Rapid changes in extracellular glutamate induced by natural arousing stimuli and intravenous cocaine in the nucleus accumbens shell and core

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Wakabayashi KT, Kiyatkin EA. Rapid changes in extracellular glutamate induced by natural arousing stimuli and intravenous cocaine in the nucleus accumbens shell and core. J Neurophysiol 108: 285–299, 2012. First published April 11, 2012; doi:10.1152/jn.01167.2011.—Glutamate (Glu) is a major excitatory neurotransmitter, playing a crucial role in the functioning of the nucleus accumbens (NAc), a critical area implicated in somatosensory integration and regulation of motivated behavior. In this study, high-speed amperometry with enzyme-based biosensors was used in freely moving rats to examine changes in extracellular Glu in the NAc shell and core induced by a tone, tail pinch (TP), social interaction with a male conspecific (SI), and intravenous (iv) cocaine (1 mg/kg). To establish the contribution of TP, SI, and cocaine, but not a tone, induced relatively large and prolonged increases in extracellular Glu levels, more predominantly in the NAc shell than core. In contrast to monophasic responses with natural stimuli, cocaine induced a biphasic Glu increase in the shell, with a transient peak during the injection and a slower postinjection peak. Therefore, Glu is phasically released in the NAc after exposure to natural arousing stimuli and cocaine; this release is rapid, stimulus dependent, and structure specific, suggesting its role in triggering neural and behavioral activation induced by these stimuli. This study also demonstrates the need for multiple in vitro and in vivo controls to reveal relatively small, highly phasic, and transient fluctuations in Glu levels occurring under behaviorally relevant conditions.

amperometry; stress; enzyme-based sensors; neural activation; glutamate release; brain temperature; metabolism

GLUTAMATE (Glu) is a major excitatory neurotransmitter essential for maintaining and regulating central activational processes. Despite extensive research regarding the action of Glu on central neurons (Krnjevic 1970; Moul 2009), the functional role of this rapid neurotransmitter in behavioral processes remains unclear. Although in vivo microdialysis has been widely used for measuring changes in Glu transmission (e.g., Tossman et al. 1986; You et al. 2001), the low temporal resolution of this technique places significant constraints on revealing rapid drug- and behavior-associated fluctuations, despite recent advances in rapid detection (Perry et al. 2009). In contrast, electrochemistry has excellent temporal resolution, but its measurement selectivity in vivo has always been problematic; concomitant changes in physical and chemical factors in the environment can confound the current changes induced by the oxidation of the substance of interest. While Glu-selective, enzyme-based electrochemical sensors have been developed (Cooper and Pritchard 1994; Hu et al. 1994; Wilson and Gifford 2005) and their selectivity validated by further work (Agnesi et al. 2010; Hascup et al. 2010; Oldenziel et al. 2006; Rutherford et al. 2007), the use of this technique in awake, behaving animals has thus far been limited (Dash et al. 2009; John et al. 2008; Naylor et al. 2011).

In the present study, enzyme-based, Glu-selective sensors coupled with high-speed amperometry were used to examine fluctuations in extracellular Glu levels induced in the nucleus accumbens (NAc) by natural arousing stimuli and intravenous (iv) cocaine in awake, freely moving male rats. The NAc is a critical brain area involved in somatosensory integration, behavioral regulation, and mediating the reinforcing properties of addictive drugs, including cocaine (Kalivas 2004; Mogenson et al. 1980; Robinson and Berridge 1993; Wise and Bozarth 1987). The NAc receives abundant Glu input from the hippocampus, amygdala, and thalamus as well as prefrontal, cingulate, and temporal cortices (Alheid and Heimer 1988). This Glu input appears to be the primary cause of the excitation of accumbal neurons induced by various sensory stimuli and occurring during motivated behavior (Carelli and West 1991; Rebec 2006). Although relatively homogeneous in morphology and neurophysiological characteristics (Meredith et al. 1992; O’Donnell and Grace 1993), the NAc is subdivided into two distinct compartments (shell and core) that differ in afferent input (Heimer et al. 1997; Willuhn et al. 2003), efferent connections (Zahn 2000), and the presumed role in behavioral regulation (Cardinal et al. 2003; Wheeler and Carelli 2009). Since Glu input to these two compartments could be an important factor determining differences in their functional role, our recordings were conducted in both the NAc shell and core.

To evaluate Glu responses induced by natural arousing and pharmacological challenges, we used a 5-s tone, 3-min tail pinch (TP), 3-min social interaction with a novel male conspecific of similar age and weight (SI), and iv cocaine injection at a low dose optimal for drug self-administration (1 mg/kg). To assess the relationships between stimulus-induced changes in Glu and motor activity, we also concurrently measured locomotion. While all these stimuli induce neural activation, each of them differs in its valence, arousing potential, and the pattern of evoked behavioral responses. Since these arousing stimuli can also induce multiple physical and chemical changes in the brain, parallel recordings were made with enzyme-free Glu-null (Glu0) sensors, enabling us to differentiate between...
Glu and confounding nonspecific factors. Since any chemical reaction is modulated by temperature and both cocaine and the arousing stimuli used in this study induce relatively large brain temperature increases (see Kiyatkin 2010 for review), in a parallel experiment using a similar protocol we also examined stimulus-induced changes in NAc temperature to establish the contribution of this biological factor to Glu-related electrochemical currents.

MATERIALS AND METHODS

Subjects and surgical preparations. Thirty male Long-Evans rats (Charles River Laboratories) weighing 460 ± 50 g at the time of testing were used in this study. Rats were individually housed in a climate-controlled animal colony maintained on a 12:12-h light-dark cycle (lights on at 0700), with food and water available ad libitum. All procedures were approved by the National Institute on Drug Abuse-Intramural Research Program (NIDA-IRP) Animal Care and Use Committee and complied with the procedures were approved by the National Institute on Drug Abuse-committee and complied with the Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 86-23).

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Figure 1. A: results of prerecording (Pre) and postrecording (Post) calibration of glutamate (Glu) and Glu-null (Glu0) sensors used in this study. The graph shows mean ±SE changes in current (nA) induced in vitro by 3 repeated applications of Glu (each 10 μM) before and after in vitro recordings; the last data point shows the increase in current after the addition of 250 μM ascorbate. In both tests the currents grew linearly (r = 0.99, P < 0.001) with increasing Glu concentration ([Glu]), and both the basal currents and the sensor’s sensitivity to Glu decreased after ~9-h in vivo recordings.

