Independent Component Analysis of Optical Recordings from the Seaslug *Tritonia*

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Abstract

Independent component analysis (ICA) was used to separate action potential trains recorded with voltage-sensitive dyes in the isolated brain preparation of the seaslug *Tritonia*. It was assumed that the membrane potential of each neuron was an approximately independent source and that detectors (photodiodes) recorded linear mixtures of sources. These assumptions appear to be reasonable as ICA outperformed existing methods for separating spike trains in optical recording data. Spike trains, artifacts, and noise were assigned to different output channels (independent components). The ICA analysis method was also less labor intensive than other methods.

Introduction

Experimental approaches to the study of population coding require the collection and analysis of action potential trains from many neurons simultaneously. This may be accomplished with arrays of microelectrodes (Kruger, 1983; Wilson and McNaughton, 1993; Nordhausen et al. 1996; Nicolelis et al. 1997) or with voltage-sensitive dyes (Cohen and Lesher, 1986; Nakashima et al. 1992). One problem faced by researchers recording populations of neurons is the assignment of action potentials to individual neurons. In general, multiple neurons can be recorded by each detector and activity from one neuron can often be found on multiple detectors (Figure 1).

The most common approach to solving the assignment problem involves two distinct steps. First, action potentials are found on each detector separately, and a variety of techniques for doing so have been reported (Abeles and Goldstein, 1977; Andresen et al. 1979; Jansen and Ter Maat, 1992; Yamada et al. 1992; Lewicki, 1994; Sahani et al. 1997). Typically, this involves clustering around certain features of the action potentials such as spike height and width, or template matching of the entire waveform. After action potential detection, events that are coincident on multiple detectors are assigned to a single spike train (Meister et al. 1994; Yamada et al. 1992). Various levels of automation have been achieved, but most assignment procedures still require a human expert at one or more steps.

The spatial distribution of action potentials recorded on multiple detectors contains more information than simple coincidence. For example, McNaughton et al. (1983) simultaneously recorded action potentials from several neurons on two closely spaced electrodes. Spike trains were then separated based on the ratio of their heights

