Modafinil Reverses Phencyclidine-Induced Deficits in Cognitive Flexibility, Cerebral Metabolism, and Functional Brain Connectivity

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Objective: In the present study, we employ mathematical modeling (partial least squares regression, PLSR) to elucidate the functional connectivity signatures of discrete brain regions in order to identify the functional networks subserving PCP-induced disruption of distinct cognitive functions and their restoration by the procognitive drug modafinil. Methods: We examine the functional connectivity signatures of discrete brain regions that show overt alterations in metabolism, as measured by semiquantitative 2-deoxyglucose autoradiography, in an animal model (subchronic phencyclidine [PCP] treatment), which shows cognitive inflexibility with relevance to the cognitive deficits seen in schizophrenia. Results: We identify the specific components of functional connectivity that contribute to the rescue of this cognitive inflexibility and to the restoration of overt cerebral metabolism by modafinil. We demonstrate that modafinil reversed both the PCP-induced deficit in the ability to switch attentional set and the PCP-induced hypometabolism in the prefrontal (anterior prelimbic) and retrosplenial cortices. Furthermore, modafinil selectively enhanced metabolism in the medial prelimbic cortex. The functional connectivity signatures of these regions identified a unifying functional subsystem underlying the influence of modafinil on cerebral metabolism and cognitive flexibility that included the nucleus accumbens core and locus coeruleus. In addition, these functional connectivity signatures identified coupling events specific to each brain region, which relate to known anatomical connectivity. Conclusions: These data support clinical evidence that modafinil may alleviate cognitive deficits in schizophrenia and also demonstrate the benefit of applying PLSR modeling to characterize functional brain networks in translational models relevant to central nervous system dysfunction.

Key words: attentional set shifting/2-deoxyglucose autoradiography/functional connectivity

Introduction

Identifying alterations in functional brain circuitry in response to pharmacological treatments and asserting their predictive validity in disease models remains a major challenge in translational neuroscience. One particularly limiting factor in elucidating these alterations includes the limited application of mathematical modeling algorithms to translational brain imaging data. For example, since its development by Sokoloff et al.,1 the 2-deoxyglucose (2-DG) imaging method has been used to investigate the influence of diverse experimental manipulations including drug treatment,2–4 genetic manipulation,5 and central nervous system (CNS) lesion6 on metabolism in discrete brain regions of interest (RoI) as a reflection of functional activity. In contrast to these diverse experimental applications, the methods applied in the analysis of 2-DG data have been strictly confined to the direct statistical comparison of overt alterations in metabolism within each RoI across experimental groups. Given that no brain region functions independently and that the rate of metabolism in each RoI is likely to be influenced by that in several others, it is surprising that available mathematical algorithms that model multiple, complex relationships have not been applied to 2-DG data. One such multivariate mathematical model, partial least squares regression (PLSR), may prove particularly useful in modeling the functional connectivity between brain regions because the method can simultaneously model the relationship between multiple explanatory and dependent variables.7 Indeed, the value of applying PLSR to characterize regional functional connectivity from human brain imaging data has long been recognized8 and has been applied under diverse experimental conditions.9–13 However, this analytical approach is yet to be applied to functional brain imaging data gained in a preclinical context.
Here we apply the PLSR method to 2-DG imaging data from a translational animal model relevant to schizophrenia. Rats treated subchronically with the N-methyl-D-aspartate (NMDA) receptor antagonist phencyclidine (PCP) display cognitive deficits (including impaired set shifting), neurochemical and receptor changes, and alterations in cerebral metabolism (hypometabolism in the prefrontal cortex [PFC], “hypofrontality”) that parallel those in schizophrenia.14–18 Furthermore, the relevance of this pharmacological model to schizophrenia is also supported by the observation that human chronic PCP users display cognitive deficits19 and hypofrontality20,21 similar to that found in this disorder. While the ability to switch attentional set is dependent upon PFC integrity,22–24 one must remember that this region does not function independently but as part of a complex, extensively interconnected functional network that subserves this task. This is evident from studies demonstrating the necessary integrity of other regions, including the nucleus accumbens,25 mediodorsal thalamic nucleus,26 and posterior parietal cortex27 in efficient set shifting. The identity of all the brain regions involved in set shifting remains to be fully elucidated.

At present, there are no prescribed drugs to treat the negative symptoms and cognitive deficits in schizophrenia, and currently prescribed antipsychotics have little efficacy against these symptoms.28,29 Recently, the psychostimulant modafinil has been reported to improve cognitive deficits in schizophrenic patients.30–33 This presents us with the opportunity to validate the subchronic PCP model as a translational model of the cognitive and cerebral metabolic deficits in schizophrenia. Here we test the ability of modafinil to reverse PCP-induced deficits in cognitive flexibility and overt alterations in cerebral metabolism. Furthermore, we apply PLSR to 2-DG data to elucidate the functional subsystems that mediate cognitive flexibility through their disruption by subchronic PCP treatment and their reinstatement by modafinil.

Methods

Animals

All experiments were completed using male Lister Hooded rats (Harlan-Olac, Bicester, UK) under standard conditions (21°C, 45%–65% humidity, 12-h dark/light cycle [lights on 0600 h]). Animals for the 2-DG experiment were singly housed, whereas those for the behavioral experiment were housed in pairs. Animals were maintained on a diet of 15–17 g of food per animal per day for a period of 2 weeks prior to the commencement of the experimental procedures. All animals gained weight over the period of food restriction and weighted between 304 and 379 g at the start of the experiment (not less than 85% ad libitum body weight). Monitoring of body weight was performed regularly (usually every second day), and drinking water was available ad libitum. All experimental manipulations were carried out at least 1 week after entry into the facility, and all experiments were carried out under the Animals (Scientific Procedures) Act 1986.

Animals received either subchronic treatment with vehicle (0.9% saline, intraperitoneally [i.p.]) or 2.58 mg kg^-1 PCP.HCl (i.p., Sigma-Aldrich, Dorset, UK) once daily for 5 consecutive days (between 0900 and 1100 h). Animals involved in the attentional set-shifting task (ASST) underwent task habituation 48 hours after the final administration of their subchronic treatment. Seventy-two hours after the final administration of the subchronic treatment, animals were tested in the ASST or underwent the semiquantitative 2-DG autoradiography protocol. This ensures that any pretreatment effects observed are independent of the acute effects of PCP. Animals treated subchronically with vehicle received acute vehicle treatment only (control; Vehicle.Vehicle; ASST, n = 19; 2-DG, n = 7). Animals who received subchronic PCP treatment acutely received either vehicle (5% methylcellulose, perorally [p.o.]) or modafinil (64 mg kg^-1 in 5% methylcellulose, p.o.). Therefore, PCP-treated animals were randomly assigned to 1 of 2 treatment groups, subchronic PCP and acute vehicle (PCP.Vehicle; ASST, n = 14; 2-DG, n = 9) or subchronic PCP and acute modafinil (PCP.Modafinil; ASST, n = 10; 2-DG, n = 7).

