doi:10.1093/brain/aws068 Brain 2012: 135; 1436–1445 | 1436

BRAIN
A JOURNAL OF NEUROLOGY

Effective connectivity of AKT1-mediated dopaminergic working memory networks and pharmacogenetics of anti-dopaminergic treatment

Hao Yang Tan,[1] Anthony G. Chen,[1] Bhaskar Kolachana,[1] Jose A. Apud,[1] Venkata S. Mattay,[1] Joseph H. Callicott,[1] Qiang Chen[1,2] and Daniel R. Weinberger[1,2]

1 Clinical Brain Disorders Branch, Genes Cognition and Psychosis Program, Division of Intramural Research Programs, National Institute of Mental Health, Bethesda, MD 20892, USA
2 Lieber Institute for Brain Development, Johns Hopkins Medical Campus, 855 Wolfe Street, Baltimore, MD 21205, USA

Correspondence to: Hao Yang Tan,
Clinical Brain Disorders Branch, Genes Cognition and Psychosis Program,
Division of Intramural Research Programs,
National Institute of Mental Health,
Bethesda, MD 20892, USA
E-mail: haoyangtan@gmail.com

Correspondence may also be addressed to: Daniel R. Weinberger. E-mail: drweinberger@libd.org

Working memory is a limited capacity system that integrates and manipulates information across brief periods of time, engaging a network of prefrontal, parietal and subcortical brain regions. Genetic control of these heritable brain processes have been suggested by functional genetic variations influencing dopamine signalling, which affect prefrontal activity during complex working memory tasks. However, less is known about genetic control over component working memory cortical–subcortical networks in humans, and the pharmacogenetic implications of dopamine-related genes on cognition in patients receiving anti-dopaminergic drugs. Here, we examined predictions from basic models of dopaminergic signalling in cortical and cortical–subcortical circuitries implicated in dissociable working memory maintenance and manipulation processes. We also examined pharmacogenetic effects on cognition in the context of anti-dopaminergic drug therapy. Using dynamic causal models of functional magnetic resonance imaging in normal subjects ($n = 46$), we identified differentiated effects of functional polymorphisms in *COMT*, *DRD2* and *AKT1* genes on prefrontal–parietal and prefrontal–striatal circuits engaged during maintenance and manipulation, respectively. Cortical synaptic dopamine monitored by the COMT Val158Met polymorphism influenced prefrontal control of both parietal processing in working memory maintenance and striatal processing in working memory manipulation. DRD2 and AKT1 polymorphisms implicated in DRD2 signalling influenced only the prefrontal–striatal network associated with manipulation. In the context of anti-psychotic drugs, the DRD2 and AKT1 polymorphisms altered dose-response effects of anti-psychotic drugs on cognition in schizophrenia ($n = 111$). Thus, we suggest that genetic modulation of DRD2–AKT1-related prefrontal–subcortical circuits could at least in part influence cognitive dysfunction in psychosis and its treatment.

Keywords: antipsychotics; functional MRI; prefrontal cortex; schizophrenia; striatum

Abbreviations: AKT1 = RAC-alpha serine/threonine-protein kinase; COMT = catechol-O-methyltransferease; DRD2 = dopamine receptor D2

Received October 14, 2011. Revised December 28, 2011. Accepted January 19, 2012. Advance Access publication April 23, 2012

Introduction

Working memory, a limited capacity system that integrates and manipulates information across brief periods of time, critically engages a network of prefrontal, parietal and subcortical regions, and underlies important aspects of general intellectual function (Duncan *et al*., 2000; Gray *et al*., 2003). Working memory is dysfunctional in schizophrenia (Weinberger *et al*., 1986) and is related to its genetics from family and twin studies (Goldberg *et al*., 1993; Cannon *et al*., 2000; Egan *et al*., 2001*a*; Callicott *et al*., 2003; Toulopoulou *et al*., 2010). Executive sub-processes of working memory engaging manipulation rather than simple maintenance of information appears more vulnerable in neuropsychiatric disease states (Goldberg *et al*., 1993; Silver *et al*., 2003; Tan *et al*., 2005) and is associated with dysfunction in dorsolateral prefrontal cortex (Weinberger *et al*., 1986; Barch *et al*., 2001; Cannon *et al*., 2005; Tan *et al*., 2005). While prefrontal brain activity during working memory is under genetic control (Koten *et al*., 2009) less is known about the specific genetic underpinning of working memory sub-processes. The role of non-D2 and D2 dopamine signalling in respective cortical–cortical and cortical–striatal circuits have been richly modelled in the basic literature (O'Reilly, 2006; O'Reilly and Frank, 2006), However, their fine-grained circuit characteristics in human brain has been largely unexplored, particularly at the level of causally related patterns of distributed neural circuit activity. Moreover, to the extent genetic variation affects working memory brain circuit function, it will be important to know how this might extend to illness-related cognitive dysfunction and treatment.

We engaged a hypothesis-driven strategy to study component dopamine signalling processes in human cortical–subcortical networks engaging dissociable working memory processes. The aim was to examine, using dynamic causal models of realistic neural function and communication in functional MRI (Friston *et al*., 2003; David *et al*., 2008), how genes that monitor non-D2 and D2 aspects of dopamine signalling influence brain network connectivity. Functional genetic variation in *catechol-O-methyltransferease* (*COMT*; Egan *et al*., 2001*b*; Chen *et al*., 2004), *dopamine receptor D2* (*DRD2*; Zhang *et al*., 2007; Bertolino *et al*., 2009) and *RAC-alpha serine/threonine-protein kinase* (*AKT1*; Emamian *et al*., 2004; Harris *et al*., 2005; Tan *et al*., 2008; Giovannetti *et al*., 2010), each known to influence the biology of dopamine signalling, served as probes of dopamine signalling processes in human brain networks. We then examined pharmacogenetic effects of these same gene variants on cognitive dysfunction in patients with schizophrenia who received anti-dopaminergtic treatment.

It has been proposed that D2-mediated prefrontal–striatal gating of new information might be important in executive aspects of working memory updating and manipulation (O'Reilly, 2006; O'Reilly and Frank, 2006), and that this could at least partly underlie cognitive dysfunction in schizophrenia. The common mechanism of anti-psychotic efficacy is also the modulation of D2 function. Functional genetic variants influencing synaptic dopamine bioavailability (*COMT* Val158Met; Egan *et al*., 2001*b*; Chen *et al*., 2004), D2-receptor function (*DRD2* rs1076560; Zhang *et al*., 2007; Bertolino *et al*., 2009) and Akt1 abundance implicated in downstream D2-signal transduction (*AKT1* rs1130233; Emamian *et al*., 2004; Beaulieu *et al*., 2005; Harris *et al*., 2005; Tan *et al*., 2008; Giovannetti *et al*., 2010) have each been repeatedly found to affect prefrontal blood oxygen level-dependent activity in working memory, consistent with the complex role of prefrontal dopamine in human working memory. The most replicated evidence for prefrontal working memory effects probably exists for the COMT Val158Met variation (Mier *et al*., 2010). However, each of the above AKT1 and DRD2 variants have also been consistently associated with prefrontal brain activation changes during working memory in replicated imaging data sets (Zhang *et al*., 2007; Tan *et al*., 2008; Bertolino *et al*., 2009).

In this study, we extended these observations to further characterize how these dopamine-related genes may influence dissociable working memory sub-processes engaging prefrontal control over distributed information processing circuitry in parietal cortex and subcortical regions. We used dynamic causal models (Friston *et al*., 2003) of functional MRI data to define cortical–cortical and cortical–subcortical networks selectively engaged during maintenance and manipulation operations in working memory.

