients with Parkinson's disease, before and after withdrawal of their dopamine agonist medication and in comparison with age-matched healthy control subjects (Chapter 6). These results showed multiple 'normalising' effects of DA withdrawal on cortico-subcortical RSN connectivity patterns, perhaps reflecting the 'non-normalising' cognitive effects of DA medication that are reported with increasing consistency in some PD patients (Dagher and Robbins, 2009). In addition, resting connectivity within striatal circuitry, found to be aberrant in PD, was associated with measures of impulsive decision-making in healthy control subjects *only*.

7.2 – General conclusions

Broadly, the current work indicates that resting-state FMRI data analysis techniques sensitive to large-scale neural network activity and connectivity modulations can provide systems-level signatures of neurochemical modulations relevant for clinical-psychopharmacological applications. In terms of more specific neurobiological conclusions that can be drawn from this thesis, it has been shown that diverse effects of drug manipulations targeting primarily

dopaminergic and, to some extent, also cholinergic (via nicotinic receptors) neurochemical systems can be characterised and monitored in this way. The findings with respect to dopamine modulations reveal associations within known major (mesolimbic and cortico-subcortical) dopaminergic pathways, but they are not concentrated exclusively therein. This distributed array of systems-level dopaminergic effects overlaps somewhat with those found to be modulated by nicotine, hinting at novel possibilities for therapeutic targets in neuropsychiatric disorders with a likely molecular basis, perhaps realisable via future investigation of neuromodulatory treatments targeting one or more key nodes of large-scale, higher-order, cortico-subcortical *and* cortico-cortical networks. Such investigations could assess either pharmacological, electrical/magnetic stimulation or even cognitive interventions (among others), or a mixture thereof. Indeed, it may be useful to compare the ability of multiple approaches to modulate activity or connectivity within reward processing and/or cognitive control neurocircuitry of interest, in order to classify ‘what works for whom’ in treating diverse symptomatology within specific patient populations and sub-populations.

It is apparent from the findings across all chapters that a major application area for further development of the techniques employed here is in the study of addictive and impulse control disorders. However, the extensive variability that is expressed across patients diagnosed with such conditions, for example in terms of symptom severity, comorbidity or risk factors for relapse, may hinder progress in this field. Bearing this in mind, a specific strength of the current findings for delineating some of this individual variability may stem from the multiple molecular pathways implicated here. In particular, the ability to

monitor systemic neuromodulations in both dopaminergic and cholinergic pathways provides insight into distinct, distributed brain networks underlying (among other functions) reward/motivational and attentional/concentration processes, respectively. In this way, it is apparent that brain network functional connectivity measures can provide markers of highly diverse symptoms, which, nonetheless, can characterise similar diagnoses or disorders. Future investigations might also harness alternative neurochemical modulations, for example of serotonergic, noradrenergic, glutamatergic or GABAergic systems, which are known to interact with those studied here and are equally fundamental to mechanisms of neuronal communication driving similar functional processes of higher-level cognition and motivated behaviour (e.g., Wonnacott et al., 2006; Dayan and Huys, 2009; Moran et al., 2011).

Continued refinement and combination of the techniques outlined here, within studies examining both healthy and neuropsychiatric populations, will undoubtedly be beneficial in addressing key computational, cognitive and clinical neuroscientific questions; further enabling the effective and efficient classification and manipulation of a range of normal and abnormal neural conditions. However, a number of critical challenges, both interpretative and methodological, remain to be addressed in the continuation of this work.

7.3 – Caveats, controversies and future directions

7.3.1 – Cognitive and behavioural relevance of RSNs

The study of RSNs has revealed many interesting observations of the way in which spontaneous connectivity patterns alter under different conditions¹⁹. Nonetheless, the concrete behavioural meaning of these inherent processes, seemingly fundamental to the human neural functional architecture, remains elusive. Task-based fMRI studies have provided insight into the functions of select brain regions surpassing pre-selected statistical thresholds in line with derived task models. However, these models may lack sensitivity to variability in between-region or between-network signalling in complex cognitive states, such as characterises neuropsychiatric disorders. Fortunately, it is rapidly becoming clear that the same large-scale systems that underpin these variable states can be characterised in the absence of specific tasks, making initial conceptual trends towards dichotomising ‘experimental’ and ‘resting’ states as describing fundamentally unrelated cognitive conditions increasingly outmoded (e.g., Fox and Raichle, 2007; Poldrack et al., 2009; Smith et al., 2009; Northoff et al., 2010a; Van Dijk et al., 2010). However, the unrestricted nature of resting-state data obviously engenders something of an interpretative minefield (Buckner and Vincent, 2007; Morcom and Fletcher, 2007; Raichle and Snyder, 2007). To better classify the fundamental human neural functional architecture for nosological applications, it may therefore be beneficial to adapt *task-based* approaches in order to complement and bolster the interpretations garnered from studies of *spontaneous* activity patterns, or RSNs. Hints at how such an approach might

¹⁹Portions of this section are adapted from a related publication; Cole DM, Smith SM, Beckmann CF (2010a) Advances and pitfalls in the analysis and interpretation of resting-state fMRI data. *Front Syst Neurosci* 4:8.

develop come from studies employing meta-analytic techniques (e.g., Smith et al., 2009; Laird et al., 2011) and those augmenting the experimental method to explore (comparatively) subject-driven, ecologically valid ‘mental states’, rather than experimenter-derived, model-conditional variability (e.g., Harrison et al., 2008b; Poldrack et al., 2009; Shirer et al., 2012). These two approaches will likely be complementary, as the historic tendency for fMRI studies to avoid reporting or discussing task-related *deactivations* places limits on the information available to meta-analytic approaches comparing resting-state and task-activation studies (Smith et al., 2009).

Although task-informed fMRI approaches may help to develop interpretations related to RSN function, an experimental trend for successive acquisitions within the same scanning session raises questions of the potential for order effects. In this thesis, no such order effects were identified and reciprocal task-rest interactions were not examined as effects of interest, although several recent studies have demonstrated direct evidence of the subsequent influence exerted by task performance and related evoked neural activity over network activity in resting periods, and *vice versa* (for reviews see Northoff et al., 2010a, 2010b). Indeed, the existence and strength of two-way interactions between task and spontaneous/RSN activity have been linked to adaptive learning (Albert et al., 2009; Lewis et al., 2009), response to or recovery from high cognitive load (Pyka et al., 2009; Van Dijk et al., 2010) and individual differences in (Fox et al., 2007; Hesselmann et al., 2008; Hasson et al., 2009; Sadaghiani et al., 2009), or fine-tuning of (Singh and Fawcett, 2008), cognitive performance. Early evidence has even emerged for specific neurochemical and neurobiological correlates of similar phenomena, established using magnetic

resonance spectroscopy (Duncan et al., 2011) and indicating a potential novel avenue of investigation for neuropsychopharmacological research. Further study is required to establish the full range and nature of task-rest interactive relationships over varying periods of time and, further, to probe their biological relevance.

7.3.2 – Applications and extensions

Following initial evidence of the potential utility of RSN measures for characterising a number of neuropsychiatric disorders, applications have extended rapidly to investigate markers or mechanisms of individual differences, disease, development, neuroplasticity and (to some extent) treatment efficacy. Of particular importance for clinical applications and drug discovery is the fact that, relative to task-fMRI, resting-state investigations require minimal cognitive model compliance. Therefore, they allow for the study of differences in brain dynamics and spatial signatures in non-normal/research-atypical populations, including infants, sedated/unconscious subjects or those with severe cognitive or physical impairments, as well as for task-unbiased study of human and non-human subjects administered with established or novel pharmaceutical agents. To date, initial evidence for the diagnostic and prognostic applicability of these measures comes primarily from targeted studies in small-to-intermediately sized clinical (or pre-clinical) populations. However, large ‘proof-of-concept’ studies with high statistical power (see e.g., Biswal et al., 2010) and ongoing meta-analytic research (Smith et al., 2009; Laird et al., 2011) could make valuable contributions to the field if extended appropriately to such targeted application areas. The ability to simultaneously test multiple brain systems, afforded by the

methods presented in this thesis, is also important in contexts of disease. For example, PD has varied comorbid presentations and known secondary effects of treatment, which may be differentially represented across multiple neurobiological networks and systems. Ideally, future applied research will also be bolstered by the integration and association of information gathered across multiple levels of biological complexity (i.e., from the genetic level, through molecular, cellular and discrete neuronal assembly levels to that of distributed, large-scale brain networks).

‘Multi-level’ research spanning the (perhaps rather biophysically detached) realms of genes and systems-level circuitry, for example, has associated specific allele variants implicated in neurodegeneration (Filippini et al., 2009), neurodevelopment (Thomason et al., 2009; Wiggins et al., 2012) and cognitive function (Liu et al., 2010; Gordon et al., 2012) with RSN functional connectivity patterns (interpreted as phenotypes) in healthy subjects. Persuasively, characterisation of similar neural connectivity patterns extends into domains of actual clinical presentation, for example in Alzheimer’s disease (Greicius et al., 2004; Rombouts et al., 2005; Wang et al., 2007a) and other neurodegenerative diseases (Seeley et al., 2009; Kwak et al., 2010), normal aging (Andrews-Hanna et al., 2007; Damoiseaux et al., 2008; Dosenbach et al., 2010), as well as multiple neuropsychiatric (Liu et al., 2006; Greicius et al., 2007; Salvador et al., 2007; Jafri et al., 2008) and neurodevelopmental disorders (Zang et al., 2007; Castellanos et al., 2008; Di Martino et al., 2009; Paakki et al., 2010).

Intra- and inter-RSN connectivity patterns of the DMN, in particular, have been implicated in a diverse range of brain functions and disorders (for reviews see Buckner et al., 2008; Greicius, 2008; Broyd et al., 2009), although other RSNs

are also widely implicated. While providing some encouragement, these diverse findings also highlight that much work is yet required to gauge not just the *sensitivity*, but also the *selectivity*, of RSN and other imaging ‘markers’. The work presented here, by bridging molecular- and systems-level phenomena (and considered in combination with other multi-level research), goes some way towards achieving this important goal.

Crucially, it is yet to be determined whether or not RSNs can provide imaging markers, at the level of individual cases, of healthy or aberrant processing relevant for *both variability in and disorders of* behaviour, personality or cognitive functioning, as well as any associated responses to clinical or experimental intervention. These potential shortcomings have been widely noted and have implications for the utility of such imaging techniques in, among other things, patient stratification and personalised medicine (Greicius, 2008; Matthews et al., 2011; Linden, 2012). Despite such concerns, pseudo-diagnostic information associating RSN function with within-subject dynamic, intervention-related changes in neurophysiology or behaviour are already apparent, for example in fields of learning (Albert et al., 2009; Lewis et al., 2009) and (to some extent) pharmacological intervention (Anand et al., 2005; Cole et al., 2010b; see also Chapter 3). Additionally, preliminary evidence exists for RSN-related metrics as potential screening devices, notably in Alzheimer’s disease (Rombouts et al., 2005; Fleisher et al., 2009) and epilepsy (Zhang et al., 2012). However, it must be noted that the majority of studies investigating the diagnostic value of RSNs in neuropsychiatric disorders do not account for tissue micro-structural differences between groups or subjects that could limit the ability to make purely ‘functional’ interpretations (Voets et al., 2012).

It is apparent that more efficient versions and better targeted applications of the methods described here and elsewhere in the literature are required, particularly for the purposes of evaluating and predicting markers and mechanisms relevant for neuropsychiatric, clinical-psychopharmacological and drug development domains (see also Chapter 1 and section 3.4.4 for discussion of additional caveats affecting pharmacological fMRI studies in general).

Fundamentally, many of the major barriers to establishing reliable and useful imaging biomarkers, a comprehensive neurocognitive functional ontology or “human (functional) connectome” (Sporns et al., 2005; Biswal et al., 2010; Van Essen and Ugurbil, 2012), can be reduced to a single source: the complexity of the human brain. This inherent complexity fuels expansion of the range of techniques with which neuroscientists study the brain, affording great potential for technical and conceptual divergences to develop and, ironically, therefore fostering the difficulties facing usefully directed research in this area. The limitations and opportunities for refinement in various analytical and theoretical subdivisions of brain connectivity research (of which the study of RSNs is a subtopic) have been summarised by a number of excellent recent reviews (Fox and Greicius, 2010; Rowe, 2010; Friston, 2011; Valdes-Sosa et al., 2011; Wig et al., 2011; Smith, 2012). Across these reviews, a number of concerns with the current research trends are consistently identified. These include a general need to reinforce the importance of hypothesis-driven science in what can often be a highly exploratory field of study, along with specialised topical issues that may be addressed under the umbrella term of ‘refining definitions’ of the data.

7.3.3 – Refining definitions (i): Brain network spatiotemporal variability

A passing glance through the RSN literature can identify inconsistencies across studies in terms of the spatial patterns identified and labelled as specific networks. For example, prior studies have proposed that distinct patterns of functional connectivity exist, which share some spatial overlap in or around their key nodes, but can be associated with different aspects of attention, emotion and cognitive control (Fox et al., 2006; Dosenbach et al., 2007; Seeley et al., 2007; Vincent et al., 2008). Although it is likely that functional dissociations partly drive these results, some of this spatial variability may be due to subjective factors introduced into the analysis, such as the choice of seed for ‘network’ definition in SCA (see Fig. 2.1 and section 2.1.2) or fixing the model order in ICA (see section 2.1.3). In an ICA context, to reiterate, there is potential for ‘splitting’ or ‘fusing’ of network units identified by increasing or reducing, respectively, the number of component outputs (Smith et al., 2009; Abou-Elseoud et al., 2010). Methodological considerations aside, it is highly likely that spatial co-activation patterns identified as distinct RSNs will subserve a complex range of functions even when ‘fractionated’ (i.e., at their least macroscopic/at high model orders), particularly those with nodes in higher association and midline cortices, such as sub-systems of the DMN, SENs and other frontoparietal networks (Fox et al., 2006; Seeley et al., 2007; Andrews-Hanna et al., 2010; Laird et al., 2011; Leech et al., 2011). Indeed, currently it is unclear whether or not it is possible to delineate fully and classify the connectivity patterns of spatially segregated nodes of each of these RSNs in terms of a small number of discrete cognitive and behavioural functions or dysfunctions (e.g., Mennes et al., 2010; Menon, 2011).

