Schizophrenia Bulletin vol. 39 no. 2 pp. 366–377, 2013
doi:10.1093/schbul/sbr144
Advance Access publication November 22, 2011

Subanaesthetic Ketamine Treatment Alters Prefrontal Cortex Connectivity With Thalamus and Ascending Subcortical Systems

Neil Dawson[*,1–3], **Brian J. Morris**[1,4], **and Judith A. Pratt**[1–3]

[1]Psychiatric Research Institute of Neuroscience in Glasgow (PsyRING), University of Glasgow, Glasgow, G12 8QQ, UK; [2]Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS), University of Strathclyde, 161 Cathedral Street, Glasgow, G4 0NR, UK; [3]Centre for Neuroscience, University of Strathclyde (CeNsUS), Glasgow, UK; [4]Institute of Neuroscience and Psychology, College of Medical and Veterinary Life Sciences, University of Glasgow, Glasgow, UK

*To whom correspondence should be addressed; tel: +44-(0)141-330-6604, fax: +44-(0)141-330-5659, e-mail: neil.dawson@strath.ac.uk

Background: **Acute treatment with subanaesthetic doses of NMDA receptor antagonists, such as ketamine, provides a translational model with relevance to many of the symptoms of schizophrenia. Previous studies have focused specifically on the prefrontal cortex (PFC) because this region is implicated in many of the functional deficits associated with this disorder and shows reduced activity (hypofrontality) in schizophrenia patients. Chronic NMDA antagonist treatment in rodents can also induce hypofrontality, although paradoxically acute NMDA receptor antagonist administration induces metabolic hyperfrontality.** ***Methods:*** **In this study, we use 2-deoxyglucose imaging data in mice to characterize acute ketamine-induced alterations in regional functional connectivity, a deeper analysis of the consequences of acute NMDA receptor hypofunction.** ***Results:*** **We show that acute ketamine treatment increases PFC metabolic activity while reducing metabolic activity in the dorsal reticular thalamic nucleus (dRT). This is associated with abnormal functional connectivity between the PFC and multiple thalamic nuclei, including the dRT, mediodorsal (MDthal), and anteroventral (AVthal) thalamus. In addition, we show that acute NMDA receptor blockade alters the functional connectivity of the serotonergic (dorsal raphe [DR]), noradrenergic (locus coeruleus [LC]), and cholinergic (vertical limb of the diagonal band of broca [VDB]) systems.** ***Conclusions:*** **Together with other emerging data, these findings suggest that the reticular nucleus of the thalamus, along with the diffusely projecting subcortical aminergic/cholinergic systems, represent a primary site of action for ketamine in reproducing the diverse symptoms of schizophrenia. Our results also demonstrate the added scientific insight gained by characterizing the functional connectivity of discrete brain regions from brain imaging data gained in a preclinical context.**

Key words: NMDA receptor/2-deoxyglucose autoradiographic imaging/partial least squares regression

Introduction

Schizophrenia is a chronic psychiatric disorder characterized by positive symptoms (eg, hallucinations), negative symptoms (eg, blunted affect), and cognitive deficits (eg, executive functioning and memory). Schizophrenia involves dysfunction in a diffuse network of brain regions, including the prefrontal and temporal cortices, hippocampus, and thalamic areas[1] (and supplementary references *S1, *S2). Arguably, the main focus of attention has been the prefrontal cortex (PFC), where converging metabolic, neurochemical, and behavioral evidence support impaired function in the disease. Reduced PFC metabolism (hypofrontality) in chronically ill patients[2] (and supplementary references *S3, *S4), reduced levels of gamma amino butyric acid (GABAergic) interneuron markers (eg, parvalbumin [PV]) detected postmortem[3] (and supplementary references *S5, *S6), and deficits in PFC-dependent cognitive tasks[4] (and sup plementary references *S7, *S8) all imply that the PFC is one of the most affected brain regions in schizophrenia.

While the molecular basis of schizophrenia is incompletely understood, compelling evidence supports a role for NMDA receptor (NMDAR) hypofunction. Both acute and repeated exposure to NMDAR antagonists can induce schizophrenia-like symptoms in humans[5] (and supplementary references *S9, *S10) and acute administration of the NMDAR antagonist ketamine exacerbates symptoms in schizophrenic patients[6] (and supplementary references *S11, *S12). Furthermore, in preclinical studies, NMDAR hypoactivation induces behavioral deficits with translational relevance to schizophrenia. In rodents,

acute NMDAR blockade induces deficits in prepulse inhibition[7] (and supplementary references *S13, *S14), memory[8] (and supplementary reference *S15), social interaction, and behavioral flexibility[9,10] (and supple mentary references *S16–*S20) that show similarities to the symptoms of schizophrenia. Furthermore, repeated exposure of rats to the NMDAR antagonist phencyclidine (PCP) induces cognitive deficits, along with alterations in brain function and neurochemistry, that parallel those seen in schizophrenia[7,9–12] (and supplementary reference *S21).

Numerous clinical and preclinical studies have been dedicated to elucidating the effect of acute NMDAR blockade on brain functioning[13] (and supplementary references *S22–*S29). However, there is still uncertainty concerning the mechanisms through which NMDAR antagonists produce a schizophrenia-like syndrome. The prevailing view is that they act primarily in the PFC, blocking tonically active NMDARs on GABAergic interneurons that suppress pyramidal neuron output. This reduces GABAergic output resulting in the disinhibition of pyramidal neuron firing, which in turn increases PFC-thalamic circuit activity and PFC glutamate release, leading to PFC hypermetabolism and impaired functioning[14] (and supplementary reference *S30). The paradox this creates considering the hypofrontality characteristic of long-term schizophrenia has not been resolved. Equally, NMDAR antagonist infusion into the PFC can not mimic their ability to increase PFC glutamate release when administered systemically[15] (and supplementary references *S31, *S32), suggesting that a primary site of action lies outwith the PFC.

The insight gained from preclinical studies of the effect of acute NMDAR blockade on brain function has been limited by the reductionist approach used, considering only overt alterations in functioning in discrete brain regions of interest (RoI). The analysis of functional brain imaging data gained in a preclinical setting is lagging behind that of clinical data, where the benefit of applying functional connectivity analysis has long been recognized[16] (and supplementary references *S33–*S35). In this context, the ability to determine the functional interactions between brain regions provides a more holistic appreciation of the actions of interventions on neural circuitry. Our recent studies using functional connectivity analysis in preclinical models have demonstrated the added insight gained when taking such an approach.[10] Here, we characterize the impact of an acute subanaesthetic dose of ketamine on brain function in mice by using ^{14}C-2-deoxyglucose autoradiographic imaging. We not only consider ketamine-induced alterations in overt regional metabolism but also alterations in regional functional connectivity, to provide greater insight into the nature of ketamine-induced alterations in brain functioning that have translational relevance to schizophrenia.

Methods

Animals

All experiments were completed using male C57BL/6J mice (aged 8–9 wk, Harlan-Olac Ltd), group housed (5–6 per cage) under standard conditions (21°C, 45%–65% humidity, 12-h dark/light cycle [lights on 0600 h]). Experiments were carried out in compliance with the Animals (Scientific Procedures) Act 1986. Access to food was restricted 4–5 hours prior to ^{14}C-2-deoxyglucose (^{14}C-2-DG) imaging to obviate the potential influence of ketamine on plasma glucose levels.

Semi-Quantitative ^{14}C-2-Deoxyglucose Autoradiographic Imaging

Local cerebral glucose utilization (LCGU) was determined 1 minute after treatment with 30 mg.kg^{-1} ketamine (Sigma-Aldrich; in 2 ml kg^{-1} saline, interperitoneally [i.p.], n = 9) or physiological saline (2 ml kg^{-1}, i.p., n = 9) in accordance with published protocols[10] (and supplementary reference *S36). The subanaesthetic dose of ketamine used in this study was based on that used in similar preclinical brain imaging studies (supplementary references *S24, *S26, *S27, *S37, *S38). Mice were injected with 4.625 MBq kg^{-1} of ^{14}C-2-DG (Perkin-Elmer) over 10 seconds before being returned to their home cage. Forty-five minutes after isotope injection animals were decapitated and a terminal blood sample collected by torso inversion. The brain was dissected out then frozen in isopentane (−40°C) and stored at −80°C until sectioning. Blood samples were centrifuged to separate the plasma and aliquots were removed for the determination of plasma glucose (10 μl) and ^{14}C (20 μl) concentrations by semiautomated glucose oxidase assay (Beckman) and liquid scintillation analysis (Packard), respectively.

