Jr., Eds. (Dekker, New York, 1975), pp. 171–190; B. J. Sahakian and T. W. Robbins, *Psychopharmacologia* **45**, 115 (1975).
11. P. Cuatrecasas and M. Hollenberg, *Adv. Protein Chem.* **30**, 251 (1976); P. A. Insel, M. E. Maguire, A. G. Gilman, H. R. Bourne, P. Coffino, K. L. Melmon, *Mol. Pharmacol.* **12**, 1062 (1976); M. E. Charness, D. B. Bylund, B. S. Beckman, M. D. Hollenberg, S. H. Snyder, *Life Sci.* **19**, 243 (1976).
12. I. Creese, D. R. Burt, S. H. Snyder, in preparation.
13. We thank J. Ryan, C. Kaufmann, G. Mack for technical assistance and Janssen Pharmaceutica for the generous gift of [^{3}H]haloperidol. Supported by PHS grant MH-18501, NIMH research scientist development award MH-33128 to S.H.S., PHS fellowships NS-01654 to D.R.B. and DA-05328 to I.C., and grants from the John H. Hartford Foundation and the Scottish Rite Foundation.
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Antigen-Antibody Reactions in Rat Brain Sites Induce Transient Changes in Drinking Behavior

Abstract. *The septum or hypothalamus of rat brain was injected through implanted cannulas with antibody against membrane antigens in the rat brain or with antibody against exogenous soluble antigens (such as ovalbumin) followed by the specific antigen. Both immunological systems produced a moderate but highly significant decrease in drinking by thirsty rats. This phenomenon is suggested as an experimental model for behavioral disorders resulting from nondegenerative, immunological processes in the brain.*

Intracranial injection of antiserum against brain has been employed since 1900 (*1*) to explore possible roles of immunological reactions in neural dysfunction and behavioral disorders. More recent investigation of immunological reactions in the brain has included the search for specific brain antigens and their localization (*2, 3*), the behavioral effects (*4, 5*) and the electrophysiological effects (*4, 6*) of antibody against brain, and the behavioral effects of active immunization of animals with brain antigens (*7*).

The objective of our study has been to elucidate the mechanisms by which immunological reactions in the brain affect behavior. Three broad categories of immunological processes are considered. One is neural cell damage by activated cytolytic pathways such as complement or by degenerative inflammatory lesions (*8*). Another is direct modification of neural cell function, either stimulation by cell surface "activation" or inhibition by blockade of neurochemical receptor sites. The third immunological process proposed is noncytotoxic release of pharmacologically active substances that affect neurotransmission (*9*).

Two types of immune reaction systems were employed. One was rabbit antibody against rat brain tissue antigens. The other was an immunochemically defined and quantifiable reaction with rabbit antibody and a crystalline antigen unrelated to brain. The antiserums against brain are operationally defined since they could be expected to have a variety of antibody specificities including some for generalized rat tissue antigens, for serum proteins, and perhaps for the adjuvant employed. The control reaction systems consisted of hen egg ovalbumin (OA) and human serum albumin (HSA) and their respective rabbit antiserums.

If a behavioral effect is found with antibody against brain, the simplest assumption is that antibody has combined with a substance on a cell surface and has thereby initiated cellular damage or has otherwise modified normal cell function. Determining the effect of a defined reaction system with an antigen not found in the rat brain provides either a control for the rabbit immunoglobulin preparation or a demonstration that an antibody-antigen reaction in the brain can indirectly alter neural function.

We have chosen a relatively simple behavioral response system—drinking by water-deprived rats—to assess the effects of the immunological reaction. By contrast, all previous experiments conducted for this purpose have demonstrated impaired performance of learned or conditioned responses. A change in any behavior can serve to detect an antibody effect and, with conventional controls, perhaps demonstrate the specificity of the antibody for brain antigens. The investigation of mechanisms, however, depends upon a predictable relationship between behavior and its physical substrate processes. We chose the drinking response because much is known about the neural substrates and the neurochemical modulation of water intake (*10*). The use of this simple indicator of neural function allows us to relate the changes in response patterns induced by immunological reactions to regulatory processes and pathways already established by neuroanatomical localization studies using electrical and chemical stimulation techniques and lesions.

