

NEUROCHEMICAL AND NEUROPHARMACOLOGICAL IMAGING IN SCHIZOPHRENIA

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Over the last 15 years, the ability to measure specific molecules and proteins in the living human brain underwent enormous developments, opening direct windows into neurotransmitter functions and cellular processes associated with health and disease. These techniques are based either on the injection of radioactive moieties whose distribution is recorded with positron emission tomography (PET) or single photon emission computed tomography (SPECT), or on the direct detection of molecules based on their intrinsic magnetic properties with magnetic resonance spectroscopy (MRS).

Although lacking the level of resolution of postmortem studies and limited by the relatively small number of targets that can be currently studied *in vivo*, these imaging techniques provide unique opportunities for elucidation of pathophysiology associated with neuropsychiatric conditions. *In vivo* imaging techniques enable studying patients with well-documented psychopathology, relating biochemical observations to psychiatric symptoms and cognitive processes, and clarifying abnormalities associated with the disease as opposed to its treatment. Neurochemical imaging allows longitudinal studies to investigate mechanisms of actions and consequences of treatments, as well as to characterize neurochemical abnormalities in relation to treatment response and illness outcome. Neurochemical imaging further provides insight as to the pathophysiologic bases of alterations measured with flow and metabolism imaging studies, and can provide a direct link with animal models of the illness. Moreover, these techniques enable the study of patient's relatives, in order to clarify the endophenotypes associated with illness vulnerability. These potentialities are discussed in this chapter.

This chapter also critically summarizes results obtained using neurochemical imaging techniques in schizophrenia research, and the various insights on the pathophysiology and treatment of schizophrenia gained by these results. We first consider PET and SPECT investigations, and then MRS studies.

PET AND SPECT NEUROCHEMICAL IMAGING

The principles of PET and SPECT neurochemical imaging are reviewed elsewhere in this volume. Numerous PET and SPECT radiotracers are currently available to study key proteins in the living brain, such as receptors, transporters, and enzymes. Regarding schizophrenia, the majority of clinical investigations studied various aspects of dopaminergic transmission. Dopamine (DA) D2 receptors were the first neuroreceptors visualized in the living human brain (1). Since then, several DA-related radiotracers have been developed, allowing the study of many aspects of dopaminergic transmission (DA synthesis, DA release, D1 and D2 receptors, DA transporters). Given the availability of these tools and the important role that DA transmission is believed to play in schizophrenia, it is not surprising that most of the research effort focused on this system. Despite marked limitations, these studies provide a relatively consistent picture suggesting that schizophrenia, at least during periods of clinical exacerbation, is associated with dysregulation of DA transmission.

Imaging DA Transmission Parameters In Schizophrenia

The classic DA hypothesis of schizophrenia, formulated over 30 years ago, proposed that a hyperactivity of the dopaminergic transmission is associated with this illness (2,3).

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This hypothesis was essentially based on the observation that all antipsychotic drugs provided at least some degree of D2 receptors blockade, a proposition that is still true today (4,5). As D2 receptor blockade is most effective against positive symptoms, the DA hyperactivity model appeared to be most relevant to the pathophysiology of positive symptoms. The fact that sustained exposure to DA agonists such as amphetamine can induce a psychotic state characterized by some salient features of positive symptoms of schizophrenia (emergence of paranoid delusions and hallucinations in the context of a clear sensorium) also contributed to the idea that positive symptoms might be due to sustained excess dopaminergic activity (6,7).

These pharmacologic effects indeed suggest, but do not establish, a dysregulation of DA systems in schizophrenia. For example, these observations are also compatible with the hypotheses that DA activity per se would be normal and increased only relatively to other systems that may be deficient, such as the glutamatergic or serotonergic system (8,9). Under these conditions, D2 receptor blockade would reestablish a compromised balance between dopaminergic and glutamatergic or serotonergic tone. Thus, these pharmacologic observations do not necessarily imply a specific disturbance of DA activity per se in the brain of patients with schizophrenia. Indeed, documentation of abnormalities of DA function in postmortem studies in schizophrenia has remained elusive (10–12). Because positive symptoms are mostly prominent in young patients and their intensity decreases with age, the ability to detect their biochemical correlates in postmortem studies (generally performed in older subjects) may be limited.

On the other hand, negative and cognitive symptoms are generally resistant to treatment by antipsychotic drugs. Functional brain imaging studies suggested that these symptoms are associated with prefrontal cortex (PFC) dysfunction (13). Studies in nonhuman primates demonstrated that deficits in DA transmission in PFC induce cognitive impairments reminiscent of those observed in patients with schizophrenia (14), suggesting that a deficit in DA transmission in the PFC might be implicated in cognitive impairments presented by these patients (15,16). In addition, a recent postmortem study described abnormalities of DA terminals in the PFC associated with schizophrenia (17). Thus, a current view on DA and schizophrenia is that subcortical mesolimbic DA projections might be hyperactive (resulting in positive symptoms) and that the mesocortical DA projections to the PFC are hypoactive (resulting in negative symptoms and cognitive impairment). Furthermore, these two abnormalities might be related, as the cortical DA system generally exerts an inhibitory action on subcortical DA systems (18,19).

The advent in the early 1980s of techniques based on PET and SPECT to measure indices of DA activity in the living human brain held considerable promise for investigating these questions.

Striatal D2 Receptor Density

Striatal D2 receptor density in schizophrenia has been extensively studied with PET and SPECT imaging. Studies comparing parameters of D2 receptor binding in patients with schizophrenia and healthy controls ($n = 16$ studies) are listed in Table 59.1, and included a total of 228 patients (102 were neuroleptic-naive, and 126 were neuroleptic-free for variable periods of time). These patients were compared to 213 controls, matched for age and sex. Ten studies used PET and six studies used SPECT. Radiotracers included butyrophenones ($[^{11}\text{C}]\text{N-methyl-spiperone}$, $[^{11}\text{C}]\text{NMSP}$, $n = 3$, and $[^{76}\text{Br}]\text{bromospiperone}$, $n = 3$), benzamides ($[^{11}\text{C}]\text{raclopride}$, $n = 3$, and $[^{123}\text{I}]\text{IBZM}$, $n = 5$) or the ergot derivative $[^{76}\text{Br}]\text{lisuride}$, $n = 2$). A variety of methods and outcome measures were used to estimate D2 receptors density. Six studies used empirical ratio methods, i.e., the ratio of striatal to cerebellar activities at a given time after single bolus injection of the radiotracer. Five studies used model-based methods to measure the binding potential (BP), which is equal to the product of receptor density (B_{max}) and affinity ($1/K_d$). Five studies reported both B_{max} and K_d .

Only two out of 13 studies detected a significant elevation of D2 receptor density parameters at a level $p < .05$. However, metaanalysis of the 16 studies reveals a small (on the order of 13%) but significant elevation of D2 receptors in patients with schizophrenia. If D2 receptor density did not differ between patients and controls (null hypothesis), one would expect approximately 50% of the studies to report lower D2 receptor levels in schizophrenics compared to controls. Instead, 12 out of 16 studies reported an increase (although not significant in 10 out of 12 cases), two reported no change, and only two studies reported a decrease in patients compared to controls. This distribution is unlikely ($p < .05$, sign test) under the null hypothesis. Moreover, under the null hypothesis, the effect sizes [mean value in schizophrenic group – mean value in control group/standard deviation (SD) in control group] should be distributed around 0. The average effect size of the 16 studies was 0.57 ± 0.78 (SD), and the probability to yield such effect size under the null hypothesis is again lower than .05. The aggregate magnitude of this elevation is thus 57% of the SD of controls. Given an average control SD of 23%, the effect is about 13%. To detect an effect size of 0.50 at the .05 significance level with a power of 80%, a sample of 64 patients and 64 controls would be needed. Clearly, none of the studies included enough patients to detect this small effect with appropriate power. Another observation is that the variability in the patient sample was larger than in the control sample in 13 out of 15 studies, which was also significant ($p < .05$, sign test). The average variance ratio (SD schizophrenics/SD controls) was 1.47 ± 0.58 . The larger variance in patients compared to controls further increases the sample size needed to detect this small group difference with reasonable power.

TABLE 59.1. IMAGING STUDIES OF D₂ RECEPTOR PARAMETERS IN DRUG NAIVE AND DRUG FREE PATIENTS WITH SCHIZOPHRENIA

Class	Radiotracer	Radiotracer	Study	Controls (n)	Patients (n)	Patients (DN/DF)	Method	Outcome	Controls (n Mean ± SD) ^a	Patients (n Mean ± SD) ^a	p	Effect Size ^b	Ratio SD
Butyrophenones	[¹¹ C]NMSP	[¹¹ C]NMSP	Wong et al. (20)	11	15 (10/5)		Kinetic	B _{max}	100 ± 50	253 ± 105	<.05	3.06	2.10
	[⁷⁶ Br]SPI	[⁷⁶ Br]SPI	Crawley et al. (21)	8	16 (12/4)		Ratio	S/C	100 ± 14	111 ± 12	<.05	0.79	0.86
	[⁷⁶ Br]SPI	[⁷⁶ Br]SPI	Blin et al. (201)	8	8 (0/8)		Ratio	S/C	100 ± 14	104 ± 14	NS	0.28	1.00
	[⁷⁶ Br]SPI	[⁷⁶ Br]SPI	Martinot et al. (202)	12	12 (0/12)		Ratio	S/C	100 ± 11	101 ± 15	NS	0.14	1.41
	[¹¹ C]NMSP	[¹¹ C]NMSP	Tune et al. (203)	17	10 (8/2)		Kinetic	B _{max}	100 ± 80	173 ± 143	.08	0.91	1.79
Benzamides	[¹¹ C]NMSP	[¹¹ C]NMSP	Nordstrom et al. (204)	7	7 (7/0)		Kinetic	B _{max}	100 ± 25	133 ± 63	NS	1.33	2.50
	[¹¹ C]Raclopride	[¹¹ C]Raclopride	Farde et al. (205)	20	18 (18/0)		Equilib.	B _{max}	100 ± 29	107 ± 18	NS	0.23	0.63
	[¹¹ C]Raclopride	[¹¹ C]Raclopride	Hietala et al. (206)	10	13 (0/13)		Equilib.	B _{max}	100 ± 22	112 ± 43	NS	0.55	1.99
	[¹²³ I]IBZM	[¹²³ I]IBZM	Pilowsky et al. (207)	20	20 (17/3)		Ratio	S/FC	100 ± 8	99 ± 7	NS	-0.07	0.82
	[¹²³ I]IBZM	[¹²³ I]IBZM	Laruelle et al. (39)	15	15 (1/14)		Equilib.	BP	100 ± 26	115 ± 33	NS	0.56	1.25
	[¹²³ I]IBZM	[¹²³ I]IBZM	Knable et al. (208)	16	21 (1/20)		Equilib.	BP	100 ± 29	97 ± 38	NS	-0.12	1.31
	[¹¹ C]Raclopride	[¹¹ C]Raclopride	Breier et al. (38)	12	11 (6/5)		Equilib.	BP	100 ± 18	100 ± 30	NS	0.02	1.69
	[¹²³ I]IBZM	[¹²³ I]IBZM	Abi-Dargham et al. (40)	15	15 (2/13)		Equilib.	BP	100 ± 20	102 ± 49	NS	0.09	2.50
	[¹²³ I]IBZM	[¹²³ I]IBZM	Abi-Dargham et al. (45)	18	18 (8/10)		Equilib.	BP	100 ± 13	104 ± 14	NS	0.33	1.11
	Ergot alk.	[⁷⁶ Br]Lisuride	[⁷⁶ Br]Lisuride	Martinot et al. (209)	14	19 (10/9)		Ratio	S/C	100 ± 10	104 ± 12	NS	0.45
[⁷⁶ Br]Lisuride		[⁷⁶ Br]Lisuride	Martinot et al. (210)	10	10 (2/8)		Ratio	S/C	100 ± 10	100 ± 13	NS	0.00	1.29

^a Mean normalized to mean of control subjects.

^b Effect size calculated as (mean patients – mean controls) / SD controls.

BP, binding potential; DF, drug free; DN, drug naive; IBZM, iodobenzamide; NMSP, N-methyl-spiperone; NS, not significant.

No clinical correlates of increased D2 receptor binding parameters has been reliably identified. Thus, the simplest conclusions from these studies are that patients with schizophrenia show a modest elevation in D2 receptor density parameters of undetermined clinical significance, that all studies were underpowered, and that positive results occasionally reported (20,21) are due to a sampling effect. These conclusions are reached under the assumptions that all studies measured parameters from the “same” D2 receptors population.

However, there is another way to look at these data. Studies performed with butyrophenones ($n = 6$) have an effect size of 1.08 ± 1.06 ($n = 6$), whereas studies performed with other ligands (benzamides and lisuride, $n = 10$) have an effect size of 0.20 ± 0.26 , a difference that is significant ($p = .022$). This observation suggests that schizophrenia might be associated with an increase in butyrophenone binding and no change in benzamide or lisuride binding.

Unfortunately, no studies have been reported in which the same subjects were scanned with both ligands. Such a study is warranted to directly test this view. Nevertheless, several hypotheses have been advanced to account for the existence of a differential increase in [^{11}C]NMSP binding *in vivo* in patient with schizophrenia in the face of normal benzamide binding. Because [^{11}C]raclopride and [^{123}I]IBZM binds to D2 and D3 receptors whereas [^{11}C]NMSP binds to D2, D3, and D4 receptors, this difference could reflect a selective elevation of D4 receptors in schizophrenia (22). This hypothesis has not been substantiated. The density of D4 receptors is negligible in the striatum, and, when measured with a specific ligand, not different in postmortem striatal samples from patients with schizophrenia and controls (23). Another hypothesis derives from the observation that D2 receptors, like several G-protein-coupled receptors, exist in monomers, dimers, and other oligomeric forms (24–27). Photoaffinity labeling experiments suggested that butyrophenones detect only monomers, whereas benzamides detect both monomers and dimers. Thus, increased butyrophenone binding and normal benzamide binding might reflect a higher monomer/dimer ratio in schizophrenia. This interesting hypothesis warrants further exploration. A third proposition evolved around the idea that the binding of these ligands would display different vulnerability to competition by endogenous DA (28,29). This proposition was based on two assumptions: (a) the concentration of DA in the proximity of D2 receptors might be higher in patients compared to controls, and (b) [^{11}C]NMSP might be less affected than [^{11}C]raclopride or [^{123}I]IBZM binding by endogenous DA competition. It follows that D2 receptor density measured *in vivo* with [^{11}C]raclopride and [^{123}I]IBZM would be “underestimated” to a greater extent in patients with schizophrenia than in control subjects. This hypothesis played

an important role in bringing the endogenous competition concept to the attention of the imaging field.

Striatal DA Presynaptic Function

Imaging studies of presynaptic DA function in schizophrenia included measurements of dihydroxyphenylalanine (DOPA) decarboxylase activity, DA release at baseline and following pharmacologic challenges with amphetamine, and DA transporters (DAT). These studies are summarized in Table 59.2.