Further to inconsistencies in the spatial patterns identified as distinct RSNs, it is evident that spatiotemporal connectivity relationships can also vary *between* networks. There is, for example, a great deal of variability in the reported literature in terms of the degree (and direction) of correlations (and anti-correlations) observed between the DMN and multiple, inconsistently-identified ‘task-positive’ networks, or SENs. As discussed, these observations can have functionally relevant associations (Kelly et al., 2008; Cole et al., 2010b). They also re-emphasise the existence and potential importance of connectivity nonstationarity, which can be observed both within and between distinct networks (Chang and Glover, 2010; Kiviniemi et al., 2011; Hutchison et al., 2012; Smith et al., 2012a). Recent analysis of resting-state BOLD fMRI data acquired at ultra-high temporal resolution provides further evidence that these unitary dissociations may be functionally relevant, reflecting transient associations of certain brain regions with more than one ‘network’ during a given time period (Smith et al., 2012a). Future applied work should attempt more directly to address the nonstationary dynamic nature of RSN connectivity associations and the factors that modulate this, over time or across conditions, as it may be a critical component of their psychological relevance and, therefore, their pertinence to neuropsychiatric functional pathology (Smith, 2012).

7.3.4 – Refining definitions (ii): Neuronal interpretations of BOLD RSNs

In humans, attempts to more directly link BOLD signal fluctuations to neuronal population activity have employed simultaneous fMRI and EEG data acquisitions. Some of these studies report evidence, in the resting state, of associations between RSN network activity and specific power profiles within

broader EEG frequency spectra (e.g., Laufs et al., 2003; Mantini et al., 2007). However, with respect to caveats regarding the frequency-specificity of resting FMRI data (see section 1.4.2) and, moreover, their somewhat indirect relationship with broadband EEG spectra, it remains unclear if low-frequency BOLD oscillations can be interpreted as relating directly to the oscillatory activity of neuronal assemblies. On the one hand, valuable multimodal research with BOLD FMRI and direct electrophysiological recordings, for example in the primary sensory or motor cortices of non-human primates (Logothetis et al., 2001; Goense and Logothetis, 2008; Shmuel and Leopold, 2008) or human neurosurgical patients (He et al., 2008), encourages inferred associations between activity in these two data types, despite their characteristic differences in terms of temporal resolution and underlying neurophysiological causes. Indeed, with signals providing representations of basic sensory, motor or perceptual information processing, it may be the case that straightforward comparisons can be made across imaging modalities. On the other hand, given that neural activity across a broad range of oscillatory frequencies is believed to contribute to multi-faceted cognitive functioning (Varela et al., 2001; Buzsaki and Draguhn, 2004), drawing similar conclusions about possible interactions between low-frequency oscillations measured by FMRI and higher frequency neuronal oscillatory activity, measurable via both EEG (e.g., Laufs, 2008) and direct cortical electrophysiological recording methods (e.g., Conner et al., 2011), is considerably more complex. As a notable and informative example of such potential complications, Conner and colleagues recently highlighted that BOLD signal dynamics (in this case task-related percent signal change) vary across the

brain space (i.e., across different lobes) in terms of their specific associations with spectral activity from local field potential recordings (Conner et al., 2011).

One suggested analytical improvement for reconciling the measurements made by simultaneous resting-state EEG and fMRI recordings lies in the study of EEG ‘microstates’. Relative to power spectra distributions in specific frequency bands, these transient events are shown to couple more closely in the time domain with the activity of a select few RSNs identified in contemporaneous resting-state fMRI data (Musso et al., 2010). This approach can be useful for exploring the nonstationary temporal qualities that undoubtedly are expressed by neural activity and connectivity patterns over multiple scales (Popa et al., 2009; Chang and Glover, 2010; Van de Ville et al., 2010; Conner et al., 2011; Smith et al., 2012a). However, the scalp-based recording of these high-speed data makes them difficult to fully co-localise with more than a few of the spatially specific, but temporally blurred, RSN measurements attainable with BOLD fMRI (Musso et al., 2010). Nevertheless, convincing evidence of the neurophysiological relevance of RSNs has been provided recently through comparison with magnetoencephalography (MEG) data acquired in the resting state. Specifically, the application of spatial and temporal ICA techniques, respectively, to fMRI and MEG data modalities (Brookes et al., 2011), reveals high spatial convergence between the macroscopic patterns of spontaneously co-activating BOLD fMRI signals (representing multiple RSNs) and those of coherent neural signals in specific frequency bands measurable with MEG. This builds on earlier evidence showing that, as with EEG, accounting for transient neural states, or nonstationarity, seemingly enhances the detection of fMRI-like RSNs with MEG (de Pasquale et al., 2010). These collective resting-state MEG findings herald

promising methodological advances, as the spatial source localisation techniques applicable to MEG data are far more accurate than those applied to EEG data. The apparent frequency-dependent nature of these MEG-derived spatial networks is, it must be noted, still somewhat debatable and further investigation is required of their dynamics (i.e., spatial comparison with fMRI requires temporal smoothing and simultaneous temporal comparison of the two data types is not possible with current technologies), between-network coupling and relevance for cognition and behaviour (Brookes et al., 2011). Ultimately, however, further development of multimodal neuroimaging and electrophysiological techniques, associated analysis approaches and convergent findings should offer greater insight into the neural communication mechanisms and psychological functions of large-scale brain network connectivity patterns.

7.3.5 – Refining definitions (iii): Correlation and causality

Significant correlations (e.g., between the connectivity strength of a given RSN and behavioural or personality measures, or even neurobiological variables such as receptor availability) certainly encourage conclusions of large-scale network (RSN) function being relevant to higher cognitive or fundamental neuronal functions, as well as clinical presentations or treatment responsiveness. However, without direct evidence of the modulatory influence of variability in one metric on that of another, one can only speculate about the precise order of events and causal relationships between the nodes in this dynamic processing stream.

Unlike ‘effective connectivity’ measures, functional connectivity measures provide no information about the potential influence exerted by activity in one

brain region on that in another (Friston, 1994, 2011). Therefore, interest is growing in the potential to exploit effective connectivity techniques for defining directional, causal relationships in resting-state fMRI data. Until recently, it was widely assumed that fMRI alone might be unsuitable for attributing causality to spontaneous activity in this way (Ramsey et al., 2010; Smith, 2012). However, the application of advanced mathematical network models to both simulated and ‘real’ BOLD data has, even more recently, provided surprisingly optimistic assessments for *some, but by no means all*, of the network modelling techniques available and increasingly applied to such data (Friston et al., 2011; Li et al., 2011; Smith et al., 2011; Smith, 2012). However, it is also acknowledged that much room for improvement is available, in the context of techniques that show methodological promise, to actually provide measures of large-scale network causality and directionality that are interpretable in terms of psychological processes (for review see Smith, 2012). In particular, it has been noted that analysis techniques based on the temporal lag between spatially distinct signals, such as Granger causality methods (which, due to their ease of use, have been among the most commonly applied to fMRI data), are technically unsuitable for the purposes of assessing directionality in resting-state fMRI data acquired with current technology and, therefore, the results of such studies should be reported and interpreted with prudence (Smith et al., 2011, 2012b). Perhaps, therefore, further work is required using alternative network modelling techniques that *do not rely on* measuring temporal lag (see, e.g., Smith, 2012), before interpretations of ‘true’ (within- or between-network) connectivity dependencies (Sridharan et al., 2008; Uddin et al., 2009) and theoretical models based on these, with wide-ranging, potentially clinical, implications (Menon, 2011), can be accepted as

relating to anything other than incidental network- or node-specific over- or under-activity.

A promising current area of research is focussed on the merger between emerging concepts of ‘computational psychiatry’ (Montague et al., 2012) and dominant approaches to cognitive neuroscience, for example ‘neuroeconomic’ theories of decision-making, reward processing and social behaviour relevant for neuropsychiatric pathology (Behrens et al., 2009; Kishida et al., 2010; Pine et al., 2010; Summerfield et al., 2011). It remains to be established whether or not RSN metrics can be integrated with these concepts. However, if this were possible, it could enhance the amount of causal/directional connectivity information obtainable from RSNs and thus serve to solidify their status as useful intermediate phenotypes, or imaging ‘biomarkers’.

In this thesis, large-scale network functional connectivity patterns varied and showed modulation in biologically plausible fashions, in line with dopamine receptor availability (Chapter 4) and dopamine-dependent linear and nonlinear drug response profiles (Chapters 5 and 6), as well as in correlation with certain patterns of behaviour (Chapters 3, 5 and 6). A growing body of theoretical and empirical evidence supports the notion that modulatory neurobiological systems, such as the dopaminergic neurotransmitter system, operate on the basis of computational mechanisms that inform their behavioural outputs in line with mathematical models of learning and principles of probability (e.g., Braver et al., 1999; Montague et al., 2004; Dayan and Huys, 2009; Pine et al., 2010; Moran et al., 2011; Friston et al., 2012). Future attempts to relate RSN functional connectivity phenomena more directly *and causally* to individual differences relevant for neuropsychiatric and psychopharmacological applications may

therefore benefit from integration with computational and biophysical modelling methods. Such models could conceivably be applied to resting-state BOLD fMRI data through, for example, the use of anatomically or physiologically informed priors at various levels of neurobiological complexity (Fernandez Galan, 2008; Honey et al., 2009; den Ouden et al., 2010; Friston et al., 2011; Hunt et al., 2012). The application of advanced causal models to ‘real’ data, combined with accurate information about the spontaneous dynamics of neurochemical and electrophysiological processes, as well as the effects of these factors on the measurable haemodynamics (including, e.g., accounting for potential nonlinear connectivity relationships between distributed regional signals and nonlinear *coupling* associations between haemodynamics and neural activity; Smith, 2012), would offer a powerful tool to explore the meaning of complex human brain functions, supported by large-scale networks, that are difficult to probe comprehensively within fully model-based paradigms.

Remnants of confusion clearly still surround causal connectivity relationships in resting-state fMRI data. For this reason, information provided by multimodal (and multi-level; see sections 7.3.2 and 7.3.6) approaches to measuring and interpreting spontaneous network activity and connectivity patterns will continue to provide important insights into these data in the near-to-medium term. For example, via combining resting-state (and task) fMRI with such varied techniques as structural connectivity (Honey et al., 2009) and covariance (Seeley et al., 2009) MRI metrics, contemporaneous/consecutive EEG/MEG acquisitions (see section 7.3.4), transcranial magnetic stimulation (Eldaief et al., 2011), optogenetic (Lee et al., 2010) or, most relevant to the experimental work described in this thesis, pharmacological manipulations, it

should be possible to relate regional connectivity modulations more directly to distinct ‘downstream’ or ‘upstream’ modulations in other regions (or network nodes). In lieu of a field-wide consensus on the ideal model for large-scale brain network (RSN) connectivity, these technically advanced approaches will help to describe systems-level connectivity relationships in terms of ‘real’ neuronal cause and effect processes (e.g., excitation, inhibition and equilibrium) at multiple levels of neurobiological complexity, as well as their impact on cognitive and behavioural functions, dysfunctions and responsiveness to experimental intervention.

7.3.6 – Future impact

The results presented in this thesis provide reassuring support for the useful application of functional network analysis methodology to address questions relevant for clinical decision-making and drug development domains. One of the most important future goals for this line of research will be to develop the multiple types of fMRI functional connectivity analytical techniques discussed (e.g., subject-specific RSN connectivity characterisation, connectivity-based functional parcellation and others), in order to enable combination with the rich data pool available from other connectivity-sensitive modalities (e.g., electrophysiology, structural neuroimaging). This integrative approach should enable ‘connectivity-based fingerprinting’ (Leergaard et al., 2012), or profiling of population and subjective connectivity characteristics across multiple levels of biological complexity, which could be further combined with non-imaging state- and trait-level information (e.g., behavioural/personality data compilations, genotyping).

In concert with this consolidation of functional information, it would be useful for future research to build on the results of the study described here in Chapter 4, by continuing to investigate the molecular neurobiological and neurochemical underpinnings of large-scale systems-level connectivity (Cole et al., 2012a). Indeed, this combined PET and fMRI work (see also Hahn et al., 2012, for an equivalent approach; and see Heinz et al., 2004, for a related approach) paves the way for future experiments and research programmes, using multimodal neuroimaging (and techniques sensitive to other levels of biological complexity; see e.g., Meyer-Lindenberg, 2009; Montague et al., 2012) to map fully the associations between multiple, distributed and specific neuroreceptor subtypes and large-scale functional (and anatomical) network connectivity across the whole brain. In effect, this work would serve as an *in vivo*, dynamic, in human extension to prior post-mortem tract-tracing and autoradiography research, predominantly conducted in primates, comparing anatomical connectivity and receptor binding data (e.g., Kotter et al., 2001; Zilles and Amunts, 2009). Initial experiments could map the receptor and other molecular ‘profiles’ of distinct large-scale functional network (RSN) connectivity patterns. Guided by these results, further applied extensions could then assess modulatory effects of targeted pharmacological (and perhaps pharmacogenetic) manipulations on these same systems. The potential for this kind of research to cover and map a more comprehensive range of ‘multi-level’ associations has, for both methodological and financial reasons, thus far been grossly under-explored. Such an approach is also more compatible with the likely ‘state-dependent’ temporal/biophysical relationship between molecular and systems-level (and consequently behavioural) phenomena than studies attempting to assess direct

associations between the latter dynamic functional *states* (as measured by BOLD fMRI) and genetic *trait* information.

Ultimately, through additional higher-level clustering of the functional, neurochemical and other data characteristics resulting from this integrative approach into various ‘classes’ and ‘sub-classes’, it should be possible to generate novel hypotheses related to stratification and prediction of the responses of state-level phenomena with respect to specific neurobiological traits or neuromodulatory interventions. These hypotheses could then be verified at the level of groups or individual subjects/patients in experimental or clinical settings. It is only with such ambitious and multi-level approaches to probing and characterising the complex signals generated by the human brain that we can hope to achieve a comprehensive mechanistic understanding of its functions, dysfunctions, resilience and manipulability.

