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Research Report

High-frequency stimulation of the subthalamic nucleus increases glutamate in the subthalamic nucleus of rats as demonstrated by *in vivo* enzyme-linked glutamate sensor

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ABSTRACT

Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is an effective therapy for Parkinson's disease; however, the mechanism whereby DBS ameliorates the symptoms of Parkinson's disease remains an area of intense research. In the present study, we investigated the hypothesis that the neurotransmitter glutamate is released within the STN during high-frequency stimulation (HFS) of the STN. Direct measurements of extracellular glutamate concentration in the STN were made using a dual enzyme-based electrochemical sensor. The studies were carried out in ketamine/xylazine anesthetized rats placed in a Kopf stereotaxic head frame. Various electrical stimulations (100-μs cathodic pulses; 100–3000 μA; 10- to 1000-Hz frequency; 5-s to 60-min stimulus durations) using bipolar stimulating electrodes were delivered to the STN. Stimulation of the STN elevated the concentration of glutamate in the STN. The concentration of glutamate rose quickly during HFS, remained elevated for the duration of stimulation, and descended slowly towards baseline upon cessation of stimulation. Elevation of the extracellular concentration of glutamate in the STN may be an important mechanism whereby DBS in the STN improves the symptoms of Parkinson's disease. Furthermore, our data argue against the hypothesis that DBS works primarily by electrotonic inhibition of the stimulated structure.

1. Introduction

High-frequency stimulation (HFS) of the subthalamic nucleus (STN) is an effective deep brain stimulation (DBS) procedure for the treatment for Parkinson's disease (Benabid et al., 2000) and has also been used to treat intractable epilepsy (Loddenkemper et al., 2001; Chabardés et al., 2002). Because the effects of HFS in the STN are similar to those of a surgical lesion within the thalamus — both treatments suppress tremor, for example (Bergman et al., 1990), DBS has been thought to

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Abbreviations: DBS, deep brain stimulation; $EAAT_1$, excitatory amino-acid transporter 1; $EAAT_2$, excitatory amino-acid transporter 2; HFS, high-frequency stimulation; 6-OHDA, 6-hydroxydopamine; STN, subthalamic nucleus; SNc, substansia nigra pars compacta

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silence neurons of the stimulated structure (Benazzouz et al., 1995; Lozano et al., 2002). However, we and others have previously reported that HFS caused excitation of neurons in the STN (Garcia et al., 2003; Lee et al., 2003, 2004) and during simulated DBS using HFS in a thalamic brain slice (Anderson et al., 2004). Glutamate is the predominant excitatory neurotransmitter in the STN, and the depolarizing effects of HFS were eliminated by glutamate receptor antagonists *in vitro* in the rat (Anderson et al., 2004; Lee et al., 2004), supporting the hypothesis that HFS in the STN results in glutamate release. However, the extracellular concentration glutamate and the dynamics of HFS-mediated glutamate release and glutamate removal have not yet been measured directly in the STN.

Microdialysis is frequently used to monitor extracellular glutamate levels (Windels et al., 2003, 2005; Boulet et al., 2006), but it has poor temporal resolution. Therefore, an improved detection technique is required to detect HFS-mediated glutamate release. We employed an enzyme-linked glutamate sensor system to measure extracellular glutamate levels directly (Kohno et al., 1998; Oldenziel et al., 2004). This biosensor contains glutamate oxidase, which catalyzes the formation of hydrogen peroxide on contact with glutamate (Oldenziel et al., 2004). The biosensor uses a platinum–iridium electrode to measure the amount of hydrogen peroxide produced from glutamate. Therefore, the glutamate measurement is indirect, but the amount of hydrogen peroxide produced is proportional to the glutamate concentration in the region adjacent to the sensor tip. The biosensor is substrate specific and is relatively insensitive to interfering substances such as ascorbate. The response of the electrode is fast, and the electrode is easily calibrated. Using this electrode, we tested the hypothesis that HFS of the STN increases the extracellular concentration of glutamate within the STN. This is the first step in our larger goal of determining whether excitatory neurotransmitter release may be an important mechanism by which DBS modulates activity of neurons within the STN and other parts of the basal ganglia and ameliorates the symptoms of Parkinson's disease (Lee et al., 2006). The extracellular glutamate concentration in the STN was significantly elevated during and after HFS, and these data provide new *in vivo* electrochemical evidence that the mechanism of action of DBS applied in the STN may be mediated through release of glutamate and other neurotransmitters (Windels et al., 2003; Lee et al., 2004, 2006; Boulet et al., 2006).

