References and Notes

1. To be described as superstitious, behavior should meet several criteria: It should be skeletal behavior that topographically and temporally resembles operant behavior, and that could in fact be an operant response (that is, be sensitive to reinforcement contingencies). But unlike operant behavior, it must have no programmed relation to reinforcer delivery. This definition excludes "adjunctive" activities [J. L. Falk, *Physiol. Behav.* **6**, 577 (1971)] on temporal grounds. Adjunctive behaviors usually occur after reinforcement, whereas operant responses occur before reinforcement. The definition also disqualifies activities which, like grooming, are refractory to ordinary operant conditioning procedures [S. J. Shettleworth, in *Constraints on Learning*, R. Hinde and J. S. Hinde, Eds. (Academic Press, New York, 1973)]. The criterion of nonnecessity eliminates from consideration activities in or near the area of reinforcement delivery, as at least some of this behavior is necessary for the discovery and ingestion of the reinforcer. Finally, on a logical basis, superstition is not implied merely because some observed response is not part of an experimental contingency. Such a definition rests on the premise that all behavior is either operant or superstitious; its use is certain to spuriously reveal superstitions in all animals in all reinforcement situations.
2. B. F. Skinner, *J. Exp. Psychol.* **38**, 168 (1948).
3. J. E. R. Staddon and V. L. Simmelhag [*Psychol. Rev.* **78**, 3 (1971)] have concluded that the emergence and persistence of superstitious activities result from nonspecific inducement by the schedule of reinforcement.
4. J. E. R. Staddon and S. L. Ayers, *Behaviour* **54**, 26 (1975).
5. L. D. Devenport, paper presented in an animal learning session of the meeting of the Western Psychological Association, San Francisco, Calif., 19 to 22 April 1978.
6. Although mammalian and avian brains are difficult to compare, the area described by those who recognize an avian hippocampal homolog is vestigial or rudimentary in relation to the rat's massive structure [C. J. Herrick, *Neurological Foundations of Animal Behavior* (Holt, New York, 1924), p. 212; J. W. Papez, *Comparative Neurology* (Crowell, New York, 1929), p. 410; E. C. Crosby, B. R. DeJonge, R. C. Schneider, in *Evolution of the Forebrain*, R. Hassler and H. Stephan, Eds. (Plenum, New York, 1967)].
7. If superstitious behavior is induced by a schedule (*3*), the hippocampus may be implicated, as at least one type of schedule-induced rat behavior, polydipsia, is modulated by the hippocampus [L. D. Devenport, *J. Comp. Physiol. Psychol.* **92**, 651 (1978)].
8. Female Sprague-Dawley rats weighed about 250 g at time of surgery. Hippocampal lesions were made by placing a No. 1 stainless steel insect pin insulated with epoxylite, except for 0.5 mm at the tip, at eight sites. With the skull horizontal the stereotaxic coordinates were as follows (in centimeters); 0.25 posterior to bregma, 0.15 and 0.25 lateral to the left and right of the midsagittal suture, 0.34 and 0.30 below the surface of the skull. The remaining four lesions were at 0.45 posterior, 0.45 lateral, 0.7 and 0.36 deep. Anodal current (1.5 mA) was passed for 30 seconds at each of the four anterior placements and for 40 seconds at each of the posterior sites. Three animals with sham lesions were treated identically, but no current was passed. Histological analysis (*7*) revealed lesions that destroyed from 55 to 88 percent of total hippocampal volume. Extrahippocampal damage was minimal and involved the corpus collosum and neocortex overlying the dorsal hippocampus. Some thalamic damage (lateral nucleus and lateral geniculate) was present in two animals but was unrelated to their behavior.
9. J. D. Green and A. Arduini, *J. Neurophysiol.* **17**, 533 (1954).
10. With the skull horizontal, stereotaxic coordinates for the 250-g females were 0.85, 0.90, 0.95, and 1.0 cm anterior to interaural line. Placements were made on the midline by gently displacing the midsagittal sinus. The electrode tip was lowered in each case to 0.5 cm below the surface of the skull. Anodal current (1.5 mA) was passed for 10 seconds at each site. Two animals had sham operations. Histological analysis revealed discrete lesions within the medial septal nuclei. Laterally, they extended to the border of, and in three cases damaged, the medial aspect of the lateral septal nuclei. In two animals the lesions extended about 0.5 anterior to the tip of the medial nucleus.
11. Other research has corroborated this finding (*5*). As it is frequently reported that rats with hippocampal lesions extinguish more slowly than shams, this tendency might have a parallel with superstitiously maintained responses. Recent findings from yoked-control experiments, in which the effects of previous response-reinforcer dependencies can be properly compared across hippocampal and sham groups, have shown that superstitious responses also persevere during extinction. This study also demonstrated, however, that the perseverative tendency is attributable to lesion-induced deficits in response variability rather than an overall inability to withhold behavior (L. D. Devenport and F. A. Holloway, in preparation).
12. P. E. Gay, *Behav. Biol.* **20**, 534 (1977).
13. J. E. R. Staddon, in *Handbook of Operant Behavior*, W. K. Honig and J. E. R. Staddon, Eds. (Prentice-Hall, Englewood Cliffs, N.J., 1977).
14. The findings presented here have now been obtained with male subjects and variable-interval schedules (L. D. Devenport, paper presented at the Annual Meeting of the American Association for the Advancement of Science, Houston, Tex., 3 to 8 January 1979). The rate of superstitious responses under these conditions was more than twice that observed for the fixed-interval schedule used here. Also, animals with lesions and sham lesions that were trained to bar press for pellets on a variable-interval 100-second schedule have been shifted to the same rate of response-independent reinforcement. Animals with sham lesions stopped responding, but those with lesions maintained steady rates (L. D. Devenport and F. A. Holloway, in preparation).