References

- Aarsland D, Larsen JP, Lim NG, Janvin C, Karlsen K, Tandberg E, Cummings JL (1999) Range of neuropsychiatric disturbances in patients with Parkinson's disease. *J Neurol Neurosurg Psychiatry* 67:492-496.
- Abou Elseoud A, Littow H, Remes J, Starck T, Nikkinen J, Nissila J, Timonen M, Tervonen O, Kiviniemi V (2011) Group-ICA Model Order Highlights Patterns of Functional Brain Connectivity. *Front Syst Neurosci* 5:37.
- Abou-Elseoud A, Starck T, Remes J, Nikkinen J, Tervonen O, Kiviniemi V (2010) The effect of model order selection in group PICA. *Hum Brain Mapp* 31:1207-1216.
- Achard S, Bullmore E (2007) Efficiency and cost of economical brain functional networks. *PLoS Comput Biol* 3:e17.
- Achard S, Salvador R, Whitcher B, Suckling J, Bullmore E (2006) A resilient, low-frequency, small-world human brain functional network with highly connected association cortical hubs. *J Neurosci* 26:63-72.
- Adelstein JS, Shehzad Z, Mennes M, DeYoung CG, Zuo XN, Kelly C, Margulies DS, Bloomfield A, Gray JR, Castellanos FX, Milham MP (2011) Personality is reflected in the brain's intrinsic functional architecture. *PLoS One* 6:e27633.
- Ahissar E, Vaadia E, Ahissar M, Bergman H, Arieli A, Abeles M (1992) Dependence of cortical plasticity on correlated activity of single neurons and on behavioral context. *Science* 257:1412-1415.
- Albert NB, Robertson EM, Miall RC (2009) The resting human brain and motor learning. *Curr Biol* 19:1023-1027.
- Alexander GE, DeLong MR, Strick PL (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu Rev Neurosci* 9:357-381.
- Allman JM, Watson KK, Tetreault NA, Hakeem AY (2005) Intuition and autism: a possible role for Von Economo neurons. *Trends Cogn Sci* 9:367-373.
- Allman JM, Tetreault NA, Hakeem AY, Manaye KF, Semendeferi K, Erwin JM, Park S, Goubert V, Hof PR (2010) The von Economo neurons in frontoinsular and anterior cingulate cortex in great apes and humans. *Brain Struct Funct* 214:495-517.
- Anand A, Li Y, Wang Y, Wu J, Gao S, Bukhari L, Mathews VP, Kalnin A, Lowe MJ (2005) Antidepressant effect on connectivity of the mood-regulating circuit: an fMRI study. *Neuropsychopharmacology* 30:1334-1344.
- Andersson JLR, Jenkinson M, Smith S (2007) Non-linear registration aka spatial normalisation. *FMRIB Technical Report TR07JA2*.
- Andrews-Hanna JR, Reidler JS, Sepulcre J, Poulin R, Buckner RL (2010) Functional-anatomic fractionation of the brain's default network. *Neuron* 65:550-562.
- Andrews-Hanna JR, Snyder AZ, Vincent JL, Lustig C, Head D, Raichle ME, Buckner RL (2007) Disruption of large-scale brain systems in advanced aging. *Neuron* 56:924-935.

- Appel-Cresswell S, de la Fuente-Fernandez R, Galley S, McKeown MJ (2010) Imaging of compensatory mechanisms in Parkinson's disease. *Curr Opin Neurol* 23:407-412.
- Atzori G, Lemmonds CA, Kotler ML, Durcan MJ, Boyle J (2008) Efficacy of a nicotine (4 mg)-containing lozenge on the cognitive impairment of nicotine withdrawal. *J Clin Psychopharmacol* 28:667-674.
- Baler RD, Volkow ND (2006) Drug addiction: the neurobiology of disrupted self-control. *Trends Mol Med* 12:559-566.
- Baliki MN, Geha PY, Apkarian AV, Chialvo DR (2008) Beyond feeling: chronic pain hurts the brain, disrupting the default-mode network dynamics. *J Neurosci* 28:1398-1403.
- Beaver JD, Long CJ, Cole DM, Durcan MJ, Bannon LC, Mishra RG, Matthews PM (2011) The Effects of nicotine replacement on cognitive brain activity during smoking withdrawal studied with simultaneous fMRI/EEG. *Neuropsychopharmacology* 36:1792-1800.
- Beaver JD, Searle G, Nichols T, Cole DM, Plisson C, Andorn A, Bani M, Ghibellini G, Iavarone L, Tziortzi A, Shotbolt P, Lee Y, Bifone A, Merlo-Pich E, Laruelle M, Gunn R, Matthews PM, Rabiner EA. Dopamine D3 receptor availability is associated with amygdala reactivity to reward. Under review.
- Bechara A, Damasio H, Damasio AR (2000) Emotion, decision making and the orbitofrontal cortex. *Cereb Cortex* 10:295-307.
- Beckmann CF (2012) Modelling with independent components. *Neuroimage* 62:891-901.
- Beckmann CF, Smith SM (2004) Probabilistic independent component analysis for functional magnetic resonance imaging. *IEEE Trans Med Imaging* 23:137-152.
- Beckmann CF, Smith SM (2005) Tensorial extensions of independent component analysis for multisubject FMRI analysis. *Neuroimage* 25:294-311.
- Beckmann CF, Jenkinson M, Smith SM (2003) General multilevel linear modeling for group analysis in FMRI. *Neuroimage* 20:1052-1063.
- Beckmann CF, DeLuca M, Devlin JT, Smith SM (2005) Investigations into resting-state connectivity using independent component analysis. *Philos Trans R Soc Lond B Biol Sci* 360:1001-1013.
- Beckmann CF, Mackay CE, Filippini N, Smith SM (2009) Group comparison of resting-state FMRI data using multi-subject ICA and dual regression. *Neuroimage* 47 Suppl 1:S148.
- Behrens TE, Hunt LT, Rushworth MF (2009) The computation of social behavior. *Science* 324:1160-1164.
- Behrens TE, Hunt LT, Woolrich MW, Rushworth MF (2008) Associative learning of social value. *Nature* 456:245-249.
- Bell SL, Taylor RC, Singleton EG, Henningfield JE, Heishman SJ (1999) Smoking after nicotine deprivation enhances cognitive performance and decreases tobacco craving in drug abusers. *Nicotine Tob Res* 1:45-52.
- Bi G, Poo M (1999) Distributed synaptic modification in neural networks induced by patterned stimulation. *Nature* 401:792-796.
- Bi G, Poo M (2001) Synaptic modification by correlated activity: Hebb's postulate revisited. *Annu Rev Neurosci* 24:139-166.

- Bianciardi M, Fukunaga M, van Gelderen P, Horovitz SG, de Zwart JA, Duyn JH (2009a) Modulation of spontaneous fMRI activity in human visual cortex by behavioral state. *Neuroimage* 45:160-168.
- Bianciardi M, Fukunaga M, van Gelderen P, Horovitz SG, de Zwart JA, Shmueli K, Duyn JH (2009b) Sources of functional magnetic resonance imaging signal fluctuations in the human brain at rest: a 7 T study. *Magn Reson Imaging* 27:1019-1029.
- Birn RM, Murphy K, Bandettini PA (2008) The effect of respiration variations on independent component analysis results of resting state functional connectivity. *Hum Brain Mapp* 29:740-750.
- Birn RM, Diamond JB, Smith MA, Bandettini PA (2006) Separating respiratory-variation-related fluctuations from neuronal-activity-related fluctuations in fMRI. *Neuroimage* 31:1536-1548.
- Biswal B, Yetkin FZ, Haughton VM, Hyde JS (1995) Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Reson Med* 34:537-541.
- Biswal BB et al. (2010) Toward discovery science of human brain function. *Proc Natl Acad Sci U S A* 107:4734-4739.
- Boileau I, Payer D, Houle S, Behzadi A, Rusjan PM, Tong J, Wilkins D, Selby P, George TP, Zack M, Furukawa Y, McCluskey T, Wilson AA, Kish SJ (2012) Higher binding of the dopamine D3 receptor-preferring ligand [11C]-(+)-propyl-hexahydro-naphtho-oxazin in methamphetamine polydrug users: a positron emission tomography study. *J Neurosci* 32:1353-1359.
- Bokde AL, Tagamets MA, Friedman RB, Horwitz B (2001) Functional interactions of the inferior frontal cortex during the processing of words and word-like stimuli. *Neuron* 30:609-617.
- Boly M, Phillips C, Tshibanda L, Vanhaudenhuyse A, Schabus M, Dang-Vu TT, Moonen G, Hustinx R, Maquet P, Laureys S (2008) Intrinsic brain activity in altered states of consciousness: how conscious is the default mode of brain function? *Ann N Y Acad Sci* 1129:119-129.
- Botvinick MM (2007) Conflict monitoring and decision making: reconciling two perspectives on anterior cingulate function. *Cogn Affect Behav Neurosci* 7:356-366.
- Braver TS, Barch DM, Cohen JD (1999) Cognition and control in schizophrenia: a computational model of dopamine and prefrontal function. *Biol Psychiatry* 46:312-328.
- Bressler SL (1995) Large-scale cortical networks and cognition. *Brain Res Brain Res Rev* 20:288-304.
- Brookes MJ, Woolrich M, Luckhoo H, Price D, Hale JR, Stephenson MC, Barnes GR, Smith SM, Morris PG (2011) Investigating the electrophysiological basis of resting state networks using magnetoencephalography. *Proc Natl Acad Sci U S A* 108:16783-16788.
- Broyd SJ, Demanuele C, Debener S, Helps SK, James CJ, Sonuga-Barke EJ (2009) Default-mode brain dysfunction in mental disorders: a systematic review. *Neurosci Biobehav Rev* 33:279-296.
- Brozoski TJ, Brown RM, Rosvold HE, Goldman PS (1979) Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. *Science* 205:929-932.

- Buckholtz JW, Treadway MT, Cowan RL, Woodward ND, Li R, Ansari MS, Baldwin RM, Schwartzman AN, Shelby ES, Smith CE, Kessler RM, Zald DH (2010) Dopaminergic network differences in human impulsivity. *Science* 329:532.
- Buckner RL, Vincent JL (2007) Unrest at rest: default activity and spontaneous network correlations. *Neuroimage* 37:1091-1096; discussion 1097-1099.
- Buckner RL, Andrews-Hanna JR, Schacter DL (2008) The brain's default network: anatomy, function, and relevance to disease. *Ann N Y Acad Sci* 1124:1-38.
- Buckner RL, Snyder AZ, Shannon BJ, LaRossa G, Sachs R, Fotenos AF, Sheline YI, Klunk WE, Mathis CA, Morris JC, Mintun MA (2005) Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. *J Neurosci* 25:7709-7717.
- Bullmore E, Sporns O (2009) Complex brain networks: graph theoretical analysis of structural and functional systems. *Nat Rev Neurosci* 10:186-198.
- Burton EJ, McKeith IG, Burn DJ, Williams ED, O'Brien JT (2004) Cerebral atrophy in Parkinson's disease with and without dementia: a comparison with Alzheimer's disease, dementia with Lewy bodies and controls. *Brain* 127:791-800.
- Bush G, Luu P, Posner MI (2000) Cognitive and emotional influences in anterior cingulate cortex. *Trends Cogn Sci* 4:215-222.
- Buzsaki G, Draguhn A (2004) Neuronal oscillations in cortical networks. *Science* 304:1926-1929.
- Cai X, Kim S, Lee D (2011) Heterogeneous coding of temporally discounted values in the dorsal and ventral striatum during intertemporal choice. *Neuron* 69:170-182.
- Caine SB, Koob GF (1993) Modulation of cocaine self-administration in the rat through D-3 dopamine receptors. *Science* 260:1814-1816.
- Calhoun VD, Adali T, Pekar JJ (2004a) A method for comparing group fMRI data using independent component analysis: application to visual, motor and visuomotor tasks. *Magn Reson Imaging* 22:1181-1191.
- Calhoun VD, Pekar JJ, Pearlson GD (2004b) Alcohol intoxication effects on simulated driving: exploring alcohol-dose effects on brain activation using functional MRI. *Neuropsychopharmacology* 29:2097-2017.
- Calhoun VD, Adali T, Pearlson GD, Pekar JJ (2001) A method for making group inferences from functional MRI data using independent component analysis. *Hum Brain Mapp* 14:140-151.
- Camchong J, MacDonald AW, 3rd, Nelson B, Bell C, Mueller BA, Specker S, Lim KO (2011) Frontal hyperconnectivity related to discounting and reversal learning in cocaine subjects. *Biol Psychiatry* 69:1117-1123.
- Castellanos FX, Margulies DS, Kelly C, Uddin LQ, Ghaffari M, Kirsch A, Shaw D, Shehzad Z, Di Martino A, Biswal B, Sonuga-Barke EJ, Rotrosen J, Adler LA, Milham MP (2008) Cingulate-precuneus interactions: a new locus of dysfunction in adult attention-deficit/hyperactivity disorder. *Biol Psychiatry* 63:332-337.
- Chang C, Glover GH (2010) Time-frequency dynamics of resting-state brain connectivity measured with fMRI. *Neuroimage* 50:81-98.

