Table 1. Characterization and analysis of complex polar lipids of *Methanospirillum hungatei* (Fig. 1).

Item	PGL-I (1)	PGL-II (3)	DGT-I (2)	DGT-II (4)	DGD-I (5)	DGD-II (6)	PG (7)
TLC, R_f*	0.34	0.46	0.73	0.83	0.68	0.78	0.61
Weight (percent of total lipid)	50	14	0.5	0.2	17	2	5
Molar ratios†							
P/tetraether‡	1	1					
P/diether§							1
Hexose/tetraether	2	2	2	2			
Hexose/diether					2	2	
Hexose/*P*	2	2					

*Thin-layer chromatography (TLC) on silica gel G in a mixture of chloroform, methanol, diethylamine, and water (110:50:8:3.5, by volume). †Lipid-*P* and hexose were determined as described (*9*). ‡Tetraether is 2,3,2′,3′-tetra-*O*-16″,16‴-biphytanyl-di-*sn*-glycerol (*7, 16*). §Diether is 2,3-di-*O*-phytanyl-*sn*-glycerol (*6*).

meric configuration of the sugar residues was established by ^{13}C NMR as α-glucopyranosyl (99.1 ppm) and β-galactofuranosyl (106.7 ppm) in PGL-I; in PGL-II, both residues were β-galactofuranosyl groups (109.0 and 108.4 ppm). These assignments were supported by the ^1H NMR spectra and by molecular rotation data calculated by Hudson's isorotation rule.

In PGL-I and PGL-II, both sugars must be located on one side of the tetraether and the glycerophosphate group on the other (Fig. 1) since, after hydrolysis, completely methylated PGL-I and PGL-II yielded only the free tetraether, whereas DGT-I and DGT-II yielded only the monomethyl tetraether derivative. The glycerophosphate group has the *sn*-3 configuration and is linked to the tetraether by a phosphodiester bond, as shown by the quantitative release of glycerophosphate [59 percent β isomer, 41 percent *sn*-3 isomer (*11, 12*)] by alkaline hydrolysis. The *sn*-3-glycerophosphate residue in PGL-I and PGL-II is enantiomeric with that of the corresponding residue in Gram-positive bacterial phosphoglycolipids (*11, 12*).

The PG component (compound **7** in Fig. 1) was identified as the 2,3-di-*O*-phytanyl-*sn*-1-glycerophosphoryl-*sn*-1′-glycerol, diasteriomeric with that (compound **8** in Fig. 1) found in extreme halophiles (*6*).

On the basis of the above results, the structure of each polar lipid component can be assigned as shown in Fig. 1. The structures of PGL-I and PGL-II suggest that they behave as covalently bonded asymmetric lipid bilayers in methanogen membranes, as was proposed by Langworthy (*7, 17*) for the lipids of thermoacidophiles. Such a structure would impart rigidity and stability to the membranes not possible with a conventional bilayer structure.

Further investigation is required to determine the biosynthetic pathway for these polar lipids, their distribution within the cell and also the orientation of the asymmetric PGL-I and PGL-II within the membranes. Our findings strengthen the concept of a phylogenetic relationship between methanogens, extreme halophiles, and certain thermoacidophiles (*3, 5, 17, 18*).

