Imaging Changes in Glutamate Transmission In Vivo with the Metabotropic Glutamate Receptor 5 Tracer [^{11}C] ABP688 and *N*-Acetylcysteine Challenge

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Background: An imaging method to probe glutamate levels in vivo would allow the study of glutamate transmission in disease states and in response to therapeutic interventions. Here we demonstrate the feasibility of this approach for the first time using positron emission tomography and [^{11}C] ABP688, a radiotracer for an allosteric site on the metabotropic glutamate receptor 5.

Methods: We conducted two sets of experiments in anesthetized baboons: test and retest without pharmacologic challenge and in combination with *N*-acetylcysteine (NAC), a promoter of the cystine-glutamate antiporter that increases extrasynaptic glutamate release. The goal was to assess whether NAC-induced changes in [^{11}C] ABP688 binding potential, ΔBP_{ND}, could be detected above the noise in the measurement.

Results: Linear mixed modeling comparing ΔBP_{ND} from test–retest to ΔBP_{ND} from NAC challenge across all brain regions showed a highly significant effect of treatment [$F(1,40) = 21.2, p < .001$]. ΔBP_{ND} was significantly different from zero following NAC [$F(1,20) = 76.6, p < .001$] but not after test–retest studies.

Conclusions: NAC induced decrease in [^{11}C] ABP688 ΔBP_{ND} may be the result of allosteric modulation, although other mechanisms may be at play. We outline steps needed to replicate and validate this method as a new tool to measure in vivo glutamate transmission.

Key Words: ABP688, allosteric modulator, glutamate transmission, mGluR5, *N*-acetylcysteine, test–retest

Imaging acute fluctuations in endogenous dopamine using positron emission tomography (PET) has been a major development in the field of imaging and has provided tools for the understanding of dopamine alterations in schizophrenia (1) and addiction (2). Abnormal glutamate transmission has been implicated in both schizophrenia and addiction by multiple lines of research, and interventions targeting the glutamate system are in development. However, there are no imaging techniques capable of measuring acute fluctuations in endogenous levels of glutamate in vivo. One major difficulty has been the lack of radiotracers for a glutamate receptor that are sensitive to changes in glutamate levels induced by a challenge drug, as shown for dopamine (3).

The glutamate system can be divided into a synaptic and nonsynaptic system (4,5). Extracellular glutamate levels are regulated by reuptake through the glutamate transporter (GLT1) and the glutamate-aspartate transporter on glial cells (6). The reuptake of glutamate by GLT1 and glutamate-aspartate transporter separates the nonsynaptic from the synaptic glutamate system by preventing "spill-in and spill-out" between compartments (4, 7–10). In the nonsynaptic system, glutamate is released from glial cells through the cystine-glutamate antiporter. The antiporter is widely distributed in the nucleus accumbens, ventral tegmental area, prefrontal cortex, amygdala, and cerebral cortex where it is expressed on glial cells, neurons, and vascular endothelial cells (11,12). The antiporter plays an important role not only for glutamate release but also cysteine-dependent glutathione synthesis, which protects the cell against oxidative stress. Administration of *N*-acetylcysteine (NAC) facilitates the activity of the cystine-glutamate antiporter and increases glutamate release and glutathione synthesis (13).

Here we present our study assessing the initial feasibility of using [^{11}C] ABP688 (three-(6-methyl-pyridine-2-ylethynyl)-cyclohex-2-enone-*O*-^{11}C-methyloxime), a specific radiotracer for an allosteric site on the metabotropic glutamate receptor 5 (mGluR5) (14), in combination with NAC, to assess NAC-induced changes in [^{11}C] ABP688 binding in anesthetized monkeys. To determine whether an effect of NAC could be demonstrated above any noise in the measurement, we compared the change in [^{11}C] ABP688 binding between baseline and NAC challenge conditions to the change between test and retest without pharmacologic challenge.

Methods and Materials

Subjects

Four adult male baboons (*Papio anubis*, baboons A, B, C, and D, 24 ± 4 kg) were scanned. Baboons A, B, and C were scanned under test–retest conditions; A, B, and D were scanned before and after pharmacologic challenge. All study procedures were approved by the Institutional Animal Care and Use Committees of Columbia University and New York State Psychiatric Institute.

