for this tRNA component in this tissue remains intact and appears to function independently of exogenously added N^6-(Δ^2-isopentenyl)adenosine.

The tRNA, however, can serve as a source of these two modified nucleosides, and any degradation of tRNA would result in their release. Although alternative pathways of synthesis for these compounds may exist, Hall (*19*) has suggested that the rate of release from tRNA and the rate of metabolism of such growth regulatory compounds could constitute a means for maintenance of a physiologically active level in the tissue.

WILLIAM H. DYSON, C. M. CHEN
SYED N. ALAM, ROSS H. HALL
Department of Biochemistry, McMaster University, Hamilton, Ontario
C. I. HONG, GIRISH B. CHHEDA
Roswell Park Memorial Institute, Buffalo, New York

References and Notes

1. R. H. Hall, *Progr. Nucleic Acid Res. Mol. Biol.* **10**, 57 (1970).
2. D. J. Armstrong, F. Skoog, L. H. Kirkegaard, A. E. Hampel, R. M. Bock, I. Gillam, G. M. Tener, *Proc. Nat. Acad. Sci. U.S.* **63**, 504 (1969).
3. F. Fittler and R. H. Hall, *Biochem. Biophys. Res. Commun.* **25**, 441 (1966).
4. M. L. Gefter and R. L. Russell, *J. Mol. Biol.* **39**, 145 (1969).
5. G. B. Chheda, R. H. Hall, D. I. Magrath, M. P. Schweizer, L. Stasiuk, P. R. Taylor, *Biochemistry* **8**, 3278 (1969).
6. M. P. Schweizer, G. B. Chheda, L. Baczynskyj, R. H. Hall, *ibid.*, p. 3283.
7. S. Takemura, M. Murakami, M. Miyazaki, *J. Biol. Chem. Japan* **65**, 553 (1969).
8. H. Ishikura, Y. Yamada, K. Murao, M. Saneyoshi, S. Nishimura, *Biochem. Biophys. Res. Commun.* **37**, 990 (1969).
9. This strain of tobacco tissue requires neither auxin nor cytokinin and was obtained from J. E. Fox, University of Kansas, from normal pith callus of *Nicotiana tobaccum*, variety Wisconsin 38.
10. A. R. Bellamy and R. K. Ralph, *Methods Enzymol.* **12B**, 156 (1968).
11. One A_{260} unit is defined as the quantity of material needed to give an absorbance of 1.0 at 260 nm when dissolved in 1 ml of solvent.
12. Soya bean tissue, derived from the cotyledon and requiring both an auxin and a cytokinin for growth, was used as the test organism. The tissue originated in C. O. Miller's laboratory and came to us via J. E. Fox. Pieces of tissue (2 mg), previously maintained on the basal medium plus 0.5 mg of benzyladenine per liter, were placed, three to five to a flask, on basal medium containing various concentrations of the compounds to be tested. After incubation in the dark at 25°C for 25 to 50 days the pieces of tissue were weighed, and their fresh weights were compared to those of controls grown on basal medium devoid of cytokinins.
13. For bioassay purposes, a basal medium was used, similar to that described by J. E. Fox [*Physiol. Plant.* **16**, 793 (1963)] with the addition of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ and $CuSO_4$ at 0.1 and 0.23 mg/liter, respectively.
14. G. B. Chheda, *Life Sci.* **8**, part 2, 979 (1969).
15. D. J. Armstrong, W. J. Burrows, F. Skoog, K. L. Roy, D. Söll, *Proc. Nat. Acad. Sci. U.S.* **63**, 834 (1969).
16. G. B. Chheda and C. I. Hong, in preparation.
17. N. P. Kefford, J. A. Zwar, M. I. Bruce, in *Biochemistry and Physiology of Plant Growth Substances*, F. Wightman and G. Setterfield, Eds. (Runge Press, Ottawa, 1968), p. 61.
18. C. M. Chen and R. H. Hall, *Phytochemistry* **8**, 1987 (1969).
19. R. H. Hall, in *Biochemistry and Physiology of Plant Growth Substances*, F. Wightman and G. Setterfield, Eds. (Runge Press, Ottawa, 1968), p. 47.
20. Supported by grants from the National Cancer Institute of Canada and the Medical Research of Canada (MT-2738), the National Science Foundation (6B-8690), and the U.S. Public Health Service (CA 10835). We acknowledge the assistance of Mrs. Lily Dyson and Mrs. G. R. Chen.

