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Journal of Neuroscience Methods 146 (2005) 61–68

JOURNAL OF NEUROSCIENCE METHODS

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Bootstrap resampling method to estimate confidence intervals of activation-induced CBF changes using laser Doppler imaging

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Received 10 September 2004; received in revised form 14 January 2005; accepted 19 January 2005

Abstract

Laser Doppler imaging (LDI) signal and noise characteristics can vary significantly depending upon the underlying vascular caliber. Further, noise characteristics are not constant over time (non-stationary) and can vary during resting and activated conditions in a typical experiment. Since only a limited number of images can be acquired in a single run, concatenation of data from similar experimental trials becomes necessary which can induce further variation in temporal noise due to instrumental response. In conventional statistical analysis methods such as cross-correlation, a fixed significance threshold is generally used (for the entire image) to detect activation assuming constant noise over time and a normal distribution. As a consequence, statistical significance can become strong or weak due to temporal differences in baseline LD noise, which can possibly deviate from a normal distribution. The main emphasis of this study was the application of bootstrap resampling in conjunction with cross-correlation to estimate the confidence intervals on a pixel-by-pixel basis to avoid distributional specifications on the additive measurement error leading to reliable whisker activation-induced CBF changes. At a 95% confidence level, bootstrap resampling followed by confidence intervals for the correlation coefficient distribution increased the number of active pixels by almost 45% when compared to conventional cross-correlation. These pixels were mostly confined to areas with intermediate and large baseline LD flux with considerable deviation from normality. It is suggested that confidence intervals of the bootstrap estimates can lead to unbiased detection of CBF change in the cerebral cortex, particularly in regions with large temporal variation in noise and low CNR.

Keywords: CBF; LDI; LDF; Rat; Barrel cortex; Bootstrap; Resampling

1. Introduction

Laser Doppler flow (LDF) technique has been used extensively to characterize hemodynamic response to functional activation in animal models. Though well established, LDF measurements are limited by its spatial resolution to one or two probes that have a sampling volume of 1–2 mm^3 (Haberl et al., 1989). These single probe measurements cannot account for spatial variations within or between different brain regions caused by stimulus-induced changes (Braverman et al., 1990). Laser Doppler imaging (LDI) systems with mechanically driven optics enable the measurement of blood flow change by scanning in a two-dimensional raster pattern (line-by-line) (Wardell et al., 1993, 1994). A significant improvement in LDI over LDF is that LDI measures blood flow from every point in the image to generate a planar surface map of laser Doppler flux representing cerebral blood flow.

The LDI technique has been used on a number of physiological systems including skin, skeletal muscle and brain to study blood flow (Bray et al., 2003; Lauritzen and Fabricius, 1995; Linden et al., 1995; Sommer et al., 2003; Troilius et al., 1992). In currently available commercial LDI scanners, a temporal resolution in the range of a few seconds can be achieved. However, a limitation of this system is its ability to collect a limited number of sequential images in a single 'run' and a temporal resolution in the range of tens of seconds, which is not very optimal in functional imaging terms. Since perfusion images with substantial spatial resolution and reproducibility can be obtained, the LDI technique

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0165-0270/$ – see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.jneumeth.2005.01.021

has been useful in micro imaging studies in small animal models (Ances et al., 1999). Recently we have applied the LDI technique to study negative CBF responses adjoining positively activated areas in response to whisker stimulation in the rat model (Kannurpatti and Biswal, 2004).