Radiochemistry

The ABP688 precursor, three-(6-methyl-pyridine-2-ylethynyl)-cyclohex-2-enone-oxime, was purchased from Texas Biochemicals (College Station, Texas). [^{11}C]ABP688 was synthesized using a modified literature method (14). [^{11}C]CO_2 was converted to [^{11}C]CH_3I by lithium aluminium hydride reduction and hydroiodic acid iodination; [^{11}C]CH_3I was converted to [^{11}C] methyl triflate ([^{11}C] CH_3OTf) by sweeping [^{11}C]CH_3I vapor through a heated glass column containing silver-triflate-impregnated graphitized carbon. ABP688 was labeled with ^{11}C by reacting the oxime precursor (desmethyl-ABP688) in acetone with [^{11}C] CH_3OTf in the presence of base at

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Table 1. Scan Parameters

	f_p	V_{ND}	ID (mCi)	IM (μg)	SA (Ci/mmol)	CL (L/h)
Test Baseline	2.2 ± .4 %	3.31 ± .43	3.62 ± .25	.51 ± .16	1576 ± 323	27.40 ± 9.20
Retest	1.9 ± .5 %	3.25 ± .64	3.34 ± .38	.65 ± .18	1172 ± 389	27.01 ± 7.87
t test (*p*)	.19	.88	.53	.09	.06	.91
NAC Baseline	1.9 ± .8 %	2.88 ± .28	2.47 ± .37	.44 ± .25	1458 ± 811	24.92 ± 5.27
Post NAC	1.8 ± .6 %	2.89 ± .37	2.45 ± .93	.61 ± .24	943 ± 616	24.26 ± 4.47
t test (*p*)	.41	.96	.97	.05	.05	.89

CL, radioligand peripheral clearance; f_p, plasma free fraction; ID, injected radioactivity dose; IM, injected mass; NAC, *N*-acetylcysteine; SA, specific activity; V_{ND}, cerebellum distribution volume.

room temperature. The product was purified by semipreparative high-performance liquid chromatography using a reversed-phase column (Prodigy C18, Phenomenex, Torrance, California; 10 × 250 mm; 10 μm; mobile phase, acetonitrile: .1 mol/L ammonium formate, 50:50; flow rate, 10 mL/min); retention time was 9 min. The high-performance liquid chromatography solvent was removed by trapping the tracer in a C-18 Sep-Pak. [^{11}C]ABP688 was formulated for administration in 10 mL ethanol in saline (v/v = 1:9).

Experimental Procedures

Animals were scanned under isoflurane anesthesia as previously described (15). Scans were performed on an HR+ scanner (Siemens, Knoxville, Tennessee), in three-dimensional mode. Dynamic data were acquired for 90 min following a 30-sec intravenous bolus of [^{11}C]ABP688. Data acquisition, image reconstruction, collection, and analysis of arterial plasma data and plasma free fraction of [^{11}C]ABP688 were performed as previously described (15). On each study day, two scans were acquired. For test–retest, a baseline scan was acquired followed by a 30-min interval followed by the retest scan. For NAC challenge, a baseline scan was acquired, followed by a 60-min intravenous infusion of NAC (50 mg/kg dissolved in saline), followed by a postchallenge scan.

Data Analysis

For the PET data, decay-corrected reconstructed data were coregistered to anatomic magnetic resonance imaging scans previously acquired for each animal, using maximization of mutual information as implemented in SPM2 (16).

Regions of interest were drawn on the magnetic resonance imaging scans and transferred to coregistered PET images. Included regions were the associative, sensorimotor, and ventral subregions of the striatum, cingulate, hippocampus, thalamus, and frontal, occipital, parietal, and temporal cortices. Cerebellum was included as a reference region. Time activity curves were generated from regions of interest data and fitted to a two-tissue compartment model with arterial plasma input. Total distribution volume (V_T) was computed from the estimated rate constants. BP_{ND}, the binding potential relative to the nondisplaceable compartment (17), was computed as

$$\frac{V_T(ROI)}{V_T(CER)} - 1$$

The percentage change across conditions in BP_{ND} (ΔBP_{ND}) was computed as

$$\Delta BP_{ND} = \frac{BP_{ND}(Post)}{BP_{ND}(Baseline\ scan)} - 1$$

Statistics

Scan parameters were assessed across conditions by two-tailed paired *t* tests. To test for differences between test–retest and NAC challenge in ΔBP_{ND}, a linear mixed model was applied. *p* values less than .05 were considered statistically significant.

Results

Scan parameters are presented in Table 1. There were no significant differences in plasma free fraction, cerebellum distribution volume, injected radioactivity dose, or radioligand peripheral clearance across conditions. Injected mass was higher in the second scan for both conditions but remained below 1 μg for all scans. Importantly, there was no difference between baseline and challenge conditions in cerebellar V_{ND}, demonstrating the appropriateness of the cerebellum as a reference tissue.