The experiments reported here demonstrate that the completely exogenous immune reaction induces a behavioral effect similar to that of the antibody against brain. The results and the analysis of our data in the light of reported effects of lesions, electrical stimulation, and chemical treatments lead us to prefer noncytotoxic release over the cell damage or receptor blockade models.

Antiserums prepared in rabbits against immunogenic fractions of rat brain and control antiserums against HSA and OA were processed to give immunoglobulin concentrates (*11*). Preparations were injected into the rat brain through cannulas implanted in septal or perifornical hypothalamic sites (*12*) involved in the regulation of drinking behavior (*13, 14*).

Charles River CD male rats (250 to 300 g) with intracranial cannulas were adapted to a 23-hour water deprivation schedule. This schedule continued for at least 10 days when each animal's daily water intake had stabilized at a characteristic level within a group range of 20 to 30 ml/day. The 1-hour daily drinking period began at 4 p.m., 2 hours before the onset of the dark period of the light-dark cycle. Purina Lab Chow was continuously available and animals were handled daily before the drinking period. Tests were separated by at least 1 week to allow animals affected by the previous experiment to reestablish normal baseline drinking. The effect of the test substance was assessed by comparing intake on day −1, a baseline measure, with that of day 0, on which the test substance was injected, and days 1 and 2, during which the course of recovery to baseline behavior was charted.

Screening rests were performed to identify active antiserums. Preparations of immunoglobulin from rabbits immunized against various brain fractions were administered in the septal site in two 2.5-μl injections with a 20- to 30-minute interval; the second injection immediately preceded the afternoon drinking period. Depression of drinking was observed following treatment with antiserums against a septal microsomal fraction, against a cerebellar microsomal fraction, and against a cortical microsomal fraction. Two antiserums against soluble fractions of septal and cerebellar homogenates, respectively, had several antibodies distinguishable by immunoelectrophoresis but, when tested in the same animals, produced minimal or no behavioral responses. The antiserum having the most pronounced effect was one against a septal microsomal fraction. The immunoglobulin from this antiserum was used in the experiments reported below as a representative antibody against brain and has been designated antibody against rat brain membrane (aRBM).

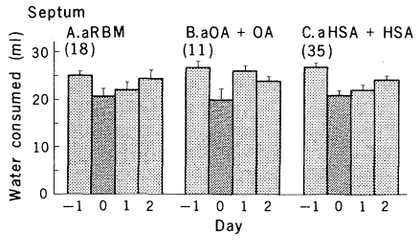

Fig. 1. Mean total water consumed ± standard error of mean (S.E.M.) during a 1-hour daily test period. Dark bars represent consumption on day of treatment, 6 hours after administration of immune reaction systems in the septum. The number of animals tested is shown in parentheses; data have been pooled from separate tests (see text). Protein concentrations in injections were as follows: (A) aRBM, 23 mg/ml; (B) aOA, 23 mg/ml, OA, 4.0 mg/ml (12 times antibody equivalence); and (C) aHSA, 24 mg/ml; HSA, 2.5 mg/ml (12 times antibody equivalence).

The most characteristic phenomena in the response to aRBM in these tests were the delay of maximal effect until the following day and the complete recovery by the second or third day. In experiments reported here, treatment was administered 6 hours before the drinking period to reduce any effects of handling and to obtain a more pronounced effect on the test day.

In the first experiment the effects of aRBM on drinking in thirsty rats were compared to the effects of a defined reaction system, antibody against HSA (aHSA) or against OA (aOA) followed by its specific antigen. Animals having received aRBM were also tested with the aHSA and aOA systems. An additional group of 17 rats was tested with only the aHSA system to control for the effects of multiple injections. Their responses were indistinguishable from those of animals receiving repeated injections and the data were combined. The mean water consumption for each treatment over the 4 days of record is shown in Fig. 1. One-way analysis of variance for repeated measures followed by selected Tukey tests (days 0 and −1) indicated that both aRBM ($P < .05$) and the defined reactions systems, aHSA + HSA ($P < .01$) and aOA + OA ($P < .01$), depressed drinking in thirsty rats to a significant and equivalent degree. The maximum response was observed on day 0 (6 hours) and often persisted until day 1 (32 hours). Recovery from treatment was always seen and was usually complete by day 2 (54 hours). When the noncross-reacting system aOA + HSA was tested in five animals whose water intake was depressed more than 25 percent by the specific aHSA + HSA reaction, no effect on the drinking response was seen. This test was a control for protein concentration, injection volume, and nonrelevant specificities (such as to mycobacterial antigens used as adjuvant).