- Christakou A, Brammer M, Rubia K (2011) Maturation of limbic corticostriatal activation and connectivity associated with developmental changes in temporal discounting. *Neuroimage* 54:1344-1354.
- Christova P, Lewis SM, Jerde TA, Lynch JK, Georgopoulos AP (2011) True associations between resting fMRI time series based on innovations. *J Neural Eng* 8:046025.
- Cilia R, van Eimeren T (2011) Impulse control disorders in Parkinson's disease: seeking a roadmap toward a better understanding. *Brain Struct Funct* 216:289-299.
- Cohen AL, Fair DA, Dosenbach NU, Miezin FM, Dierker D, Van Essen DC, Schlaggar BL, Petersen SE (2008) Defining functional areas in individual human brains using resting functional connectivity MRI. *Neuroimage* 41:45-57.
- Cohen MX, Krohn-Grimberghe A, Elger CE, Weber B (2007) Dopamine gene predicts the brain's response to dopaminergic drug. *Eur J Neurosci* 26:3652-3660.
- Cole DM, Smith SM, Beckmann CF (2010a) Advances and pitfalls in the analysis and interpretation of resting-state FMRI data. *Front Syst Neurosci* 4:8.
- Cole DM, Beckmann CF, Long CJ, Matthews PM, Durcan MJ, Beaver JD (2010b) Nicotine replacement in abstinent smokers improves cognitive withdrawal symptoms with modulation of resting brain network dynamics. *Neuroimage* 52:590-599.
- Cole DM, Beckmann CF, Oei NY, Both S, van Gerven JM, Rombouts SA. Linear and 'inverted U-shaped' effects of dopamine modulation on resting-state network connectivity. Under review.
- Cole DM, Beckmann CF, Searle GE, Plisson C, Tziortzi AC, Nichols TE, Gunn RN, Matthews PM, Rabiner EA, Beaver JD (2012a) Orbitofrontal connectivity with resting-state networks is associated with midbrain dopamine D3 receptor availability. *Cereb Cortex* 22:2784-2793.
- Cole DM, Oei NY, Soeter RP, Both S, van Gerven JM, Rombouts SA, Beckmann CF (2012b) Dopamine-dependent architecture of cortico-subcortical network connectivity. *Cereb Cortex* [Epub ahead of print] doi:10.1093/cercor/bhs136.
- Conner CR, Ellmore TM, Pieters TA, DiSano MA, Tandon N (2011) Variability of the relationship between electrophysiology and BOLD-fMRI across cortical regions in humans. *J Neurosci* 31:12855-12865.
- Cools R (2006) Dopaminergic modulation of cognitive function-implications for L-DOPA treatment in Parkinson's disease. *Neurosci Biobehav Rev* 30:1-23.
- Cools R, D'Esposito M (2011) Inverted-U-shaped dopamine actions on human working memory and cognitive control. *Biol Psychiatry* 69:e113-125.
- Cools R, Sheridan M, Jacobs E, D'Esposito M (2007) Impulsive personality predicts dopamine-dependent changes in frontostriatal activity during component processes of working memory. *J Neurosci* 27:5506-5514.
- Cordes D, Haughton VM, Arfanakis K, Wendt GJ, Turski PA, Moritz CH, Quigley MA, Meyerand ME (2000) Mapping functionally related regions of brain with functional connectivity MR imaging. *AJNR Am J Neuroradiol* 21:1636-1644.

- Cordes D, Haughton VM, Arfanakis K, Carew JD, Turski PA, Moritz CH, Quigley MA, Meyerand ME (2001) Frequencies contributing to functional connectivity in the cerebral cortex in "resting-state" data. *AJNR Am J Neuroradiol* 22:1326-1333.
- Crow TJ (1980) Molecular pathology of schizophrenia: more than one disease process? *Br Med J* 280:66-68.
- Dagher A, Robbins TW (2009) Personality, addiction, dopamine: insights from Parkinson's disease. *Neuron* 61:502-510.
- Dalaker TO, Larsen JP, Bergsland N, Beyer MK, Alves G, Dwyer MG, Tysnes OB, Benedict RH, Kelemen A, Bronnick K, Zivadinov R (2009) Brain atrophy and white matter hyperintensities in early Parkinson's disease(a). *Mov Disord* 24:2233-2241.
- Dalley JW, Fryer TD, Brichard L, Robinson ES, Theobald DE, Laane K, Pena Y, Murphy ER, Shah Y, Probst K, Abakumova I, Aigbirhio FI, Richards HK, Hong Y, Baron JC, Everitt BJ, Robbins TW (2007) Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. *Science* 315:1267-1270.
- Damasio AR, Tranel D, Damasio H (1990) Individuals with sociopathic behavior caused by frontal damage fail to respond autonomically to social stimuli. *Behav Brain Res* 41:81-94.
- Damoiseaux JS, Rombouts SA, Barkhof F, Scheltens P, Stam CJ, Smith SM, Beckmann CF (2006) Consistent resting-state networks across healthy subjects. *Proc Natl Acad Sci U S A* 103:13848-13853.
- Damoiseaux JS, Beckmann CF, Arigita EJ, Barkhof F, Scheltens P, Stam CJ, Smith SM, Rombouts SA (2008) Reduced resting-state brain activity in the "default network" in normal aging. *Cereb Cortex* 18:1856-1864.
- Davis KL, Kahn RS, Ko G, Davidson M (1991) Dopamine in schizophrenia: a review and reconceptualization. *Am J Psychiatry* 148:1474-1486.
- Dayan P, Huys QJ (2009) Serotonin in affective control. *Annu Rev Neurosci* 32:95-126.
- De Luca M, Beckmann CF, De Stefano N, Matthews PM, Smith SM (2006) fMRI resting state networks define distinct modes of long-distance interactions in the human brain. *Neuroimage* 29:1359-1367.
- de Pasquale F, Della Penna S, Snyder AZ, Lewis C, Mantini D, Marzetti L, Belardinelli P, Ciancetta L, Pizzella V, Romani GL, Corbetta M (2010) Temporal dynamics of spontaneous MEG activity in brain networks. *Proc Natl Acad Sci U S A* 107:6040-6045.
- Dehaene S, Le Clec HG, Poline JB, Le Bihan D, Cohen L (2002) The visual word form area: a prelexical representation of visual words in the fusiform gyrus. *Neuroreport* 13:321-325.
- den Ouden HE, Daunizeau J, Roiser J, Friston KJ, Stephan KE (2010) Striatal prediction error modulates cortical coupling. *J Neurosci* 30:3210-3219.
- Di Martino A, Shehzad Z, Kelly C, Roy AK, Gee DG, Uddin LQ, Gotimer K, Klein DF, Castellanos FX, Milham MP (2009) Relationship between cingulo-insular functional connectivity and autistic traits in neurotypical adults. *Am J Psychiatry* 166:891-899.
- Diaconescu AO, Menon M, Jensen J, Kapur S, McIntosh AR (2010) Dopamine-induced changes in neural network patterns supporting aversive conditioning. *Brain Res* 1313:143-161.

- Dickson JW, Gerstein GL (1974) Interactions between neurons in auditory cortex of the cat. *J Neurophysiol* 37:1239-1261.
- Ding JR, Liao W, Zhang Z, Mantini D, Xu Q, Wu GR, Lu G, Chen H (2011) Topological fractionation of resting-state networks. *PLoS One* 6:e26596.
- Doria V, Beckmann CF, Arichi T, Merchant N, Groppo M, Turkheimer FE, Counsell SJ, Murgasova M, Aljabar P, Nunes RG, Larkman DJ, Rees G, Edwards AD (2010) Emergence of resting state networks in the preterm human brain. *Proc Natl Acad Sci U S A* 107:20015-20020.
- Dosenbach NU, Fair DA, Miezin FM, Cohen AL, Wenger KK, Dosenbach RA, Fox MD, Snyder AZ, Vincent JL, Raichle ME, Schlaggar BL, Petersen SE (2007) Distinct brain networks for adaptive and stable task control in humans. *Proc Natl Acad Sci U S A* 104:11073-11078.
- Dosenbach NU, Nardos B, Cohen AL, Fair DA, Power JD, Church JA, Nelson SM, Wig GS, Vogel AC, Lessov-Schlaggar CN, Barnes KA, Dubis JW, Feczko E, Coalson RS, Pruett JR, Jr., Barch DM, Petersen SE, Schlaggar BL (2010) Prediction of individual brain maturity using fMRI. *Science* 329:1358-1361.
- Duff EP, Trachtenberg AJ, Mackay CE, Howard MA, Wilson F, Smith SM, Woolrich MW (2012) Task-driven ICA feature generation for accurate and interpretable prediction using fMRI. *Neuroimage* 60:189-203.
- Duncan NW, Enzi B, Wiebking C, Northoff G (2011) Involvement of glutamate in rest-stimulus interaction between perigenual and supragenual anterior cingulate cortex: a combined fMRI-MRS study. *Hum Brain Mapp* 32:2172-2182.
- Efron B (2004) Large-scale simultaneous hypothesis testing: the choice of a null hypothesis. *J Am Stat Assoc* 99:96-104.
- Eldaief MC, Halko MA, Buckner RL, Pascual-Leone A (2011) Transcranial magnetic stimulation modulates the brain's intrinsic activity in a frequency-dependent manner. *Proc Natl Acad Sci U S A* 108:21229-21234.
- Erhardt EB, Rachakonda S, Bedrick EJ, Allen EA, Adali T, Calhoun VD (2011) Comparison of multi-subject ICA methods for analysis of fMRI data. *Hum Brain Mapp* 32:2075-2095.
- Ersche KD, Turton AJ, Pradhan S, Bullmore ET, Robbins TW (2010) Drug addiction endophenotypes: impulsive versus sensation-seeking personality traits. *Biol Psychiatry* 68:770-773.
- Esposito F, Scarabino T, Hyvarinen A, Himberg J, Formisano E, Comani S, Tedeschi G, Goebel R, Seifritz E, Di Salle F (2005) Independent component analysis of fMRI group studies by self-organizing clustering. *Neuroimage* 25:193-205.
- Evans AH, Pavese N, Lawrence AD, Tai YF, Appel S, Doder M, Brooks DJ, Lees AJ, Piccini P (2006) Compulsive drug use linked to sensitized ventral striatal dopamine transmission. *Ann Neurol* 59:852-858.
- Evenden JL (1999) Varieties of impulsivity. *Psychopharmacology (Berl)* 146:348-361.
- Everitt BJ, Robbins TW (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nat Neurosci* 8:1481-1489.
- Fahn S (2008) The history of dopamine and levodopa in the treatment of Parkinson's disease. *Mov Disord* 23 Suppl 3:S497-508.

- Fair DA, Dosenbach NU, Church JA, Cohen AL, Brahmbhatt S, Miezin FM, Barch DM, Raichle ME, Petersen SE, Schlaggar BL (2007) Development of distinct control networks through segregation and integration. *Proc Natl Acad Sci U S A* 104:13507-13512.
- Fernandez Galan R (2008) On how network architecture determines the dominant patterns of spontaneous neural activity. *PLoS One* 3:e2148.
- Filippini N, MacIntosh BJ, Hough MG, Goodwin GM, Frisoni GB, Smith SM, Matthews PM, Beckmann CF, Mackay CE (2009) Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. *Proc Natl Acad Sci U S A* 106:7209-7214.
- Fleisher AS, Sherzai A, Taylor C, Langbaum JB, Chen K, Buxton RB (2009) Resting-state BOLD networks versus task-associated functional MRI for distinguishing Alzheimer's disease risk groups. *Neuroimage* 47:1678-1690.
- Fox MD, Raichle ME (2007) Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat Rev Neurosci* 8:700-711.
- Fox MD, Greicius M (2010) Clinical applications of resting state functional connectivity. *Front Syst Neurosci* 4:19.
- Fox MD, Snyder AZ, Vincent JL, Raichle ME (2007) Intrinsic fluctuations within cortical systems account for intertrial variability in human behavior. *Neuron* 56:171-184.
- Fox MD, Zhang D, Snyder AZ, Raichle ME (2009) The global signal and observed anticorrelated resting state brain networks. *J Neurophysiol* 101:3270-3283.
- Fox MD, Corbetta M, Snyder AZ, Vincent JL, Raichle ME (2006) Spontaneous neuronal activity distinguishes human dorsal and ventral attention systems. *Proc Natl Acad Sci U S A* 103:10046-10051.
- Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME (2005) The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc Natl Acad Sci U S A* 102:9673-9678.
- Frank MJ, Claus ED (2006) Anatomy of a decision: striato-orbitofrontal interactions in reinforcement learning, decision making, and reversal. *Psychol Rev* 113:300-326.
- Fransson P (2005) Spontaneous low-frequency BOLD signal fluctuations: an fMRI investigation of the resting-state default mode of brain function hypothesis. *Hum Brain Mapp* 26:15-29.
- Fransson P, Marrelec G (2008) The precuneus/posterior cingulate cortex plays a pivotal role in the default mode network: Evidence from a partial correlation network analysis. *Neuroimage* 42:1178-1184.
- Fransson P, Skiold B, Horsch S, Nordell A, Blennow M, Lagercrantz H, Aden U (2007) Resting-state networks in the infant brain. *Proc Natl Acad Sci U S A* 104:15531-15536.
- Friston KJ (1994) Functional and effective connectivity in neuroimaging: a synthesis. *Hum Brain Mapp* 2:56-78.
- Friston KJ (2011) Functional and effective connectivity: a review. *Brain Connect* 1:13-36.
- Friston KJ, Frith CD, Liddle PF, Frackowiak RS (1993) Functional connectivity: the principal-component analysis of large (PET) data sets. *J Cereb Blood Flow Metab* 13:5-14.

- Friston KJ, Li B, Daunizeau J, Stephan KE (2011) Network discovery with DCM. *Neuroimage* 56:1202-1221.
- Friston KJ, Buechel C, Fink GR, Morris J, Rolls E, Dolan RJ (1997) Psychophysiological and modulatory interactions in neuroimaging. *Neuroimage* 6:218-229.
- Friston KJ, Shiner T, FitzGerald T, Galea JM, Adams R, Brown H, Dolan RJ, Moran R, Stephan KE, Bestmann S (2012) Dopamine, affordance and active inference. *PLoS Comput Biol* 8:e1002327.
- Froeliger B, Gilbert DG, McClernon FJ (2009) Effects of nicotine on novelty detection and memory recognition performance: double-blind, placebo-controlled studies of smokers and nonsmokers. *Psychopharmacology (Berl)* 205:625-633.
- Fukunaga M, Horovitz SG, van Gelderen P, de Zwart JA, Jansma JM, Ikonomidou VN, Chu R, Deckers RH, Leopold DA, Duyn JH (2006) Large-amplitude, spatially correlated fluctuations in BOLD fMRI signals during extended rest and early sleep stages. *Magn Reson Imaging* 24:979-992.
- Gerstein GL, Perkel DH (1969) Simultaneously recorded trains of action potentials: analysis and functional interpretation. *Science* 164:828-830.
- Gevins AS, Doyle JC, Cuttillo BA, Schaffer RE, Tannehill RS, Bressler SL (1985) Neurocognitive pattern analysis of a visuospatial task: rapidly-shifting foci of evoked correlations between electrodes. *Psychophysiology* 22:32-43.
- Gjedde A, Kumakura Y, Cumming P, Linnet J, Moller A (2010) Inverted-U-shaped correlation between dopamine receptor availability in striatum and sensation seeking. *Proc Natl Acad Sci U S A* 107:3870-3875.
- Glahn DC, Winkler AM, Kochunov P, Almasy L, Duggirala R, Carless MA, Curran JC, Olvera RL, Laird AR, Smith SM, Beckmann CF, Fox PT, Blangero J (2010) Genetic control over the resting brain. *Proc Natl Acad Sci U S A* 107:1223-1228.
- Glover GH, Li TQ, Ress D (2000) Image-based method for retrospective correction of physiological motion effects in fMRI: RETROICOR. *Magn Reson Med* 44:162-167.
- Goense JB, Logothetis NK (2008) Neurophysiology of the BOLD fMRI signal in awake monkeys. *Curr Biol* 18:631-640.
- Goldberg TE, Greenberg RD, Griffin SJ, Gold JM, Kleinman JE, Pickar D, Schulz SC, Weinberger DR (1993) The effect of clozapine on cognition and psychiatric symptoms in patients with schizophrenia. *Br J Psychiatry* 162:43-48.
- Goldstein RZ, Volkow ND (2011) Dysfunction of the prefrontal cortex in addiction: neuroimaging findings and clinical implications. *Nat Rev Neurosci* 12:652-669.
- Gordon EM, Stollstorff M, Devaney JM, Bean S, Vaidya CJ (2012) Effect of dopamine transporter genotype on intrinsic functional connectivity depends on cognitive state. *Cereb Cortex* 22:2182-2196.
- Graff-Guerrero A, Willeit M, Ginovart N, Mamo D, Mizrahi R, Rusjan P, Vitcu I, Seeman P, Wilson AA, Kapur S (2008) Brain region binding of the D2/3 agonist [¹¹C]-(+)-PHNO and the D2/3 antagonist [¹¹C]raclopride in healthy humans. *Hum Brain Mapp* 29:400-410.

