Local Cerebral Blood Flow Increases During Auditory and Emotional Processing in the Conscious Rat

Abstract. *Local cerebral blood flow was measured in rats by the ^{14}C-labeled iodoantipyrine technique with quantitative autoradiography during the processing of environmental stimuli. Presentation of a tone increased blood flow in the auditory but not the visual pathway. When the animal had previously been conditioned to fear the tone, blood flow additionally increased in the hypothalamus and amygdala. Local cerebral blood flow can thus be used to detect patterns of cerebral excitation associated with transient (30- to 40-second) mental events in experimental animals.*

Until recently, the localization in brain of regions in which neuronal activity is modified during mental processing or behavior has been based on recordings of electrical activity (*1*). While electrophysiological methods allow the analysis of the temporal relationship between neural and psychological events, they are limited in that relatively few brain regions can be examined simultaneously.

The 2-deoxy-D-glucose (2DG) autoradiographic technique for detecting local changes in cerebral metabolism that accompany neural activity has provided a new approach to the problem of functional cerebral localization in experimental animals (*2*). Since stimulus conditions must be maintained for 30 to 45 minutes, however, the 2DG method is suitable only for examining the neuronal correlates of mental or behavioral activities that can be effectively sustained in a prolonged steady state.

Local cerebral blood flow (LCBF), under most conditions (*3*), is coupled to local energy metabolism (*4*) and has thus been used effectively in humans to detect changes in regional brain activation during mental processing and behavior (*5*). In experimental animals, early findings suggested that sensory stimulation might increase LCBF in sensory pathways, but the procedures proved technically cumbersome (*6*). Although practical methods for autoradiographically measuring LCBF in animals have been developed (*7, 8*), results to date indicating global activation of the brain during rapid eye movement sleep (*9*) and decreases in LCBF secondary to hypocapnia during intense physical stress (*10*) have not demonstrated select activation of neural pathways by natural stimuli in conscious animals. This is unfortunate since LCBF can be measured within 30 to 40 seconds in experimental animals (*11*), a time suitable for examining neural activity associated with evanescent mental or behavioral events.

We therefore sought to determine whether increases in LCBF, and thus in local brain activity, could be detected during the processing of transient environmental stimuli in conscious rats. We examined whether LCBF, measured by the ^{14}C-labeled iodoantipyrine (IAP) technique (*8*), would increase in the primary stations of the auditory pathway during acoustic stimulation and whether LCBF would additionally increase in brain regions implicated in emotional behavior when the animal had previously been conditioned to fear the acoustic stimulus.

Male Sprague-Dawley rats (300 to 350 g) were randomly assigned to one of three groups: (i) unstimulated controls ($N = 5$); (ii) naïve rats exposed to the tone only during the infusion of the tracer (tone alone, $N = 5$); and (iii) rats subjected to fear conditioning before being exposed to the tone during tracer infusion (conditioned, $N = 6$). Each rat was adapted to a Plexiglas tube, in which it fit comfortably, for 2 hours each day for three consecutive days (*12*). After being removed from the tube on day 3, the rat was either returned to its home cage or placed in a conditioning chamber (Colbourn Instruments). Animals in the emotional conditioning group were then subjected to 30 trials of classical fear conditioning during which the conditioned emotional stimulus (an auditory tone of 800 Hz and 80 dB, presented for 10 seconds) was reliably followed by the unconditioned stimulus (a 1.5-mA electric footshock presented for 0.5 second). This procedure is effective in establishing conditioned fear responses in several strains of rats (*13*). After the 30 trials, the animal was returned to its home cage.

On day 4, under light halothane anesthesia (1.5 percent in 100 percent O_2), polyethylene catheters were rapidly implanted in both femoral arteries and veins and the rat was placed in the tube. After recovery from the anesthesia (4 hours), the tube was transferred to a sound-attenuating box equipped with an acoustic speaker. The catheters were

Table 1. Local cerebral blood flow and physiological measures during acoustic and affective processing. Values represent means ± standard errors. Data were evaluated by two-way analyses of variance (repeated measures, with structures the repeated measure) and post hoc comparison of means by the Dunn statistic. Significant differences are indicated relative to the control group. Both raw scores and square root transformed scores were analyzed; the pattern of significance was the same in each case.

