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Technical note

Wireless voltammetry recording in unanesthetised behaving rats

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Abstract

In vivo voltammetry is a valuable technique for rapid measurement of dopamine in the brain of freely behaving rats. Using a conventional voltammetry system, however, behavioural freedom is restricted by cables connecting the head assembly to the measurement system. To overcome these difficulties, we developed a wireless voltammetry system utilizing radio waves. This system consisted of a potentiostat and transmitter system that was mounted on the back of the rat, and a receiver and analysis system. A single-step pulse (100–250 mV) was applied at 4 Hz after an activation pulse to a carbon fibre recording electrode (diameter: 7 μm). Measurement of dopamine (detection limit: 2.7×10^{-7} M) was demonstrated *in vitro*. *In vivo* experiment was performed at least 1 week after the recording electrode was implanted in the rat striatum. Administration of 2-phenylethylamine to rats increased dopamine signal current, which was consistent with the result in the microdialysis measurement. During a resident–intruder test, dopamine signal current in a resident rat increased upon introduction of an intruder rat. These results show that the present wireless system is useful for a long-term measurement of dopamine in behaving rats.

Keywords: In vivo voltammetry; Telemetry; Cordless; Remote; Dopamine; Social interaction; 2-Phenylethylamine

1. Introduction

In vivo voltammetry has become a powerful method of recording changes in extracellular transmitter concentration in unrestrained animals (Kissinger et al., 1973; Horikawa et al., 1997; Hoffman and Gerhardt, 1998; Crespi et al., 2001; Hopwood and Stamford, 2001; Robinson et al., 2001), allowing examination of amounts of extracellular transmitter similar to microdialysis, but with higher time resolution. With recent increases in time resolution and the demonstration of stable recording over very long-term studies (>1 year; Nakazato and Akiyama, 2002; Nakazato, 2005), in vivo voltammetry has become an important recording technique in studies of learning (Richardson and Gratton, 1996; Roitman et al., 2004; Nakazato, 2005) and drug addiction (Kiyatkin and Stein, 1994, 1995; Phillips et al., 2003; Cheer et al., 2007).

One of the remaining limitations with both conventional voltammetry and microdialysis is the presence of cables connecting the head assembly to the measuring system. The widely used phrase ‘‘freely behaving animals’’ includes the caveat ‘‘insofar as the cables connecting the head assembly to the measurement system allow’’ for conventional voltammetry and microdialysis systems. Specifically, these cables prohibit recording under conditions that may introduce mechanical noise (e.g., very active movement), restrictive twisting of or damage to the cables (e.g., another animal chewing or becoming tangled), or movement through a tunnel or running wheel.

To address these problems, De Simoni et al. (1990) developed a telemetry voltammetry system using optoelectronic transmission, and measured 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindole-3-acetic acid (5HIAA) every 2 min. Imeri et al. (1994) applied the same system to study the sleep–wake cycle in the serotonergic system for 24 h in the rat. The system used two-way wireless interconnections, one for sending the voltage ramp for oxidation from a main unit (on a desk) to a satellite unit (on the animal) and the other for sending measured current from the satellite to the main unit. However, Crespi et al.

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(2004) pointed out that the two-way design would cause variations of voltage at the level of the recording electrode when the first-way channel was interrupted. The susceptibility to the interruption in the first channel would inhibit the system to work stably at a higher sampling frequency.

Two more wireless voltammetry systems were developed in 2004, one using Bluetooth digital telemetry (Garris et al., 2004) and another using infrared transmission (Crespi et al., 2004). In both systems, oxidation waveforms were generated in the satellite unit on the animal side, and consequently much better temporal resolution of 100 ms was achieved. The first one (Garris et al., 2004) was sensitive to dopamine using fast scan cyclic voltammetry, and was used in experiments with an anesthetised rat. However, the system was a large prototype that was mounted on a board of 23 cm × 19 cm, and thus was not actually mounted on a behaving animal.

The second (Crespi et al., 2004) was very small and lightweight, was sensitive to both dopamine and serotonin using chronoamperometry and differential pulse voltammetry, and recorded stable signals in experiments with rats. However, the system was not actually applied to *in vivo* measurements in awake behaving animals. Because of the use of infrared transmission, the system was vulnerable to the interruption of the infrared transmission when an obstacle, such as a hand, was interposed between the main and the satellite system (Crespi et al., 2004).

In this study, we aimed at developing a wireless voltammetry system that would be small and light enough to be fixed to an unanesthetised behaving rat, while maintaining high sensitivity to dopamine, sub-second time resolution, and very long-term (for several months) capability of a conventional wired voltammetry system that we developed in our laboratory (Nakazato and Akiyama, 1999). To overcome difficulties in the previous wireless systems, we generated voltage waveforms in a remote unit on the animal and used radio waves, rather than the infrared radiation, to mediate measured current from the remote unit to a home-base unit.

In the present report, we describe the wireless system and demonstrate the performance of the system first in the measurement of dopamine *in vitro*, second in the measurement of changes in dopamine *in vivo* in rats in response to administration of a releaser of dopamine, and lastly in the measurement of changes in dopamine in response to the introduction of an intruder rat (resident–intruder test).