Frozen brains were coronally sectioned (20 μm) in a cryostat (−20°C). Three consecutive sections were retained from every 60 μm, thaw mounted onto slide covers and rapidly dried on a hot plate (70°C). Autoradiograms were generated by apposing these sections, along with ^{14}C-standards (40–1069 nCi g^{-1} tissue equivalents; Amersham International) to X-ray film (Kodak, SB-5) for 5 days. Autoradiographic images were analyzed by computer-based image analysis (MCID/M5+). The local isotope concentration for each RoI was derived from the optical density of autoradiographic images relative to that of the coexposed ^{14}C standard. Sixty-four anatomically distinct RoI were measured with reference to a stereotactic mouse brain atlas (supplementary reference *S39). LCGU in each RoI was determined because the ratio of ^{14}C present in that region relative to the average ^{14}C concentration in the whole brain of the same animal, referred to as the ^{14}C-2-DG uptake ratio. Whole brain average ^{14}C levels were determined as the average ^{14}C

concentration across all sections in which a RoI was measured.

Data Analysis

Statistical Analysis of Ketamine-Induced Alterations in Overt Regional Metabolism. In keeping with previous studies, the significance of overt LCGU differences between experimental groups in discrete RoI were assumed to represent independent variables (supplementary reference *S40). Significant overt alterations in LCGU between groups were analyzed by *t* test, as were the plasma variables for animals involved in the study (see online supplementary table S1). Significance was set at $P < .05$.

Functional Brain Connectivity Analysis Using PLSR. Ketamine-induced alterations in RoI functional connectivity were considered using partial least squares regression (PLSR). The application of the PLSR algorithm to ^{14}C-2-DG data and its interpretation has been previously outlined,[10] and the algorithm is discussed in detail in Wold, 1995 (supplementary reference *S41) and Wold et al., 2001 (supplementary reference *S42). Data were analyzed using the PLSR module in XLSTAT 2010 (Addinsoft). PLSR is widely applied in human neuroimaging to determine the connectivity of brain regions to a defined "seed" region[16] (and supplementary references *S43–*S46). We take a similar approach in this study where only selected "seed" regions, in which ketamine induced a significant overt alteration in LCGU, are considered. In this way, ketamine-induced alterations in PFC connectivity were considered by determining the functional connectivity of the anterior prelimbic (PrL) and medial prelimbic (layer 2, mPrL2) cortex. The functional connectivity of the dorsal reticular (dRT), mediodorsal (MDthal), and anteroventral (AVthal) thalamus were considered when investigating ketamine-induced alterations in thalamic connectivity. Ketamine-induced alterations in the connectivity of the serotonergic (5-HT), noradrenergic (NA), and cholinergic (ACh) neurotransmitter systems were considered in terms of the functional connectivity of the dorsal raphe (DR), locus coeruleus (LC), and vertical band of broca (VDB), respectively. The functional connectivity of each "seed" region (dependent variable in the PLSR model) to all of the other RoI measured (explanatory variables; 63) was considered in terms of the variable importance to the projections (VIP) statistic. Within each experimental group and for each "seed" region, a significant functional connection between brain regions was considered to exist if the 95% CI for the VIP statistic exceeded 0.8 because this threshold denotes a considerable contribution of an explanatory variable to the dependent variable in PLSR models (supplementary reference *S42). The SD and CI for the VIP statistic were estimated by jackknifing. The significance of ketamine-induced alterations in functional connectivity (the VIP statistic) was analyzed using *t* test with Bonferroni correction for multiple comparisons. Significance was set at $P < .05$.

Results

Ketamine-Induced Alterations in Overt Regional Cerebral Metabolism

Representative pseudocolor autoradiograms showing ketamine-induced alterations in LCGU are shown in Figure 1. Ketamine treatment produced both significant increases and decreases in LCGU on a region-dependent basis, in 30 of the RoI analyzed (table 1; figure 2). In particular, ketamine treatment induced hyperfrontality, an increased rate of metabolism in the PFC, an effect present in all subfields of the prelimbic (anterior [PrL] and medial [mPrL1, mPrL2, and mPrL3]) cortex. In addition, ketamine induced significant hypermetabolism in the entorhinal cortex (EC), hippocampal subiculum (Sub), nucleus accumbens core (NacC), substantia nigra pars compacta (SNC), and mamillary body (MB). While ketamine treatment did not significantly alter overt metabolism in the CA1 subfield of the hippocampus as a whole (all cell layers), ketamine did significantly increase cerebral metabolism in the ventral portion of CA1 subfield, thought to be the CA1 molecular layer (saline-treated: 1.09 ± 0.03, ketamine-treated: 1.28 ± 0.05, $P < .01$), as previously reported (supplementary reference *S25, *S28). To ensure consistent measurement across all hippocampal subfields, the functional connectivity of CA1, and all other hippocampal subfields, was defined by that of the whole subfield measure. In contrast to pronounced ketamine-induced hyperfrontality, ketamine treatment also induced significant widespread hypometabolism in thalamic nuclei (anteroventral [AVthal], mediodorsal [MDthal], dorsal reticular [dRT], and ventrolateral [VL]). Ketamine treatment also induced significant hypometabolism in RoI that are the origin of serotonergic (5-HT), noradrenergic (NA), and cholinergic (ACh) innervation to the forebrain including hypometabolism in the raphe (dorsal [DR], medial [MR]), LC, and the diagonal band of broca (horizontal [HDB], vertical [VDB]), respectively. In addition, ketamine induced hypometabolism in the habenula (medial [mHab], lateral [LHab]), medial geniculate (MG), tegmental nuclei (dorsal [DTg], ventral [VTg]), inferior colliculus (IC), hippocampal CA3 subfield, and retrosplenial cortex (RSC).

Ketamine-Induced Alterations Regional Functional Connectivity

Prefrontal Cortex. The functional connectivity of the anterior prelimbic (PrL) and medial prelimbic (layer 2 [mPrL2]) cortex were considered when characterizing ketamine-induced alterations in PFC connectivity