- Greicius M (2008) Resting-state functional connectivity in neuropsychiatric disorders. *Curr Opin Neurol* 21:424-430.
- Greicius MD, Krasnow B, Reiss AL, Menon V (2003) Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proc Natl Acad Sci U S A* 100:253-258.
- Greicius MD, Srivastava G, Reiss AL, Menon V (2004) Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. *Proc Natl Acad Sci U S A* 101:4637-4642.
- Greicius MD, Supekar K, Menon V, Dougherty RF (2009) Resting-state functional connectivity reflects structural connectivity in the default mode network. *Cereb Cortex* 19:72-78.
- Greicius MD, Kiviniemi V, Tervonen O, Vainionpaa V, Alahuhta S, Reiss AL, Menon V (2008) Persistent default-mode network connectivity during light sedation. *Hum Brain Mapp* 29:839-847.
- Greicius MD, Flores BH, Menon V, Glover GH, Solvason HB, Kenna H, Reiss AL, Schatzberg AF (2007) Resting-state functional connectivity in major depression: abnormally increased contributions from subgenual cingulate cortex and thalamus. *Biol Psychiatry* 62:429-437.
- Guillin O, Diaz J, Carroll P, Griffon N, Schwartz JC, Sokoloff P (2001) BDNF controls dopamine D3 receptor expression and triggers behavioural sensitization. *Nature* 411:86-89.
- Gunn RN, Lammertsma AA, Hume SP, Cunningham VJ (1997) Parametric imaging of ligand-receptor binding in PET using a simplified reference region model. *Neuroimage* 6:279-287.
- Gurevich EV, Bordelon Y, Shapiro RM, Arnold SE, Gur RE, Joyce JN (1997) Mesolimbic dopamine D3 receptors and use of antipsychotics in patients with schizophrenia. A postmortem study. *Arch Gen Psychiatry* 54:225-232.
- Gusnard DA, Raichle ME (2001) Searching for a baseline: functional imaging and the resting human brain. *Nat Rev Neurosci* 2:685-694.
- Gusnard DA, Akbudak E, Shulman GL, Raichle ME (2001) Medial prefrontal cortex and self-referential mental activity: relation to a default mode of brain function. *Proc Natl Acad Sci U S A* 98:4259-4264.
- Habas C, Kamdar N, Nguyen D, Prater K, Beckmann CF, Menon V, Greicius MD (2009) Distinct cerebellar contributions to intrinsic connectivity networks. *J Neurosci* 29:8586-8594.
- Hahn A, Wadsak W, Windischberger C, Baldinger P, Hoflich AS, Losak J, Nics L, Philippe C, Kranz GS, Kraus C, Mitterhauser M, Karanikas G, Kasper S, Lanzenberger R (2012) Differential modulation of the default mode network via serotonin-1A receptors. *Proc Natl Acad Sci U S A* 109:2619-2624.
- Hahn B, Ross TJ, Yang Y, Kim I, Huestis MA, Stein EA (2007) Nicotine enhances visuospatial attention by deactivating areas of the resting brain default network. *J Neurosci* 27:3477-3489.
- Hahn B, Ross TJ, Wolkenberg FA, Shakleya DM, Huestis MA, Stein EA (2009) Performance effects of nicotine during selective attention, divided attention, and simple stimulus detection: an fMRI study. *Cereb Cortex* 19:1990-2000.

- Hampson M, Peterson BS, Skudlarski P, Gatenby JC, Gore JC (2002) Detection of functional connectivity using temporal correlations in MR images. *Hum Brain Mapp* 15:247-262.
- Harrison BJ, Pujol J, Lopez-Sola M, Hernandez-Ribas R, Deus J, Ortiz H, Soriano-Mas C, Yucel M, Pantelis C, Cardoner N (2008a) Consistency and functional specialization in the default mode brain network. *Proc Natl Acad Sci U S A* 105:9781-9786.
- Harrison BJ, Pujol J, Ortiz H, Fornito A, Pantelis C, Yucel M (2008b) Modulation of brain resting-state networks by sad mood induction. *PLoS One* 3:e1794.
- Hasson U, Nusbaum HC, Small SL (2009) Task-dependent organization of brain regions active during rest. *Proc Natl Acad Sci U S A* 106:10841-10846.
- Hayasaka S, Laurienti PJ (2010) Comparison of characteristics between region- and voxel-based network analyses in resting-state fMRI data. *Neuroimage* 50:499-508.
- He BJ, Snyder AZ, Zempel JM, Smyth MD, Raichle ME (2008) Electrophysiological correlates of the brain's intrinsic large-scale functional architecture. *Proc Natl Acad Sci U S A* 105:16039-16044.
- Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO (1991) The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. *Br J Addict* 86:1119-1127.
- Hebb DO (1949) *The organization of behavior*. New York: Wiley & Sons.
- Heidbreder CA, Newman AH (2010) Current perspectives on selective dopamine D(3) receptor antagonists as pharmacotherapeutics for addictions and related disorders. *Ann N Y Acad Sci* 1187:4-34.
- Heinz A, Siessmeier T, Wrase J, Hermann D, Klein S, Grusser SM, Flor H, Braus DF, Buchholz HG, Grunder G, Schreckenberger M, Smolka MN, Rosch F, Mann K, Bartenstein P (2004) Correlation between dopamine D(2) receptors in the ventral striatum and central processing of alcohol cues and craving. *Am J Psychiatry* 161:1783-1789.
- Heishman SJ, Taylor RC, Henningfield JE (1994) Nicotine and smoking: a review of effects on human performance. *Exp Clin Psychopharmacol* 2:345-395.
- Helmich RC, Derikx LC, Bakker M, Scheeringa R, Bloem BR, Toni I (2010) Spatial remapping of cortico-striatal connectivity in Parkinson's disease. *Cereb Cortex* 20:1175-1186.
- Hesselmann G, Kell CA, Eger E, Kleinschmidt A (2008) Spontaneous local variations in ongoing neural activity bias perceptual decisions. *Proc Natl Acad Sci U S A* 105:10984-10989.
- Himberg J, Hyvarinen A, Esposito F (2004) Validating the independent components of neuroimaging time series via clustering and visualization. *Neuroimage* 22:1214-1222.
- Honey CJ, Sporns O, Cammoun L, Gigandet X, Thiran JP, Meuli R, Hagmann P (2009) Predicting human resting-state functional connectivity from structural connectivity. *Proc Natl Acad Sci U S A* 106:2035-2040.
- Honey GD, Suckling J, Zelaya F, Long C, Routledge C, Jackson S, Ng V, Fletcher PC, Williams SC, Brown J, Bullmore ET (2003) Dopaminergic drug effects on physiological connectivity in a human cortico-striato-thalamic system. *Brain* 126:1767-1781.
- Hong LE, Gu H, Yang Y, Ross TJ, Salmeron BJ, Buchholz B, Thaker GK, Stein EA (2009) Association of nicotine addiction and nicotine's actions with

- separate cingulate cortex functional circuits. *Arch Gen Psychiatry* 66:431-441.
- Horovitz SG, Fukunaga M, de Zwart JA, van Gelderen P, Fulton SC, Balkin TJ, Duyn JH (2008) Low frequency BOLD fluctuations during resting wakefulness and light sleep: a simultaneous EEG-fMRI study. *Hum Brain Mapp* 29:671-682.
- Horovitz SG, Braun AR, Carr WS, Picchioni D, Balkin TJ, Fukunaga M, Duyn JH (2009) Decoupling of the brain's default mode network during deep sleep. *Proc Natl Acad Sci U S A* 106:11376-11381.
- Horwitz B, Grady CL, Haxby JV, Schapiro MB, Rapoport SI, Ungerleider LG, Mishkin M (1992) Functional associations among human posterior extrastriate brain regions during object and spatial vision. *J Cogn Neurosci* 4:311-322.
- Housden CR, O'Sullivan SS, Joyce EM, Lees AJ, Roiser JP (2010) Intact reward learning but elevated delay discounting in Parkinson's disease patients with impulsive-compulsive spectrum behaviors. *Neuropsychopharmacology* 35:2155-2164.
- Huettel SA, Song AW, McCarthy G (2004) Functional magnetic resonance imaging. Sunderland: Sinauer Associates.
- Hughes JR (1992) Tobacco withdrawal in self-quitters. *J Consult Clin Psychol* 60:689-697.
- Hughes JR, Hatsukami D (1986) Signs and symptoms of tobacco withdrawal. *Arch Gen Psychiatry* 43:289-294.
- Hughes JR, Higgins ST, Bickel WK (1994) Nicotine withdrawal versus other drug withdrawal syndromes: similarities and dissimilarities. *Addiction* 89:1461-1470.
- Hunt LT, Kolling N, Soltani A, Woolrich MW, Rushworth MF, Behrens TE (2012) Mechanisms underlying cortical activity during value-guided choice. *Nat Neurosci* 15:470-476, S471-473.
- Hutchison RM, Mirsattari SM, Jones CK, Gati JS, Leung LS (2010) Functional networks in the anesthetized rat brain revealed by independent component analysis of resting-state fMRI. *J Neurophysiol* 103:3398-3406.
- Hutchison RM, Womelsdorf T, Gati JS, Everling S, Menon RS (2012) Resting-state networks show dynamic functional connectivity in awake humans and anesthetized macaques. *Hum Brain Mapp* [Epub ahead of print] doi:10.1002/hbm.22058.
- Iannetti GD, Wise RG (2007) BOLD functional MRI in disease and pharmacological studies: room for improvement? *Magn Reson Imaging* 25:978-988.
- Iida M, Iida H, Dohi S, Takenaka M, Fujiwara H (1998) Mechanisms underlying cerebrovascular effects of cigarette smoking in rats in vivo. *Stroke* 29:1656-1665.
- Insel TR, Sahakian BJ (2012) Drug research: a plan for mental illness. *Nature* 483:269.
- Izquierdo A, Suda RK, Murray EA (2004) Bilateral orbital prefrontal cortex lesions in rhesus monkeys disrupt choices guided by both reward value and reward contingency. *J Neurosci* 24:7540-7548.

- Izquierdo A, Suda RK, Murray EA (2005) Comparison of the effects of bilateral orbital prefrontal cortex lesions and amygdala lesions on emotional responses in rhesus monkeys. *J Neurosci* 25:8534-8542.
- Jafri MJ, Pearlson GD, Stevens M, Calhoun VD (2008) A method for functional network connectivity among spatially independent resting-state components in schizophrenia. *Neuroimage* 39:1666-1681.
- Jenkinson M, Bannister P, Brady M, Smith S (2002) Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage* 17:825-841.
- Jezzard P, Matthews PM, Smith SM (2001) *Functional MRI: an introduction to methods*. Oxford: Oxford University Press.
- Jubault T, Gagnon JF, Karama S, Pitto A, Lafontaine AL, Evans AC, Monchi O (2011) Patterns of cortical thickness and surface area in early Parkinson's disease. *Neuroimage* 55:462-467.
- Kanwisher N, McDermott J, Chun MM (1997) The fusiform face area: a module in human extrastriate cortex specialized for face perception. *J Neurosci* 17:4302-4311.
- Kayser AS, Allen DC, Navarro-Cebrian A, Mitchell JM, Fields HL (2012) Dopamine, corticostriatal connectivity, and intertemporal choice. *J Neurosci* 32:9402-9409.
- Kelly AM, Uddin LQ, Biswal BB, Castellanos FX, Milham MP (2008) Competition between functional brain networks mediates behavioral variability. *Neuroimage* 39:527-537.
- Kelly C, de Zubicaray G, Di Martino A, Copland DA, Reiss PT, Klein DF, Castellanos FX, Milham MP, McMahon K (2009) L-dopa modulates functional connectivity in striatal cognitive and motor networks: a double-blind placebo-controlled study. *J Neurosci* 29:7364-7378.
- Kenney JW, Gould TJ (2008) Modulation of hippocampus-dependent learning and synaptic plasticity by nicotine. *Mol Neurobiol* 38:101-121.
- Khalili-Mahani N, Zoethout RM, Beckmann CF, Baerends E, de Kam ML, Soeter RP, Dahan A, van Buchem MA, van Gerven JM, Rombouts SA (2012) Effects of morphine and alcohol on functional brain connectivity during "resting state": A placebo-controlled crossover study in healthy young men. *Hum Brain Mapp* 33:1003-1018.
- Kim EJ, Sidhu M, Gaus SE, Huang EJ, Hof PR, Miller BL, Dearmond SJ, Seeley WW (2012) Selective frontoinsula von Economo neuron and fork cell loss in early behavioral variant frontotemporal dementia. *Cereb Cortex* 22:251-259.
- Kirby KN, Petry NM, Bickel WK (1999) Heroin addicts have higher discount rates for delayed rewards than non-drug-using controls. *J Exp Psychol Gen* 128:78-87.
- Kishida KT, King-Casas B, Montague PR (2010) Neuroeconomic approaches to mental disorders. *Neuron* 67:543-554.
- Kiviniemi V, Kantola JH, Jauhiainen J, Hyvarinen A, Tervonen O (2003) Independent component analysis of nondeterministic fMRI signal sources. *Neuroimage* 19:253-260.
- Kiviniemi V, Vire T, Remes J, Elseoud AA, Starck T, Tervonen O, Nikkinen J (2011) A Sliding Time-Window ICA Reveals Spatial Variability of the Default Mode Network in Time. *Brain Connect* 1:339-347.