DOPA Decarboxylase Activity

Five studies reported rates of DOPA decarboxylase in patients with schizophrenia, using [^{18}F]DOPA (30–33) or [^{11}C]DOPA (34) (Table 59.2). Four out of five studies reported increased accumulation of DOPA in the striatum of patients with schizophrenia, and the combined analysis yielded an effect size of 0.92 ± 0.45 , which is significantly different from zero ($p = .01$). The variability of the DOPA accumulation was larger in the schizophrenic group compared to the control group. Several of these studies reported the observation of high DOPA accumulation in psychotic paranoid patients, and low accumulation in patients with negative or depressive symptoms and catatonia. Although the relationship between DOPA decarboxylase and DA synthesis rate is unclear (DOPA decarboxylase is not the rate-limiting step of DA synthesis), these observations are compatible with higher DA synthesis activity of DA neurons in schizophrenia, at least in subjects experiencing psychotic symptoms.

Amphetamine-Induced DA Release

As discussed above, endogenous DA competition is a source of errors for *in vivo* measurement of D2 receptors. On the other hand, the recognition of this phenomenon implies that D2 receptor imaging, combined with pharmacologic manipulation of DA release, could provide a functional evaluation of DA presynaptic activity. Indeed, over the last decade, numerous groups demonstrated that acute increase in synaptic DA concentration is associated with decreased *in vivo* binding of [^{11}C]raclopride and [^{123}I]IBZM. These interactions have been demonstrated in rodents, nonhuman primates, and humans, using a variety of methods to increase synaptic DA [amphetamine, DAT blockers, levodopa (L-DOPA), nicotine agonists, serotonin receptor subtype 2A (5-HT_{2A}) antagonists, direct electrical stimulation of DA neurons] (see ref. 35 for review of this abundant literature). It has also been consistently observed that the *in vivo* binding of spiperone and other butyrophenones is not as affected as the binding of benzamides by acute fluctuations in endogenous DA levels (35).

The decrease in [^{11}C]raclopride and [^{123}I]IBZM *in vivo*

TABLE 59.2. IMAGING STUDIES OF PRESYNAPTIC DA PARAMETERS IN DRUG NAIVE AND DRUG FREE PATIENTS WITH SCHIZOPHRENIA

Parameter	Study	Controls (n)	Patients (n) (DN/DF)	Radiotracer (/Challenge)	Method	Outcome	Controls (n Mean ± SD) ^a	Patients (n Mean ± SD) ^a	p	Effect Size ^b	Ratio SD
DOPA decarboxylase activity	Reith et al. (30)	13	5 (4/1)	[¹⁸ F]DOPA	Kinetic	k ₃	100 ± 23	120 ± 15	<.05	0.91	0.68
	Hietala et al. (31)	7	7 (7/0)	[¹⁸ F]DOPA	Graphical	K _i	100 ± 11	117 ± 20	<.05	1.54	1.82
	Dao-Castellana et al. (32)	7	6 (2/4)	[¹⁸ F]DOPA	Graphical	K _i	100 ± 11	103 ± 40	NS	0.30	3.80
	Lindstrom et al. (34)	10	12 (10/2)	[¹¹ C]DOPA	Graphical	K _i	100 ± 17	113 ± 12	<.05	0.77	0.70
Amphetamine-induced DA release	Hietala et al. (33)	13	10 (10/0)	[¹⁸ F]DOPA	Graphical	K _i	100 ± 14	115 ± 28	<.05	1.09	1.25
	Laruelle et al. (39)	15	15 (2/13)	[¹²³ I]BZM/ amphetamine	Equilibrium	Delta BP	100 ± 113	271 ± 221	<.05	1.51	1.95
	Breier et al. (38)	18	18 (8/10)	[¹¹ C]raclopride/ amphetamine	Equilibrium	Delta BP	100 ± 43	175 ± 82	<.05	1.73	1.90
Baseline DA concentration	Abi-Dargham et al. (40)	16	21 (1/20)	[¹²³ I]BZM/ amphetamine	Equilibrium	Delta BP	100 ± 88	194 ± 145	<.05	1.07	1.64
	Abi-Dargham et al. (45)	18	18 (8/10)	[¹²³ I]BZM/ α-MPT	Equilibrium	Delta BP	100 ± 78	211 ± 122	<.05	1.43	1.57
DAT density	Laakso et al. (211)	9	9 (9/0)	[¹⁸ F]CFT	Ratio	S/C	100 ± 12	101 ± 13	<.05	0.11	1.06
	Laruelle et al. (46)	22	22 (2/20)	[¹²³ I]CIT	Equilibrium	BP	100 ± 17	93 ± 20	<.05	-0.43	1.21

^a Mean normalized to mean of control subjects.

^b Effect size calculated as (mean patients – mean controls) / SD controls.

DAT, dopamine transporter; DF, drug free; DN, drug naive; DOPA, dihydroxyphenylalanine; MPT, methyl-para-tyrosine.

binding following acute amphetamine challenge has been well validated as a measure of the change in D2 receptor stimulation by DA due to amphetamine-induced DA release. Manipulations that are known to inhibit amphetamine-induced DA release, such as pretreatment with the DA synthesis inhibitor α -methyl-para-tyrosine (α -MPT) or with the DAT blocker GR12909 also inhibit the amphetamine-induced decrease in [123 I]IBZM or [11 C]raclopride binding (36,37). These experiments support the assumption that the amphetamine effect on [11 C]raclopride and [123 I]IBZM binding is mediated by DA release. Combined microdialysis and imaging experiments in primates demonstrated that the magnitude of the decrease in ligand binding was correlated with the magnitude of the increase in extracellular DA induced by the challenge (37,38), suggesting that this noninvasive technique provides an appropriate measure of the changes in synaptic DA levels.

Three out of three studies demonstrated that amphetamine-induced decrease in [11 C]raclopride or [123 I]IBZM binding was elevated in untreated patients with schizophrenia compared to well-matched controls (38–40). A significant relationship was observed between magnitude of DA release and transient induction or worsening of positive symptoms. The increased amphetamine-induced DA release was observed in both male and female patients, and in both first-episode/drug-naive patients and patients previously treated by antipsychotic drugs (41). Combined analysis of the results of two studies revealed that patients who were experiencing an episode of illness exacerbation (or a first episode of illness) at the time of the scan showed elevated amphetamine-induced DA release, whereas patients in remission showed DA release values not different from those of controls (41). This exaggerated response of the DA system to amphetamine exposure did not appear to be a nonspecific effect of stress, as higher self-reports of anxiety before the experiments were not associated with larger effect of amphetamine. Furthermore, nonpsychotic subjects with unipolar depression, who reported levels of anxiety similar to, if not higher than, the schizophrenic patients at the time of the scan, showed normal amphetamine-induced displacement of [123 I]IBZM (42).

These findings were generally interpreted as reflecting a larger DA release following amphetamine in the schizophrenic group. Another interpretation of these observations would be that schizophrenia is associated with increased affinity of D2 receptors for DA. Development of D2 receptors imaging with radiolabeled agonists is needed to settle this issue (42). Another limitation of this paradigm is that it measures changes in synaptic DA transmission following a nonphysiologic challenge (i.e., amphetamine) and do not provide any information about synaptic DA levels at baseline, i.e., in the unchallenged state.

Baseline DA Release

Several laboratories reported that, in rodents, acute depletion of synaptic DA is associated with an acute increase in the *in vivo* binding of [11 C]raclopride or [123 I]IBZM to D2 receptors (see ref. 35 for review). The increased binding was observed *in vivo* but not *in vitro*, indicating that it was not due to receptor up-regulation (43), but to removal of endogenous DA and unmasking of D2 receptors previously occupied by DA. The acute DA depletion technique was developed in humans using α -MPT to assess the degree of occupancy of D2 receptors by DA (43,44). Using this technique, higher occupancy of D2 receptor by DA was recently reported in patients with schizophrenia experiencing an episode of illness exacerbation, compared to healthy controls (45). Again assuming normal affinity of D2 receptors for DA, the data are consistent with higher DA synaptic levels in patients with schizophrenia. This observation was present in both first-episode/drug-naive and previously treated patients.

Following DA depletion, higher D2 receptor availability was observed in patients with schizophrenia compared to controls. This observation supported the proposition that, in schizophrenia, elevated D2 receptor density might be masked by DA occupancy when imaging studies are performed with ligands vulnerable to endogenous competition (28). However, the increase in D2 receptors measured with [123 I]IBZM in DA-depleted patients was moderate (12%), suggesting that other factors than vulnerability to endogenous DA competition are involved in the butyrophenone-benzamides binding differences discussed above.

Interestingly, higher occupancy of D2 receptors by DA in patients with schizophrenia was not associated with the intensity of positive symptoms, but was predictive of good therapeutic response of these symptoms following 6 weeks of treatment with atypical antipsychotic medications. The fact that high levels of synaptic DA at baseline predicted better or faster response to atypical antipsychotic drugs suggested that the D2 receptor blockade induced by these drugs remains a key component of their initial mode of action.

DA Transporters

The data reviewed above are consistent with higher DA output in the striatum of patients with schizophrenia, which could be explained by increased density of DA terminals. Because striatal DATs are exclusively localized on DA terminals, this question was investigated by measuring binding of [123 I]-CIT (46) or [18F]CFT (48) in patients with schizophrenia. Both studies reported no differences in DAT binding between patients and controls. In addition, Laruelle et al. (46) reported no association between amphetamine-induced DA release and DAT density. Thus, the increased presynaptic output suggested by the studies reviewed above

does not appear to be due to higher terminal density, an observation consistent with postmortem studies that failed to identify alteration in striatal DAT binding in schizophrenia (47–52).

Subcortical DA Dysregulation as a Failure of Inhibitory Pathways

Although the studies reviewed above generally confirmed the classic DA hypothesis of schizophrenia, it is important to examine these results in light of the more recent views of schizophrenia as a neurodevelopmental illness, involving dysconnectivity of multiple cortico-subcortical and intracortical networks. Although it cannot be definitively ruled out that the DA dysregulation revealed by these studies would stem from a primary abnormality of DA neurons, it seems more likely that these abnormalities are a consequence of cortico-subcortical dysconnectivity. Moreover, given the weight of evidence implicating PFC connectivity as a central deficient node in the schizophrenic brain, it is tempting to speculate that dysregulation of DA neurons' firing activity might stem from a failure of the PFC to regulate this process. In fact, it has long been hypothesized that dysregulation of subcortical DA function in schizophrenia may be secondary to a failure of the PFC to adequately control subcortical dopaminergic function (53,54).

Activity of midbrain DA neurons is under dual influence of PFC via an activating pathway (the “accelerator”) and an inhibitory pathway (“the brake”), allowing fine-tuning of dopaminergic activity by the PFC (55). The activating pathway is provided by direct glutamatergic projections onto the dopaminergic cells. The inhibitory pathway is provided by glutamatergic projections to midbrain γ -aminobutyric acid (GABA)ergic interneurons or striatomesencephalic GABA neurons. The inhibition of dopaminergic cell firing following amphetamine is an important feedback mechanism by which the brain reduces the effect of amphetamine on DA release. The inhibition of dopaminergic cell firing induced by amphetamine is mediated both by stimulation of presynaptic D2 autoreceptors and by stimulation of this inhibitory pathway (56). Following administration of amphetamine (i.e., under conditions in which the inhibitory pathway should be activated), *N*-methyl-D-aspartate (NMDA) receptor blockade results in a failure of activation of the inhibitory pathway, resulting in exaggerated amphetamine-induced DA release (57). Kegeles et al. (58) recently confirmed this mechanism in humans: ketamine pretreatment significantly enhanced amphetamine-induced decrease in [123 I]IBZM BP, from $-5.5 \pm 3.5\%$ under control conditions to $-12.8 \pm 8.8\%$ under ketamine pretreatment ($p = .023$). The increase in amphetamine-induced DA release induced by ketamine (greater than two-fold) was comparable in magnitude to the exaggerated response seen in patients with schizophrenia. These data are

consistent with the hypothesis that the alteration of DA release revealed by the amphetamine challenge in schizophrenia results from a disruption of glutamatergic neuronal systems regulating dopaminergic cell activity and are consistent with the hypothesis that schizophrenia might be associated with NMDA receptor hypofunction (59–61).

The failure of glutamatergic control of DA release might stem from mechanisms other than NMDA hypofunction. For example, glutamatergic projections from the PFC to the ventral tegmental area (VTA) are under tonic inhibition by prefrontal GABA and DA activity (see ref. 62 and references therein). It follows that deficits in GABAergic or dopaminergic function in the PFC (both deficits also implicated in schizophrenia) are expected to have similar consequences to an NMDA deficiency on the subcortical DA response to amphetamine. Thus, in patients with schizophrenia, various or multiple mechanisms (NMDA receptor hypofunction, GABAergic or dopaminergic deficits in the PFC) may lead to the dysregulation of subcortical DA revealed by the amphetamine challenge.

As reviewed below, direct evidence has been provided that disinhibition of subcortical DA activity is associated with prefrontal pathology in schizophrenia. In patients with schizophrenia, low *N*-acetylaspartate (NAA) concentration in the dorsolateral prefrontal cortex (DLPFC), a marker of DLPFC pathology, is associated with increased amphetamine-induced DA release (63). Studies in primates have documented the consequences of neurodevelopmental alteration in PFC connectivity on subcortical DA release (64, 65). Adult rhesus monkeys with neonatal ablation of the amygdala-hippocampal formation within 3 weeks of birth exhibit lower NAA concentration in the PFC and abnormal relationships between prefrontal and subcortical DA functions; whereas local perfusion of amphetamine into the PFC induced a decrease in striatal DA in control monkeys and in monkeys with adult lesions, PFC amphetamine perfusion increased striatal DA release in monkeys with neonatal lesions. This study documents that dysregulation of subcortical DA function might be a delayed and enduring consequence of neurodevelopmental abnormalities of PFC connectivity.

Positive Symptoms and Neuroplasticity

The fluctuating nature of the DA abnormalities documented by the imaging studies reviewed above implies that some level of neuroplasticity is involved in the emergence of this increased subcortical DA activity during episodes of illness exacerbation (66). Neurochemical sensitization of mesolimbic DA systems has been proposed by several authors as one mechanism that might underlie the progression of a “silent” vulnerability into an overt symptomatology (67–71). Sensitization of DA systems is a positive feedback loop, in which increased DA activity leads to more DA

activity (70,72). During late adolescence, the failure of cortical development associated with schizophrenia liability might limit the brain capacity to modulate stress-related increased activity of mesolimbic DA neurons. This failure of normal homeostatic and buffering mechanisms would result in an increased vulnerability of DA neurons to develop a process of endogenous sensitization, a response not observed in humans under normal circumstances. The endogenous sensitization process drives the prodromal and initial phases of the illness, characterized by increased DA activity and culminating in the expression of positive symptoms. Sustained D2 receptor blockade interrupts this positive feedback loop. Upon neuroleptic discontinuation, the brain becomes again vulnerable to the stress-induced re-emergence of this endogenous sensitization process and clinical relapse.

