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Use of [^{14}C]-2-deoxyglucose to detect regional brain activities associated with fearful behavior in wild Norway rats

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The 2-deoxyglucose method was used to compare regional brain activities of unrestrained wild Norway rats engaged in fear-based defensive behavior (n = 8), and that of solitary controls (n = 8). After infusion with 100 micrograms/kg of (^{14}C)-deoxyglucose via jugular catheters, experimental rats spent the 45-min uptake period in flight, boxing, and defensive attack to painless threat stimuli. Coronal sections of brains were exposed to X-ray film, and the resultant global maps of regional brain activity for the two groups were quantitated by high-resolution fiber optic densitometry at 86 cerebral points and analyzed statistically by computer. Significant group differences in regional brain glucose uptake were found at 16 loci. The considerable agreement between these structures and those previously identified in the literature as involved in defensive behavior provides evidence for the potential of this method of investigation of brain correlates of specific behavioral patterns.

Until fairly recently, brain energy metabolism in vivo was studied primarily by way of the nitrous oxide technique of Kety and Schmidt (1948), which measures average rates of energy metabolism for the brain as a whole. It was found that overall cerebral metabolic rates were constant for a number of conditions, including deep sleep, performance of mental arithmetic, schizophrenia, drug-induced sedation, tranquilization, and psychosis. It varied only with structural or functional alterations, such as occur in postnatal development, senility, anesthesia, and convulsive states (Kety, 1950; Lassen, 1959; Sokoloff, 1960, 1969).

However, newer developments have made possible the resolution of regional cerebral energy metabolism. These have confirmed a close relationship between local blood flow and regional brain functional activity (Plum, Gjedde, & Samson, 1976; Sokoloff, Revich, Kennedy, Des Rosiers, Patlak, Pettigrew, Sakurada, & Shinohara, 1977). One such method, developed in Sokoloff's laboratory (Sokoloff, 1981; Sokoloff et al., 1977) permits the 100-micrometer resolution of brain regional activities by playing an elegant biochemical trick on the brain.

While an animal is maintained in the state to be investigated, a tracer amount of [^{14}C]-2-deoxyglucose (DG) is injected. As the DG crosses the blood brain barrier, along with glucose, both are phosphorylated by hexokinase at a local rate proportional to that brain region's nervous activity. The next glycolytic enzyme, glucose phosphate isomerase, is unable to convert the deoxyglucose-6-phosphate (DG 6-P) from the aldose to the ketose form (fructose), due to the absence of the requisite 2-hydroxyl. Because 2-deoxyglucose-6-phosphate is not a substrate for G6-P dehydrogenase (Sols & Crane, 1954) and because there is very little DG 6-phosphatase activity in brain, the $t_1/_2$ for DG 6-P is about 6 h (Sokoloff et al., 1977). It therefore accumulates regionally at a rate proportionate to the local brain work load associated with the brain state under study, until by about 30 min when essentially all blood DG has been cleared. After this time, the brain activities of the animal may be altered or even terminated without

A preliminary report of this work has been presented elsewhere, in Morton, Blanchard, Diamond, Blanchard, Lee, Pang, Olipares, Hanohano, Cabebe, and Chan (1983) K. Pang was supported during the course of this work by a Minorities Access to Research Careers Fellowship NIGMS 5T32 GM07684.

erasing the indelible [^{14}C] DG 6-P deposits recording the global regional activities of the brain performing the selected tasks during the experimental period. This record may be transcribed from brain slices directly onto X-ray film for visualization and quantitative analysis.

The DG method has been successful in demonstrating that although whole-brain metabolism is fairly constant, striking regional differences in local brain activity exist in conscious animals (Sokoloff, 1981). These in part corresponded to the localization of cell bodies and associated dendritic synapses of gray matter that appear to perform significantly more metabolic work than the axonic fibers of passage in white-matter areas (Schwartz, Smith, Davidsen, Savaki, & Sokoloff, 1979). However, additional variation in these regional activities, especially in the gray-matter areas, could be produced if the animals were subjected to electrode stimulation, lesioning, drug administration, or sensory stimulation during the 45-min period following [^{14}C] DG administration (Sokoloff, 1981). The proportionality of the DG 6-P deposition in a relevant brain structure to the intensity of the stimulus given provided further support for the concept that regional brain neurotransmission activity was paralleled by local energy metabolism.

Few reports using DG to identify the brain regions participating in natural behavior or learning have appeared. One reason is that the behavior must be sufficiently robust to occur despite the handling and injection procedures by which DG is normally administered, or an alternative injection route must be used. In addition, brain changes associated with natural behaviors might be expected to be more subtle than those caused by drugs, electrical stimulation, or lesions, in terms of the intensity of regional brain activity changes. Indeed, we have found that such changes are not readily detectable by visual inspection of the exposed X-ray film, but they are sufficiently robust when groups of animals are compared by densitometry and computer analysis.