Further, in light of the importance of working memory and prefrontal systems for general intellectual function (Duncan *et al*., 2000; Gray *et al*., 2003), and the genetic findings reported here on prefrontal connectivity, we also explored the effects of these genes on cognitive deficits associated with anti-psychotic drug treatment that targets DRD2 signalling (Beaulieu *et al*., 2009). Our results elucidate the segregated circuitry of working memory processes in human brain, and the specific effects of dopamine signalling genes on these circuits. We also suggest that these gene effects may relate to the broader cognitive effects of DRD2-inhibiting drugs in patients who receive them.

Materials and methods

Subjects

The working memory functional MRI study comprised 46 unrelated right-handed healthy controls that were independent of samples used previously, which found replicated prefrontal activation effects of the same AKT1 variant (Tan *et al*., 2008). The pharmacogenetic study comprised 111 patients with schizophrenia. Research subjects were ascertained as part of the Clinical Brain Disorders Branch Sibling Study (Egan *et al*., 2001*b*). Subjects were all of European ancestry to minimize genetic heterogeneity and stratification artefacts. All subjects were between 18 and 55 years of age, with IQ $>$ 70 and gave written informed consent. Exclusion criteria were significant medical problems, history of loss of consciousness for $>$5 min, alcohol or drug abuse/dependence within the last 12 months and electroconvulsive therapy within the last 6 months. All subjects were interviewed by a psychiatrist using the Structured Interview for DSM-IV. For probands with schizophrenia, data from psychiatric records were also evaluated during diagnostic ascertainment. The study was approved by the NIMH Institutional Review Board.

DNA collection and genotyping

DNA was extracted from transformed B lymphocyte cell lines using standard procedures. All genotypes were determined using the 5′ exonuclease TaqMan® assay; SNP probe and primer sets were acquired as 'Assays on Demand' from Applied Biosystems. We genotyped the *AKT1* rs1130233 (Tan *et al.*, 2008), *COMT* Val158Met (Egan *et al.*, 2001*b*) and *DRD2* rs1076560 (Zhang *et al.*, 2007; Bertolino *et al.*, 2009) for the primary analyses reported here. Genotype accuracy was assessed by re-genotyping within a subsample, and reproducibility was routinely >99%. Genotyping completion rate was >90%.

Functional imaging of working memory function

Blood oxygen level-dependent functional MRI data were acquired from healthy individuals as they performed an event-related working memory task after brief training. An array of two or four number digits was presented for 0.5 s and held in working memory for 3–6 s (Fig. 1). Half the trials involved manipulating the numerical information in working memory, where subjects were subsequently presented with a cue for 3 s, to do a subtraction of two from one or two of the remembered array of numbers. In the other half of the trials, the cue was to continue maintaining the array of numbers without any arithmetic operation. The cues were followed by a 2 s probe, where a number appeared in one of the two or four spaces. Subjects were required to press the right button if the number corresponded correctly to the numerical answer at that position, or the left button if it was incorrect. In each of the three runs in the scanning session, there were 12 trials each of manipulation and maintenance, randomly distributed over ~5 min.

Whole-brain blood oxygen level-dependent functional MRI data were collected on a 3-T scanner (General Electric Systems) with a gradient echo–echo planar imaging pulse sequence acquisition of 24 contiguous slices (echo time = 30 ms, repetition time = 2 s, flip angle = 90°, field of view = 24 cm, matrix = 64 × 64, voxel dimensions = 3.75 × 3.75 × 6 mm). The first four scans were discarded to allow for signal saturation. Stimuli were presented via a back-projection system, and responses were recorded through a fibre-optic response box, which allowed the measurement of the accuracy and reaction time for each trial.

The functional MRI data were preprocessed and spatially normalized to a common stereotaxic space (Montreal Neurological Institute template) with SPM5 software (Wellcome Department of Cognitive Neurology, http://www.fil.ion.ucl.ac.uk/spm). Functional images for each subject were slice timing corrected, realigned to the first volume in the time series, and corrected for head motion. Images were then spatially normalized into standard stereotaxic space (Montreal Neurological Institute template) using a 12-parameter affine model. Spatial smoothing was applied with a Gaussian filter set at 8 mm full-width at half-maximum. After realignment, data sets were individually examined to ensure motion correction was less than 2 mm translation and less than 1.5° rotation, leading to the exclusion of four of the original 50 subjects. Each task-evoked stimulus was modelled as a separate delta function and convolved with a canonical haemodynamic response function, ratio normalized to the whole-brain global mean to control for systematic differences in global activity, and temporally filtered using a high-pass filter of 128 s. Task-evoked stimulus events at encoding, cued maintenance or manipulation, and at the probe phase, were modelled for correctly performed trials. Incorrect responses and residual movement parameters were also modelled as regressors of no interest. In our study, planned contrasts of interest were brain activity at the maintenance or manipulation task phases. These contrasts were subsequently taken to a second-level group analysis in which inter-subject variability was treated as a random effect. We evaluated the main effect of each maintenance or manipulation task phase at a threshold of $P < 0.05$ corrected for false discovery rate (Genovese *et al.*, 2002) within the whole-brain search volume (Supplementary Fig. 1).

Dynamic causal modelling

We used dynamic causal models (Friston *et al.*, 2003) to investigate how prefrontal, parietal and subcortical brain systems interacted with one another during working memory maintenance and manipulation, and importantly, the effects of dopamine genes on these interactions.

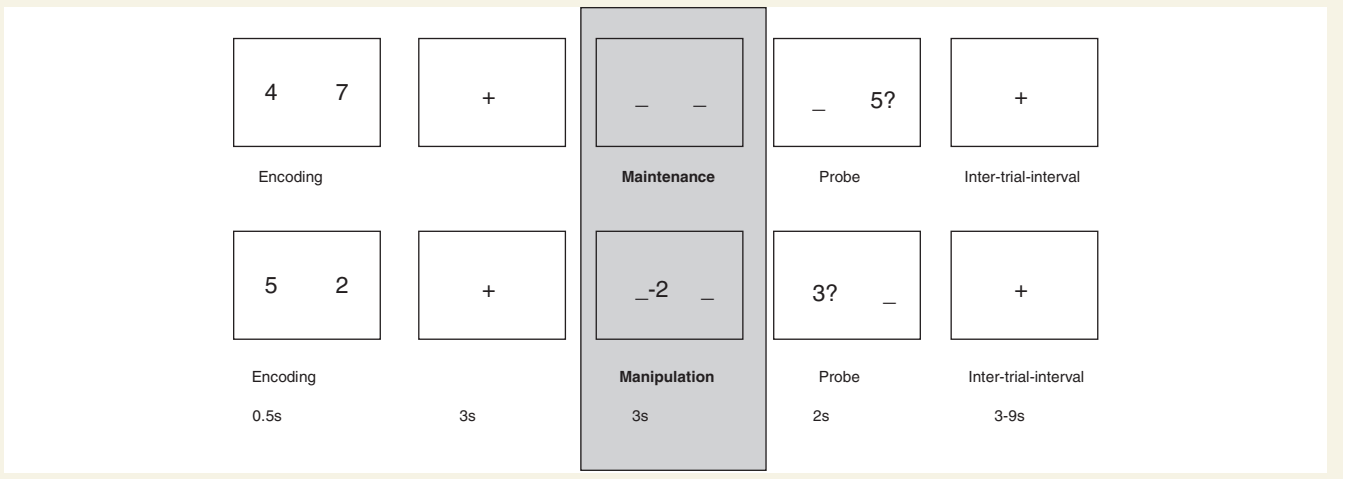

Figure 1 Event-related working memory paradigm. An array of two (*top*) or four number digits was encoded and held in working memory for 3–6 s. Half the trials involved subsequent manipulation of the numerical information in working memory, where subjects were cued to perform a subtraction of two from one (*bottom*) or two of the remembered array of numbers. In the other half of the trials, the cue was to continue maintaining the array of numbers without any arithmetic operation (*top*).