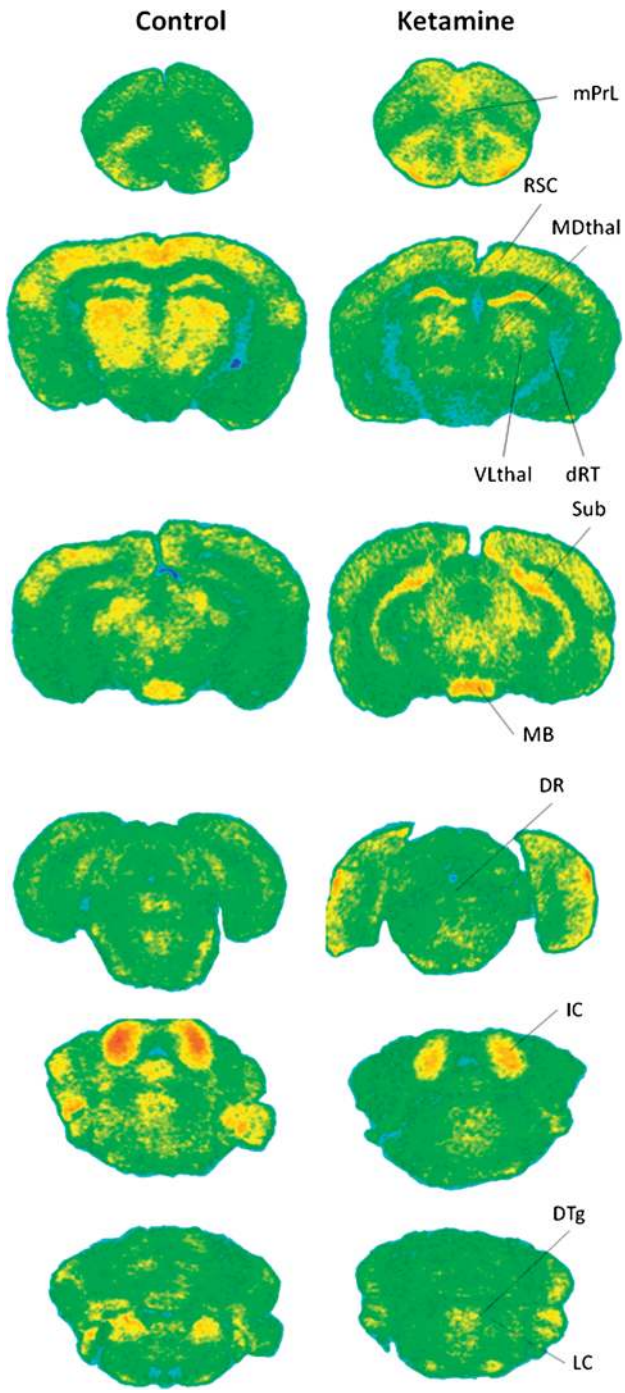

Fig. 1. Representative pseudocolor autoradiograms showing ketamine-induced alterations in overt cerebral metabolism. Acute treatment with ketamine (30 mg kg^{-1}) induces hypermetabolism in the medial prelimbic cortex (mPrL), hippocampal subiculum (Sub), and mamillary body (MB). In contrast, ketamine induces hypometabolism in multiple thalamic nuclei (dorsal reticular [dRT], mediodorsal [MDthal], and ventrolateral [VLthal]), the inferior colliculus (IC), dorsal tegmental nucleus (DTg), and locus coeruleus (LC). Higher rates of metabolism are indicated by warm colors (red/yellow), and lower rates of metabolism are indicated by colder colors (green/blue). Full data and significant ketamine-induced alterations in local cerebral glucose utilization are shown in table 1. Figure can be seen in color in *Schizophrenia Bulletin* online.

because these showed significant hypermetabolism in ketamine-treated animals (table 1; figure 2). Ketamine-induced alterations in the connectivity of these regions are summarized in figure 3, and full data are shown in the online supplementary material (tables S2 and S3). Ketamine significantly increased the functional connectivity of these PFC subfields to the dRT but decreased their connectivity to other thalamic nuclei (AMthal, MDthal, CLthal, CMthal, VMthal, and Rh). In contrast, ketamine significantly increased the connectivity of these 2 PFC subfields to other PFC subfields (lateral orbital [LO], ventral orbital [VO], PrL, mPrL1, and mPrL3). In addition, ketamine treatment significantly increased the connectivity of these 2 PFC subfields to the DR, the primary source of 5-HT innervation to the PFC (supplementary reference *S47), the nucleus accumbens shell (NacS), and ventral lateral lemniscus (VLL). By contrast, ketamine significantly decreased the connectivity of these PFC subfields to the lateral habenula (LHab).

Thalamic Nuclei. The functional connectivity of the dRT, AVthal, and MDthal were considered when characterizing ketamine-induced alterations in thalamic connectivity because ketamine induced significant hypometabolism in these nuclei (table 1; figure 2). Ketamine-induced alterations in the connectivity of these regions are summarized in figure 4, and full data are shown in the online supplementary material (tables S4–S6).

Ketamine treatment significantly increased the coupling of the dRT to multiple PFC subfields (VO, DLO, PrL, mPrL1-3, and IL) and other cortical regions (cingulate [Cg1], primary motor [M1], and piriform [Piri]). In contrast, the coupling of the dRT to subcortical structures, including the central amygdala (CeA), substantia nigra pars reticulata (SNR), lateral lemniscus (DLL and VLL), and the raphe (DR and MR) was significantly decreased by ketamine treatment.

Ketamine treatment significantly decreased MDthal connectivity to the medial PFC (mPrL1 and mPrL2) and VLL. In contrast, MDthal coupling to Cg1, NacS, MR, and other thalamic nuclei (AMthal and VMthal) was significantly increased by ketamine treatment.

Ketamine treatment significantly attenuated the functional connectivity of the AVthal to other thalamic nuclei (AMthal and vRT), the RSC, and MR. In contrast, ketamine significantly increased AVthal functional connectivity to the septum/diagonal band of broca system (lateral septum [LS], medial septum [MS], and VDB), a primary source of ACh innervation in the forebrain, the mesolimbic system (bed nucleus of the stria terminalis [BST] and Ventral tegmental area [VTA]), and the basal ganglia (dorsolateral striatum [DLST] and globus palludus [GP]).

Neuromodulatory Nuclei. Altered functional connectivity of the 5-HT, ACh, and NA neurotransmitter systems

Table 1. Ketamine-Induced Overt Alterations in Cerebral Metabolism

	Saline Mean ± SE	Ketamine Mean ± SE
Cortex		
Anterior prelimbic (PrL)	1.19 ± 0.03	1.28* ± 0.03
Frontal association (FRA)	1.24 ± 0.03	1.17 ± 0.03
Dorsolateral orbital (DLO)	1.34 ± 0.04	1.42 ± 0.02
Medial oribital (MO)	1.04 ± 0.02	1.06 ± 0.03
Ventral orbital (VO)	1.30 ± 0.03	1.37 ± 0.04
Lateral orbital (LO)	1.62 ± 0.04	1.7 ± 0.04
Medial prelimbic (layer 1) (mPrL1)	1.14 ± 0.04	1.40*** ± 0.05
Medial prelimbic (layer 2) (mPrL2)	1.17 ± 0.03	1.35** ± 0.04
Medial prelimbic (layer 3) (mPrL3)	1.10 ± 0.04	1.28** ± 0.03
Infralimbic (IL)	0.96 ± 0.02	1.01 ± 0.04
Cingulate (Cg1)	1.29 ± 0.03	1.37 ± 0.03
Primary motor (M1)	1.25 ± 0.03	1.22 ± 0.03
Retrosplenial (RSC)	1.49 ± 0.02	1.37** ± 0.03
Entorhinal (EntC)	0.92 ± 0.03	1.06** ± 0.02
Piriform (Piri)	1.65 ± 0.03	1.70 ± 0.03
Insular (InsC)	0.88 ± 0.02	0.88 ± 0.02
Thalamus		
Anteromedial (AMthal)	1.50 ± 0.04	1.42 ± 0.05
Anteroventral (AVthal)	1.61 ± 0.03	1.49** ± 0.02
Mediodorsal (MDthal)	1.32 ± 0.03	1.22* ± 0.03
Centrolateral (CLthal)	1.18 ± 0.03	1.13 ± 0.03
Centromedial (CMthal)	1.05 ± 0.03	0.99± 0.02
Ventrolateral (VLthal)	1.42 ± 0.03	1.16*** ± 0.01
Ventromedial (VMthal)	1.40 ± 0.04	1.37 ± 0.05
Reuniens (Re)	1.26 ± 0.03	1.21 ± 0.02
Rhombiod (Rh)	1.24 ± 0.04	1.17 ± 0.04
Dorsal reticular (dRT)	1.31 ± 0.03	1.12*** ± 0.01
Ventral reticular (vRT)	1.43 ± 0.02	1.39 ± 0.04
Amygdala		
Medial (meA)	0.69 ± 0.03	0.69 ± 0.03
Basolateral (BLA)	0.90 ± 0.01	0.91 ± 0.02
Central (CeA)	0.66 ± 0.01	0.67 ± 0.02
Hippocampus		
Subiculum (Sub)	1.15 ± 0.04	1.31* ± 0.03
CA1	0.95 ± 0.03	0.95 ± 0.03
CA2	0.91 ± 0.04	0.83 ± 0.03
CA3	0.69 ± 0.02	0.61* ± 0.02
Dentate gyrus (DG)	0.71 ± 0.02	0.74 ± 0.02
Septum/Diagonal Band of Broca		
Medial septum (MS)	0.89 ± 0.03	0.86 ± 0.02
Lateral septum (LS)	0.80 ± 0.02	0.76 ± 0.02
Horizontal DB (HDB)	1.02 ± 0.02	0.90** ± 0.03
Vertical DB (VDB)	0.95 ± 0.03	0.82** ± 0.03
Mesolimbic		
Bed nucleus of the stria terminalis (BST)	0.66 ± 0.01	0.59* ± 0.02
Ventral tegmental area (VTA)	1.20 ± 0.02	1.10* ± 0.03
Nucleus accumbens core (NacC)	0.92 ± 0.02	1.01* ± 0.04
Nucleus accumbens shell (NacS)	1.01 ± 0.01	1.06 ± 0.03