In the semiquantitative 2-DG imaging protocol, animals received 1 dose of 64 mg kg^-1 modafinil, or vehicle, 30 minutes before the injection of 2-DG. The semiquantitative 2-DG protocol used in this study determines the rate of metabolism in each brain region over a 45-minute period following tracer injection. In the ASST, due to the short half-life of modafinil34 and in line with previous studies using this drug in this behavioral task35, 64 mg kg^-1 modafinil was administered 30 minutes prior to the first discrimination and again 30 minutes before the fifth (intra-dimensional reversal, Reversal 2 [Rev2]) discrimination. This ensured that modafinil would be present at a pharmacologically active level over the entire period of the ASST. The dose of modafinil used in this study was based upon that used in previously published behavioral studies.35,36 Due to the technical constraints of the 2-DG technique, specifically the requirement for termination of glucose utilization measurement at exactly 45 minutes after tracer injection,1 the effects of PCP on ASST performance and brain function were investigated in 2 separate groups of animals. Animals from all experimental groups took longer than 45 minutes to complete the ASST.

Attentional Set-Shifting Task

Behavioral Apparatus. The test apparatus consisted of an adapted home cage (40 × 70 × 18 cm). One-third of the box was divided into 2 sections (left and right) by Plexiglas panels into which the ceramic bowls (7 cm diameter and 4 cm depth) for digging were placed. The
Habituation. Habituation and testing procedures for the ASST were adapted from the protocol as described by Birrell and Brown. During the habituation period, the ASST were adapted from the protocol as described Habituation and testing procedures for Habituation. The ASST were adapted from the protocol as described. During the habituation period, the ASST were adapted from the protocol as described by Birrell and Brown.22 During the habituation period, animals first learned to dig in the bowls filled with home cage sawdust in order to retrieve the food reward (1/2 HNC). Once digging behavior was reliably established in sawdust, animals were exposed to each exemplar, first in the dimension of odor and then in the dimension of texture. In each trial, both bowls presented were baited and it was ensured that each exemplar was presented on both sides of the divide. After exposure to all exemplars, rats were then trained sequentially on 2 simple discriminations (SD) within each dimension to be used within the test period (medium and odor). Exemplars in the first SD differed with regard to the odor of the medium (mint vs oregano) and the second was with regard to the texture of the medium (polystyrene vs paper confetti). The rats were trained within each SD until criterion performance was met, which was set at the completion of 6 consecutive correct trials.

Behavioral Testing. The identity of the exemplar combinations employed in the ASST and their pairings are outlined in Table 1. During the test session, rats performed a series of discriminations in the order outlined in Table 2. During the first 4 trials of each discrimination, rats were allowed to dig in both bowls, and a digging error was recorded if the first dig occurred in the nonbaited bowl. During subsequent trials, if the first dig occurred in the nonbaited (incorrect) bowl, the animal was punished by blocking access to the baited (correct) bowl and immediately terminating the trial. Such trials were classified as “punishment trials” as the animals were not allowed to retrieve the reward after digging in the incorrect bowl and received a brief time-out before the initiation of the next trial. Trials continued until the rat had reached criterion performance of 6 consecutive correct digs. Testing then progressed onto the next discrimination. The first discrimination of the testing period involved a SD between 2 bowls that differed only along the perceptual dimension of medium. After reaching criterion on this discrimination, testing progressed to the compound discrimination (CD) stage, where the correct and incorrect exemplars relevant within the SD phase were maintained but the second (irrelevant) dimension of odor was introduced. The CD was then followed by a reversal discrimination (compound discrimination reversal, Reversal 1 [Rev1]), in which the exemplars and dimensions were unchanged from the CD but the previously rewarded exemplar was now not baited and vice versa. After reaching criterion in Rev1, animals progressed to the intradimensional shift (ID), which comprised new exemplars in both the relevant and the irrelevant dimensions, with the relevant dimension remaining the same as that present in the SD, CD, and Rev1 (medium). Once animals had reached criterion on the ID, they were removed from the testing apparatus in order to receive the second dose of their acute treatment (either modafinil or vehicle). Following the second acute treatment, animals were returned to the testing apparatus and testing was reinitiated 30 minutes after this treatment. On resuming the test phase, animals underwent a second reversal (Reversal 2 [Rev2]), during which the exemplars and relevant dimension remained the same as in the ID but the rewarded bowl was reversed as in Rev1. Once animals had reached criterion on Rev2, rats went on to the extradimensional shift (ED) stage of the task. As in the ID stage of the task, the rat was presented with new exemplars for both the relevant and the irrelevant dimensions. In contrast to the ID, however, the previously relevant dimension of medium texture was irrelevant and the previously irrelevant dimension of odor became relevant. Once animals reach criterion in the ED, a final reversal discrimination (Reversal 3 [Rev3]) was completed where, as in the Rev1 and Rev2, the exemplars and dimensions remain unchanged from the previous discrimination (ED), but now the previously rewarded exemplar was not rewarded and vice versa. Because the combinations of exemplars were too numerous to permit full counterbalancing, stimuli were always presented as pairs as previously described.22

In addition to the number of trials performed to reach criteria, the number of errors committed and the number of punishment trials, where animals were not allowed to retrieve the reward after an incorrect dig, were also recorded. To further elucidate the behavioral mechanisms underlying behavioral deficits in the ASST, measures of perseverative responding and the likelihood of committing regressive errors were also characterized across the experimental groups. Perseverative responding was characterized as the proportion of trials following

<table>
<thead>
<tr>
<th>Medium Pairs</th>
<th>Odor Pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coarse tea vs fine tea</td>
<td>Cinnamon vs ginger</td>
</tr>
<tr>
<td>Sand vs grit</td>
<td>Sage vs paprika</td>
</tr>
<tr>
<td>Coarse sawdust vs fine sawdust</td>
<td>Tumeric vs cloves</td>
</tr>
</tbody>
</table>

Note: The exemplars within a dimension were presented in pairs adapted from Birrell and Brown.22
a punishment trial, in which animals failed to appropriately reorientate their digging behavior to the correct (baited) bowl. This was expressed as the number of failed reorientations an animal completed relative to the total number of punishments that animal had experienced. Only animals that had received at least one punishment trial during Rev3 were included in this analysis (Vehicle.Vehicle \( n = 15/19 \), PCP.Vehicle \( n = 12/14 \), PCP.Modafinil \( n = 10/12 \) animals in each group). The likelihood of committing regressive errors was calculated as the proportion of times an animal returned to the previously correct (now incorrect) digging strategy at each trial level (1–5) after a punishment-induced reorientation of digging behavior. A level 1 trial is the one that occurs immediately after and level 5 being 5 consecutively correct digs after punishment-induced reorientation of digging behavior. A level 1 trial is the one that occurs immediately after and level 5 being 5 consecutively correct digs after punishment-induced reorientation of digging, at which point the animal reaches trial criteria by completing 6 consecutively correct trials. The likelihood of “dropping out” at each trial level was then calculated as the number of times an animal dropped out at a given trial level relative to the total number of punishment-induced reorientations in digging behavior an animal had completed.