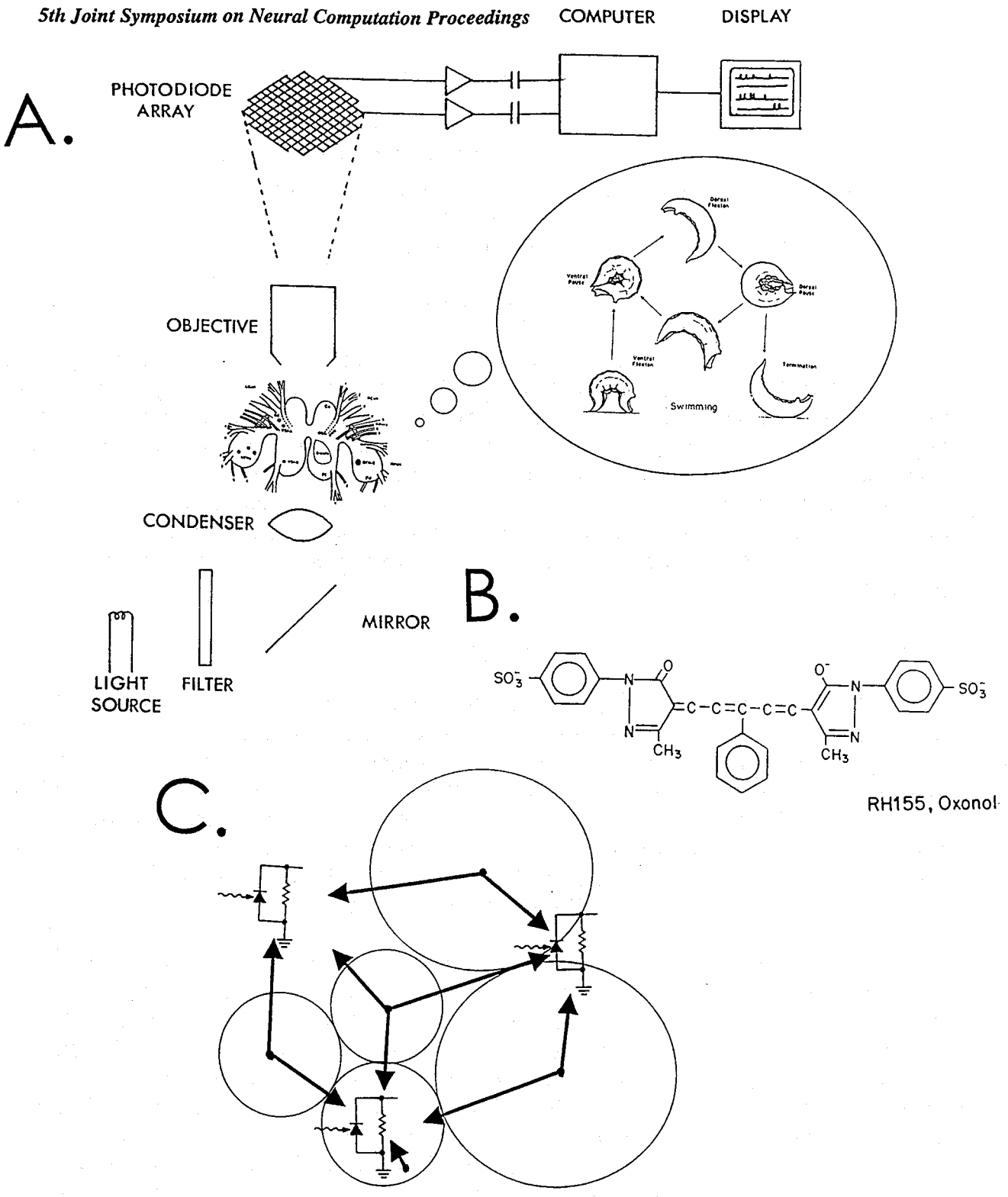


Figure 1. A. Experimental Setup. The *Tritonia* brain was bathed in a voltage sensitive dye and placed under the microscope objective for recording. Absorption changes were recorded on a 448-photodiode array, amplified, and digitized for display and analysis. A fictive swim pattern was activated by stimulation of a nerve root attached to the isolated brain. The actual swimming behavior is pictured in the balloon. B. Voltage-Sensitive Dye. RH155. C. Geometry of the Spike Sorting Task. Photodiodes record voltage changes from multiple neurons (shown as circles). One neuron is recorded on multiple detectors (arrows) so that information from more than one detector can be used to do spike sorting.

on the two electrodes. A similar approach has now been applied to tetrode recordings, which use four electrodes to detect action potentials from multiple neurons (Rebrik et al. 1997). Unfortunately, this correlational approach uses only one point--the peak--in the spike waveform. It also does not consider higher-order relationships between the detectors (see Figure 1). Clustering of the results is still performed manually (but see Zhang et al. 1997).

An alternative approach is to use the mutual information among the detectors to do the separation. Recently, a new technique called independent component analysis (ICA) has been developed to separate mixtures of signals from multiple sources (Comon, 1994; Bell and Sejnowski, 1995; Amari et al. 1996). ICA maximizes the entropy between output units in a feed-forward neural network, thus using statistical dependencies between mixtures of all orders to do the separation. If mixing is linear and sources are independent and non-Gaussian, then the outputs of an ICA network should be the unmixed sources--the membrane potentials of individual neurons in this case.