15. I thank the Department of Biology, Southern Oregon State College, for lending histological equipment, and J. Falk, R. Isaacson, L. Jarrard, and A. Zeiner, for their thoughtful comments on the manuscript. Supported by a grant from the Institutional Research Committee, Southern Oregon State College, Ashland.

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Metabolic Mapping of Functional Activity in the Hypothalamo-Neurohypophysial System of the Rat

Abstract. *Physiological stimulation of the hypothalamo-neurohypophysial system by salt loading of rats resulted in a dramatically increased glucose utilization in the posterior pituitary but not in the paraventricular or supraoptic nuclei. The good correlation between glucose utilization and neural activity in the posterior pituitary (that is, nerve terminals) contrasted with the lack of correlation in the paraventricular and supraoptic nuclei (that is, the sites of the cell bodies of the same neurons). This difference in the metabolic response to functional activity between the two regions of these neurons can be explained by the differences in surface-to-volume ratios of these regions.*

Under most normal circumstances the brain is almost entirely dependent on the utilization of glucose for its biochemical energy (*1*). Studies with the recently developed [^{14}C]deoxyglucose method (*2*) for measuring rates of glucose utilization in discrete structural and functional components of the central nervous system have demonstrated a close correlation between levels of local functional activity and local glucose utilization (*3*, *4*). Experimentally induced increases or decreases in functional activity in specific motor and sensory systems resulted in corresponding increases or decreases in glucose utilization in specific structural components of the appropriate pathways (*3*, *4*). The mechanisms underlying the relationship between functional activity and energy metabolism remain, however, essentially unknown. In which of the cellular compartments are the alterations of glucose utilization localized—glia, perikarya, axons, axonal terminals, dendrites? With which of the numerous energy-consuming processes is the functionally related component of energy metabolism associated—electrical activity; ion transport; storage, release, and reuptake of neurotransmitters; membrane and macromolecular synthesis?

The mammalian hypothalamo-neurohypophysial system appears particularly appropriate for investigation of these questions. It is a neural pathway that is anatomically well defined (*5*-*7*) and can easily be stimulated physiologically (*8*), and its output can be measured at a site separate and distinct from that of its input. Its perikarya are located in the supraoptic and paraventricular nuclei of the hypothalamus, whereas its axon terminals are situated in the posterior pituitary. We applied the [^{14}C]deoxyglucose method to study the metabolic responses of this system to osmotic and pharmacological stimulation. The results demonstrate that metabolic activity varies with functional activity in this system and that this relationship is most prominent in the axonal terminals of the neural pathway.