- Kiviniemi V, Starck T, Remes J, Long X, Nikkinen J, Haapea M, Veijola J, Moilanen I, Isohanni M, Zang YF, Tervonen O (2009) Functional segmentation of the brain cortex using high model order group PICA. *Hum Brain Mapp* 30:3865-3886.
- Klumpers LE, Cole DM, Khalili-Mahani N, Soeter RP, te Beek ET, Rombouts SARB and van Gerven JMA (2012). Manipulating brain connectivity with δ^9 -tetrahydrocannabinol: a pharmacological resting state fMRI study. *Neuroimage* 63:1701-1711.
- Koob GF, Volkow ND (2010) Neurocircuitry of addiction. *Neuropsychopharmacology* 35:217-238.
- Kotter R, Stephan KE, Palomero-Gallagher N, Geyer S, Schleicher A, Zilles K (2001) Multimodal characterisation of cortical areas by multivariate analyses of receptor binding and connectivity data. *Anat Embryol (Berl)* 204:333-350.
- Kriegeskorte N, Simmons WK, Bellgowan PS, Baker CI (2009) Circular analysis in systems neuroscience: the dangers of double dipping. *Nat Neurosci* 12:535-540.
- Krienen FM, Buckner RL (2009) Segregated fronto-cerebellar circuits revealed by intrinsic functional connectivity. *Cereb Cortex* 19:2485-2497.
- Kringelbach ML, Rolls ET (2004) The functional neuroanatomy of the human orbitofrontal cortex: evidence from neuroimaging and neuropsychology. *Prog Neurobiol* 72:341-372.
- Kunisato Y, Okamoto Y, Okada G, Aoyama S, Demoto Y, Munakata A, Nomura M, Onoda K, Yamawaki S (2011) Modulation of default-mode network activity by acute tryptophan depletion is associated with mood change: a resting state functional magnetic resonance imaging study. *Neurosci Res* 69:129-134.
- Kwak Y, Peltier S, Bohnen NI, Muller ML, Dayalu P, Seidler RD (2010) Altered resting state cortico-striatal connectivity in mild to moderate stage Parkinson's disease. *Front Syst Neurosci* 4:143.
- Laird AR, Fox PM, Eickhoff SB, Turner JA, Ray KL, McKay DR, Glahn DC, Beckmann CF, Smith SM, Fox PT (2011) Behavioral interpretations of intrinsic connectivity networks. *J Cogn Neurosci* 23:4022-4037.
- Lammertsma AA, Hume SP (1996) Simplified reference tissue model for PET receptor studies. *Neuroimage* 4:153-158.
- Lancaster JL, Woldorff MG, Parsons LM, Liotti M, Freitas CS, Rainey L, Kochunov PV, Nickerson D, Mikiten SA, Fox PT (2000) Automated Talairach atlas labels for functional brain mapping. *Hum Brain Mapp* 10:120-131.
- Langers DR (2010) Unbiased group-level statistical assessment of independent component maps by means of automated retrospective matching. *Hum Brain Mapp* 31:727-742.
- Laruelle M, Kegeles LS, Abi-Dargham A (2003) Glutamate, dopamine, and schizophrenia: from pathophysiology to treatment. *Ann N Y Acad Sci* 1003:138-158.
- Laufs H (2008) Endogenous brain oscillations and related networks detected by surface EEG-combined fMRI. *Hum Brain Mapp* 29:762-769.
- Laufs H, Krakow K, Sterzer P, Eger E, Beyerle A, Salek-Haddadi A, Kleinschmidt A (2003) Electroencephalographic signatures of attentional and cognitive

- default modes in spontaneous brain activity fluctuations at rest. *Proc Natl Acad Sci U S A* 100:11053-11058.
- Lawrence NS, Ross TJ, Stein EA (2002) Cognitive mechanisms of nicotine on visual attention. *Neuron* 36:539-548.
- Lee B, London ED, Poldrack RA, Farahi J, Nacca A, Monterosso JR, Mumford JA, Bokarius AV, Dahlbom M, Mukherjee J, Bilder RM, Brody AL, Mandelkern MA (2009) Striatal dopamine d2/d3 receptor availability is reduced in methamphetamine dependence and is linked to impulsivity. *J Neurosci* 29:14734-14740.
- Lee JH, Durand R, Gradinaru V, Zhang F, Goshen I, Kim DS, Fenno LE, Ramakrishnan C, Deisseroth K (2010) Global and local fMRI signals driven by neurons defined optogenetically by type and wiring. *Nature* 465:788-792.
- Leech R, Kamourieh S, Beckmann CF, Sharp DJ (2011) Fractionating the default mode network: distinct contributions of the ventral and dorsal posterior cingulate cortex to cognitive control. *J Neurosci* 31:3217-3224.
- Leergaard TB, Hilgetag CC, Sporns O (2012) Mapping the connectome: multi-level analysis of brain connectivity. *Front Neuroinform* 6:14.
- Levant B (1997) The D3 dopamine receptor: neurobiology and potential clinical relevance. *Pharmacol Rev* 49:231-252.
- Lewis CM, Baldassarre A, Committeri G, Romani GL, Corbetta M (2009) Learning sculpts the spontaneous activity of the resting human brain. *Proc Natl Acad Sci U S A* 106:17558-17563.
- Li R, Chen K, Fleisher AS, Reiman EM, Yao L, Wu X (2011) Large-scale directional connections among multi resting-state neural networks in human brain: a functional MRI and Bayesian network modeling study. *Neuroimage* 56:1035-1042.
- Linden DE (2012) The challenges and promise of neuroimaging in psychiatry. *Neuron* 73:8-22.
- Liu B, Song M, Li J, Liu Y, Li K, Yu C, Jiang T (2010) Prefrontal-related functional connectivities within the default network are modulated by COMT val158met in healthy young adults. *J Neurosci* 30:64-69.
- Liu H, Liu Z, Liang M, Hao Y, Tan L, Kuang F, Yi Y, Xu L, Jiang T (2006) Decreased regional homogeneity in schizophrenia: a resting state functional magnetic resonance imaging study. *Neuroreport* 17:19-22.
- Llinas R, Ribary U, Contreras D, Pedroarena C (1998) The neuronal basis for consciousness. *Philos Trans R Soc Lond B Biol Sci* 353:1841-1849.
- Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A (2001) Neurophysiological investigation of the basis of the fMRI signal. *Nature* 412:150-157.
- Lowe MJ, Mock BJ, Sorenson JA (1998) Functional connectivity in single and multislice echoplanar imaging using resting-state fluctuations. *Neuroimage* 7:119-132.
- Lu H, Zou Q, Gu H, Raichle ME, Stein EA, Yang Y (2012) Rat brains also have a default mode network. *Proc Natl Acad Sci U S A* 109:3979-3984.
- Lu J, Liu H, Zhang M, Wang D, Cao Y, Ma Q, Rong D, Wang X, Buckner RL, Li K (2011) Focal pontine lesions provide evidence that intrinsic functional connectivity reflects polysynaptic anatomical pathways. *J Neurosci* 31:15065-15071.

- Mansvelder HD, van Aerde KI, Couey JJ, Brussaard AB (2006) Nicotinic modulation of neuronal networks: from receptors to cognition. *Psychopharmacology (Berl)* 184:292-305.
- Mantini D, Perrucci MG, Del Gratta C, Romani GL, Corbetta M (2007) Electrophysiological signatures of resting state networks in the human brain. *Proc Natl Acad Sci U S A* 104:13170-13175.
- Mantini D, Gerits A, Nelissen K, Durand JB, Joly O, Simone L, Sawamura H, Wardak C, Orban GA, Buckner RL, Vanduffel W (2011) Default mode of brain function in monkeys. *J Neurosci* 31:12954-12962.
- Margulies DS, Kelly AM, Uddin LQ, Biswal BB, Castellanos FX, Milham MP (2007) Mapping the functional connectivity of anterior cingulate cortex. *Neuroimage* 37:579-588.
- Marrelec G, Kim J, Doyon J, Horwitz B (2009) Large-scale neural model validation of partial correlation analysis for effective connectivity investigation in functional MRI. *Hum Brain Mapp* 30:941-950.
- Martinez D, Carpenter KM, Liu F, Slifstein M, Broft A, Friedman AC, Kumar D, Van Heertum R, Kleber HD, Nunes E (2011) Imaging dopamine transmission in cocaine dependence: link between neurochemistry and response to treatment. *Am J Psychiatry* 168:634-641.
- Marx E, Deutschlander A, Stephan T, Dieterich M, Wiesmann M, Brandt T (2004) Eyes open and eyes closed as rest conditions: impact on brain activation patterns. *Neuroimage* 21:1818-1824.
- Mathew RJ, Wilson WH (1991) Substance abuse and cerebral blood flow. *Am J Psychiatry* 148:292-305.
- Matthews PM, Honey GD, Bullmore ET (2006) Applications of fMRI in translational medicine and clinical practice. *Nat Rev Neurosci* 7:732-744.
- Matthews PM, Rabiner I, Gunn R (2011) Non-invasive imaging in experimental medicine for drug development. *Curr Opin Pharmacol* 11:501-507.
- McAvoy M, Larson-Prior L, Nolan TS, Vaishnavi SN, Raichle ME, d'Avossa G (2008) Resting states affect spontaneous BOLD oscillations in sensory and paralimbic cortex. *J Neurophysiol* 100:922-931.
- McKeown MJ, Makeig S, Brown GG, Jung TP, Kindermann SS, Bell AJ, Sejnowski TJ (1998) Analysis of fMRI data by blind separation into independent spatial components. *Hum Brain Mapp* 6:160-188.
- Menke RA, Jbabdi S, Miller KL, Matthews PM, Zarei M (2010) Connectivity-based segmentation of the substantia nigra in human and its implications in Parkinson's disease. *Neuroimage* 52:1175-1180.
- Mennes M, Kelly C, Zuo XN, Di Martino A, Biswal BB, Castellanos FX, Milham MP (2010) Inter-individual differences in resting-state functional connectivity predict task-induced BOLD activity. *Neuroimage* 50:1690-1701.
- Menon V (2011) Large-scale brain networks and psychopathology: a unifying triple network model. *Trends Cogn Sci* 15:483-506.
- Meunier D, Ersche KD, Craig KJ, Fornito A, Merlo-Pich E, Fineberg NA, Shabbir SS, Robbins TW, Bullmore ET (2012) Brain functional connectivity in stimulant drug dependence and obsessive-compulsive disorder. *Neuroimage* 59:1461-1468.
- Meyer-Lindenberg A (2009) Neural connectivity as an intermediate phenotype: brain networks under genetic control. *Hum Brain Mapp* 30:1938-1946.

- Milenkova M, Mohammadi B, Kollwe K, Schrader C, Fellbrich A, Wittfoth M, Dengler R, Munte TF (2011) Intertemporal choice in Parkinson's disease. *Mov Disord* 26:2004-2010.
- Montague PR, Hyman SE, Cohen JD (2004) Computational roles for dopamine in behavioural control. *Nature* 431:760-767.
- Montague PR, Dolan RJ, Friston KJ, Dayan P (2012) Computational psychiatry. *Trends Cogn Sci* 16:72-80.
- Moran RJ, Symmonds M, Stephan KE, Friston KJ, Dolan RJ (2011) An in vivo assay of synaptic function mediating human cognition. *Curr Biol* 21:1320-1325.
- Morcom AM, Fletcher PC (2007) Does the brain have a baseline? Why we should be resisting a rest. *Neuroimage* 37:1073-1082.
- Mumford JA, Horvath S, Oldham MC, Langfelder P, Geschwind DH, Poldrack RA (2010) Detecting network modules in fMRI time series: a weighted network analysis approach. *Neuroimage* 52:1465-1476.
- Murphy K, Birn RM, Handwerker DA, Jones TB, Bandettini PA (2009) The impact of global signal regression on resting state correlations: are anti-correlated networks introduced? *Neuroimage* 44:893-905.
- Murphy SE, Mackay CE (2011) Using MRI to measure drug action: caveats and new directions. *J Psychopharmacol* 25:1168-1174.
- Musso F, Brinkmeyer J, Mobascher A, Warbrick T, Winterer G (2010) Spontaneous brain activity and EEG microstates. A novel EEG/fMRI analysis approach to explore resting-state networks. *Neuroimage* 52:1149-1161.
- Nagano-Saito A, Leyton M, Monchi O, Goldberg YK, He Y, Dagher A (2008) Dopamine depletion impairs frontostriatal functional connectivity during a set-shifting task. *J Neurosci* 28:3697-3706.
- Naqvi NH, Rudrauf D, Damasio H, Bechara A (2007) Damage to the insula disrupts addiction to cigarette smoking. *Science* 315:531-534.
- Newhouse PA, Potter AS, Dumas JA, Thiel CM (2011) Functional brain imaging of nicotinic effects on higher cognitive processes. *Biochem Pharmacol* 82:943-951.
- Niazy RK, Xie J, Miller K, Beckmann CF, Smith SM (2011) Spectral characteristics of resting state networks. *Prog Brain Res* 193:259-276.
- Nichols TE, Holmes AP (2002) Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum Brain Mapp* 15:1-25.
- Nieoullon A (2002) Dopamine and the regulation of cognition and attention. *Prog Neurobiol* 67:53-83.
- Nimchinsky EA, Gilissen E, Allman JM, Perl DP, Erwin JM, Hof PR (1999) A neuronal morphologic type unique to humans and great apes. *Proc Natl Acad Sci U S A* 96:5268-5273.
- Noonan MP, Walton ME, Behrens TE, Sallet J, Buckley MJ, Rushworth MF (2010) Separate value comparison and learning mechanisms in macaque medial and lateral orbitofrontal cortex. *Proc Natl Acad Sci U S A* 107:20547-20552.
- Northoff G, Qin P, Nakao T (2010a) Rest-stimulus interaction in the brain: a review. *Trends Neurosci* 33:277-284.
- Northoff G, Duncan NW, Hayes DJ (2010b) The brain and its resting state activity--experimental and methodological implications. *Prog Neurobiol* 92:593-600.