Table 1. Continued

	Saline Mean ± SE	Ketamine Mean ± SE
Basal ganglia		
Ventromedial striatum (VMST)	1.20 ± 0.02	1.27 ± 0.03
Dorsolateral striatum (DLST)	1.25 ± 0.02	1.32 ± 0.03
Substantia nigra pars compacta (SNC)	1.04 ± 0.02	0.95* ± 0.03
Substantia nigra pars reticulata (SNR)	0.84 ± 0.02	0.78 ± 0.02
Globus palludus (GP)	0.94 ± 0.01	0.89** ± 0.01
Ventral pallidum (VP)	1.12 ± 0.04	1.09 ± 0.03
Neuromodulatory		
Dorsal raphe (DR)	0.94 ± 0.03	0.81** ± 0.01
Median raphe (MR)	1.20 ± 0.03	1.06* ± 0.05
Locus coeruleus (LC)	1.03 ± 0.02	0.91** ± 0.03
Multimodal		
Lateral habenula (lHab)	1.14 ± 0.03	0.96** ± 0.03
Medial habenula (mHab)	1.28 ± 0.02	1.17** ± 0.03
Mamillary body (MM)	1.69 ± 0.03	1.94* ± 0.09
Corpus callosum (CC)	0.45 ± 0.01	0.44 ± 0.01
Dorsal tegmental nucleus (DTg)	1.35 ± 0.03	1.18*** ± 0.02
Ventral tegmental nucleus (VTg)	1.27 ± 0.03	1.07*** ± 0.03
Dorsal lateral lemniscus (DLL)	1.13 ± 0.04	1.02 ± 0.04
Ventral lateral lemniscus (VLL)	1.14 ± 0.03	1.03* ± 0.04
Inferior colliculus (IC)	2.20 ± 0.06	1.74*** ± 0.05
Medial geniculate (MG)	1.26 ± 0.01	1.00*** ± 0.04
Interpeduncular nucleus (IP)	1.54 ± 0.03	1.41 ± 0.06
Pontine nucleus (Pn)	1.02 ± 0.03	0.92 ± 0.03

Note: Ketamine-induced alterations in cerebral metabolism. Data shown as the ^{14}C-2-deoxyglucose uptake ratio. Data were analyzed by *t* test.
*$P < .05$, **$P < .01$, and ***$P < .001$ significant difference from control animals.

in ketamine-treated animals was considered in terms of DR, VDB, and LC connectivity, respectively. These regions showed significant hypometabolism in ketamine-treated animals (table 1; figure 2). Significant ketamine-induced alterations in the connectivity of these regions are summarized in figure 5, and full data are shown in the online supplementary material (tables S7–S9).

Ketamine treatment significantly enhanced the functional coupling of the DR to the PFC (LO, PrL, mPrL1, and mPrL2), nucleus accumbens (NacC and NacS), lateral lemiscus (DLL and VLL), and MR but significantly decreased DR coupling to the amygdala (CeA and meA) and thalamus (AMthal, CLthal, and CMthal).

In a similar way, the functional connectivity of the LC to the PFC (LO and mPrL2) was increased but connectivity to thalamic regions (AMthal and dRT) was significantly decreased by ketamine. In addition, LC connectivity to the dorsal tegmental nucleus (DTg) was significantly increased but connectivity to the LHab significantly decreased by ketamine treatment.

Ketamine treatment significantly enhanced the functional connectivity of the VDB to other components of the septal/diagonal band of broca system (MS and LS) and striatum (DLST and ventromedial striatum [VMST]) but decreased VDB connectivity to the AMthal and LHab. Ketamine-induced alterations in the connectivity of the VDB to the PFC were complex, with both significant increases and decreases evident on a subfield-dependent basis.

Discussion

Here, we have shown that acute treatment with a subanaesthetic dose of ketamine alters the functional connectivity of prefrontal-thalamic circuitry and of multiple neurotransmitter systems. In addition, we identified ketamine-induced alterations in LCGU including PFC hypermetabolism (hyperfrontality) and thalamic hypometabolism. These alterations in LCGU and regional functional connectivity have translational relevance to brain dysfunction in schizophrenia.

Hyperfrontality in ketamine-treated animals reflects, in part, its ability to increase PFC pyramidal neuron activity[14,15] (and supplementary references *S30–*S32, *S48). Here, we provide evidence for the first time of

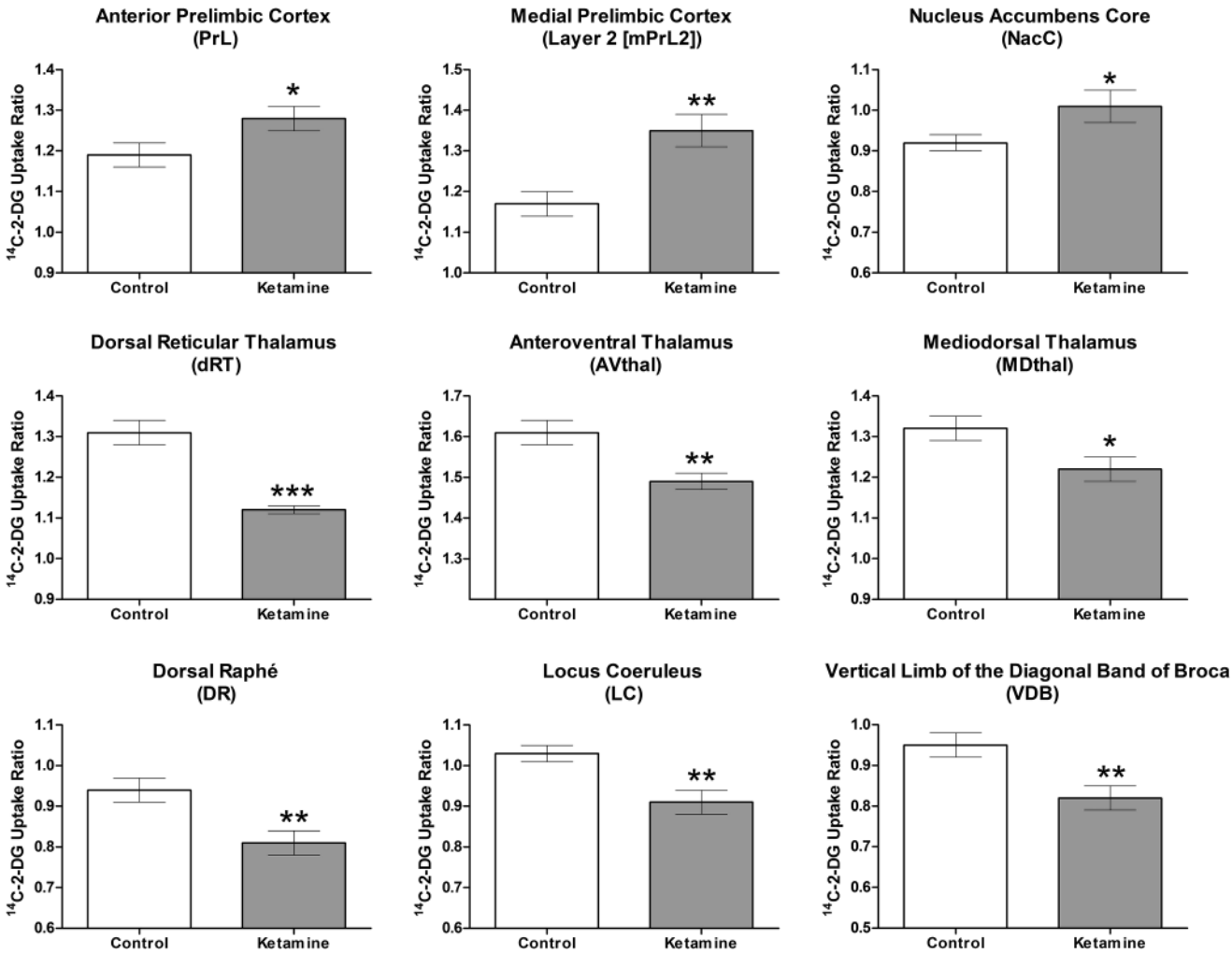

Fig. 2. Ketamine-induced alterations in overt cerebral metabolism. Data shown as the mean ± SEM ^{14}C-2-deoxyglucose uptake ratio. Data were analyzed using *t* test. *$P < .05$, **$P < .01$, and ***$P < .001$ significant difference from control animals. Group sizes: control animals $n = 9$, ketamine-treated animals $n = 9$.