It should be emphasized that the relationship between stimulation of D2 receptors and psychotic symptoms is complex and presumably also involves neuroplasticity. NMDA antagonists such as ketamine or 5-HT_{2A} agonists such as lysergic acid diethylamide (LSD) induce psychotic symptoms in healthy subjects immediately upon drug exposure. In contrast, sustained administration of DA agonists is required to induce psychotic symptoms in healthy subjects (7,73). This observation suggests that sustained overstimulation of D2 receptors leads to remodeling of prefrontal-ventrostriatal-thalamic-prefrontal loops and their modulation by hippocampal afferents projections, neuronal ensembles that are believed to underlie the psychotic experience (74,75). In the amphetamine studies, DA-mediated stimulation of D2 receptors explained only about 30% of the variance in the positive symptom change in untreated patients with schizophrenia (41), indicating that factors downstream from the DA synapse play a role in the exacerbation of these symptoms following amphetamine. In the α -MPT study, global severity of positive symptoms did not correlate with occupancy of striatal D2 receptors by DA (45), suggesting that, in some patients, the experience of positive symptoms is no longer (or has never been) dependent on DA overstimulation. Patients with psychotic symptoms in the presence of apparently normal DA function failed to show significant improvement in these symptoms following 6 weeks of D2 receptor blockade (45). Thus, although these imaging studies have generally confirmed the time-honored dopamine hypothesis of schizophrenia, they also contributed to pointing out the limitations of an oversimplified model linking psychosis and excess DA activity.

Prefrontal DA D1 Receptor Density

As discussed above, several lines of evidence from preclinical, clinical, and postmortem studies converge to suggest that a deficiency in DA transmission in the prefrontal cortex is involved in the pathophysiology of negative symptoms and cognitive impairment in schizophrenia (14,16). Fur-

thermore, a deficient prefrontal DA function is a potential mechanism to account for the subcortical DA disinhibition discussed above, as cortical DA function has an inhibitory impact on subcortical DA function (18,19). *In vivo* measurement of prefrontal DA function would provide the tools to directly test these hypothesis.

The majority of DA receptors in the PFC are of the D1 subtype (76,77). At the ultrastructural level, they are mostly located on pyramidal spines, and are mostly abundant on the distal dendrites (78–80). In postmortem studies, no evidence was found of an alteration in D1 receptors in the DLPFC of patients with schizophrenia (81,82), and the expression of the D1 receptor gene is unaltered (83). In contrast, a PET study with [¹¹C]SCH 23390 reported decreased density of D1 receptors in younger patients with schizophrenia (84). No significant differences were found in the other regions examined (anterior cingulate, temporal, occipital, and striatum). In addition, low-PFC D1 density was associated with the severity of negative symptoms and poor performance on the Wisconsin Card Sort Test (WCST). This finding is important, because it represents the first direct evidence of an association between negative symptoms, working memory deficits, and selective alteration in prefrontal DA function. However, the camera used in this study had a limited resolution, and the low specific to nonspecific ratio of [¹¹C]SCH3390 makes the measurement of D1 receptor in PFC with this ligand quite vulnerable to noise (85). Several groups are currently attempting to replicate this finding, using better cameras and a superior D1 receptor radiotracer, [¹¹C]NNC 112 (86).

In addition, measurement of receptor availability reveals only one aspect of neurotransmission. As D1 receptors are the most abundant DA receptors in the PFC, the availability of a D1 receptor radiotracer vulnerable to competition by endogenous DA (i.e., a D1 receptor “[¹¹C]raclopride”) would be invaluable to assess presynaptic DA function in the PFC. Unfortunately, such a ligand is currently lacking (87,88).

Studies of Nondopaminergic Receptors in Schizophrenia

Receptors related to the GABA and 5-HT systems have been studied *in vivo* in schizophrenia. Postmortem studies reported abnormalities of both systems in schizophrenia. A robust body of findings suggests deficiency of GABAergic function in the PFC in schizophrenia (see refs. 89 and 90 for reviews). *In vivo* evaluation of GABAergic systems in schizophrenia has so far been limited to evaluation of benzodiazepine receptor densities with SPECT and [¹²³I]iomazenil, and three out of three studies comparing patients with schizophrenia and controls reported no significant regional differences (91–93). Although some significant correlations with symptoms clusters and regional benzodiazepine densities have been observed (91,92,94,95), these relationships

have not been replicated by other studies. Thus, together, these studies are consistent with an absence of marked abnormalities of benzodiazepine receptor concentration in the cortex and patients with schizophrenia. Alterations of GABAergic systems in schizophrenia might not involve benzodiazepine receptors (96), or be restricted to certain cortical layers or classes of GABAergic cells that are beyond the resolution of current radionuclides based imaging techniques. Recent developments in GABA imaging with MRS (described below) are a promising new avenue to study *in vivo* GABAergic function in schizophrenia.

Abnormalities of 5-HT transporters (SERT), 5-HT_{2A} receptors and, more consistently, 5-HT_{1A} receptors have been described in postmortem studies in schizophrenia (see references in ref. 97). Given the relatively recent development of radiotracers to study 5-HT receptors, only a limited number of imaging studies have been published. The concentration of SERT in the midbrain measured by [¹²³I]β-CIT is unaltered in patients with schizophrenia (46). Studies with more specific ligands are warranted to assess the distribution of SERT in other brain areas, such as the PFC, where their density has been reported to be reduced in three out of four postmortem studies (97). Decrease in 5-HT_{2A} receptors has been reported in the PFC in four out of eight postmortem studies (97,98). Three PET studies in drug-naïve or drug-free patients with schizophrenia reported normal cortical 5-HT_{2A} receptor binding (98–100), whereas one study reported a significant decrease in PFC 5-HT_{2A} binding in a small group ($n = 6$) of drug-naïve schizophrenic patients (101). The most consistent abnormality of 5-HT parameters reported in postmortem studies in schizophrenia is an increase in the density of 5-HT_{1A} receptors in the PFC, reported in seven out of eight studies (97). Several groups are currently evaluating the binding of this receptor *in vivo* with PET and [¹¹C]WAY100907.

Receptor Occupancy By Antipsychotic Drugs

Maybe the most widespread use of neuroreceptor imaging in schizophrenia over the last decade has been the assessment of neuroreceptor occupancy achieved by typical and atypical antipsychotic drugs, a topic that has been the subject of recent reviews (102,103). Neuroreceptor studied included essentially D2 receptors, but also 5-HT_{2A} and D1 receptors. The main conclusions from this line of research are as follows: (a) Studies repeatedly confirmed the existence of a threshold of occupancy of striatal D2 receptors (about 80%) above which extrapyramidal side effects are likely to occur (104). (b) In general, studies failed to observe a relationship between degree of D2 receptor occupancy and clinical response (105,106). Yet, most studies were performed at doses achieving more than 50% occupancy, and the minimal level of occupancy required for therapeutic response remains undefined. Two studies performed with low doses of relatively

selective D2 receptor antagonists (haloperidol and raclopride) suggested that 50% to 60% occupancy was required to observe a rapid clinical response (107,108). (c) Clozapine, at clinically therapeutic doses, achieved only 40% to 60% D2 receptor occupancy (104,106,109), which, in conjunction with its anticholinergic properties, accounts for its low liability for extrapyramidal symptoms (EPSs). (d) Occupancy of 5-HT_{2A} receptors by “5-HT_{2A}/D2 balanced antagonists” such as risperidone does not confer protection against EPS, because the threshold of D2 receptor occupancy associated with EPS is not markedly different between these drugs and drugs devoid of 5-HT_{2A} antagonism (110–113). (e) Studies with quetiapine suggested that, at least with this agent, transient high occupancy of D2 receptors might be sufficient to elicit clinical response (114,115).

An interesting question relates to putative differences in degree of occupancy achieved by atypical antipsychotic drugs in striatal and extrastriatal areas. Pilowsky et al. (116) reported lower occupancy of striatal D2 receptors compared to temporal cortex D2 receptors in seven patients treated with clozapine, using the high-affinity SPECT ligand [¹²³I]epidipride. In contrast, typical antipsychotics were reported to achieve similar occupancy in striatal and extrastriatal areas, as measured with [¹¹C]FLB 457 (117) or [¹²³I]epidipride (118). It should be noted, however, that these very high affinity ligands do not allow accurate determination of D2 receptor availability in the striatum. In contrast, [¹⁸F]fallypride enables accurate determination of D2 receptor availability in both striatal and extrastriatal areas (119), and preliminary PET experiments in primates with [¹⁸F]fallypride indicate that clozapine and risperidone achieve similar D2 receptor occupancy in striatal and extrastriatal regions (120). Finally, it is important to point out that the most robust evidence relative to the site of therapeutic effect of antipsychotic drugs in rodents points toward the nucleus accumbens (121,122), whereas the imaging studies reviewed above contrasted striatal versus mesotemporal D2 receptor binding. Improved resolution of PET cameras currently allows dissociating signals from ventral and dorsal striatum (123,124), and it is now feasible to specifically study the clinical correlates of D2 receptor occupancy in ventral striatum in humans.

Another unresolved question is the discrepant values of D2 receptor occupancy obtained with [¹¹C]raclopride versus [¹¹C]NMSP. The haloperidol plasma concentration associated with 50% inhibition of [¹¹C]NMSP binding (3 to 5 mg/mL) (125) is ten times higher than that associated with 50% inhibition of [¹¹C]raclopride binding (0.32 ng/mL) (126). Quetiapine, at a dose of 750 mg, decreased [¹¹C]raclopride-specific binding by 51%, but failed to affect [¹¹C]NMSP-specific binding (127). These observations contribute to the debate regarding differences between benzamides and butyrophenones binding to D2 receptors.

Future Developments

Despite the remarkable achievements of the last decade, imaging neurosignaling processes with PET and SPECT in schizophrenia are still limited by the relative low number of probes available. For example, despite major research efforts, direct measurement of parameters of glutamate transmission are still not available. Radiotracers enabling evaluation of second messengers and intracellular pathways are only beginning to emerge (128). A growing collaboration between academic centers and industry currently holds the promise of increasing access to molecules for evaluation as candidate radiotracers.

Studies of the DA systems in schizophrenia illustrates how dynamic measurement of neurotransmission can be more informative than simple measurement of receptor density. With the exception of the cholinergic system (129, 130), the paradigm used with [^{11}C]raclopride and [^{123}I]IBZM has been difficult to extend to other neuroreceptor systems, maybe because the fundamental mechanisms underlying acute change in *in vivo* binding following transmitter fluctuations are still not perfectly understood (66). Additional research is warranted to better characterize the factors that confer vulnerability of radiotracers *in vivo* binding to functional status of neurotransmission.

Finally, a general limitation of these radionuclide-based techniques is the technical sophistication required as well as the high cost of these investigations. However, the growing success of PET in oncology results in a larger availability of PET cameras for neuropsychiatric clinical and basic research. This growing availability should be associated with a vigorous research effort toward the development of more F-18 based probes, since the relatively longer half-life of F-18 compared to C-11 does not require that these ligands be radiolabeled locally. For SPECT, the development of technetium-based neuroreceptor ligands (131) will further enhance the availability of these techniques to the nuclear medicine community.

MAGNETIC RESONANCE SPECTROSCOPY

Magnetic resonance spectroscopy (MRS) is a chemical assay technique. It is the only clinically available method for the direct measurement of chemical moieties in the living brain. MRS is based on the same physical principles as magnetic resonance imaging (MRI), which involves characterizing atoms and molecules based on how they interact with a magnetic field. This interaction occurs because the nuclei of atoms with an odd number of nucleons (i.e., protons and neutrons), such as ^1H , ^{19}F , ^{13}C , ^7Li , ^{23}Na , have angular momentum, so-called spin, which generates a small magnetic field around the nucleus. The spin properties of specific atoms and of the specific molecules that they compose are unique and are exploited in an MRS experiment.

When a strong external magnetic field is applied to a tissue (e.g., the main magnet of an MR scanner), nuclei with spin align themselves with the external field and assume an equilibrium state of net magnetization, which is proportional to the strength of the field and the spin properties of the nucleus. An MRS experiment involves four steps, analogous to an MRI procedure. First, specific nuclei are excited with a brief "pulse" of a radiofrequency (RF) magnetic field supplied by an RF transmitter coil. This excitation causes magnetized spins to transiently assume a higher energy state, from which they "relax" to a lower energy state of equilibrium magnetization. Because the energy states are quantized, only specific RFs will excite the nucleus to another state and only these frequencies will be emitted during relaxation ("resonance"). These resonant frequencies are unique for each atom, and vary in proportion to the strength of the external field. The motion of spins in the process of returning to equilibrium ("relaxation") induces a current in a receiver coil, which represents the MR signal.

The second step involves spatially encoding the signal so that its origin can be mapped to a particular locale in the field of view. This is accomplished with the application of linear magnetic gradients that add localizing characteristics to the signal. The third step involves translating the signal acquired over time into a representation of its component frequencies and amplitudes, the so-called Fourier transformation. The fourth step involves the mathematical and statistical analyses of the data.

In MRI, the signal used to reconstruct images is from hydrogen atoms (^1H), which are found in many molecules, but most abundantly in water and lipids. Although the signal from hydrogen contains frequencies corresponding to many different molecules, the water and lipids signals dominate and the signals from hydrogen in other molecules are ignored. MRS, however, is based on resolving these other molecular signals. These molecules are identifiable because of the phenomenon called "chemical shift." The electron cloud that surrounds the nucleus of an atom partially shields the nucleus from the external magnetic field. This shielding effect will cause the nucleus to experience a slightly different external field, and thus to resonate at a slightly shifted frequency. Because the degree of shielding varies from one molecule to another, depending on the electron sharing of the chemical bonds in the molecule, the exact shift in the resonant frequency of a target nucleus (e.g., ^1H , ^{31}P) will reflect its chemical environment. In addition to electron shielding, complex interactions between neighboring spins ("j-coupling") also may affect the resonant frequency of a nucleus in certain molecules. A Fourier transformation of the emitted signal resolves the various components of the signal into a spectrum having peaks of specific frequency and amplitude, each peak corresponding to a different chemical environment of the target nucleus. The area under the peak is directly proportional to the concentration of spins having that frequency, and thus of that specific chemi-

cal moiety. It is customary to label peaks in the spectrum based on their relative displacement (“chemical shift”) in parts per million from a standard peak (e.g., in the proton spectra, water is assigned a value of 4.75), with higher frequencies to the left. The use of relative shift units allows for peaks to be identified regardless of the strength of the magnet. This also makes it possible to compare *in vivo* and *in vitro* data to establish the chemical identity of each peak.

In schizophrenia research, MRS has been used to approach four general topics: (a) assays of brain chemicals, (b) evidence of tissue pathology, (c) functional analysis of specific neuronal populations, and (d) drug effects. Before considering the results of these efforts, it is important to consider some general methodologic issues that are germane to the MRS literature.