We have tested ICA on a data set of optical recordings made in the isolated-brain preparation of *Tritonia* (Brown et al. 1994). The *Tritonia* brain has certain properties that

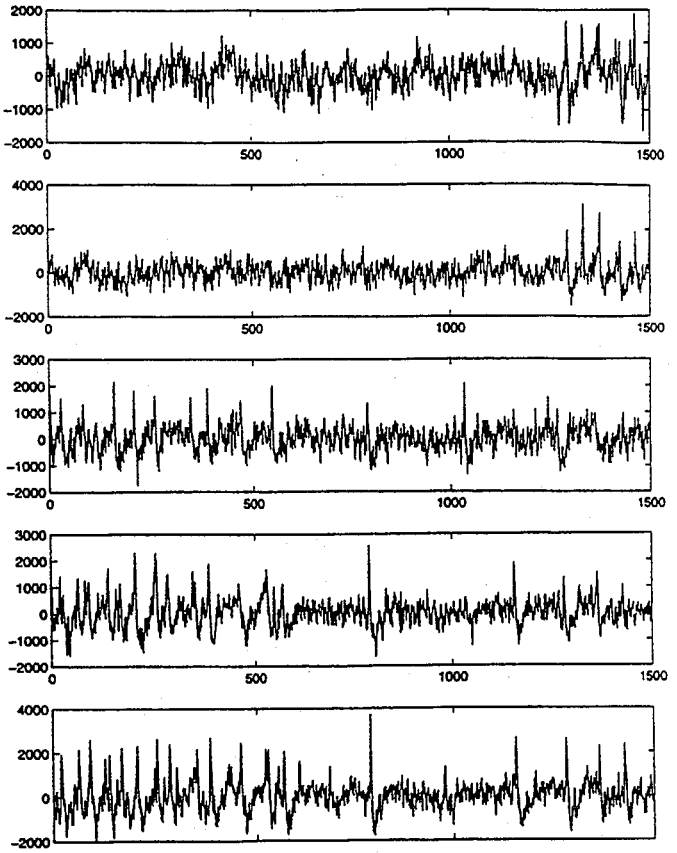


Figure 2. Raw Data. 1500 ms of raw data is shown from five nearby detectors with an arbitrary scale measuring absorption on the Y axis. Note that on the left side of the bottom three traces, there appears to be a neuron firing that shows up on all three. However, closer inspection reveals a more complicated pattern. Another unit at about 775 ms shows up on the bottom four traces, etc.

make it good for optical recording studies including large neurons, a thin outer sheath, and not too much pigment (Boyle et al. 1983). The large number of neurons recorded in a single preparations makes it a difficult test case for any separation method (Figure 2).

Methods

Tritonia isolated brain preparations were prepared as described in Dorsett et al. (1972). The isolated brain can be kept alive for several days in isolation, and a fictive swimming pattern can be activated by a few electrical pulses applied to a nerve root (Figure 1). Many neurons are inhibited during fictive swimming, which is advantageous because it lowers the background activity for optical recording. In addition, neurons in the pattern generator for swimming have the same periodicity as the swimming behavior itself and were therefore easily recognized.

The absorption dye was RH155, which has been used previously in a number of optical recording studies (e.g. Zecevic et al. 1989). Absorption changes due to action potential activity were collected on a 448-photodiode array and digitized at 1 KHz (Nakashima et al. 1992). A single fictive swim episode was collected on each 45 s trial. Typically, photodynamic damage limited the number of trials to two per preparation.