### Statistical Analysis

The trials to criterion data were analyzed using a 2-way repeated measures ANOVA with significance set at \( P < .05 \). The within-subject factor was discrimination (SD, CD, Rev1, intradimensional [ID], Rev2, extradimensional [ED], and Rev3), and the between-subject factor was drug treatment (Vehicle.Vehicle, PCP.Vehicle, and PCP.Modafinil). Significant main effects were explored using \( t \) test with Bonferroni correction for multiple comparisons, so that criterion for 5% statistical significance was set at \( P < .007 \). The ability of animals to shift attentional set was determined by the number of trials taken to reach criteria in the ED stage of the task relative to the number of trials animals required to reach criteria at the ID stage of the task. The statistical significance of the set-shifting (ED/ID) ratio between experimental groups was assessed using Mann-Whitney \( U \) test with Bonferroni correction for multiple comparisons, with 5% significance set at \( P < .017 \). Error, punishment, and perseverative responding data were analyzed using 1-way ANOVA with Tukeys post hoc test for multiple comparisons. Regressive error data were analyzed using 2-way repeated measures ANOVA with the within-subject factor as the trial level (1–5) and the between-subject factor as drug treatment group. Tukeys post hoc test was applied to investigate differences between treatment groups at each trial level. Significance was set at \( P < .05 \) throughout, and all statistical analyses were performed using SPSS (SPSS Inc, Version 17.0).

### Semiquantitative 14C-2-DG Autoradiographic Imaging

Local cerebral glucose utilization (LCGU) was determined 72 hours after the last administration of the subchronic treatment (saline or PCP) and 30 minutes after the acute treatment (modafinil or methylcellulose) in accordance with previously published protocols.\(^5\) Rats were injected i.p. with 4.625MBq kg\(^{-1} \) of \(^{14}\)C-2-DG (Sigma-Aldrich) at a steady rate over a 10-second period before being returned to their home cage. At exactly 45 minutes after isotope injection, animals were

### Table 2. Behavioral Testing Discrimination Order

<table>
<thead>
<tr>
<th>Discrimination</th>
<th>Relevant</th>
<th>Irrelevant</th>
<th>Exemplar Combinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>Medium</td>
<td></td>
<td>M1</td>
</tr>
<tr>
<td>CD</td>
<td>Medium</td>
<td>Odor</td>
<td>M1/O1</td>
</tr>
<tr>
<td>Rev1</td>
<td>Medium</td>
<td>Odor</td>
<td>M1/O2</td>
</tr>
<tr>
<td>ID</td>
<td>Medium</td>
<td>Odor</td>
<td>M2/01</td>
</tr>
<tr>
<td>Rev2</td>
<td>Medium</td>
<td>Odor</td>
<td>M2/02</td>
</tr>
<tr>
<td>ED</td>
<td>Odor</td>
<td>Medium</td>
<td>M3/03</td>
</tr>
<tr>
<td>Rev3</td>
<td>Odor</td>
<td>Medium</td>
<td>M4/03</td>
</tr>
</tbody>
</table>

Note: CD, compound discrimination; ED, extradimensional shift; ID, intradimensional shift; Rev, reversal; SD, simple discrimination.

Table illustrating the order of discriminations and the combinations of exemplars used when shifting set from medium texture to odor at the extradimensional discrimination stage. On every trial, except the SD, the pair of stimuli presented differed along both the relevant and the irrelevant dimensions. The rewarded exemplar (correct) is shown in bold and is paired with either exemplar from the irrelevant dimension. The combination of exemplars into positive (+) and negative (–) stimuli and their left-right position of presentation in the case was a pseudorandom series (Birrell and Brown).\(^22\)
decapitated and a terminal blood sample was collected by
torso inversion in heparinized weigh boats. The brain was
rapidly dissected out intact, then frozen in isopentane
(−40°C), and stored at −80°C until sectioning. Blood sam-
pies were centrifuged to separate the plasma, and aliquots
were removed for the determination of plasma glucose
(10 µl) and 14C (20 µl) concentrations by semiautomated
glucose oxidase assay (Beckman Glucose Analyzer) and
liquid scintillation analyses (Packard), respectively.

Frozen brains were sectioned (20 µm) in the coronal
plane in a cryostat (−20°C). A series of 3 consecutive sec-
tions were retained from every 200 µm, thaw mounted,
and rapidly dried on a hot plate (70°C). Autoradiograms
were generated by apposing these sections together with
precalibrated 14C-standards 40–1069 nCi g−1 tissue equiv-
alents (Amersham International, Little Chalfont, UK) to
X-ray film (Kodak, SB-5) for 5 days. Autoradiographic
images were analyzed by a computer-based image analy-
ysis system (MCID/M5+). The local isotope concentra-
tion for each brain ROI was derived from the optical
density of autoradiographic images relative to that of
the coexposed 14C-standards. Measurements were taken
from 64 anatomically distinct brain regions defined
with reference to a stereotactic mouse atlas38. The rate
of metabolism, LCGU, in each ROI was determined as
the ratio of 14C present in that region relative to the av-
erage 14C concentration in the whole brain of the same
animal and from hereon will be referred to as the 14C-2-
DG uptake ratio. Whole-brain average 14C levels
were determined from the average 14C concentration
across all sections, in which an ROI was measured.

Data Analysis

Statistical Analysis of Experimentally Induced Metabolic
Alterations. When analyzing changes in the rate of me-
tabolism between experimental groups and in keeping
with previous studies of similar experimental design, an-
atomically discrete brain regions were assumed to repre-
sent independent variables within each measure and no
correction was applied for multiple comparisons.39
14C-2-DG data were analyzed using 1-way ANOVA
with Newman-Keuls post hoc test for multiple compar-
isons across the 3 experimental groups. Plasma variables
for the 14C-2-DG study were analyzed using Student’s
t test with Bonferroni post hoc correction for multiple
comparisons. Significance was set at P < .05 throughout.

Functional Brain Connectivity Analysis Using PLSR

PLSR (also known as projection to latent structures or PLS)
is a method for relating 2 data matrices, X and Y, by a mul-
tivariate linear regression model and is particularly suited
to analyzing functional connectivity within the brain as it can
model the influence of multiple, collinear “predictor” var-
iables upon a given dependent variable. The PLS algorithm
itself is detailed and discussed in Wold (1995)40 and Wold
et al. (2001)7 and its application to the data in this study is
outlined in the supplementary section to this article. The
data presented in this article were analyzed using the
PLSR module in XLSTAT 2009 (Addinsoft, New York,
NY). PLS has previously been applied in neuroimaging
studies to investigate functional brain activity in relation
to behavior and the functional coupling of brain regions
to a defined “seed” brain region in humans.8–13 Here we
use the algorithm to characterize functional brain connec-
tivity signatures by modeling the relationship between
the rate of metabolism, as given by the 14C-2-DG uptake ratio,
in a specified seed brain region (the dependent variable)
and all the other ROI measured (explanatory variables;
63 brain regions in each analysis). The PLS models were
generated independently for each experimental group
(Vehicle, Vehicle, PCP, Vehicle, and PCP, Modafinil).

The goodness of fit statistics for each model used in the analysis
are shown in the supplementary material (table S2). The
function coupling of each explanatory brain region to
the seed brain region was taken to be reflected by the vari-
able importance for the projection (VIP) statistic. The stand-
ard deviation of the VIP statistic for each explanatory
brain region was estimated by jackknifing (“leave-one-
out” procedure). Statistical differences in the VIP for
each explanatory ROI to a given seed brain region between
experimental groups were analyzed using 1-way ANOVA
with the Newman-Keuls post hoc test, with reference to
the mean VIP, its standard deviation, and the group size
(n). Significance was set at P < .05 throughout. The func-
tional connectivity of 3 seed brain regions were analyzed in
this study, which were the retrosplenial cortex (RSC), an-
terior prelimbic cortex (PrL), and medial prelimbic cortex
(layer 1, mPrL1). These regions were chosen as seed regions
because the rate of metabolism in the first 2 regions (RSC,
PrL) is reduced by subchronic PCP treatment and corrected
by acute modafinil and in the third region (mPrL1) acute
modafinil treatment enhances metabolism (figure 3).