MRS Methodology

MRS is a methodologically complex technique that requires many quality control steps, both at the front end in data collection and at the back end in data analysis. It is not a turnkey technique, and careful scrutiny of the raw data is essential in every experiment. There are no absolute standards for the quality of spectral data, e.g., for the sharpness of a peak, or the degree of noise in the baseline. Nevertheless, spectra derived from a particular atom (e.g., ^1H or ^{19}F) have a characteristic pattern, with sharp peaks at defined relative frequencies, appropriate separation of peaks, acceptable signal to noise, and a stable baseline. Because the goal of MRS is to assay the concentrations of chemicals that are in the range of three to five orders of magnitude less abundant than water, it is necessary to make compromises to maximize the signal-to-noise ratio (SNR) in the data. One of the first decisions to be made in an MRS experiment is how to sample from the brain. Most MRS studies in the schizophrenia literature have sampled from relatively large (5 to 80 mL) single volume elements (“voxels”), though so-called MRS imaging (MRSI) approaches have also been utilized. The single-voxel approach benefits from enhanced signal-to-noise (STN) and reduced scanner time (5 to 20 minutes per voxel), but it suffers from imprecise and limited anatomic localization, contamination from signal outside of the voxel, and, most importantly, partial volume averaging. Large voxels contain multiple tissue compartments, including cerebrospinal fluid (CSF), white matter, gray matter, and vascular tissue, which have different concentrations of chemical constituents. Thus, the signals from a voxel are the average of signals from all of these compartments. Although there are statistical procedures aimed at “segmenting” these compartments within a voxel, such procedures are rough approximations (132). One of the fundamental difficulties in comparing data from single-voxel studies between subject groups and across studies in the literature is uncertainty about the comparability of voxel characteristics. ^1H -MRSI studies generally employ smaller voxels (<1.5 mL) and have

the advantage of being more anatomically precise. This means that regions based on known anatomy, rather than arbitrary voxel placement, can be compared. There also are less partial volume effects. However, because of the smaller voxels, imaging studies have reduced SNR and require longer scanner times (20 to 60 minutes for a multislice study).

Because the amplitudes and frequencies of peaks in a spectrum are critically dependent on the magnetic field surrounding a target nucleus, magnetic field uniformity within a voxel and between voxels is essential. Field homogeneity is routinely honed with “shimming” procedures. It is also possible to make additional corrections based on field mapping, which corrects for frequency shifts between voxels. Most studies in the literature have used shim procedures supplied by the manufacturer of the scanner, and it is doubtful that these procedures have been optimal and comparable across studies (133). Coil design is another important parameter in data acquisition and STN. Small surface coils have been used in many of the phosphorus spectroscopy studies, because they improve SNR. However, surface coils introduce potential variance in studies that compare signals from specific locales across individuals. This is because as transmitters, surface coils excite spins with a gradient of intensity from the surface, and again as a receiver, the sensitivity drops off across the volume of activated tissue. Clearly, differences in coil placement and in head geometry may contribute to variations in the data.

There has been much discussion in the MRS literature about absolute quantification of chemical concentrations. In practice, this is almost impossible to do. References in the schizophrenia MRS literature to studies that have employed absolute quantification are inaccurate, as all studies have employed some normalization procedure, often based on measures acquired in normal subjects. To accurately measure absolute concentration of a chemical peak, it is necessary to control for all the variables that will affect the amplitude of the peak from a given voxel. These include factors that affect field homogeneity, relaxation times of the molecule(s) responsible for the peak, transmission efficiency and reception sensitivity of the RF coil (“coil loading”), and partial volume effects. These characteristics vary across the brain within an individual and between individuals even in the same locale in the brain. Moreover, any attempt to control for these factors assumes that the voxel remains in place, i.e., that the subject does not move during the scan, which is difficult for many individuals. Thus, even with the help of absolute standards for calibration, it is very difficult to correct for differences in these parameters (134). All studies to date have employed some normalization routine, whether to noise in the baseline, to the total observed signal, to another metabolite in the spectra, or to a signal from a molecule in the spectra that is thought to be metabolically neutral (e.g., water in the ^1H spectra). Although each of these normalization procedures involves assumptions and trade-offs, they attempt to control for unavoidable varia-

tions in the local field, in coil loading, in relaxation times, and, to a lesser extent, for partial volume averaging effects. A further discussion of technical issues involved in collecting and analyzing MRS data is available in several reviews of the topic (135–137).

³¹P Spectroscopy: Assays Of Phospholipids And High Energy Phosphates

The first *in vivo* MRS study of schizophrenia was of ³¹P spectra. At clinical MR field strength (1.5 to 4.0 tesla), the ³¹P spectrum contains several peaks across a wide chemical shift range [approximately 30 parts per million (ppm)]. The major metabolite peaks represent resonances for (a) phosphomonoester (PME) compounds (at 6.5 ppm), which includes phosphocreatine, phosphorylethanolamine; (b) phosphodiester (PDE, 2.6 ppm), which include glycerolphosphocholine, glycerolphosphoethanolamine, and various membrane phospholipids; (c) phosphate residues on adenosine triphosphate (ATP) (−16.3 ppm for beta, −7.8 for alpha, and −2.7 for gamma); (d) inorganic phosphate at 4.9 ppm; and (e) phosphocreatine, which, as the chemical shift standard, has a resonance set at 0 ppm. PME concentrations, which are thought to reflect in part membrane precursors, increase with tissue growth, including in early brain development and gliomas, but also with tissue destruction, e.g., in Alzheimer's disease and HIV. In white matter, however, PMEs are metabolites of both synthesis and breakdown of sphingomyelin. PDEs are thought to reflect in part products of membrane breakdown and have been shown to be decreased in certain brain tumors and to increase shortly after birth. However, both PME and PDE peaks include other phosphorylated proteins associated with cell organelles and with membrane phospholipids that are not clearly related to membrane turnover. This fact is underscored by studies of patients with Huntington's disease (138) and of at least some patients with Alzheimer's disease (139), which have observed no abnormalities in these metabolites. Thus, changes in PME and PDE peaks are not easily interpreted. The phosphocreatine (PCr), ATP, and Pi peaks confer information about the state of high-energy phosphate metabolism and pH in the tissue. It also should be noted that because ³¹P spectra are acquired with large voxels (i.e., 15 to 80 cm³), the relative contributions of various tissue components (i.e., gray matter, white matter, neurons, glia, endothelia) to the signal is uncertain.

O'Callaghan and colleagues (140) reported the first ³¹P spectroscopy study in schizophrenia, on a sample of 18 patients and 10 control subjects. They used a surface coil to acquire data from 87-cm³ voxels arbitrarily placed in both temporal lobes. There were no differences found in any of the peaks. Pettegrew et al. (141) reported a highly cited study of 11 first-episode patients, using a surface coil placed over the front of the head. They reported that their voxel

of 20 cm³ sampled the dorsolateral prefrontal cortex, though its exact location is unclear, as subsequent reports stated that both right and left prefrontal cortices were sampled (142). With only a single 20-cm³ voxel, this would indicate a more midline localization. The investigators reported that patients had decreased PMEs and Pi, and increased PDEs and ATP. They interpreted their findings to reflect greater membrane turnover and less energy utilization, results that they speculated were consistent with hypotheses about excessive synaptic pruning and decreased frontal lobe metabolism.

Because of these initial reports, over fifteen ³¹P studies of patients with schizophrenia have appeared in the literature. Although several studies have reported partial replication of the findings of Pettegrew et al. (141,142), particularly with respect to a reduced PME peak, the majority of the reports have failed to do so. The data with respect to high-energy phosphate peaks are especially inconsistent and generally negative. Studies that have looked outside the frontal lobe, at parietal and temporal lobes and at the basal ganglia, also have yielded most often negative or inconsistent results. Several recent reviews provide tabulated summaries of this literature (143,144). The reasons for the inconsistencies are unclear. The usual explanations—differences in patient sample (e.g., medicated or unmedicated, acute or chronic), differences in acquisition parameters (e.g., surface or volume coil, single voxel, or multivoxel)—seem inadequate, as both positive and negative reports have appeared regardless of the characteristics of the samples and in the context of a variety of acquisition parameters. A more likely explanation for the inconsistencies involves the low sensitivity and reproducibility of ³¹P spectroscopy and problems in achieving a standardized placement of a region of interest (ROI) or of an acquisition plane. There are very limited data about the reliability of ³¹P measurements in patients, with one study reporting a coefficient of variation of 30% across two scans (145). Because it is impossible to accurately register single voxels or single planes to an anatomic reference, there is unavoidable error in the localization process, which is further compounded by subject motion during the scan. Large voxel sizes also introduce error in terms of partial volume effects. The small differences that have been reported in most of the positive studies, on the order of 10% to 25%, would seem especially sensitive to such methodologic difficulties.

Two recent studies are particularly noteworthy in their efforts to control for localization variance and to improve the sensitivity of the spectral data. Volz et al. (146) acquired a plane of ³¹P spectra, composed of 30 voxels of 19 cm³ each, and drew ROIs on an anatomic reference scan that was acquired in a coplanar orientation, on 11 medication-free patients, including seven acute patients who were medication naive. Though the planes are not strictly registered in space, because subject motion may have tilted their relative orientations during the scans, the authors' approach to ROI

placement is more reliable than that in earlier studies. With the exception of one mesial prefrontal voxel having a decreased PDE peak and one voxel localized to the basal ganglia having a decreased PME peak, no other cerebral differences in phospholipids were found. Four prefrontal voxels having decreases in ATP and PCr also were found. The authors argued that their data suggested decreased membrane catabolism, but, given the number of voxels analyzed, chance results cannot be excluded. In an earlier study of chronic, medicated patients, they also found a decreased prefrontal PDE peak, but the high-energy phosphate data were in the opposite direction (147). Finally, Potwarka et al. (148), using signal enhancement techniques that reduce the effects of coupling between phosphorus and proton spins, were able to separate structural membrane phospholipids from other constituents of the PME and PDE peaks, with 50-cc voxels acquired as part of a plane. Although the authors found no decrease in PMEs as reported by Pettegrew et al., they did find increases in the frontal PDE peak, but not related to membrane breakdown products such as glycerophosphocholine (GPC) and glycerolphosphoethanolamine (GPE), as suggested by Pettegrew et al. and others. Rather, the difference was reflected in the membrane phospholipid components of the peak. These differences were not found in motor or occipital cortices, providing some evidence of internal validity. This study also found no differences between patients and controls in total PMEs, again in contrast to the Pettegrew et al. study, but a component of the PME peak, phosphocholine, was reduced. Potwarka et al. (148) proposed that their data implicated membrane abnormalities selectively in DLPFC, perhaps involving pre-synaptic vesicular phospholipids. However, to confuse the story even further, Bluml et al. (149), using a similar proton decoupled ^{31}P MRS approach, reported increases in GPC and GPE acquired with a large (97-cc) voxel in the middle of the cerebrum.

Several studies have attempted to link ^{31}P data to clinical characteristics of patients, but these also have been inconsistent. For example, Deicken et al. (150) reported a correlation between prefrontal PME signals and performance on the WCST, suggesting that prefrontal membrane abnormalities were reflected in prefrontal function. However, in the same patient sample, Deicken et al. did not find an abnormality of PME signals. Volz et al. (147) could not find a correlation between any ^{31}P signals and WCST performance. Potwarka et al. (148) found a correlation between an ATP peak in right prefrontal cortex and negative symptom ratings, but similar relationships were not found in other studies (151).

It is difficult to arrive at a synthetic analysis of the ^{31}P data in schizophrenia. Technical error is probably the critical factor in the variable results that have been reported. It is doubtful that the small differences between patients and controls could escape corruption by the many methodologic limitations of the current techniques. Future studies using

higher field magnets, with better sensitivity and resolution, combined with signal enhancement and peak separation procedures may lead to more reliable methods.

Proton Spectroscopy: Assays of Cells and Cellular Metabolism

Proton spectroscopy has been a more widely applied technique in schizophrenia research and the results are much more consistent. A variety of chemicals in the proton spectrum can be assayed with clinical magnets, including several amino acids, membrane and myelin metabolites, and several high-energy substrates. Although the sensitivity of proton spectroscopy is approximately 20 times that of phosphorus, allowing for much better resolution, the metabolites of interest need to be resolved in a smaller chemical shift range (less than 10 ppm), in the presence of large concentrations of brain water (approximately 10^4 times greater concentration than the other metabolites) and mobile lipids from the skull and scalp. Until recently, most ^1H MRS techniques used special procedures to suppress the signal from water and lipids, procedures that can affect other signals. With the availability of analogue to digital converters having greater dynamic range, it is now possible to acquire the water signal and still resolve the other metabolites (152). In addition to preservation of neighboring signals, this approach also has the advantage of making the water signal available as an internal standard for normalization and potentially for tissue segmentation.

The proton spectrum is characterized by several relatively large and distinct peaks and several complexes of smaller overlapping peaks. The metabolite signals acquired with ^1H MRS vary depending on the echo time of the pulse sequence used for the acquisition. Many of the resolvable elements have short T2 (e.g., myoinositol, glutamate, glutamine, and GABA) and emit no observable signal with longer echo times. On the other hand, long echo time acquisitions produce signals from several compounds that are very distinctly resolved. The long echo time metabolite spectrum is dominated by a peak at approximately 2 ppm corresponding to the methyl group (CH_3) of several *N*-acetyl containing compounds, principally *N*-acetylaspartate (NAA) and to a small degree, *N*-acetyl aspartate glutamate (NAAG) and possibly *N*-acetylneuraminic acid (NANA) (134). NAA is an intracellular neuronal marker, found almost exclusively in mature neurons and their processes (153), with the highest concentrations in pyramidal glutamate neurons (154). NAA is the second most abundant amino acid in the brain (155). Its concentration is higher in gray matter than in white matter (156), and NAA signals increase during childhood, remaining relatively stable throughout adult life (156–158). The exact implications of changes in NAA signals is uncertain, as its cellular function is still unclear. It is synthesized in mitochondria from glutamate and either pyruvate or 3-hydroxybutyrate via L-aspartate-*N*-amino

transferase and also is a by-product of NAALadase catabolism of NAAG, which occurs within glia (159). Whether NAA signals are absolutely specific to neurons is unclear. Mature astroglia do not contain NAA, though small concentrations have been reported in oligodendroglial cultures (160). NAA is a nonspecific though highly sensitive marker of neuronal pathology. Virtually all neurologic conditions involving neuronal pathology that have been studied, including multiple sclerosis, motor neuron disease, Alzheimer's disease, Huntington's disease, cerebellar degenerations, multiple sclerosis, epilepsy, and various encephalopathies, show changes in NAA signals in the regions of brain pathology. Moreover, NAA changes are sensitive measures of dynamic neuropathologic processes (161–163), for example, correlating over time with cognitive change in Alzheimer's disease (164) and with the number of trinucleotide repeats in Huntington's disease (165). Although early studies interpreted NAA findings as indicative of cell loss, recent data have established that NAA reductions can reverse following various forms of brain damage and can change with clinical improvement and treatment (161,162,166–168). This has led to speculation that NAA reductions occur as a manifestation of changes in neuronal volume or in NAA concentrations within a neuron, perhaps reflecting reduced mitochondrial energy metabolism (159) or a change in the abundance and patterns of neuronal connectivity (169,170). It is interesting to note that in various conditions associated with tissue volume loss and reduced NAA signals (e.g., epilepsy, Alzheimer's disease, schizophrenia), these two parameters are not tightly correlated. The only condition in which NAA concentrations are increased is Canavan's disease, which involves a mutation in a gene on chromosome 17 controlling the synthesis of *N*-acetyl-L-aspartate amidohydrolase (aspartoacylase), the enzyme that breaks down NAA.

Two other prominent peaks are seen in the long echo time proton spectrum. At 3.2 ppm is a peak corresponding to the trimethylamine group of various choline (CHO)-containing compounds, mostly membrane phospholipids. This signal reflects the concentrations of several phospholipid moieties, including glycerophosphocholine, phosphocholine, and phosphatidylcholine. In pathologic conditions associated with membrane turnover or gliosis (e.g., Alzheimer's disease, gliomas, epilepsy), the CHO peak tends to be elevated. At 3.0 ppm, a peak corresponding to creatine and phosphocreatine (CRE) appears. These metabolites participate as energy buffers in many energy-consuming processes in the brain, but consistent changes in the CRE signal are generally found only in the presence of tissue loss.

