Brain Blood Flow: Alteration by Prior Exposure to a Learned Task

Abstract. *Chicks with the eyelids of one eye sutured were trained to discriminate between grains and pebbles. The learned experience was completely recognizable by the naive eye that had been occluded during training. When both eyes were opened after monocular training, the velocity of blood flow through paired left and right brain regions was identical. However, when chicks were reexposed to the discrimination situation, blood flow through the cerebral hemisphere associated with the naive eye was greater than that through the hemisphere associated with the trained eye.*

The relation between mentation and the metabolic activity of the brain has been studied recently (*1*). A major difficulty in such studies has been to arrange the "experimental" and "control" states so that the only difference between these conditions is the extent of learning or recall activity. In an ideal experiment, all other extraneous potential variables such as motor activity, endocrine level, and the surrounding environment are identical for the "experimental" and "control" situation. Any difference found between them may then be attributable to altered cerebral activity.

To minimize random variability, we have previously used the chick in studies on the effect of sensory input on brain metabolism. The avian optic nerve is completely crossed at the optic chiasm and thus each eye solely innervates the contralateral optic lobe (*2*). The cerebral hemispheres contain the major associative areas of the chick, and the secondary innervation from each eye to these hemispheres is also totally contralateral. While several small commissures connect the hemispheres, the absence in birds of a large interhemispheric commissure, such as a corpus callosum, further limits interactions between the two halves of the brain. Consequently, the metabolic changes caused by unilateral visual deprivation or stimulation are largely confined to regions contralateral to the treated eye. We have used this experimental system to show that reduction of visual input by eyelid suture results in a suppression of cerebral blood flow and glucose consumption (*3*). An increase in blood flow has also been demonstrated in regions contralateral to an eye receiving information relevant to feeding activity (*4*). The comparison of experimental and control regions derived from the same animal and consequent elimination of variability between animals enables minor metabolic changes to be readily detected.

The purpose of the study described here was to ascertain whether the act of learning in a discrimination situation could result in regional changes of cerebral metabolism. We have attempted to minimize extraneous influences so that the predominant variable becomes the meaningfulness of the afferent visual pattern. The behavioral paradigm in these experiments was essentially similar to that designed by Rogers *et al.* (*5*). This task requires that the animal learn to discriminate between food grains (about 2 mm in diameter) and similar appearing brown pebbles (about 4 mm in diameter). Grains were randomly distributed over the floor of a smooth, white circular bowl (25 cm in diameter) to a density of approximately 0.5 grain per square centimeter and stones were sprinkled among the grain at a similar density. Chicks (4 days old) of a White Leghorn strain that had been fed meagerly in their home cage prior to the experiment were placed in the white test bowl. Two or three birds were always placed in the bowl together because solitary birds experienced distress and pecked with less vigor. The targets of the first 60 pecks were scored with an event counter. Repeated pecks at the same grain or stone were only counted once. Data were tabulated as the number of errors made per trial of 20 pecks. Birds were returned to their home cage for 3 hours and then again placed in the trial bowl and the number of mistakes again recorded for three 20-peck blocks. Chicks learned to discriminate between grains and stones rapidly in the first three 20-peck trials and this learning was almost totally retained in the second three trials, 3 hours later. In agreement with the results of Hogan (*6*), we found chicks of less than 3 days of age to be almost incapable of discrimination learning related to the selection of appropriate materials in feeding.

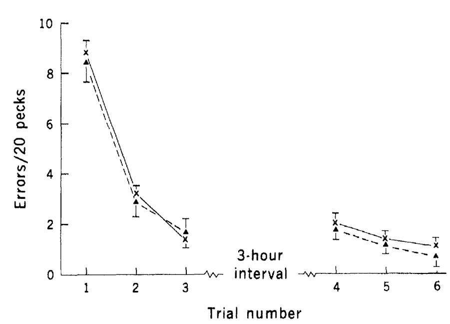

Fig. 1. Number of pecks at pebbles made by 4-day-old chicks trained to discriminate between grains and pebbles. Each trial consisted of 20 pecks. The first three trials followed each other immediately. After a 3-hour interval, the next three trials (4 to 6) were also run sequentially. (▲) Left eyelids sutured for first three trials, then sutures were removed and the right eyelids sutured for the remainder of the experiment. (x) Left eyelids sutured throughout experiment. Bars represent standard error of the mean.

Four-day-old chicks that had the lids of a single eye sutured immediately after hatching and chicks with binocular vision had very similar learning and retention abilities. In another group of 4-day-old chicks that were trained with the left eye closed immediately after hatching, the suture was removed from the left eyelids and the right eyelids were sutured immediately after the first training session. The recall session, 3 hours later, showed an almost complete transfer of learned discrimination to the naive eye (Fig. 1). Transfer of monocularly acquired responses to aversive stimuli had been reported previously for chicks (*7*), but this transfer had not always been found for less aversive stimuli (*8*). It seemed that the memory for the task was equally accessible to either eye. A report by Bell and Gibbs (*9*) indicated that such a memory in the chick is unilaterally established in the side of the brain served by the eye used in learning. Interocular transfer may be achieved by the untrained hemisphere "borrowing" information from the other hemisphere without the permanent establishment of memory by the untrained hemisphere. This report encouraged us to consider whether an underlying metabolic difference between the two hemispheres could exist, in spite of the behavioral symmetry we observed.