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References and Notes

1. G. B. Patel, L. A. Roth, L. van den Berg, D. S. Clark, *Can. J. Microbiol.* **22**, 1404 (1976).
2. A. J. B. Zehnder, B. A. Huser, T. D. Brock, K. Wuhrmann, *Arch. Microbiol.* **124**, 1 (1980).
3. L. J. Magrum, K. W. Leuhrsen, C. R. Woese, *J. Mol. Evol.* **11**, 1 (1978); M. Yaguchi, A. T. Matheson, L. P. Visentin, A. M. Zucker, in *Genetics and Evolution of RNA Polymerase, t-RNA and Ribosomes*, S. Osawa, E. Ozeki, H. Uchida, T. Yura, Eds. (Univ. of Tokyo Press, Tokyo, 1980), pp. 585–599; G. E. Fox *et al.*, *Science* **209**, 457 (1980).
4. R. A. Makula and M. E. Singer, *Biochem. Biophys. Res. Commun.* **82**, 716 (1978).
5. T. G. Tornabene and T. A. Langworthy, *Science* **203**, 51 (1979).
6. M. Kates, *Prog. Chem. Fats Other Lipids* **15**, 301 (1978).
7. T. A. Langworthy, *Biochim. Biophys. Acta* **487**, 37 (1977).
8. G. D. Sprott, R. C. McKellar, K. M. Shaw, J. Giroux, W. G. Martin, *Can. J. Microbiol.* **25**, 192 (1979).
9. M. Kates, *Techniques of Lipidology* (Elsevier/North-Holland, New York, 1972).
10. E. C. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.* **37**, 911 (1959).
11. P. Komaratat and M. Kates, *Biochim. Biophys. Acta* **398**, 464 (1975).
12. W. Fischer, I. Ischizuka, H. R. Landgraf, J. Hermann, *ibid.* **296**, 527 (1973); E. P. Kennedy, *Methods Enzymol.* **5**, 476 (1962).
13. E. Hirst and E. Percival, *Methods Carbohydr. Chem.* **5**, 287 (1965).
14. H.-J. Yang and S.-I. Hakamori, *J. Biol. Chem.* **246**, 1192 (1971).
15. P.-E. Jansson, L. Kenne, H. Liedgren, B. Lindberg, J. Lönngren, *Chem. Commun. Univ. Stockholm* **8**, 1 (1976); A. S. Rao and N. Roy, *Carbohydr. Res.* **74**, 349 (1979).
16. M. de Rosa, A. Gambacorta, J. D. Bu'Lock, *Phytochemistry* **15**, 143 (1976).
17. T. A. Langworthy, in *The Mycoplasmas* (Academic Press, New York, 1979), vol. 1, p. 495.
18. S. T. Bayley, *Trends Biochem. Sci.* **4**, 223 (1979).
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Conversion of 3T3-L1 Fibroblasts to Fat Cells by an Inhibitor of Methylation: Effect of 3-Deazaadenosine

Abstract. *3-Deazaadenosine, an inhibitor of methylation, increased the frequency of conversion of 3T3-L1 fibroblasts to fat cells in a dose-dependent manner. Once converted, the 3T3-L1 fat cells retained their adipose morphology and accumulated triglycerides even when 3-deazaadenosine was removed from the culture medium. 3-Deazaadenosine may perturb cellular methylation and thereby lead to an increase in the frequency of differentiation of 3T3-L1 fibroblasts to fat cells.*

3-Deazaadenosine (3-deaza-Ado), an adenosine analog that is not deaminated or phosphorylated, inhibits methylation reactions mediated by *S*-adenosylmethionine (AdoMet) in vivo or in vitro (*1–4*). 3-Deaza-Ado inhibits biochemical methylations by acting as either an inhibitor or a substrate of *S*-adenosylhomocysteine (AdoHcy) hydrolase (*5–7*). By inhibiting AdoHcy hydrolase with 3-deaza-Ado, the intracellular level of AdoHcy can be increased, and in the majority of cases 3-deaza-AdoHcy is also generated (*1–3, 5, 8*). Thus, the accumulation of AdoHcy or 3-deaza-AdoHcy, or both, leads to perturbation of biochemical methylations that are sensitive to inhibition by these two compounds (*1, 5, 7–9*). The biological effects of 3-deaza-Ado are varied and interesting. It can act as a potent antiviral agent (*8, 10*) and can also reverse the oncogenic transformation induced by Rous sarcoma virus (*8*). It exhibits antimalarial effects in vitro (*11*). Chemotaxis by neutrophils (*12*), phagocytosis by macrophages (*13*), lymphocyte-mediated cytolysis (*3*), capping of membrane immunoglobulin in lymphocytes (*14*), and immunoglobulin E-mediated histamine release from human basophils (*15*) are inhibited by 3-deaza-Ado. It has also been shown that 3-deaza-Ado can inhibit, rapidly and reversibly, synaptic responses between retinal neurons and muscle fibers in culture (*16*).