Fig. 1A: results of prerecording (Pre) and postrecording (Post) calibration of glutamate (Glu) and Glu-null (Glu0) sensors used in this study. The graph shows mean ±SE changes in current (nA) induced in vitro by 3 repeated applications of Glu (each 10 μM) before and after in vitro recordings; the last data point shows the increase in current after the addition of 250 μM ascorbate. In both tests the currents grew linearly (r = 0.99, P < 0.001) with increasing Glu concentration ([Glu]), and both the basal currents and the sensor’s sensitivity to Glu decreased after ~9-h in vivo recordings. Component analysis of variance (ANOVA) was performed on the data to determine the effect of time on the current (nA) induced in vitro by 3 repeated applications of Glu (each 10 μM). The ANOVA revealed a significant decrease in basal currents (Pre > Post, P < 0.001) with approximately twofold decrease in their sensitivity to both Glu and Glu0 (mean 0.69 ± 0.17 nA/250 μM). Repeated tests with lower glutamate concentrations (0.5 and 1 μM) were also conducted to determine selectivity thresholds. As shown in Fig. 1A, Glu sensors used in this study (n = 20) varied slightly in their Glu sensitivity (mean 3.21 ± 0.08 nA/μM); increasing incremental, highly linear (r = 0.99) increases in current with increases in Glu concentration ([Glu]) during pre- and postrecording calibrations. The in vitro detection limit of our electrodes was 0.02 ± 0.003 nA, thus allowing us to detect ~58 nM Glu after a single test, although the precision of Glu detection is known to increase (as √n) after repeated tests. Glu sensors showed changes in currents with addition of ascorbate (mean 0.69 ± 0.17 nA/250 μM). Electrodies (n = 5) with low sensitivity to Glu (<2.0 nA/10 μM) and/or low selectivity against ascorbate (<1:50) were not used. The average selectivity ratio of Glu sensors used for in vivo recordings in this study was 1.21 ± 0.29 (calculated as a mean of individual sensors) or 1:16 (calculated based on mean group changes in currents). Postrecording calibrations of Glu sensors (see Fig. 1A) revealed a significant decrease in basal currents (Pre = 2.60 ± 0.24 nA vs. Post = 0.51 ± 0.06 nA; P < 0.01) coupled with an approximately twofold decrease in their sensitivity to both Glu and Glu0 (mean 1.54 ± 0.08 nA/10 μM) and ascorbate (0.35 ± 0.04 nA/250 μM). This decrease in sensitivity is consistent with other studies using sensors of similar design (Behrend et al. 2009; Naylor et al. 2011) and may be due in part to fouling of the active surface (Kulagina et al. 1999) or perturbation of the enzyme layer during sensor removal from the brain. Despite decreases in sensitivity to both substances, the Glu-ascorbate

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selectivity ratio during postrecording calibration (1:111) remained relatively similar to that during prerecording calibrations.

As expected, Glu0 sensors were insensitive to Glu (0.02 ± 0.01 nA/10 μM) but showed current responses to ascorbate slightly weaker than those in Glu sensors (0.35 ± 0.02 nA/250 μM) (Fig. 1). These parameters remained virtually unchanged during postrecording in vitro tests, but, similarly to Glu sensors, basal currents (3.36 ± 0.47 nA) significantly decreased (0.44 ± 0.06 nA; P < 0.01). Importantly, basal currents generated by both Glu and Glu0 sensors were similar for both prerecording and postrecording conditions. Since our rats received cocaine injections (1 mg/kg or 0.3 μM/kg) and this drug could theoretically act directly on Glu sensors, we also tested their sensitivity to cocaine in vitro. As expected based on the sensor design, no changes in currents occurred with applications of cocaine at up to 60 μM concentration.

Since dopamine also shows phasic fluctuations following exposure to arousing stimuli and cocaine (Aragona et al. 2008; Phillips et al. 2003; Wightman et al. 2007), we also examined how dopamine at different concentrations (1 μM and 100 nM) affects currents generated by Glu and Glu0 sensors. Although Glu sensors showed increases in currents following applications of dopamine at high concentrations [0.15–0.70 nA/1 μM; mean 0.54 ± 0.12 nA/1 μM (n = 4 sensors; 16 tests)], the current change produced by dopamine at low concentrations (15–70 pA) was within the background noise. These ineffective concentrations are higher than maximal stimulus-induced dopamine increases (30–70 nM) revealed in either microdialysis (Wise et al. 1995) or electrochemical (Kiyatkin and Gratton 1994; Owesson-White et al. 2012; Wightman et al. 2007) studies. Glu0 sensors also showed current responses to dopamine, but they were slightly lower than those in Glu sensors.

Since chemical reactions including Glu oxidation are temperature dependent and arousing stimuli and cocaine both increase brain temperatures up to 1°C (Kiyatkin 2010), in a separate in vitro experiment (8 sensors, 48 measurements) we examined the influence of temperature on Glu sensitivity and basal electrochemical currents generated by Glu and Glu0 sensors. By simultaneously monitoring electrochemical currents and temperature in the testing solution, we first examined differences in current response induced by Glu at 23°C and 37°C, which correspond to standard ambient and averaged brain temperature (Kiyatkin 2010), respectively. We found that the magnitude of current response to 5 μM Glu is almost doubled within this range, from 1.65 ± 0.11 nA at 23°C to 3.04 ± 0.21 nA at 37°C, an increase of 84.10 ± 3.48%. Since NAc temperature during the behavioral experiment in well-habituated rats is ~37°C, this temperature coefficient was used in the conversion of in vivo recorded currents (nA) to Glu concentrations (nM). Second, we measured how background currents are affected by small increases and decreases in testing temperature (1–2°C), within a possible range of naturally occurring brain temperature fluctuations. In this case, we found that electrochemical currents generated by both Glu and Glu0 sensors are highly temperature dependent, showing a change of 0.091 ± 0.009 nA/0.5°C and 0.074 ± 0.006 nA/0.5°C, respectively.

**Experimental protocol.** All behavioral procedures occurred in an electrically insulated chamber (38 × 47 × 47 cm) located in a larger open-faced cabinet. The cage was illuminated continuously by a dim 20-W red light bulb, and a room-wide air filter fan provided background noise. Two speakers were placed above the cage to provide audio stimuli. The bottom of the cage was covered with wood chip bedding, which remained in place during the habituation and recording of each rat. The cage was equipped with four infrared motion detectors (Med Associates), which were used for monitoring rat locomotion. Prior to recording session, rats were habituated to the testing environment for a minimum of 6 h/day for 3 consecutive days.