The studies were carried out in adult male Sprague-Dawley rats weighing 300 to 400 g. Each animal was prepared for the experiment by surgical catheterization of one femoral artery and vein under light halothane anesthesia. While still under anesthesia, the animals were immobilized by application of a loose-fitting plaster cast over the abdominal and pelvic regions, and then at least 2 hours were allowed for complete recovery from the effects of anesthesia. The period of measurement of glucose utilization was initiated by the administration of a

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pulse of 125 μCi of 2-deoxy-D-[1-^{14}C]-glucose per kilogram of body weight through the venous catheter and was terminated by killing the animal with an intravenous injection of sodium pentobarbital 45 minutes later. The brains and pituitaries were then removed as rapidly as possible, frozen in isopentane chilled to −40°C with Dry Ice, and coated with chilled embedding medium (Lipshaw Manufacturing Co., Detroit). Serial sections 20 μm thick were prepared from the frozen tissues in a cryostat maintained at −20°C. The brain was sectioned coronally through the full extent of the supraoptic and paraventricular nuclei. The sections were dried and autoradiographed as previously described (*2*). After autoradiography the sections were stained with either cresyl violet or toluidine blue for histological identification of structures visualized in the autoradiographs.

Experiments were carried out on three groups of six rats each: control rats, which had free access to water; dehydrated rats, which were given 2 percent NaCl in the drinking water for 5 days before the experiment; and pharmacologically treated rats, which were injected with 20 mg of phenoxybenzamine per kilogram of body weight 45 to 60 minutes before administration of the [^{14}C]deoxyglucose.

Representative autoradiographs obtained from the three groups are illustrated in Fig. 1. In the brain sections from the normal control rats the loci of the supraoptic and paraventricular nuclei could be clearly identified histologically (Fig. 1B), but neither could be visualized in the autoradiographs (Fig. 1A), indicating that their rates of glucose utilization were not detectably different from those of the surrounding tissues. The optical density of the autoradiograph of the posterior pituitary was, however, significantly higher than that of the anterior pituitary surrounding it (Fig. 1A, right).

Osmotic stimulation (2 percent NaCl administration) caused a marked increase in the optical density of the posterior pituitary relative to the anterior pituitary (Fig. 1C, right), suggesting intensely increased glucose utilization in association with the high level of functional activity of the hypothalamo-neurohypophysial system previously demonstrated under similar experimental conditions by electrophysiological and biochemical methods (*7*, *9*). Despite the striking response of the posterior pituitary, however, there was no obvious autoradiographic evidence of any increased glucose utilization in the paraventricular and supraoptic nuclei during dehydration (Fig. 1C). Inasmuch as the increased functional activity of the hypothalamo-neurohypophysial system elicited by dehydration must have been initiated in the hypothalamic nuclei and projected to the posterior pituitary, it is surprising that evidence of increased glucose utilization was found only in the nerve terminals in the projection area and not in the perikarya of the pathway in the hypothalamus. It is even more surprising in view of the reported fivefold or greater increase in protein synthesis in the perikarya of the supraoptic nuclei during similar conditions of dehydration (*10*). Results similar to those illustrated in Fig. 1C were obtained when the hypothalamo-neurohypophysial system was activated by hypotension induced by hemorrhage or small intravenous doses (1 to 2 mg/kg) of phenoxybenzamine.

There are several possible explanations for the failure to detect autoradiographic evidence of increased glucose utilization in the supraoptic and paraventricular nuclei under conditions in which evidence of metabolic activation is clearly and strikingly apparent in their projection areas in the posterior pituitary. One explanation might be that the activated perikarya in the nuclei are too diffusely distributed for the present spatial resolution of the [^{14}C]deoxyglucose method. This seems unlikely, however, in view of the fact that high doses (20 mg/kg) of the α-adrenergic blocking agent phenoxybenzamine produce marked increases in glucose utilization that are clearly visible not only in the autoradio-