Here, we report the preliminary results of the adaptation of DG methodology to investigate the regional brain activities of unrestrained frightened wild rats.

METHOD

Subjects

Wild *Rattus norvegicus* males (350-400 g) trapped in cane fields near Hilo, Hawaii, were housed separately preoperatively for 2-6 months in large metal cages (48 x 40 x 20 cm) supplied with plywood nesting boxes (20 x 15 x 10 cm) and ad-lib food and water.

Procedure

All subjects were fitted with a jugular catheter (P.E. 50 tubing) that was anchored to the top of the skull and emerged through the scalp. This was done in order to permit DG administration without traumatic restraint of wild rat subjects. Each animal was then allowed 2 weeks for recovery before behavioral testing.

Isotope injections. 2-Deoxy-D-[1-^{14}C] glucose (^{14}C-DG), 61 mCi/mmole in 3% ethanol (Amersham), was lyophillized and reconstituted in .9%-w/v NaCl. Immediately preceding the behavioral experiments, 100 Ci/kg of the ^{14}C-DG was injected into the rats through the jugular catheter.

Behavioral testing. For the eight experimental rats, painless fear-inducing procedures (Blanchard, Blanchard, Lee, & Williams, 1981; Blanchard, Williams, Lee, & Blanchard, 1981) were performed immediately after ^{14}C-DG injection. This fear procedure involved a 15-min runway sequence, a 15-min barrel sequence, and a second 15-min runway sequence. The runway was created by partitioning off 15 m of a 1.5-m-wide hallway and placing within it a 8.5-m free-standing central wall to produce a 20 x .75 m runway loop. After the wild rat had been left 1 min in the runway, the experimenter entered and approached the rat at a rate of .5 m/sec, stopping when the subject either moved away from or attacked the experimenter. The experimenter then retreated to the opposite end of the runway and reapproached the subject after waiting 15 sec. This was done for a total of 35 trials.

The rat was then chased into an aluminum barrel (.5-m top diameter and 1.2-m height) that was lying on its side. The barrel was turned upright, and each subject was presented 16 times alternatively with both a vibrissae brush (2.5-cm bristles on a 2-cm^2 base, perpendicular to a 1-m handle) and to an anesthetized laboratory rat held in a holder, head exposed.

The behavioral measures recorded during the three 15-min painless fear sessions were obtained as follows: In the runway, the distance between the wild rat and the experimenter at which the subject reacted to the experimenter was recorded. If the rat fled, the flight distance was recorded. For both the runway and barrel sessions, the numbers of bites, jump attacks, vocalizations, and upright boxing toward the experimenter, vibrissae brush, or anesthetized conspecific were also recorded.

To provide nondefensive wild rat controls, the previously inserted jugular catheters of nine subjects were connected to a 20-cm cannula extension 1 h before testing. This extension ran out of the rats' home cages to a location out of view by the subject. At the beginning of the test period, ^{14}C-DG was quietly infused without physical or visual contact of control subjects and experimenter. The controls were left undisturbed in their home cages for the subsequent 45-min DG uptake period.

Brain treatment. Immediately following the behavioral experiments, the animals were given an overdose of 50-mg/ml sodium pentobarbitol, infused via catheter. The subjects were then perfused pericardially for 1 min total with 20-ml saline (.1-M sodium cacodylate, pH 7.3), followed by 40 ml of .1-M sodium cacodylate (3.75% paraformaldehyde solution, pH 7.3). The brain was then removed within 5 min, frozen in isopentane dry ice, and stored at -70°C.

Coronal brain sections, 20 micrometers thick, cut at -18°C, were finger melted onto coverslips and dehydrated 5 min on a 60°C hot plate. The coverslips were then mounted on cardboard with rubber cement and placed against Kodak SB-5 X-ray film for 3-6 weeks. A parallel histological series was mounted and stained by the Kluver-Barrera method.

X-ray film densitometry and data analysis. For each animal, 86 brain loci were analyzed by a fiber optic, two-dimensional microdensitometer developed by Morton, Martin, Chan, and Olipares (1982). The data were entered into an IBM 360 computer. To bring overall exposure differences between all animals into alignment, the 86-locus average for each animal was arbitrarily adjusted to .5 optical density units (OD) by deriving and applying an alignment factor. Then each of the animal's 86 loci were similarly aligned.

