Relationship between striatal dopamine function and hippocampal glutamate levels in subjects at ultra high risk of psychosis

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Background: Animal models of psychosis propose that striatal hyperdopaminergia is driven by abnormalities in hippocampal glutamatergic neurotransmission, but this has never been tested in humans.

Methods: Sixteen individuals with an at risk mental state for psychosis (ARMS) and 12 age and sex-matched controls underwent proton magnetic resonance spectroscopy to estimate hippocampal glutamate, and [18F]DOPA positron emission tomography to index striatal dopamine function. The relationship between hippocampal glutamate and striatal dopamine, and their relationship with prodromal symptoms, was determined using linear regression.

Results: In ARMS subjects, but not controls, there was a significant negative relationship between hippocampal glutamate levels and striatal [18F]DOPA uptake (p=0.03). Within the ARMS sample, striatal [18F]DOPA uptake was correlated with severity of abnormal beliefs (p=0.03), there was a trend for hippocampal glutamate levels to be correlated with disordered speech (p=0.06), and a trend for the interaction between hippocampal glutamate and [18F]DOPA uptake to predict later transition to psychosis (p=0.07).

Conclusions: The relationship between hippocampal glutamate and striatal dopamine systems is abnormal in people at high risk of psychosis. The degree of this abnormality may be related to the risk of transition. Drugs targeting the glutamate system before the development of psychosis might ameliorate this risk.
Introduction

Excess striatal dopamine activity in schizophrenia has been hypothesized to occur secondary to dysfunctional glutamatergic transmission (1-2). Whilst imaging studies have provided substantial evidence for both glutamatergic and dopaminergic abnormalities in schizophrenia (3), the relationship between alterations in these two neurochemical systems has yet to be directly investigated in humans.

The striatum receives extensive projections from the hippocampus, and when the glutamatergic output of the hippocampus is experimentally stimulated, both the number of spontaneously active dopamine neurons, and the amount of dopamine released in the striatum are increased (4). In patients with schizophrenia, loss of hippocampal GABAergic tone, either due to intrinsic GABAergic interneuron deficits, or to dysfunction of NMDA receptors expressed on their surface, may lead to disinhibition of hippocampal glutamatergic efferents (2, 5). In keeping with this hypothesis, reductions in both NMDA receptor binding (6) and levels of NMDAR1 subunit mRNA (7) in the hippocampus have been reported in schizophrenia.

Glutamate and dopamine dysfunction in schizophrenia may each underlie different psychopathological features of the disorder (5). Speech disturbances (incoherent speech and poverty of speech) and negative symptoms (loss of motivation, reduced emotional reactivity, social impairments) have been linked to perturbations in glutamatergic transmission (5), whereas positive symptoms (hallucinations and delusions) appear to be associated with hyperactivity of dopaminergic transmission (5). The relationship between psychotic symptoms and neurochemical dysfunction
has yet to be examined in a sample in which both glutamate and dopamine have been assessed in the same individuals.

Subjects with attenuated psychotic symptoms (an At Risk Mental State - ARMS), have a greatly increased risk of developing a psychotic disorder (8). Previous neuroimaging studies have separately shown that the ARMS is associated with alterations in hippocampal structure and function (9-10), regional changes in glutamate levels (11), and striatal hyperdopaminergia (12).

We investigated the in vivo relationship between hippocampal glutamate levels, measured using proton magnetic resonance spectroscopy (1H-MRS), and striatal pre-synaptic dopamine function, estimated with 3,4-dihydroxy-6-[18F]fluoro-L-phenylalanine ([18F]DOPA) positron emission tomography (PET), a measure of striatal dopamine synthesis capacity. We tested the hypothesis that there would be an abnormal relationship between hippocampal glutamate levels and striatal dopaminergic function in the ARMS group. We also predicted that dopamine function in the ARMS would be related to attenuated delusions and hallucinations, while glutamate levels would be related to disordered speech. Finally, we examined whether the relationship between glutamate and dopamine measures in the ARMS predicted the subsequent onset of psychosis.

