Caffeine's Attenuation of Cocaine-Induced Dopamine Release by Inhibition of Adenosine

Lauren B. Malave, MS,¹⁻³ and Patricia A. Broderick, PhD¹⁻⁴

Background: It is well known that the reinforcing properties of cocaine addiction are caused by the sharp increase of dopamine (DA) in the reward areas of the brain. However, other mechanisms have been speculated to contribute to the increase. Adenosine is one system that is associated with the sleep-wake cycle and is most important in regulating neuronal activity. Thus, more and more evidence is pointing to its involvement in regulating DA release. The current study set out to examine the role of adenosine in cocaine-induced DA release.

Methods: Increasing doses of cocaine, caffeine, and their combination, as well as, 8-cyclopentyltheophylline (CPT), an adenosine A1 antagonist (alone and in combination with cocaine) were used to denote a response curve. A novel biosensor, the BRODERICK PROBE[®] was implanted in the nucleus accumbens to image the drug-induced surge of DA release *in vivo*, in the freely moving animal in real time.

Results: Combinations of cocaine and caffeine were observed to block the increased release of DA moderately after administration of the low dose (2.5 mg/kg cocaine and 12.5 mg/kg caffeine) and dramatically after administration of the high dose (10 mg/kg cocaine and 50 mg/kg caffeine), suggesting neuroprotection. Similarly, CPT and cocaine showed a decreased DA surge when administered in combination. Thus, the low and high dose of a nonselective adenosine antagonist, caffeine, and a moderate dose of a selective adenosine antagonist, CPT, protected against the cocaine-induced DA release.

Conclusions: These results show a significant interaction between adenosine and DA release and suggest therapeutic options for cocaine addiction and disorders associated with DA dysfunction.

Introduction

ADENOSINE IS A ubiquitous purine molecule that plays a key modulatory role in both dorsal and ventral dopaminergic transmission. Adenosine has been shown to decrease neural activity. One substance that targets the adenosine system is coffee, which is a commonly ingested drink containing the active ingredient caffeine. Caffeine is a psychostimulant that promotes wakefulness by nonselectively antagonizing the adenosine A1 receptors (A1R) and A2 receptors (A2AR) in the nucleus accumbens (NAcc).^{1,2} The NAcc is the brain area involved in motivation and motor response. Similarly, cocaine is strongly associated with the NAcc. Cocaine causes a euphoric sensation by the significant increase of dopamine (DA) through the inhibition of DA transporters.³ Previous findings show that A1Rs are co-expressed with DA type 1 receptors, and A2AR with DA type 2 receptors.^{1,4}

There are observed interactions between adenosine and DA that regulate behavior.⁵⁻⁷ Activation of A1Rs and A2ARs were found to be involved in the depressant effects of caffeine on locomotion.¹ A1Rs were found to act as inhibitory G-coupled proteins that block adenylyl cyclase activity.¹ Thus, antagonizing A1Rs would block this inhibition and cause an increase in DA. On the other hand, it was discovered that caffeine blocked the inhibitory actions of adenosine on DA transmission by A2AR antagonism on GABA neurons.⁸⁻¹¹ Thus, caffeine inhibits the inhibitory actions of GABA neurons on DA neurons. In addition, it is well established that caffeine produces biphasic effects on locomotion.¹ Thus, the effects are highly dose-dependent.^{3,12,13}

Cocaine abuse is seen in more than 1.8 million Americans.¹⁴ Given the highly addictive properties of cocaine this phenomenon has become a public health concern. Mechanisms to better understand cocaine use are being intensively

Two abstracts were presented and published at the *Society for Neuroscience* as follows: (1) Broderick, P.A., Murthy, V. and Forrester, L.W., Adenosine A1 antagonist 8-cyclopentyltheophylline mediates cocaine interactions; a view from the ventrolateral nucleus accumbens. Soc Neurosci Abstr. 32: Atlanta, GA October 11-18 (2006). (2) Broderick, P.A., Ho, H., Huang, J., Wat, K., Shahidullah, J., Murthy, V., Wenning, L. and Buchheim, L., Caffeine may neuroprotect against cocaine-induced dopamine release in accumbens of female rats. Soc. Neurosci. Abstr. 33: San Diego, CA (2007).

¹Department of Physiology, Pharmacology and Neuroscience, The Sophie Davis School, The City College of New York, New York, New York.

²Department of Biology, CUNY Graduate Center, New York, New York.

³Center for Advanced Technology (CAT) CUNY, New York, New York.