Chicks had their left eyelids sewn shut at hatch, and after 4 days of meager feeding were trained in the grain and stone differentiation task. The sutured eye was then opened and chicks were returned to their home cage with both eyes open. After 3 hours, chicks were replaced in the testing bowl. This represented a recall situation for the right eye but involved the possibility of new learning by the left eye. Chicks were then allowed to feed for 5 minutes and they successfully discriminated between grains and stones. Only animals with both eyes fully open were used. The relative velocity of cerebral blood flow through left and right optic lobes and cerebral hemispheres was then determined by means of a diffusible indicator (*10*). Ten seconds after intracardiac injection of [^{14}C]antipyrine (15.6 mc/mmole) the chicks were decapitated and the brain regions were dissected, weighed, and dissolved in tissue solubilizer for assay of radioactivity. Data were expressed as counts per minute per milligram of tissue (wet weight). This figure varied severalfold between birds, probably because of the difficulty of ensuring the precise location of the injection needle within the heart. Results were calculated as the ratio of radioactivity in the right (R) brain region relative to that in the left (L) region. To avoid skewing data the natural logarithm of this ratio was determined for each bird (ln R/L). The antilogarithms of the final mean logarithmic ratio (that is, the geometric mean) constitute the data presented (Table 1). The rate of blood flow through the hemisphere contralateral to the eye exposed to the learning situation for the first time was significantly greater than that in the opposite hemisphere, whereas no such major asymmetry was detected between pairs of optic lobes.

A second series of experiments was conducted that was almost identical to that described above except that in the second 5 minutes a test bowl containing solely grains was substituted for the bowl containing the grain and stone mixture. In this case the bird's feeding did not involve any discrimination task. No significant asymmetry of blood flow was detected between pairs of brain regions under these control conditions (Table 1).

Blood flow data derived from the discrimination studies were compared to those obtained from the control series involving feeding without discrimination (Table 1). The asymmetry of blood flow between hemispheres of chicks in the discrimination situation was marginally statistically different from the corresponding values for control chicks ($P = .065$; t-test, one-tailed). This last figure for control conditions was close to unity. However, the probability that asymmetry of blood flow in experimental birds was significantly different from unity was much greater ($P < .01$, Table 1). The one-tailed t-test was used because previous experiments had suggested that there might be a positive correlation between new arousal and cerebral blood flow. The naive hemisphere would be expected to be freshly alerted by exposure to the task. The production of a difference in blood flow between the two brain hemispheres was therefore demonstrable only in the visual discrimination situation. It was possible to obtain metabolic differences in the two brain halves without concomitant asymmetry of sensory input or behavioral response. The variation in blood flow observed between pairs of hemispheres may be attributable to the different prior experiences of these regions. There is evidence that the engram for a monocularly learned behavior in the chick is confined to a single cerebral hemisphere (*9*). The excess blood flow we have observed in the "naive" hemisphere may reflect the activation of the hemisphere in which no engram is present. Such changes in the supply of nutrients to the brain could underlie the accretion of RNA and protein reported to be a consequence of environmental enrichment or behavioral challenge (*11*).

There are several suggestions of increased rates of macromolecule synthesis associated with learning phenomena (*11*), but many nonspecific factors can obscure comparison between individual animals in differing environmental situations. The changed rate of blood flow we have described cannot be attributed to variation in the intensity or quality of sensory input or to a changed humoral content of circulating hormones. Nor can the data be accounted for in terms of altered animal arousal or motor activity. Our results suggest that the presentation of a visual discrimination task can modify cerebral metabolism.

Table 1. Relative rates of blood flow in the right (R) and left (L) sides of the chick brain 10 seconds after intracardiac injection of [^{14}C]antipyrine. Blood flow rates were determined as counts per minute per milligram of tissue. The chicks had learned a discrimination task with the left eyelids sutured; 3 hours later they were reexposed to the discrimination task for 5 minutes while control birds were merely given grain. Both groups of birds had both eyes open while blood flow was determined. Results are the mean of 33 experimental or 29 control birds ± standard error.

Group	R/L	
	Cerebral hemispheres	Optic lobes
Experimental	1.15 ± .06*	1.07 ± .07†
Control	1.04 ± .04†	1.02 ± .05†

*$P < .01$ that value is significantly above unity (Student's one-tailed t-test). †Not significantly above 1.0.

STEPHEN C. BONDY
MARILYN E. HARRINGTON
Departments of Neurology and Pharmacology, University of Colorado Medical Center, Denver 80262

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12 April 1977; revised 1 August 1977

Guanosine 3′,5′-Monophosphate: A Central Nervous System Regulator of Analgesia

Abstract. *The dibutyryl derivative of guanosine 3′,5′-monophosphate (cyclic GMP), administered centrally, totally abolishes response to noxious stimuli without depressing the central nervous system. Analgesic properties of the nucleotide are not reversed by naloxone. Microinjected intracerebrally into different sites, dibutyryl cyclic GMP does not mimic the action of morphine. Pharmacological effects of dibutyryl cyclic GMP suggest that endogenous cyclic GMP modulates an inhibitory pain pathway distinct from that on which morphine acts.*

The search for the endogenous ligand of the opiate receptor has recently focused scientific interest on naturally occurring peptides (*1*) possessing the beneficial as well as the harmful properties of the opiates (*2*). We have discovered that the dibutyryl derivative of the naturally occurring cyclic nucleotide guanosine 3′,5′-monophosphate (dibutyryl cyclic GMP) administered directly into the central nervous system protects against noxious stimuli without inducing sedation, depressing respiration, or altering either awareness (as manifested by response to auditory and visual stimuli) or locomotor activity; and protects against nociperception and mortality resulting from high temperatures. Unlike those of the opiates, the analgesic properties of dibutyryl cyclic GMP are neither prevented nor reversed by naloxone.

Information on cyclic GMP has been

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