In the experiment just described the antigens of the defined reaction systems were administered in amounts about 12 times the reaction equivalent of the antibody. It was reasoned that more soluble and more dissociable antigen-antibody complexes formed in extreme antigen excess could disperse farther from the cannula tip and affect a larger area. In a subsequent study we used antigen-antibody ratios of 1, 4, 12, and 20. Antigen at 20 times antibody equivalence increased the number of animals whose drinking was depressed by 25 percent or more; this ratio was adopted as standard procedure.

When similar experiments in the hypothalamus were performed, comparable results were obtained. Figure 2 depicts the mean water intake for 18 rats after weekly intrahypothalamic injection of aHSA + HSA, aHSA + OA, or aRBM over the 4 days of record. One-way analysis of variance for repeated measures and selected Tukey tests indicated that significant depression of drinking occurred following aRBM ($P < .01$) and aHSA + HSA ($P < .01$) treatments. Mean intake after aHSA + OA was not significantly different from intake on the baseline day.

In order to determine that aRBM was indeed acting as an immune reactant to an antigen or antigens in the parenchyma of the brain, an additional group of 18 was tested with aRBM absorbed with a crude pooled homogenate of rat liver and kidney, or rat serum, or with a crude homogenate of perfused rat hypothalamus (*15*). The drinking responses of animals with hypothalamic cannulas after treatments with absorbed aRBM are shown in

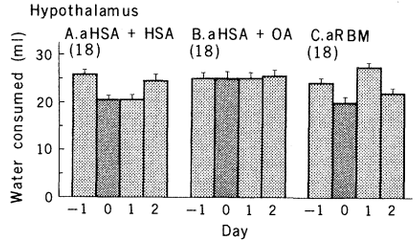

Fig. 2. Mean total water consumed ± S.E.M. during a 1-hour daily test period. Dark bars represent consumption on day of treatment, 6 hours after administration of an immune reaction system in the hypothalamus. The number of animals tested is shown in parentheses. Injections were given in successive weeks in the sequence shown to the same animals at the following protein concentrations: (A) aHSA, 27 mg/ml, HSA, 4.8 mg/ml (20 times antibody equivalence); (B) aHSA, 27 mg/ml, OA, 4 mg/ml (noncross-reacting system); and (C) aRBM, 23 mg/ml.

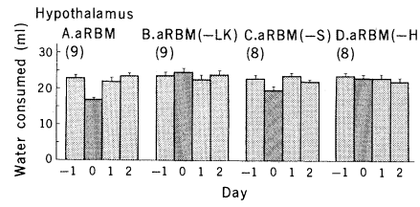

Fig. 3. Effect of selective antigen absorptions of aRBM on induced depression of drinking in water-deprived rats. The immunoglobulin preparation was (A) unabsorbed; (B) absorbed with a mixture of liver and kidney homogenates (−LK); (C) absorbed with rat serum (−S); or (D) absorbed with homogenate of perfused hypothalamus (−H). Antiserum was absorbed before immunoglobulin preparation. Immunoglobulin solutions (10 mg/ml) were administered in two 2.5-μl injections. The experiments were performed at weekly intervals in the sequence shown in the same group of animals.

Fig. 3. The antibody activity expressed as reduced water intake was completely removed by absorption with the hypothalamus preparations but not by absorption with serum. It was also completely removed by the liver-kidney preparation; thus, it appears that the active antibody in aRBM is not specific to brain although it is specific to some tissue antigen.