16 June 1970; revised 31 July 1970

Amygdaloid Nucleus: New Afferent Input from the Vomeronasal Organ

Abstract. *Terminal degeneration stained by the Fink-Heimer technique was found in the medial and cortical amygdaloid nuclei in a discrete zone after lesions were inflicted in the accessory olfactory bulb but not after lesions were made in the main olfactory bulb in the rabbit. Since the accessory olfactory bulb receives the endings of the vomeronasal nerve, the mediocortical complex of the amygdala is the central projection area for the vomeronasal sensory organ. The vomeronasal organ is seen as having new potential significance in sexual and feeding behavior because the cortical amygdaloid nucleus projects to the anterior, medial hypothalamus and the ventromedial nucleus.*

Bilateral ablation of the medial preoptic-anterior hypothalamic zone abolishes copulatory behavior in the male rat (*1*). Ablation of the olfactory bulb also abolishes or impairs copulatory activity in some mammals (*2*). Among the structures likely to receive a projection from the olfactory bulb in the rat, only the posteromedial part of the cortical amygdaloid nucleus projects to the medial preoptic-anterior hypothalamic zone (*3*). However, the olfactory bulb does not actually project to the posteromedial part of the cortical amygdaloid nucleus (*4*). How, then, may ablation of the olfactory bulb impair copulation? We have evidence that the posteromedial part of the cortical amygdaloid nucleus provides a link in a chemoreceptive pathway arising from the vomeronasal organ of the snout (*5*). The peripheral part of the vomeronasal pathway is adjacent to the olfactory pathway (*6*) and is, therefore, vulnerable to injury by ablation of the olfactory bulb.

The vomeronasal organ (*5*) is a tubular sac which opens by way of a small pore either into the anterior, basal corner of the nasal chamber or into the anterior end of the oral cavity by way of the nasopalatine canal or into both oral and nasal cavities in some species. The organs, one on each side, are lined with an epithelium resembling the olfactory epithelium and, by analogy with the latter, probably have a chemoreceptive function. The openings of the vomeronasal organs are so situated that they may allow entry of moisture-borne substances picked up by the snout or taken into the mouth. The nuzzling or licking activities common to many mammals (*7*) during sexual behavior, for example, may favor stimulation of the vomeronasal organ. The vomeronasal nerve connects the vomeronasal organ to the accessory olfactory bulb (*6*), which lies in a depression on the dorsal surface of the main olfactory bulb in many mammals (Fig. 1, left). In its course to the accessory olfactory bulb, the vomeronasal nerve comes to lie closely adjacent to the main olfactory nerves and main olfactory bulb (*6*).

Although the peripheral morphology of the vomeronasal system as described above has been known for a long time, the central connections of the mammalian accessory olfactory bulb defied analysis until now (*8*). Previous investigators (*9*), who used the conventional Nauta-Gygax techniques after making lesions of the olfactory formation, were unable to detect any difference in the distribution of degenerating fibers of the lateral olfactory tract which could be attributed to damage to the accessory olfactory bulb. It now appears that in the rabbit much of the accessory olfactory bulb projection becomes thinly myelinated or unmyelinated a few millimeters before the fibers leave their main course in the lateral olfactory tract to enter their area of termination. The poorly myelinated segment of the accessory olfactory bulb efferents is readily detected, however, in sections of rabbit brain stained by the Fink-Heimer method (*10*).

We first determined the projection of the main olfactory bulb in the rabbit by charting the terminal degeneration, as revealed by the Fink-Heimer method (*10*), in seven young, female, New Zealand white rabbits which had survived surgical destruction of an olfactory bulb

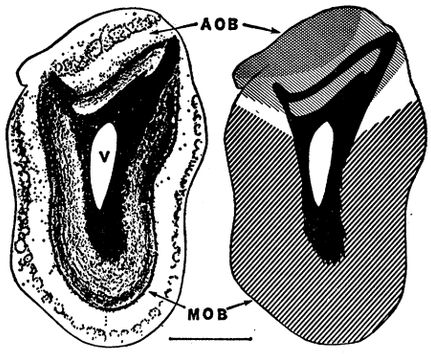

Fig. 1. Cross-sectional views of the main (*MOB*) and accessory (*AOB*) olfactory bulb of the rabbit. Left side: section stained for myelinated axons. Right side: tracing of the same section showing regions typically destroyed by large lesions of the accessory olfactory bulb (light, oblique hatch); small lesions of the accessory olfactory bulb (cross-hatch); or large lesions restricted to the main olfactory bulb (heavy, oblique hatch). *V*, ventricle. Scale, 1 mm.

for 4 to 6 days. Even the largest lesions in this series (Fig. 1) did not damage the accessory olfactory bulb (*11*). Briefly, heavy deposits of terminal degeneration were found in various parts of the pyriform lobe and olfactory tubercle, but very little degeneration was observed in the amygdala. What little was found there was sharply confined to an anterolateral strip of the cortical amygdaloid nucleus. We subsequently operated on another series of animals