ketamine-induced hypofunction in the dorsal reticular thalamus (dRT) and of altered functional connectivity between the PFC and dRT, thereby providing new insight into the neurobiological mechanisms important in the NMDA-receptor hypofunction hypothesis of schizophrenia. The RT largely consists of GABAergic neurons (supplementary reference *S49) with a particularly high level of fast spiking PV positive GABAergic neurons (supplementary reference *S50). The RT is involved in both the top-down regulation of information processing, such as in attentional processing (eg, rapidly moving attention between external stimuli based on decisions made in the PFC) (supplementary reference *S51, *S52) and in bottom-up processing, including sensory gating[17] and sleep spindle generation.[18] Not only are these processes disrupted by NMDAR antagonists[19] (and supplementary references *S53, *S54) but they are also disrupted in schizophrenia[20] (and supplementary references *S55, *S56). The RT receives direct glutamatergic innervation from the PFC[21] (and supplementary reference *S57). Neurons from the RT send inhibitory GABAergic innervation to other thalamic nuclei, including the MDthal and AMthal[21] (and supplementary references *S58–*S60), that in turn innervate the PFC and send collaterals to the RT. Our finding of ketamine-induced dRT hypoactivity is intriguing given that NMDARs contribute to basal synaptic transmission in this region. For both prefrontal-reticular and thalamo-reticular afferents, NMDAR antagonists block a proportion of excitatory postsynaptic potentials under resting conditions[19] (and supplementary reference *S60). Tonic activity of NMDARs in the RT is further supported by recent evidence showing that NMDAR antagonists hyperpolarize RT neurons,[22] probably due to the presence of NMDARs containing the NR2C subunit, which show little voltage-dependent block at resting membrane potentials. Thus, ketamine-induced hypometabolism in the dRT likely represents the local inhibition of afferent glutamatergic drive mediated

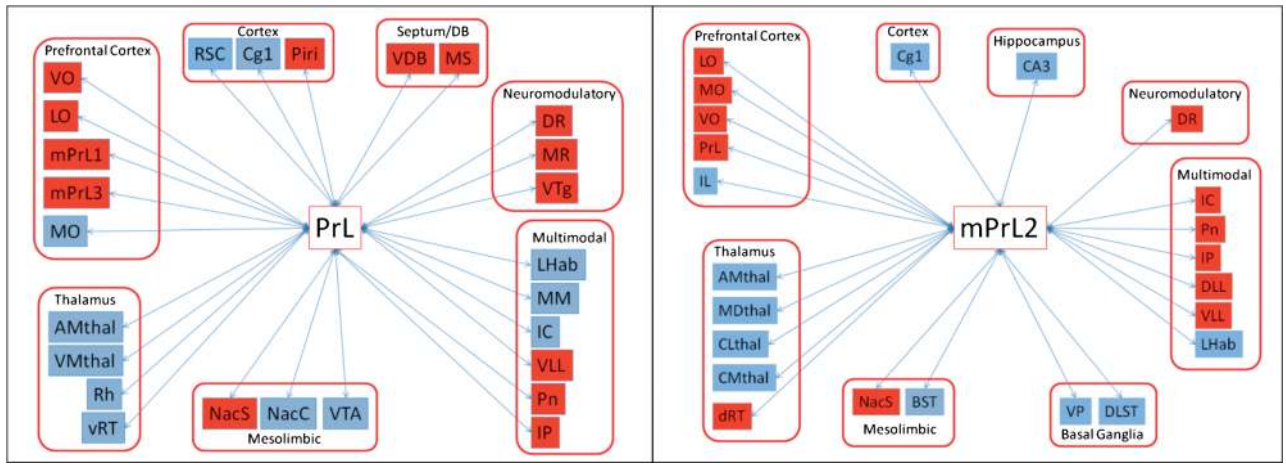

Fig. 3. Ketamine-induced alterations in the functional connectivity of the anterior prelimbic (PrL) and medial prelimbic (layer 2, [mPrL2]) PFC subfields. Only regions where the 95% CI of the variable importance to the projections statistic exceeded 0.80, in either experimental group, were considered to be functionally connected to the "seed" region. Ketamine-induced alterations in functional connectivity were analyzed using *t* test with Bonferroni post hoc correction. Significance was set a $P < .05$. Red (dark gray in print version) denotes a significant increase, whereas blue (light gray in print version) denotes a significant decrease, in the strength of a given functional connection in ketamine-treated animals. Full data are shown in the supplementary material (tables S2 and S3). Figure can be seen in color in *Schizophrenia Bulletin* online.

via NMDARs. The data also suggested that there is a weakening of thalamo-reticular connectivity, in contrast to a strengthening of prefrontal-reticular connectivity, after acute ketamine. This suggests that ketamine may preferentially block thalamo-reticular NMDAR stimulation. Indeed, there is evidence to support the presence of NR2C subunits at thalamo-reticular synapses (supplementary reference *S60). Hence, both overt changes in LCGU and functional connectivity support a rapid action of ketamine on the RT. Reduced and mistimed firing of dRT neurons will reduce inhibition of thalamic relay neurons, resulting in elevated thalamocortical firing which would contribute to PFC hypermetabolism (figure 6). Ketamine may also act directly on PFC GABAergic interneurons to increase PFC metabolism, although a preferential action of NMDAR antagonists on these neurons is currently a matter of debate.[14,23]

However, our and other emerging data support the complementary concept that NMDAR antagonists can disrupt PFC function via a primary site of action in the RT. Evidence supporting this concept includes the observation that systemic but not PFC application of NMDAR antagonists increases PFC glutamate[15] (and supplementary references *S31, *S32). In addition, while decreased PV expression in the PFC is a robust characteristic of postmortem tissue from schizophrenia patients, repeated NMDAR antagonist administration reduces PV expression in the RT prior to that in the PFC.[24] Furthermore, electrophysiological phenomena generated by the RT (gamma oscillations and sleep spindles) are disrupted at an early stage in schizophrenia[25] (and supplementary references *S61, *S62), supporting RT dysfunction as an early event in the disorder. Hence, our results challenge the tacit assumption that NMDAR

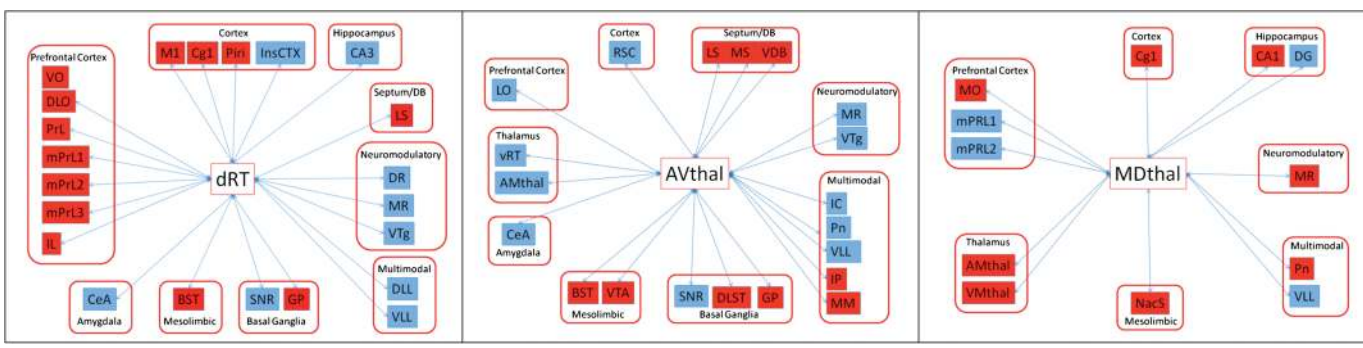

Fig. 4. Ketamine-induced alterations in the functional connectivity of the dorsal reticular (dRT), Anteroventral (AVthal), and Mediodorsal (MDthal) thalamic nuclei. Only regions where the 95% CI of the variable importance to the projections statistic exceeded 0.80, in either experimental group, were considered to be functionally connected to the "seed" region. Ketamine-induced alterations in functional connectivity were analyzed using *t* test with Bonferroni post hoc correction. Significance was set a $P < .05$. Red (dark gray in print version) denotes a significant increase, whereas blue (light gray in print version) denotes a significant decrease, in the strength of a given functional connection in ketamine-treated animals. Full data are shown in the supplementary material (tables S4, S5, and S6). Figure can be seen in color in *Schizophrenia Bulletin* online.