Results

Attentional Set-Shifting Task

Subchronic PCP treatment impairs the ability of animals
to switch attentional set, and this deficit in cognitive flex-
ibility is reversed by acute modafinil treatment. Repeated
measures ANOVA revealed a significant effect of discrimi-
nation phase (F(6,204) = 35.401, P < .001) but not
pretreatment (F(1,34) = 1.618, P = .212) on the number of
trials required to reach criteria during the ASST. This
suggests that animals from all experimental groups
find some discrimination phases harder to perform than
others during the ASST, but the ability of animals to
complete the ASST is not, overall, affected by PCP pre-
treatment. However, a significant pretreatment × dis-

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significantly alters the ability of animals to complete specific discrimination phases of the ASST. The suggestion that animals find some discriminations harder to complete in the ASST than others was confirmed by the finding that animals from all experimental groups required significantly more trials to reach criterion at the ED stage relative to the ID discrimination (table 3; \( P < .05 \), \( t \) test with Bonferroni correction). This also confirms that animals in all groups formed an attentional set toward the relevant dimension at the discrimination stages prior to the ED. The ability of PCP pretreatment to impede performance on a discrimination phase-dependent basis was confirmed by a significant increase in the number of trials required to reach criterion at the ED stage relative to the ID stage (increased ED/ID set-shifting ratio; figure 1; \( P = .049 \), Mann-Whitney \( U \) test with Bonferroni correction) in PCP-experienced (PCP.Vehicle) animals in comparison to controls (Vehicle.Vehicle). This confirms that these animals were significantly impaired in their ability to switch attentional set. This impairment was effectively reversed by acute modafinil treatment because the ability of PCP-experienced animals to switch attentional set when given acute modafinil was significantly lower than PCP-experienced animals who received acute vehicle (\( P = .0438 \), Mann-Whitney \( U \) test, Bonferroni adjusted) but was not significantly different from control (Vehicle.Vehicle) animals (\( P = 2.731 \), Mann-Whitney, Bonferroni adjusted).

The ability of PCP pretreatment to impede performance at specific discrimination stages was also apparent during the Rev3 where subchronic PCP treatment induced a significant deficit in reversal learning (figure 2A; \( P = .001 \), \( t \) test Bonferroni adjusted). This deficit was not reversed by acute modafinil treatment because PCP-pretreated animals who received modafinil remained significantly different from control (\( P = .008 \), \( t \) test, Bonferroni adjusted) but not from PCP-treated animals given acute vehicle (PCP.Vehicle; \( P = .816 \), \( t \) test, Bonferroni adjusted). In accordance with this observation, PCP-experienced animals were found to commit significantly more errors (1-way ANOVA with Tukeys post hoc test, \( P < .05 \), \( q = 3.537 \); figure 2C) during this discrimination. However, this did not result in PCP-experienced animals receiving significantly more punishment than control animals (figure 2D). This suggested that PCP-experienced animals respond to punishment in the same way as control animals. Indeed, there was no evidence that PCP-experienced animals displayed significantly greater perseverative responding than control animals during this reversal discrimination (figure 2E). In all experimental groups, animals reversed their digging behavior in the trial immediately after a punishment to the correct bowl approximately 70%-80% of the time. The chance of perseverative responding in the trial immediately after punishment was approximately 16% in control animals, 10% in PCP.Vehicle animals, and 25% in PCP.Modafinil animals. The increased likelihood of PCP-experienced animals committing regressive errors appears to underlie

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### Table 3. Effect of Phencyclidine (PCP) and Modafinil on the Attentional Set-Shifting Task

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Subchronic</th>
<th>Acute</th>
<th>Simple Discrimination</th>
<th>Compound Discrimination</th>
<th>Rev1</th>
<th>ID</th>
<th>Rev2</th>
<th>ED</th>
<th>Rev3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>Vehicle</td>
<td></td>
<td>10.47 ± 0.59</td>
<td>7.53 ± 0.38</td>
<td>14.37 ± 0.79</td>
<td>12.16 ± 0.88</td>
<td>17.84 ± 1.29</td>
<td>17.99± ± 1.23</td>
<td>14.58 ± 0.89</td>
</tr>
<tr>
<td>PCP</td>
<td>Vehicle</td>
<td></td>
<td>9.71 ± 0.71</td>
<td>6.57 ± 0.19</td>
<td>16.57 ± 1.37</td>
<td>10.57 ± 0.82</td>
<td>15.07 ± 0.96</td>
<td>20.79± ± 1.70</td>
<td>21.21* ± 1.22</td>
</tr>
<tr>
<td>PCP</td>
<td>Modafinil</td>
<td></td>
<td>8.33 ± 0.43</td>
<td>7.85 ± 0.79</td>
<td>15.70 ± 1.51</td>
<td>13.43 ± 2.15</td>
<td>15.99 ± 0.94</td>
<td>18.06* ± 1.69</td>
<td>20.72* ± 1.77</td>
</tr>
</tbody>
</table>

Notes: ED, extradimensional; ID, intradimensional; Rev, reversal. Data shown as the number of trials required to reach a criteria of 6 correct consecutive trials for each discrimination following subchronic pretreatment with vehicle (saline, \( n = 19 \)) or PCP. PCP animals receive either acute vehicle (\( n = 14 \)) or modafinil (\( n = 12 \)). In all treatment groups, the intradimensional shift (ID) took significantly less trials to complete than the extradimensional shift (\( * \) denotes \( P < .05 \) significant difference from ID, \( t \) test with Bonferroni correction). PCP-experienced animals required significantly more trials to reach criteria during Rev3 as compared with control (Vehicle.Vehicle) animals (\( * \) denotes \( P < .05 \) significant difference from control, \( t \) test with Bonferroni correction).
the deficits in reversal learning during Rev3 because they were significantly more likely to drop out of correct digging behavior following a punishment-induced reorientation of digging behavior (figure 2B; significant pretreatment main effect, repeated measures ANOVA, $F_{1(34)} = 4.188$, $P = .049$). While the increased frequency of dropout in PCP-treated animals appeared to be most evident in trials 2 and 3 after punishment-induced reorientation of behavior, there was no statistical evidence for this (repeated measures ANOVA, pretreatment $\times$ trial interaction was not significant ($P = .892$, $F(4,136) = 0.287$) and there was no significant effect following Tukey's post hoc analysis for each trial level independently). This suggests that the enhanced drop out of PCP-pretreated animals from correct responding following punishment-induced reorientation was a more general effect than one that could be localized to a specific trial. Importantly, in all experimental groups, the likelihood of dropping out of correct digging behavior significantly decreases as animals make a greater number of sequentially correct digs (trial-level main effect, repeated measures ANOVA, $P < .001$, $F_{4(136)} = 5.98$). In this way, in vehicle animals, the likelihood of making an incorrect dig in trial 1 after reorientation is approximately 9% (square root [$\sqrt{.09} = 0.3$]), whereas the likelihood of an incorrect dig at trial 5, following 5 consecutively correct digs after punishment, is approximately 0.5% ($\sqrt{.0025} = 0.07$) (figure 2B).