BP_{ND} is presented in Table 2. Linear mixed modeling comparing ΔBP_{ND} from test–retest to ΔBP_{ND} from the NAC challenge across all

Table 2. BP_{ND} and ΔBP_{ND} ± SD in All Regions

	Test–Retest				NAC Challenge			
	Test	Retest	ΔBP_{ND}	*p*	Baseline	NAC	ΔBP_{ND}	*p*
AST	.84 ± .07	.86 ± .11	2% ± 11%	.790	1.01 ± .21	.88 ± .13	−12% ± 4%	.095
SMST	.55 ± .23	.57 ± .28	2% ± 7%	.535	.55 ± .41	.54 ± .36	2% ± 18%	.829
VST	.74 ± .33	.79 ± .47	3% ± 16%	.573	.95 ± .48	.75 ± .39	−20% ± 8%	.094
Cingulate	.75 ± .03	.79 ± .13	5% ± 14%	.577	.91 ± .16	.76 ± .10	−16% ± 5%	.062
Frontal Cortex	.73 ± .07	.76 ± .13	5% ± 20%	.728	.90 ± .08	.75 ± .12	−16% ± 11%	.107
Hippocampus	.85 ± .08	.84 ± .19	−2% ± 14%	.917	1.07 ± .34	.91 ± .25	−14% ± 5%	.107
Occipital Cortex	.51 ± .05	.52 ± .04	4% ± 17%	.819	.63 ± .10	.49 ± .02	−21% ± 13%	.158
Parietal Cortex	.56 ± .19	.57 ± .28	−2% ± 21%	.818	.70 ± .28	.57 ± .21	−16% ± 8%	.121
Temporal Cortex	.92 ± .14	.98 ± .31	5% ± 20%	.622	1.21 ± .32	1.04 ± .25	−13% ± 9%	.167
Thalamus	.52 ± .16	.53 ± .14	3% ± 11%	.870	.49 ± .07	.46 ± .08	−6% ± 12%	.461

p values are paired *t* tests for the change between test and retest or baseline and NAC in $n = 3$ subjects for each region.

AST, associative striatum; BP_{ND}, binding potential relative to the nondisplaceable compartment; NAC, *N*-acetylcysteine; SMST, sensorimotor striatum; VST, ventral striatum.

10 measured regions showed a highly significant effect of treatment [$F(1,40) = 21.2$, $p < .001$]. ΔBP_{ND} was significantly different from zero following NAC [$F(1,20) = 76.6$, $p < .001$] but not for test–retest. Effects of region and region by treatment interaction were not statistically significant.

Discussion

In this report, we present the first demonstration of the effect of a pharmacologic challenge on the binding of a PET glutamate receptor tracer. We believe the change seen is due to increased glutamate levels, but future studies are needed to confirm this. We propose that the NAC-induced increase in extrasynaptic glutamate levels may have produced an affinity shift in the binding of the tracer to the allosteric site, but many other mechanisms may be at play and will need to be considered in future studies. An allosteric modulation may be the simplest explanation, because BP_{ND} is proportional to the affinity of the radiotracer for the binding site, a decrease in BP_{ND} is consistent with an allosteric interaction in which the affinity of [^{11}C] ABP688 for mGluR5 is reduced compared with baseline. Although this is a proposed interpretation, much work is necessary to confirm the nature of the interaction and to rule out other potential explanations. However, the study reported here is an encouraging step to justify this validation work.

[^{11}C] ABP688 BP_{ND} was significantly decreased following NAC, but it was not different between test and retest, indicating that the observed difference following NAC was not likely due to mass carryover or some other effect of scan order and is robust enough to be detected reliably above noise level in the measurement. Furthermore, the magnitude of the decrease was in the range observed with the amphetamine challenge and [^{11}C] raclopride. The effect was consistently detected across all three animals, with some variation in the magnitude of the change. It was also detected in all measured regions except for the sensorimotor striatum or posterior putamen. The reasons for the lack of effect in this area are unclear because there are no studies showing the normal distribution of the antiporter pump in the brain to suggest regional differences.