⁴Department of Neurology, NYU Langone Medical Center, New York, New York.

examined. Animal studies have shown that pharmacologically altering adenosine affects the locomotor reinforcing and sensitizing properties of cocaine.^{9,15} A1R antagonists have been shown to augment cocaine-seeking behavior in a dose-specific manner.^{3,13} Thus, the previous data suggesting that caffeine, via adenosine, strongly interacts with cocaine and may provide future pharmacotherapies.

This study set out to directly measure DA neurotransmission in the ventrolateral NAcc produced by cocaine, caffeine, and 8-cyclopentyltheophylline (CPT), an adenosine A1 antagonist. In addition, the combination of cocaine with caffeine and cocaine with CPT were studied. Low and high doses of cocaine and caffeine were used to test the dose-dependent effect on DA release.⁵

Detection of neurotransmission was done using neuromolecular imaging (NMI) with novel biosensors: The BRODERICK PROBE[®], *in vivo* within seconds, on line and in real time.^{16,17} This biotechnological advance is based on conventional electrochemistry.¹⁸ We focused on imaging DA directly in ventrolateral NAcc. The ventrolateral NAcc was selected because DA axons, which project from the ventral tegmental area, terminate on neurons in silver intensification procedures and also indicate high concentrations of DA in ventrolateral NAcc.^{5,19} Herein, NMI provided a close cause and effect relationship between drug and brain.

Materials and Methods

Neuromolecular imaging

NMI has made significant advances in the field of electrochemical methods. Specifically, (a) formulations and detection capabilities of biosensors are different. We embedded a series of saturated and unsaturated fatty acid and lipid surfactant assemblies into carbon-paste-based biosensors in a range of concentrations to allow advanced detection capabilities, for example, selective imaging of ascorbic acid, DA, serotonin (5-HT), Homovanillic acid, L-Tryptophan (L-Trp), and peptides, such as dynorphin and somatostatin²⁰ (b) with NMI bio-

sensors, there is no need for cumbersome head stages as are needed by conventional *in vivo* voltammetric and microvoltammetric methods because NMI biosensors have low resistance properties, (c) NMI biosensors are resistant to bacterial growth, (d) unlike carbon fiber biosensors, NMI biosensors do not form gliosis, that is, scar tissue that impedes detection of neurotransmitters, causing electrochemical signals to decay, and (e) like other carbon-paste-based biosensors, NMI biosensors respond to the lipid matrix of the brain by enhancing electron transfer kinetics; this property improves the sensitivity, selectivity, and operational stability of the biosensors, allowing the detection of reliable electrochemical signals that are long-lasting.^{16,17,19,21–24} Procedure and biosensor specifications are previously discussed.^{18,25}

In this article, results from NMI laurate biosensors are presented. Lauric acid has a hydrophobic head, hydrophilic tail, and acts as a surfactant to reduce surface tension. The surfactant, lauric acid, also acts to assist the migration of molecules to form an oriented, adsorbed film on the interfacial surface of the indicator sensor. This mechanism is a key characteristic for electron transfer kinetics exhibited by NMI biosensor subtypes. Figure 1 shows a schematic diagram of a BRODERICK PROBE biosensor with specifications (a) and scans (b–c).

Animals and surgical procedures

These studies are approved by the National Institutes of Health (NIH) in accordance with the Institutional Animal Care and Use Committee (IACUC) of the City College New York, the City University of New York. To begin these studies, we purchase male, Sprague Dawley, laboratory rats (*rattus norvegicus*) from Charles River Laboratories, Kingston, NY. When the animals arrive at our facility, they are housed in our Marshak Vivarium for about 1 week before surgery begins to allow animals to become acclimated to their environment. Animals are fed Purina Rat Chow and water *ad libitum*. A 12 hour dark–light cycle is maintained both in the Vivarium and in Dr. Broderick's research laboratory where studies take place, to maintain animals' circadian rhythms.

FIG. 1. (a) Schematic of the BRODERICK PROBE[®] biosensor with specifications. (b, c) scans depicting neurotransmitter detection *in vivo*. Neuromolecular imaging recordings of neurotransmitters are drawn from raw data. (b) shows serotonin (5-HT) and L-Tryptophan (L-Trp). (c) shows ascorbic acid, dopamine (DA), and 5-HT. The y axis shows current changes in nanoamperes (nA) from baseline. The x axis shows applied potential in subunits of Volts (millivolts). Each neurochemical exhibits electron transfer properties at a specific oxidation potential. Current is proportional to the concentration of each neurochemical as described by the Cottrell equation.