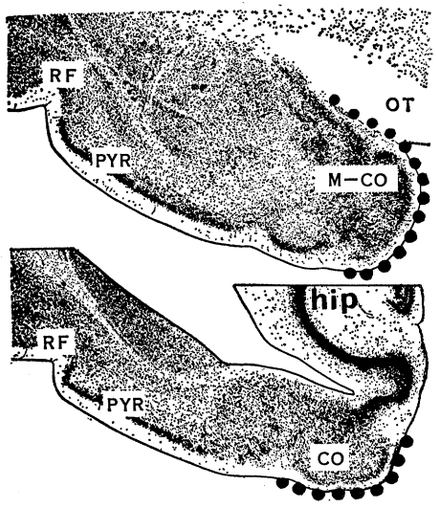

Fig. 2. Two cross sections through the pyriform lobe at anterior (upper) and posterior (lower) levels of the amygdaloid nucleus of the rabbit. Cresyl violet stain. *CO*, cortical amygdaloid nucleus; *hip*, hippocampus; *M-CO*, mediocortical complex. *OT*, optic tract; *PYR*, prepyriform and periamygdaloid cortex; *RF*, rhinal fissure. Large black dots indicate the area in which the secondary vomeronasal fibers (accessory bulb efferents) terminate.

in order to damage the accessory olfactory bulb. The accessory olfactory bulb of the rabbit is located on the dorsal surface of the olfactory formation well anterior to the frontal pole and is readily identifiable on inspection. The lesions were inflicted from above under full visual control and were made either by mechanical avulsion or by application of a heated probe. Optimum results were obtained in animals surviving 4 days (eight specimens). In every case except one, the mitral cell layer of the accessory olfactory bulb was damaged and rather heavy terminal degeneration was found in a discrete segment of the amygdaloid complex (Figs. 2 and 3). There was usually also some degeneration diffusely and lightly sprinkled in those parts of the pyriform lobe cortex and olfactory tubercle to which the olfactory bulb projected because the lesions disturbed the main olfactory bulb to various degrees. The heavy terminal degeneration was always located in the molecular layer of the medial and cortical amygdaloid nuclei (mediocortical complex) and was always sharply restricted to the particular locus illustrated. There was no overlap between this locus and the locus of terminal degeneration observed after lesions were made in the main olfactory bulb in the first experiment.

The size of the lesions of the accessory olfactory bulb varied. Large lesions (two specimens, 4 days' survival) involved tissue belonging to the main olfactory bulb and anterior olfactory nucleus (Fig. 1). Small lesions (five specimens, 4 days' survival) damaged the mitral cell layer of the accessory olfactory bulb and inflicted slight, variable damage to efferent fibers from the main olfactory bulb or adjacent parts of the anterior olfactory nucleus. In each case, however, regardless of whether efferent fibers of the olfactory bulb were involved, the extent of the area of heavy terminal degeneration was the same. What differed among the cases was the density of the diffusely sprinkled degeneration found in the areas to which the main olfactory bulb projects. That degeneration was more noticeable in the larger lesions but it was never heavy or concentrated (*12*). In one case, which serves as a sham operation, only the glomerular layer of the accessory olfactory bulb was damaged by the heated probe; the mitral cells were unaffected. In that case, no terminal degeneration was found outside of the accessory olfactory bulb.

These data and data on the further

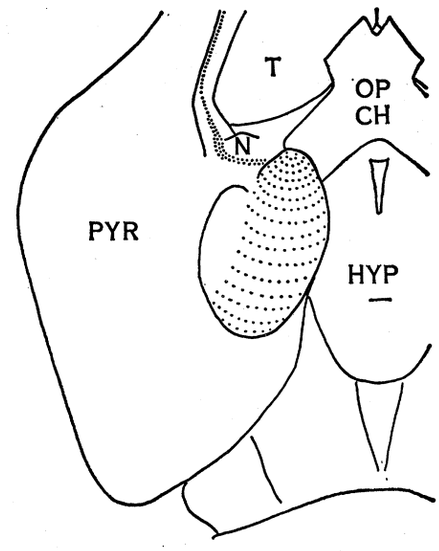

Fig. 3. Drawing of ventral surface of posterior two-thirds of right pyriform lobe in the rabbit. *HYP*, hypothalamus; *N*, nucleus of the lateral olfactory tract; *OP CH*, optic chiasma; *PYR*, pyriform lobe; *T*, olfactory tubercle. The stipple along the lateral olfactory tract lateral to the olfactory tubercle indicates the path of the secondary vomeronasal fibers from the accessory olfactory bulb. These fibers terminate in the mediocortical region of the amygdala as indicated by the contoured rows of dots.