Methods
Sixteen subjects with attenuated psychotic symptoms, who met ARMS criteria (8), were compared with 12 healthy control subjects. The data were collected as part of studies approved by the South London and Maudsley NHS Trust/Institute of
Psychiatry research ethics committee and the Administration of Radioactive Substances Advisory Committee (ARSAC). All subjects provided informed written consent to participate.

**Clinical and neuropsychological measures**

All subjects were assessed at the time of first clinical presentation with the Comprehensive Assessment of At Risk Mental States (CAARMS) (8). Subjects were then followed up clinically for 2 years. Transition to psychosis was determined according to the CAARMS criteria (8).

**1H-MRS data acquisition**

Proton MRS data were acquired at presentation on a General Electric (Milwaukee, USA) HDx 3T MR scanner, using a body coil for transmit and 8-channel coil for receive. A whole brain 3D pure coronal inversion recovery prepared spoiled gradient echo (IR-SPGR) scan was used for voxel localization and volume correction. The hippocampal voxel (20mm x 20mm x 15mm) was placed in the left hemisphere, with placement being standardized by matching to a comparison image. A single spectrum was obtained using Point Resolved Spectroscopy (PRESS) acquisition (TE=30ms, TR=3000ms, 96 averages).

**1H-MRS spectrum analysis**

All spectra were analyzed, generating water-scaled metabolite levels, using LCModel version 6.1-4F (13). The IR-SPGR images were segmented into grey and white matter and cerebral spinal fluid (CSF) to allow correction of the spectroscopy results for partial volume CSF contamination. Metabolite levels were divided by the brain tissue
(grey plus white matter) content of the voxel in each subject. Poorly fitted metabolite peaks (Cramer-Rao minimum variance bounds estimated by LCModel >20%) were excluded from further analysis.

**PET data acquisition**

PET imaging was performed at clinical presentation using an ECAT/EXACT3D 966 (Siemens/CTI, Knoxville, Tennessee) tomograph (spatial resolution: 4.8 (0.2) mm; sensitivity: 69 cps/Bq/mL). To reduce the formation of radiometabolites all subjects received carbidopa (150 mg) and entacapone (400 mg) orally 1 hour before imaging. A 5-minute transmission image was obtained before radiotracer injection to correct for attenuation and scatter. Approximately 150 MBq of [18F]-DOPA was administered by bolus intravenous injection 30 seconds after the start of the PET imaging. Emission data were acquired for 95 minutes and reconstructed using a 3D re-projection algorithm.

**PET image processing**

Nonattenuation corrected dynamic images were de-noised, and individual frames were realigned to a single frame acquired 8 minutes after [18F]DOPA injection using a mutual information algorithm (14). The transformation parameters were then applied to the corresponding attenuation-corrected frames, and the realigned frames were combined to create a movement-corrected dynamic image for analysis. Standardized regions in Montreal Neurologic Institute (MNI) space were defined in the cerebellum (the reference region) and striatum using a probabilistic atlas (15). As hippocampal efferents project to the ipsilateral but not the contralateral striatum in primates (16), left-sided hippocampal pathology would be predicted to relate
primarily to left striatal dopamine function. Therefore, [18F]DOPA influx rate constants (Ki values) were calculated, using Patlak graphical analysis, for the left striatum only.

**Statistical analysis**

All statistical analyses were performed using the R statistical programming language version 2.9 (17). Data were checked for normality of distribution and equality of variance. Group differences in the relationship between striatal [18F]DOPA uptake and hippocampal glutamate were determined using a general linear model to examine the group by covariate (group-mean centered hippocampal glutamate) interaction, with striatal [18F]DOPA Ki as the dependent variable. In ARMS subjects, relationships between left striatal [18F]DOPA uptake or left hippocampal glutamate and the three main CAARMS outcome measures (severity of abnormal thought content, abnormal perceptions and speech abnormalities) were explored using linear regression with model simplification by backwards elimination. The relationship of dopamine and glutamate to later psychosis was investigated by logistic regression.