Histological examination of sections (hematoxylin-eosin stain) of the cannula tracks and the reaction area at the cannula tips revealed that repeated testing produces a well-circumscribed inflammation site (0.25 to 1.0 mm, mean = 0.6 mm). Lymphohistiocytic infiltration of the perimeter and, in most cases, an aggregation of polymorphonuclear leukocytes in the open space at the cannula tip are apparent, but the occurrence of any specific cell type did not correlate reliably with the behavioral data. Evidence for any extensive lysis or necrosis like that described for electrolytic lesions (*16*) was not found.

The major result of this study is that a similar behavioral change is induced by two different types of immunological reactions. It is quite possible that antibody against brain cell fractions react directly with some antigen on neural cell membranes, but such activity cannot be proposed for the defined soluble antigen-antibody systems. The most reasonable mode of action for the soluble systems is the release of physiologically active substances from cells by one of a variety of mechanisms known to occur in tissues when an antigen reacts with cytophilic antibody (*8, 9*). In the periphery histamine is the biogenic amine most frequently associated with anaphylactic reactions. The injection of histamine (25 to 100 μg) into the septum of 12 rats that had responded to aHSA + HSA failed to

alter drinking behavior. Subsequent experiments were designed to implicate these reactions in the effects of transmitter substances or analogs known to be associated with appetitive behavior.

Analysis of our behavioral data in the light of reported effects of lesions, electrical stimulation, and chemical treatments lends further support to the neurochemical release model and appears to rule out a neural cell damage model for the effects we observe. This conclusion is based on the following facts. (i) Depression of drinking occurs only upon retreatment with immunoactive materials even though a significant inflammatory site has developed. (ii) While we see depression of drinking, lesions of the septum produce extensive and persistent hyperdipsia (*13, 17*). (iii) Electrical stimulation of the septum is reported to reduce water intake in rats on a 23-hour water deprivation schedule (*18*) to about the same degree that we observe with an immune reaction. (iv) Most important, in subsequent experiments we have found that defined immune reactions in hypothalamic sites stimulate eating in free-feeding, free-drinking animals (*19*), whereas lesions of the lateral hypothalamus in the placement region we employed produce aphagia in free-feeding rats (*20*). (v) Eating is elicited in free-feeding animals when norepinephrine is introduced into the lateral hypothalamus (*21*), and norepinephrine also depresses drinking in rats on a 23-hour water deprivation schedule (*15, 22*)—both effects obtained by defined antigen-antibody reactions (*19*). (vi) Excessive drinking in satiated rats is induced by carbachol in the perifornical hypothalamus (*23*), an effect that is antagonized by norepinephrine (*22*) and by aHSA + HSA (*19*).

In summary, the effects of immunological reaction systems in this region of the brain were qualitatively similar, both in direction and range, to the effects of locally applied norepinephrine. In the case of the soluble defined reaction systems the release of norepinephrine might be accounted for by a local anaphylaxis-like reaction. Antibody against rat brain membrane, however, could depress drinking by a different mechanism. By direct immunochemical binding, aRBM might functionally suppress cells which are normally activated during deprivation. In the nondeprived state the release of norepinephrine by the exogenous soluble systems would elicit excessive eating, an effect which we have reported (*19*). The mechanism proposed above for aRBM, on the other hand, should not elicit excessive eating in the nondeprived state since the affected neurons would be quiescent. We would therefore expect a dissociation of these two immunological reactions with regard to eating behavior in sated rats. While these experiments suggest that norepinephrine is released, they do not exclude the release of other active substances that, in other regions of the brain, may be more effective in disrupting a characteristic behavioral regulation (*24*). Our experiments are based on a general hypothesis that a wide variety of behavioral dysfunction, perhaps some of clinical significance, may be caused by otherwise mundane immune responses.