At the beginning of each experimental session, rats were minimally anesthetized (<2 min) with isoflurane and a calibrated sensor was inserted into the brain through the guide cannula. Then the rat was placed into the testing chamber, and the sensor was connected to the potentiostat (model 3104, Pinnacle Technology) via an electrically shielded flexible cable and a multichannel electrical swivel. Additionally, the injection port of the jugular catheter on the head mount was connected to a plastic catheter extension, which passed through a liquid swivel to the outside of the recording chamber, thus allowing stress-free drug delivery and minimizing possible detection of the iv drug injection by the rat. Testing began a minimum of 2 h after insertion of the sensor when the baseline current had relatively stabilized (see RESULTS). Rats were exposed to four types of stimuli: 5-s tone (880 Hz, 75 dB), Tone, 3-min TP, 3-min SI, and iv injection of cocaine (1 mg/kg in 0.2 ml saline delivered over 20 s). For the TP, a wooden clothespin was attached to the base of the tail; in contrast to studies that employed a metal clamp, this is a milder form of TP that is nonharmful to the animal. For SI trials, a novel male rat was introduced into the cage for 3 min. Each rat was tested with all types of stimuli twice per session (except for a single tone); each test was presented in random order and separated by at least 45 (for TP and SI) or 60 (for cocaine) min. The dose of cocaine used is optimal for self-administration in rats (Pickens and Thompson 1968) and induces clear behavioral and physiological effects (Brown and Kiyatkin 2005). The 60-min postinjection interval is approximately five to six times longer than the half-life of cocaine with iv administration (Tsibulsky and Norman 1999) and sufficiently long enough for all basic physiological parameters to return to baseline—the time between two cocaine injections was at least 2.5 h. Thus during each session the rat was tested with a tone (which was always the first stimulus of a session) and two pairs of arousing stimuli (TP and SI) and received two cocaine injections. At the end of each session, rats were lightly anesthetized (0.5–0.6 ml Equithesin over 2 min via iv injection) and disconnected from the potentiostat, and the biosensor was removed for postrecording calibration. Rats were allowed to recover from anesthesia, and their jugular catheters were flushed with sterile saline before they were returned to the animal colony. Rats with bilateral implants (n = 14) that underwent a second day of testing were given a minimum of 1 day of recovery between recording sessions. In rats tested twice, the order of natural stimuli presentation was reversed.

**Brain temperature recordings.** Since temperature affects electrochemical currents and brain temperature fluctuates within 2–3°C within the normal physiological continuum (see Kiyatkin 2010 for review), in a separate experiment (6 rats) we examined changes in NAc temperatures induced by our testing stimuli. As described in detail elsewhere (Brown and Kiyatkin 2005), rats were implanted with miniature copper-constantan thermocouple electrodes in the NAc shell and other body locations and tested in the same experimental protocol as in our electrochemical studies. Although the use of Glu sensors makes it possible to exclude all nonspecific physical and chemical contributions to currents recorded by Glu sensors, NAc temperature recordings were important for revealing the role of naturally occurring brain temperature fluctuations as a factor affecting electrochemical recordings in behavioral experiments.

**Sensor placement verification.** Within 2 days of the final experimental session, rats were deeply anesthetized with an iv injection of Equithesin (1.5–2.0 ml) and trascendally perfused initially with room-temperature PBS (pH 7.4) followed by 10% formalin. Brains were cryoprotected with an 18% sucrose solution for 2 days and then sectioned on a cryostat to a thickness of 45 μm and stained with cresyl violet. The location of the sensors was verified with the stereotaxic atlas of Paxinos and Watson (1998). Electrochemical data were accepted only with sensors histologically verified to be located in either the NAc shell or the NAc core (Fig. 1B).

**Data analysis.** Electrochemical data were collected at 1 Hz and analyzed with two time resolutions. Slow current changes were analyzed with 1-min quantification bins for 5 min before and 20, 40, and 60 min after Tone, TP, and SI, and cocaine injection, respectively. Rapid current changes were analyzed with 4-s bins for 30 s before and 360 s after both natural and drug stimuli. We found that the 4-s time
resolution is optimal for detecting rapid changes in currents within the timescale of our stimuli (3–5 min) while simultaneously reducing the contribution of background noise. Since the baseline currents slightly varied in amplitude between individual electrodes and showed a slight gradual decrease within a session, the stimulus-induced changes in current were transformed into relative changes, taking a basal value (8 and 30 s before stimulus for rapid and slow changes, respectively) as 0 nA. Since stimulus-induced current changes were detected with both Glu and Glu0 sensors, current differentials (mean group changes generated by Glu sensors minus mean group changes generated by Glu0 sensors) were calculated to reveal the contribution of Glu to observed current changes. These data were calibrated in nanomolar Glu concentrations based on in vitro sensor sensitivity and adjusted by the known temperature coefficient (see RESULTS). Although some of our rats had bilateral implants and a within-subject double-sensor recording (Glu-Glu0) could be viewed as the best approach to reveal a specific Glu contribution to currents detected by Glu sensors, only one sensor was used in each recording session and current differentials were determined by analyzing the mean data obtained with each type of sensor. This approach appears to be the most appropriate with the sensors of this design (relatively large size, inbuilt reference electrode), because Glu and Glu0 sensors during simultaneous use have different baseline currents and may electrically cross talk with each other, because of the relatively close between-sensor distance, thus affecting the measurements of each sensor.

To evaluate the dynamics of Glu levels, data for each stimulus were also analyzed as the rate of change (a difference between each consecutive current value). For each response we also determined latency to increase as the first time point with significant change versus baseline.

In addition to stimulus-related changes in electrochemical currents, we also analyzed slow, tonic changes in baseline currents detected by Glu and Glu0 sensors over the entire recording interval. These data were used to evaluate the origins of the slow negative current trend observed in vivo during behavioral experiments and estimate basal levels of Glu in the NAc shell and core.

One- and two-way repeated-measures (RM) ANOVAs were used for evaluating statistical significance of changes in current and differences in current dynamics produced by Glu and Glu0 sensors, where appropriate. Locomotor data were collected as the number of photobeam breaks per 10 s and analyzed as 1-min bins with standard statistical techniques (1-way ANOVA for repeated measures, followed by Fisher or Dunnett test). Temperature data were collected with 10-s time resolution and analyzed as relative changes at a 1-min timescale. Standard correlative and regression analyses were used to determine the relationships between specific and nonspecific changes in electrochemical currents and changes in locomotion and brain temperature. Data are represented as means ± SE, and the minimal level of significant difference was set as $P < 0.05$.

RESULTS

Data sample. Data with Glu sensors were obtained from 15 rats, each tested during 1 ($n = 4$) or 2 ($n = 11$) experimental sessions; a total of 110 individual tests (70 in the shell and 40 in the core) were analyzed. Glu0 sensors were used in 9 rats (3 with double and 6 with single implants); 71 individual tests (36 in the shell and 35 in the core) were analyzed. In each rat, sensors were histologically verified to be located in either NAc shell or core (see Fig. 1B).