	Group		
	Quiet ($N = 5$)	Acoustic stimulation ($N = 5$)	Emotional stimulation ($N = 6$)
	Blood flow (milliliters per 100 g per minute)		
Auditory system			
Cochlear nucleus	163 ± 13	216 ± 20*	252 ± 13*
Superior olive	167 ± 15	225 ± 18*	250 ± 11*
Inferior colliculus	258 ± 8	320 ± 36*	336 ± 32*
Medial geniculate	177 ± 12	217 ± 19	223 ± 9
Auditory cortex	197 ± 17	266 ± 18*	278 ± 16*
Visual system			
Superior colliculus	181 ± 10	187 ± 15	201 ± 9
Lateral geniculate	150 ± 15	178 ± 13	182 ± 12
Visual cortex	155 ± 11	159 ± 11	166 ± 8
Limbic system			
Septum	83 ± 7	116 ± 13	119 ± 11
Hippocampus	102 ± 10	115 ± 8	118 ± 7
Entorhinal cortex	102 ± 12	114 ± 8	112 ± 10
Hypothalamus	113 ± 5	145 ± 9	158 ± 7*
Amygdala: medial	92 ± 4	126 ± 8	141 ± 9*
lateral	107 ± 7	121 ± 12	143 ± 9
White matter			
Corpus callosum	50 ± 11	64 ± 5	59 ± 10
	Physiological measures		
Arterial pressure (mm-Hg)	123 ± 4.3	119 ± 1.0	127 ± 2.3
O_2 pressure (mm-Hg)	88.0 ± 1.4	88.7 ± 4.3	85.0 ± 4.2
CO_2 pressure (mm-Hg)	40.1 ± 0.9	42.6 ± 0.07	40.6 ± 1.5
*p*H	7.37 ± 0.01	7.39 ± 0.02	7.35 ± 0.05
Hematocrit	46.2 ± 2.2	47.5 ± 2.2	47.5 ± 0.92

*$P < 0.05$.

accessible from the rear. Blood gases and hematocrit were measured. For unstimulated controls, IAP (100 μCi/kg) was infused under quiet conditions. For animals in the tone-alone and conditioned groups, the tone (*14*) was presented throughout the 30-second infusion, during which timed arterial samples were taken. After 30 seconds the rat was killed and the brain was removed and prepared for autoradiography. The autoradiograms were subjected to a computer-based image analysis (*3, 15*). LCBF was calculated from the arterial concentration time course and local tissue concentration of IAP (*3, 8, 10, 11, 15*). The brain regions (*3*) selected for analysis included nuclei along the primary auditory and visual pathways and structures implicated in emotional behavior (Table 1).

Blood gases, hematocrit, and arterial pressure, measured immediately before isotope infusion, did not differ among the three groups (Table 1).

In quiet, unstimulated rats, LCBF varied over a fivefold range, being lowest in the white matter (corpus callosum) and highest in the inferior colliculus. Our values for LCBF were mostly within 5 to 10 percent of those reported by others in conscious rats (*8*).

The presentation of a tone to naïve animals resulted in significant increases in LCBF in most stations of the auditory pathway (Table 1 and Fig. 1). The average LCBF in the auditory structures was approximately 130 percent that of unstimulated control. In contrast, LCBF did not change significantly in the visual pathways or in the other brain regions measured.

In rats previously conditioned to fear the tone, LCBF increased in the same auditory structures, to approximately 141 percent of control (Table 1). However, blood flow also increased significantly in the hypothalamus (medial and lateral nuclei sampled collectively; 139 percent of unstimulated control) and in the medial amygdala (153 percent of unstimulated control) in the emotionally conditioned animals.

We have demonstrated in conscious rats that LCBF increases in select and predictable neural pathways during the processing of environmental stimuli. During acoustic stimulation with a pure auditory tone, LCBF increased 25 to 35 percent in most stations of the auditory pathway, as classically determined by anatomical, electrophysiological, and behavioral methods (*16*). The failure of blood flow in the auditory thalamic relay nucleus, the medial geniculate body, to increase significantly could reflect the fact that medial geniculate neurons fire less frequently after the onset of a pure tone than do neurons in the inferior colliculus (*17*). Studies with the 2DG method have similarly shown that during photic stimulation the increase in metabolic activity in the lateral geniculate is less than in the superior colliculus (*18*). That LCBF did not increase in visual structures during acoustic stimulation in our study demonstrates that the changes in LCBF along the auditory pathway are specific to the stimulated modality.