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References and Notes

1. C. Delezenne, *Ann. Inst. Pasteur* **14**, 686 (1900), cited along with other early work in review by B. D. Janković in *Macromolecules and Behavior*, J. Gaito, Ed. (Appleton-Century-Crofts, New York, ed. 2, 1972), pp. 100–130.
2. H. Hydèn and B. S. McEwen, *Proc. Natl. Acad. Sci. U.S.A.* **55**, 354 (1966); E. D. Day and S. H. Appel, *J. Immunol.* **104**, 710 (1970); A. Orosz, J. Hamori, A. Falus, E. Madurasz, I. Lakos, G. Adam, *Nature (London) New Biol.* **245**, 18 (1973); A. van NieuwAmerongen and P. A. Roukem, *J. Neurochem.* **23**, 85 (1974); A. I. Matus, *J. Neurocytol.* **4**, 47 (1975).
3. S. E. Karpiak, L. Graf, M. M. Rapport, *Science* **194**, 735 (1976).
4. L. Mihailović and B. D. Janković, *Neurosci. Res. Progr. Bull.* **3**, 8 (1965); S. E. Karpiak, Jr., M. M. Rapport, F. P. Bowen, *Neuropsychologia* **12**, 313 (1974); S. E. Karpiak, M. Serokosz, M. M. Rapport, *Brain Res.* **102**, 313 (1976).
5. R. G. Heath and I. M. Krupp, *Am. J. Psychiatry* **123**, 12 (1967); B. D. Janković, L. Radić, R. Veskov, J. Horvat, *Nature (London)* **218**, 270 (1968); L. Mihailović, I. Divac, K. Mitrović, D. Milošević, B. D. Janković, *Exp. Neurol.* **24**, 325 (1969); S. E. Karpiak, Jr., and M. M. Rapport, *Brain Res.* **92**, 405 (1975).
6. L. Mihailović and B. D. Janković, *Nature (London)* **192**, 665 (1961); ———, D. Cupic, *Brain Res.* **32**, 97 (1971); R. G. Heath, I. M. Krupp, L. W. Byers, J. I. Liljekvist, *Arch. Gen. Psychiatry* **16**, 10 (1967); *ibid.*, p. 24; E. T. Segura, *Acta Physiol. Lat. Am.* **20**, 284 (1970); S. E. Karpiak, F. P. Bowen, M. M. Rapport, *Brain Res.* **59**, 303 (1973); R. E. Garey, R. G. Heath, J. W. Harper, *Biol. Psychiatry* **8**, 75 (1975).
7. C. F. C. MacPherson, *Can. Psychiatr. Assoc. J.* **15**, 641 (1970); ——— and R. P. N. Shek, *Exp. Neurol.* **29**, 1 (1970).
8. E. L. Becker, *Adv. Immunol.* **13**, 267 (1971).
9. ———, *ibid.* **17**, 94 (1973).
10. A. N. Epstein, H. R. Kissileff, E. Stellar, Eds., *The Neuropsychology of Thirst: New Findings and Advances in Concepts* (Winston, Washington, D.C., 1973).
11. Rat brains were perfused with phosphate-buffered saline (PBS) (0.15*M* NaCl in 0.01*M* sodium phosphate buffer at *p*H 7.2); septal, cortical, and cerebellar tissue was excised, weighed, and frozen. Tissues were disrupted in SKM buffer (0.4*M* sucrose, 0.025*M* KCl, 0.01*M* $MgCl_2$, 0.085*M* tris, *p*H 7.8) (one part tissue per ten parts buffer, weight to volume) with a Teflon pestle in a Potter-Elvehjem homogenizer at 1000 rev/min for 30 seconds at 5°C. Homogenates were centrifuged at 15,000*g* for 10 minutes, and the supernatant was then centrifuged at 100,000*g* for 90 minutes. The pellet was resuspended in PBS and sonicated three times for 20 seconds. For immunization with complete Freund's adjuvant, the pellet (crude microsomal membrane fraction) was resuspended to the homogenate volume; the soluble extract was used as recovered. Male New Zealand White rabbits (1.7 to 2.3 kg) were injected at two sites intramuscularly and two sites subcutaneously with 0.5 ml of emulsion per site. After 6 weeks, booster injections were administered intravenously with soluble extracts, and subcutaneously in incomplete adjuvant in the case of the pellet suspensions. Rabbits were bled weekly for testing. Antiserums to HSA and OA were produced by similar protocols, using five-times crystallized antigens (Pentex). Antibody production was monitored by immunoelectrophoresis of rat serum proteins, soluble rat brain antigens, and soluble rat liver antigens. Response to membrane antigens was only presumptive, and collection bleeding was initiated when antibody to some soluble tissue antigen was detected. Immunoglobulin concentrates from antiserums were prepared by precipitation with 35 percent saturated $(NH_4)_2SO_4$, dialysis against PBS, and adjustment of protein concentration to 25 to 35 mg/ml (determined by ultraviolet absorbency at 280 nm, $E^{1\%}_{1cm} = 14$) for storage at − 20°C.