Tonic changes in electrochemical currents detected by Glu and Glu0 sensors: specific and nonspecific contributions. As shown in Fig. 2, basal oxidation currents recorded by Glu sensors slowly decreased during the course of the entire experiment (~8 h); these changes were similar in both accumbal compartments in both amplitude and rate and were combined together for analysis. A similar, time-dependent decrease in current, with no differences in the shell and core, was also detected by Glu0 electrodes; these data were also combined. Since both types of electrodes were exposed in vivo to the same physical and chemical influences except for Glu, their difference reflects the contribution of Glu. Thus, taking into account substrate sensitivity of Glu sensors (3.21 nA/10 μM) corrected for 37°C (5.91 nA/10 μM), it appears that basal levels of extracellular Glu in the NAc are 0.96 μM, with similar values in both its compartments (~150 min after sensor insertion in the brain, just before the start of behavioral tests). Although basal currents for both Glu and Glu0 sensors slowly decreased during the session, the decrease was similar in both groups, suggesting that extracellular Glu levels in the NAc remain relatively stable during long-term behavioral experiments (0.84 μM at the final recording point, ~500 min after implantation). Although the decreases in currents recorded by both Glu and Glu0 sensors were exponential within the entire experiment, within the time of our behavioral tests (150–500 min; vertical dashed lines in Fig. 2) they could be adequately described by a linear correlation ($r = −0.940$ and $−0.967$ for Glu and Glu0 currents, respectively). Electrochemical currents recorded by Glu0 sensors in vitro at 22°C also exponentially decreased during a similar recording interval. In this case, absolute values of currents were much lower than those recorded by the same electrodes in vivo and their difference gradually decreased during the session (from 1.65 nA at 150 min to 0.94 nA at 500 min). This current difference reflects total nonspecific chemical and physical contributions to electrochemical currents recorded by Glu sensors—notably, these
nonspecific contributions to the current are proportionally much greater than those contributed by Glu.

Changes in electrochemical currents recorded in NAc shell by Glu and Glu0 sensors after exposure to natural arousing stimuli. To examine how natural arousing stimuli affect extra-cellular Glu levels in the NAc shell, we calculated mean changes in electrochemical currents recorded by Glu and Glu0 sensors after exposure to Tone, TP, and SI. Since there were no evident changes in current responses elicited by these stimuli at the first and second presentations and during the first and second recording sessions, they were combined. Figure 3 shows original examples of changes in electrochemical currents detected by Glu and Glu0 sensors in the NAc shell after TP. First, these data were analyzed at low resolution (1-min bin) to represent changes within the entire recording interval.

Both TP and SI but not Tone induced relatively large changes in electrochemical currents (0.10–0.12 nA) detected by Glu sensors (Fig. 4, A, D, and G). The currents rapidly increased at the start of stimulus presentation, peaked around its end, and slowly decreased below the prestimulus baseline. These changes were even slightly larger and poststimulation current decrease disappeared when the data were corrected for a tonic drift in baseline (hatched lines in Fig. 4D). However, broadly similar changes in currents were also detected by the Glu0 sensors after exposure to these stimuli (see also original examples in Fig. 3). Two-way RM ANOVA revealed that both TP and SI induce significant changes in electrochemical currents detected by Glu and Glu0 sensors (main effect of time: TP \( F_{1,144} = 17.28, \) SI \( F_{6,144} = 32.78; \) both \( P < 0.05 \)) and that the Glu and Glu0 currents differed significantly from each other for the first 13 min of TP and 6 min of SI (main effect of Glu-Glu0; TP \( F_{1,29} = 4.64, \) SI \( F_{1,24} = 6.23; \) Glu-Glu0 \( \times \) time interaction: TP \( F_{13,377} = 2.08, \) SI \( F_{6,144} = 2.25; \) all \( P < 0.05 \)). By using current differentials, we found that the actual changes in [Glu] are relatively small (\( \sim 60–100 \) nM), more transient, and different for TP and SI (Fig. 4, E and H). Compared with SI, TP induced larger increases in [Glu] and its levels gradually increased during the 3-min duration of procedure, peaked at its end, and slowly decreased toward baseline. These increases were significant for 3–11 min [Fisher least significant difference (LSD) post hoc test, \( P < 0.05; \) Fig. 4E]. The phasic nature of TP-induced Glu increase can also be seen more clearly by analyzing the rate of Glu change; levels began to increase during the first minute (\( \sim 17 \) nM/min), further increased during the next 3 min, and then rapidly declined to baseline (Fig. 4F). Although SI induced a slightly lower increase in [Glu] (Fig. 4H; \( \sim 70 \) nM) than TP, its levels peaked more rapidly (i.e., during the first minute of the stimulus, \( \sim 70 \) nM/min; Fig. 4I). In this case, Glu increase was significant for the first 6 min from the stimulation onset (Fisher LSD post hoc test, \( P < 0.05 \)). Current differentials revealed a second increase in [Glu] that occurred from \( \sim 9 \) to 18 min after SI, but this post-SI increase did not reach significance and its reliability is currently unclear. Although electrochemical currents recorded by Glu and Glu0 sensors decreased after tone presentation (Fig. 4A), these currents did not differ significantly (no significant interaction with a 2-way ANOVA), suggesting no changes in [Glu] (Fig. 4, B and C). Of note, the decrease in current baseline was slightly steeper during the tone stimulus than during TP and SI because the tone was always presented first and earliest within an experiment, when tonic decrease in baseline currents was more pronounced. Although our rats were exposed to TP and SI up to four times, there were no consistent changes, either decreases or increases, in both Glu and motor responses.

While both SI and TP evoked behavioral responses, these responses differed qualitatively. Typically during a TP test, rats began to chew on the clothespin applied to the tail and engage in forward locomotion after a variable (20–60 s) period of inactivity. In contrast, during SI tests the presentation of the conspecific resulted in a more complex, highly variable series of behaviors, which included direct interactions such as sniff-
ing, chasing, touching, and mounting. While generally these interactions commenced slowly and peaked at the end of the test, the transition from inactivity to response was often less distinct during a SI test than a TP test. In both SI and TP tests, rats remained active for \( \frac{30}{30} \) min after the onset of the stimulus.