Surgery begins with an intraperitoneal (ip) injection of the anesthetic pentobarbital Na (50 mg/kg in a dilute 6% solution). Laurate biosensors are inserted stereotaxically (Kopf Stereotaxic) within NAcc (AP = +2.5; ML = +2.6; DV = -7.3) and an Ag/AgCl microreference and stainless steel micro-auxiliary are placed in contact with dura. Indicator laurate biosensors are held in place with Splintline Acrylic (Lang Dental). Temperature is continuously monitored with a rectal probe and thermometer (Fisher Sci.); temperature is maintained at $37.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ with an aquamatic K module heating pad (Amer. Hosp. Supply). Pinnal, corneal, and leg flexion responses are monitored throughout surgery and supplemental doses of Na pentobarbital are administered to maintain adequate pharmacokinetic induction and depth of anesthesia. Physiological saline (based on animal body weight) is injected at the completion of surgery to maintain proper electrolyte and volumetric status. The total time for surgery is two to three hrs. Animals are individually housed after surgery and recover from surgery with food and water *ad lib* before the experimental studies begin.

Study design

NMI electrochemical signals for neurochemicals are separately recorded at distinct oxidation potentials, within seconds and sequentially. Recordings are repeated every 5 minutes for a period of 2 hours before drug(s) are administered. Concentrations of neurotransmitters were directly correlated to their wavelength peak.

Drugs and doses

There were five treatment groups: cocaine, caffeine, CPT, cocaine/caffeine, and cocaine/CPT. There were 2 dose groups: low and high doses of cocaine, caffeine, and cocaine/caffeine. On the other hand, there was one dose for CPT and combination of cocaine/CPT. Doses were chosen based on peak effects from previous literatures.^{10,11,26} Sigma Aldrich, supplied all drugs and solutions were dissolved in distilled water per body weight of animal. The doses are described in Table 1.

Confirmation of biosensor placement

Placement within NAcc is confirmed by the potassium ferrocyanide blue dot method (specifications: current in mA, 30; time in seconds, 40).

TABLE 1. SPECIFIED DRUGS AND DOSES USED THROUGHOUT THE EXPERIMENT

Drug	Dose
Cocaine	LD: 2.5 mg/kg HD: 10 mg/kg
Caffeine	LD: 12.5 mg/kg HD: 50 mg/kg
CPT	4.8 mg/kg
Cocaine and Caffeine combination	LD: 2.5 mg/kg cocaine/12.5 mg/kg caffeine HD: 10 mg/kg cocaine/50 mg/kg caffeine
Cocaine and CPT combination	5 mg/kg cocaine/4.8 mg/kg CPT

CPT, 8-cyclopentyltheophylline; HD, high dose; LD, low dose.

FIG. 2. Concentration of DA release in the nucleus accumbens (NAcc) was measured over a 2-hour period after acute administration of cocaine, caffeine, and combination of both. The median is shown above. Baseline neurotransmitter release was adjusted to 100 and the data reported are a percentage over that. DA concentrations for low (LD; 2.5 mg/kg cocaine and 12.5 mg/kg caffeine), and high (HD; 10 mg/kg cocaine and 50 mg/kg caffeine) dose were shown for cocaine, caffeine, and combination of both. Significance was denoted by $p < 0.005$. *Significant to low dose (LD) cocaine and LD combination groups, ^significant to HD caffeine and HD combination groups.

Statistics

Multi Analysis of Variance (ANOVA) as studied by the Statistica Software program is used to determine statistically significant differences in neurochemical responses after the administration of drug(s) within and between the major

FIG. 3. The effect of A1R antagonism on DA release in the NAcc. Values were averages over the 2 hours shown above and same as described in Figure 2. DA release is shown for cocaine at 5 mg/kg dose, CPT 4.8 mg/kg dose, and a combination of both. Significance was measured according to $p < 0.005$. *Significant to all other groups. A1R, A1 receptor.

groups (dose and drug) studied. *Bonferroni* and *Fisher post hoc* tests performed statistical analyses within each individual group. Alpha significance is set at the $p=0.05$ level.