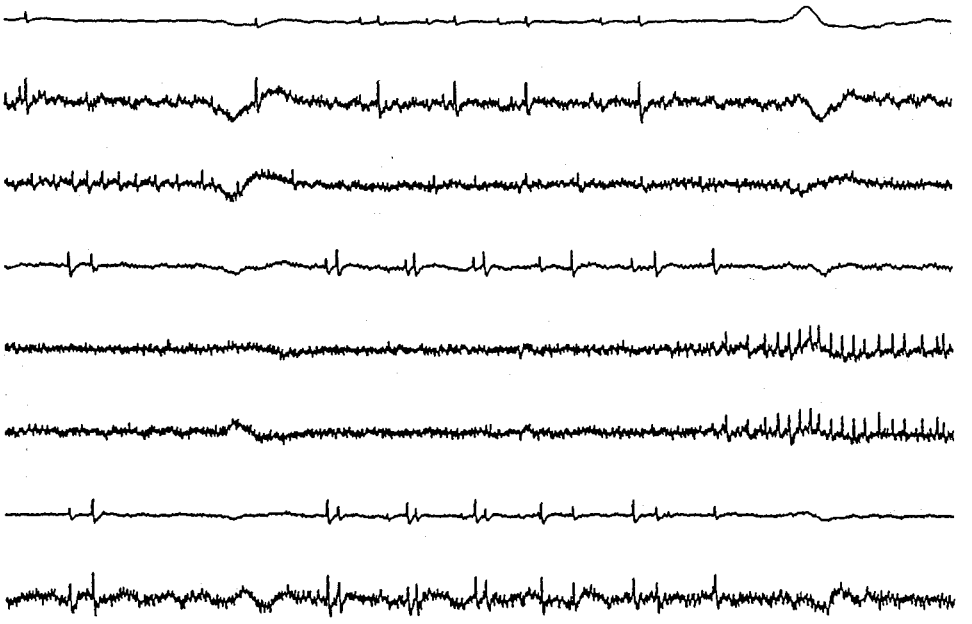
ICA was performed on all 448 channels simultaneously or on overlapping subsets of channels (piecewise ICA). We were able to use piecewise ICA because a single neuron typically only appeared on between 1 and 9 contiguous detectors. Subsets of neurons were merged in a final ICA step to eliminate redundancies. No differences were found in the results from the two methods.

Results

The primary result was that ICA separated action potentials from multiple-detector arrays into individual spike trains (Figure 3). Action potentials from an individual neuron were assigned to a single output channel indicating that the assumptions of the linear-mixing model were reasonable. Artifacts were also separated out, though it appeared that the same artifact sometimes appeared on multiple output channels. (We did not explore this further because clean separation of artifacts was not a goal of the analysis.)

We compared ICA to existing methods for processing optical recording data of this type (Yamada et al. 1992; Yamada, unpublished). ICA corrected several types of errors. First, when spikes from two action potential trains overlap, old methods sometimes assigned the spike to one action potential train or the other. When at least one of the spikes appeared on a second input channel, ICA assigned one spike to each action potential train. Second, the old method tended to assign one or a few action potentials from certain neurons to separate channels, which resulted in a large number of action potential trains with one or a few spikes. Sometimes these could be fixed manually in the old method, but ICA never made this type of over separation error. Finally, ICA made fewer mistakes--as judged by experienced users of the old method--

A.



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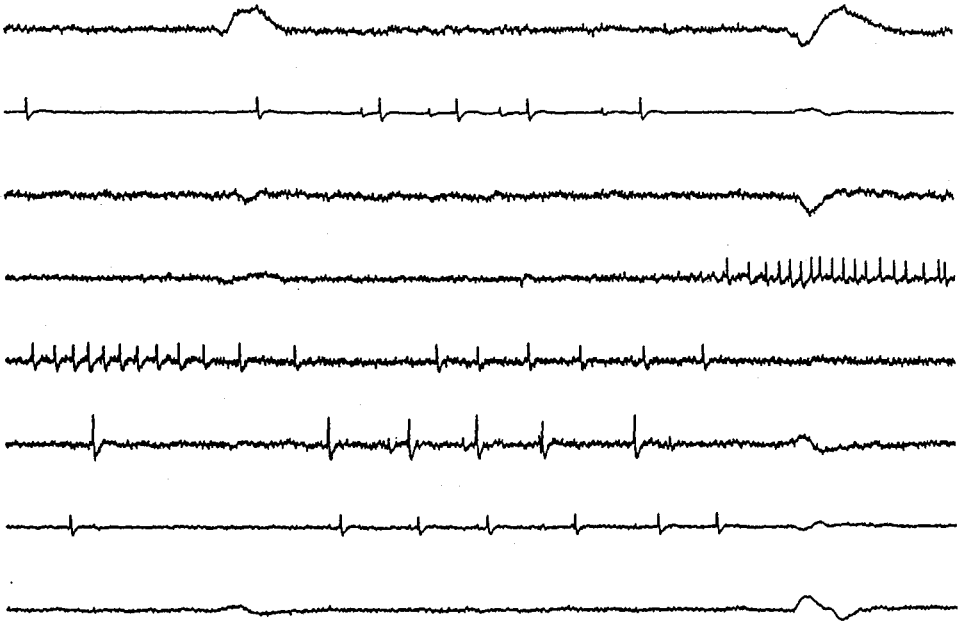

Figure 3. A. Raw Data. A five second epoch of eight selected channels from one experiment are shown. B. After ICA. Spike trains and artifacts have been separated into separate channels.

in the most fundamental assignment problem, for example separating two action potential trains that appear on two or more detectors.

