Multimodality Multidimensional Image Analysis of Cortical and Subcortical Plasticity in the Rat Brain

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Abstract—In this work, we developed and implemented a multimodality multidimensional imaging system which is capable of generating and displaying anatomical and functional images of selected structures and processes within a vertebrate’s central nervous system (CNS). The functional images are generated from [14C]-2-deoxy-D-glucose (2DG) autoradiography whereas the anatomic images are derived from cytochrome oxidase (CO) histochemistry. This multi-modality imaging system has been used to study mechanisms underlying information processing in the rat brain. We have applied this technique to visualize and measure the plasticity (deformation) observed in the rat’s whisker system due to neonatal lesioning of selected peripheral sensory organs. Application of this imaging system revealed detailed alignment of the sections and to avoid propagation of errors is capable of registering images with subpixel accuracy. It uses a technique which employs extrinsic fiduciary marks for alignment and registration problems. We developed an image registration technique that employs extrinsic fiduciary marks for alignment and is capable of registering images with subpixel accuracy. It uses the information from all available fiduciary marks to promote alignment of the sections and to avoid propagation of errors across a serial data set.

Keywords—Multimodality imaging, Image registration, Brain mapping, Brain plasticity, Rat brain, Imaging

INTRODUCTION

In diagnostic and research applications, the interpretation of brain images is greatly enhanced when different data sets are compared in the same coordinate system. The ability to integrate information collected by different imaging modalities has great potential for answering basic and clinical problems in the neurosciences. For instance, imaging modalities like positron emission tomography (PET), single photon emission computed tomography (SPECT), and functional magnetic resonance imaging (fMRI) can provide information about brain function, allowing the study of hemodynamics, pharmacokinetics, and metabolism in vivo. However, although these functional imaging modalities often exhibit excellent contrast between different tissues, they suffer from low signal-to-noise ratio (SNR) and poor spatial resolution. Therefore, the understanding of brain function is greatly enhanced when functional images are complemented with the underlying anatomy visible on other imaging modalities, such as magnetic resonance imaging (MRI) and computerized tomography (CT).

Some examples involving multimodality imaging of the brain can be found in the work of Apicella et al. (1); the combination of CT, MRI, and PET images by Pelizzari et al. (19); the use of stereotactic coordinate systems for anatomical-functional correlative studies by Evans et al. (8); and the work of Ogawa et al. (18), which employs MRI and fMRI. All of the above-mentioned investigators use multimodality imaging systems to obtain better anatomical localization of functional activity in the brain. This information has been used in radiation therapy, surgical planning, cognitive studies, and brain mapping.

Multimodality three-dimensional imaging has become increasingly important in clinical environments, particularly for the diagnosis and treatment of brain disorders like epilepsy, Alzheimer’s disease, and multiple sclerosis. Its importance to neuroscience has also been demonstrated (7,9,20). In this paper, we introduce a multimodality imaging system applied to vertebrate brain mapping. This imaging system is capable of handling a massive number of misaligned sliced data and providing relevant anatomical and functional information on macroscopic structures in two- and three-dimensional space.

This multimodality multidimensional imaging system has been used to study the plasticity (deformation) phenomena in the rat’s vibrissal representation after neonatal lesion of the sensory organ. The functional (metabolic) images are obtained with the [14C]-2-deoxy-D-glucose (2DG) autoradiographic technique after Sokoloff et al.
correlated with the stimulus. After that time, the animal is sacrificed, the brain removed and frozen to prevent diffusion of the tracer. The brain is then thin sectioned in a cryostat and the sections placed in contact with photographic emulsion, most often X-ray film. The radioactive decay of the isotope causes a latent image to form on the film through an interaction with the silver halide crystals in the emulsion."