Semiquantitative $^{14}$C-2-DG Autoradiography

In parallel to its ability to reverse discrete aspects of PCP-induced cognitive dysfunction, acute modafinil treatment effectively reversed distinct PCP-induced alterations in overt cerebral metabolism. Subchronic PCP treatment produced significant, regionally selective, alterations in the overt rate of cerebral metabolism in 9 of the 62 regions analyzed (table 4; figure 3). In particular, subchronic PCP treatment induced hypofrontality, a reduced rate of metabolism in prefrontal cortical regions including the anterior PrL and orbital (lateral [LO] and dorsolateral [DLO]) cortices, and a reduced metabolism in discrete thalamic nuclei (dorsal reticular thalamus [dRT] and centromedial thalamus [CM]). In addition to PFC structures, PCP treatment also produced hypometabolism in the RSC, which is part of the cingulate cortex (Cg1), the dorsolateral striatum (DLST), and mammillary body (MB). In only one region, the bed nucleus of the stria terminalis (BST), was a significant increase in metabolism found. The alterations in overt cerebral metabolism induced by subchronic PCP treatment were highly localized and metabolism remained unaltered in the majority of RoI investigated including all subfields of the hippocampus, the amygdala nuclei, components of the basal ganglia, and septal nuclei. The PCP-induced alterations in cerebral metabolism found in this study are consistent with those previously reported when applying the quantitative 2-DG autoradiographic imaging protocol to animals treated using the same PCP treatment regime.\[15\]

Acute treatment of PCP-experienced animals with modafinil produced a significant increase in cerebral metabolism in 3 RoI, all of which were cortical. Modafinil treatment significantly increased metabolism in the anterior PrL and RSC of PCP-experienced animals to a level similar to that in control (Vehicle.Vehicle) animals (table 4; figure 3). In addition, acute modafinil treatment produced an increase in metabolism in the mPrL1 that resulted in a significant hypermetabolism relative to both control and PCP-experienced (PCP.Vehicle) animals. Despite its effectiveness in reversing PCP-induced hypometabolism in the PrL and RSC, acute modafinil treatment failed to correct the PCP-induced hypermetabolism observed in the orbital cortex (LO and DLO), the CM or dRT thalamic nuclei, the DLST, and in the MB. The rate of metabolism in each of these brain regions remained significantly lower than that found in control animals but was not significantly lower than that found in PCP-experienced (PCP.Vehicle) animals. In addition, modafinil failed to correct the PCP-induced hypermetabolism observed in the BST. These results suggest that acute modafinil is able to reverse some but not all the alterations in cerebral metabolism induced by subchronic PCP treatment.

Functional Brain Connectivity Assessed Using PLSR

The relevant statistics supporting the appropriateness of each of the PLSR models used to generate the VIP statistic for the different seed brain regions and experimental groups are shown in the supplementary material (table S2). These statistics show that for each experimental group and seed brain region, a PLSR model that adequately fits and models that data was generated. The 3 seed regions selected for analysis were the anterior PrL and RSC where modafinil was shown to reverse PCP-induced hypometabolism and the mPrL1 where modafinil produces a significant hypermetabolism in PCP-experienced animals.

Anterior Prelimbic Cortex. Subchronic PCP treatment significantly altered the functional connectivity of the PrL (figure 4 and tables S3 and S6 in the supplementary material), which included the dissociation of functional activity in this region from that in several components of the mesolimbic system (nucleus accumbens core [NacC], nucleus accumbens shell [NacS], and BST), the amygdala (basolateral amygdala and central amygdala [CeA]), and habenula (lateral habenula and medial habenula [mHab]) nuclei. In addition, PrL function was also dissociated from that in the rhomboid nucleus of the thalamus, the median raphe (MR), and several other cortical regions (frontal association area [FRA], infralimbic [IL], and entorhinal cortex [EC]). In contrast, functional
connectivity to multiple hippocampal subfields (subiculum [Sub], cornu ammonis subfield 1 [CA1], and cornu ammonis subfield 3 [CA3]), the septum (lateral septum [LS]), and many other cortical areas (including mPrL1, RSC, DLO, and Cg1) was significantly enhanced by subchronic PCP treatment.

In parallel to the restoration of PCP-induced hypometabolism in PrL, modafinil normalized discrete PCP-induced alterations in the functional connectivity of this brain region. These functional coupling events may be relevant to both the normalization of overt hypometabolism in this region and of set-shifting capabilities in PCP-treated animals by modafinil. These restoration events were seen with multiple components of the mesolimbic system (NacC, NacS, and BST), the locus coeruleus (LC), the CA1 subfield of this hippocampus, the mHab, MR, and cortical regions (primary motor cortex [M1] and Cg1). In addition to these normalization events, it is also possible that alterations in functional connectivity that are specifically altered by modafinil treatment may also play a role in normalizing PrL function in PCP-treated animals. These functional events included enhanced coupling of the PrL to the orbital cortex (ventral orbital [VO]), centromedial thalamus (CM), medial amygdala (MeA), and dorsal tegmental nucleus (DTg). In contrast, functional connectivity of the PrL was significantly decreased with multiple components of the basal ganglia (globus pallidus [GP], substantia nigra pars reticulata [SNR], and Substantia nigra pars compacta [SNC]), insular cortex, anteroventral thalamus, the ventral tegmental area (VTA), and medial septum (MS) by modafinil treatment in comparison to both control and PCP-experienced (PCP.Vehicle) animals.

Fig. 2. (A) Phencyclidine (PCP)-Experienced Animals Display a Reversal Deficit That Specifically Manifests at the Third Reversal (Rev3) That Is Not Reversed by Acute Modafinil Treatment. †Denotes significant difference from control (Vehicle.Vehicle) animals at the same discrimination stage (t-test with Bonferroni post hoc correction). Data for errors, punishments, perseverative responding, and regressive errors were square root (sqrt) transformed to give the data a normal distribution (B) PCP-experienced animals commit a significantly higher level of regressive errors, as evidenced by a higher proportion of dropout across all trial levels following punishment-induced reorientation of digging behavior (main pretreatment effect, 2-way repeated measures ANOVA, $F_{(1,34)} = 4.188, P = .049$). (C) PCP-treated animals commit more errors in Rev3 (1-way ANOVA; PCP main effect $F_{(2,41)} = 3.275, P = .048$). *Denotes significant ($P < .05$) difference from control (Vehicle.Vehicle) animals (post hoc Tukeys test, $P < .05, q = 3.537$). Despite a trend toward a higher number of errors in PCP.Modafinil-treated animals in comparison to control animals, these failed to reach significance ($P > .05, q = 2.143$). (D) PCP-treated animals do not experience significantly more punishments in Rev3 (1-way ANOVA $F_{(2,41)} = 1.597, P = .2148$). (E) PCP pretreatment did not significantly alter the likelihood of perseverative responding in Rev3. Animals in all groups perform below the 50% chance level (sqrt 0.5 = 0.7) of remaining with the incorrect bowl following punishment.
Table 4. Phencyclidine (PCP)- and Modafinil-Induced Alterations in Cerebral Metabolism