Further clarification of rapid Glu fluctuations was obtained when TP and SI data were analyzed at high temporal resolution (Fig. 5). At this time resolution, it is clear that the stimuli induce different dynamics in [Glu]. While electrochemical currents generated by both Glu and Glu0 sensors increased immediately after the initiation of TP and differed from each other for the entire post-TP period (Fig. 5A; main effect of time: \( F_{90,2610} = 29.60 \); Glu-Glu0: \( F_{1,29} = 4.78 \); Glu-Glu0 \times time interaction: \( F_{90,2610} = 3.64 \); all \( P < 0.05 \)), post hoc tests revealed that the Glu current did not diverge consistently from the Glu0 current until 86 s after the start of the stimulus but then remained elevated for the entire duration of the observation period. This indicates that [Glu] began to rise after the onset of the TP, peaked within seconds after its end, and began slowly decreasing but remained for some time afterwards (Fig. 5B). While SI induced significant changes in electrochemical currents for both Glu and Glu0 sensors (main effect of time: \( F_{25,600} = 9.85 \); \( P < 0.05 \)), in contrast to TP, the increase detected by Glu sensors was more rapid and greater than that detected by Glu0 sensors, with a significant difference at 4 – 8 s after the introduction of the conspecific rat (Fig. 5C; main effect of Glu-Glu0: \( F_{1,24} = 5.21 \); Glu-Glu0 \times time interaction: \( F_{25,600} = 1.56 \); both \( P < 0.05 \)). In this case, [Glu] increased much more rapidly to its maximum (\( \sim 50 \) nM) and was maintained at this level for the first 94 s of the SI (Fig. 5D; Fisher LSD post hoc test, \( P < 0.05 \)). Although it appears that [Glu] remained high for some time after the SI, because of increased variability in both data sets Glu levels were only significantly higher during the beginning of the SI.

Changes in electrochemical currents recorded in NAc core by Glu and Glu0 sensors after exposure to natural arousing stimuli. Recordings from the NAc core (Fig. 6) produced markedly different results than in the shell. When analyzed with low temporal resolution, current changes detected by Glu and Glu0 sensors were virtually identical in their amplitude and time course for all three stimuli (Fig. 6, A, D, and G), resulting in no significant changes in [Glu] for Tone and TP (Fig. 6, B and E). A very small, but significant current difference was found only for SI for 2 min after its onset (Fig. 6H; main effect of Glu-Glu0: \( F_{1,19} = 7.89 \); time: \( F_{2,38} = 20.41 \); Glu-Glu0 \times time interaction: \( F_{2,38} = 3.28 \); all \( P < 0.05 \)). In this case, Glu levels weakly but significantly increased (\( \sim 40 \) nM) during the
first 2 min after the start of stimulus. This transient effect resulted from a rapid rise in [Glu] at the first minute of SI (Fig. 6; Fisher LSD post hoc test, \( P < 0.05 \)).

Although there was an overall lack of significance in response to natural stimuli in the core at 1-min time resolution, the initial component of [Glu] increase seemed to resemble that of the shell. However, when data were analyzed at high temporal resolution, virtually no changes in Glu were found in the core (Fig. 7). Although TP induced rapid increases in electrochemical currents (main effect of time: TP \( F_{90,1890} = 43.07, P < 0.05 \)), there was little difference between the currents measured by the Glu and Glu0 sensors, resulting in no significant changes in [Glu] (Fig. 7, A and B). Only a small difference in currents was found for the first 58 s after the onset of SI (Fig. 7D; main effect of time: \( F_{16,304} = 5.00, \) Glu-Glu0; \( F_{16,19} = 9.94; \) Glu-Glu0 \( \times \) time interaction: \( F_{16,304} = 1.76; \) both \( P < 0.05 \)), resulting in a weak, transient rise in [Glu] at the onset of SI (Fisher LSD post hoc test, \( P < 0.05 \)).

Cocaine-induced changes in electrochemical currents recorded in NAc shell and core by Glu and Glu0 sensors. Figure 8 shows changes in electrochemical currents recorded by Glu and Glu0 sensors in the NAc shell (Fig. 8A) and core (Fig. 8D) after an iv injection of cocaine at 1-min time resolution. Similarly to natural arousing stimuli, cocaine induced significant increases in electrochemical current detected by Glu and Glu0 sensors in both the shell and the core (Fig. 8, A and D; main effect of time: shell \( F_{38,1064} = 11.46, P < 0.05 \); core \( F_{38,570} = 4.47, P < 0.05 \)), but the currents measured by the Glu and Glu0 sensors differed only in the shell for the first 38 min (Fig. 8, A and D; shell: Glu-Glu0 \( \times \) time interaction \( F_{38,1064} = 1.47, P < 0.05 \); Glu-Glu0 not significant (n.s.), core: Glu-Glu0, Glu-Glu0 \( \times \) time interaction, both n.s.), resulting in an increase in [Glu] that began during the first minute after the injection start and became significant from the 3rd minute to the 8th minute after injection (Fig. 8B; Fisher LSD post hoc, \( P < 0.05 \)). While Glu levels remained positive for \( \sim 20 \) min after injection, they gradually decreased below baseline, suggesting a possible reboundlike Glu decrease from \( \sim 30 \) min after injection. It should be noted that despite a consistent decrease in the current mean and corresponding differential, this [Glu] decrease is less reliable than the early immediate Glu increase during the first 10 min after injection because of the greater variability in the current values detected by both types of sensors (see large SE in Fig. 8A).

Although both natural arousing stimuli and cocaine increased Glu levels in the NAc shell, the dynamics of [Glu] after an iv cocaine injection differed. In contrast to monophasic increases in [Glu] seen with natural arousing stimuli, the initial increase in [Glu] seen after cocaine injection appears to have two components as can be seen in the [Glu] curve (Fig. 8B) and, somewhat more clearly, in the rate of concentration change (Fig. 8C). In contrast to the dynamic changes in [Glu] in the shell, no significant changes in Glu were seen in the NAc core (Fig. 8E), although changes in currents detected by Glu sensors were slightly larger for \( \sim 20 \) min after cocaine injection (Fig. 8D).

The dual-component increase in NAc shell [Glu] seen at 1-min resolution can be clearly distinguished at 4-s resolution (Fig. 9, A and B). At this time resolution, it is very evident that Glu levels in the shell began to increase within the 20 s of the injection (Fig. 9A; time: \( F_{90,2520} = 4.14; \) Glu-Glu0 \( \times \) time interaction: \( F_{90,2520} = 1.64, \) Glu-Glu0 n.s.), with the first significant point at 14 s after the start of the injection (Fig. 9B; Fisher LSD post hoc test, \( P < 0.05 \)). Glu levels continued to increase further, peaking (\( \sim 60 \) nM) around the end of injection and slowly decreasing afterward to levels of no significance. However, from 80–90 s, Glu levels began to increase again, becoming significant from 126 s and continuing to rise beyond the 6-min analysis interval. Although no significant differences in currents detected by Glu and Glu0 sensors were found in the core at a slow temporal resolution, it is possible that small [Glu] changes could be revealed with higher time resolution analysis. However, virtually no rapid changes in Glu were found in the NAc core, where both current (Fig. 9C; all effects
are not significant) and concentration (Fig. 9D) responses were much weaker than in the NAc shell.

