Electrochemical evaluations of physiological and drug-induced fluctuations in extracellular glutamate in freely moving rats

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Introduction
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 [1,2] and the role of dynamic changes in GLU receptors of different subtypes [3-5], our knowledge on physiological and drug-induced fluctuations in GLU release remains unclear. In vivo microdialysis has been widely used for measuring changes in GLU transmission [6,7], but the low temporal resolution of this technique places significant constraints on revealing rapid drug- and behavior-associated fluctuations. In contrast, electrochemistry has excellent temporal resolution, but its measurement selectivity in vivo has always been problematic.

This report presents our recent data, in which high-speed amperometry coupled with enzyme-based, GLU-selective sensors was used in freely moving rats. First, we consider methodological aspects of GLU evaluations in awake, freely moving rats and focus on multiple controls to make these evaluations reliable. Second, we present data on physiological fluctuations in extracellular GLU induced by arousing stimuli in the nucleus accumbens (NAcc) and show their structural specificity for shell and core compartments. Third, we show changes in extracellular GLU induced in the NAcc by intravenous (iv) injections of cocaine and nicotine, two widely used addictive drugs. Finally, we demonstrate that GLU responses undergo consistent changes following drug experience. These data are considered with respect to drug learning and its possible role in the development of addictive behavior.

Methods
In this study, we used commercially prepared enzyme-based GLU sensors (Pinnacle Technology, Inc.), with an active electrode (~1-mm length, 180 µm diameter) incorporated with integrated Ag/AgCl reference electrode. They were inserted into previously stereotaxically implanted BAS cannulae to reach the structures of interest (NAcc, core or shell; ventral tegmental area or VTA). Recordings were conducted in freely moving rats during a single ~8-h session, when the rat was exposed to 5-s audio stimulus, 3-min tail-pinches (TP), 3-min social interaction with another male (SI), and iv injections of either cocaine (1 mg/kg, over 20 s) or nicotine (30 µg/kg, over 20 s). Sensors were calibrated in vitro both before and after in vivo recordings and only those showing high substrate sensitivity and selectivity were used in vivo. Identical recordings were
conducted with GLU-null sensors (lacking glutamate oxidase), which have a similar design and sensitivity to all physical and chemical factors except GLU.

Electrochemical data were sampled at 1 Hz and analyzed with both slow (1-min) and rapid (4-s) time resolution. Because of the sensor design, recordings with GLU-null sensors were conducted in different animals using the same experimental protocol. The method and protocol has been recently described in detail elsewhere [8].

Results and Discussion

*Rapid, structure-specific changes in NAcc extracellular GLU induced by natural arousing stimuli and iv cocaine.*

TP and SI but not a tone induced rapid, relatively large and prolonged current increases detected by GLU sensors. However, GLU-null sensors also detected significant current increases. Subtraction of these non-specific changes revealed that both these procedures induce very rapid, much smaller, and transient increases in extracellular GLU levels (50-75 nM), more predominantly in the NAcc shell than core. In contrast to monophasic responses induced by natural stimuli, cocaine elicited a biphasic GLU increase in the shell, with a transient peak during the injection (latency, ~10 s; magnitude, ~50 nM) and a slower and more prolonged post-injection elevation. While the initial, rapid component of cocaine-induced GLU response remained stable following subsequent cocaine injections, the second peak became larger with drug experience. Our preliminary experiments with cocaine-methiodide, which cannot cross the blood-brain barrier, suggest peripheral action of cocaine as the trigger of the initial GLU release peak. In contrast to much slower and more prolonged direct actions of cocaine on brain neurons, it also acts on neural substrates of visceral sensory nerves densely innervating blood vessels [9]. Current changes detected by GLU-null sensors following natural arousing and pharmacological stimulation were slower and more prolonged and they were tightly correlated with brain temperature increases induced by all these procedures [10]. Therefore, GLU is phasically released in the NAcc following 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, and specifically the use of parallel recordings with enzyme-free GLU-null sensors, to reveal relatively small, highly phasic, and transient fluctuations in GLU levels occurring under behaviorally relevant conditions.

*Iv nicotine induces rapid GLU release in the NAcc and VTA*

Our previous studies [11] revealed that iv nicotine at low, self-administering doses (10-30 µM) induces rapid neural activation as evidenced by desynchronization of electrical activity in the cortex and VTA as well as neck EMG activation. By monitoring GLU currents from the NAcc shell and VTA, we show in this study that iv nicotine also induces rapid GLU release in both structures. Consistent with rapid time-course of EEG desynchronization, GLU release began within the injection duration, was relatively large (as a mean
stronger than that induced by cocaine) but transient, and showed consistent increases following repeated drug injections. Similar to cocaine, experiments with nicotine suggest the importance of parallel recordings with GLU-null sensors, which provide a valuable tool to exclude all chemical and physical contributions to GLU currents and reveal less profound but rapid changes in extracellular GLU. These data also suggest that in addition to the direct action of nicotine on centrally located nicotinic receptors, neural effects could occur very rapidly via drug’s action on peripherally located nicotinic receptors on the afferents of sensory nerves, involving GLU transmission.

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References