At short echo time, several other peaks are observed, in addition to the peaks that persist into the long echo time spectra. The most studied of these is the myoinositol peak (3.6 ppm) and a peak complex (called tGlx) at around 2.2 to 2.4 and 3.75 ppm corresponding to overlapping signals from glutamate, glutamine, and GABA. Myoinositol is a

hexol present in high concentrations in human brain, and accounts for most of the myoinositol peak, though other complex inositol phosphates also contribute to the signal. Some of these inositol phosphates may represent second-messenger signaling molecules that may vary with the state of cellular activity. The myoinositol peak, however, tends to change in conditions associated with active membrane turnover and gliosis, and is consistently increased in Alzheimer's disease. The glutamate and GABA peaks include soluble forms of these amino acids involved both in neurotransmission and in peptide synthesis. Glutamine is an intermediary in glial-based recycling of the carbon skeletons of these amino acids, and has been proposed as a more sensitive marker of turnover of the glutamate amino acid pool.

Evidence from ^1H MRS of Neuronal Pathology

Nasrallah et al. (171) reported the first ^1H MRS study of both mesial temporal lobes in schizophrenia, an investigation of 11 chronic patients and 11 controls, using a 12-cm³ voxel. They found decreases in NAA peaks in both voxels, with the difference on the right significant at the .05 level, and on the left at the .06 level. Since the report of Nasrallah et al., over 20 ^1H MRS studies of patients with schizophrenia have appeared. Most of them have addressed metabolite changes, primarily NAA, in the frontal and temporal lobes. Table 59.3 summarizes studies of frontal and temporal lobe NAA signals. Several recent reviews describe these reports in greater detail (143,144,172). Although most of these studies have involved single voxel data, with the inherent methodologic issues described above, several recent relatively high resolution (approximately 1-mL voxel) multivoxel and multislice imaging studies (MRSI) have also been reported. Reliability data for NAA are much superior to those of other metabolites in either the proton or the phosphorus spectra; for example, the coefficient of variation for repeat studies in patients using an MRSI technique is on the order of 10% (173). It is interesting in this regard that all of the MRSI studies to date that have examined cortical regions in the frontal and temporal lobes have reported reduced NAA signals in these regions (174–179). These studies are exemplified by a series of reports from Bertolino and colleagues (174–177) using a technique that acquires over 700 1.4-mL voxels in approximately 25 minutes. The technique allows for registration of spectroscopic and anatomic images, for reliable and anatomically correct ROI definition, and for relatively diminished partial volume effects. The studies of Bertolino et al. included chronic medicated patients, unmedicated and several neuroleptic-naive patients, and childhood-onset patients. In each of these samples, NAA signals were reduced selectively and bilaterally in dorsolateral prefrontal and perihippocampal cortices. Using a similar technical approach in studies of chronic patients, Deicken et al.

TABLE 59.3. ¹H MRS STUDIES OF FRONTAL AND TEMPORAL LOBE NAA SIGNALS IN SCHIZOPHRENIA

Study	Method	Sample Size Patients/Controls	Temporal Lobe	Frontal Lobe	Other
Nasrallah et al. (171)	12 cc voxel	11/11	↓	—	
Choe et al. (212)	8 cc voxel	23/10	—	↓	↑ tGlx
Renshaw et al. (213)	8 cc voxel	13/15	↓	—	First episode patients
Yurgelun-Todd et al. (214)	8 cc voxel	16/14	↓	—	Mainly extrahippocampal voxel
Buckley et al. (215)	11 cc voxel	28/20	NC	↓	
Maier et al. (216)	4–9 cc voxel	25/32	↓	—	
Maier and Ron (217)	5–7 cc voxel	26/38	↓	—	No differential change w/aging
Fukuzako et al. (218)	27 cc voxel	30/30	↓	NC	No abnormalities in drug naive patients; white matter voxel in frontal lobe
Stanley et al. (184)	8 cc voxel	29/24	—	NC	↑ glutamine in chronic patients
Bartha et al. (183)	4.5 cc voxel	10/10	NC	—	First episode, mesial frontal voxel; increased glutamine
Cecil et al. (219)	4.5–8 cc voxel	10/24	↓	↓	Drug naive patients
Brooks et al. (220)	8 cc voxel	16/12	↓	↓	Childhood schizophrenia
Thomas et al. (221)	8 cc voxel	13/12	↓	↓	Childhood schizophrenia
Bertolino et al. (174)	MRSI 1.4 cc/voxel	10/10	↓	↓	Chronic patients
Bertolino et al. (175)	MRSI 1.4 cc/voxel	12/12	↓	↓	Medication-free patients
Bertolino et al. (176)	MRSI 1.4 cc/voxel	14/14	↓	↓	Childhood onset schizophrenia
Callicott et al. (177)	MRSI 1.4 cc/voxel	103/71	↓	↓	Hippocampal NAA decreased—prefrontal NAA unchanged in healthy siblings
Deicken et al. (179)	MRSI 1.3 cc/voxel	23/18	↓	—	No change in hippocampal volume
Deicken et al. (178)	MRSI 1.3 cc/voxel	24/15	—	↓	
Heimberg et al. (186)	8 cc ³ voxel	13/142	NC	NC	White matter frontal voxel; temporal voxel excluded hippocampus
Kegeles et al. (143)	1.4 cc multivoxel	10/10	NC	—	Poor reliability [cv >30%]
Fukuzako et al. (222)	8 cc ³ voxel	64/51	↓	—	
Block et al. (198)	30 cc ³ voxel	25/19	—	↓	No abnormality in healthy relatives
Bartha et al. (185)	6 cc ³ voxel	11/11	NC	—	No glutamine differences

↓, reduced; ↑, increased; —, not assayed; MRSI, magnetic resonance spectroscopy imaging; NAA, *N*-acetylaspartate; NC, no change.

replicated these results (178,179), and also found reduced NAA signals in cingulate cortex (180) and in thalamus (181), which were not found in the Bertolino et al. studies. The only imaging study that reported no decrease in gray matter NAA did not regionally parcellate the cortex, and reported only an average measure for the entire cortex of the top half of the brain (182). Indeed, this result is consistent with the findings of Bertolino et al., in which NAA levels throughout most regions of brain were not different between patients and controls. Although most of the single voxel studies also found reductions in NAA signals in frontal and temporal lobes, the few negative studies (183–187) may reflect methodologic variance related to small sample sizes or to voxel placement, etc. The single voxel studies even of NAA signals also tend to have much less reliable data [e.g., a coefficient of variation (cv) in a negative study by Kegeles et al. (187) of greater than 30%]. It is clear from this literature that reduced NAA peaks are found in the frontal and

temporal lobes of many patients with schizophrenia (see table for a summary).

There have been few studies of other peaks in the proton spectra, and the results have been inconclusive. Because of interest in glutamate and cortical function in schizophrenia, several groups have attempted to measure the tGlx peak, using quantitation methods based on a priori knowledge of the relative contributions of the metabolite components, but the reliability of the method is limited [e.g., cv >50% (183)]. Stanley et al. (184) found increased glutamine signals in chronically treated patients, but no differences in acute patients. Bartha et al. (183) reported increased glutamine in cingulate cortex of a group of ten patients. Rakow et al. (188) reported decreased tGlx in dorsolateral prefrontal cortex and Kegeles et al. (187) reported no changes in mesial temporal lobe. The data with choline and creatine peaks have generally been negative, and the occasional positive result has been inconsistent across studies.

There has been considerable interest in trying to understand the meaning of NAA decreases in patients with schizophrenia. The NAA changes have consistently been shown not to correlate with stage of illness, with medication status, and with length of illness. Moreover, hippocampal and prefrontal volume do not appear to correlate either. In the Bertolino et al. (175) study of unmedicated patients, several of the subjects had been chronically psychotic and untreated for over 10 years, and no relationship between NAA signals and length of untreated psychosis was found. These data suggest that NAA reductions are not the result of a linearly progressive pathologic process. They also provide evidence against the notion that chronic untreated psychosis is “neurotoxic.” Because postmortem studies do not provide convincing evidence of neuronal loss or of neuronal degeneration in the areas where NAA signals are consistently reduced (i.e., hippocampal formation and prefrontal cortices) (189), the NAA changes probably reflect more subtle aspects of neuronal biology (see below). As such, they are unique *in vivo* evidence of cellular changes probably restricted to neurons in the schizophrenic brain. Although NAA reductions are found in both medicated and unmedicated patients, the assumption that NAA changes are independent of medical treatment may be incorrect. Bertolino et al. (167) recently reported that NAA signals in dorsolateral prefrontal cortex increase slightly but significantly after only several weeks of neuroleptic treatment. This slight increase is further evidence that NAA levels may reflect dynamic neuronal events, and is consistent with evidence that other pharmacologic treatments can alter NAA signals [e.g., lithium (190)]. Although NAA changes clearly occur independent of neuroleptic treatment, the neuroleptic effect illustrates that physiologic factors may contribute to the variations in NAA signals.

NAA Signals and Abnormalities of Specific Functional Systems

Because NAA signals originate perhaps exclusively in neurons, principally glutamate neurons, it is possible to probe the functional connectivity of cortical glutamate neurons with NAA as a surrogate marker of the integrity or activity of such connectivity. This has been done in animals and in human studies. In animals, optic nerve injury reduces NAA concentrations in the lateral geniculate (191), and prefrontal cortical injury reduces NAA signals in basal ganglia, suggestive of loss of afferent terminals or transynaptic cellular changes (169). Bertolino et al. (170) measured NAA signals in DLPFC of adult monkeys that had undergone mesial temporal lobe removals as neonates and in monkeys that had undergone temporal lobe removals as adults. Reduced NAA signals were found only in the animals with neonatal removals, suggesting that NAA could reflect more complex plastic neuronal modifications, perhaps at the level of local

circuit architecture, than simply loss of afferent terminals. Similar results have also been observed in rodents (192).

This evidence of relationships between NAA signals and connections between neurons led to a series of studies aimed at testing hypotheses about the centrality of prefrontal connectivity in the pathophysiology of schizophrenia (193). Bertolino et al. (63,194,195) used NAA signals as a marker of glutamate projection neuronal function/integrity in predicting the activity of distributed neuronal systems implicated in positive and negative symptoms. NAA signals selectively in DLPFC were found to predict the availability of DA receptors in the striatum in patients with schizophrenia, assayed with radionuclide imaging (194). Specifically, lower DLPFC NAA predicted greater availability of DA receptors in an unstimulated, resting state, speculated to reflect less DA release and cell firing. The same measure, i.e., low NAA, also predicted the exaggerated response to amphetamine found in striatum with radioreceptor imaging (63). These data suggested that NAA in DLPFC predicted the steady-state and stimulus-induced responses of dopamine neurons in the ventral brainstem. Similar relationships were not found for any other cortical regions or in normal controls, implicating the neuronal pathology associated with the illness as instrumental in constraining these relationships. Although DA release was inferred indirectly from radioligand binding availability, the assumptions were confirmed in studies of monkeys undergoing *in vivo* microdialysis, in which NAA concentrations in DLPFC directly predicted DA release in the striatum (194).

NAA signals in DLPFC also were found in patients with schizophrenia to selectively predict the activation of the distributed working memory cortical network, studied both with PET (195) and with functional MRI (fMRI) (196). These data suggest that prefrontal glutamate neurons, by virtue of their intracortical connectivities, modulate the capacity and efficiency of distributed cortical networks involved in working memory. Working memory deficits have been consistently linked to other aspects of the negative symptoms of schizophrenia, and in fact NAA signals in DLPFC also specifically predict negative symptoms ratings in patients (197).

These various clinical studies of phenomena predicted by NAA signals in DLPFC converge on a tantalizing interpretation of these various findings—that NAA reductions reflect subtle cellular pathology of intrinsic DLPFC neurons and their local circuitry that, by virtue of intracortical and corticofugal projections, modify the activity of distributed cortical networks and of DA neurons, implicated in the negative and positive symptoms of schizophrenia, respectively. Thus, DLPFC neurons appear to be an effector neuronal population that is associated with the manifest biology of the illness. It is interesting to note that NAA signals in hippocampal formation, the other region consistently implicated in studies of patients with schizophrenia, do not show these predictable relationships. A study by Callicott et al.

(177) may shed some light on this apparent inconsistency. Callicott et al. studied 60 healthy siblings of patients with schizophrenia and found that although NAA signals were also reduced in the hippocampal formation of the healthy siblings, changes in DLPFC were not found. These data suggest that consistent with other evidence of hippocampal functional abnormalities in relatives of patients with schizophrenia (e.g., memory deficits, P-50-evoked potentials), NAA changes in the hippocampal formation may reflect biology of genetic risk. In contrast, consistent with the predictable relationships of NAA in DLPFC with other functional systems implicated in schizophrenia, NAA changes in DLPFC reflect biology of manifest illness. The lack of a difference in frontal lobe NAA in healthy relatives of patients with schizophrenia has recently been reported by another group as well (198). Therefore, these results have added hippocampal NAA measures to the list of potential phenotypic markers of genetic risk for further exploration in genetic studies of mental illness.

Future Developments

MRS is a rapidly evolving technology and its future applications in schizophrenia research should lead to important discoveries. New developments in the near future will likely emerge from methodologic advances leading to improved sensitivity and resolution, measurement of novel chemical moieties, indexing neuronal metabolism, and characterizing drug effects. The availability of high-field human magnets (3 to 7 tesla) will substantially improve the STN of MRS and allow for improved reliability and resolution. At the National Institute of Mental Health (NIMH), proton spectral images are currently acquired at 3 T with 0.7-mL voxels, and the cv of repeat NAA measurements in the hippocampus is about 3%. Various hardware upgrades have also made it possible to shim individual slices rather than slabs of tissue, and to acquire both early and late echo spectra within the same acquisition, without suppressing the water signal. Use of techniques to control for motion effects (e.g., navigator signals) may further improve reliability and make it possible to avoid lipid suppression approaches, as well. The improved STN and signal acquisition of new methods will result in more sensitive and reliable acquisition of other spectral peaks. This will improve the potential reliability of phosphorus moieties in the proton spectrum and will make calculations of the components of the tGlx peak more reliable. Preliminary results using spectral editing approaches to the GABA peak suggest that clinically meaningful data about GABA metabolism can be derived from this peak (199). Greater sensitivity and SNR also will permit spectral analyses of externally administered molecules. C13 glucose can be given as a glucose load and the fate of the C13 carbon skeleton tracked over time as changing concentrations of moieties in the C13 spectra (200). This may provide near-real-time information about glucose metabolism, and

also potentially about the turnover of several neurotransmitters. Preliminary studies of fluorinated compounds, such as fluoxetine and fluphenazine, have demonstrated the feasibility of measuring the concentrations of such compounds in brain. The potential applications of such measurements will become clearer in the context of improved methodology. Finally, the demonstration that NAA changes with antipsychotic drug treatment represents the first *in vivo* evidence of an intracellular effect of these drugs. Future studies will aim to understand the basis for this change, as well as other factors that affect NAA signals.

