Neuroscience 134 (2005) 301–315

REAL-TIME AND AUTOMATIC SORTING OF MULTI-NEURONAL ACTIVITY FOR SUB-MILLISECOND INTERACTIONS *IN VIVO*

S. TAKAHASHI* AND Y. SAKURAI

Department of Psychology, Graduate School of Letters, Kyoto University, Kyoto 606-8501, Japan

Abstract—Recent *in vitro* electrophysiological studies have revealed that neighboring interneurons interact with each other in a sub-millisecond time range via gap junctions and that individual dendritic compartments generate local excitation spikes and back-propagated spikes within a single-neuron. However, most *in vivo* electrophysiological studies using behaving animals only focus on activity rates of single-neurons and/or large neuronal populations without considering the potential role of such sub-millisecond interactions among neurons. This neglect is due to the limitation of ordinary *in vivo* multi-neuronal recording and spike sorting techniques applied to behaving animals. Though independent component analysis (ICA) is a powerful method to overcome certain limitations, ICA has a serious problem in that the number of single-electrodes (microwires) must be more than the number of single-neurons to be recorded. Our recently-developed method has solved this limitation of ICA, but a few problems have remained: the computational load is heavy, the method can be used only for off-line, not real-time, processing, and the electrode-neuron drift problem remains unsolved. In this paper, solving all these problems, we introduce a novel system consisting of automatic and real-time spike sorting with ICA in combination with a newly developed multi-electrode, *dodecatrode*. The system has the potential to answer some important neurobiological questions that have not been explored in *in vivo* electrophysiological experiments: how sub-millisecond interactions between closely neighboring single-neurons act in freely behaving animals. The system promises to be a bridge connecting electrophysiological studies *in vitro* and *in vivo*.

Key words: spike sorting, independent component analysis, dodecatrode.

Recent *in vitro* electrophysiological studies have revealed that closely neighboring interneurons interact with each other in a sub-millisecond time range via gap junctions (Galarreta and Hestrin, 2001a) and that individual dendritic compartments generate local excitation spikes and back-propagated spikes within a single-neuron (Stuart et al., 1997). However, most *in vivo* electrophysiological studies using behaving animals only focus on the properties of single-neurons and/or large neuronal populations without considering the potential role of such sub-millisecond activity interaction among neurons. This neglect is due to the limitation of ordinary *in vivo* multi-neuronal recording and spike sorting technique applied to behaving animals. Three serious problems cause the limitation. First, the spike waveforms of individual neurons sometime overlap on a common electrode when two or more neurons fire coincidently or when two neurons are electrically coupled via gap junctions. Most ordinary spike sorting techniques for multi-neuronal recording cannot separate such overlapping spikes. This is referred to as the "spike-overlapping problem" (Lewicki, 1994; Takahashi et al., 2003a,b). Second, the spike waveforms of single-neurons, especially in the presence of complex spike bursts, are often non-stationary (Fee et al., 1996b). This causes spike-isolation errors when researchers employ template-matching methods on the assumption of stationary waveforms. This is called the "non-stationary spike waveform problem." The third problem is the "electrode-neuron drift problem" where the relationship between the electrode and the recorded neurons sometime drift slowly during recording sessions because the brain tissue does not stabilize in response to pressure from the electrode (Lewicki, 1998). Though some recent techniques for spike sorting (Lewicki, 1994; Fee et al., 1996a; Kaneko et al., 1999; Huang and Miller, 2004) have solved some of the problems, no single technique can solve all three problems.

Independent component analysis (ICA) (Comon, 1994) is a method that was originally developed for solving blind source separation (BSS) problems. The basic procedure of BSS is to unmix N independent signals that have been linearly mixed onto N channels with an unknown mixing matrix. The only assumption underlying ICA is that the unknown sources are statistically independent, thus making ICA a powerful method to solve both the spike-overlapping and non-stationary waveform problems (Takahashi et al., 2002). However, ICA has a serious limitation that the number of single-electrodes (microwires) must be more than the number of single-neurons to be recorded. Our recent study (Takahashi et al., 2003a,b; Sakurai et al., 2004) overcame this limitation of ICA and introduced a unique method, suggesting that the combination of ICA and k-means clustering can solve both the spike-overlapping and non-stationary waveform problems and, at the same time, can record more neurons than the number of microwires. The only remaining problem is that the computational load is heavy and the method can be used only for off-line, not real-time, spike sorting. This also leaves the

*Corresponding author. Tel: +81-75-753-2452; fax: +81-75-753-2452.
E-mail address: susumu.takahashi@bun.kyoto-u.ac.jp (S. Takahashi).
Abbreviations: A/D, analog-to-digital; BMI, brain–machine interface; BSS, blind source separation; FET, field-effect transistor; FS cell, fast-spiking cell; ICA, independent component analysis; ICBV, independent component basis vector; PCA, principal component analysis; RASICA, real-time and automatic sorting with independent component analysis; SNR, signal-to-noise ratio; S1 cortex, primary somatosensory cortex.