Future studies are necessary to validate this observation, and our proposed interpretation of allosteric modulation includes replication of this initial observation, confirmation that the change in binding of [^{11}C]ABP688 is not the result of a direct displacement by either NAC or glutamate (neither of these has been shown to bind to the allosteric site on the mGluR5 to which the tracer binds), altering levels of glutamate using other challenges to provide additional proof of concept, and comparing various levels of change in glutamate levels measured with microdialysis in primates to ΔBP_{ND} measured with PET in the same subjects to demonstrate an association between the two measurements, as shown for imaging dopamine transmission (18). Furthermore, observing this effect in awake subjects will be crucial to justify further validation work and to rule out an interaction between the anesthesia, used here in nonhuman primates, and the sensitivity of the antiporter pump. If these validation studies are positive and the proposed mechanism is confirmed, [^{11}C]ABP688 PET imaging with a pharmacologic challenge will be a potential tool for detecting changes in glutamate levels in vivo. Furthermore, validation of the method in humans will allow testing in clinical populations in which alterations in glutamate transmission are suspected, such as addiction, anxiety disorders, and schizophrenia.

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1. Kegeles LS, Abi-Dargham A, Frankle WG, Gil R, Cooper TB, Slifstein M, *et al.* (2010): Increased synaptic dopamine function in associative regions of the striatum in schizophrenia. *Arch Gen Psychiatry* 67:231–239.
2. Volkow ND, Fowler JS, Wang GJ (2003): The addicted human brain: Insights from imaging studies. *J Clin Invest* 111:1444–1451.
3. Laruelle M (2000): Imaging synaptic neurotransmission with in vivo binding competition techniques: A critical review. *J Cereb Blood Flow Metab* 20:423–451.
4. Huang YH, Bergles DE (2004): Glutamate transporters bring competition to the synapse. *Curr Opin Neurobiol* 14:346–352.
5. Kalivas PW (2009): The glutamate homeostasis hypothesis of addiction. *Nat Rev Neurosci* 10:561–572.
6. Gegelashvili G, Schousboe A (1998): Cellular distribution and kinetic properties of high-affinity glutamate transporters. *Brain Res Bull* 45:233–238.
7. Arnth-Jensen N, Jabaudon D, Scanziani M (2002): Cooperation between independent hippocampal synapses is controlled by glutamate uptake. *Nat Neurosci* 5:325–331.
8. Tanaka K (2000): Functions of glutamate transporters in the brain. *Neurosci Res* 37:15–19.
9. Huang YH, Sinha SR, Tanaka K, Rothstein JD, Bergles DE (2004): Astrocyte glutamate transporters regulate metabotropic glutamate receptor-mediated excitation of hippocampal interneurons. *J Neurosci* 24:4551–4559.
10. Yang Y, Gozen O, Watkins A, Lorenzini I, Lepore A, Gao Y, *et al.* (2009): Presynaptic regulation of astroglial excitatory neurotransmitter transporter GLT1. *Neuron* 61:880–894.
11. Burdo J, Dargusch R, Schubert D (2006): Distribution of the cystine/glutamate antiporter system xc-in the brain, kidney, and duodenum. *J Histochem Cytochem* 54:549–557.
12. Knackstedt LA, LaRowe S, Mardikian P, Malcolm R, Upadhyaya H, Hedden S, *et al.* (2009): The role of cystine-glutamate exchange in nicotine dependence in rats and humans. *Biol Psychiatry* 65:841–845.
13. Rathinam ML, Watts LT, Stark AA, Mahimainathan L, Stewart J, Schenker S, Henderson GI (2006): Astrocyte control of fetal cortical neuron glutathione homeostasis: Up-regulation by ethanol. *J Neurochem* 96:1289–1300.
14. Ametamey SM, Kessler LJ, Honer M, Wyss MT, Buck A, Hintermann S, *et al.* (2006): Radiosynthesis and preclinical evaluation of C-11-ABP688 as a probe for imaging the metabotropic glutamate receptor subtype 5. *J Nucl Med* 47:698–705.
15. Slifstein M, Suckow RF, Javitch JA, Cooper T, Lieberman J, Abi-Dargham A (2010): Characterization of in vivo pharmacokinetic properties of the dopamine D1 receptor agonist DAR-0100A in nonhuman primates using PET with [(11)C] NNC112 and [(11)C] raclopride. *J Cereb Blood Flow Metab* 2011:293–304.
16. Friston KJ, Holmes AP, Worsley KJ, Poline J-P, Frith CD, Frakowiak RSJ (1995): Statistical parametric maps in functional imaging: A general linear approach. *Hum Brain Mapp* 2:189–210.
17. Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, *et al.* (2007): Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *J Cereb Blood Flow Metab* 27:1533–1539.
18. Laruelle M, Iyer RN, al-Tikriti MS, Zea-Ponce Y, Malison R, Zoghbi SS, *et al.* (1997): Microdialysis and SPECT measurements of amphetamine-induced dopamine release in nonhuman primates. *Synapse* 25:1–14.