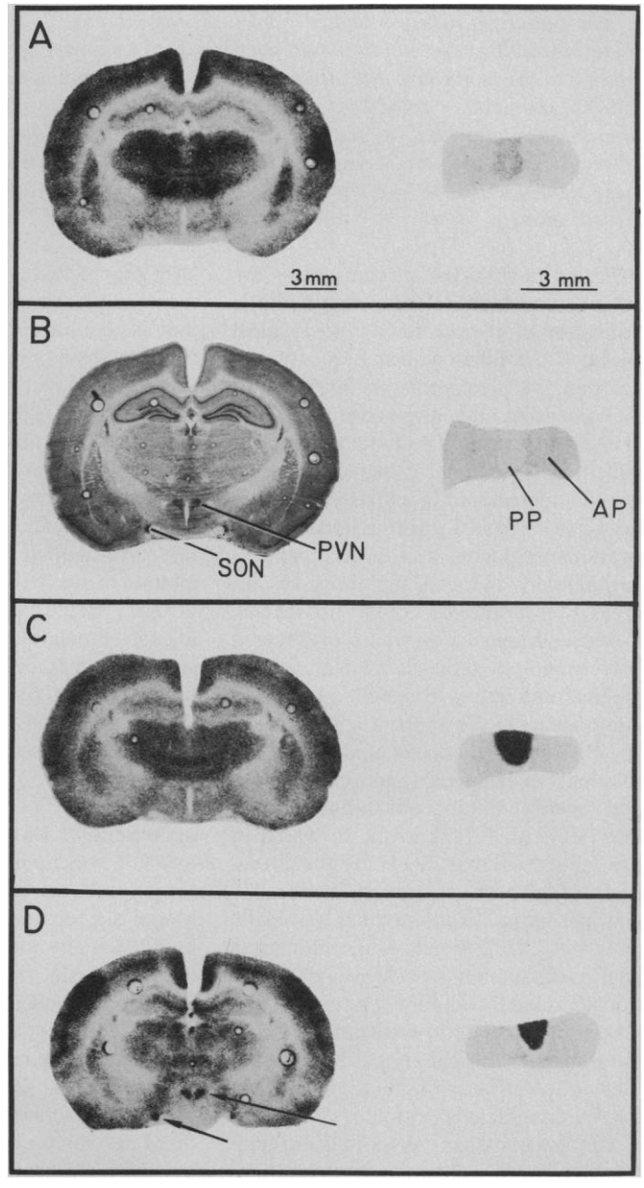

Fig. 1. [^{14}C]Deoxyglucose autoradiographs and stained histological sections of coronal brain sections (left) and pituitary sections (right). The autoradiographs in (A) are characteristic of control rats, which were allowed to drink water freely. The photographs in (B) illustrate the positions of the supraoptic (*SON*) and paraventricular (*PVN*) nuclei in the brain section shown in (*A*) after cresyl violet (Nissl) staining. The positions of the posterior pituitary (*PP*) and anterior pituitary (*AP*) are illustrated on the right side in (B) after toluidine blue staining. (C) Autoradiographs of brain and pituitary typical of dehydrated rats, which were given 2 percent NaCl to drink for 5 days. Note the intense labeling in the posterior pituitary, without comparable change in the SON or PVN. (D) Autoradiographs characteristic of normal rats given an intravenous injection of an α-blocker, phenoxybenzamine (20 mg/kg), approximately 45 to 60 minutes before injection of [^{14}C]deoxyglucose. Note the dramatic increase in labeling of the SON, PVN, and PP.

graphs of the posterior pituitary but in those of the supraoptic and paraventricular nuclei as well (Fig. 1D). Intravenous administration of other α-adrenergic blocking agents, such as phentolamine and yohimbine, produced similar results, whereas β-adrenergic blocking agents had no such effects (*11*).