ICA, as used in this study, could not separate action potential trains from two or more neurons that appeared on only one diode (see Figure 3). However, ICA removed artifacts and reduced noise so that it was valuable as a preprocessing step for spike-sorting methods that separate action-potential trains from a single mixture (Yamada et al. 1992, Lewicki, 1994). Sometimes very small action potentials were also lost, though this problem was corrected when we used the extended ICA algorithm for sub-Gaussian sources (Lee and Sejnowski, 1997). Extended ICA is computationally more intensive than normal ICA, and the improvement in separation was marginal.

We also compared ICA results to several other methods which use information from multiple detectors simultaneously to sort spikes. Principal component analysis (PCA) performed poorly except in the simplest cases of a single neuron on multiple channels. In general, variance tended to be spread across many principal components so that dimensionality reduction with PCA was also dangerous. The JADE algorithm (Cardoso and Souloumiac, 1993) which uses fourth-order moments of the data, was comparable to ICA at sorting action potentials trains in one example. A wavelet algorithm (Koehler and Orglmeister, 1998) did not perform as well on the separation, but did separate out many of the action potential trains and was computationally more efficient than the ICA and JADE algorithms.

Discussion

In summary, ICA provides an excellent method to process multiple-detector recordings of action potential trains from multiple neurons. Action-potential trains from individual neurons were assigned to separate channels, artifacts were removed, and noise was reduced. Overall, ICA performed better than existing methods for processing this type of data. Indeed, ICA appeared to outperform human experts at this task, although this was impossible to say with certainty on this (real) data since there was no independent measure of accuracy.

Already, our method can save many hours, perhaps days, of manual data analysis for each experiment. At present, human input is only required when the resulting spike trains are sorted into the categories of "only one spike train per output channel" or "more than one spike train per channel." This is necessary because channels that obviously contain action potentials from two or more neurons need to be separated by other methods (Yamada et al. 1992; Lewicki, 1994). It should be possible to fully automate this process so that the input is raw data, and the output is raster diagrams of action potential trains.

As mentioned earlier, when two or more spike trains were recorded on only one detector, ICA assigned them to a single output channel. As formulated by Bell and Sejnowski (1995), ICA requires at least as many mixtures as there are sources and is also unable to separate, for example, three neurons detected by two electrodes. Assignment

of one action potential train may be split between two output channels in this case. It may be possible to use overcomplete ICA (Lewicki and Sejnowski, 1997) to separate the spike trains when there are more neurons than detectors.

We are currently investigating several other applications of ICA relevant to the recording of neuronal populations. First, spike activity recorded by arrays of microelectrodes face the same problems as multiple-unit, multiple-detector optical recordings, and we are using ICA to sort action potential trains acquired by electrode arrays. Second, it may be possible to modify ICA to cluster action potentials from different neurons that appear on a single detector (the problem discussed above). There is information in both the spatial distribution of action potentials across the detectors and the shape of the action potentials in a single detector. Ideally, this information would all be used simultaneously in a spatio-temporal ICA algorithm to accomplish the spike sorting task. Third, we are working toward an on-line implementation of ICA for real-time acquisition of spike times from multiple neurons. The wavelet algorithm was the only one tested so far that has enough computational efficiency for on-line spike sorting. Since ICA did a better job at sorting spikes, we are currently testing other algorithms for doing ICA (e.g. Hyvarinen and Oja, 1997). Finally, after action potentials have been sorted, it will be of interest to study the independent components of the spike time data.

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