Dynamic causal models enable the estimation of the strength and direction of these regional interactions. Using Bayesian parameter estimation, the observed blood oxygen level-dependent responses were compared with that predicted by a neurobiologically plausible model. This model comprised parameters describing how regional neural activity and their interactions are influenced by external inputs (e.g. cued maintenance or manipulation of information), as well as describing how these neuronal effects are biophysically linked to form the blood oxygen level-dependent responses. In constructing the dynamic causal models at working memory maintenance and manipulation, we first selected time series from regions of interest in the left dorsolateral prefrontal cortex, parietal cortex and subcortical region that were robustly engaged in these working memory processes in each subject. The three regions of interest were within Brodmann area (BA) 9 and 46 for dorsolateral prefrontal cortex, BA 7 and 40 for parietal cortex, and the thalamus (for maintenance) and striatum (for manipulation), as defined by the Wake Forest University Pickatlas (http://www.fmri.wfubmc.edu/download.htm). From each individual subjects' task-specific *t*-contrast map, we extracted the time series from the median activation voxel within each of these three regions of interest that met an activation threshold of $P < 0.005$ uncorrected at the individual subject level, as well as being within 10 mm of a group level peak at $P < 0.05$ corrected for whole-brain false discovery rate (Supplementary Fig. 1). We then built a set of all plausible dynamic causal models in which the three regions could interact, and tested which was optimal in describing the observed blood oxygen level-dependent data. In constraining the alternative models accounting for task-related activation in the three nodes, each model was required to meet all the following conditions: task-related information entered the system directly at one or more of the nodes; task-related connectivity occurred at two or more different pairs of nodes to reach activation in all three nodes; and each source node in a connected pair either received task information directly or a target node through a connection from another source node. These constraints resulted in 282 models, the simplest with task information entering at one node and two modulatory connections from that source node to the remaining two nodes; the most complex model with inputs into all three nodes and bidirectional connections between them.

Bayesian model selection in dynamic causal model (Penny *et al*., 2004; Stephan *et al*., 2007) was then used to determine the optimal model. This procedure approximated the 'model evidence' (i.e. the probability $p(y|m)$ of the data y given a particular model m) using the more conservative of the Bayesian information criterion or Akaike information criterion to compute a Bayes factor, $p(y|m1)/p(y|m2)$, i.e. the evidence ratio of any two models being compared (Penny *et al*., 2004). At each maintenance and manipulation task for each subject, we obtained pairwise comparisons between all models, and computed the group Bayes factor across subjects (Stephan *et al*., 2007) to select the optimal model for that working memory task.

From the optimal dynamic causal models describing maintenance and manipulation in working memory, we then examined task-related modulation of regional connectivity across subjects, and the effect of dopamine genes. A one-sample *t*-test (two-tailed) was used to establish the main effect of connectivity across subjects at $P < 0.05$ Bonferroni corrected. Significantly implicated connections between dorsolateral prefrontal cortex and parietal cortex at maintenance, and between dorsolateral prefrontal cortex and the striatum at manipulation were then examined in relation to hypothesized relationships to functional genotypes in the dopamine genes. In the genetic tests, we assumed a dominant model to avoid sparse cell sizes. Given basic models of non-D2 and D2 effects in maintenance and manipulation aspects of working memory (Miller and Cohen, 2001; O'Reilly and Frank, 2006), we hypothesized that cortical–cortical roles of *COMT* but not *AKT1* and *DRD2* would occur in working memory maintenance, and cortical–subcortical roles of *COMT*, *AKT1* and *DRD2* in working memory manipulation. A nominal significance of $P < 0.05$ was set for testing this reduced set of specific functional genetic and neural connectivity relationships. Permutation tests were then conducted to estimate the false positive rate at which the hypothesized set of genetic relationships could occur. These were performed over 1 million random label changes of subject identity within this sample, keeping constant the genotype labels to maintain the same genotype frequencies.

Pharmacogenetic association in schizophrenia

We studied the effect of functional genetic variation in the *COMT*, *DRD2* and *AKT1* genes on anti-psychotic drug action in patients with schizophrenia who received these drugs ($n = 111$). We examined if these genetic variants interacted with anti-psychotic dose effects to impact disease-related IQ changes. Illness-associated reduction in IQ scores relative to premorbid IQ is a well-documented characteristic of patients with schizophrenia (Weickert *et al*., 2000) and reflects general cognitive concomitants of illness, and predicts treatment outcome and disability (Green, 1996). We obtained the current IQ estimate from a four-subtest version of the Wechsler Adult Intelligence Scale-Revised (WAIS-R), comprising the Arithmetic, Digit Symbol Substitution, Picture Completion and Similarities subtests (Weickert *et al*., 2000). The estimate of premorbid IQ was based on the Reading subtest of the Wide Range Achievement Test (WRAT-R). The Reading subtest is a valid reflection of preserved abilities acquired before the onset of illness (Dalby and Williams, 1986; Weickert *et al*., 2000). The pharmacogenetic interactions across the functional *COMT*, *AKT1* and *DRD2* genes on the effect of anti-psychotic dose on disease-related IQ, were tested at a nominal significance of $P < 0.05$. We also explored if anti-psychotic dose, duration of illness, gender or symptoms severity confounded the effect on disease-related IQ.

Results

Demographic and behavioural effects in working memory

In the functional MRI study of healthy subjects ($n = 46$), no demographic differences were found across *COMT* Val/Met, *AKT1* rs1130233 and *DRD2* rs1076560 genotypes in terms of gender, age or IQ ($n = 46$, $P > 0.14$). Demographic and genotype frequencies of samples studied are further detailed in Supplementary Table 1. While there was the expected main effect of working memory task phase, with the more difficult working memory manipulation task having lower accuracy and slower reaction time than the maintenance task (accuracy $P < 0.001$, reaction time $P < 0.001$), there were no significant differences across genotypes in working memory performance in either maintenance or manipulation tasks during functional MRI (Supplementary Table 2; $P > 0.25$). Thus, genetic differences in functional MRI reported subsequently reflect neural information processing and not task performance *per se*.

Dynamic causal models of prefrontal–subcortical–parietal networks during working memory maintenance and manipulation events

Across each of the working memory maintenance and manipulation events, subjects robustly increased blood oxygen level-dependent activity in the left dorsolateral prefrontal cortex, parietal cortex and subcortical brain regions ($P < 0.05$ corrected for false discovery rate; Supplementary Fig. 1). We focused on the left hemisphere because of its dominant role in the processing of symbolic numerical information engaged in the numerical working memory task (Dehaene *et al*., 2003). During working memory maintenance, the optimal model (i.e. highest group Bayes factor score with a log odds ratio of this model at 17.1 relative to the second highest model; Fig. 2) engaged working memory information input to the dorsolateral prefrontal cortex, and task-related changes in prefrontal-to-parietal and parietal-to-thalamic neural connections (Fig. 2). In particular, the maintenance task engaged 'excitatory' prefrontal-to-parietal connectivity (parameter estimates 0.45 + SE 0.011, $P < 0.005$ Bonferroni corrected). On the other hand, the best model for working memory manipulation (with log odds ratio of 34.5 relative to the second highest model; Fig. 2) engaged task inputs to the dorsolateral prefrontal cortex and striatum, and top-down task-modulated connections from dorsolateral prefrontal cortex to striatum and from dorsolateral prefrontal cortex to parietal cortex (Fig. 3). In particular, the manipulation task modulated 'inhibitory' prefrontal-to-striatal connectivity (parameter estimates −0.10 + SE 0.003, $P < 0.01$ Bonferroni corrected).