A large number of different radiotracers can be employed in functional mapping using the above protocol. One commonly employed tracer is \([^{14}\text{C}]\)-2-deoxy-D-glucose—2DG for short—developed by Sokoloff et al. (24) for the purpose of studying local cerebral metabolism \textit{in vivo}. This analog tracer of glucose is transported into the brain by the same carrier as glucose and, similarly, is subsequently phosphorylated by hexokinase; however, the product of this reaction—\([^{14}\text{C}]\)-deoxyglucose-6-phosphate—is essentially trapped inside the brain for the duration of the study. This radioactive product thus builds up in proportion to the intensity of the applied stimulus, quantitatively marking anatomical sites of functional activity.

The 2DG method can provide information concerning functional organization of large arrays of neurons. For example, it is possible to study the functional connectivity of an entire sensory pathway, such as that of the rat’s whisker system, within an individual animal. Such mapping is possible because of the close link between brain metabolism, which in the adult is almost entirely dependent upon glucose, and neural function (11, 24). In the case of mapping studies, the increased neural activity produces an increased demand for, and uptake of, glucose as well as \([^{14}\text{C}]\)-2DG. Thus, structures of the activated neural system become more densely labeled than those of non-stimulated systems.

**FUNCTIONAL AND ANATOMICAL IMAGING IN EXPERIMENTAL BIOLOGY**

**Functional Imaging**

In our work, we employ quantitative autoradiography as the functional imaging modality and cytochrome oxidase staining as our anatomical imaging source. Quantitative autoradiography can measure many functional parameters on a regional basis in the brain, including local hemodynamics, metabolism, and pharmacokinetics. The general procedure was best summarized by McEachron et al. (17): “[It] involves the injection of a radiolabelled compound followed by a predetermined waiting period during which the compound is distributed throughout the body. In most instances, an environmental or pharmacological stimulus is applied during the waiting period and changes in the pattern of the radiotracer’s distribution are correlated with the stimulus. After that time, the animal is sacrificed, the brain removed and frozen to prevent diffusion of the tracer. The brain is then thin sectioned in a cryostat and the sections placed in contact with photographic emulsion, most often X-ray film. The radioactive decay of the isotope causes a latent image to form on the film through an interaction with the silver halide crystals in the emulsion.”

The anatomical images used in our experiments were generated with cytochrome oxidase histochemistry (27). Cytochrome oxidase is an endogenous enzyme found in neurons. Its usage is intimately associated with the neurons’ metabolic machinery, which is in turn closely related to the levels of neuronal activity. CO belongs to a group of energy-deriving enzymes. They are responsible for electron transport and oxidative phosphorylation, yielding adenosine triphosphate (ATP). ATP is related to essential processes such as protein synthesis, maintenance of the resting membrane potential, and rapid axoplasmic transport within neurons; therefore, it can be reasoned that more active neurons would be more vigorously involved with the above processes and, as a consequence, would develop more active cytochrome systems (27).

Previous studies (27) have indicated that the detection of cytochrome oxidase enzymes in neurons of the central nervous system (CNS) correlates directly with the morphology of the structures being studied. Therefore, the histological sections that produced the autoradiographic images can be stained to detect cytochrome oxidase enzymes to produce anatomical (or morphological) descriptors of functional processes.

Figure 1 shows a two-dimensional (2-D) multimodality image of a coronal section through a rat’s brain, which was submitted to stimulation of a selected structure in the sensory system (Experimental Design). The image on the left was generated with the 2DG autoradiographic technique and represents rates of glucose utilization. The image on the right, produced with CO histochemistry, is the corresponding anatomical representation of the 2DG image.

Imaging anatomical and metabolic (functional) structures and/or processes within the vertebrate’s CNS becomes possible through the combination of the above-mentioned techniques. However, multidimensional representations of both metabolism and anatomy, particularly autoradiograms and the corresponding histological sections, still remain a big challenge. Some of the problems are associated with tissue degeneration after exposure to radiotracers and during sectioning, resulting in poor staining quality of these tissues and, consequently, low-quality (anatomical) images. On the functional imaging side, the