Relationships between changes in Glu levels, nonspecific contributions, locomotion, and brain temperature. Figure 10 shows the relationships between changes in extracellular [Glu] levels in both compartments of the NAc (Fig. 10, A, D, and G) and locomotor responses (Fig. 10, B, E, and H) induced by TP, SI, and cocaine. Since Tone did not induce evident changes in either parameter, data for this stimulus are not shown. Figure 10, C, F, and I, show mean changes in electrochemical currents detected by Glu<sub>0</sub> sensors superimposed on changes in NAc temperature induced by these procedures.

TP, SI, and cocaine significantly increased locomotor activity (1-way RM ANOVA: \( F_{22,990} = 5.52 \), \( F_{19,885} = 11.24 \), and \( F_{19,180} = 9.76 \), respectively; each \( P < 0.001 \)). Compared with almost no activity in baseline, locomotion rapidly increased after exposure to each stimulus, peaked at 3–4 min, and slowly decayed afterwards; the pattern and magnitude of changes were similar for all three stimuli. Changes in locomotion correlated significantly with Glu increases in the NAc shell (TP \( r = 0.876 \), SI \( r = 0.753 \), cocaine \( r = 0.849 \); each \( P < 0.01 \)).

Each type of arousing stimulus induced significant increases in the electrochemical currents detected by Glu<sub>0</sub> sensors (one-way RM ANOVA: TP \( F_{20,800} = 15.30 \), SI \( F_{18,720} = 20.81 \), cocaine \( F_{14,770} = 8.07 \); each \( P < 0.001 \); see Fig. 10, C, F, and I). Since these currents had a similar time course in both the shell and the core, they were combined into one group and corrected by the known negative baseline drift of the Glu<sub>0</sub> electrodes found in vivo (see Fig. 2). In all cases, this nonspecific component was slower and more prolonged than those of [Glu]. In the cases of TP and SI, nonspecific currents gradually increased during stimulation, peaked at \(~8–10\) min, and slowly decreased toward baseline. The increase was delayed, slightly smaller but not longer after cocaine injection. The nonspecific currents were highly correlated with changes in NAc temperature (Fig. 10, C, F, and I), which increased significantly after exposure to each stimulus (TP \( F_{17,557} = 27.9 \), SI \( F_{17,557} = 22.0 \), cocaine \( F_{17,557} = 32.1 \); each \( P < 0.001 \)). For the entire recording interval, the correlation was exceptionally strong for TP and SI (\( r = 0.920 \) and 0.973, respectively; \( P < 0.001 \)) and much weaker for cocaine (\( r = 0.591 \)). While for both TP and SI mean changes in nonspecific current and NAc temperature are almost fully superimposable within the entire recording interval (Fig. 10, C and F), the positive relationship between cocaine-induced current increase and temperature, albeit very high during the initial, ascending part of the effect (\( r = 0.958 \), \( P < 0.001 \)), became weaker as time from the injection increased.
DISCUSSION

In this study, enzyme-based Glu-selective biosensors coupled with amperometry were used to examine how natural arousing stimuli and iv cocaine at a low, behaviorally active dose affect extracellular Glu levels in two primary subdivisions of the NAc—the shell and the core—in freely moving rats. These Glu sensors were selective to Glu in vitro both before and after in vivo recordings, and our initial assumption was
that the oxidation currents recorded by these sensors provide a reliable measure of fluctuations in [Glu]. However, the use of Glu0 sensors as well as several other control measurements revealed that only a minor component of the stimulus-induced current changes generated by Glu sensors are related to alterations in extracellular Glu levels. These specific Glu changes were relatively small in amplitude (40–100 nM), highly phasic in nature, structure specific, and different with respect to each stimulus. We also confirmed, in this study, that naturally occurring brain temperature fluctuations provide a major contribution to changes in electrochemical currents recorded in awake, behaving animals by even highly selective and selective Glu sensors. Therefore, despite clear advantages of electrochemistry for detecting rapid fluctuations in Glu levels under behaviorally relevant conditions, great caution and multiple controls are essential to make these measurements reliable.

Reliability of Glu measurements in behaving animals: critical methodological and technical issues. Traditionally, the development of a highly substrate-sensitive and -selective biosensor has been viewed as the most critical factor determining the accuracy of electrochemical measurement of neuroactive substances. With respect to Glu, such sensors were developed in the 1990s (Cooper and Pritchard 1994; Hu et al. 1994; Qin et al. 2008; Wilson and Gifford 2005) and their use was validated by further in vivo work (Hascup et al. 2010; Oldenziel et al. 2006), including experiments in awake, behaving animals (Dash et al. 2009; John et al. 2008; Naylor et al. 2011; Uslaner et al. 2011). As confirmed in this study, Glu sensors produced by Pinnacle Technology showed in vitro high substrate sensitivity (~0.6 nA/1 μM) and reasonably high selectivity against possible interferents, particularly ascorbate and dopamine. Moreover, as confirmed by postrecording in vitro calibrations, these sensors remained Glu sensitive and selective after the in vivo experiment. Consistent with previous reports with the same (Behrend et al. 2009; John et al. 2008) or different (Dash et al. 2009; Gourine et al. 2008; Hascup et al. 2010; Rutherford et al. 2007) designs of Glu sensors, postrecording Glu selectivity of our sensors dropped almost twofold, reflecting mainly contamination of their active area by brain substances, most notably by albumin. Taking into account all these factors, it was reasonable to assume that the stimulus-induced changes in oxidation currents detected in the in vivo experiment reflect fluctuations in extracellular Glu levels.

Although both natural arousing stimuli and iv cocaine induced rapid, relatively strong, and prolonged changes in Glu currents, large changes in currents were also detected by Glu0 sensors that were identical in their design but lacked Glu oxidase, the enzyme that converts Glu into an oxidizable substance. Despite relatively large data samples in each group, only relatively minor differences were found in the current dynamics between Glu and Glu0 sensors. Moreover, these differences were evident immediately after the start of stimulation and particularly with rapid time course analyses. Therefore, changes in the current measured by Glu sensors during the behavioral experiment depend significantly on other chemical and physical factors, with only a minor component reflecting alterations in extracellular Glu. On the other hand, stimulus-specific, graded current responses detected by Glu0 sensors suggest that these stimuli induce certain physical and chemical changes in the brain environment. While the oxidation of other chemical substances such as ascorbate and dopamine is usually viewed as a primary source of these nonspecific current changes, our control experiments with direct monitoring of NAc temperature as well as several in vitro tests revealed that naturally occurring alterations in brain temperature play a major role in generating these relatively slow current fluctuations. First, changes in electrochemical currents detected in the NAc by Glu0 sensors showed very tight correlation with NAc temperature within the entire recording intervals (Fig. 10). Second, basal currents detected by both Glu and Glu0 sensors in vitro were highly temperature sensitive, suggesting that relatively small increases in brain temperature induced by all arousing stimuli (0.6–1.0°C) result in corresponding increases.
Finally, the current response induced by Glu in vitro was also strongly dependent on temperature, almost doubling between 22–23°C (standard ambient temperature of in vitro sensitivity testing) and 37°C [the averaged basal temperature of ventrally located brain structures in awake, quietly resting rats (Kiyatkin 2010)]. The latter finding suggests that in vivo Glu concentrations would be overestimated if the sensors’ sensitivity was determined at room temperature. To account for this, all our data with current differentials were adjusted for the difference in temperature when calculating their concentration values. Thus systematically controlling for nonspecific factors revealed that the real changes in extracellular Glu following arousing stimulation are rapid, very transient in nature, and relatively small in magnitude (up to 80–100 nM).