Functional genetic variation impacting prefrontal circuitry during working memory maintenance and manipulation

We next examined the influence of the three functional genetic variants involved in aspects of dopamine signalling on the prefrontal–subcortical–parietal connectivity networks during segregated working memory maintenance and manipulation events. At the maintenance task phase, *COMT*-Met individuals, associated with putatively greater cortical synaptic dopamine bioavailability (Egan *et al*., 2001*b*; Chen *et al*., 2004; Papaleo *et al*., 2008), showed relatively stronger task-modulated prefrontal-to-parietal cortical excitatory effective connectivity relative to COMT Val-homozygotes ($t = 1.94$, $P < 0.05$; Fig. 3). The effects of

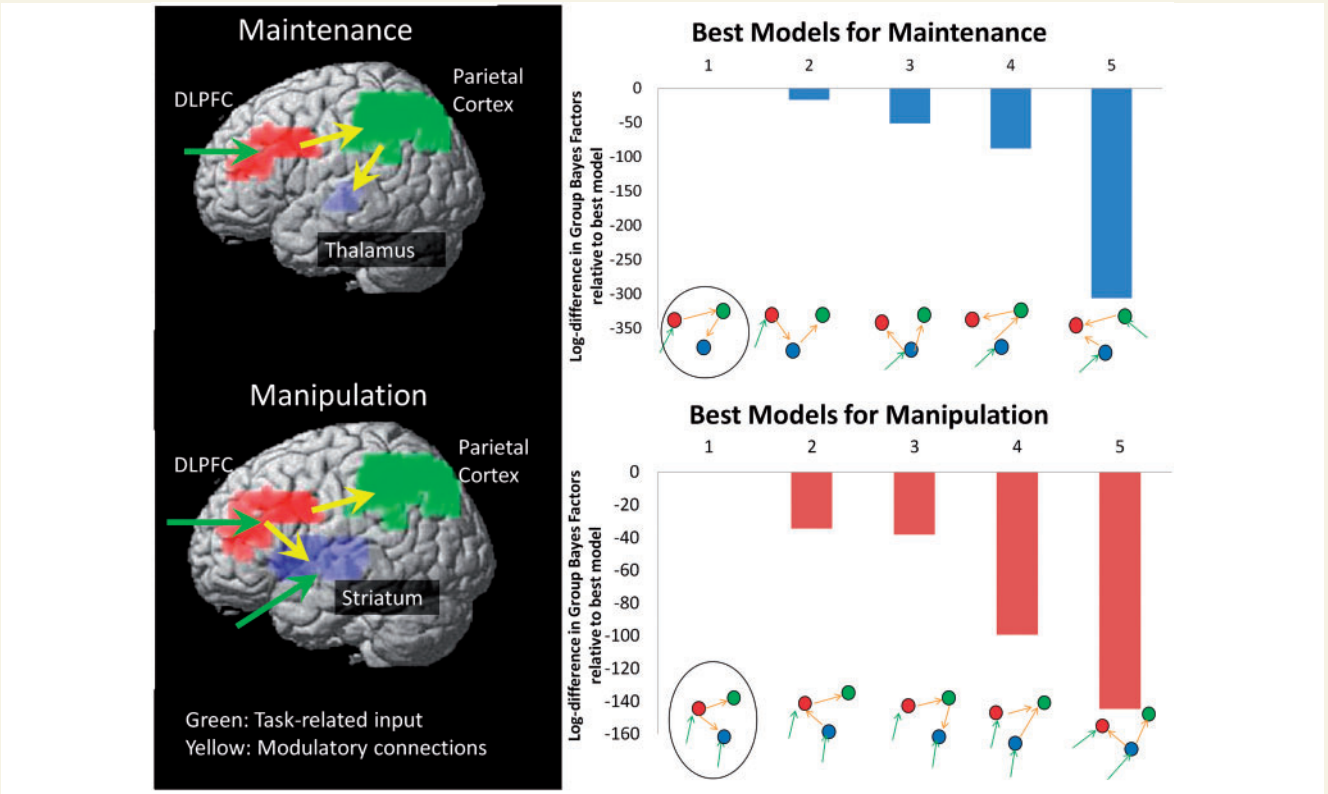

Figure 2 Dynamic causal models of working memory sub-processes. *Left*: Optimal models for working memory maintenance and manipulation projected onto dorsolateral prefrontal cortex (DLPFC, red), parietal (green) and subcortical (blue) regions activated by the task. Green arrows represent task-related information input into the model, and yellow arrows represent task-modulated changes in effective connectivity. *Right*: Log-difference in the Group Bayes Factors of the top five models for working memory maintenance and manipulation relative to the best model (circled). Each model's nodes, connections and inputs are similarly colour-coded as that on the *left*.

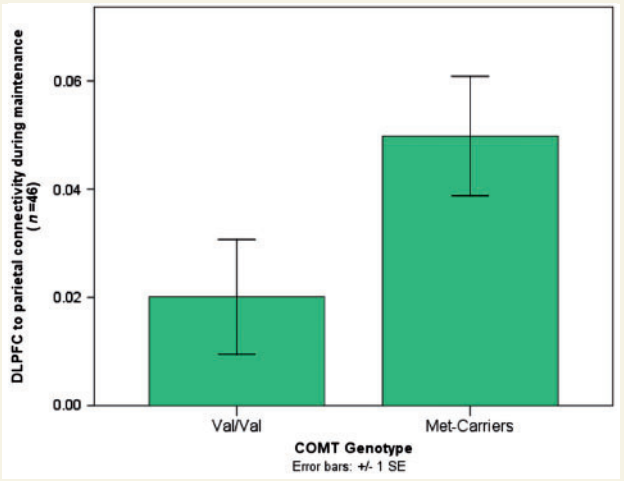

Figure 3 Dorsolateral prefrontal cortex to parietal connectivity induced by working memory maintenance. During working memory maintenance events, dorsolateral prefrontal cortex (DLPFC) to parietal 'excitatory' effective connectivity was increased in relation to *COMT* Met-carriers > Val/Val ($P < 0.05$). Error bars are +1 standard error (SE).

DRD2 and *AKT1* functional variants were not significant in predicting cortical effective connectivity.

At working memory manipulation, COMT-Met individuals had relatively stronger task-modulated prefrontal-to-striatal inhibitory effective connectivity ($t = 2.57$, $P < 0.014$; Fig. 4). DRD2 rs1076560 GG-versus T-carrier effects ($t = 2.17$, $P < 0.035$) and AKT1 rs1130233 GG-versus A-carrier effects ($t = 2.34$, $P < 0.028$) were also independently associated with relatively stronger inhibitory prefrontal-to-striatal effective connectivity. As the pattern of genetic results at working memory maintenance and manipulation differentiated non-D2 and D2-related signalling effects at the respective fronto-parietal and fronto-striatal processes, we further examined the rate at which this set of findings could arise by chance in our data. Permutation tests of over 1 million replicates suggest that the conservative rate of false positivity was $P < 3 \times 10^{-6}$.

DRD2 and AKT1 pharmacogenetic effects on cognitive changes associated with anti-dopaminergic treatment

The genetic effects on brain function that we observed would be expected to have implications for pharmacological interventions that target the same genes. IQ and working memory are linked (Duncan *et al*., 2000; Gray *et al*., 2003) and we recapitulated this in our own imaging data in healthy individuals ($r = -0.35$, $P < 0.007$; Supplementary Fig. 2). We thus further investigated the effects of genetic variation in COMT, DRD2 and AKT1 at the level of putative DRD2–AKT1 anti-psychotic drug actions (Beaulieu *et al*., 2009) in patients with schizophrenia ($n = 111$) who received these drugs.

