Functional neuroimaging as a direct probe of the effects of gonadal steroid hormones on the brain

There is considerable evidence from animal studies that gonadal steroid hormones modulate neuronal activity and affect behavior. In humans, however, the behavioral and cognitive evidence has not been conclusive, and, until recently, there have been few direct neurophysiological data. Functional brain imaging offers unique opportunities to characterize in humans the effects of gonadal steroid hormones on basic neurobiological parameters, such as neuronal metabolism and neurochemical systems, and to clarify the interactions between these hormones and cognition and mood regulation in health and disease. The most commonly used tools within the considerable armamentarium available for such research and the parameters of neural function that they can access are briefly reviewed here.

Measurable parameters of brain function

No single parameter completely, or even best, describes the functional status of the brain. Any measurement of brain “activity” subsumes a complex set of biochemical and physiological phenomena subserving diverse neuronal activities, such as cellular homeostasis, neuronal excitation and inhibition, maintenance of membrane potentials, and plastic change at the cellular or subcellular level. The choice of which parameter to measure in a given study must be guided by the particular research question, and the use of multiple imaging methods to obtain information about several different parameters in the same patients is perhaps the most informative approach (Figure 1).

Measures of general neuronal activity

The idea of measuring regional cerebral blood flow (rCBF, with positron emission tomography [PET] and IV H215O or inhaled 15O2 or C15O) or blood oxygenation level (with functional magnetic resonance imaging [fMRI]) to assess neural activity is well grounded in a firm theoretical base beginning with observations in the late 1800s that an augmented level of tissue function is sustained by increasing the rate of oxygen consumption and, therefore, the flow of oxygenated blood to the tissue (in this case, brain). Because these parameters can be measured in less than a minute and repeatedly, they are well suited to delineating the cerebral concomitants of transient mental phenomena such as cognition and emotion.

The brain’s energy requirements, among the highest of any organ system, are normally provided by blood glucose. The PET [18F]fluorodeoxyglucose (FDG) technique for measuring local cerebral metabolic rate of glucose (CMRGlu) is based on the fact that deoxyglucose and glucose are transported across the blood–brain barrier by the same carrier, but in cerebral tissues they are phosphorylated to deoxyglucose-6-phosphate and glucose-6-phosphate, respectively, which have differing fates. The latter is metabolized to CO2 and water, while the former is “trapped” in neurons long enough to be imaged, if radiolabeled. The long measurement period of this method (a 20- to 30-min scan carried out 30 to 40 min postinjection, when CMRGlu is assumed to have reached a steady state) limits its temporal resolution and sensitivity to cognitive and acute pharmacological activations; it is, therefore, best suited to providing detailed, quantitative maps of trait-like brain functional characteristics, as opposed to mental states.

Measurement of neurochemical systems

Using PET to image and quantify the functional activity of various neurochemical system components (eg, neuro-
receptors and enzymes) has much in common with autoradiography and in vitro receptor-binding techniques. A specific ligand (or binding agent) is labeled with a positron emitter and injected into the subject and the anatomical distribution of the radioligand in the brain is determined with PET. A quantitative estimate of specific receptor binding can be achieved by compartmental modeling to account for the kinetic behavior of the ligand between extra- and intracerebral plasma and tissue, as well as nonspecific binding and extraneuronal concentration. Alternatively, and more simply, the radioligand concentration in a brain area known to have little or no specific binding (eg, the cerebellum for dopamine receptors) can be used to estimate nonspecific binding. PET ligands are available for dopamine, opiate, serotonin, benzodiazepine, and other receptors. Cerebral concentration and distribution of neurotransmitter turnover and enzymes can also be measured using ligands, such as [11C]clorgiline and L-[11C]deprenyl, irreversible inhibitors of monoamine oxidase (MAO) for mapping MAOA and MAOB, respectively, and [11C]dihydroxyphenylalanine ([11C]DOPA), an analog of the dopamine precursor. Distribution and kinetics of pharmacological agents such as [11C]chlorpromazine, [11C]benztropine, and [11C]cocaine can also be determined. Ligands specific to gonadal steroid hormones that cross the blood–brain barrier have yet to be developed.

Magnetic resonance spectroscopy (MRS) is a chemical assay technique for measuring chemical moieties in the living brain. 31P spectroscopy measures high energy compounds and phospholipids (eg, phosphomonoesters and phosphodiesters, ATP, phosphocreatine), which reflect the energy state of neurons and constituents such as membrane precursors. 1H spectroscopy can detect amino acids, energy substrates, and membrane and myelin metabolites. Its greatest application has been to measure N-acetyl aspartate, an intracellular neuronal marker and sensitive indicator of neuronal pathology.