While the use of Glu0 sensors was essential to reveal the true contribution of Glu to stimulus-induced changes in electrochemical currents, this approach also allowed us to estimate the basal levels of Glu in the NAc, which were ~1 μM in both shell and core compartments. Interestingly, these values are within the range measured in the same structures by microdialysis (0.6–1.4 μM; Miguens et al. 2011; Rahman and Bardo 2008; Saulskaya and Michailova 2002) but typically lower than those reported in electrochemical studies (1–45 μM; Dash et al. 2009; Hascup et al. 2010; Oldenziel et al. 2006; Thomas et al. 2009). While higher estimates of basal Glu levels and the magnitudes of stimulus-induced Glu responses in some studies could also result from the use of sensors of different size and design, as smaller sensors may generate higher values. However, the reasons for such high variability in electrochemical estimates are currently unclear and require further study.

Basal Glu levels could also be affected by local structural damage that always occurs during the insertion of any sensor in brain tissue. Although this influence should be time dependent, basal levels of Glu calculated in this study were relatively stable, with only 10–20% decrease at the end of an ~8-h experiment (see Fig. 2). Therefore, although postrecording in vitro calibrations revealed an almost twofold decrease in sensor sensitivity, it is unlikely that the sensitivity of our sensors seriously deteriorated within the recording session. Most likely, the drop seen during postrecording calibration may have resulted from disrupting the active surface of the sensor via contamination or damage while removing the sensor from the brain.

**Rapid Glu release: sensory stimulation, arousal, and the behavioral response.** The NAc receives dense Glu input from multiple sources, and this Glu input appears to be the primary force for the excitation of accumbal neurons induced by various sensory stimuli and occurring during movements (Carelli and West 1991; Rebec 2006). Although these neuronal activations are mimicked by iontophoretically applied Glu (Kiyatkin and Rebec 1999), suggesting Glu release as their cause, our present study directly supports this mechanism. Consistent with short-latency excitations induced in most dorsal and ventral striatal neurons by sensory stimuli (Carelli and West 1991; Kiyatkin and Brown 2007; Schneider 1991), extracellular Glu levels began to increase rapidly after the start of TP and
SI, suggesting sensory input as the cause of this phasic Glu release. These changes were stronger and significant in the NAc shell, but a similar but weaker response was also observed in the NAc core. In contrast to the tone that did not affect both [Glu] and locomotor activity, TP and SI induced strong locomotor activation, which was tightly related to Glu increases in the NAc shell. Therefore, rapid Glu release could be an essential mechanism underlying stimulus-induced neural activation, which results in motor activation.

Although both TP and SI induced significant changes in [Glu] in NAc shell, their patterns differed. The SI-induced Glu release occurred more rapidly, peaking at the first minute after the conspecific was introduced to the cage (see Fig. 4f) but not sustained despite a much stronger and more prolonged motor activation and brain hyperthermic response (Fig. 10). In contrast, TP induced less rapid but more prolonged Glu release that was maintained within the entire 3 min of exposure but rapidly stopped at its offset (Fig. 4f). While the initial component of Glu release in this case was weaker in magnitude than that seen during SI (~20 nM/min vs. 70 nM/min), because of its longer duration TP-induced elevation in Glu levels was larger and more prolonged. Indeed, differences in the Glu response between SI and TP could be related to the differences in the behavioral response induced by each stimulus. In most SI trials, rats began engaging in a complex, highly variable series of interactions, including sniffing, chasing, touching, and mounting, often as soon as the conspecific was introduced to the rat being recorded. In marked contrast, the TP elicited a much more uniform behavioral response. In general, rats froze for approximately a minute before clearly transitioning to escape-type behaviors including forward locomotion and chewing on the clothespin applied to the tail. Thus these distinct patterns of Glu release could be related to differences in the source and activity of incoming Glu afferents, resulting in stimulus- and activity-specific alterations in neuronal activity, contributing to previously assumed differences in the behavioral roles of these two accumbal compartments (Cardinal et al. 2003; Wheeler and Carelli 2009). While the mechanisms underlying the greater Glu responses in the shell versus the core remain unclear, the NAc shell also shows larger dopamine responses to primary reinforcing and conditioned stimuli (Caccipaglia et al. 2012) as well as cocaine (Aragona et al. 2008; Phillips et al. 2003). Our data may suggest an interrelated link between Glu and dopamine release in processing these stimuli, specifically in the shell.

The rapid, transient increases in Glu levels observed in this study are consistent with the general knowledge on this rapid neurotransmitter, which is phasically released from axonal terminals within Glu synapses and rapidly removed from synaptic receptor sites by powerful reuptake mechanisms. Although uptake mechanisms prevent significant diffusion of Glu out of synapses (Barbour and Haussler 1997; Clements 1996), Glu is still present in the extracellular space at much lower concentrations affecting the extrasynaptic pool of Glu receptors (Bergles et al. 1999; Diamond 2002). In addition to neuronal Glu release, neuronal activation also triggers slower and more prolonged Glu release from astrocytes via a calcium-dependent mechanism (Miele et al. 1996). Therefore, changes in Glu levels, especially their slower components, could reflect both neuronal and nonneuronal Glu release, related to its tonic action as a neuromodulator (Fillenz 2005).