- O'Doherty J, Critchley H, Deichmann R, Dolan RJ (2003) Dissociating valence of outcome from behavioral control in human orbital and ventral prefrontal cortices. *J Neurosci* 23:7931-7939.
- O'Doherty J, Kringelbach ML, Rolls ET, Hornak J, Andrews C (2001) Abstract reward and punishment representations in the human orbitofrontal cortex. *Nat Neurosci* 4:95-102.
- O'Doherty JP (2004) Reward representations and reward-related learning in the human brain: insights from neuroimaging. *Curr Opin Neurobiol* 14:769-776.
- O'Reilly JX, Beckmann CF, Tomassini V, Ramnani N, Johansen-Berg H (2010) Distinct and overlapping functional zones in the cerebellum defined by resting state functional connectivity. *Cereb Cortex* 20:953-965.
- O'Sullivan SS, Wu K, Politis M, Lawrence AD, Evans AH, Bose SK, Djamshidian A, Lees AJ, Piccini P (2011) Cue-induced striatal dopamine release in Parkinson's disease-associated impulsive-compulsive behaviours. *Brain* 134:969-978.
- Ogawa S, Lee TM, Kay AR, Tank DW (1990) Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *Proc Natl Acad Sci U S A* 87:9868-9872.
- Owen AM, James M, Leigh PN, Summers BA, Marsden CD, Quinn NP, Lange KW, Robbins TW (1992) Fronto-striatal cognitive deficits at different stages of Parkinson's disease. *Brain* 115 (Pt 6):1727-1751.
- Paakki JJ, Rahko J, Long X, Moilanen I, Tervonen O, Nikkinen J, Starck T, Remes J, Hurtig T, Haapsamo H, Jussila K, Kuusikko-Gauffin S, Mattila ML, Zang Y, Kiviniemi V (2010) Alterations in regional homogeneity of resting-state brain activity in autism spectrum disorders. *Brain Res* 1321:169-179.
- Parrott AC, Roberts G (1991) Smoking deprivation and cigarette reinstatement: effects upon visual attention. *J Psychopharmacol* 5:404-409.
- Parrott AC, Garnham NJ, Wesnes K, Pincock C (1996) Cigarette smoking and abstinence: comparative effects upon cognitive task performance and mood state over 24 hours. *Hum Psychopharmacol* 11:391-400.
- Patenaude B, Smith SM, Kennedy DN, Jenkinson M (2011) A Bayesian model of shape and appearance for subcortical brain segmentation. *Neuroimage* 56:907-922.
- Patton JH, Stanford MS, Barratt ES (1995) Factor structure of the Barratt impulsiveness scale. *J Clin Psychol* 51:768-774.
- Pawela C, Biswal B (2011) Brain connectivity: a new journal emerges. *Brain Connect* 1:1-2.
- Pessiglione M, Seymour B, Flandin G, Dolan RJ, Frith CD (2006) Dopamine-dependent prediction errors underpin reward-seeking behaviour in humans. *Nature* 442:1042-1045.
- Peters J, Buchel C (2010) Episodic future thinking reduces reward delay discounting through an enhancement of prefrontal-mediotemporal interactions. *Neuron* 66:138-148.
- Petry NM (2001) Pathological gamblers, with and without substance use disorders, discount delayed rewards at high rates. *J Abnorm Psychol* 110:482-487.
- Pine A, Shiner T, Seymour B, Dolan RJ (2010) Dopamine, time, and impulsivity in humans. *J Neurosci* 30:8888-8896.

- Plisson C, Huiban M, Pampols-Maso S, Singleton G, Hill SP, Passchier J (2012) Automated preparation of the dopamine D2/3 receptor agonist ligand [11C]-(+)-PHNO for human PET imaging studies. *Appl Radiat Isot* 70:380-387.
- Pluck GC, Brown RG (2002) Apathy in Parkinson's disease. *J Neurol Neurosurg Psychiatry* 73:636-642.
- Poldrack RA, Halchenko YO, Hanson SJ (2009) Decoding the large-scale structure of brain function by classifying mental States across individuals. *Psychol Sci* 20:1364-1372.
- Popa D, Popescu AT, Pare D (2009) Contrasting activity profile of two distributed cortical networks as a function of attentional demands. *J Neurosci* 29:1191-1201.
- Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE (2012) Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *Neuroimage* 59:2142-2154.
- Pyka M, Beckmann CF, Schoning S, Hauke S, Heider D, Kugel H, Arolt V, Konrad C (2009) Impact of working memory load on fMRI resting state pattern in subsequent resting phases. *PLoS One* 4:e7198.
- Rabiner EA, Laruelle M (2010) Imaging the D3 receptor in humans in vivo using [11C](+)-PHNO positron emission tomography (PET). *Int J Neuropsychopharmacol* 13:289-290.
- Rabiner EA, Slifstein M, Nobrega J, Plisson C, Huiban M, Raymond R, Diwan M, Wilson AA, McCormick P, Gentile G, Gunn RN, Laruelle MA (2009) In vivo quantification of regional dopamine-D3 receptor binding potential of (+)-PHNO: Studies in non-human primates and transgenic mice. *Synapse* 63:782-793.
- Raichle ME, Mintun MA (2006) Brain work and brain imaging. *Annu Rev Neurosci* 29:449-476.
- Raichle ME, Snyder AZ (2007) A default mode of brain function: a brief history of an evolving idea. *Neuroimage* 37:1083-1090; discussion 1097-1089.
- Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL (2001) A default mode of brain function. *Proc Natl Acad Sci U S A* 98:676-682.
- Ramsey JD, Hanson SJ, Hanson C, Halchenko YO, Poldrack RA, Glymour C (2010) Six problems for causal inference from fMRI. *Neuroimage* 49:1545-1558.
- Reynolds JN, Wickens JR (2002) Dopamine-dependent plasticity of corticostriatal synapses. *Neural Netw* 15:507-521.
- Riba J, Kramer UM, Heldmann M, Richter S, Munte TF (2008) Dopamine agonist increases risk taking but blunts reward-related brain activity. *PLoS One* 3:e2479.
- Robbins TW (2000) Chemical neuromodulation of frontal-executive functions in humans and other animals. *Exp Brain Res* 133:130-138.
- Robinson S, Basso G, Soldati N, Sailer U, Jovicich J, Bruzzone L, Kryspin-Exner I, Bauer H, Moser E (2009) A resting state network in the motor control circuit of the basal ganglia. *BMC Neurosci* 10:137.
- Roiser JP, Elliott R, Sahakian BJ (2012) Cognitive mechanisms of treatment in depression. *Neuropsychopharmacology* 37:117-136.

- Rombouts SA, Barkhof F, Goekoop R, Stam CJ, Scheltens P (2005) Altered resting state networks in mild cognitive impairment and mild Alzheimer's disease: an fMRI study. *Hum Brain Mapp* 26:231-239.
- Rombouts SA, Damoiseaux JS, Goekoop R, Barkhof F, Scheltens P, Smith SM, Beckmann CF (2009) Model-free group analysis shows altered BOLD FMRI networks in dementia. *Hum Brain Mapp* 30:256-266.
- Roosendaal SD, Schoonheim MM, Hulst HE, Sanz-Arigita EJ, Smith SM, Geurts JJ, Barkhof F (2010) Resting state networks change in clinically isolated syndrome. *Brain* 133:1612-1621.
- Rowe JB (2010) Connectivity Analysis is Essential to Understand Neurological Disorders. *Front Syst Neurosci* 4.
- Sadaghiani S, Hesselmann G, Kleinschmidt A (2009) Distributed and antagonistic contributions of ongoing activity fluctuations to auditory stimulus detection. *J Neurosci* 29:13410-13417.
- Saini S, DeStefano N, Smith S, Guidi L, Amato MP, Federico A, Matthews PM (2004) Altered cerebellar functional connectivity mediates potential adaptive plasticity in patients with multiple sclerosis. *J Neurol Neurosurg Psychiatry* 75:840-846.
- Sala-Llorch R, Pena-Gomez C, Arenaza-Urquijo EM, Vidal-Pineiro D, Bargallo N, Junque C, Bartres-Faz D (2012) Brain connectivity during resting state and subsequent working memory task predicts behavioural performance. *Cortex* 48:1187-1196.
- Salvador R, Suckling J, Schwarzbauer C, Bullmore E (2005) Undirected graphs of frequency-dependent functional connectivity in whole brain networks. *Philos Trans R Soc Lond B Biol Sci* 360:937-946.
- Salvador R, Martinez A, Pomarol-Clotet E, Sarro S, Suckling J, Bullmore E (2007) Frequency based mutual information measures between clusters of brain regions in functional magnetic resonance imaging. *Neuroimage* 35:83-88.
- Schafer M, Rujescu D, Giegling I, Guntermann A, Erfurth A, Bondy B, Moller HJ (2001) Association of short-term response to haloperidol treatment with a polymorphism in the dopamine D(2) receptor gene. *Am J Psychiatry* 158:802-804.
- Schleim S, Roiser JP (2009) FMRI in translation: the challenges facing real-world applications. *Front Hum Neurosci* 3:63.
- Schultz W (2002) Getting formal with dopamine and reward. *Neuron* 36:241-263.
- Searle G, Beaver JD, Comley RA, Bani M, Tziortzi A, Slifstein M, Mugnaini M, Griffante C, Wilson AA, Merlo-Pich E, Houle S, Gunn R, Rabiner EA, Laruelle M (2010) Imaging dopamine D3 receptors in the human brain with positron emission tomography, [¹¹C]PHNO, and a selective D3 receptor antagonist. *Biol Psychiatry* 68:392-399.
- Seeley WW, Crawford RK, Zhou J, Miller BL, Greicius MD (2009) Neurodegenerative diseases target large-scale human brain networks. *Neuron* 62:42-52.
- Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, Kenna H, Reiss AL, Greicius MD (2007) Dissociable intrinsic connectivity networks for salience processing and executive control. *J Neurosci* 27:2349-2356.
- Sesack SR, Grace AA (2010) Cortico-Basal Ganglia reward network: microcircuitry. *Neuropsychopharmacology* 35:27-47.

- Shannon BJ, Raichle ME, Snyder AZ, Fair DA, Mills KL, Zhang D, Bache K, Calhoun VD, Nigg JT, Nagel BJ, Stevens AA, Kiehl KA (2011) Premotor functional connectivity predicts impulsivity in juvenile offenders. *Proc Natl Acad Sci U S A* 108:11241-11245.
- Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar GC (1998) The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* 59 Suppl 20:22-33;quiz 34-57.
- Shehzad Z, Kelly AM, Reiss PT, Gee DG, Gotimer K, Uddin LQ, Lee SH, Margulies DS, Roy AK, Biswal BB, Petkova E, Castellanos FX, Milham MP (2009) The resting brain: unconstrained yet reliable. *Cereb Cortex* 19:2209-2229.
- Shirer WR, Ryali S, Rykhlevskaia E, Menon V, Greicius MD (2012) Decoding subject-driven cognitive states with whole-brain connectivity patterns. *Cereb Cortex* 22:158-165.
- Shmuel A, Leopold DA (2008) Neuronal correlates of spontaneous fluctuations in fMRI signals in monkey visual cortex: Implications for functional connectivity at rest. *Hum Brain Mapp* 29:751-761.
- Shotbolt P, Tziortzi AC, Searle GE, Colasanti A, van der Aart J, Abanades S, Plisson C, Miller SR, Huiban M, Beaver JD, Gunn RN, Laruelle M, Rabiner EA (2012) Within-subject comparison of [(11)C]-(+)-PHNO and [(11)C]raclopride sensitivity to acute amphetamine challenge in healthy humans. *J Cereb Blood Flow Metab* 32:127-136.
- Shulman GL, Fiez JA, Corbetta M, Buckner RL, Miezin FM, Raichle ME, Petersen SE (1997) Common blood flow changes across visual tasks: II. Decreases in cerebral cortex. *J Cogn Neurosci* 9:648-663.
- Singh KD, Fawcett IP (2008) Transient and linearly graded deactivation of the human default-mode network by a visual detection task. *Neuroimage* 41:100-112.
- Smith SM (2002) Fast robust automated brain extraction. *Hum Brain Mapp* 17:143-155.
- Smith SM (2012) The future of FMRI connectivity. *Neuroimage* 62:1257-1266.
- Smith SM, Fox PT, Miller KL, Glahn DC, Fox PM, Mackay CE, Filippini N, Watkins KE, Toro R, Laird AR, Beckmann CF (2009) Correspondence of the brain's functional architecture during activation and rest. *Proc Natl Acad Sci U S A* 106:13040-13045.
- Smith SM, Miller KL, Salimi-Khorshidi G, Webster M, Beckmann CF, Nichols TE, Ramsey JD, Woolrich MW (2011) Network modelling methods for FMRI. *Neuroimage* 54:875-891.
- Smith SM, Miller KL, Moeller S, Xu J, Auerbach EJ, Woolrich MW, Beckmann CF, Jenkinson M, Andersson J, Glasser MF, Van Essen DC, Feinberg DA, Yacoub ES, Ugurbil K (2012a) Temporally-independent functional modes of spontaneous brain activity. *Proc Natl Acad Sci U S A* 109:3131-3136.
- Smith SM, Bandettini PA, Miller KL, Behrens TE, Friston KJ, David O, Liu T, Woolrich MW, Nichols TE (2012b) The danger of systematic bias in group-level FMRI-lag-based causality estimation. *Neuroimage* 59:1228-1229.
- Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, Bannister PR, De Luca M, Drobnjak I, Flitney DE, Niazy RK, Saunders J,