connections of the primary olfactory cortex (*3*, *13*) and amygdala (*3*) suggest that the main olfactory and the vomeronasal systems provide parallel, separate routes of chemosensory influence into the hypothalamus. The main olfactory pathway includes certain specific parts of the pyriform lobe cortex and subsequently enters the lateral preoptic area and lateral hypothalamus. The vomeronasal pathway includes the posteromedial part of the cortical amygdaloid nucleus and subsequently enters the medial preoptic area and the medial hypothalamus. This interpretation suggests to us that in experiments in which ablations of the olfactory bulb affected copulatory behavior, interruption of the vomeronasal-medial hypothalamic axis may well have been a critical factor. Since the cortical amygdaloid nucleus projects also to the ventromedial nucleus of the hypothalamus, it is likely that the vomeronasal system plays some role in feeding reactions as well as in sexual behavior.

Not all adult mammals possess a vomeronasal organ or accessory olfactory bulb, however. Higher primates and some bats, for example, lack these structures. It would, therefore, be interesting to know whether in such species the olfactory bulb provides an afferent

supply for the mediocortical amygdaloid field or whether some other arrangement exists. Application of the new, Fink-Heimer technique to appropriate species and careful observation of the distribution of terminal degeneration in and about the amygdaloid complex should readily resolve this question.

SARAH S. WINANS*
FRANK SCALIA
Department of Anatomy, Downstate Medical Center, Brooklyn, New York

References and Notes

1. L. Heimer and K. Larsson, *Brain Res.* **3**, 248 (1966/1967); A. Soulairac and M.-L. Soulairac, *Ann. Endocrinol. (Paris)* **17**, 731 (1956).
2. M. R. Murphy and G. E. Schneider, *Science* **167**, 302 (1970); L. Heimer and K. Larsson, *Physiol. Behav.* **2**, 207 (1967). In the rabbit [C. M. Brooks, *Amer. J. Physiol.* **120**, 544 (1937)], ablation of the olfactory bulb was effective in retarding copulation by the male only after extensive neocortical ablation.
3. C. Leonard, *Anat. Rec.* **106**, 337 (1970).
4. L. Heimer, *J. Anat.* **103**, 413 (1968).
5. V. Negus, *Comparative Anatomy of the Nose and Paranasal Sinuses* (Livingstone, London, 1958), p. 103.
6. R. E. McCotter, *Anat. Rec.* **6**, 299 (1912).
7. M. R. Murphy and G. E. Schneider, *Science* **167**, 302 (1970); F. A. Beach and A. Merari, *J. Comp. Physiol. Psychol.* **70**, 1 (1970).
8. Before the report of L. Heimer [*Ann. N.Y. Acad. Sci.* **167**, 129 (1969)] on the central connections of the accessory olfactory bulb in the tegu lizard, the only well-documented report known to the authors on this subject in in lower forms was the Golgi study of C. J. Herrick in the frog [C. J. Herrick, *J. Comp. Neurol.* **33**, 213 (1921)]. In the tegu, the accessory olfactory bulb projects to the nucleus sphericus of the posterior telencephalon and in the frog it projects to the so-called amygdala. The latter finding has been confirmed in limited observations in our own laboratory in material studied by the Fink-Heimer method (F. Scalia, in preparation). Neither the reptilian nucleus sphericus nor the amygdala of the frog have clearly identifiable counterparts in the mammalian brain as studied by descriptive morphological analysis. Our study on the projections of the accessory olfactory bulb of the rabbit may provide a valid bridge, however, between the mammalian and inframammalian morphology.
9. See review by F. Scalia, *Brain Behav. Evol.* **1**, 101 (1968); also see A. H. M. Lohman, *Acta Anat.* **53** (suppl. 49) 1 (1963).
10. R. P. Fink and L. Heimer, *Brain Res.* **4**, 369 (1967).
11. F. Scalia and S. Winans, in preparation.
12. In considering our data, we have argued that even in those cases of lesions of the accessory olfactory bulb in which no apparent damage was done to the neighboring efferent fibers of the main olfactory bulb, it ought to be assumed nevertheless that those fibers were somehow injured until evidence to the contrary is obtained. If that assumption were warranted and if the main olfactory bulb does contribute to the synapses in the mediocortical amygdaloid nuclei, then there ought to have been some indication of terminal degeneration in that part of the amygdala whenever a lesion of the olfactory bulb resulted in degeneration of fibers passing close enough to the accessory olfactory bulb (two cases, 4 days' survival) to have been injured by a lesion of the accessory olfactory bulb. As already stated, however, in no case in which the accessory olfactory bulb was not damaged was degeneration observed in the mediocortical amygdaloid zone illustrated in Figs. 2 and 3. Therefore, the assumption, even if warranted, is of little significance.
13. T. P. S. Powell, W. M. Cowan, G. Raisman, *J. Anat.* **99**, 791 (1965).
14. We thank Carlyle Clement for histological assistance and Jack Illari for photography. Supported by PHS grant NB-06577.
* Present address: Department of Anatomy, Medical Science II, University of Michigan, Ann Arbor 48104.