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>PCP. Vehicle</th>
<th>PCP. Modafinil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>%</td>
</tr>
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<td><strong>Cortex</strong></td>
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<td>Prelimbic</td>
<td>1.27 ± 0.03</td>
<td>1.15* ± 0.03</td>
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<td>1.75 ± 0.03</td>
<td>1.55** ± 0.04</td>
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<td>1.13 ± 0.03</td>
<td>1.15 ± 0.04</td>
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<tr>
<td>Dorso-lateral orbital</td>
<td>1.53 ± 0.05</td>
<td>1.35** ± 0.05</td>
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<td>Medial prefrontal cortex (Layer 1) (mPrL1)</td>
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<tr>
<td>Medial prefrontal cortex (Layer 2)</td>
<td>1.26 ± 0.02</td>
<td>1.29 ± 0.02</td>
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<td>Medial prefrontal cortex (Layer 3)</td>
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<td>Infralimbic cortex</td>
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<td>1.02 ± 0.02</td>
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<tr>
<td>Primary motor</td>
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<td>1.41 ± 0.04</td>
<td>6</td>
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<tr>
<td>Retrosplenial</td>
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<td>1.43** ± 0.02</td>
<td>−7</td>
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<tr>
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<td>Piriform</td>
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<tr>
<td>Insular</td>
<td>1.07 ± 0.03</td>
<td>1.15 ± 0.03</td>
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<td>1.23** ± 0.03</td>
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<td>Central</td>
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<tr>
<td><strong>Amygdala</strong></td>
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<td>Basolateral</td>
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<tr>
<td>Central</td>
<td>0.63 ± 0.02</td>
<td>0.68 ± 0.03</td>
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<tr>
<td>Medial</td>
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<tr>
<td><strong>Basal ganglia</strong></td>
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<td>Globus pallidus</td>
<td>0.80 ± 0.02</td>
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<tr>
<td>Dorso-lateral</td>
<td>1.26 ± 0.02</td>
<td>1.18* ± 0.02</td>
<td>−6</td>
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<td>Ventromedial</td>
<td>1.11 ± 0.03</td>
<td>1.11 ± 0.03</td>
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<td>Substantia nigra pars compacta</td>
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<td>Substantia nigra pars reticulata</td>
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<td>0.76 ± 0.03</td>
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<td><strong>Hippocampus</strong></td>
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<tr>
<td>Subiculum</td>
<td>1.08 ± 0.03</td>
<td>1.05 ± 0.02</td>
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<td>Dentate gyrus</td>
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<td>0.79 ± 0.01</td>
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<tr>
<td>CA1</td>
<td>0.93 ± 0.03</td>
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<td>CA2</td>
<td>0.85 ± 0.03</td>
<td>0.82 ± 0.03</td>
<td>−4</td>
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<tr>
<td>CA3</td>
<td>0.80 ± 0.02</td>
<td>0.82 ± 0.02</td>
<td>3</td>
</tr>
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<td><strong>Septum</strong></td>
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<td></td>
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<tr>
<td>Medial</td>
<td>0.83 ± 0.04</td>
<td>0.81 ± 0.03</td>
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<tr>
<td>Lateral</td>
<td>0.80 ± 0.03</td>
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<td><strong>Diagonal band of broca</strong></td>
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<tr>
<td>Vertical band</td>
<td>1.10 ± 0.02</td>
<td>1.04 ± 0.02</td>
<td>−5</td>
</tr>
<tr>
<td>Horizontal band</td>
<td>1.03 ± 0.03</td>
<td>1.02 ± 0.02</td>
<td>−1</td>
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<tr>
<td><strong>Mesolimbic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleus accumbens core</td>
<td>0.93 ± 0.03</td>
<td>0.96 ± 0.03</td>
<td>4</td>
</tr>
<tr>
<td>Nucleus accumbens shell</td>
<td>0.94 ± 0.04</td>
<td>1.00 ± 0.04</td>
<td>7</td>
</tr>
<tr>
<td>Ventral tegmental area</td>
<td>0.96 ± 0.04</td>
<td>1.05 ± 0.05</td>
<td>10</td>
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<tr>
<td>Bed nucleus of the stria terminalis</td>
<td>0.59 ± 0.02</td>
<td>0.66** ± 0.01</td>
<td>12</td>
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</tbody>
</table>
While modafinil was able to reverse some PCP-induced alterations in functional coupling of the PrL, others remained intact, including a PCP-induced enhancement of functional coupling to the DLO and RSC, the CA3 subfield of the hippocampus, and the ventral pallidum (VP). In addition, modafinil further enhanced PCP-induced alterations in the functional coupling of the PrL to the mPrL1, LS, CeA, and centrolateral nucleus of the thalamus. These functional coupling events are unlikely to contribute to PCP-induced deficits in PrL function and its reversal by modafinil treatment.

**Retrosplenial Cortex.** Subchronic PCP treatment significantly altered the functional connectivity of the RSC (figure 4 and tables S4 and S6 in the supplementary material), which included the dissociation of RSC functional activity from that in several thalamic (anteromedial thalamus [AM], ventrolateral [VL], and ventromedial [VM]) nuclei, the medial prelimbic cortex (mPrL1 and mPrL2), and the LC. In contrast, functional connectivity of the RSC with multiple subfields of the PFC (PrL, DLO, LO, and FRA), the hippocampus (dentate gyrus [DG] and CA3), the mesolimbic system (VTA and NacC), striatum (dorsal, DLST and ventral, VMST), the vertical limb of the diagonal band of broca (VDB), and dorsal raphé (DR) was enhanced by subchronic PCP treatment.

In parallel to the restoration of PCP-induced hypometabolism in the RSC, modafinil normalized discrete PCP-induced alterations in the functional connectivity of this brain region. Modafinil normalized the functional connectivity of the RSC to the mPrL1, AM, ventromedial striatum (VMST), NacC, CeA, and the DG of the hippocampus in PCP-experienced animals. In addition to these normalization events, PCP-induced alterations in functional connectivity that are reversed by modafinil treatment, which then significantly exceeded the level of functional coupling seen in control animals, may also be relevant to the normalization of RSC function. This pattern of altered functional connectivity was observed with the mPrL3, DLST, the LC, VDB, and the dRT.

Significant alterations in functional coupling specific to modafinil treatment may also contribute to the restoration of RSC function in PCP-treated animals. Such events included the enhanced functional connectivity of the RSC with the medio orbital cortex, EC, GP, and ventral tegmental nuclei and a decreased functional connectivity with the hippocampal Sub, substantia nigra (both SNC and SNR), DTg, and BST.

While modafinil was able to reverse some PCP-induced deficits, others remained intact, including a PCP-induced enhancement of functional coupling to the PrL, CA3 subfield of the hippocampus (CA3), and VTA. PCP-induced decreases in functional connectivity that were not reversed by modafinil included coupling to the VL and VM thalamic nuclei, MeA, and M1. In addition, modafinil further enhanced PCP-induced deficits in the functional coupling of the RSC to the DR and ventral lateral lamniscus. These functional coupling events are unlikely to contribute to PCP-induced deficits in RSC functioning and their reversal by modafinil treatment.
Medial Prelimbic Cortex (Layer 1). Subchronic PCP treatment significantly altered the functional connectivity of the mPrL1 (figure 4 and tables S4 and S6 in the supplementary material), which included the dissociation of functional activity in this region from that in the hippocampus (CA1 and CA3), striatum (VMST), thalamic nuclei (AM and ventral reticular), the MeA, and LC. In contrast, functional coupling of mPrL1 to all other components of the media PFC (mPFC [mPrL2, mPrL3 and IL]), MS, ventral limb of the diagonal band of broca (VDB), and VP was enhanced by subchronic PCP treatment. While these alterations in functional coupling were significant, they did not produce an overt significant alteration in metabolism in the mPRL1.