Illness-related IQ change did not relate to the chlorpromazine-equivalent anti-psychotic dose, duration of illness, gender or Positive and Negative Syndrome Scale-positive, -general or -negative scores ($P > 0.18$). These variables did not differ across *COMT*, *AKT1* or *DRD2* genetic variants ($P > 0.56$). *COMT*, *AKT1* and *DRD2* genotypes were also independent ($P > 0.6$). However, we found the hypothesized *AKT1* and *DRD2*, but not the *COMT* ($P > 0.8$) pharmacogenetic effects with anti-psychotic dose on IQ change. Patients with the *AKT1* A-allele, associated with relatively reduced AKT1 expression and reduced prefrontal–subcortical inhibitory effective connectivity, had less IQ change but only in association with higher anti-psychotic doses (accounting for ~6.3% of variance in IQ change). Patients with the *AKT1* GG-alleles did not show the dose–IQ relationship (*AKT1* × dose interaction: $\chi^2(1) = 6.34$, $P = 0.012$; Fig. 5A). For *DRD2*, we similarly found that patients with the T-allele associated with relatively dysregulated DRD2 function and reduced prefrontal–subcortical inhibitory effective connectivity had less IQ change in association with higher anti-psychotic doses (accounting for ~13% of variance in IQ change). This effect was also not apparent in patients with the *DRD2* major allele homozygotes (*DRD2* × dose interaction: $\chi^2(1) = 4.96$, $P = 0.034$; Fig. 5B).

Discussion

Studying dynamic causal models of brain circuits (Friston *et al*., 2003) has revealed insights into working memory task-specific patterns of regional neural communication and their genetic control. The D2-AKT1 pathway genes that affect this communication also appear relevant in the pharmacology of cognitive deficits associated with anti-dopaminergic therapy at least in patients with schizophrenia. In this study, we first made a comprehensive search through the model space connecting the entry of working memory-specific information and their effects on integrated neural activity in prefrontal, parietal and subcortical regions using a task that reliably dissociates maintenance from manipulation sub-processes in working memory. Models with the best fit to the acquired data were those where working memory sub-processes engaged prefrontal control of neural activity in distributed brain regions, e.g. parietal cortex and striatum, consistent with the primary role of the prefrontal cortex in working memory, and in coordinating information storage and manipulation (Fuster, 1997).

Further, during the maintenance of information in working memory, the dynamic causal models suggest that prefrontal cortex integrated working memory-related inputs and controlled the engagement of excitatory parietal neural activity in mediating this temporary information storage. These findings are consistent with the hypothesized presence of reverberatory excitatory connections that keep information active in distributed cortical neural circuits during working memory (Wang and O'Donnell, 2001). The strength of these connections was found to be modulated by cortical synaptic dopamine availability indexed by *COMT* Val158Met, where individuals who were *COMT* Val-homozygotes associated with reduced synaptic cortical dopamine availability (Egan *et al*., 2001*b*; Chen *et al*., 2004;

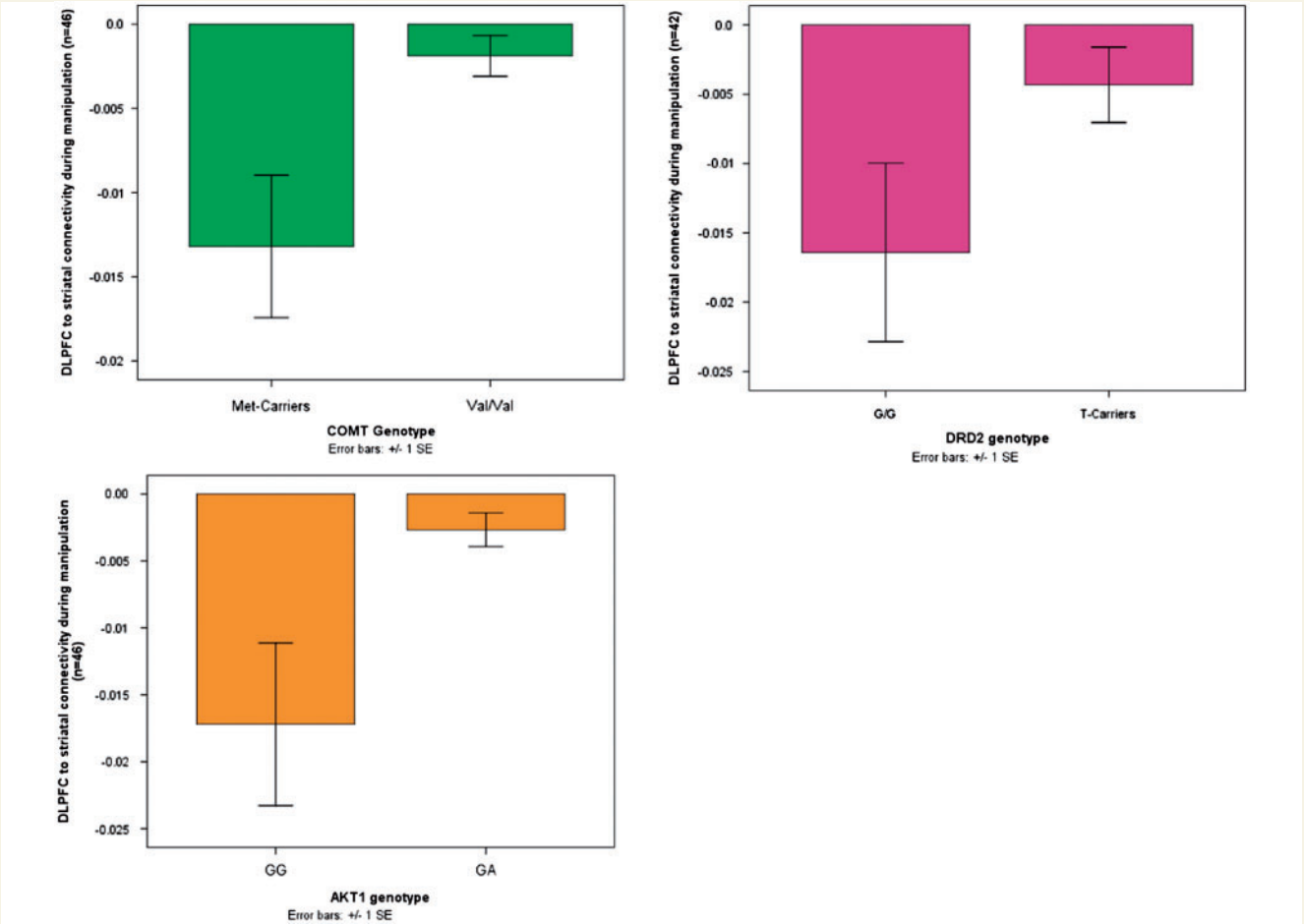

Figure 4 Dorsolateral prefrontal cortex to subcortical connectivity induced by working memory manipulation. Dorsolateral prefrontal cortex (DLPFC) to striatal 'inhibitory' effective connectivity was accentuated in *COMT*-Met relative to Val/Val ($P < 0.014$), in relation to *AKT1* rs1130233 GG versus A-carrier ($P < 0.028$), and in relation to *DRD2* rs1076560 GG versus T-carriers ($P < 0.035$). Error bars are +1 standard error (SE).

Papaleo *et al*., 2008; Slifstein *et al*., 2008) had relatively reduced prefrontal-to-parietal connectivity. This is consistent with conceptualizations of reduced neural signal-to-noise tuning in these individuals (Williams and Goldman-Rakic, 1995; Winterer and Weinberger, 2004), and consequent reduced interregional neural coherence during information storage.