11 June 1970

Hemagglutination Assay for Antigen and Antibody Associated with Viral Hepatitis

Abstract. *Hemagglutination assays are described for measuring hepatitis-associated Australia antigen and antibody. Red cells coated with isolated antigen, with chromic chloride as a coupling agent, are used for detection of antibodies. Detection of the antigen in serums depends on inhibition of hemagglutination. The test has the sensitivity and rapidity of the best tests available, is simpler to perform, and lends itself to large-scale screening of blood donors.*

The finding of a specific antigen, popularly known as Australia antigen (*1*), in the serum of many patients with viral hepatitis has led to evaluation of a variety of techniques to provide a simple, rapid, and sensitive test for diagnosing hepatitis and detecting blood donors who may transmit the disease (*2–6*). At present complement fixation (CF) procedures appear to be most sensitive and rapid, immunodiffusion (ID) techniques are simplest but least sensitive and slowest (*7*), and electrophoretic modifications of precipitin techniques are as rapid as CF but are relatively insensitive (*5*). We now describe a hemagglutination (HA) technique for measuring hepatitis-associated antigen (HAA), which is as sensitive and rapid as the CF technique for detecting HAA and is easy to perform with reagents that can be standardized for use over long periods. The technique is more sensitive than CF and at least as sensitive as radioimmunoassay (*6*) for detecting antibody to HAA. In the HA test the agglutinogen consists of inert indicator red cells coated with isolated HAA with chromic chloride as a coupling agent (*8*). The agglutinator is antibody to HAA, and detection of HAA in serums depends on inhibition of agglutination (*9*).

Plasma samples from asymptomatic carriers of HAA were used to isolate purified antigen. Isolation was carried out by combined rate and isopycnic zonal centrifugation (*10*) with cesium chloride density gradients (*11*). The purified preparations were dialyzed extensively against 0.85 percent sodium chloride (saline) at 4°C. The Kjeldahl nitrogen content of the purified HAA used was 27 μg/ml, and the optical density at 280 nm wavelength was 0.445. This antigen preparation gave a single band in agarose at a maximum dilution of 1:2 with antiserum to HAA, and when concentrated tenfold gave no precipitin band with rabbit antiserum against whole human plasma.

Indicator cells were prepared from group O human blood collected in ethylenediaminetetraacetate. Cells were stored at 4°C for no more than a week prior to being coated with HAA. Red cells were washed four times with saline and used as a 40 percent suspension in saline. To coat the cells, 0.025 ml of cell suspension was mixed with 0.075 ml of purified HAA, and 0.025 ml of 1.25 m*M* chromic chloride was added. The mixture was agitated gently in a glass tube (10 by 75 mm) at room temperature for 5 minutes. Cells were then washed four times with saline and used as a 0.2 percent suspension in phosphate-buffered saline, *p*H 7.3, containing 0.5 percent bovine serum albumin (BSA), 0.0025 percent polyvinyl pyrrolidone (PVP), and a 1 : 20,000 dilution of Tween 80 (*12*). These conditions of coating were found to be optimum by a "checkerboard" test with different dilutions of HAA and chromic chloride (*9*).

Hemagglutination reactions for detection and titration of antibodies were carried out in V-shaped microtiter plates (*13*). The specificity of antibody to HAA was established by hemagglutination inhibition (HAI) with purified HAA and known HAA-positive serums and failure to inhibit agglutination with known HAA-negative serums (*12*).

In testing for HAA, ten times the minimum amount of antibody that gave a positive agglutination was mixed with an equal volume of various dilutions of test serum, and then HAA-coated cells were added. The antiserum to HAA used had a titer of 10,000 in HA, 480 by CF, and 8 by ID. Serum containing HAA used as a known positive control was from a patient with serum hepatitis and had a titer of 1280 by HAI, 1000 by CF, and 8 by ID. Serum from a normal individual that had contained no antigen by these tests was used as a negative control.