In the case of the mPrL1 region, functional coupling events specifically altered by modafinil treatment are of particular interest because these may contribute to the hypermetabolism in this region induced by modafinil. This included the enhanced coupling of the FRA and DR to the mPrL1 in PCP-experienced animals treated with modafinil. In addition, the PCP-induced alteration in functional coupling of the mPrL1 to the AM was not only reversed by modafinil but also exceeded that in control animals. This may also contribute to the enhancing metabolism in the mPrL1 of PCP-experienced animals treated with modafinil. Furthermore, it is also possible that normalization events may also contribute to the increased rate of metabolism in PCP-experienced animals treated acutely with modafinil. Such events were seen with multiple subfields of the medial PFC (mPrL2 and IL), the NacS, the MS, and LC.

**Discussion**

The present study demonstrates that, in addition to overt alterations in metabolism, the functional connectivity signatures of the PrL (PrL and mPrL1) and RSC are altered in an animal model of schizophrenia, which displays cognitive inflexibility. Furthermore, we have identified the discrete components of this altered functional connectivity rescued by modafinil treatment that may contribute to its ability to restore cognitive flexibility and the rate of overt cerebral metabolism in these regions.

Here we confirm previous reports of reduced cognitive flexibility, as evidenced by the reduced ability to switch attentional set, and hypofrontality in rats treated subchronically with PCP. These behavioral and functional deficits are akin to those seen in schizophrenia. While there are some inconsistencies on the detection of both baseline and task-activated hypofrontality in schizophrenia, with positive and negative findings as well as a contrasting hyperfrontality reported in some cases.
studies, 2 recent meta-analyses of the available data support both baseline and task-activated hypofrontality in schizophrenia. Hypofrontality in schizophrenia has been proposed to underlie, or at least provide an accurate biomarker of, many of the cognitive deficits reported in this disorder. However, more recently, the hypothesis of PFC “inefficiency” has been proposed, which suggests that compromised functioning of the PFC in schizophrenia may result in either hyperfrontality or hypofrontality dependent upon the cognitive task involved and the relative level of task demand. Either way, hyper- or hypofrontality are likely to reflect compromised functional integration between neuronal activity within the PFC and the other components of the distributed functional brain networks involved in the completion of the cognitive task at hand. A primary focus of this article has been to elucidate the functional brain networks that are involved, along with the PFC, in the cognitive inflexibility seen in schizophrenia. Interestingly, schizophrenic patients fail to adequately activate PFC regions during the Wisconsin Card Sorting Task (WCST), which measures cognitive flexibility and is akin to the ASST paradigm used in this study, further highlighting the central role of these regions in cognitive flexibility. Furthermore, the degree of this hypofrontality has also been shown to correlate with the degree of neuropsychological impairment in this task. However, the exact relationship between overt PFC metabolism and cognitive flexibility may be more complex, although still consistent with the PFC inefficiency hypothesis. For example, in contrast to the effects of prolonged NMDA antagonist exposure, acute administration of the NMDA receptor antagonist ketamine induces both hyperfrontality while also producing similar deficits in cognitive flexibility in the WCST. This further highlights the complex relationship between PFC metabolism and cognitive flexibility. Hence, considering PFC function in the context of functional brain networks may be key to understanding its role in cognitive flexibility. Either way, these observations, along with those from preclinical studies in which PFC function is disrupted, strongly implicate the PFC in the control of behavioral flexibility and in contributing to the deficits in cognitive flexibility seen in schizophrenia. The subchronic PCP treatment regime outlined in this article clearly recapitulates both the cognitive inflexibility and the PFC dysfunction reported in schizophrenia.

Importantly, our results suggest that altered functional connectivity in brain networks and the dysfunction of multiple brain regions, rather than the discrete dysfunction of the PFC alone, underlie the cognitive inflexibility seen in PCP-treated animals and its reversal by modafinil. For example, in addition to the hypofrontality found in this model, this is the first study to report hypometabolism in the RSC, a finding consistent with the nonlethal injury of glutamatergic pyramidal neurones in this region following NMDA receptor antagonist exposure and its reversal by modafinil.
with emerging reports of hypometabolism in this region in schizophrenia. There are several mechanisms through which NMDA receptor antagonists could induce nonlethal neuronal injury in cortical regions including through the disinhibition of thalamic glutamatergic projections to the cortex, by the inhibition of B-aminobutyric acid (GABA)ergic neurones located in thalamic regions, as well as through the direct inhibition of cortical GABAergic interneurones resulting in the disinhibition of cortical pyramidal cells. This suggests that compromised pyramidal neuron integrity may contribute to the cortical hypometabolism seen in PCP-treated animals. In addition, subchronic PCP treatment has been shown to result in compromised GABAergic interneuron integrity in both cortical and thalamic regions, and these alterations parallel those seen in the brains of schizophrenics. The compromised integrity of both glutamatergic and GABAergic neurones in these brain regions is likely, in part, to contribute to their hypometabolism because these neurotransmitters have a central role in governing the coupling of cerebral metabolism to neuronal activity. In addition, the finding that repeated PCP treatment results in decreased cortical glutamatergic neurotransmission further highlights glutamatergic hypofunction as a mechanism contributing to the cortical hypometabolism seen in PCP-treated animals.

While previous studies have shown that modafinil reverses PCP-induced deficits in set shifting, ours is the first to elucidate the alterations in cerebral metabolism that underlie this, namely, the reversal of PCP-induced hypometabolism in the PrL and RSC and the activation of the mPrL1 by modafinil. These alterations in metabolism bare a remarkable resemblance to those reported in schizophrenic patients where modafinil-induced increases in cingulate cortex and dorsolateral PFC, a region analogous to the mPFC in rodents, metabolism correlate with its enhancement of cognitive performance. In addition, we have been able to elucidate the alterations in the functional connectivity of these regions, through the application of the PLSR algorithm, that may contribute to the cognitive inflexibility seen in PCP-experienced rats and its reversal by modafinil. PLSR analysis represents one algorithm from a range of statistical multivariate modeling techniques that may be used as part of a battery of algorithms to elucidate functional connectivity alterations in functional brain networks. Through the application of the PLSR algorithm, we identified a common functional subsystem, with both the PrL and the RSC, which included the NacC and LC, involved in the reversal of PCP-induced alterations in cognitive flexibility by modafinil. Interestingly, the same pattern of altered functional connectivity between the NacC and LC and the mPrL1 is also shown across the experimental groups. The importance of the NacC and LC in subserving cognitive flexibility is supported by previous studies showing that lesioning or inactivating either of these regions results in set-shifting deficits similar to those in PCP-treated animals.