12. Cannulas were made from 23-gauge hypodermic needles with a hub length of 9 mm and a screw-top closure fitted with a thin wire stylet to prevent clogging when not in use. Unilateral implantation was performed using the *Atlas of Coordinates of the Albino Rat*, by W. J. S. Kreig (Johnson Scientific Instrument Co., Berwyn, Ill., 1946). The rat's head was positioned in the stereotaxic apparatus with the top of the incisor bar 3.1 mm above the interaural line and the following coordinates were employed: (i) medial septum: 0.5 mm posterior to bregma, 0.5 mm lateral to midline, 5.5 mm deep from surface of skull; (ii) hypothalamus: 1.3 mm lateral to midline at the anterior-posterior level of bregma, 8.2 mm deep from surface of skull.
13. J. Harvey and H. Hunt, *J. Comp. Physiol. Psychol.* **59**, 49 (1965) E. M. Blass and D. G. Hanson, *ibid.* **70**, 87 (1970).
14. S. P. Grossman, *Science* **132**, 301 (1960); *J. Comp. Physiol. Psychol.* **58**, 194 (1964); A. E. Fisher and J. N. Coury, *Science* **138**, 691 (1962); G. Singer and S. Armstrong, *J. Comp. Physiol. Psychol.* **85**, 453 (1973).
15. Three-step absorptions of antiserum against brain tissue were carried out before immunoglobulin preparation. Absorption with rat serum was performed with 0.1, 0.1, and 1.0 mg of protein per milliliter of antiserum; with rat liver and kidney or hypothalamus homogenate 2, 2, and 4 mg of protein per milliliter of antiserum was used.
16. G. Wolf and L. V. DiCara, *Exp. Neurol.* **23**, 529 (1969).
17. R. J. Carey, *Physiol. Behav.* **4**, 759 (1969).
18. T. B. Wishart and G. J. Mogenson, *Physiol. Behav.* **5**, 1399 (1970).
19. C. A. Williams and N. Schupf-Smith, *Fed. Proc.* **32**, 304 (abstr.) (1973); N. Schupf-Smith and C. A. Williams, *Neurosci. Abstr.* (1975), p. 688.
20. P. Teitelbaum and A. N. Epstein, *Psychol. Rev.* **69**, 74 (1962).
21. D. A. Booth, *Science* **158**, 515 (1967); J. L. Slangen and N. E. Miller, *Physiol. Behav.* **4**, 543 (1969); S. Armstrong and G. Singer, *Pharmacol. Biochem. Behav.* **2**, 811 (1974); S. F. Leibowitz, *Physiol. Behav.* **14**, 731 (1975); *ibid.*, p. 743.
22. G. Singer and J. Kelley, *Physiol. Behav.* **8**, 885 (1972).
23. S. P. Grossman, *Am. J. Physiol.* **202**, 872 (1962); *ibid.*, p. 1230.
24. We do not suggest that nonreversible or even progressive immune lesions do not occur but we would expect them to have different behavioral effects. We administer our active substances to a site completely healed after surgery so endogenous humoral substances such as complement should not be present in amounts significantly greater than normal. Thus, we found no degenerative reactions of sort described by L. M. Davidoff, B. C. Seegal, and D. Seegal [*J. Exp. Med.* **55**, 163 (1932)] after hypodermic injection of foreign antigens into the brain, where bleeding would be expected at the site. Nor would we expect the cellular breakdown reported by E. De Robertis, E. Lapetina, J. Peccisaavedra, and E. S. Soto [*Life Sci.* **5**, 1979 (1966)] and E. De Robertis, E. Lapetina, and F. Wald [*Exp. Neurol.* **21**, 322 (1968)], when antibody against brain and complement were added to neural cells in vitro.
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