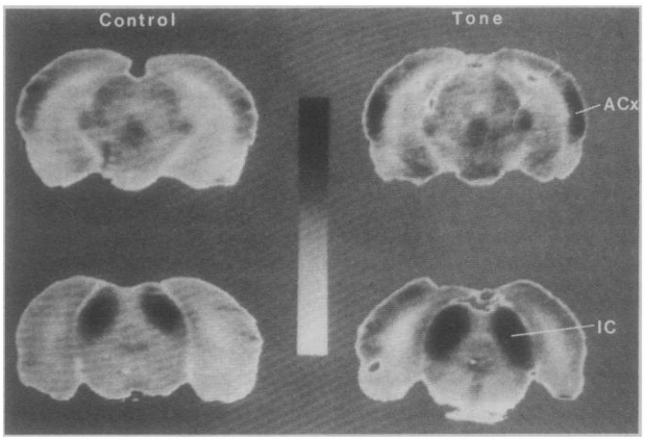

Fig. 1. Quantitative autoradiographic images illustrating LCBF in auditory nuclei during pure tone stimulation. Original autoradiograms were digitized, and the densities (IAP concentration in nanocuries per gram) were converted into actual LCBF values (milliliters per 100 g per minute) through the use of a calibration scale (center). The scale directly indicates LCBF values (white, 0; black, 350), allowing a direct comparison of LCBF in the four images. The images illustrate selective increases in LCBF in the inferior colliculus (*IC*) (bottom) and auditory cortex (*ACx*) (top) in an animal receiving tone stimulation relative to an unstimulated control.

When the acoustic stimulus had been endowed with aversive properties through conditioning, blood flow increased not only in auditory structures but also increased as much as 53 percent in regions of the amygdala and hypothalamus. These findings are consistent with the results of studies showing deficits in conditioned emotional responses after lesions of either the medial or lateral hypothalamus, as well as the medial amygdala (*19*). Moreover, reciprocal connections exist between the medial amygdala and the medial hypothalamus (*20*) and amygdalar-hypothalamic connections have been implicated in aversive conditioning (*21*) and in the expression of autonomic responses associated with the defense reaction elicited by stimulation of the amygdala (*22*). The failure of differences between the tone alone and fear conditioned groups to reach significance could reflect the elicitation of unconditioned fear by the novel tone.

These data thus demonstrate that it is possible to detect in experimental animals increases in LCBF during the processing of short-lived (30- to 40-second) environmental stimuli. Since LCBF is usually (*3*) coupled with local energy metabolism, which in turn reflects local neural activity (*4*), the observed increases in LCBF can be assumed to indicate increases in regional neural activity. Although the spatial resolution of this method is less than that of the 2DG technique, the primary nuclear groups of the brain can be identified (*3, 11, 15*). The IAP technique thus provides a practical and promising approach to the problem of localizing functional neuronal activity coupled with short-lived changes in mental processing and behavior in experimental animals.

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was again modified (*8*) to use iodoantipyrine, a freely diffusable, nonvolatile substance which has proven to be an effective tracer for quantitative measurements of LCBF (*3, 8, 10, 11, 15*).
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12. It is necessary to restrain conscious animals studied by the IAP technique. During tracer infusion, timed arterial blood samples must be taken from a very short catheter (approximately 3 cm maximum external to the artery) to determine blood-tracer concentration curves accurately (*11*). We familiarized each subject with the restraint tubes in order to minimize the stress of restraint. That the animals were comfortable and not stressed by the restraint procedure is indicated by the blood gas values (Table 1), which were within normal limits for all three groups.
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Epstein-Barr Virus: Inhibition of Replication by Three New Drugs

Abstract. *Epstein-Barr virus (EBV) is the cause of infectious mononucleosis and is associated with three human malignancies. Acyclovir [9-(2-hydroxyethoxymethyl)guanine], the first clinically useful drug effective against replication of EBV, is without effect against latent or persistent EBV infection. Three nucleoside analogs, E-5-(2-bromovinyl)-2'-deoxyuridine, 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine, and 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-methyluracil are potent inhibitors of EBV replication in vitro. Moreover, in contrast to the reversibility of viral inhibition by Acyclovir, these three drugs have prolonged effects in suppressing viral replication even after the drugs are removed from persistently infected cell cultures.*