**Results**

The relationship between hippocampal glutamate and striatal [18F]DOPA Ki differed significantly between groups (F$_{2,25}$=3.377; p=0.05) (Figure 1), with a negative correlation in ARMS subjects (n=16, r=-0.538, p=0.031), but no correlation in controls (n=12 r=0.046; p=0.89).

Linear regression of hippocampal glutamate levels on CAARMS symptoms in the ARMS sample after model simplification revealed a trend for lower glutamate levels
to be related to abnormalities of speech production ($F_{1,14}=4.215$, $p=0.059$). Higher levels of striatal [18F]DOPA were significantly associated with abnormalities of thought content ($F_{1,14}=6.268$, $p=0.025$).

Subsequent to scanning, four (25%) of the ARMS sample underwent transition to frank psychosis. There was a trend for the interaction between hippocampal glutamate levels and striatal [18F]DOPA Ki in ARMS subjects to predict the later onset of psychosis ($p=0.075$). This trend was stronger when both ARMS and control subjects were included in the analysis ($p=0.058$). No group differences in [18F]DOPA Ki in the left striatum ($t_{29}=0.589$; ns), or in left hippocampal glutamate were detected, however ($t_{26}=0.325$; ns).

**Discussion**

These findings provide the first *in vivo* evidence that the coupling between hippocampal glutamate and striatal dopamine activity is altered in people with prodromal symptoms of psychosis (2). They also suggest that this abnormal relationship may be a risk marker for later transition to a full-blown psychotic disorder. As hippocampal glutamate levels were also related to the severity of speech abnormalities, and presynaptic dopamine synthesis capacity predicted the severity of abnormal beliefs, the data also support the hypothesis that glutamate and dopamine dysfunction are related to distinct types of psychotic phenomena (5).

The altered relationship between hippocampal glutamate and striatal [18F-]DOPA uptake in ARMS subjects relative to controls suggests that there may be a dysfunctional signaling pathway between the hippocampus and striatum in these
subjects. Whilst, as previously reported (11), mean hippocampal glutamate levels did not differ significantly between ARMS subjects and controls, putative deficits in hippocampal NMDA receptor activity (6-7), or GABA interneuron function (2), in the ARMS could lower the responsiveness of hippocampal GABAergic interneurons to presynaptic glutamate. This would result in increased activity in hippocampal efferents, with the net effect of increased striatal dopamine neuron activity (Figure 2) (2, 4). Alternatively, as suggested elsewhere (1-2, 4), dopamine neurons in patients with schizophrenia and ARMS subjects may be more sensitive to alterations in glutamatergic drive from the hippocampus than in controls (Figure 2).

In conclusion, these data indicate that the relationship between hippocampal glutamatergic and striatal dopaminergic function may be abnormal in people at high risk of psychosis. Drugs targeting this abnormal coupling, perhaps through promotion of glutamatergic transmission at NMDA receptors (18-19), may have potential as a means of reducing the risk of psychosis in individuals at high risk.
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References


Figure Legends

Figure 1. Relationship between left hippocampal glutamate levels (institutional units) and left striatal 18F-DOPA uptake (Ki/min) in ARMS subjects and controls. ARMS subjects who underwent transition to psychosis subsequent to scanning (t) are depicted by filled triangles.
Figure 2: Diagram showing putative mechanism underlying the relationship between hippocampal glutamate and striatal dopamine in individuals at high risk of schizophrenia. In the hippocampus, reduced GABAergic interneuron activity, in response to hippocampal glutamate, disinhibits glutamatergic pyramidal neurons (A), leading to increased glutamate release from their terminals in the striatum (B). This results in disinhibition (C), and increased activity (D) of dopaminergic neurons in the midbrain projecting to the striatum. Midbrain dopaminergic neurons may also be at increased sensitivity to changes in inhibitory tone (C) in these individuals.