2. Materials and methods

2.1. Wireless voltammetry system

2.1.1. Hardware

The system consisted of two systems, a potentiostat and transmitter system (a remote system) that carried out voltammetry with recording, reference and auxiliary electrodes, and a receiver and analysis system (a home-base system) that received data sent out from the remote system (Fig. 1). On the remote system, the voltage potential was applied to a carbon recording electrode via a potentiostat unit. Controlling signals were sent to the potentiostat from the CPU (PIC16F876; Microchip Technology Inc.) via a digital–analogue converter (AD557; Analogue-Devices Inc., Japan; Fig. 1B). The specific voltage potential was applied between the references and recording electrodes. The resulting

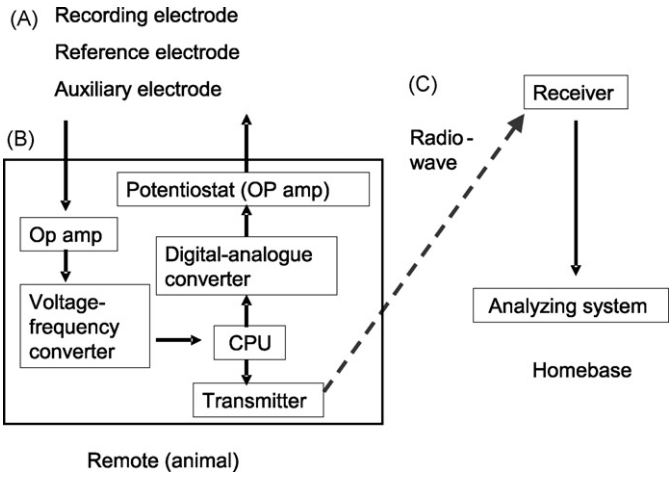

Fig. 1. Diagram of the wireless voltammetry system. (A) A recording electrode, a reference electrode, and an auxiliary electrode were placed in the recording chamber with 100 ml PBS *in vitro* or in the rat brain *in vivo*. (B) Remote system. Digital signals from the CPU were converted to analogue voltage by a digital–analogue converter. The specific voltage potential was applied using a potentiostat between the references and recording electrodes. The resulting current was converted to voltage via an operational amplifier, and then changed to pulses via a voltage–frequency converter. The pulse frequency was calculated by the CPU and transmitted by the transmitter as a radio signal. (C) Home-base system. The transmitted signal was received using a radio-wave receiver, and the data were analyzed using a microcomputer.

current (signal current) was converted to voltage via an operational amplifier (Intersil, CA, USA), and then changed to pulses via a voltage–frequency converter (AD654; Analog-Devices Inc.). The pulse frequency was calculated by the CPU and then transmitted as a radio wave between 259.45 and 264.85 MHz with a channel interval of 50 kHz (HRF-260T; Hertz Electric Co., Japan). The signal was received remotely via a CPU-controlled receiver (HRF-260R; Hertz Electric Co., Japan) that was connected via an RS-232C interface to a computer for data analysis (Fig. 1C). All electronic parts of the remote portion of the system were powered by a rechargeable battery (7.2 V, NP-FF51; Sony, Japan) that allowed for more than 8 h of continuous measurements before requiring recharging. The remote system consisted of two parts, each size was 7 cm × 4 cm × 2 cm (Fig. 2A), and the total weight with the battery was 90 g. The remote system was mounted on the back of the animal by inserting each part into a pair of bags on a harness (Fig. 2C).

2.1.2. Measurement of dopamine signal current

The paradigm used to measure dopamine signal current was as described previously (Nakazato and Akiyama, 1997, 1999). It consisted of a square-wave activation pulse (±2000 mV) and then a single-step measurement pulse (Fig. 3). The activation and measurement pulses were applied every 250 ms during measurements. To measure dopamine signal current, the potential was maintained at 100 mV and then stepped to 250 mV. Chemicals electrochemically oxidized between 100 and 250 mV were detected as the subtraction of the current at 100 mV from that of 250 mV (inset, Fig. 3). It is worth noting that chemicals other than dopamine, such as DOPAC and ascorbate, would be oxidized in the voltage range. By using these parameters, however, the ratio of *in vitro* sensitivity to dopamine, DOPAC and ascorbate is reported to be around 2000:20:1 (Nakazato and Akiyama, 1997). Thus, if the same amount of dopamine, DOPAC and ascorbate increased in a short-time period, changes in the subtracted signal current largely reflect increase in dopamine. Accordingly, we term the subtracted current as dopamine signal current.

2.2. In vitro voltammetry measurement

The recording electrode consisted of a pulled glass capillary tube containing a carbon fibre (7 μm in diameter, Toho Tenax Co., Tokyo, Japan; 500 μm of carbon fibre protruded from the pulled capillary tube tip) (Nakazato and Akiyama, 1997). The electrodes were initially activated (polished) *in vitro*