In manipulating information in working memory, the dynamic causal models suggest engagement of net prefrontal inhibitory control of striatal neural function. This is remarkably consistent with conceptualizations of prefrontal-driven modulatory connections with the dopamine-rich striatal regions in basic models (Saunders *et al*., 1998; Akil *et al*., 2003), as well as the role these prefrontal–striatal control functions might play in regulating striatal dopamine to allow optimal gating, updating and transformation of new information in working memory (Miller and Cohen, 2001; Meyer-Lindenberg *et al*., 2005; O'Reilly and Frank, 2006). As could be predicted from the putative DRD2 involvement in these gating functions (O'Reilly, 2006; O'Reilly and Frank, 2006), the prefrontal–striatal effective connectivity during working memory manipulation was influenced by overall cortical synaptic dopamine availability indexed by *COMT* Val158Met (Egan *et al*., 2001*b*; Chen *et al*., 2004; Slifstein *et al*., 2008), and by specific DRD2 function indexed by rs1076560 (Zhang *et al*., 2007; Bertolino *et al*., 2009). Genetic probes of relatively reduced cortical dopamine and dysregulated DRD2 function were independently associated with deficits in prefrontal–striatal effective connectivity. Moreover, to the extent that AKT1 critically couples downstream of DRD2 function (Beaulieu *et al*., 2007), these connectivity effects should also be influenced by Akt1 expression indexed by the *AKT1* rs1130233 variant (Harris *et al*., 2005; Tan *et al*., 2008; Giovannetti *et al*., 2010). Correspondingly, we found that the *AKT1* allele associated with its reduced expression, risk for schizophrenia and prefrontal deficits (Emamian *et al*., 2004; Harris *et al*., 2005; Schwab *et al*., 2005; Tan *et al*., 2008; Giovannetti *et al*., 2010), was also associated with connectivity deficits between the prefrontal and subcortical regions during working memory manipulation. In sum, the genetic associations with circuit-based connectivity models are strikingly consistent with data from prior human and basic animal experiments, which predict that COMT would be relevant to primarily cortical

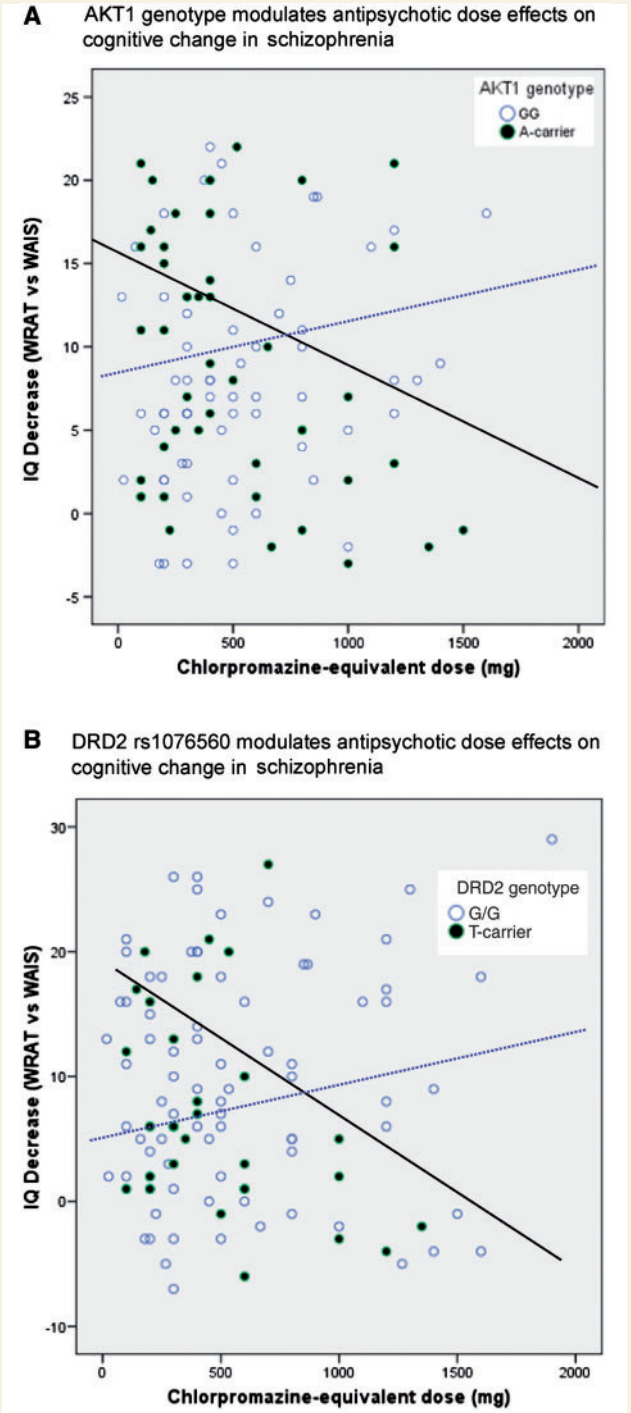

Figure 5 *AKT1* and *DRD2* genotype modulation of the anti-psychotic dose relationships with cognitive deficits in schizophrenia. (**A**) Filled circles represent patients with the *AKT1* A-carriers, who showed less illness-related IQ change in association with higher chlorpromazine-equivalent anti-psychotic doses (dark line, $P < 0.05$). Open circles represent patients with the *AKT1* GG-allele, who did not show the dose–IQ relationship. (**B**) Filled circles represent patients with the *DRD2* T-carriers, who similarly had less illness-related IQ change in association with higher anti-psychotic doses (dark line, $P < 0.05$). The open circles here represent patients with the *DRD2* major allele homozygotes, who did not show the dose–IQ relationship. WAIS = Wechsler Adult Intelligence Scale; WRAT = Wide Range Achievement Test.

operations (Slifstein *et al*., 2008; Käenmäki *et al*., 2010), and that D2-AKT1 would engage cortical and striatal dopamine operations (Lai *et al*., 2006; Beaulieu *et al*., 2007; Cools *et al*., 2009). These differentiated prefrontal connectivity findings in working memory support and further extend previous data of localized prefrontal activation changes in working memory associated with the same COMT, AKT1 and DRD2 genetic variants (Egan *et al*., 2001*b*; Zhang *et al*., 2007; Tan *et al*., 2008; Bertolino *et al*., 2009; Mier *et al*., 2010).

In view of the emerging data implicating DRD2–AKT1 not only in the dynamics of working memory but also attention (Blasi *et al*., 2011) and episodic memory (Bertolino *et al*., 2008; Tan *et al*., 2011), we addressed the potentially broader clinical relevance of these genetic effects on general cognition and IQ in the context of pharmacological therapy that targets the same dopamine signalling system. It follows that cognitive changes associated with pharmacological modulation of DRD2 and AKT1 could be influenced by these same genes. These same prefrontal–striatal processes are important in general cognition and IQ (Duncan *et al*., 2000; Gray *et al*., 2003). IQ is affected in and genetically linked to schizophrenia (Goldberg *et al*., 1995; Cannon *et al*., 2000; Egan *et al*., 2001*a*; Toulopoulou *et al*., 2010), and is diminished from premorbid levels in treated patients (Weickert *et al*., 2000). In the pharmacogenetic interactions, we found that higher chlorpromazine-equivalent anti-psychotic doses were associated with reduced IQ change, but apparently only in those carrying the disadvantageous *AKT1* or *DRD2* alleles. There was no pharmacogenetic interaction for *COMT*. Ostensibly, these pharmacogenetic interactions suggest that only patients with more dysregulated DRD2 function or deficits in AKT1 expression benefited cognitively from higher anti-psychotic doses. These findings are remarkably consistent with anti-psychotic drug actions and their resultant effects on D2-blockade and AKT1 activation (Beaulieu *et al*., 2007). The pharmacogenetic data further parses genetically selective pharmacological outcomes, hereto not known to impact cognitive deficits in schizophrenia and in association with anti-dopaminergic drug treatment.