Results

DA release: cocaine and caffeine

Both cocaine and caffeine increased DA release above baseline release (set at 100). An overall factorial ANOVA showed a drug $\times$ dose effect [$F(2, 18)=6.3032$, $p<0.008417$]. As expected, cocaine linearly increased DA in a dose-dependent manner. *Fisher post hoc* analysis revealed a significant increase from low dose to high dose of cocaine ($p<0.0023$). On the other hand, caffeine decreased DA release from low dose to the high-dose group ($p<0.0072$). There were no significant differences in DA release within the combination group ($p<0.5798$). However, there was an imperative decrease from the cocaine group to the combination group during the high dose ($p<0.0040$). Additionally, there was a significant decrease in DA release from the cocaine high-dose group compared with the caffeine high-dose group ($p<0.0072$). Furthermore, in the low-dose groups, caffeine showed significantly higher DA release when compared with the combination group ($p<0.03484$) (Fig. 2).

DA release: cocaine and CPT

All groups increased DA release over baseline. DA release was lower in the combination group compared with the cocaine and CPT groups. One-way univariate ANOVA test showed a drug effect [$F(2, 12)=17.383$, $p<0.00004$]. A strict *post hoc Bonferroni* pairwise test revealed a significant decrease from the cocaine group to the combination group ($p<0.0399$) and from the CPT group to the combination group ($p<0.00003$). DA release was similar for both the cocaine and CPT group (Fig. 3).

Discussion

A surge of DA in the reward centers of the brain is a strengthening aspect in addiction. Increased concentration of DA is correlated to spikes of action potentials.³ Alcohol and drugs of abuse all have this spike of DA release in the NAcc. Past studies have shown DA concentrations *in vivo* via microdialysis. However, the BRODERICK PROBE biosensor is the first direct NMI that detects DA release on line and in real time.^{18,20,24} DA release occurs in a dose-dependent manner. Cocaine enhanced the amount of DA in the synapse allowing for more activation on postsynaptic cells. Cocaine induced a linear increase of DA release from the low dose to the high dose, as expected.

Caffeine has nonselective properties on A1Rs and A2ARs. Previous studies have revealed that caffeine elicits an inverted “U-shaped” dose–response curve.^{3,11–13} Our results showed that at the low dose a ceiling effect of DA release occurred while DA release was reduced after the high dose. The increase in DA release over baseline shows a potential interaction between DA and adenosine.

Conditioned placed preference studies have shown an additive effect of a combination of cocaine and caffeine at low doses.²⁷ However, high doses of this combination immediately eliminated this cocaine seeking behavior.¹ Similarly, our results show strong neuroprotective behavior following

high doses of caffeine and cocaine on DA release. Following administration of 50 mg/kg, caffeine blocked this cocaine-induced increase in DA release. Previous studies have shown that at doses exceeding 50 mg/kg, other mechanisms of action come into play as well, such as inhibition of phosphodiesterase and release of intracellular calcium.²⁸ Furthermore, we speculate that at high doses of caffeine, adenosine receptors are all inhibited inducing a “selective” antagonist characteristic, and thus, a protective effect. This effect can be further examined using selective adenosine receptor antagonism to determine the specific adenosine receptor responsible for this response.

To further explore the mechanisms behind this neuroprotective behavior we tested the adenosine A1 selective antagonist. Our results showed strong evidence of the involvement of A1Rs. Both cocaine and CPT induced an increase in DA release in the NAcc. However, after combination of both cocaine and CPT, release was significantly decreased. Acting presynaptically on autoreceptors could cause the CPT-induced increase in DA release since A1Rs are coupled to D1 autoreceptors.²⁹ The combination of both caused a reduced DA release effect. Past studies have shown that A1Rs are activated first by caffeine, while higher doses then activate A2ARs.³⁰ A1Rs are thought to be associated with the tolerance aspect of caffeine; A2ARs have been extensively shown to be involved in the release of DA.^{1,31–34} Thus, our results propose a necessary role of A1Rs as well. Other doses should be used to create a dose–response curve. Overall, this study provides valuable insight into the relationship between DA and adenosine.

Conclusion

These results are important findings for therapeutic options in cocaine addiction suggesting treatment therapies with adenosine antagonism at high doses to block the euphoric effect associated with increased DA release. Additionally, adenosine may be a potential option in other mental disorders or diseases associated with DA dysfunction. Parkinson's disease has been shown to benefit from pharmacological alterations to adenosine. Additionally, a schizophrenic phenotype is induced by cocaine administration in laboratory animals. Thus, adenosine may have an underlying role in schizophrenia that has not been previously explored since schizophrenia is still a highly elusive disorder.