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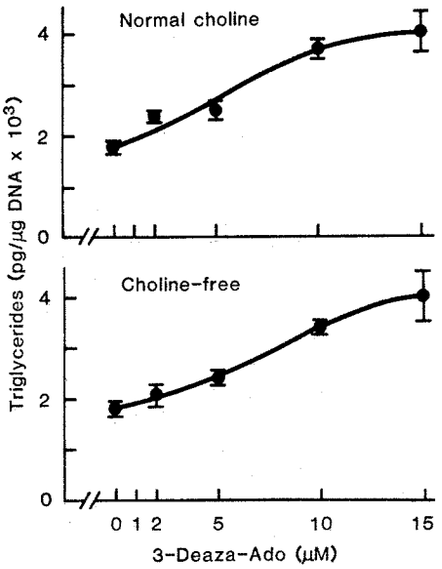

Fig. 1. Effect of 3-deaza-Ado on the accumulation of triglycerides in 3T3-L1 cells cultured for 10 days with or without choline in the medium. Normal choline in the culture medium was 28.5 μM. Each data point represents the mean for five cultures; bars show standard errors.

A subline of mouse fibroblasts (3T3-L1) in the confluent state can undergo spontaneous differentiation to become fat cells that resemble mature mammalian adipocytes, both morphologically and biochemically (*17*). The frequency of conversion to fat cells is increased by chemical agents such as prostaglandin $F_{2\alpha}$ and 1-methyl-3-isobutylxanthine (*18, 19*) or high insulin levels in the culture medium (10 μg/ml) (*19*). I report here that 3-deaza-Ado can increase the frequency of conversion of 3T3-L1 fibroblasts to fat cells.

For these studies, 3T3-L1 fibroblasts (American Type Culture Collection) were grown in Dulbecco-Vogt modified Eagle's medium supplemented with 10 percent fetal calf serum, penicillin (100 unit/ml), and streptomycin (125 μg/ml). The cells were grown at 37°C in 5 percent CO_2 and were fed every other day. Three to four days after 2×10^5 cells were seeded in 25-cm^2 flasks, the cells were about confluent. At this time, 3-deaza-Ado was added to induce conversion to fat cells. Triglyceride levels in cells were determined with a triglyceride kit (Sigma Chemical Company) based on a coupled-enzyme method (*20*). The cells were scraped into Dulbecco's phosphate-buffered saline and sonicated. Cellular DNA content was determined by the procedure of Burton (*21*), and total carnitine was assayed by the radioactive method of Pande and Parvin (*22*).

When cultured in the presence of 3-deaza-Ado for 10 days, the 3T3-L1 fibroblasts were converted to fat cells in a dose-dependent manner (Fig. 1). As judged by staining with Oil Red O, more than 90 percent were converted to fat cells at 10 μM 3-deaza-Ado. The conversion took place regardless of the choline concentration in the medium. Ten days after treatment, the cellular concentration of triglycerides (per microgram of DNA) was about 1800 pg for the control, about 3500 pg at 10 μM 3-deaza-Ado, and about 4000 pg at 15 μM 3-deaza-Ado (Fig. 1). Next, the 3T3-L1 fibroblasts were cultured with 10 μM 3-deaza-Ado for 10 days, and then the drug treatment was discontinued for the following 10 days. Triglycerides remained largely unaltered when the cells were cultured further in the absence of 3-deaza-Ado (Fig. 2). Apparently, once converted, the 3T3-L1 fat cells could retain their adipocyte morphology and triglycerides. If the cells were cultured continuously in the presence of 10 μM 3-deaza-Ado for 20 days, the level of triglycerides increased further to about 10,000 pg per microgram of DNA. The finding that choline did not affect the conversion of 3T3-L1 fibroblasts to fat cells suggests that the accumulation of triglycerides in the differentiated cells is not equivalent to fatty liver formation caused by choline deficiency (*23*).