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REFERENCES

1. Wagner H Jr, Burns HD, Dannals RF, et al. Imaging dopamine receptors in the human brain by positron tomography. *Science* 1983;221:1264–1266.
2. Rossum V. The significance of dopamine receptor blockade for the mechanism of action of neuroleptic drugs. *Arch Int Pharmacodyn Ther* 1966;160:492–494.
3. Carlsson A, Lindqvist M. Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. *Acta Pharmacol Toxicol* 1963;20:140–144.
4. Seeman P, Lee T. Antipsychotic drugs: direct correlation between clinical potency and presynaptic action on dopamine neurons. *Science* 1975;188:1217–1219.
5. Creese I, Burt DR, Snyder SH. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science* 1976;19:481–483.
6. Connell PH. *Amphetamine psychosis*. London: Chapman and Hill, 1958.
7. Angrist BM, Gershon S. The phenomenology of experimentally induced amphetamine psychosis—Preliminary observation. *Biol Psychiatry* 1970;2:95–107.
8. Meltzer H. Clinical studies on the mechanism of action of clozapine: the dopamine-serotonin hypothesis of schizophrenia. *Psychopharmacology* 1989;99:S18–S27.
9. Carlsson A. The current status of the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology* 1988;1:179–186.
10. Laruelle M. Imaging dopamine transmission in schizophrenia. A review and meta-analysis. *Q J Nucl Med* 1998;42:211–221.
11. Davis KL, Kahn RS, Ko G, et al. Dopamine in schizophrenia: a review and reconceptualization. *Am J Psychiatry* 1991;148:1474–1486.
12. Harrison PJ. The neuropathology of schizophrenia. A critical review of the data and their interpretation. *Brain* 1999;122:593–624.
13. Weinberger DR, Berman KF. Prefrontal function in schizophrenia: confounds and controversies. *Philos Trans R Soc Lond [B]* 1996;351:1495–1503.
14. Goldman-Rakic PS, Selemon LD. Functional and anatomical aspects of prefrontal pathology in schizophrenia. *Schizophr Bull* 1997;23:437–458.
15. Weinberger DR. Implications of the normal brain development

- for the pathogenesis of schizophrenia. *Arch Gen Psychiatry* 1987;44:660–669.
16. Knable MB, Weinberger DR. Dopamine, the prefrontal cortex and schizophrenia. *J Psychopharmacol* 1997;11:123–131.
 17. Akil M, Pierri JN, Whitehead RE, et al. Lamina-specific alterations in the dopamine innervation of the prefrontal cortex in schizophrenic subjects. *Am J Psychiatry* 1999;156:1580–1589.
 18. Deutch AY. Prefrontal cortical dopamine systems and the elaboration of functional corticostriatal circuits: implications for schizophrenia and Parkinson's disease. *J Neural Transm Gen Sect* 1993;91:197–221.
 19. Wilkinson LS. The nature of interactions involving prefrontal and striatal dopamine systems. *J Psychopharmacol* 1997;11:143–50.
 20. Wong DF, Wagner HN, Tune LE, et al. Positron emission tomography reveals elevated D2 dopamine receptors in drug-naive schizophrenics. *Science* 1986;234:1558–1563.
 21. Crawley JC, Owens DG, Crow TJ, et al. Dopamine D2 receptors in schizophrenia studied *in vivo*. *Lancet* 1986;2:224–225.
 22. Seeman P, Guan HC, Van Tol HHM. Dopamine D4 receptors elevated in schizophrenia. *Nature* 1993;365:411–445.
 23. Lahti RA, Roberts RC, Cochrane EV, et al. Direct determination of dopamine D-4 receptors in normal and schizophrenic postmortem brain tissue: a [H-3]NGD-94-1 study. *Mol Psychiatry* 1998;3:528–533.
 24. Ng GY, O'Dowd BF, Caron M, et al. Phosphorylation and palmitoylation of the human D2L dopamine receptor in Sf9 cells. *J Neurochem* 1994;63:1589–1595.
 25. Ng GY, O'Dowd BF, Lee SP, et al. Dopamine D2 receptor dimers and receptor-blocking peptides. *Biochem Biophys Res Commun* 1996;227:200–204.
 26. Zawarynski P, Tallerico T, Seeman P, et al. Dopamine D2 receptor dimers in human and rat brain. *FEBS Lett* 1998;441:383–386.
 27. Lee SP, O'Dowd BF, Ng GY, et al. Inhibition of cell surface expression by mutant receptors demonstrates that D2 dopamine receptors exist as oligomers in the cell [In Process Citation]. *Mol Pharmacol* 2000;58:120–128.
 28. Seeman P, Guan H-C, Niznik HB. Endogenous dopamine lowers the dopamine D2 receptor density as measured by [3H]raclopride: implications for positron emission tomography of the human brain. *Synapse* 1989;3:96–97.
 29. Seeman P. Brain dopamine receptors in schizophrenia: PET problems. *Arch Gen Psychiatry* 1988;45:598–560.
 30. Reith J, Benkelfat C, Sherwin A, et al. Elevated dopa decarboxylase activity in living brain of patients with psychosis. *Proc Natl Acad Sci USA* 1994;91:11651–11654.
 31. Hietala J, Syvalahti E, Vuorio K, et al. Presynaptic dopamine function in striatum of neuroleptic-naive schizophrenic patients. *Lancet* 1995;346:1130–1131.
 32. Dao-Castellana MH, Paillere-Martinot ML, Hantraye P, et al. Presynaptic dopaminergic function in the striatum of schizophrenic patients. *Schizophr Res* 1997;23:167–174.
 33. Hietala J, Syvalahti E, Vilkmann H, et al. Depressive symptoms and presynaptic dopamine function in neuroleptic-naive schizophrenia. *Schizophr Res* 1999;35:41–50.
 34. Lindstrom LH, Gefvert O, Hagberg G, et al. Increased dopamine synthesis rate in medial prefrontal cortex and striatum in schizophrenia indicated by L-(beta-¹¹C) DOPA and PET [In Process Citation]. *Biol Psychiatry* 1999;46:681–688.
 35. Laruelle M. Imaging synaptic neurotransmission with *in vivo* binding competition techniques: a critical review. *J Cereb Blood Flow Metab* 2000;20:423–451.
 36. Villemagne VL, Wong DF, Yokoi F, et al. GBR12909 attenuates amphetamine-induced striatal dopamine release as measured by [(11)C]raclopride continuous infusion PET scans. *Synapse* 1999;33:268–273.
 37. Laruelle M, Iyer RN, Al-Tikriti MS, et al. Microdialysis and SPECT measurements of amphetamine-induced dopamine release in nonhuman primates. *Synapse* 1997;25:1–14.
 38. Breier A, Su TP, Saunders R, et al. Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. *Proc Natl Acad Sci USA* 1997;94:2569–2574.
 39. Laruelle M, Abi-Dargham A, van Dyck CH, et al. Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug free schizophrenic subjects. *Proc Natl Acad Sci USA* 1996;93:9235–9240.
 40. Abi-Dargham A, Gil R, Krystal J, et al. Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. *Am J Psychiatry* 1998;155:761–767.
 41. Laruelle M, Abi-Dargham A, Gil R, et al. Increased dopamine transmission in schizophrenia: relationship to illness phases. *Biol Psychiatry* 1999;46:56–72.
 42. Hwang DR, Kegeles LS, Laruelle M. [¹¹C]N-propyl-apomorphine. A positron-labeled dopamine agonist for PET imaging of D2 receptors. *Nucl Med Biol* 2000;27:533–539.
 43. Laruelle M, D'Souza CD, Baldwin RM, et al. Imaging D-2 receptor occupancy by endogenous dopamine in humans. *Neuropsychopharmacology* 1997;17:162–174.
 44. Fujita M, Verhoeff NP, Varrone A, et al. Imaging extrastriatal dopamine D(2) receptor occupancy by endogenous dopamine in healthy humans. *Eur J Pharmacol* 2000;387:179–188.
 45. Abi-Dargham A, Rodenhiser J, Printz D, et al. Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. *Proc Natl Acad Sci USA* 2000;97:8104–8109.
 46. Laruelle M, Abi-Dargham A, van Dyck C, et al. Dopamine and serotonin transporters in patients with schizophrenia: an imaging study with [(123)I]beta-CIT. *Biol Psychiatry* 2000;47:371–379.
 47. Hirai M, Kitamura N, Hashimoto T, et al. [3H]GBR-12935 binding sites in human striatal membranes: binding characteristics and changes in parkinsonians and schizophrenics. *Jpn J Pharmacol* 1988;47:237–243.
 48. Czudek C, Reynolds GP. [3H] GBR 12935 binding to the dopamine uptake site in post-mortem brain tissue in schizophrenia. *J Neural Transm* 1989;77:227–230.
 49. Pearce RK, Seeman P, Jellinger K, et al. Dopamine uptake sites and dopamine receptors in Parkinson's disease and schizophrenia. *Eur Neurol* 1990;30(suppl 1):9–14.
 50. Joyce JN, Lexow N, Bird E, et al. Organization of dopamine D1 and D2 receptors in human striatum: receptor autoradiographic studies in Huntington's disease and schizophrenia. *Synapse* 1988;2:546–557.
 51. Chinaglia G, Alvarez FJ, Probst A, et al. Mesostriatal and mesolimbic dopamine uptake binding sites are reduced in Parkinson's disease and progressive supranuclear palsy: a quantitative autoradiographic study using [3H]mazindol. *Neuroscience* 1992;49:317–327.
 52. Knable MB, Hyde TM, Herman MM, et al. Quantitative autoradiography of dopamine-D1 receptors, D2 receptors, and dopamine uptake sites in postmortem striatal specimens from schizophrenic patients. *Biol Psychiatry* 1994;36:827–835.
 53. Weinberger DR, Berman KF, Zec RF. Physiological dysfunction of dorsolateral prefrontal cortex in schizophrenia: I. Regional cerebral blood flow evidence. *Arch Gen Psychiatry* 1986;43:114–124.
 54. Grace AA. Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. *Neuroscience* 1991;41:1–24.
 55. Carlsson A, Waters N, Carlsson ML. Neurotransmitter interac-

- tions in schizophrenia—therapeutic implications. *Biol Psychiatry* 1999;46:1388–1395.
56. Bunney BS, Aghajanian GK. d-Amphetamine-induced depression of central dopamine neurons: evidence for mediation by both autoreceptors and a striato-nigral feedback pathway. *Nauyn Schmiedebergs Arch Pharmacol* 1978;304:255–261.
 57. Miller DW, Abercrombie ED. Effects of MK-801 on spontaneous and amphetamine-stimulated dopamine release in striatum measured with *in vivo* microdialysis in awake rats. *Brain Res Bull* 1996;40:57–62.
 58. Kegeles L, Abi-Dargham A, Zea-Ponce Y, et al. Modulation of amphetamine-induced striatal dopamine release by ketamine in humans: implications for schizophrenia. *Biol Psychiatry* 2000;48:627–640.
 59. Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia. *Arch Gen Psychiatry* 1995;52:998–1007.
 60. Jentsch JD, Roth RH. The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology* 1999;20:201–225.
 61. Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry* 1991;148:1301–1308.
 62. Karreman M, Moghaddam B. The prefrontal cortex regulates the basal release of dopamine in the limbic striatum: an effect mediated by ventral tegmental area. *J Neurochem* 1996;66:589–598.
 63. Bertolino A, Breier A, Callicott JH, et al. The relationship between dorsolateral prefrontal neuronal N-acetylaspartate and evoked release of striatal dopamine in schizophrenia. *Neuropsychopharmacology* 2000;22:125–132.
 64. Saunders RC, Kolachana BS, Bachevalier J, et al. Neonatal lesions of the medial temporal lobe disrupt prefrontal cortical regulation of striatal dopamine. *Nature* 1998;393:169–171.
 65. Bertolino A, Saunders RC, Mattay VS, et al. Altered development of prefrontal neurons in rhesus monkeys with neonatal mesial temporo-limbic lesions: a proton magnetic resonance spectroscopic imaging study. *Cereb Cortex* 1997;7:740–748.
 66. Laruelle M. The role of endogenous sensitization in the pathophysiology of schizophrenia: implications from recent brain imaging studies. *Brain Res Rev* 2000;31:371–384.
 67. Lieberman JA, Kinon BL, Loebel AD. Dopaminergic mechanisms in idiopathic and drug-induced psychoses. *Schizophr Bull* 1990;16:97–110.
 68. Glenthøj BY, Hemmingsen R. Dopaminergic sensitization: implications for the pathogenesis of schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* 1997;21:23–46.
 69. Lieberman JA, Sheitman BB, Kinon BJ. Neurochemical sensitization in the pathophysiology of schizophrenia: deficits and dysfunction in neuronal regulation and plasticity. *Neuropsychopharmacology* 1997;17:205–229.
 70. Robinson TE, Becker JB. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. *Brain Res Rev* 1986;11:157–198.
 71. Pierce RC, Kalivas PW. A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. *Brain Res Rev* 1997;25:192–216.
 72. Kalivas PW, Sorg BA, Hooks MS. The pharmacology and neural circuitry of sensitization to psychostimulants. *Behav Pharmacol* 1993;4:315–334.
 73. Griffith JD, Cavanaugh J, Held J, et al. Dextroamphetamine: evaluation of psychomimetic properties in man. *Arch Gen Psychiatry* 1972;26:97–100.
 74. O'Donnell P, Grace AA. Dysfunctions in multiple interrelated systems as the neurobiological bases of schizophrenic symptom clusters. *Schizophr Bull* 1998;24:267–283.
 75. Grace AA, Moore H, O'Donnell P. The modulation of corticoaccumbens transmission by limbic afferents and dopamine: a model for the pathophysiology of schizophrenia. *Adv Pharmacol* 1998;42:721–724.
 76. De Keyser J, Ebinger G, Vauquelin G. Evidence for a widespread dopaminergic innervation of the human cerebral neocortex. *Neurosci Lett* 1989;104:281–285.
 77. Hall H, Sedvall G, Magnusson O, et al. Distribution of D1- and D2-dopamine receptors, and dopamine and its metabolites in the human brain. *Neuropsychopharmacology* 1994;11:245–256.
 78. Bergson C, Mrzljak L, Smiley JF, et al. Regional, cellular, and subcellular variations in the distribution of D1 and D5 dopamine receptors in primate brain. *J Neurosci* 1995;15:7821–7836.
 79. Smiley JF, Levey AI, Ciliax BJ, et al. D1 dopamine receptor immunoreactivity in human and monkey cerebral cortex: predominant and extrasynaptic localization in dendritic spines. *Proc Natl Acad Sci USA* 1994;91:5720–5724.
 80. Krimer LS, Jakab RL, Goldman-Rakic PS. Quantitative three-dimensional analysis of the catecholaminergic innervation of identified neurons in the macaque prefrontal cortex. *J Neurosci* 1997;17:7450–7461.
 81. Laruelle M, Casanova M, Weinberger D, et al. Postmortem study of the dopaminergic D1 receptors in the dorsolateral prefrontal cortex of schizophrenics and controls. *Schizophr Res* 1990;3:30–31.
 82. Knable MB, Hyde TM, Murray AM, et al. A postmortem study of frontal cortical dopamine D1 receptors in schizophrenics, psychiatric controls, and normal controls. *Biol Psychiatry* 1996;40:1191–1199.
 83. Meador-Woodruff JH, Haroutunian V, Powchik P, et al. Dopamine receptor transcript expression in striatum and prefrontal and occipital cortex. Focal abnormalities in orbitofrontal cortex in schizophrenia. *Arch Gen Psychiatry* 1997;54:1089–1095.
 84. Okubo Y, Suhara T, Suzuki K, et al. Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET. *Nature* 1997;385:634–636.
 85. Karlsson P, Farde L, Halldin C, et al. D1-dopamine receptors in schizophrenia examined by PET. *Schizophr Res* 1997;24:179.
 86. Abi-Dargham A, Martinez D, Mawlawi O, et al. Measurement of striatal and extrastriatal dopamine D1 receptor binding potential with [¹¹C]NNC 112 in humans: validation and reproducibility. *J Cereb Blood Flow Metab* 2000;20:225–243.
 87. Abi-Dargham A, Simpson N, Kegeles L, et al. PET studies of binding competition between endogenous dopamine and the D1 radiotracer [¹¹C]NNC 756. *Synapse* 1999;32:93–109.
 88. Chou YH, Karlsson P, Halldin C, et al. A PET study of D1-like dopamine receptor ligand binding during altered endogenous dopamine levels in the primate brain. *Psychopharmacology* 1999;146:220–227.
 89. Lewis DA. GABAergic local circuit neurons and prefrontal cortical dysfunction in schizophrenia. *Brain Res Rev* 2000;31:270–6.
 90. Benes FM. Emerging principles of altered neural circuitry in schizophrenia. *Brain Res Rev* 2000;31:251–269.
 91. Busatto GF, Pilowsky LS, Costa DC, et al. Correlation between reduced *in vivo* benzodiazepine receptor binding and severity of psychotic symptoms in schizophrenia. *Am J Psychiatry* 1997;154:56–63.
 92. Verhoeff NP, Soares JC, D'Souza CD, et al. [¹²³I]Iomazenil SPECT benzodiazepine receptor imaging in schizophrenia. *Psychiatry Res* 1999;91:163–173.
 93. Abi-Dargham A, Laruelle M, Krystal J, et al. No evidence of altered *in vivo* benzodiazepine receptor binding in schizophrenia. *Neuropsychopharmacology* 1999;20:650–661.
 94. Schröder J, Bubeck B, Demisch S, et al. Benzodiazepine receptor