- Vickers J, Zhang Y, De Stefano N, Brady JM, Matthews PM (2004) Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage* 23 Suppl 1:S208-219.
- Sokoloff P, Diaz J, Le Foll B, Guillin O, Leriche L, Bezard E, Gross C (2006) The dopamine D3 receptor: a therapeutic target for the treatment of neuropsychiatric disorders. *CNS Neurol Disord Drug Targets* 5:25-43.
- Sporns O, Tononi G, Kotter R (2005) The human connectome: A structural description of the human brain. *PLoS Comput Biol* 1:e42.
- Sridharan D, Levitin DJ, Menon V (2008) A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. *Proc Natl Acad Sci U S A* 105:12569-12574.
- Staley JK, Mash DC (1996) Adaptive increase in D3 dopamine receptors in the brain reward circuits of human cocaine fatalities. *J Neurosci* 16:6100-6106.
- Stam CJ, Reijneveld JC (2007) Graph theoretical analysis of complex networks in the brain. *Nonlinear Biomed Phys* 1:3.
- Starck T, Remes J, Nikkinen J, Tervonen O, Kiviniemi V (2010) Correction of low-frequency physiological noise from the resting state BOLD fMRI--Effect on ICA default mode analysis at 1.5 T. *J Neurosci Methods* 186:179-185.
- Summerfield C, Behrens TE, Koechlin E (2011) Perceptual classification in a rapidly changing environment. *Neuron* 71:725-736.
- Sutherland MT, McHugh MJ, Pariyadath V, Stein EA (2012) Resting state functional connectivity in addiction: Lessons learned and a road ahead. *Neuroimage* 62:2281-2295.
- Thomason ME, Yoo DJ, Glover GH, Gotlib IH (2009) BDNF genotype modulates resting functional connectivity in children. *Front Hum Neurosci* 3:55.
- Tinaz S, Courtney MG, Stern CE (2011) Focal cortical and subcortical atrophy in early Parkinson's disease. *Mov Disord* 26:436-441.
- Tohka J, Foerke K, Aron AR, Tom SM, Toga AW, Poldrack RA (2008) Automatic independent component labeling for artifact removal in fMRI. *Neuroimage* 39:1227-1245.
- Tomasi D, Volkow ND (2012) Abnormal functional connectivity in children with attention-deficit/hyperactivity disorder. *Biol Psychiatry* 71:443-450.
- Tost H, Braus DF, Hakimi S, Ruf M, Vollmert C, Hohn F, Meyer-Lindenberg A (2010) Acute D2 receptor blockade induces rapid, reversible remodeling in human cortical-striatal circuits. *Nat Neurosci* 13:920-922.
- Tziortzi AC, Searle GE, Tzimopoulou S, Salinas C, Beaver JD, Jenkinson M, Laruelle M, Rabiner EA, Gunn RN (2011) Imaging dopamine receptors in humans with [11C]-(+)-PHNO: dissection of D3 signal and anatomy. *Neuroimage* 54:264-277.
- Uddin LQ, Kelly AM, Biswal BB, Xavier Castellanos F, Milham MP (2009) Functional connectivity of default mode network components: correlation, anticorrelation, and causality. *Hum Brain Mapp* 30:625-637.
- Valdes-Sosa PA, Roebroeck A, Daunizeau J, Friston K (2011) Effective connectivity: influence, causality and biophysical modeling. *Neuroimage* 58:339-361.
- Van de Ville D, Britz J, Michel CM (2010) EEG microstate sequences in healthy humans at rest reveal scale-free dynamics. *Proc Natl Acad Sci U S A* 107:18179-18184.

- Van Dijk KR, Sabuncu MR, Buckner RL (2012) The influence of head motion on intrinsic functional connectivity MRI. *Neuroimage* 59:431-438.
- Van Dijk KR, Hedden T, Venkataraman A, Evans KC, Lazar SW, Buckner RL (2010) Intrinsic functional connectivity as a tool for human connectomics: theory, properties, and optimization. *J Neurophysiol* 103:297-321.
- Van Essen DC, Ugurbil K (2012) The future of the human connectome. *Neuroimage* 62:1299-1310.
- van Gaalen MM, Unger L, Jongen-Relo AL, Schoemaker H, Gross G (2009) Amphetamine decreases behavioral inhibition by stimulation of dopamine D2, but not D3, receptors. *Behav Pharmacol* 20:484-491.
- Varela F, Lachaux JP, Rodriguez E, Martinerie J (2001) The brainweb: phase synchronization and large-scale integration. *Nat Rev Neurosci* 2:229-239.
- Varoquaux G, Baronnet F, Kleinschmidt A, Fillard P, Thirion B (2010) Detection of brain functional-connectivity difference in post-stroke patients using group-level covariance modeling. *Med Image Comput Comput Assist Interv* 13:200-208.
- Vincent JL, Kahn I, Snyder AZ, Raichle ME, Buckner RL (2008) Evidence for a frontoparietal control system revealed by intrinsic functional connectivity. *J Neurophysiol* 100:3328-3342.
- Vincent JL, Snyder AZ, Fox MD, Shannon BJ, Andrews JR, Raichle ME, Buckner RL (2006) Coherent spontaneous activity identifies a hippocampal-parietal memory network. *J Neurophysiol* 96:3517-3531.
- Vincent JL, Patel GH, Fox MD, Snyder AZ, Baker JT, Van Essen DC, Zempel JM, Snyder LH, Corbetta M, Raichle ME (2007) Intrinsic functional architecture in the anaesthetized monkey brain. *Nature* 447:83-86.
- Voets NL, Beckmann CF, Cole DM, Hong S, Bernasconi A, Bernasconi N (2012) Structural substrates for resting network disruption in temporal lobe epilepsy. *Brain* 135:2350-2357.
- Voets NL, Adcock JE, Stacey R, Hart Y, Carpenter K, Matthews PM, Beckmann CF (2009) Functional and structural changes in the memory network associated with left temporal lobe epilepsy. *Hum Brain Mapp* 30:4070-4081.
- Volkow ND, Fowler JS, Wang GJ, Swanson JM (2004) Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. *Mol Psychiatry* 9:557-569.
- Volkow ND, Gur RC, Wang GJ, Fowler JS, Moberg PJ, Ding YS, Hitzemann R, Smith G, Logan J (1998) Association between decline in brain dopamine activity with age and cognitive and motor impairment in healthy individuals. *Am J Psychiatry* 155:344-349.
- Volkow ND, Tomasi D, Wang GJ, Fowler JS, Telang F, Goldstein RZ, Alia-Klein N, Woicik P, Wong C, Logan J, Millard J, Alexoff D (2011) Positive emotionality is associated with baseline metabolism in orbitofrontal cortex and in regions of the default network. *Mol Psychiatry* 16:818-825.
- Volkow ND, Chang L, Wang GJ, Fowler JS, Ding YS, Sedler M, Logan J, Franceschi D, Gatley J, Hitzemann R, Gifford A, Wong C, Pappas N (2001) Low level of brain dopamine D2 receptors in methamphetamine abusers: association with metabolism in the orbitofrontal cortex. *Am J Psychiatry* 158:2015-2021.

- Volkow ND, Fowler JS, Logan J, Alexoff D, Zhu W, Telang F, Wang GJ, Jayne M, Hooker JM, Wong C, Hubbard B, Carter P, Warner D, King P, Shea C, Xu Y, Muench L, Apelskog-Torres K (2009) Effects of modafinil on dopamine and dopamine transporters in the male human brain: clinical implications. *JAMA* 301:1148-1154.
- Wallace DL, Vytlačil JJ, Nomura EM, Gibbs SE, D'Esposito M (2011) The dopamine agonist bromocriptine differentially affects fronto-striatal functional connectivity during working memory. *Front Hum Neurosci* 5:32.
- Walton ME, Behrens TE, Buckley MJ, Rudebeck PH, Rushworth MF (2010) Separable learning systems in the macaque brain and the role of orbitofrontal cortex in contingent learning. *Neuron* 65:927-939.
- Wang K, Liang M, Wang L, Tian L, Zhang X, Li K, Jiang T (2007a) Altered functional connectivity in early Alzheimer's disease: a resting-state fMRI study. *Hum Brain Mapp* 28:967-978.
- Wang L, Zang Y, He Y, Liang M, Zhang X, Tian L, Wu T, Jiang T, Li K (2006) Changes in hippocampal connectivity in the early stages of Alzheimer's disease: evidence from resting state fMRI. *Neuroimage* 31:496-504.
- Wang Z, Faith M, Patterson F, Tang K, Kerrin K, Wileyto EP, Detre JA, Lerman C (2007b) Neural substrates of abstinence-induced cigarette cravings in chronic smokers. *J Neurosci* 27:14035-14040.
- Watson DJ, Loiseau F, Ingallinesi M, Millan MJ, Marsden CA, Fone KC (2012) Selective blockade of dopamine D(3) receptors enhances while D(2) receptor antagonism impairs social novelty discrimination and novel object recognition in rats: a key role for the prefrontal cortex. *Neuropsychopharmacology* 37:770-786.
- Weintraub D, Koester J, Potenza MN, Siderowf AD, Stacy M, Voon V, Whetteckey J, Wunderlich GR, Lang AE (2010). Impulse control disorders in Parkinson disease: a cross-sectional study of 3090 patients. *Arch Neurol* 67:589-595.
- Wig GS, Schlaggar BL, Petersen SE (2011) Concepts and principles in the analysis of brain networks. *Ann N Y Acad Sci* 1224:126-146.
- Wiggins JL, Bedoyan JK, Peltier SJ, Ashinoff S, Carrasco M, Weng SJ, Welsh RC, Martin DM, Monk CS (2012) The impact of serotonin transporter (5-HTTLPR) genotype on the development of resting-state functional connectivity in children and adolescents: A preliminary report. *Neuroimage* 59:2760-2770.
- Wilcox CE, Teshiba TM, Merideth F, Ling J, Mayer AR (2011) Enhanced cue reactivity and fronto-striatal functional connectivity in cocaine use disorders. *Drug Alcohol Depend* 115:137-144.
- Willeit M, Ginovart N, Graff A, Rusjan P, Vitcu I, Houle S, Seeman P, Wilson AA, Kapur S (2008) First human evidence of d-amphetamine induced displacement of a D2/3 agonist radioligand: A [11C]-(+)-PHNO positron emission tomography study. *Neuropsychopharmacology* 33:279-289.
- Williams D, Tijssen M, Van Bruggen G, Bosch A, Insola A, Di Lazzaro V, Mazzone P, Oliviero A, Quartarone A, Speelman H, Brown P (2002) Dopamine-dependent changes in the functional connectivity between basal ganglia and cerebral cortex in humans. *Brain* 125:1558-1569.
- Wilson AA, McCormick P, Kapur S, Willeit M, Garcia A, Hussey D, Houle S, Seeman P, Ginovart N (2005) Radiosynthesis and evaluation of [11C]-(+)-4-

- propyl-3,4,4a,5,6,10b-hexahydro-2H-naphtho[1,2-b][1,4]oxazin-9 -ol as a potential radiotracer for in vivo imaging of the dopamine D2 high-affinity state with positron emission tomography. *J Med Chem* 48:4153-4160.
- Wink AM, Bullmore E, Barnes A, Bernard F, Suckling J (2008) Monofractal and multifractal dynamics of low frequency endogenous brain oscillations in functional MRI. *Hum Brain Mapp* 29:791-801.
- Wise RA (2004) Dopamine, learning and motivation. *Nat Rev Neurosci* 5:483-494.
- Wolf ND, Sambataro F, Vasic N, Frasch K, Schmid M, Schonfeldt-Lecuona C, Thomann PA, Wolf RC (2011) Dysconnectivity of multiple resting-state networks in patients with schizophrenia who have persistent auditory verbal hallucinations. *J Psychiatry Neurosci* 36:366-374.
- Wonnacott S, Barik J, Dickinson J, Jones IW (2006) Nicotinic receptors modulate transmitter cross talk in the CNS: nicotinic modulation of transmitters. *J Mol Neurosci* 30:137-140.
- World Health Organization (2008) The global burden of disease: 2004 update.
- Worsley KJ, Friston KJ (1995) Analysis of fMRI time-series revisited--again. *Neuroimage* 2:173-181.
- Wu T, Wang L, Chen Y, Zhao C, Li K, Chan P (2009) Changes of functional connectivity of the motor network in the resting state in Parkinson's disease. *Neurosci Lett* 460:6-10.
- Xu J, Mendrek A, Cohen MS, Monterosso J, Rodriguez P, Simon SL, Brody A, Jarvik M, Domier CP, Olmstead R, Ernst M, London ED (2005) Brain activity in cigarette smokers performing a working memory task: effect of smoking abstinence. *Biol Psychiatry* 58:143-150.
- Yan C, Liu D, He Y, Zou Q, Zhu C, Zuo X, Long X, Zang Y (2009) Spontaneous brain activity in the default mode network is sensitive to different resting-state conditions with limited cognitive load. *PLoS One* 4:e5743.
- Zald DH, Cowan RL, Riccardi P, Baldwin RM, Ansari MS, Li R, Shelby ES, Smith CE, McHugo M, Kessler RM (2008) Midbrain dopamine receptor availability is inversely associated with novelty-seeking traits in humans. *J Neurosci* 28:14372-14378.
- Zang Y, Jiang T, Lu Y, He Y, Tian L (2004) Regional homogeneity approach to fMRI data analysis. *Neuroimage* 22:394-400.
- Zang YF, He Y, Zhu CZ, Cao QJ, Sui MQ, Liang M, Tian LX, Jiang TZ, Wang YF (2007) Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI. *Brain Dev* 29:83-91.
- Zhang D, Snyder AZ, Fox MD, Sansbury MW, Shimony JS, Raichle ME (2008) Intrinsic functional relations between human cerebral cortex and thalamus. *J Neurophysiol* 100:1740-1748.
- Zhang J, Cheng W, Wang Z, Zhang Z, Lu W, Lu G, Feng J (2012) Pattern classification of large-scale functional brain networks: identification of informative neuroimaging markers for epilepsy. *PLoS One* 7:e36733.
- Zhang Y, Brady M, Smith S (2001) Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. *IEEE Trans Med Imaging* 20:45-57.
- Zilles K, Amunts K (2009) Receptor mapping: architecture of the human cerebral cortex. *Curr Opin Neurol* 22:331-339.

- Zou QH, Zhu CZ, Yang Y, Zuo XN, Long XY, Cao QJ, Wang YF, Zang YF (2008) An improved approach to detection of amplitude of low-frequency fluctuation (ALFF) for resting-state fMRI: fractional ALFF. *J Neurosci Methods* 172:137-141.
- Zuo XN, Kelly C, Adelstein JS, Klein DF, Castellanos FX, Milham MP (2010a) Reliable intrinsic connectivity networks: test-retest evaluation using ICA and dual regression approach. *Neuroimage* 49:2163-2177.
- Zuo XN, Di Martino A, Kelly C, Shehzad ZE, Gee DG, Klein DF, Castellanos FX, Biswal BB, Milham MP (2010b) The oscillating brain: complex and reliable. *Neuroimage* 49:1432-1445.