L-Carnitine, a postulated carrier for fatty acid transport, at concentrations as high as 100 μM has no effect on the conversion of 3T3-L1 fibroblasts to fat cells by 15 μM 3-deaza-Ado (data not shown). Nor was there a difference in carnitine concentration between the control and treated cells; the values observed (per microgram of DNA) were 14.6 ± 0.7 pmole for the control and 14.2 ± 0.5, 14.3 ± 0.9, and 14.3 ± 2.1 pmole for cells treated with 5, 10, and 15 μM 3-deaza-Ado, respectively, for 10 days. Therefore, 3-deaza-Ado probably did not inhibit the AdoMet-dependent methylation of lysine. The methylation of lysine to trimethyllysine is one of the steps in carnitine biosynthesis (*24*). 3-Deaza-Ado, at 10 or 15 μM, had no apparent effect on logarithmic growth of 3T3-L1 cells. In fact, at confluence, the number of cells in the treated cultures was about twice that in the untreated ones. Two hours after treatment with 10 μM 3-deaza-Ado, a tenfold rise in the cellular level of AdoHcy and formation of 3-deaza-AdoHcy at a concentration that is 40 times the normal AdoHcy level have been observed in 3T3-L1 fibroblasts (*25*).

3-Deaza-Ado has been shown to have no effect on the binding of hormones or polypeptides to receptors of cells or on subsequent internalization (*12, 26*). Although 3-deaza-Ado can inhibit adenylate cyclase in vitro (*1*), it has no effect on levels of cyclic adenosine monophosphate in intact cells (*27*). However, it is a very potent inhibitor of phospholipid methylation (*1, 2, 4*). 3-Deaza-Ado may perturb cellular methylation and subsequently trigger an increase in the frequency of differentiation of 3T3-L1 fibroblasts to fat cells. Use of 3-deaza-Ado may help elucidate some underlying biochemical mechanisms of cellular obesity.

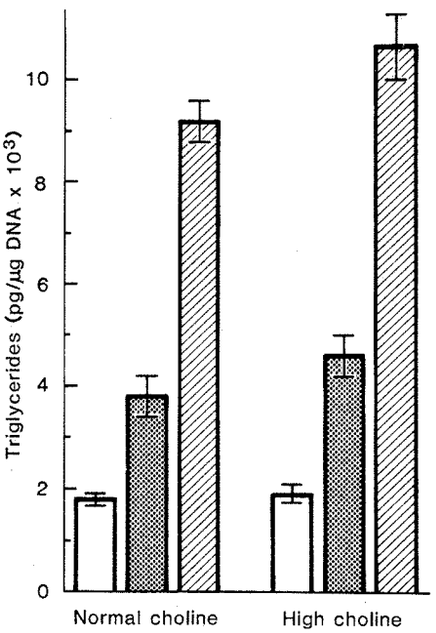

Fig. 2. Accumulation of triglycerides in 3T3-L1 cells treated with 10 μM 3-deaza-Ado for 10 days and then cultured for another 10 days in its absence (dotted bar) and in cells treated with 10 μM 3-deaza-Ado continuously for 20 days (hatched bar). The control (open bar) consisted of cells cultured in the absence of 3-deaza-Ado for 20 days. Normal choline was 28.5 μM and high choline was 1.79 mM. Each data point represents the mean for five cultures; bars show standard errors.