Nevertheless, there are limitations to the data that merit comment. We have suggested that the pharmacogenetic data on IQ relate biologically to working memory and fronto-striatal circuitry (Duncan *et al*., 2000; Gray *et al*., 2003). However, other brain networks influenced by DRD2 or AKT1 engaging attentional (Blasi *et al*., 2011) and memory processes (Bertolino *et al*., 2008) in IQ may also contribute to these effects. Future imaging work would be needed to directly map these specific circuits to pharmacogenetic effects in patients with schizophrenia. The clinical data are also cross-sectional and thus causative inferences are debatable. In the interpretation of the pharmacogenetic data, we have reasonably assumed that the common anti-psychotic mechanism across these subjects involves at least DRD2 blockade. However, we cannot disambiguate the possibility that higher anti-psychotic doses contributed instead to deterioration in those carrying the *AKT1* or *DRD2* major allele-homozygotes. Similarly, we will not be able to disambiguate the possibility that patients carrying the *AKT1*-A or *DRD2*-T alleles were more resilient to side effects of higher anti-psychotic doses relative to the major allele homozygotes. Still, the pharmacogenetic finding remains that an

over-representation of patients on higher doses of anti-psychotic drugs differed in their apparent cognitive response according to *AKT1* or *DRD2* genotype.

We have performed a number of hypothesis-driven tests in this report. The main working memory task and connectivity effects were statistically corrected within imaging space using conservative and well-validated imaging statistics (Penny *et al*., 2004; Stephan *et al*., 2007). Then, these served as specific loci for subsequent genetic interrogation. The genetic tests assumed a dominant model to avoid sparse cell sizes and were not individually controlled for multiple testing. These limitations may be mitigated because the genetic tests were not exploratory, but were a set of findings that converged from several theoretical and experimental models. Biological assays of these same genetic variants with the same genetic models have repeatedly implicated these variants on gene expression or function for *COMT* (Egan *et al*., 2001*b*; Chen *et al*., 2004; Papaleo *et al*., 2008; Slifstein *et al*., 2008), *DRD2* (Zhang *et al*., 2007; Bertolino *et al*., 2009) and *AKT1* (Tan *et al*., 2008; Blasi *et al*., 2011). The same functional variants and genetic models affected prefrontal physiology in working memory (Egan *et al*., 2001*b*; Meyer-Lindenberg *et al*., 2005; Tan *et al*., 2008; Bertolino *et al*., 2009; Mier *et al*., 2010). In this context, permutation tests on our new findings of effective connectivity on models of segregated non-D2 and D2 dopaminergic mechanisms of working memory (O'Reilly, 2006; Frank *et al*., 2007) provide further assurance that the hypothesis-driven set of genetic associations are statistically conservative. Future work in larger samples might be necessary to investigate genetic interactions of these genes in segregated working memory circuits, and associated pharmacological function.

Conclusion

We have examined multiple levels of AKT1-dopaminergic brain function in healthy individuals and in patients with schizophrenia. Functional variants in *COMT, DRD2* and *AKT1* influenced working memory task-specific prefrontal control of distributed neural circuitry. Cortical synaptic dopamine availability indexed by *COMT* Val158Met influenced dynamic causal models of prefrontal influence on parietal neural connectivity as information is maintained in working memory. On the other hand, manipulating information in working memory engaged prefrontal control of striatal circuitry, which was also influenced by *COMT*, as well as by variants impacting DRD2 function and AKT1 expression. This is consistent with the mechanistic role these D2-related genes play in fronto-striatal cognitive control (Alexander *et al*., 1986; O'Reilly and Frank, 2006; Tan *et al*., 2008). Extending to cognitive deficits in patients with schizophrenia and anti-dopaminergic drug treatment, we found that the same *DRD2* and *AKT1* variants modulated the cognitive response according to anti-psychotic dosage, underlining the potential therapeutic relevance of these genetic pathways. Thus, we suggest that genetic perturbations in dopaminergic signalling impact distributed prefrontal brain systems that may, at least in part, be relevant to individual variation in the cognitive outcome of anti-dopaminergic treatment.

Funding

This work was funded by the National Institute of Mental Health Intramural Research Program.

Supplementary material

Supplementary material is available at *Brain* online.

References

Akil M, Kolachana BS, Rothmond DA, Hyde TM, Weinberger DR, Kleinman JE. Catechol-O-methyltransferase genotype and dopamine regulation in the human brain. J Neurosci 2003; 23: 2008–13.

Alexander GE, DeLong MR, Strick PL. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu Rev Neurosci 1986; 9: 357–81.

Barch DM, Carter CS, Braver TS, Sabb FW, MacDonald A, Noll DC, et al. Selective deficits in prefrontal cortex function in medication-naive patients with schizophrenia. Arch Gen Psychiatry 2001; 58: 280–8.

Beaulieu J-M, Gainetdinov RR, Caron MG. The Akt-GSK-3 signaling cascade in the actions of dopamine. Trends Pharmacol Sci 2007; 28: 166.

Beaulieu J-M, Gainetdinov RR, Caron MG. Akt/GSK3 Signaling in the Action of Psychotropic Drugs. Annu Rev Pharmacol Toxicol 2009; 49: 327–47.

Beaulieu JM, Sotnikova TD, Marion S, Lefkowitz RJ, Gainetdinov RR, Caron MG. An Akt/β-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. Cell 2005; 122: 261–73.

Bertolino A, Di Giorgio A, Blasi G, Sambataro F, Caforio G, Sinibaldi L, et al. Epistasis between dopamine regulating genes identifies a nonlinear response of the human hippocampus during memory tasks. Biol Psychiatry 2008; 64: 226–34.

Bertolino A, Fazio L, Caforio G, Blasi G, Rampino A, Romano R, et al. Functional variants of the dopamine receptor D2 gene modulate prefronto-striatal phenotypes in schizophrenia. Brain 2009; 132: 417.

Blasi G, Napolitano F, Ursini G, Taurisano P, Romano R, Caforio G, et al. DRD2/AKT1 interaction on D2 c-AMP independent signaling, attentional processing, and response to olanzapine treatment in schizophrenia. Proc Natl Acad Sci USA 2011; 108: 1158–63.

Callicott JH, Egan MF, Mattay VS, Bertolino A, Bone AD, Verchinksi B, et al. Abnormal fMRI Response of the dorsolateral prefrontal cortex in cognitively intact siblings of patients with schizophrenia. Am J Psychiatry 2003; 160: 709–19.

Cannon TD, Glahn DC, Kim J, Van Erp TGM, Karlsgodt K, Cohen MS, et al. Dorsolateral prefrontal cortex activity during maintenance and manipulation of information in working memory in patients with schizophrenia. Arch Gen Psychiatry 2005; 62: 1071–80.

Cannon TD, Huttunen MO, Lonnqvist J, Tuulio-Henriksson A, Pirkola T, Glahn D, et al. The inheritance of neuropsychological dysfunction in twins discordant for schizophrenia. Am J Hum Genet 2000; 67: 369.

Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, et al. Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet 2004; 75: 807–21.

Cools R, Frank MJ, Gibbs SE, Miyakawa A, Jagust W, D'Esposito M. Striatal dopamine predicts outcome-specific reversal learning and its sensitivity to dopaminergic drug administration. J Neurosci 2009; 29: 1538–43.

Dalby JT, Williams R. Preserved reading and spelling ability in psychotic disorders. Psychol Med 1986; 16: 171–5.

David O, Guillemain I, Saillet S, Reyt S, Deransart C, Segebarth C, et al. Identifying neural drivers with functional MRI: an electrophysiological validation. PLoS Biol 2008; 6: e315.

Dehaene S, Piazza M, Pinel P, Cohen L. Three parietal circuits for number processing. Cogn Neuropsychol 2003; 20: 487–506.

Duncan J, Seitz RJ, Kolodny J, Bor D, Herzog H, Ahmed A, et al. A neural basis for general intelligence. Science 2000; 289: 457–60.