- distribution and diazepam binding in schizophrenia: an exploratory study. *Psychiatr Res Neuroimaging* 1997;68:125–131.
95. Ball S, Busatto GF, David AS, et al. Cognitive functioning and GABAA/benzodiazepine receptor binding in schizophrenia: a ¹²³I-iodozepam SPET study. *Biol Psychiatry* 1998;43:107–117.
 96. Benes FM, Wickramasinghe R, Vincent SL, et al. Uncoupling of GABA(A) and benzodiazepine receptor binding activity in the hippocampal formation of schizophrenic brain. *Brain Res* 1997;755:121–129.
 97. Abi-Dargham A, Krystal J. Serotonin receptors as target of antipsychotic medications. In: Lidow MS, eds. *Neurotransmitter receptors in actions of antipsychotic medications*. Boca Raton, FL: CRC Press LLC, 2000:79–107.
 98. Lewis R, Kapur S, Jones C, et al. Serotonin 5-HT₂ receptors in schizophrenia: a PET study using [18F]setoperone in neuroleptic-naïve patients and normal subjects. *Am J Psychiatry* 1999;156:72–78.
 99. Trichard C, Paillere-Martinot ML, Attar-Levy D, et al. No serotonin 5-HT_{2A} receptor density abnormality in the cortex of schizophrenic patients studied with PET. *Schizophr Res* 1998;31:13–17.
 100. Okubo Y, Suhara T, Suzuki K, et al. Serotonin 5-HT₂ receptors in schizophrenic patients studied by positron emission tomography. *Life Sci* 2000;66:2455–2464.
 101. Ngan ET, Yatham LN, Ruth TJ, et al. Decreased serotonin 2A receptor densities in neuroleptic-naïve patients with schizophrenia: a PET study using [(18F)]setoperone. *Am J Psychiatry* 2000;157:1016–1018.
 102. Kapur S, Zipursky RB, Remington G. Clinical and theoretical implications of 5-HT₂ and D₂ receptor occupancy of clozapine, risperidone, and olanzapine in schizophrenia. *Am J Psychiatry* 1999;156:286–293.
 103. Nyberg S, Nilsson U, Okubo Y, et al. Implications of brain imaging for the management of schizophrenia. *Int Clin Psychopharmacol* 1998;13(suppl 3):S15–20.
 104. Farde L, Nordström AL, Wiesel FA, et al. Positron emission tomography analysis of central D₁ and D₂ dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. *Arch Gen Psychiatry* 1992;49:538–544.
 105. Wolkin A, Barouche F, Wolf AP, et al. Dopamine blockade and clinical response: evidence for two biological subgroups of schizophrenia. *Am J Psychiatry* 1989;146:905–908.
 106. Pilowsky LS, Costa DC, Ell PJ, et al. Clozapine, single photon emission tomography, and the D₂ dopamine receptor blockade hypothesis of schizophrenia. *Lancet* 1992;340:199–202.
 107. Nordstrom AL, Farde L, Wiesel FA, et al. Central D₂-dopamine receptor occupancy in relation to antipsychotic drug effects: a double-blind PET study of schizophrenic patients. *Biol Psychiatry* 1993;33:227–235.
 108. Kapur S, Zipursky R, Jones C, et al. Relationship between dopamine D₂ occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. *Am J Psychiatry* 2000;157:514–520.
 109. Nordstrom AL, Farde L, Nyberg S, et al. D₁, D₂, and 5-HT₂ receptor occupancy in relation to clozapine serum concentration: a PET study of schizophrenic patients [see comments]. *Am J Psychiatry* 1995;152:1444–1449.
 110. Nyberg S, Farde L, Eriksson L, et al. 5-HT₂ and D₂ dopamine receptor occupancy in the living human brain. A PET study with risperidone. *Psychopharmacology* 1993;110:265–272.
 111. Kapur S, Remington G, Zipursky RB, et al. The D₂ dopamine receptor occupancy of risperidone and its relationship to extrapyramidal symptoms: a PET study. *Life Sci* 1995;57:L103–107.
 112. Knable MB, Heinz A, Raedler T, et al. Extrapyramidal side effects with risperidone and haloperidol at comparable D₂ receptor occupancy levels. *Psychiatr Res Neuroimaging* 1997;75:91–101.
 113. Kapur S, Zipursky RB, Remington G, et al. 5-HT₂ and D₂ receptor occupancy of olanzapine in schizophrenia: a PET investigation. *Am J Psychiatry* 1998;155:921–8.
 114. Gefvert O, Bergstrom M, Langstrom B, et al. Time course of central nervous dopamine-D₂ and 5-HT₂ receptor blockade and plasma drug concentrations after discontinuation of quetiapine (Seroquel) in patients with schizophrenia. *Psychopharmacology (Berl)* 1998;135:119–126.
 115. Kapur S, Zipursky R, Jones C, et al. A positron emission tomography study of quetiapine in schizophrenia: a preliminary finding of an antipsychotic effect with only transiently high dopamine D₂ receptor occupancy. *Arch Gen Psychiatry* 2000;57:553–559.
 116. Pilowsky LS, Mulligan RS, Acton PD, et al. Limbic selectivity of clozapine. *Lancet* 1997;350:490–491.
 117. Farde L, Suhara T, Nyberg S, et al. A PET study of [C-11]FLB 457 binding to extrastriatal D₂-dopamine receptors in healthy subjects and antipsychotic drug-treated patients. *Psychopharmacology* 1997;133:396–404.
 118. Bigliani V, Mulligan RS, Acton PD, et al. *In vivo* occupancy of striatal and temporal cortical D₂/D₃ dopamine receptors by typical antipsychotic drugs. [¹²³I]epidepride single photon emission tomography (SPET) study. *Br J Psychiatry* 1999;175:231–238.
 119. Abi-Dargham A, Hwang DR, Huang Y, et al. Reliable quantification of both striatal and extrastriatal D₂ receptors in humans with [18F]fallypride. *J Nucl Med* 2000;41:139P.
 120. Mukherjee J, Christian BT, Narayan TK, et al. Measurement of striatal and extrastriatal D₂ receptor occupancy by clozapine and risperidone using [18F]fallypride and PET. *Neuroimage* 2000;11:S53.
 121. Robertson GS, Matsumura H, Fibiger HC. Induction patterns of Fos-like immunoreactivity in the forebrain as predictors of atypical antipsychotic activity. *J Pharmacol Exp Ther* 1994;271:1058–1066.
 122. Deutch AY, Lee MC, Iadarola MJ. Regionally specific effects of atypical antipsychotic drugs on striatal fos expression: the nucleus accumbens shell as a locus of antipsychotic action. *Mol Cell Neurosci* 1992;3:332–341.
 123. Martinez D, Hwang DR, Broft A, et al. PET imaging of amphetamine-induced endogenous dopamine release in mesolimbic and nigro-striatal dopamine systems in humans. *J Nucl Med* 2000;41:105P.
 124. Drevets WC, Price JC, Kupfer DJ, et al. PET measures of amphetamine-induced dopamine release in ventral versus dorsal striatum. *Neuropsychopharmacology* 1999;21:694–709.
 125. Wolkin A, Brodie JD, Barouche F, et al. Dopamine receptor occupancy and plasma haloperidol levels. *Arch Gen Psychiatry* 1989;46:482–484.
 126. Fitzgerald PB, Kapur S, Remington G, et al. Predicting haloperidol occupancy of central dopamine D₂ receptors from plasma levels. *Psychopharmacology (Berl)* 2000;149:1–5.
 127. Hagberg G, Gefvert O, Bergstrom M, et al. N-[¹¹C]methylspiperone PET, in contrast to [¹¹C]raclopride, fails to detect D₂ receptor occupancy by an atypical neuroleptic. *Psychiatry Res* 1998;82:147–160.
 128. Lourenco CM, DaSilva JN, Warsh JJ, et al. Imaging of cAMP-specific phosphodiesterase-IV: comparison of [¹¹C]rolipram and [¹¹C]Ro 20-1724 in rats. *Synapse* 1999;31:41–50.
 129. Suhara T, Inoue O, Kobayashi K, et al. An acute effect of triazolam on muscarinic cholinergic receptor binding in the human brain measured by positron emission tomography. *Psychopharmacology (Berl)* 1994;113:311–317.
 130. Ding YS, Logan J, Bermel R, et al. Dopamine receptor-mediated

- regulation of striatal cholinergic activity: positron emission tomography studies with norchloro[18F]fluoroepibatidine. *J Neurochem* 2000;74:1514–1521.
131. Kung MP, Stevenson DA, Plossl K, et al. [99mTc]TRODAT-1: a novel technetium-99m complex as a dopamine transporter imaging agent. *Eur J Nucl Med* 1997;24:372–380.
 132. Lundbom N, Barnett A, Bonavita S, et al. MR image segmentation and tissue metabolite contrast in 1H spectroscopic imaging of normal and aging brain. *Magn Reson Med* 1999;41:841–845.
 133. Spielman DM, Adalsteinsson E, Lim KO. Quantitative assessment of improved homogeneity using higher-order shims for spectroscopic imaging of the brain. *Magn Reson Med* 1998;40:376–382.
 134. Varho T, Komu M, Sonninen P, et al. A new metabolite contributing to N-acetyl signal in 1H MRS of the brain in Salla disease [published erratum appears in *Neurology* 1999;53(5):1162]. *Neurology* 1999;52:1668–1672.
 135. Salibu NaMAB. *Clinical MR spectroscopy*. New York: Wiley-Liss, 1998.
 136. Gunther H. *NMR spectroscopy: basic principle, concepts and applications in chemistry*, second ed. New York: Wiley, 1995.
 137. Gadian DG. *NMR and its application to living systems*, second ed. New York: Oxford University Press, 1995.
 138. Bottomley PA. Human *in vivo* NMR spectroscopy in diagnostic medicine: clinical tool or research probe? *Radiology* 1989;170:1–15.
 139. Murphy DG, Bottomley PA, Salerno JA, et al. An *in vivo* study of phosphorus and glucose metabolism in Alzheimer's disease using magnetic resonance spectroscopy and PET. *Arch Gen Psychiatry* 1993;50:341–349.
 140. O'Callaghan E, Redmond O, Ennis R, et al. Initial investigation of the left temporoparietal region in schizophrenia by 31P magnetic resonance spectroscopy. *Biol Psychiatry* 1991;29:1149–1152.
 141. Pettegrew JW, Keshavan MS, Panchalingam K, et al. Alterations in brain high-energy phosphate and membrane phospholipid metabolism in first-episode, drug-naive schizophrenics. A pilot study of the dorsal prefrontal cortex by *in vivo* phosphorus 31 nuclear magnetic resonance spectroscopy [see comments]. *Arch Gen Psychiatry* 1991;48:563–568.
 142. Pettegrew JW, Keshavan MS, Minshew NJ. 31P nuclear magnetic resonance spectroscopy: neurodevelopment and schizophrenia. *Schizophr Bull* 1993;19:35–53.
 143. Kegeles LS, Shungu DC, Anjilvel S, et al. Hippocampal pathology in schizophrenia: magnetic resonance imaging and spectroscopy studies. *Psychiatry Res* 2000;98:163–175.
 144. Keshevan MS, Stanley JA, Pettegrew J. Magnetic resonance spectroscopy in schizophrenia: methodological issues and findings-Part II. *Biol Psychiatry* 2000;48:369–380.
 145. Kato Tea. Phosphorus-31 magnetic resonance spectroscopy and ventricular enlargement in bipolar disorder. *Psychiatry Research: Neuroimaging* 1994;55:41–50.
 146. Volz HR, Riehemann S, Maurer I, et al. Reduced phosphodiesterases and high-energy phosphates in the frontal lobe of schizophrenic patients: a (31)P chemical shift spectroscopic-imaging study. *Biol Psychiatry* 2000;47:954–961.
 147. Volz HP, Hubner G, Rzanny R, et al. High-energy phosphates in the frontal lobe correlate with Wisconsin Card Sort Test performance in controls, not in schizophrenics: a 31-phosphorus magnetic resonance spectroscopic and neuropsychological investigation. *Schizophr Res* 1998;31:37–47.
 148. Potwarka JJ, Drost DJ, Williamson PC, et al. A 1H-decoupled 31P chemical shift imaging study of medicated schizophrenic patients and healthy controls. *Biol Psychiatry* 1999;45:687–693.
 149. Bluml S, Tan J, Harris K, et al. Quantitative proton-decoupled 31P MRS of the schizophrenic brain *in vivo*. *J Comput Assist Tomogr* 1999;23:272–275.
 150. Deicken RF, Merrin EL, Floyd TC, et al. Correlation between left frontal phospholipids and Wisconsin Card Sort Test performance in schizophrenia. *Schizophr Res* 1995;14:177–181.
 151. Stanley JA, Williamson PC, Drost DJ, et al. An *in vivo* study of the prefrontal cortex of schizophrenic patients at different stages of illness via phosphorus magnetic resonance spectroscopy. *Arch Gen Psychiatry* 1995;52:399–406.
 152. van der Veen JW, et al. Proton MR spectroscopic imaging without water suppression. *Radiology* 2000;217:296–300.
 153. Urenjak J, Williams SR, Gadian DG, et al. Proton nuclear magnetic resonance spectroscopy unambiguously identifies different neural cell types. *J Neurosci* 1993;13:981–989.
 154. Moffett JR, Namboodiri MA. Differential distribution of N-acetylaspartylglutamate and N-acetylaspartate immunoreactivities in rat forebrain. *J Neurocytol* 1995;24:409–433.
 155. Tallan HH. Studies on the distribution of N-acetyl-aspartic acid in brain. *J Biol Chem* 1957;224:41–45.
 156. Kreis DG. Absolute quantitation of water and metabolites in the human brain. II. Metabolite concentrations. *J Magn Reson* 1993;102:9–19.
 157. Huppi PS, Posse S, Lazeyras F, et al. Magnetic resonance in preterm and term newborns: 1H-spectroscopy in developing human brain. *Pediatr Res* 1991;30:574–578.
 158. Hashimoto T, Tayama M, Miyazaki M, et al. Developmental brain changes investigated with proton magnetic resonance spectroscopy. *Dev Med Child Neurol* 1995;37:398–405.
 159. Clark JB. N-acetyl aspartate: a marker for neuronal loss or mitochondrial dysfunction. *Dev Neurosci* 1998;20:271–276.
 160. Bhakoo KK, Pearce D. *In vitro* expression of N-acetyl aspartate by oligodendrocytes: implications for proton magnetic resonance spectroscopy signal *in vivo*. *J Neurochem* 2000;74:254–262.
 161. De Stefano N, et al. Chemical pathology of acute demyelinating lesions and its correlations with disability. *Ann Neurol* 1995;38:901–909.
 162. Hugg JW, Kuzniecky RI, Gilliam FG, et al. Normalization of contralateral metabolic function following temporal lobectomy demonstrated by 1H magnetic resonance spectroscopic imaging. *Ann Neurol* 1996;40:236–239.
 163. Jenkins BG, Klivenyi P, Kustermann E, et al. Nonlinear decrease over time in N-acetyl aspartate levels in the absence of neuronal loss and increases in glutamine and glucose in transgenic Huntington's disease mice. *J Neurochem* 2000;74:2108–2119.
 164. Doraiswamy PM, Charles HC, Krishnan KR. Prediction of cognitive decline in early Alzheimer's disease [letter]. *Lancet* 1998;352:1678.
 165. Jenkins BG, Rosas HD, Chen YC, et al. 1H NMR spectroscopy studies of Huntington's disease: correlations with CAG repeat numbers. *Neurology* 1998;50:1357–1365.
 166. Vion-Dury J, Nicoli F, Salvan AM, et al. Reversal of brain metabolic alterations with zidovudine detected by proton localized magnetic resonance spectroscopy [letter]. *Lancet* 1995;345:60–61.
 167. Bertolino A, et al. The effect of treatment with antipsychotic drugs on brain N-acetylaspartate measures in patients with schizophrenia. *Biol Psychiatry* 2001;49:39–46.
 168. Roffman JL, Lipska BK, Bertolino A, et al. Local and downstream effects of excitotoxic lesions in the rat medial prefrontal cortex on *In vivo* 1H-MRS signals. *Neuropsychopharmacology* 2000;22:430–439.
 169. Bertolino A, Saunders RC, Mattay VS, et al. Altered development of prefrontal neurons in rhesus monkeys with neonatal mesial temporo-limbic lesions: a proton magnetic resonance spectroscopic imaging study. *Cerebral Cortex* 1997;7:740–748.