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References and Notes

1. P. K. Chiang and G. L. Cantoni, *Biochem. Pharmacol.* **28**, 1897 (1979).
2. P. K. Chiang, Y. S. Im, G. L. Cantoni, *Biochem. Biophys. Res. Commun.* **94**, 174 (1980).
3. T. P. Zimmerman, G. Wolberg, G. S. Duncan, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 6220 (1978); R. T. Borchardt, *J. Med. Chem.* **23**, 347 (1980).
4. F. Hirata, J. Axelrod, F. T. Crews, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4813 (1979); F. Hirata and J. Axelrod, *Science* **209**, 1082 (1980).
5. P. K. Chiang, H. H. Richards, G. L. Cantoni, *Mol. Pharmacol.* **13**, 939 (1977).
6. H. H. Richards, P. K. Chiang, G. L. Cantoni, *J. Biol. Chem.* **253**, 4476 (1978).
7. G. L. Cantoni and P. K. Chiang, in *Natural Sulfur Compounds*, D. Cavallini, G. E. Gaull, V. Zappia, Eds. (Plenum, New York, 1980), pp. 67-80.
8. J. P. Bader, N. R. Brown, P. K. Chiang, G. L. Cantoni, *Virology* **89**, 494 (1978).
9. Y. S. Im, P. K. Chiang, G. L. Cantoni, *J. Biol. Chem.* **254**, 11047 (1979).

10. P. K. Chiang, G. L. Cantoni, J. P. Bader, W. M. Shannon, H. J. Thomas, J. A. Montgomery, *Biochem. Biophys. Res. Commun.* **82**, 417 (1978).
11. W. Trager, M. Tershakovec, P. K. Chiang, G. L. Cantoni, *Exp. Parasitol.* **50**, 83 (1980).
12. P. K. Chiang, K. Venkatasubramanian, H. H. Richards, G. L. Cantoni, E. Schiffman, in *Transmethylation*, E. Usdin, R. T. Borchardt, C. R. Creveling, Eds. (Elsevier/North-Holland, New York, 1979), pp. 164–172.
13. E. J. Leonard, A. Skeel, P. K. Chiang, G. L. Cantoni, *Biochem. Biophys. Res. Commun.* **84**, 102 (1978).
14. J. Braun, F. S. Rosen, E. R. Unanue, *J. Exp. Med.* **151**, 174 (1980).
15. Y. Morita, P. K. Chiang, R. P. Siraganian, *Biochem. Pharmacol.*, in press.
16. J. M. Thompson, P. K. Chiang, R. R. Ruffalo, Jr., G. L. Cantoni, M. Nirenberg, *Soc. Neurosci. Abstr.* **5**, 747 (1979).
17. H. Green and O. Kehinde, *Cell* **1**, 113 (1974).
18. T. R. Russell and R.-J. Ho, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4516 (1976).
19. J. C. Mackall, A. K. Student, S. E. Polakis, M. D. Lane, *J. Biol. Chem.* **251**, 6462 (1976).
20. J. K. Pinter, J. A. Hayashi, J. A. Watson, *Arch. Biochem. Biophys.* **121**, 404 (1967).
21. K. Burton, *Biochem. J.* **62**, 315 (1956).
22. S. V. Pande and R. Parvin, *J. Biol. Chem.* **255**, 2994 (1980).
23. R. E. Olson, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **30**, 131 (1971); B. Lombardi, *ibid.*, p. 139; S. Mookerjea, *ibid.*, p. 143.
24. R. A. Cox and C. G. Hoppel, *Biochem. J.* **142**, 699 (1974); W. K. Paik and S. Kim, *Protein Methylation* (Wiley, New York, 1980).
25. A. Guranowski, J. A. Montgomery, G. L. Cantoni, P. K. Chiang, *Biochemistry* **20**, 110 (1981).
26. H. T. Haigler, M. C. Willingham, I. Pastan, *Biochem. Biophys. Res. Commun.* **94**, 630 (1980).
27. T. P. Zimmerman, R. D. Deeprose, G. Wolberg, G. S. Duncan, *Biochem. Pharmacol.* **28**, 2375 (1979); Y. S. Im, R. R. Aksamit, P. K. Chiang, G. L. Cantoni, in preparation.
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Opiate Receptor Gradients in Monkey Cerebral Cortex: Correspondence with Sensory Processing Hierarchies