Although our data generally concur with microdialysis evaluations of basal Glu levels, they suggest that stimulus-induced phasic increases in Glu levels are in fact relatively small in amplitude, within 40–100 nM. Other studies using enzyme-based sensors report widely different concentrations of Glu, with some reporting levels much higher than those found here. For example, a rapid, ~86 μM jump in Glu levels was reported in the striatum at the start of TP in awake rats (Rutherford et al. 2007). Relatively large fluctuations in [Glu] (~1–10 μM) were also reported in the cortex after spontaneous transitions within the sleep-wake cycle (Dash et al. 2009; John et al. 2008; Naylor et al. 2011) and in the NAc shell after a systemic injection of MK-801, a noncompetitive NMDA antagonist (Uslaner et al. 2011). In contrast, with simultaneous two-sensor (Glu-Glu) recordings, only ~25–35 nM phasic increases in [Glu] were detected during breathing in the medulla of anesthetized rats (Gourine et al. 2008). Several factors could explain these highly divergent results. As clearly demonstrated in this study, current measurements conducted without accounting for nonspecific contributors could lead to an overly high estimation of Glu levels. Moreover, overestimation of Glu responses could also arise from the artificially decreased sensitivity values when in vitro sensitivity tests are conducted at room temperature. Finally, the amplitude and time course of Glu fluctuations could depend significantly on the size of the sensor and the area of active enzyme layer. Larger sensors, like those used in our current study, integrate signals from multiple release sites, sampling Glu release across a relatively large area but as a consequence decreasing a signal’s amplitude and slowing its time course. While it is certainly plausible that smaller-size sensors could more closely approach release sites and therefore produce larger and more rapid Glu current changes, much lower absolute currents generated by these sensors make them much more susceptible to extraneous electrical artifacts. Additionally, reducing the sampling area provides a much more spatially localized sample of Glu fluctuations, which may differ dramatically from site to site within the structure as a whole. Therefore, the data reported in this study are valid for this type of sensor with a relatively large sampling area, while measurements conducted with much smaller sensors could reveal quantitatively different results.

Cocaine: sensory effects, arousal, and behavioral activation. Despite indirect evidence suggesting that Glu is involved in mediating acute cocaine’s effects and cocaine addiction (Kalivas 2004; Schmidt and Pierce 2010), microdialysis with rapid detection has not revealed consistent changes in Glu levels in both the NAc shell (Venton et al. 2006) and dorsal striatum (Ferrario et al. 2008) following iv cocaine injections in drug-naive awake rats. Small increases in the NAc core have been reported with traditional microdialysis after large-dose systemic cocaine injections (Reid et al. 1997; Smith et al. 1995; Zhang et al. 2001). However, cocaine at the same systemic doses increased Glu levels in the core of drug-experienced rats (Pierce et al. 1996). Relatively weak increases in Glu levels were also found in the NAc shell and core during cocaine self-administration behavior in rats, but no changes were seen after passive cocaine injections in drug-naive conditions (Suto et al. 2010).

Consistent with these data, relatively weak, phasic Glu increases were found in the NAc shell, with no changes in the core. Moreover, the pattern of this response differed from those induced by natural arousing stimuli. In contrast to monophasic
increases typical of both natural arousing stimuli, the cocaine-induced Glu increase had two components, with an initial, rapid phase that began during the injection and a second, slower, more prolonged, and variable phase that matched the time course (~20 min) of locomotor activation. The cocaine-induced increases in Glu levels were exceptionally rapid, with a significant increase at 10 s after the start of a 20-s injection and a peak immediately after the injection end. While such a rapid appearance of a central response to a peripherally administered drug could be considered surprising, it agrees with our recent data suggesting that cocaine induces rapid excitatory effects in the brain via activation of peripherally located neural substrates and rapid neural transmission via visceral somatosensory pathways. Consistent with this mechanism and the rapid rise of [Glu] in the NAc shell, impulse activity of accumbal neurons phasically increases (Kiyatkin and Brown 2007) and cortical EEG is desynchronized (Kiyatkin and Smirnov 2010) well within the duration of iv cocaine injection. Both latter effects are resistant to dopamine receptor blockade, suggesting their nondopamine nature, and are mimicked by cocaine methiodide, which cannot cross the blood-brain barrier (Shriver and Long 1971), suggesting its peripheral triggering. While an excitatory signal triggered from the periphery and arriving to the brain via neural pathways could be the cause of rapid, strong, and brief Glu release seen during the iv injection, the second, more variable phase of the Glu response could reflect a direct action of cocaine in the brain. Its slower appearance, longer duration, larger magnitude, and correlation with locomotor activation are consistent with this pharmacological mechanism.

Nonspecific biological contributions to Glu currents: advantages of high-speed electrochemistry and its essential limitations. In contrast to microdialysis, which is based on direct measurement of chemical substances sampled from the brain, any electrochemical technique relies on measurement of currents produced by oxidation of the substance of interest. This is true for both electroactive substances (i.e., monoamines and their metabolites, ascorbate, urate), which generate currents during their oxidation and reduction by an applied voltage, and nonelectroactive substances (i.e., Glu, glucose), which are detected by employing specific enzymes. Theoretically, when sensors’ sensitivity and selectivity is determined, changes in oxidation currents recorded in vivo could provide a quantifiable measure of the target molecule’s concentration.

However, this study clearly demonstrates that a significant portion of the electrochemical current measured by the Glu sensors, and specifically their slow, tonic components, result from nonspecific chemical and physical contributions. Nevertheless, these contributions reflect certain biological changes that result from both the oxidation of other neurochemical substances and alterations in brain physical environment. Surprisingly, electrochemical currents recorded by Glu sensors showed an exceptionally tight correlation with changes in brain temperature (r = 0.97–0.99), suggesting this variable as a major nonspecific biological contributor to changes in electrochemical currents obtained in behavioral experiments. The importance of this variable was also confirmed by our in vitro tests, which revealed clearly detectable increases in electrochemical currents with small temperature increases. Therefore, despite high substrate sensitivity, Glu probes are also highly sensitive temperature sensors. While temperature per se is a direct contributor to changes in electrochemical currents, transient brain hyperthermia induced by arousing stimuli and cocaine is a manifestation of metabolic brain activation that is associated with changes in various neuroactive substances and metabolites, some of them themselves being electroactive (i.e., DOPAC, uric acid, ascorbate). These latter substances are present in the brain in large concentrations, showing slower and weaker changes that could correlate with relatively slow but prolonged brain temperature increases. Therefore, these chemical changes are another source of additional nonspecific contributions to electrochemical currents recorded in behavioral experiments. Although creating supersensitive and superselective sensors is usually viewed as the way to further increase the reliability of neurochemical measurements, brain temperature fluctuation is a basic physiological phenomenon and it is principally impossible to make temperature-independent electrochemical sensors. Therefore, the use of Glu sensors and parallel differential recordings from the same brain locations appears to be the only way to provide reliable and accurate Glu measurements under behaviorally relevant conditions. This reliability is greater for detecting rapid changes, the main advantage of electrochemistry, and weaker for evaluating slow, tonic changes, which could be affected by other biological factors.

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AUTHOR CONTRIBUTIONS

REFERENCES


GLUTAMATE, SENSORY STIMULI AND COCAINE