Computer-assisted group averages for each locus were next

Table 1
Averages of Fear-Associated Behaviors Elicited From Wild *Rattus Norvegicus* During the 45 Min Following [^{14}C]-2-Deoxyglucose Administration

Session	Number of Trials	Boxing Posture	Bite Attacks	Jump Attacks	Vocalizations
Runway (1-15 min)	35	6.0	20.2	25.7	24.0
Barrel (16-30 min)					
Brush Stimuli	16	15.7	15.8	14.3	15.0
Anesthetized Rat Stimuli	16	14.2	15.5	15.8	14.7
Runway (31-45 min)	35	12.0	22.6	20.3	14.0

Note–Values are averages (n = 8). For experimental procedures, see Method and Blanchard et al. (1981).

obtained, along with standard deviations. The experimental group's optical density averages for all loci were then compared with those of the control groups, and significant differences assessed by use of student's t test corrected for multiple comparisons (Barr, Goodnight, & Sall, 1979).

RESULTS

The behaviors observed in the experimental rats during the 45-min fear sessions are shown in Table 1. Substantial fear-associated behavior was seen in these sessions.

Table 2 contains the 16 brain regions showing significant differences ($p < .05$) in regional glucose uptake between the fearful and the control rats. The brain loci whose activity in fearful rats significantly ($p < .05$) exceeded that of the control rats were: pretectum, habenula, dorsal central gray, cerebellar cortex, prefrontal cortex, perifornical hypothalamus, and lateral preoptic area. The brain loci whose activity was significantly ($p < .05$) lower in fearful rats were: substantia nigra pars compacta, reticular formation, pyriform cortex, basolateral amygdala, internal capsule, hippocampal commissure, body of corpus callosum, trigeminal sensory nucleus, and spinal tract of Nu. V.

Table 2
Brain Areas Showing Significant ($p < .05$) Differences (in Percentages) in Regional Glucose Uptake

Locus	Difference	t	df	p
Spinal tract of V	−45	4.26	11	.001
Trigeminal sensory nucleus	−44	2.97	10	.014
Body corpus callosum	−35	3.25	11	.008
Hippocampal commissure	−26	3.23	9	.010
Pyriform cortex	−16	2.33	11	.040
Internal capsule	−16	2.33	11	.024
Reticular formation	−14	3.23	11	.008
Basolateral amygdala	−14	3.10	11	.010
Substantia nigra pars compacta	−13	3.43	11	.006
Lateral preoptic area	14	2.78	11	.018
Perifornical hypothalamus	15	2.36	11	.037
Prefrontal	16	3.23	11	.008
Cerebellar cortex (maximum)	18	3.06	11	.011
Dorsal central gray	18	2.70	11	.021
Habenula	19	2.17	11	.052
Pretectum	20	2.60	11	.021

Note–Difference = [locus average for fear groups (n = 8)/locus average for control group (n = 9)] × 100, t (student t test).

DISCUSSION

The control group of the present study was given the isotope through jugular catheters without handling in order to minimize injection trauma. This was necessitated because wild rat subjects are very defensive to human handling and the usual direct painless catheter injection procedures would have elicited in the control rats a feaful state similar to that of experimentals.

Another issue to be considered in interpretation of the data is that no effort was made to equate experimental and control activity patterns. The rationale for this decision was simply that there is no known nontraumatic method of producing in control rats an activity pattern equivalent to that of defensive animals. Future research may involve attempts to elicit activity patterns in controls that are equivalent in terms of quantity if not of quality, but it was felt that the attempt to do so with the present wild rat subjects would be more likely to add problems of interpretation than to solve them. Accordingly, the present comparisons are between brains of rats that are both frightened and displaying species-typical activities and those that are calm and relatively inactive.

A final consideration is that traditional procedures for the determination of absolute glucose uptake per gram of brain per minute could not be used here, because they require restraint of the animal during the behavioral period (Sokoloff et al., 1977). However, such data are used in the Sokoloff procedure only to calculate a constant, employed uniformly to convert the already quantitative individual regional activities of each subject to pmoles of glucose consumed per gram of brain per minute. This element of the DG method is not required to quantitatively compare relative regional activities of brains, and its omission is thus not crucial in the present study.

It is notable that many of the areas showing densitometric changes during defense, such as the dorsal central gray, habenula, pretectum, prefrontal cortex, lateral preoptic area, basolateral amygdala, and various hypothalamic sites, are among those that have been previously associated with alterations of defense (Ursin, 1981). It is also notable that they generally agree with results of regional activity changes in fearful mice (Morton, Blanchard, Lee, Hanohano, Cabebe, & Blanchard, Note 1), with the exception that the smaller mouse brain does not permit as great a resolution of regional optical densities.