The hallmark of herpesviruses is their ability to cause latent and persistent as well as active infections. For the Epstein-Barr virus (EBV) there are cell systems in vitro that are counterparts of these three virologic states. In all of the diseases associated or caused by EBV infection—infectious mononucleosis, Burkitt's lymphoma, nasopharyngeal carcinoma and, as discovered recently, certain immunoblastic sarcomas that arise in allograft recipients (*1*)—the virus occurs in the pathologic cells in one, and often more than one, of these three states. In the past 2 years we have shown that Acyclovir (ACV) [9-(2-hydroxyethoxymethyl)guanine] (*2*) has potent effects against EBV infections in vitro when there is active replication of the virus (*3–5*). However, ACV has no effect in vitro on latent EBV infection, nor does the drug promise to be of much use in persistent low-level infection with EBV (*6*). Inasmuch as latent and persistent infection may be key elements of the oncogenic and chronic disease states associated with EBV, we started searching for other antiviral compounds active against EBV with the specific goal of identifying compounds that might have more prolonged effects than ACV.

Since all herpesviruses induce the formation of new viral DNA polymerases, we thought that drugs inhibitory to herpes simplex virus (HSV) might also inhibit EBV infection. We therefore selected several new nucleoside analogs for testing. Among the most promising were E-5-(2-bromovinyl)-2'-deoxyuridine (BVDU) (*7–10*), 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (FIAC) (*11–13*), and 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-methyluracil (FMAU) (*11, 13*). All three compounds showed anti-EBV activity and had persistent effects in EBV-producing cell cultures.

We tested these compounds on EBV DNA replication in a virus-producing cell line (P3HR-1) and in Raji cells, a latently infected cell line, after superinfection with P3HR-1 virus. Cells were grown in RPMI 1640 medium (*14*). P3HR-1 cells spontaneously and continuously replicate large numbers of linear EBV genomes, synthesize early antigen (EA) and virus capsid antigen (VCA), and make virus particles. The level of cells showing spontaneous induction of virus replication fluctuates cyclically in 3 to 10 percent of the cells under our culture conditions. A steady-state mode of cell growth was established (*15*). Briefly, cells were seeded at a density of 4×10^5 to 6×10^5 cells per milliliter and counted daily until they reached 2×10^6 cells per milliliter. During this interval the cells were growing in a strictly exponential fashion.

The cells were harvested and resuspended in fresh RPMI 1640 medium containing the concentration of the drug to be tested, then incubated at 37°C for 5 days. The cells were harvested, and EBV genome copies per cell were determined by complementary RNA–DNA hybridization with an EBV-specific complementary RNA probe (*5, 14*). Table 1 shows that BVDU, FIAC, and FMAU,

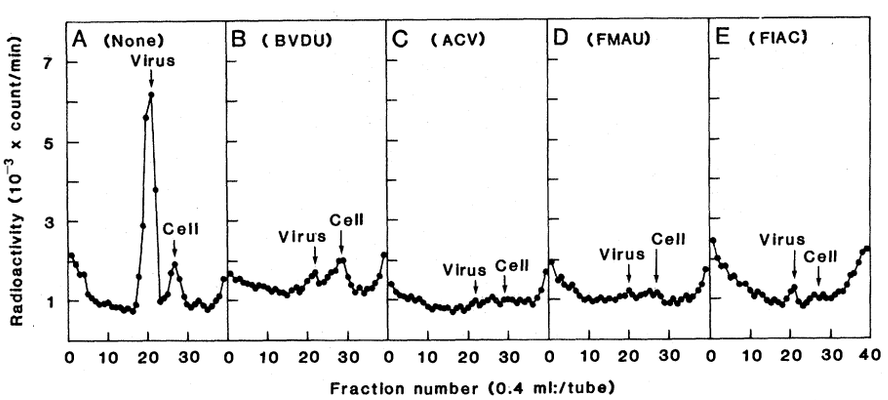

Fig. 1. Inhibition of EBV DNA replication by BVDU, FIAC, FMAU, and ACV in superinfected Raji cells. EBV DNA replication was determined by measuring the incorporation of ^{32}P into viral DNA and analyzing the DNA by cesium chloride density gradient centrifugation. The drug concentrations used were (A) no drug, (B) 20 μ*M* BVDU, (C) 100 μ*M* ACV, (D) 5 μ*M* FMAU, and (E) 10 μ*M* FIAC.