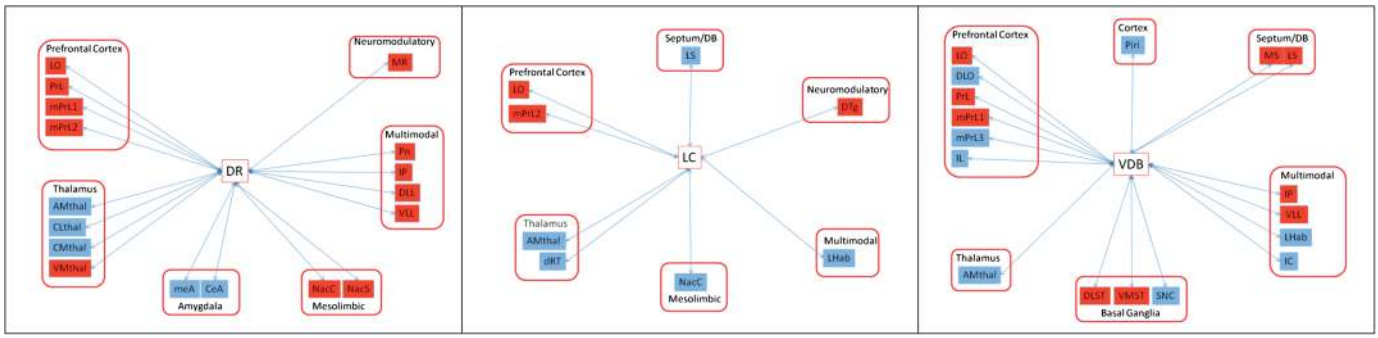

Fig. 5. Ketamine-induced alterations in the functional connectivity of the dorsal raphe (DR), locus coeruleus (LC), and vertical limb of the diagonal band of broca (VDB). Only regions where the 95% CI of the variable importance to the projections statistic exceeded 0.80, in either experimental group, were considered to be functionally connected to the "seed" region. Ketamine-induced alterations in functional connectivity were analyzed using *t* test with Bonferroni post hoc correction. Significance was set a $P < .05$. Red (dark gray in print version) denotes a significant increase, whereas blue (light gray in print version) denotes a significant decrease, in the strength of a given functional connection in ketamine-treated animals. Full data are shown in the supplementary material (tables S7, S8, and S9). Figure can be seen in color in *Schizophrenia Bulletin* online.

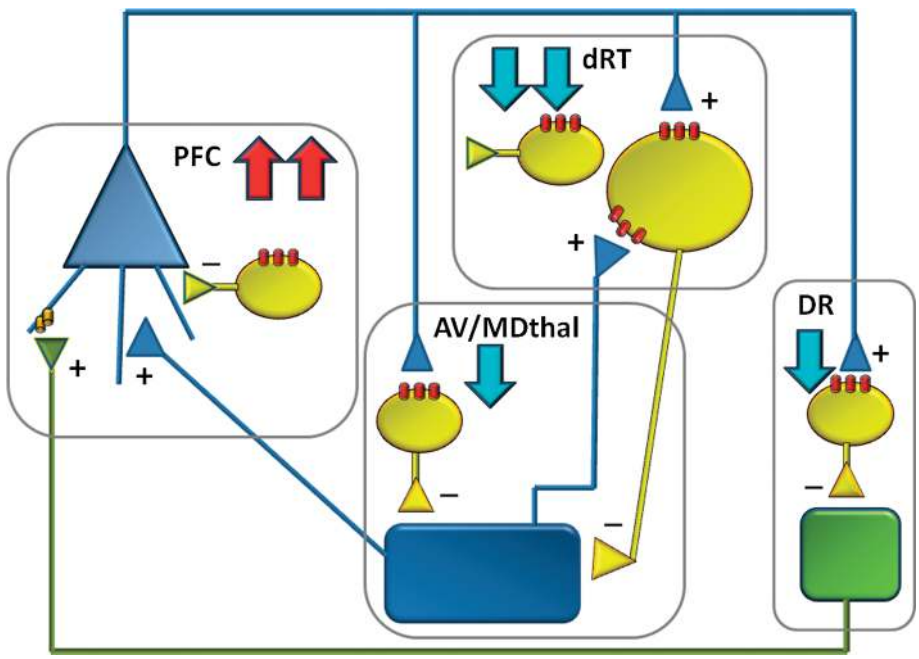

Fig. 6. A new hypothesis for extended neurocircuitry involved in ketamine-induced prefrontal cortex (PFC) hypermetabolism. Acute ketamine treatment alters cerebral metabolism in 2 closed neural circuits involving (1) the reticular thalamus-anteroventral/mediodorsal thalamic nuclei and PFC (dRT-AV/MDthal-PFC) and (2) the dorsal raphe and PFC (DR-PFC). Ketamine inhibits tonically-active NMDARs (red) on dRT GABAergic neurons (yellow online, light gray in print), including those projecting to the AV/MDthal, and NMDARs on local GABAergic interneurons (yellow online, light gray in print) in each region. This results in reduced cerebral metabolism in the dRT, the thalamic nuclei to which the dRT projects (AV/MDthal), and the DR. The inhibition of dRT GABAergic neurons results in the disinhibition of AV/MDthal glutamatergic projections (blue online, dark gray in print) to the PFC, increasing PFC pyramidal neuron activity and inducing hypermetabolism. The excitatory feedback of glutamateric PFC pyramidal neurons (blue online, dark gray in print) onto dRT GABAergic neurons is blocked by the antagonism of dRT NMDARs. In addition, the excitatory feedback of PFC projections onto GABAergic interneurons in the AV/MDthal is blocked by the antagonism of NMDARs on these neurons. Both of these mechanisms prevent the negative feedback inhibition of the AV/MDthal glutamatergic projection to the PFC, in an attempt to reduce PFC activity. In a similar way, the negative feedback of the PFC glutamatergic projection to the DR is blocked by the action of ketamine on DR GABAergic interneurons, resulting in the disinhibition of the DR-PFC serotonergic (green online, mid gray in print) projection and a dramatic elevation of PFC 5-HT levels. In turn, enhanced 5-HT levels in the PFC contribute to pyramidal cell excitation through the activation of 5-HT_{2A} receptors (orange receptors). Key: Yellow neurons: GABAergic, Blue neurons: glutamatergic; Green neurons: serotonergic. NMDA receptors: red; 5-HT_{2A} receptors: orange. Large arrows denote alterations in overt local cerebral glucose utilization (blue online, light gray in print: decrease; red online, dark gray in print: increase). Figure can be seen in color in *Schizophrenia Bulletin* online.

antagonists promote a schizophrenia-like state by acting principally in the PFC. Instead, they suggest that the ability of NMDAR antagonists to suppress RT activity may contribute to their ability to model many of the symptoms of schizophrenia. This hypothesis clearly warrants further systematic investigation.