A more likely explanation for the apparent discrepancy in the metabolic responses of the hypothalamic nuclei and their projection areas in the posterior pituitary to stimulation by dehydration may be in their anatomical properties. The surface-to-volume ratios of the nerve terminals in the posterior pituitary are considerably greater than those of the cell bodies in the supraoptic and paraventricular nuclei, and equivalent impulse activity would therefore be expected to lead to greater increases in energy metabolism in the nerve terminals than in the cell bodies (*12, 13*). Indeed, the energy metabolism of any region may represent primarily the metabolic activities of the nerve terminals and synaptic elements within it. For example, the glucose utilization in the hypothalamic nuclei may reflect mainly the synaptic input and interneuronal activity of these nuclei and not its output (that is, the magnocellular neurons' firing rates). Indeed, the metabolic activation produced by high doses of phenoxybenzamine in the supraoptic and paraventricular nuclei may be a reflection of increased synaptic activity rather than a direct activation of the perikarya. There is already evidence from studies of the visual system of the monkey that it is the neuropil of layer 4 that has the highest rate of glucose utilization in the striate cortex and is the portion most metabolically responsive to alterations in visual input (*4*). The excellent correlation between functional activity and glucose utilization in the posterior pituitary, which is also composed primarily of small unmyelinated axons and nerve terminals (*13*), in contrast to the poor correlation in the cell bodies of the pathway in the hypothalamic nuclei may represent another example of the same phenomenon.

On the basis of this reasoning, the [^{14}C]deoxyglucose method may provide a unique approach for the study of afferent pathways in the mammalian brain. In any neural pathway where the site of input (nucleus containing the cell bodies and dendrites) and the site of output (region containing the nerve terminals) are known, it should be possible to electrically stimulate specific, putative afferent pathways and evaluate whether [^{14}C]-deoxyglucose uptake increases at the input site. Increased [^{14}C]deoxyglucose uptake at the input site would then provide evidence for the presence of the afferent pathway, and evaluation of [^{14}C]deoxyglucose uptake changes at the output site would provide information on whether the specific afferent pathway is inhibitory or excitatory.

WILLIAM J. SCHWARTZ
CAROLYN B. SMITH, LESLIE DAVIDSEN
HELEN SAVAKI, LOUIS SOKOLOFF
Laboratory of Cerebral Metabolism, National Institute of Mental Health, Bethesda, Maryland 20014
MARINA MATA
DAVID J. FINK, HAROLD GAINER
Section on Functional Neurochemistry, Behavioral Biology Branch, National Institute of Child Health and Human Development, Bethesda