2. Results

2.1. *Stereotaxic placements of STN stimulating electrodes and glutamate sensors*

The oxidation current generated by glutamate release was calibrated *in vitro* with known concentrations of glutamate before the start of each experiment. A typical calibration response is shown in Fig. 1A. In this example, the sensor sensitivity was 0.039±0.001 nA/μM glutamate (mean± standard error). Each sensor was calibrated on each day of use. The locations of the stimulating electrode and glutamate sensor are shown schematically in Fig. 1C along with

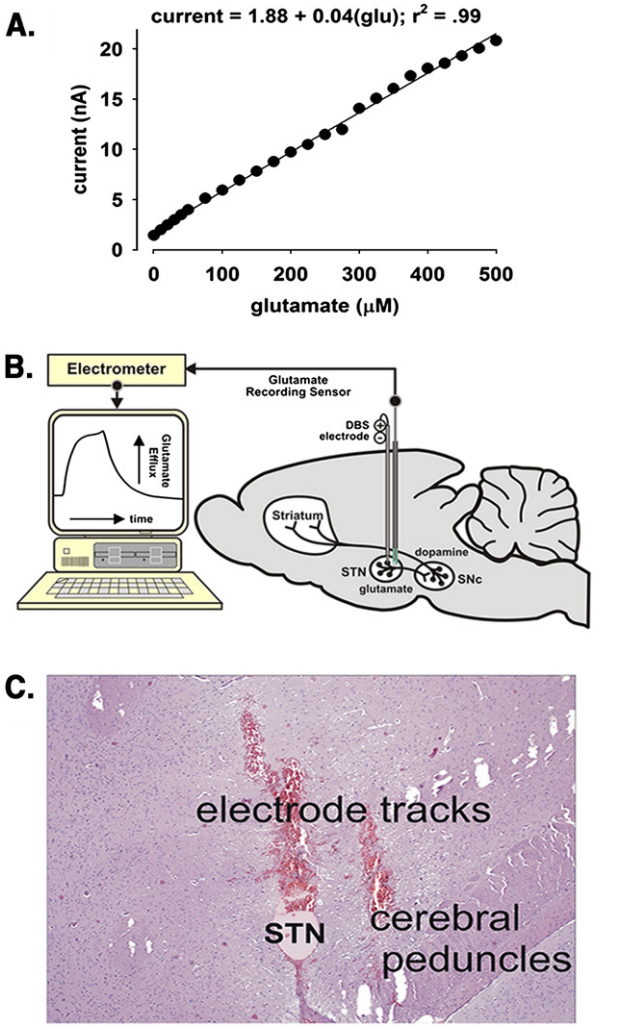

Fig. 1 – (A) An example of a calibration curve for the glutamate biosensor. (B) Schematic sagittal view of the rat brain showing the location of the biosensor within the STN. (C) An example of hematoxylin and eosin stained section through the STN showing the electrode tracks and location of the electrode and biosensor within the STN.

an example of the typical change in extracellular glutamate in response to HFS.

To test the hypothesis that glutamate levels are increased during HFS of the STN, we measured the extracellular concentration of glutamate during and after HFS of the STN. We tested varying durations of 100-Hz stimulation from 10 to 3600 s. We tested at least three of the four stimulus durations (10 s and 5, 10 and 60 min) in each animal (all at 300 μA and a 100-μs pulse width), but all durations could not be tested in each animal. Typical stimulation-evoked changes in the extracellular glutamate concentration from a single animal are shown in Fig. 2A, and the summary of all responses from 11 animals is shown in Fig. 2B. The peak amplitudes of the glutamate concentration were similar among animals after similar durations of HFS. The data from 10 animals were fitted to an exponential growth curve (Fig. 2B). The concentration of glutamate rose to a maximum value, and the plateau glutamate concentration (~500 μM) was achieved after