**Available methods for functional brain imaging**

**Positron emission tomography**

PET involves the administration of cyclotron-produced radioisotopes such as 18F, 15O, and 11C. These atoms emit very short-lived positrons that are rapidly annihilated, producing two characteristic photons of equal energy that can be simultaneously detected by a ring of crystals outside the head. PET’s spatial resolution is 3 to 6 mm, and it is sensitive enough for sequential scans of very short duration (eg, 5 to 20 s) to be acquired, providing detailed information about the kinetic behavior of the radiotracer. This has been particularly important in studying neuroreceptors, and allows rapid measurement.
of the very short-lived $^{15}$O blood flow agents (e.g., total scanning time of 40 s for H$_2^{15}$O). The major disadvantage of PET lies in its invasiveness and expense, and in the limitations of short-lived radiotracers. For fully quantitative studies, radial arterial lines are necessary to continuously monitor the amount of radioisotope being delivered to the brain by the arterial blood supply. However, for paired studies, in which one is mainly interested in the difference between the two data sets or their relative values, adequate information can be obtained by simply comparing the regional count rates between the two (Figure 2), obviating the need for the arterial catheter.

*Magnetic resonance imaging*

MRI is based on the behavior of atoms with unpaired protons in a magnetic field. Most commonly, the signal from $^1$H, most abundant in water and lipids, is used to construct images. During the last decade, the traditional use of MRI for anatomical imaging expanded to probe biochemistry and blood flow through two major advances, spectroscopy and ultrafast imaging. The latter takes advantage of the different magnetic properties of oxyhemoglobin and deoxyhemoglobin to rapidly measure blood oxygenation in the brain (the blood oxygen-level dependent [BOLD] method). Because of the improved temporal and spatial resolution of this technique, and because it has no radiation exposure (and, thus, the potential for unlimited repeated scans on a single individual under different cognitive, pharmacological, or hormonal states), it is now the premier imaging tool for research on cognitive neuroscience and clinical neuropsychiatry. MRS detects and resolves the smaller signals from other elements such as $^{31}$P and $^{23}$Na. Like nuclear magnetic resonance (NMR) spectroscopy, the unique chemical shifts in the spectra of various molecules provide the signal used to create images.

**An application: gonadal steroid hormones and brain function**

To directly study the effects of the gonadal steroid hormones estrogen and progesterone on brain function in humans, we used an incisive hormonal manipulation protocol based on ovarian suppression induced by the
gonadotropin-releasing hormone agonist leuprolide acetate (Lupron) with $^{15}$O PET to measure rCBF in young women during three pharmacologically controlled hormonal conditions spanning 4 to 5 months:

(i) ovarian suppression induced by leuprolide acetate;
(ii) leuprolide acetate plus estradiol replacement; and
(iii) leuprolide acetate plus progesterone replacement.

Estradiol and progesterone were administered separately and in a double-blind, crossover design. We found that when the endogenous ovarian hormones, estrogen and progesterone, of young women were pharmacologically ablated, the neurophysiological response to performing a frontal lobe task (the Wisconsin Card Sorting Test [WCS]) was attenuated, and the typically seen frontal lobe activation virtually disappeared. When either estrogen or progesterone was pharmacologically “added back” to the hypogonadism produced by leuprolide acetate, the activation pattern in response to the cognitive challenge of the WCS normalized and the prefrontal activation was reestablished (Figure 3). These data directly demonstrate that gonadal steroid hormones affect cognitively related neural activity. They also illustrate how functional neuroimaging can provide a framework for understanding the neurobiological mechanisms underlying gender-related brain features as well as hormone-related neuropsychiatric and neuropsychological disorders.

**REFERENCE**

1. Berman KF, Schmidt PJ, Rubinow DR, et al. Modulation of cognition-specific cortical activity by gonadal steroids: a positron-emission tomography study in women. Proc Natl Acad Sci U S A. 1997;94:8836-8841.

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**Figure 3.** Regional cerebral blood flow (rCBF) group average activation maps for 11 women during three different hormonal states. **Top:** Activation (red voxels) during the Wisconsin Card Sorting (WCS) test; the arrow shows that the characteristic prefrontal activation typically seen with this task was abolished during leuprolide acetate alone (ie, in the absence of estrogen and progesterone) and was reestablished when estrogen or progesterone was replaced. **Bottom:** In contrast, primary visual and motor activation in the same women during the same scanning sessions, was unaltered, demonstrating that the finding in the prefrontal cortex was not due to purely vascular effects and that it was associated relatively specifically to the cognitive system accessed by the WCS.

Modified from reference 1: Berman KF, Schmidt PJ, Rubinow DR, et al. Modulation of cognition-specific cortical activity by gonadal steroids: a positron-emission tomography study in women. Proc Natl Acad Sci U S A. 1997;94:8836-8841. Copyright © 1997, National Academy of Sciences of the United States of America.