Evidence supporting a role for the LC in set shifting is particularly strong because this region provides noradrenergic (NA) innervation to the cortex and deafferentation of these projections reduces cortical NA levels and produces a set shifting deficit. In addition, enhanced coupling between the LC and the cortical regions in modafinil-treated animals is consistent with the ability of modafinil to increase LC neuronal activity, inhibit NA uptake, and thus elevate extracellular NA. The involvement of the NacC in this functional subsystem is more complex because although effective coupling between the nucleus accumbens (NaC) and PFC has been shown to be essential for effective set shifting, the mechanism by which modafinil achieves this enhanced coupling in conjunction with enhanced cognitive flexibility remains unclear. Modafinil has previously been shown to enhance NaC dopamine (DA) levels. Increased DA neurotransmission in the NaC impairs set shifting, suggesting that the regulation of DA neurotransmission in this region is unlikely to be a primary mechanism by which modafinil improves cognitive flexibility. However, the ability of modafinil to increase DA levels in the PFC may contribute to its ability to improve cognitive flexibility in PCP-treated animals. Decreased levels of DA have been reported in the PFC of both primates and rats following repeated PCP exposure. Decreasing DA levels in the PFC of rats has also been shown to impair the ability to switch attentional set, whereas inhibiting catechol-O-methyltransferase, the enzyme responsible for the break down of DA, significantly increases PFC DA levels and the ability of rats to switch attentional set. In this study, we found that modafinil enhanced the functional coupling of PFC regions (in particular the PrL) to the substantia nigra (SNR/SNC) and VTA, which provide DA innervation to the PFC. This supports the suggestion that the regulation of DA neurotransmission in the PFC may be one mechanism through which modafinil improves cognitive flexibility in PCP-treated animals.

In addition to identifying this core functional subsystem, functional connectivity analysis also provides evidence for connectivity alterations specific to each seed brain region that relate to known anatomical connectivity. For example, the restoration of functional coupling between the AM and the RSC by modafinil in PCP-treated animals has an anatomical basis and may contribute to the restoration of metabolism in this region by modafinil. The anterior thalamic nuclei provide direct glutamatergic innervation to the RSC. The disinhibition of these projections, as a result of local GABAergic neuron inhibition in the thalamus, is a primary mechanism by which nonlethal neuronal damage occurs in the RSC following NMDA receptor antagonist administration. This mechanism may contribute to both the
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reduced metabolism in the RSC and also to the uncoupling of RSC-AM functional connectivity in PCP-treated animals. Modafinil may restore metabolism in the RSC by the direct stimulation of the AM-RSC glutamatergic projection as modafinil enhances thalamic glutamate levels, and this projection is known to be regulated by glutamatergic transmission in the thalamus. Importantly, functional connectivity analysis also appears to differentiate the relative importance of different neurotransmitter systems in mediating the effects of modafinil in a brain region-dependent manner. Thus, a possible role for serotonin (5-hydroxytryptamine, 5-HT) in mediating the effects of modafinil was supported for both prelimbic cortical regions but not for the RSC. Modafinil reversed PCP-induced uncoupling of the MR from the PrL and enhances coupling of the DR to the mPrL1. The functional coupling of these nuclei to the RSC is not altered by modafinil. These observations are consistent with the ability of modafinil to enhance 5-HT neurotransmission in frontal cortical regions. Enhanced 5-HT neurotransmission in prelimbic regions may contribute to the increased metabolism in these regions following modafinil because it has been shown to reduce cortical GABA levels through a 5-HT2 receptor-dependent mechanism. Interestingly, PFC 5-HT2 receptors also have a role in regulating DA neurotransmission in the nucleus accumbens, and this receptor subtype has been shown to be downregulated in the PFC of rats treated with the subchronic PCP regime used in this study. Therefore, the increased coupling of the raphé to the PrL as a result of enhanced activation of 5-HT2 receptors in this region may, in turn, contribute to the increased functional connectivity between these regions and the NacC following modafinil treatment. Despite these observations, the relationship between 5-HT neurotransmission in the PFC and cognitive flexibility remains unclear because chronic 5-HT depletion in these regions does not affect set shifting. However, chronic alterations in 5-HT are unlikely to model the effect of acute alterations in 5-HT neurotransmission because plasticity changes are likely to occur, including alterations in 5-HT2 receptor expression. The possible involvement of the acute regulation of PFC 5-HT neurotransmission in cognitive flexibility requires further investigation.

In addition to deficits in set shifting, subchronic PCP treatment also induced a deficit at the third reversal learning stage (Rev3). This suggests that this translational model not only has relevance to the set-shifting deficits but may also model the reported reversal learning deficits seen in schizophrenia. While PCP-induced deficits in reversal learning are not commonly reported when using the ASST experimental paradigm, they have been reported in studies utilizing alternative behavioral tests. Furthermore, the observed reversal learning deficit in PCP-treated animals is also consistent with the functional imaging deficits we identified in these animals. In particular, hypometabolism in the orbitofrontal cortex (OFC) of PCP-treated animals is consistent with the observation that lesioning this region induces reversal learning deficits in the ASST and other behavioral paradigms. While PCP-induced deficits in set shifting were amenable to modafinil treatment, deficits in reversal learning were not. In parallel, hypometabolism in the OFC (LO and VO), the dRT and centromedial (CM) thalamus, DLST, and MB were not reversed by modafinil. The sustained hypometabolism in the OFC seems particularly pertinent given the established role of this region in reversal learning. The involvement of the OFC in determining this deficit in PCP-treated animals is also consistent with the behavioral mechanism underlying it. The OFC contributes to efficient reversal learning through a form of working memory involving the online retention of associated outcomes with certain actions, enabling the detection of, and modification of behavior following, a shift in reward contingencies. In PCP-treated animals, regressive errors, the inability to maintain a new strategy following the initial reorientation of behavior, rather than perseverative errors, the inability to efficiently reorientate behavior, appear to underlie the reversal deficit. This is consistent with previous reports showing that regressive rather than perseverative errors underlie the reversal learning deficit induced by OFC lesioning. In addition, regressive errors induced by OFC inactivation only become apparent under conditions of increased task demand, which parallels our findings in PCP-treated animals. In the ASST protocol, Rev1 is the first discrimination in which animals experience a shift in stimulus-reward contingencies during the test. While this represents their first experience of such a shift, we found no evidence to suggest that PCP-treated animals found completing this discrimination harder than control animals, as previously reported. The successful completion of Rev2 carries a higher cognitive load than Rev1 because animals are aware that reward contingencies can change in the form of either a reversal, as experienced in Rev1, or as an ID. The cognitive load of Rev3 is higher still because at this discrimination, animals must choose from 3 possible responding strategies following a shift in the stimulus-reward contingency. They may consider the alteration to be an ID, ED, or, correctly, a reversal. The mediation of reversal learning by the OFC, and other PFC regions, is regulated by local serotonergic and cholinergic but not dopaminergic or NA neurotransmission. Since modafinil treatment increases serotonin, DA, and noradrenaline levels in the PFC without affecting acetylcholine, the inability of modafinil to correct PCP-induced OFC hypometabolism may relate to its inability to affect cholinergic neurotransmission. Therefore, OFC cholinergic neurotransmission may represent a target for the normalization of OFC.
metabolism and the reversal learning deficit in PCP-treated animals.

In conclusion, taking a combined translational and mathematical modeling approach to investigate the functional connectivity signatures of brain regions in relation to alterations in cerebral metabolism and behavior (eg, cognitive flexibility) provides added scientific insight into the mechanism of CNS system dysfunction and drug action in schizophrenia. In addition, these functional connectivity alterations appear to have a firm basis in previous established knowledge including that of anatomical connectivity, lesioning studies, and drug pharmacology.

Supplementary Material
Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

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