In conclusion, these results provide further insights in understanding the neurochemistry of cocaine, presently focusing on the adenosine system and its potential neuroprotective behavior. Furthermore, we suggest potential treatment insight/options for mental disorders involving malfunction of the DA neurocircuits in concert with adenosine interaction.

Acknowledgments

Lauren Malave, B.S., is a candidate for the Master's Degree in Biology, CCNY, CUNY. Lauren is performing research in Dr. Broderick's laboratory in the Department of Physiology, Pharmacology, and Neuroscience at the Sophie Davis School of Biomedical Education, CCNY, CUNY. Dr. Broderick is Principal Investigator, Medical Professor and Mentor.

The authors wish to thank the Broderick Brain Foundation, the F.M. Kirby Foundation, the Center for Advanced

Technology CUNY, the City College of New York, and the MacKenzie Foundation for funding support for our laboratory during these studies.

Author Disclosure Statement

No competing financial interests exist.

References

- Fredholm BB, Battig K, Holmen J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol Rev.* 1999;51:83–133.
- Lazarus M, Shen H-Y, Cherasse Y, Qu W-M, Huang Z-L, Bass CE, Winsky-Sommerer R, Semba K, Fredholm BB, Boison D, Hayaishi O, Urade Y, Chen J-F. Arousal effect of caffeine depends on adenosine A2A receptors in the shell of the nucleus accumbens. *J Neurosci.* 2011;31:10067–10075.
- Koob GF, Nestler EJ. The neurobiology of drug addiction. *J Neuropsychiatry Clin Neurosci.* 1997;9:482–497.
- Ongini E, Fredholm BB. Pharmacology of adenosine A2A receptors. *Trends Pharmacol Sci.* 1996;17:364–372.
- Phelix CF, Broderick PA. Light microscopic immunocytochemical evidence of converging serotonin and dopamine terminals in ventrolateral nucleus accumbens. *Brain Res Bull.* 1995;37:37–40.
- Powell KR, Koppelman LF, Holtzman SG. Differential involvement of dopamine in mediating the discriminative stimulus effects of low and high doses of caffeine in rats. *Behav Pharmacol.* 1999;10:707–716.
- Wang JH, Short J, Ledent C, Lawrence AJ, Buuse Mvd. Reduced startle habituation and prepulse inhibition in mice lacking the adenosine A2A receptor. *Behav Brain Res.* 2003;143:201–207.
- Ferré S, O'Connor WT, Fuxe K, Ungerstedt U. The striopallidal neuron: A main locus for adenosine-dopamine interactions in the brain. *J Neurosci.* 1993;13:5402–5406.
- Filip M, Frankowska M, Zaniowska M, Przegalinski E, Muller CE, Agnati L, Franco R, Roberts DC, Fuxe K. Involvement of adenosine A2A and dopamine receptors in the locomotor and sensitizing effects of cocaine. *Brain Res.* 2006;1077:67–80.
- Solinas M, Ferré S, You Z-B, Karcz-Kubicha M, Popoli P, Goldberg SR. Caffeine induces dopamine and glutamate release in the shell of the nucleus accumbens. *J Neurosci.* 2002;22:6321–6324.
- Solinas M, Ferré S, Antoniou K, Quarta D, Justinova Z, Hockemeyer J, Pappas LA, Segal PN, Wertheim C, Muller CE, Goldberg SR. Involvement of adenosine A1 receptors in the discriminative-stimulus effects of caffeine in rats. *Psychopharmacology (Berl.)* 2005;179:576–586.
- Jacobs BL, Azmitia EC. Structure and function of the brain serotonin system. *Physiol Rev.* 1992;72:165–229.
- Kuzmin A, Johansson B, Zvartau EE, Fredholm BB. Caffeine, acting on adenosine A(1) receptors, prevents the extinction of cocaine-seeking behavior in mice. *J Pharmacol Exp Ther.* 1999;290:535–542.
- SAMSHA. Depression and the initiation of cigarette, alcohol, and other drug use among young adults. *The NSDUH report.* 2012; November 15.
- Marcellino D, Roberts DCS, Navarro G, Filip M, Agnati L, Lluís C, Franco R, Fuxe K. Increase in A_{2A} receptors in the nucleus accumbens after extended cocaine self-administration and its disappearance after cocaine withdrawal. *Brain Res.* 2007;1143:208–220.