In this study, we have systematically characterized the effects of ketamine on LCGU in a wide range of thalamic nuclei and have shown significant ketamine-induced hypometabolism in multiple thalamic nuclei (dRT, AVthal, MDthal, and VLthal [table 1]). Previous semiquantitative ^{14}C-2-DG studies by Duncan's group (supplementary references *S37, *S27) have reported ketamine-induced increases in ^{14}C-2-DG uptake in the AVthal, but they did not examine the dRT and found no significant alteration in the MDthal. In contrast to previous semiquantitative ^{14}C-2-DG imaging (supplementary references *S24, *S27) and blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) (supplementary reference *S38) studies, we found that RSC metabolism was not significantly increased by ketamine. One potential explanation for these disparities is that previous semiquantitative ^{14}C-2-DG studies have characterized the ^{14}C-2-DG signature over a short-time frame following tracer injection (5 min). Using a shorter time frame for the characterization of drug-induced alterations in LCGU may preclude the detection of significant reductions in LCGU because high residual levels of the unmetabolized tracer remain in the brain. More importantly, at shorter time frames, the ^{14}C-2-DG signal represents a composite of drug-induced alterations in both cerebral glucose utilization and blood flow (supplementary reference *S63) rather than glucose utilization alone. This is an important issue to consider in light of the ability of ketamine to induce pronounced alterations in cerebral blood flow (supplementary references *S64, *S65) and the ability of NMDAR antagonists to uncouple the relationship between cerebral blood flow and glucose utilization (supplementary references *S65, *S66). This mechanism may also contribute to the disparities between ketamine-induced alterations in brain functioning as detected by ^{14}C-2-DG and BOLD fMRI imaging (supplementary references *S26, *S38). Interestingly, isofluorane anesthesia is capable of completely ablating ketamine-induced alterations in LCGU as detected by ^{14}C-2-DG imaging, but BOLD responses appear to be maintained (supplementary reference *S26) suggesting that ketamine-induced alterations in the BOLD signal may involve effects on blood flow that are not necessarily coupled to cerebral metabolism. The ability of ketamine to modify the relationship between cerebral metabolism and blood flow requires further systematic investigation and has relevance to the interpretation of brain imaging techniques used to assess the impact of this drug on brain functioning. Despite localized disparities between the effects of ketamine on cerebral metabolism as detected previously by others using ^{14}C-2-DG and BOLD imaging, the majority of the overt alterations in metabolism detected in our study are similar to those previously reported.

In addition to ketamine-induced hypometabolism in thalamic regions, this is the first study identifying ketamine-induced hypometabolism and altered functional connectivity in multiple neuromodulatory regions (DR, LC, and VDB). Interestingly, both the LC and raphe express high levels of the NR2C NMDAR subunit (supplementary references *S67, *S68). Hence, overt hypometabolism in these regions is consistent with a direct effect of ketamine on these tonically active NMDARs. As alterations in LCGU largely reflect the metabolic demand of local synapses and not soma (supplementary references *S69, *S70), it is unlikely that hypometabolism in these regions directly reflects the activity of their projecting neurons. Rather, it might reflect the decreased activity of synaptic projections to these regions or that of local interneurons.

The enhanced connectivity of the DR to the PFC and nucleus accumbens and its reduced connectivity to the amygdala and thalamic nuclei suggest that 5-HT neurotransmission is profoundly altered during ketamine treatment. The enhanced connectivity of DR to the PFC and nucleus accumbens parallels, the observation that 5-HT levels are enhanced in these regions following acute NDMAR blockade[26] (and supplementary references *S71, *S72). The ability of ketamine to increase PFC 5-HT levels may contribute to its ability to induce PFC hypermetabolism, altered PFC connectivity, and to disrupt PFC-dependent cognitive processes. 5-HT may increase functional activity in the PFC via activation of 5-HT$_2$ receptors present on pyramidal cells,[27] by reducing their responsiveness to GABA[28] and also through increased glutamate release in the PFC, partially mediated by 5-HT$_{2A}$ receptors.[29] Furthermore, enhanced raphe-PFC coupling may contribute to the functional dissociation of the PFC from that of innervating thalamic nuclei in ketamine-treated animals, particularly the dissociation of PFC-MDthal connectivity (figure 3) because neuronal activity in the PFC elicited by MDthal stimulation is attenuated by the stimulation of the raphe.[30] Interestingly, PFC 5-HT neurotransmission is directly implicated in many PFC-dependent cognitive processes disrupted by ketamine treatment including cognitive flexibility[31] (and supplementary reference *S73), attentional processing[32] (and supplementary reference *S74), and working memory.[33] Overall, our data suggest that altered 5-HT neurotransmission in the PFC may be an important mechanism by which ketamine disrupts cognitive functions relevant to schizophrenia. This is further supported by the observation that targeting 5-HT neurotransmission can restore NMDAR antagonist-induced alterations in behavior[34] (and supplementary reference *S75).

The enhanced connectivity of the LC to the PFC and decreased connectivity to discrete thalamic nuclei (AMthal and dRT) and the nucleus accumbens suggest that NA neurotransmission is disrupted by ketamine treatment. Enhanced LC-PFC connectivity (figure 5) is

consistent with the drug's ability to enhance PFC NA levels (supplementary reference *S76) and may contribute to the ability of ketamine to disrupt PFC function. The ability of ketamine to influence LC-PFC connectivity may contribute to its impact on PFC-dependent cognitive processes including behavioral flexibility and working memory which are regulated by PFC NA neurotransmission[35] (and supplementary references *S77–*S79). Interestingly, moderate increases in PFC NA are associated with improved performance, whereas large increases result in deficits in PFC-dependent cognitive tasks. Recently, this has been attributed to the differential occupation of PFC α1- and α2-adrenoreceptors during moderate and high NA levels.[36] Given the dramatic increase in PFC NA following acute ketamine treatment (413% increase following 100 mg kg^{-1}) (supplementary reference *S76), it is likely that this contributes to PFC-dependent cognitive dysfunction following acute ketamine. Targeting PFC NA neurotransmission as a potential therapeutic target for the alleviation of the cognitive deficits in schizophrenia certainly warrants further investigation.

In ketamine-treated animals the connectivity of the VDB, a major source of ACh innervation in the forebrain, to the striatum (DLST and VMST), and septum (LS and MS) was significantly increased. In addition, PFC-VDB connectivity was altered by ketamine treatment in a complex manner (figure 5). Dense reciprocal projections exist between the PFC and the septal/diagonal band of broca system[37] (and supplementary reference *S80), and these are central to the regulation of hippocampal and PFC ACh neurotransmission. The ability of ketamine to enhance functional coupling of the VDB to specific PFC subfields (mPrL1, PrL, and LO) may related to its ability to increase extracellular ACh in the PFC[38] (and supplementary reference *S81). Enhanced ACh release in the PFC during ketamine treatment could contribute to its ability to disrupt attention and short-term/working memory[39] (and supplementary references *S82–*S86). Alternatively, dysfunctional regulation of VDB ACh neurons that innervate the hippocampus, onto which PFC neurons directly project (supplementary reference *S80), as supported by altered PFC-VBD functional connectivity, could result in abnormal hippocampal ACh neurotransmission following ketamine treatment. While this contention is not supported by any significant alteration VDB-hippocampal coupling in our study, evidence does suggest that hippocampal ACh neurotransmission is enhanced following acute ketamine.[40] This could contribute to the effect of ketamine on the generation of hippocampal theta rhythms. The influence of ketamine on ACh neurotransmission is further complicated by its direct action at a range of cholinergic receptors (supplementary reference *S87), which could also contribute to its ability to produce schizophrenia-like symptoms.

In conclusion, our data suggest that subanaesthetic doses of ketamine are capable of modeling the diverse symptoms of schizophrenia by producing widespread alterations in cerebral metabolism and by altering the functional connectivity of multiple diverse neural systems. In particular, we identified altered functional connectivity in a neural subsystem comprised the reticular thalamus-anterior/mediodorsal thalamus-PFC (dRT-AMthal/MDthal-PFC [figure 6]), suggesting that disruptions in this neural circuitry may be central to the ability of ketamine to model schizophrenia. Furthermore, we identified alterations in the functional connectivity of multiple neuromodulatory systems that also have relevance to the symptoms of this disorder. In addition to providing dramatic new insight into the mechanisms whereby NMDAR antagonists induce a schizophrenia-like condition, these results may also illuminate the processes involved in the early stages of disease development and progression.

Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org. *S denotes additional supporting reference in supplementary material.

Funding

Psychiatric Research Institute of Neuroscience in Glasgow (PsyRING): a joint initiative between the University of Glasgow, University of Strathclyde, and the National Health Service (NHS) Greater Glasgow and Clyde.

Acknowledgments

The authors have declared that there are no conflicts of interest in relation to the subject of this study.

References

1. Lynall ME, Bassett DS, Kerwin R, et al. Functional connectivity and brain networks in schizophrenia. *J Neurosci*. 2010;30:9477–9487.
2. Hill K, Mann L, Laws KR, Stephenson CM, Nimmo-Smith I, McKenna PJ. Hypofrontality in schizophrenia: a meta-analysis of functional imaging studies. *Acta Psychiatr Scand*. 2004;110:243–256.
3. Benes FM, McSparren J, Bird ED, SanGiovanni JP, Vincent SL. Deficits in small interneurones in prefrontal and cingulate cortices of schizophrenic and schizoaffective patients. *Arch Gen Psychiatry*. 1991;48:996–1001.
4. Joyce E, Hutton S, Mutsatsa S, Gibbins H, Webb E, Paul S. Executive dysfunction in first-episode schizophrenia and relationship to duration of untreated psychosis: the West London Study. *Br J Psychiatry*. 2002;181:s38–s44.
5. Krystal JH, Karper LP, Seibyl JP, et al. Subanaesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive and neuroendocrine responses. *Arch Gen Psychiatry*. 1994;51:199–214.