Egan MF, Goldberg TE, Gscheidle T, Weirich M, Rawlings R, Hyde TM, et al. Relative risk for cognitive impairments in siblings of patients with schizophrenia. Biol Psychiatry 2001a; 50: 98–107.

Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, et al. Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. Proc Natl Acad Sci USA 2001b; 98: 6917–22.

Emamian ES, Hall D, Birnbarum MJ, Karayiogou M, Gogos JA. Convergent evidence for impaired AKT1-GSK3b signaling in schizophrenia. Nat Genet 2004; 36: 131–7.

Frank MJ, Moustafa AA, Haughey HM, Curran T, Hutchison KE. Genetic triple dissociation reveals multiple roles for dopamine in reinforcement learning. Proc Natl Acad Sci USA 2007; 104: 16311–6.

Friston KJ, Harrison L, Penny W. Dynamic causal modelling. NeuroImage 2003; 19: 1273.

Fuster JM. The prefrontal cortex - anatomy, physiology, and neuropsychology of the frontal lobe. Lippincott-Raven 1997.

Genovese CR, Lazar NA, Nichols T. Thresholding of statistical maps in functional imaging using the false discovery rate. Neuroimage 2002; 15: 870–8.

Giovannetti E, Zucali PA, Peters GJ, Cortesi F, D'Incecco A, Smit EF, et al. Association of Polymorphisms in AKT1 and EGFR with Clinical Outcome and Toxicity in Non-Small Cell Lung Cancer Patients Treated with Gefitinib. Mol Cancer Therapeutics 2010; 9: 581–93.

Goldberg TE, Torrey EF, Gold JM, Bigelow LB, Ragland RD, Taylor E, et al. Genetic risk of neuropsychological impairment in schizophrenia: a study of monozygotic twins discordant and concordant for the disorder. Schizophrenia Res 1995; 17: 77–84.

Goldberg TE, Torrey EF, Gold JM, Ragland JD, Bigelow LB, Weinberger DR. Learning and memory in monozygotic twins discordant for schizophrenia. Psychol Med 1993; 23: 71–85.

Gray JR, Chabris CF, Braver TS. Neural mechanisms of general fluid intelligence. Nat Neurosci 2003; 6: 316.

Green MF. What are the functional consequences of neurocognitive deficits in schizophrenia? Am J Psychiatry 1996; 153: 321–30.

Harris SL, Gil G, Robins H, Hu W, Hirshfield K, Bond E, et al. Detection of functional single-nucleotide polymorphisms that affect apoptosis. Proc Natl Acad Sci USA 2005; 102: 16297–302.

Käenmäki M, Tammimäki A, Myöhänen T, Pakarinen K, Amberg C, Karayiorgou M, et al. Quantitative role of COMT in dopamine clearance in the prefrontal cortex of freely moving mice. J Neurochem 2010; 114: 1745–55.

Koten JW, Wood G, Hagoort P, Goebel R, Propping P, Willmes K, et al. Genetic contribution to variation in cognitive function: an fMRI study in twins. Science 2009; 323: 1737–40.

Lai WS, Xu B, Westphal KGC, Paterlini M, Olivier B, Pavlidis P, et al. Akt1 deficiency affects neuronal morphology and predisposes to abnormalities in prefrontal cortex functioning. Proc Natl Acad Sci USA 2006; 103: 16906–11.

Meyer-Lindenberg A, Kohn PD, Kolachana B, Kippenhan S, McInerney-Leo A, Nussbaum R, et al. Midbrain dopamine and prefrontal function in humans: interaction and modulation by COMT genotype. Nat Neurosci 2005; 8: 594–6.

Mier D, Kirsch P, Meyer-Lindenberg A. Neural substrates of pleiotropic action of genetic variation in COMT: a meta-analysis. Mol Psychiatry 2010; 15: 918–27.

Miller EK, Cohen JD. An integrative theory of prefrontal cortex function. Annu Rev Neurosci 2001; 24: 167–202.

O'Reilly RC. Biologically based computational models of high-level cognition. Science 2006; 314: 91–4.

O'Reilly RC, Frank MJ. Making working memory work: a computational model of learning in the prefrontal cortex and basal ganglia. Neural Comput 2006; 18: 283–328.

Papaleo F, Crawley JN, Song J, Lipska BK, Pickel J, Weinberger DR, et al. Genetic dissection of the role of catechol-O-methyltransferase in cognition and stress reactivity in mice. J Neurosci 2008; 28: 8709–23.

Penny WD, Stephan KE, Mechelli A, Friston KJ. Comparing dynamic causal models. NeuroImage 2004; 22: 1157.

Saunders RC, Kolachana BS, Bachevalier J, Weinberger DR. Neonatal lesions of the medial temporal lobe disrupt prefrontal cortical regulation of striatal dopamine. Nature 1998; 393: 169.

Schwab SG, Hoefgen B, Hanses C, Hassenbach MB, Albus M, Lerer B, et al. Further evidence for association of variants in the AKT1 gene with schizophrenia in a sample of European sib-pair families. Biol Psychiatry 2005; 58: 446.

Silver H, Feldman P, Bilker WB, Gur RC. Working memory deficit as a core neuropsychological dysfunction in schizophrenia. Am J Psychiatry 2003; 160: 1809–16.

Slifstein M, Kolachana B, Simpson EH, Tabares P, Cheng B, Duvall M, et al. COMT genotype predicts cortical-limbic D1 receptor availability measured with 11C NNC112 and PET. Mol Psychiatry 2008; 13: 821–7.

Stephan KE, Weiskopf N, Drysdale PM, Robinson PA, Friston KJ. Comparing hemodynamic models with DCM. NeuroImage 2007; 38: 387.

Tan HY, Chen AG, Chen Q, Browne LB, Verchinski BA, Kolachana B, et al. Epistatic interactions of AKT1 on human medial temporal lobe biology and pharmacogenetic implications. Mol Psychiatry 2011; doi: 10.1038/mp.2011.91 (In press).

Tan HY, Choo WC, Fones CSL, Chee MWL. fMRI study of maintenance and manipulation processes within working memory in first-episode schizophrenia. Am J Psychiatry 2005; 162: 1849–58.

Tan HY, Nicodemus KK, Chen Q, Li Z, Honea R, Brooke J, et al. Genetic variation in AKT1 is linked to dopamine-associated prefrontal cortical structure and function in humans. J Clin Investig 2008; 118: 2200–8.

Toulopoulou T, Goldberg TE, Mesa IR, Picchioni M, Rijsdijk F, Stahl D, et al. Impaired intellect and memory: a missing link between genetic risk and schizophrenia? Arch Gen Psychiatry 2010; 67: 905–13.

Wang J, O'Donnell P. D1 Dopamine receptors potentiate NMDA-mediated excitability increase in layer V prefrontal cortical pyramidal neurons. Cereb Cortex 2001; 11: 452–62.

Weickert TW, Goldberg TE, Gold JM, Bigelow LB, Egan MF, Weinberger DR. Cognitive impairments in patients with schizophrenia displaying preserved and compromised intellect. Arch Gen Psychiatry 2000; 57: 907.

Weinberger DR, Berman K, Zec R. Physiological dysfunction of dorsolateral prefrontal cortex in schizophrenia: I. Regional cerebral blood flow evidence. Arch Gen Psychiatry 1986; 43: 114–24.

Williams GV, Goldman-Rakic PS. Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. Nature 1995; 376: 572–5.

Winterer G, Weinberger DR. Genes, dopamine and cortical signal-to-noise ratio in schizophrenia. Trends Neurosci 2004; 27: 683–90.

Zhang Y, Bertolino A, Fazio L, Blasi G, Rampino A, Romano R, et al. Polymorphisms in human dopamine D2 receptor gene affect gene expression, splicing, and neuronal activity during working memory. Proc Natl Acad Sci USA 2007; 104: 20552–7.