- Broderick PA, Pacia SV. Identification, diagnosis, and treatment of neuropathologies, neurotoxicities, tumors and brain and spinal cord injuries using electrodes with microvoltammetry. US patent Application 20120029331. 2006.
- Broderick PA, Pacia SV. Identification, diagnosis, and treatment of neuropathologies, neurotoxicities, tumors and brain and spinal cord injuries using electrodes with microvoltammetry. US patent No: 7,112,319. 2007.
- Broderick P, Ho H, Wat K, Murthy V. Laurate biosensors image brain neurotransmitters in vivo: can an antihypertensive medication alter psychostimulant behavior. *Sensors.* 2008;8:4033–4061.
- Broderick PA, Phelix CFI. Serotonin (5-HT) within dopamine reward circuits signals open-field behavior. II. Basis for 5-HT-DA interaction in cocaine dysfunctional behavior. *Neurosci Biobehav Rev.* 1997;2:227–260.
- Broderick PA. Microelectrodes and their use in an electrochemical arrangement with telemetric application. U.S. Patent # 5. 1999;938:903.
- Broderick PA. Distinguishing *in vitro* electrochemical signatures for norepinephrine and dopamine. *Neurosci Lett.* 1988;95:275–280.
- Broderick PA. Cathodic Electrochemical Current Arrangement with Telemetric Application. U.S. Patent # 4. 1989;883:057.
- Broderick PA. Characterizing stearate probes *in vitro* for the electrochemical detection of dopamine and serotonin. *Brain Res.* 1989;495:115–121.
- Broderick PA. Microelectrodes and their use in cathodic electrochemical current arrangement with telemetric application. U.S. Patent # 5. 1995;433:710.
- Saleem W, Broderick PA. Biomarkers for brain disorder electrochemically detected by BRODERICK PROBE® microelectrodes/biosensors. *J Biosens Bioelectron.* 2013; S12.
- Karcz-Kubicha M, Antoniou K, Terasmaa A, Quarta D, Solinas M, Justinova Z, Pezzola A, Reggio R, Muller C, Fuxe K, Goldberg S, Popoli P, Ferré S. Involvement of Adenosine A1 and A2A Receptors in the Motor Effects of Caffeine after its Acute and Chronic Administration. *Neuropsychopharmacology.* 2003;28:1281–1291.
- Bedingfield JB, King DA, Holloway FA. Cocaine and caffeine: conditioned place preference, activity, and additivity. *Pharmacol Biochem Behav.* 1998;61:291–296.
- Daly JW, Fredholm BB. Caffeine—an atypical drug of dependence. *Drug Alcohol Dependence.* 1998;51:199–206.
- Rung J, Rung E, Helgeson L, Johansson A, Svensson K, Carlsson A, Carlsson M. Effects of (–)-OSU6162 and ACR16 on motor activity in rats, indicating a unique mechanism of dopaminergic stabilization. *J Neural Transm.* 2008;115:899–908.
- Sergi Ferré MO, Xavier Guitart. Paraxanthine: Connecting Caffeine to Nitric Oxide Neurotransmission. *J Caffeine Res.* 2013;3:72–78.
- Halldner L, Aden U, Dahlberg V, Johansson B, Ledent C, Fredholm BB. The adenosine A1 receptor contributes to the stimulatory, but not the inhibitory effect of caffeine on locomotion: a study in mice lacking adenosine A1 and/or A2A receptors. *Neuropharmacology.* 2004;46:1008–1017.
- Kaplan GB, Greenblatt DJ, Kent MA, Cotreau MM, Arcelin G, Shader RI. Caffeine-induced behavioral stimulation is

- dose-dependent and associated with A1adenosine receptor occupancy. *Neuropsychopharmacology*. 1992;6:145–153.
33. Kuzmin A, Johansson B, Semenova S, Fredholm BB. Differences in the effect of chronic and acute caffeine on self-administration of cocaine in mice. *Eur J Neurosci*. 2000;12:3026–3032.
34. Quarta D, Ferre S, Solinas M, You ZB, Hockemeyer J, Popoli P, Goldberg SR. Opposite modulatory roles for adenosine A1 and A2A receptors on glutamate and dopamine release in the shell of the nucleus accumbens. Effects of chronic caffeine exposure. *J Neurochem*. 2004;88:1151–1158.

Address correspondence to:
Patricia A. Broderick, PhD

*Department of Physiology, Pharmacology and Neuroscience
The Sophie Davis School of Biomedical Education
The City College of New York
160 Convent Avenue
Harris Hall Rm. 01
New York, NY 10031*

E-mail: broderick@med.cuny.edu