The general compatability of these data with previous literature on the brain correlates of fear or defense provides further evidence of the validity of the method used in conjunction with natural behaviors. The use of this method should thus permit a much more comprehensive analysis of brain activity patterns underlying specific behaviors than has ever been attempted before.

REFERENCE NOTE

1. Morton, B. E., Blanchard, R. J., Lee, E. M. C., Hanohano, D., Cabebe, L., & Blanchard, D. C. *Use of [^{14}C]-deoxyglucose to detect regional brain activities associated with aggressive and defensive behavior in mice.* Paper presented at the meeting of the International Society for Research on Aggression, Wheaton, Massachusetts, 1981.

REFERENCES

BARR, A. J., GOODNIGHT, J. H., & SALL, J. P. *SAS user's guide.* Raleigh, N.C: SAS Institute, 1979.

BLANCHARD, D. C., BLANCHARD, R. J., LEE, E. M. C., & WILLIAMS, G. Taming of the wild Norway rat following lesions in the basal ganglia. *Physiology & Behavior,* 1981, **27**, 995-1000.

BLANCHARD, D. C., WILLIAMS, G., LEE, E. M. C., & BLANCHARD, R. J. Taming of the wild *Rattus norvegicus* by lesions of the mesencephalic central gray. *Physiological Psychology,* 1981, **9**, 157-163.

KETY, S. S. Circulation and metabolism of the human brain in health and disease. *American Journal of Medicine,* 1950, **8**, 205-217.

KETY, S. S., & SCHMIDT, C. E. The nitrous oxide method for the quantitative determination of cerebral blood flow in man: Theory, procedure and normal values. *Journal of Clinical Investigation,* 1948, **27**, 476-483.

LASSEN, N. A. Cerebral blood flow and oxygen consumption in man. *Physiological Reviews,* 1959, **39**, 183-238.

MORTON, B. E., BLANCHARD, R. J., DIAMOND, M., BLANCHARD, D. C., LEE, E. M. C., PANG, K., OLIPARES, H., HANOHANO, D., CABEBE, C., & CHAN, C. Use of [^{14}C]-2-deoxyglucose to detect regional brain activities associated with fearful aggressive and copulatory behaviors in male rodents. In F. W. Stratman, D. L. F. Lennon, & R. N. Zalten (Eds.), *Biochemistry of metabolic processes.* New York: Elsevier, 1983.

MORTON, B. E., MARTIN, C., CHAN, D., & OLIPARES, H. Inexpensive fiber optic two dimensional densitometer for the high resolution quantitation of autoradiogram brain densities. *Journal of Neuroscience Methods,* 1982, **6**, 113-120.

PLUM, F., GJEDDE, A., & SAMSON, F. E. Neuroanatomical function mapping by the radioactive 2-deoxy-D-glucose method. *Neuroscience Research Program Bulletin,* 1976, **14**, 457-518.

SCHWARTZ, W. J., SMITH, C. B., DAVIDSEN, L., SAVAKI, L., & SOKOLOFF, L. Metabolic mapping of functional activity in the hypothalamoneurohypophyseal system in the rat. *Science,* 1979, **205**, 723-725.

SOKOLOFF, K. Metabolism of the central nervous system *in vivo.* In J. Field, H. W. Magoun, & V. E. Hall (Eds.), *Handbook of physiology-neurophysiology* (Vol. 3). Washington, D.C: American Physiological Society, 1960.

SOKOLOFF, L. Cerebral circulation and behavior in man: Strategy and findings. In A. J. Mandel & M. P. Mandel (Eds.), *Psychochemical research in man.* New York: Academic Press, 1969.

SOKOLOFF, L. Localization of functional activity in the central nervous system by measurement of glucose utilization with radioactive deoxyglucose. *Journal of Cerebral Blood Flow and Metabolism,* 1981, **1**, 7-36.

SOKOLOFF, L., REVICH, M., KENNEDY, C., DES ROSIERS, M. G., PATLAK, C. S., PETTIGREW, X. P., SAKURADA, O., & SHINOHARA, M. The [^{14}C] deoxyglucose method for measurement of local cerebral glucose utilization: Theory, procedure, and normal values in the conscious and anesthetized albino rat. *Journal of Neurochemistry,* 1977, **28**, 897-916.

SOLS, A., & CRANE, R. K. Substrate specificity of brain hexokinase. *Journal of Biological Chemistry,* 1954, **210**, 581-595.

URSIN, H. Neuroanatomical basis of aggression. In P. F. Brain & D. Benton (Eds.), *Multidisciplinary approaches to aggressive research.* Amsterdam: Elsevier, 1981.

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