References and Notes

1. L. Sokoloff, in *Basic Neurochemistry*, G. J. Siegel, R. W. Albers, R. Katzman, B. W. Agranoff, Eds. (Little, Brown, Boston, 1976), p. 388.
2. L. Sokoloff, M. Reivich, C. Kennedy, M. H. Des Rosiers, C. S. Patlak, K. D. Pettigrew, O. Sakurada, M. Shinohara, *J. Neurochem.* **28**, 897 (1977).
3. C. Kennedy, M. H. Des Rosiers, J. W. Jehle, M. Reivich, F. Sharpe, L. Sokoloff, *Science* **187**, 850 (1975); F. R. Sharp, J. S. Kauer, G. M. Shepherd, *Brain Res.* **98**, 596 (1975); F. Plum, A. Gjedde, F. E. Samson, Eds., *Neurosci. Res. Program Bull.* **14**, 457 (1976); L. Sokoloff, *J. Neurochem.* **29**, 13 (1977).
4. C. Kennedy, M. H. Des Rosiers, O. Sakurada, M. Shinohara, M. Reivich, J. W. Jehle, L. Sokoloff, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4230 (1976).
5. The mammalian hypothalamo-neurohypophysial system is composed of magnocellular neurosecretory cells with perikarya located in the supraoptic and paraventricular nuclei and nerve terminals located in the posterior pituitary [see H. Heller, *Handb. Physiol.* **4**, 103 (1974)]. Two distinct cell types, vasopressin- and oxytocin-secreting cells, are present in this system and have been identified physiologically (*6, 7*) and immunocytochemically [E. A. Zimmerman, A. G. Robinson, M. K. Husain, M. Acosta, A. G. Frantz, W. H. Sawyer, *Endocrinology* **95**, 931 (1974); F. Vandesande and K. Dierickx, *Cell Tissue Res.* **164**, 153 (1975)].
6. D. A. Poulain, J. B. Wakerley, R. E. J. Dyball, *Proc. R. Soc. London Ser. B* **196**, 367 (1977).
7. M. J. Brimble and R. E. J. Dyball, *J. Physiol. (London)* **271**, 253 (1977).
8. The oxytocin cells in the supraoptic and paraventricular nuclei selectively respond (by increased spike activity and oxytocin release) to suckling during the milk ejection reflex [J. B. Wakerley and D. W. Lincoln, *J. Endocrinol.* **57**, 477 (1973); D. W. Lincoln and J. B. Wakerley, *J. Physiol. (London)* **242**, 533 (1974)]. Vasopressin cells respond most selectively to hemorrhage as a stimulus [(*6*); J. B. Wakerley, D. A. Poulain, R. E. J. Dyball, B. A. Cross, *Nature (London)* **258**, 82 (1975)]. Both cell types respond vigorously to hyperosmotic stimuli (*6, 7*).
9. R. E. J. Dyball and P. S. Pountney, *J. Endocrinol.* **56**, 91 (1973); J. K. Walters and G. I. Hatton, *Physiol. Behav.* **13**, 661 (1974).
10. H. Gainer, Y. Sarne, M. J. Brownstein, *J. Cell Biol.* **73**, 366 (1977).
11. H. Savaki, unpublished data. It should be pointed out that the injection of phenoxybenzamine into rats (1.5 mg/kg) itself causes considerable vasopressin release [T. E. Bridges and N. A. Thorn, *J. Endocrinol.* **48**, 265 (1970)]. However, α-adrenergic antagonists, such as phenoxybenzamine and phentolamine, also inhibit specific stimulation-induced release of oxytocin and vasopressin [Bridges and Thorn, *ibid.*; E. Tribollet, G. Clarke, J. J. Dreifuss, D. W. Lincoln, *Brain Res.* **142**, 69 (1978)].
12. The posterior pituitary represents a uniquely high concentration of axon terminals. Morphometric studies of rat neurohypophysis indicated that more than 42 percent of the total volume of this tissue is composed of axon terminals [J. J. Nordmann, *J. Anat.* **123**, 213 (1977)].
13. Previous studies have shown that the increase in energy metabolism (oxygen consumption) in nervous tissue in response to electrical activity is due principally to the activity of the sodium pump in restoring the ionic gradients [J. M. Ritchie, *J. Physiol. (London)* **188**, 309 (1967); P. De Weer, in *Physiology*, Ser. 1, vol. 3, *Neurophysiology*, C. C. Hunt, Ed. (Butterworth, London, 1975)]. In any nerve process, the increase in concentration of intracellular sodium (or decrease in potassium) per impulse is an inverse function of the diameter of that process [for quantitative relations see P. Greengard and J. M. Ritchie, in *Handbook of Neurochemistry*, A. Lajtha, Ed. (Plenum, New York, 1971), vol. 5A, pp. 317-335]. Hence, neural tissues containing fibers of smaller diameter (or fibers with greater surface-to-volume ratios) will need to pump more sodium ions per impulse and consequently will utilize more oxygen and glucose per impulse than tissues of equal mass (or volume) containing fibers of larger diameter. We examined the relationship of [^{14}C]deoxyglucose uptake to impulse activity in a preparation of rat posterior pituitary. Under conditions of depolarizing stimulation, glucose utilization was increased and this increase could be blocked by ouabain. The incremental increase in glucose utilization accompanying membrane depolarization was primarily a function of the increased activity of the sodium pump (M. Mata, D. J. Fink, H. Gainer, C. B. Smith, L. Davidsen, H. Savaki, W. J. Schwartz, L. Sokoloff, *J. Neurochem.*, in press).

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Lead Enhancement of Lithium-Induced Polydipsia

Mailman *et al.* (*1*) concluded in the abstract of their report that their data were "evidence that there may be permanent neural changes induced by postnatal exposure to lead that are manifested by pharmacological challenge with lithium." The report documents that massive oral doses of lead administered postnatally lead to subsequent enhancement of lithium-induced polydipsia. Urine osmolality was not given so that we have no idea of the role of antidiuretic hormone in this syndrome. Sodium excretion and free water clearance are similarly unrecorded. Moreover, no information is given about renal histology or lead content. The report documents only that the polydipsia was not due to changes in the renin-angiotensin system.

Acute lead intoxication in the young is well known to produce a proximal tubular transport defect (*2*). This could lead to proximal renal sodium wasting which might show up in the final urine as an increased sodium supply to the diluting segment of the distal nephron or as natriuresis. In either case the polydipsia would be the result of lead nephropathy

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