6. Lahti AC, Weiler MA, Michaelidis T, Parwani A, Tamminga CA. Effects of ketamine in normal and schizophrenic volunteers. *Neuropsychopharmacology*. 2001;25:455–467.
7. Egerton A, Reid L, McGregor S, Cochran SM, Morris BJ, Pratt JA. Subchronic and chronic PCP treatment produces temporally distinct deficits in attentional set shifting and prepulse inhibition in rats. *Psychopharmacology*. 2008;198: 37–49.
8. Pitsikas N, Boultadakis A, Sakellaridis N. Effects of sub-anaesthetic doses of ketamine on rats' spatial and non-spatial recognition memory. *Neuroscience*. 2008;154:454–460.
9. Sams-Dodd F. Phencyclidine in the social interaction test: an animal models of schizophrenia with face and predictive validity. *Rev Neurosci*. 1999;10:59–90.
10. Dawson N, Thompson RJ, McVie A, Thomson DM, Morris BJ, Pratt JA. Modafinil reverses phencyclidine-induced deficits in cognitive flexibility, cerebral metabolism and functional brain connectivity [published online ahead of print September 1, 2011]. *Schizophr Bull*. doi:10.1093/schbull/sbq090.
11. Cochran SM, Kennedy M, McKerchar CE, Steward LJ, Pratt JA, Morris BJ. Induction of metabolic hypofunction and neurochemical deficits after chronic intermittent exposure to phencyclidine: differential modulation by antipsychotic drugs. *Neuropsychopharmacology*. 2003;28:265–275.
12. Morris BJ, Cochran SM, Pratt JA. PCP: from pharmacology to modelling schizophrenia. *Curr Opin Pharmacol*. 2005;5:101–106.
13. Langsjo JW, Salmi E, Kaisti KK, et al. Effects of subanaesthetic Ketamine on regional cerebral glucose metabolism in humans. *Anesthesiology*. 2004;100:1065–1071.
14. Homayoun H, Moghaddam B. NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. *J Neurosci*. 2007;27:11496–11500.
15. Lopez-Gil X, Babot Z, Amargos-Bosch M, Sunol C, Artigas F, Adell F. Clozapine and haloperidol differently suppress the MK-801-increased glutamatergic and serotonergic transmission in the medial prefrontal cortex of the rat. *Neuropsychopharmacology*. 2007;32:2087–2097.
16. McIntosh AR, Bookstein FL, Haxby JV, Grady CL. Spatial pattern analysis of functional brain images using partial least squares. *Neuroimage*. 1996;3:143–157.
17. Krause M, Hoffman WE, Hajos M. Auditory sensory gating in hippocampus and reticular thalamic neurones in anaesthetized rats. *Biol Psychiatry*. 2003;53:244–253.
18. McCormick DA, Bal T. Sleep and arousal: thalamocortical mechanisms. *Annu Rev Neurosci*. 1997;20:185–212.
19. Jacobsen RB, Ulrich D, Hugenard JR. $GABA_B$ and NMDA receptors contribute to spindle-like oscillations in rat thalamus in vitro. *J Neurophysiol*. 2001;86:1365–1375.
20. Ferrarelli F, Huber R, Peterson MJ, et al. Reduced sleep spindle activity in schizophrenic patients. *Am J Psychiatry*. 2007;164:483–492.
21. Zikopoulos B, Barbas H. Prefrontal projections to the thalamic reticular nucleus form a unique circuit for attentional mechanisms. *J Neurosci*. 2006;26:7348–7361.
22. Zhang Y, Llinas RR, Lisman JE. Inhibition of NMDARs in the nucleus reticularis of the thalamus produces delta frequency bursting. *Front Neural Circuits*. 2009;3:1–9.
23. Rotaru DC, Yoshino H, Lewis DA, Ermentrout GB, Gonzalez-Burgos G. Glutamate receptor subtypes mediating synaptic activation of prefrontal cortex neurons: relevance for schizophrenia. *J Neurosci*. 2011;31:142–156.
24. Cochran SM, Fujimara M, Morris BJ, Pratt JA. Acute and delayed effects of phencyclidine upon mRNA levels of markers of glutamatergic and GABAergic neurotransmitter function in the rat brain. *Synapse*. 2002;46:206–214.
25. Ferrarelli F, Tononi G. The thalamic reticular nucleus and schizophrenia. *Schizophr Bull*. 2011;37:306–315.
26. Yan QS, Reith MEA, Jobe P, Dailey JW. Dizocilpine (MK-801) increases not only dopamine but also serotonin and norepinephrine transmissions in the nucleus accumbens as measured by microdialysis in freely moving rats. *Brain Res*. 1997;765:149–158.
27. Aghajanian GK, Marek GJ. Serotonin model of schizophrenia: emerging role of glutatmate mechanisms. *Brain Res Rev*. 2000;31:302–312.
28. Feng J, Cai X, Zhao JH, Yan Z. Serotonin receptors modulate $GABA_A$ receptor channels through the activation of anchored protein kinase C in prefrontal cortical neurones. *J Neurosci*. 2001;21:6502–6511.
29. Aghajanian GK, Marek GJ. Serotonin, via 5-HT_{2A} receptors, increases EPSCs in layer V pyramidal cells of the prefrontal cortex by an asynchronous mode of glutamate release. *Brain Res*. 1999;825:161–171.
30. Mantz J, Godbout R, Tassin JP, Glowinski J, Thierry AM. Inhibition of spontaneous and evoked unit activity in the rat medial prefrontal cortex by mesencephalic raphé nuclei. *Brain Res*. 1990;524:22–30.
31. Clarke HF, Walker SC, Crofts HS, Dalley JW, Robbins TW, Roberts AC. Prefrontal serotonin depletion affects reversal learning but not attentional set shifting. *J Neurosci*. 2005;25:532–538.
32. Winstanley CA, Theobald DEH, Dalley JW, Cardinal RN, Robbins TW. Double dissociation between serotonergic and dopaminergic modulation of medial prefrontal and orbitofrontal cortex during a test of impulsive choice. *Cereb Cortex*. 2006;16:106–114.
33. Williams GV, Rao SG, Goldman-Rakic PS. The physiological role of 5-HT_{2A} receptors in working memory. *J Neurosci*. 2002;22:2843–2854.
34. Varty GB, Bakshi VP, Geyer MA. M100907, a serotonin 5-HT_{2A} receptor antagonist and putative antipsychotic, blocks dizocilpine-induced prepulse inhibition deficits in Sprague-Dawley and Wistar rats. *Neuropsychopharmacology*. 1999;20:311–321.
35. Lapiz MDS, Morilak DA. Noradrenergic modulation of cognitive function in rat medial prefrontal cortex as measured by attentional set shifting capability. *Neuroscience*. 2006;137:1039–1049.
36. Ramos BP, Arnsten AFT. Adrenergic pharmacology and cognition: focus on prefrontal cortex. *Pharmacol Ther*. 2007; 113:523–536.
37. Gaykema RPA, Luiten PGM, Nyakas C, Traber J. Cortical projection patterns of the medial septum-diagonal band complex. *J Comp Neurol*. 1990;293:103–124.
38. Nelson CL, Burk JA, Bruno JP, Sarter M. Effects of acute and systemic administration of ketamine on prefrontal acetylcholine release and sustained attention performance in rats. *Psychopharmacology*. 2002;161:168–179.
39. Broersen LM, Heisbroek RPW, de Bruin JPC, Uylings HDB, Olivier B. The role of the medial prefrontal cortex in short-term memory functioning: further support for the involvement of cholinergic, rather than dopaminergic mechansism. *Brain Res*. 1995;674:221–229.
40. Sato K, Wu J, Kikuchi T, Wang Y, Watanabe I, Okumaura F. Differential effects of ketamine and pentobarbitone on acetylcholine release from the rat hippocampus and striatum. *Br J Anaesth*. 1996;77:381–384.