Traditionally activation maps (location of stimulation-induced neuronal functional responses) are statistically determined by a *t*-test, *F*-test or cross-correlation. Subsequently a significance threshold for the *t*-value, *F*-value or the correlation coefficient is used to represent activation. The most commonly used statistics is the cross-correlation analysis where an idealized reference waveform representing the "ON/OFF" cycle of the stimulus is cross-correlated with every time-course of the image signal on a pixel-by-pixel basis. Subsequently a fixed significance threshold of the correlation coefficient is applied to the correlation map. Pixels that have a signal time-course similar to the reference waveform have a high correlation value and all pixels with high correlation coefficient or above a significant threshold are considered active. However, in the above methods it is assumed that variance in each pixel is constant over time which need not necessarily be true. Further, in case of LDI studies, data from several similar experimental runs would be necessary to obtain sufficient number of sampling points. Variation in instrumental response can also induce variation in the temporal noise. Thus, departure from the above assumptions would lead to sub-optimal detection of activation. To improve statistical reliability, the bootstrap technique, a commonly used resampling procedure was used to estimate the confidence interval of LDI parameters. In bootstrap resampling, parts of the original data set are randomly selected to empirically generate pseudo sample data sets that can reproduce the population distribution exactly as the number of resamples grow. The specific parameter of interest for the pseudo-set can then be determined. This procedure is repeated a large number of times with a different sample being analyzed each time. As a consequence, pixel-wise statistical characteristics or parameters of interest can be determined. The resulting advantage of bootstrap over other parametric statistical methods is that activation maps can be generated with all pixels having the same confidence interval such that the statistical power in pixels with equivalent increase in LD flux due to task would not be decreased due to violations in distributional assumptions.

2. Methods

2.1. Surgical preparation

Sprague–Dawley rats (250–300 g, $n = 5$) from Taconic Labs, Germantown, NY were anesthetized with urethane (1.2 g/kg i.p.). Additional bolus of anesthesia (20% of initial dose) was injected at later times if blood pressure increased to a tail pinch during the protocol. A rectal probe was used to monitor the body temperature while the animal was maintained at 37.0 ± 0.5 using a homeothermic feedback heating system (Baxter K-MOD100, Gaymar Industries). The femoral arteries were cannulated with PE50 tubing for monitoring mean arterial blood pressure (MAP) and blood gas sampling. The rats were endotracheally intubated and administered with a single dose of gallamine triethiodide (250 mg/kg i.p.), a muscle paralysis agent, prior to mechanical ventilation. Arterial blood pressure, end-tidal CO_2 and inspired oxygen concentration were continuously monitored (8100 Poet Plus Vital Signs Monitor, Criticare Systems Inc., Wisconsin). The physiological parameters under the ventilator settings used with room air were: $P_aO_2 = 93.8 \pm 7.1$ mmHg, $P_aCO_2 = 35 \pm 3$ mmHg, $pH = 7.4 \pm 0.06$ and $MAP = 92.2 \pm 7.1$ mmHg. The study protocol followed institutional guidelines and was approved by the Animal Research Center of our institution.

The head was secured to an adjustable stereotaxic frame and the scalp was retracted from the fronto-parietal cortex region of the intact cranium by a midline incision. The temporalis muscle was disconnected from the skull and an area of 5 mm × 5 mm (2 mm posterior and 5 mm lateral to the bregma) enclosing the whisker barrel on either hemisphere was thinned to translucency (Kannurpatti and Biswal, 2004). The underlying vasculature was visible when washed with saline. The stereotaxic frame was tilted by 30° on the midline axis to align the whisker barrel cortex normal to the laser beam.

2.2. Laser Doppler imaging

Laser Doppler imaging was carried out using the Moor LDI device (Moor Instruments, Sussex, UK). The method uses the principle of monochromatic light incident on tissue being scattered by moving red blood cells and as a consequence is frequency broadened. The frequency-broadened light together with laser light scattered from static tissue is photo-detected and the resulting photocurrent is processed to provide a blood flow measurement. Blood flow measured by the laser Doppler technique usually termed LDI flux is a quantity proportional to the product of the average speed of the blood cells and their number concentration.

A plane mirror in the device was used to direct the beam from a low power (2 mW ± 20%, 632.8 nm) He–Ne laser onto the tissue surface and also collect the scattered Doppler shifted beam from the tissue, which was focused onto a photodetector. The mirror controlled by a motor enabled the laser beam to scan in a raster pattern across the surface of the tissue and the signal proportional to the tissue perfusion at each measurement point was calculated and stored. When the scanning procedure was completed, the Doppler shift was processed to build up a color-coded flux image of blood flow. The Moor LDI image can be generated in a matrix of upto 256 × 256 pixels covering an area of 5 cm × 5 cm with a maximum distance of 1 m between the head of the scanner and the tissue surface imaged. Shortening the distance between the imaged tissue and the scanner head to about 20–30 cm results in the reduction of the cross-sectional area of the laser beam