170. Bertolino A, Roffman JL, Lipska BK, et al. Postpubertal emergence of N-acetylaspartate decreases in prefrontal cortex of rats with neonatal hippocampal damage. *Submitted*.
171. Nasrallah HA, Skinner TE, Schmalbrock P, et al. Proton magnetic resonance spectroscopy (1H MRS) of the hippocampal formation in schizophrenia: a pilot study. *Br J Psychiatry* 1994; 165:481–485.
172. Bertolino A, Weinberger DR. Proton magnetic resonance spectroscopy in schizophrenia. *Eur J Radiol* 1999;30:132–141.
173. Bertolino A, Callicott JH, Nawroz S, et al. Reproducibility of proton magnetic resonance spectroscopic imaging in patients with schizophrenia. *Neuropsychopharmacology* 1998;18:1–9.
174. Bertolino A, Nawroz S, Mattay VS, et al. Regionally specific pattern of neurochemical pathology in schizophrenia as assessed by multislice proton magnetic resonance spectroscopic imaging. *Am J Psychiatry* 1996;153:1554–1563.
175. Bertolino A, Callicott JH, Elman I, et al. Regionally specific neuronal pathology in untreated patients with schizophrenia: a proton magnetic resonance spectroscopic imaging study. *Biol Psychiatry* 1998;43:641–648.
176. Bertolino A, Kumra S, Callicott JH, et al. Common pattern of cortical pathology in childhood-onset and adult-onset schizophrenia as identified by proton magnetic resonance spectroscopic imaging. *Am J Psychiatry* 1998;155:1376–1383.
177. Callicott JH, Egan MF, Bertolino A, et al. Hippocampal N-acetyl aspartate in unaffected siblings of patients with schizophrenia: a possible intermediate neurobiological phenotype [published erratum appears in *Biol Psychiatry* 1999;45(2):following 244]. *Biol Psychiatry* 1998;44:941–950.
178. Deicken RF, Zhou L, Corwin F, et al. Decreased left frontal lobe N-acetylaspartate in schizophrenia. *Am J Psychiatry* 1997; 154:688–690.
179. Deicken RF, Pegues M, Amend D. Reduced hippocampal N-acetylaspartate without volume loss in schizophrenia. *Schizophr Res* 1999;37:217–223.
180. Deicken RF, Zhou L, Schuff N, et al. Proton magnetic resonance spectroscopy of the anterior cingulate region in schizophrenia. *Schizophr Res* 1997;27:65–71.
181. Deicken RF, Johnson C, Eliaz Y, et al. Reduced concentrations of thalamic N-acetylaspartate in male patients with schizophrenia. *Am J Psychiatry* 2000;157:644–647.
182. Lim KO, Adalsteinsson E, Spielman D, et al. Proton magnetic resonance spectroscopic imaging of cortical gray and white matter in schizophrenia. *Arch Gen Psychiatry* 1998;55:346–352.
183. Bartha R, Williamson PC, Drost DJ, et al. Measurement of glutamate and glutamine in the medial prefrontal cortex of never-treated schizophrenic patients and healthy controls by proton magnetic resonance spectroscopy. *Arch Gen Psychiatry* 1997;54:959–965.
184. Stanley JA, Williamson PC, Drost DJ, et al. An *in vivo* proton magnetic resonance spectroscopy study of schizophrenia patients. *Schizophr Bull* 1996;22:597–609.
185. Bartha R, al-Semaan YM, Williamson PC, et al. A short echo proton magnetic resonance spectroscopy study of the left mesial-temporal lobe in first-onset schizophrenic patients. *Biol Psychiatry* 1999;45:1403–1411.
186. Heimberg C, Komoroski RA, Lawson WB, et al. Regional proton magnetic resonance spectroscopy in schizophrenia and exploration of drug effect. *Psychiatry Res* 1998;83:105–115.
187. Kegeles LS, Humaran TJ, Mann JJ. *In vivo* neurochemistry of the brain in schizophrenia as revealed by magnetic resonance spectroscopy. *Biol Psychiatry* 1998;44:382–398.
188. Rakow RL, et al. N-acetylaspartate reduction in dorsolateral prefrontal cortex of patients with schizophrenia as revealed by short-echo time 1H-MRS. *Biol Psychiatry* 1999;45:121S.
189. Weinberger DR. Cell biology of the hippocampal formation in schizophrenia. *Biol Psychiatry* 1999;45:395–402.
190. Moore GJ, Bechuk JM, Hasnat K, et al. Lithium increases N-acetyl-aspartate in the human brain: *in vivo* evidence in support of bcl-2's neurotrophic effects? [In Process Citation]. *Biol Psychiatry* 2000;48:1–8.
191. Rango M, Spagnoli D, Tomei G, et al. Central nervous system trans-synaptic effects of acute axonal injury: a 1H magnetic resonance spectroscopy study. *Magn Reson Med* 1995;33: 595–600.
192. Bertolino A, Roffman JL, Lipska BK, et al. Postpubertal emergence of prefrontal neuronal deficits and altered dopaminergic behaviors in rats with neonatal hippocampal lesions. *Soc Neurosci* 1999.
193. Weinberger DR, Lipska BK. Cortical maldevelopment, antipsychotic drugs, and schizophrenia: a search for common ground. *Schizophr Res* 1995;16:87–110.
194. Bertolino A, Knable MB, Saunders RC, et al. The relationship between dorsolateral prefrontal N-acetylaspartate measures and striatal dopamine activity in schizophrenia [see comments]. *Biol Psychiatry* 1999;45:660–667.
195. Bertolino A, Esposito G, Callicott JH, et al. Specific relationship between prefrontal neuronal N-acetylaspartate and activation of the working memory cortical network in schizophrenia [see comments]. *Am J Psychiatry* 2000;157:26–33.
196. Callicott JH, Bertolino A, Matley VS, et al. Physiological dysfunction of the dorsolateral prefrontal cortex in schizophrenia revisited. *Cerebral Cortex* 2000;10:1078–1092.
197. Callicott JH, Bertolino A, Egan MF, et al. A selective relationship between prefrontal N-acetylaspartate measures and negative symptoms in schizophrenia. *Am J Psychiatry* 2000;157: 1646–1651.
198. Block W, Bayer TA, Tepest R, et al. Decreased frontal lobe ratio of N-acetyl aspartate to choline in familial schizophrenia: a proton magnetic resonance spectroscopy study [In Process Citation]. *Neurosci Lett* 2000;289:147–151.
199. Mattson RH, Petroff O, Rothman D, et al. Vigabatrin: effects on human brain GABA levels by nuclear magnetic resonance spectroscopy. *Epilepsia* 1994;35:S29–32.
200. Sibson NR, Shen J, Mason GF, et al. Functional energy metabolism: *in vivo* 13C-NMR spectroscopy evidence for coupling of cerebral glucose consumption and glutamatergic neuronal activity. *Dev Neurosci* 1998;20:321–330.
201. Blin J, Baron JC, Cambon H, et al. Striatal dopamine D2 receptors in tardive dyskinesia: PET study. *J Neurol Neurosurg Psychiatry* 1989;52:1248–1252.
202. Martinot J-L, Peron-Magnan P, Huret J-D, et al. Striatal D2 dopaminergic receptors assessed with positron emission tomography and 76-Br-bromospiperone in untreated patients. *Am J Psychiatry* 1990;147:346–350.
203. Tune LE, Wong DF, Pearlson G, et al. Dopamine D2 receptor density estimates in schizophrenia: a positron emission tomography study with ¹¹C-N-methylspiperone. *Psychiatry Research* 1993;49:219–237.
204. Nordstrom AL, Farde L, Eriksson L, et al. No elevated D2 dopamine receptors in neuroleptic-naive schizophrenic patients revealed by positron emission tomography and [¹¹C]N-methylspiperone [see comments]. *Psychiatry Res* 1995;61:67–83.
205. Farde L, Wiesel F, Stone-Elander S, et al. D2 dopamine receptors in neuroleptic-naive schizophrenic patients. A positron emission tomography study with [¹¹C]raclopride. *Arch Gen Psychiatry* 1990;47:213–219.
206. Hietala J, Syvälahti E, Vuorio K, et al. Striatal D2 receptor characteristics in neuroleptic-naive schizophrenic patients studied with Positron Emission Tomography. *Arch Gen Psychiatry* 1994;51:116–123.
207. Pilowsky LS, Costa DC, Ell PJ, et al. D2 dopamine receptor binding in the basal ganglia of antipsychotic-free schizophrenic

- patients. An I-123-IBZM single photon emission computerized tomography study. *Br J Psychiatry* 1994;164:16–26.
208. Knable MB, Egan MF, Heinz A, et al. Altered dopaminergic function and negative symptoms in drug-free patients with schizophrenia. [¹²³I]-iodobenzamide SPECT study. *Br J Psychiatry* 1997;171:574–577.
 209. Martinot JL, Paillère-Martinot ML, Loch C, et al. The estimated density of D2 striatal receptors in schizophrenia. A study with positron Emission tomography and 76Br-bromolisuride. *Br J Psychiatry* 1991;158:346–350.
 210. Martinot JL, Paillère-Martinot ML, Loch H C, et al. Central D2 receptors and negative symptoms of schizophrenia. *Br J Pharmacol* 1994;164:27–34.
 211. Laakso A, Vilkkumäki H, Alakare B, et al. Striatal dopamine transporter binding in neuroleptic-naive patients with schizophrenia studied with positron emission tomography. *Am J Psychiatry* 2000;157:269–271.
 212. Choe BY, Kim KT, Suh TS, et al. 1H magnetic resonance spectroscopy characterization of neuronal dysfunction in drug-naive, chronic schizophrenia. *Acad Radiol* 1994;1:211–216.
 213. Renshaw PF, Yurgelun-Todd DA, Tohen M, et al. Temporal lobe proton magnetic resonance spectroscopy of patients with first-episode psychosis. *Am J Psychiatry* 1995;152:444–446.
 214. Yurgelun-Todd DA, Renshaw PF, Gruber SA, et al. Proton magnetic resonance spectroscopy of the temporal lobes in schizophrenics and normal controls. *Schizophr Res* 1996;19:55–59.
 215. Buckley PF, Moore C, Long H, et al. 1H-magnetic resonance spectroscopy of the left temporal and frontal lobes in schizophrenia: clinical, neurodevelopmental, and cognitive correlates. *Biol Psychiatry* 1994;36:792–800.
 216. Maier M, Ron MA, Barker GJ, et al. Proton magnetic resonance spectroscopy: an *in vivo* method of estimating hippocampal neuronal depletion in schizophrenia [published erratum appears in *Psychol Med* 1996;26(4):877]. *Psychol Med* 1995;25:1201–1209.
 217. Maier M, Ron MA. Hippocampal age-related changes in schizophrenia: a proton magnetic resonance spectroscopy study. *Schizophr Res* 1996;22:5–17.
 218. Fukuzako H, Takeuchi K, Hokazono Y, et al. Proton magnetic resonance spectroscopy of the left medial temporal and frontal lobes in chronic schizophrenia: preliminary report. *Psychiatry Res* 1995;61:193–200.
 219. Cecil KM, Lenkinski RE, Gur RE, et al. Proton magnetic resonance spectroscopy in the frontal and temporal lobes of neuroleptic naive patients with schizophrenia. *Neuropsychopharmacology* 1999;20:131–140.
 220. Brooks WM, Hodde-Vargas J, Vargas LA, et al. Frontal lobe of children with schizophrenia spectrum disorders: a proton magnetic resonance spectroscopic study. *Biol Psychiatry* 1998;43:263–269.
 221. Thomas MA, Ke Y, Levitt J, et al. Preliminary study of frontal lobe 1H MR spectroscopy in childhood-onset schizophrenia. *J Magn Reson Imaging* 1998;8:841–846.
 222. Fukuzako H, Kodama S, Fukuzako T, et al. Subtype-associated metabolite differences in the temporal lobe in schizophrenia detected by proton magnetic resonance spectroscopy. *Psychiatry Res* 1999;92:45–56.