0306-4522/05$30.00+0.00
doi:10.1016/j.neuroscience.2005.03.031

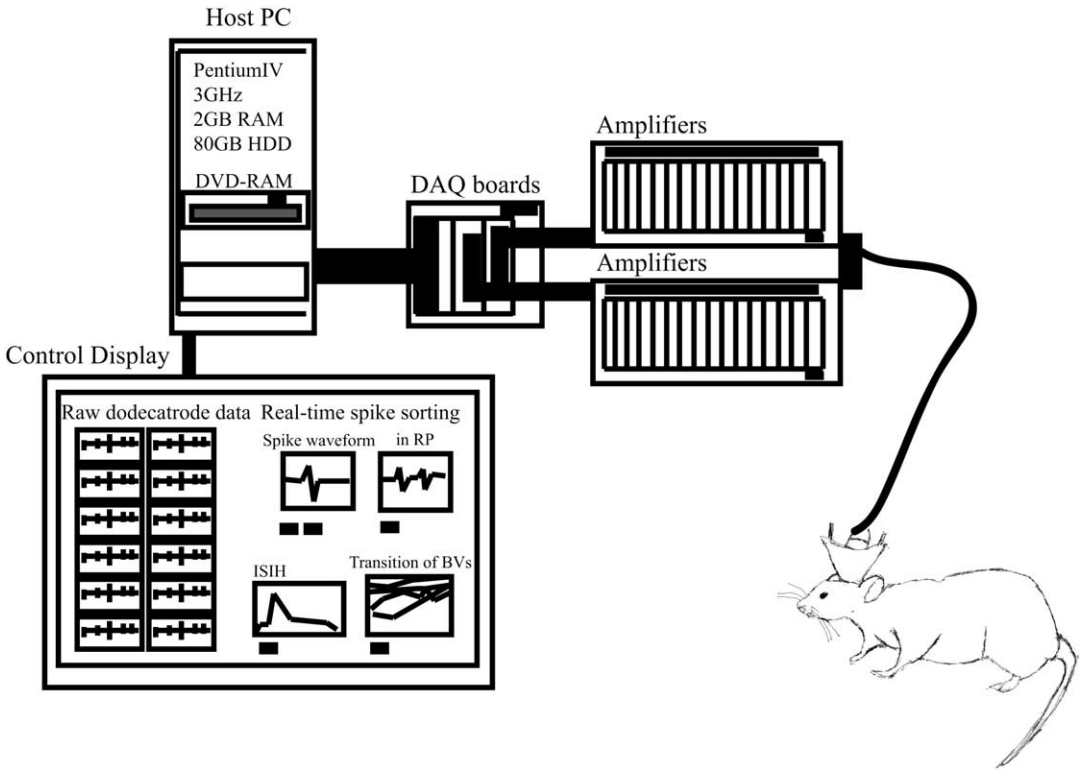

Fig. 1. A schematic diagram of the system for the RASICA. A host computer for the RASICA controls the one A/D board and data storage, transmitted from the A/D board to the personal computer via the high speed bus, and is responsible for the real-time spike sorting with ICA (see Experimental Procedures). The data files are written to backup DVD-RAMs in a DVD drive.

electrode-neuron drift problem unsolved. The sub-millisecond neuronal interactions are inevitably influenced by a little contamination. Therefore, we must solve all three problems described above because it is possible that many neurons fire simultaneously and exhibit complex spike bursts during sharp wave-associated population events (Harris et al., 2000), and that the electrode-neuron drift forces spike sorting methods to misclassify clusters.

Here, solving all three problems, we introduce an automatic system for real-time multi-neuronal spike sorting in combination with newly developed multi-electrodes and ICA. We show that it can be applied to investigate sub-millisecond processing of information by closely neighboring neurons in freely behaving animals.

EXPERIMENTAL PROCEDURES

Electrode

We bundle and twist 12 8 μm diameter insulated tungsten microwires (California Fine Wire, Grover Beach, CA, USA) and insert them into the independent movable microdrives. We call the electrode consisting of the 12 microwires a "dodecatrode." Each uninsulated end of the dodecatrode is fixed with a small connector, fixed with conductive epoxy, and the whole assembly sealed and fixed in place with dental acrylic. The tip is freshly cut at a right angle using sharp surgical scissors before each penetration. The tip is not plated and the impedance is approximately 1 MΩ at 1 kHz. The diameter of a dodecatrode is around 70 μm. Two dodecatrodes are implanted per rat and the distance between the dodecatrodes is ~0.5 mm. Ground wires are also soldered to free pins in the plastic connector.

System for spike sorting

We developed a system for real-time and automatic sorting with ICA and call it "RASICA." The ICA we employ for RASICA was reported to be a powerful method to solve both the spike-overlapping and non-stationary waveform problems (Takahashi et al., 2002, 2003a,b; Sakurai et al., 2004). In the most complete configuration (Fig. 1), RASICA allows simultaneous sampling from a maximum of two dodecatrodes. In this configuration, head stages, containing 24 field-effect transistors (FETs, Toshiba, Tokyo, Japan) are used to connect the 24-channel plastic connectors cemented in the animal's head to the preamplifiers (Nihon Koden, Kyoto, Japan). In all recordings, the FETs are set as source followers. The preamplifiers contain differential OP-Amps (gain 10). Their output signals are transmitted, through cables, to differential amplifiers. The analog signals are filtered (bandpass 500 Hz–10 kHz). The RASICA converts the filtered analog signals to digital ones using one 12-bit analog-to-digital (A/D) converter (National Instruments, Austin, TX, USA), which simultaneously digitize the waveforms defining extracellular action potentials at 25 kHz. After A/D conversion, the signals are routed to the real-time spike-sorting program with ICA on a PC host computer. During the experiment, the time of occurrence of each of the valid spikes for all 24 channels is written to the hard disk of the PC host. Digitized samples of the spike waveforms are also recorded periodically and stored for verifying the validity of spike isolation performance. The host PC is a single Pentium IV personal computer with hyper-threading (3GHz, with 2 GB of RAM and 80 GB of disk space) running LabVIEW (National Instruments, Austin, TX, USA) in the Windows XP operating system (Microsoft, Seattle, USA). It