Abstract. *In order to obtain information on the possible functions of endogenous opiates in the primate cerebral cortex, we assessed the distribution of μ-like opiate receptors (which selectively bind ^{3}H-labeled naloxone) and δ-like opiate receptors (which selectively bind ^{3}H-labeled D-Ala^2, D-Leu^5-enkephalin) throughout the cerebral cortex of the rhesus monkey. Stereospecific [^{3}H]naloxone binding sites increased in a gradient along hierarchically organized cortical systems that sequentially process modality-specific sensory information of a progressively more complex nature. Specific [^{3}H]enkephalin binding sites, in contrast, were relatively evenly distributed throughout the cerebral cortex. These results, in combination with electrophysiological studies of monkeys and humans, suggest that μ-like opiate receptors may play a role in the affective filtering of sensory stimuli at the cortical level, that is, in emotion-induced selective attention.*

Despite evidence that the cerebral cortex contains opiate receptors (*1*, *2*) and opiate peptides (*3*) and responds electrically to opiate compounds (*4*), information regarding the functional significance of cortical opiate systems is scarce. Clues to such functions might be obtained by analyzing the distribution of opiate receptors in structurally or functionally defined systems of the cerebral cortex of the rhesus monkey (*Macaca mulatta*), a species used frequently in neuroanatomical, neurophysiological, and neurobehavioral analyses of cortical function. Although previous studies have provided some information on the regional distribution of opiate receptors in the monkey and the human cortex (*5*), the dissections were not complete, and selective assay conditions for opiate receptor subtypes were not used. We now report that while specific [^{3}H]enkephalin binding hardly varies in different parts of the cortex, opiate receptors labeled by [^{3}H]naloxone (*6*) increased in a gradient along hierarchically organized cortical systems, which sequentially process modality-specific sensory information of a progressively more complex nature.

Rhesus monkeys (young adults, two male and two female, weighing 5 to 7 kg) were rapidly anesthetized with sodium pentothal (125 mg in 1.5 ml saline, intrahepatically) followed by sodium pentobarbital (35 mg per kilogram of body weight, injected intraperitoneally). Cerebral circulation and respiration were maintained while a craniotomy was performed. The brain was removed within 12 minutes after sedation and chilled on crushed ice. The boundaries of the cortical dissection were determined on the basis of the cytoarchitectonic map of von Bonin and Bailey (*7*); the areas obtained are listed according to both anatomical name and cytoarchitectonic designation (Table 1). Tissues were immediately frozen on dry ice and stored at −85°C until assayed.

Tissue was homogenized in 50 volumes of ice-cold 0.05*M* tris HCl buffer (*p*H 7.55 at 4°C) through the use of a Polytron tissue disruptor (setting 6 for 15 seconds) and then centrifuged at 4°C at 10,000*g*. The pellets were resuspended in 2.5 volumes of fresh buffer. To measure stereospecific [^{3}H]naloxone binding, 100 μl of the tissue homogenate was added to tubes containing (in tris) 100 m*M* NaCl, 1 n*M* [^{3}H]naloxone (New England Nuclear; 50 Ci/mmole), and 1 μ*M* dextrallorphan or 1 μ*M* levallorphan in a final volume of 500 μl. After the tubes were incubated at 4°C for 60 minutes, the reactions were stopped by filtration under low vacuum through Whatman GF/B filters. The tubes were washed twice and the filters once with 5-ml portions of ice-cold tris; the filters were placed in vials, 10 ml of Aquassure (New England Nuclear) was added, and the radioactivity was measured by liquid scintillation spectrometry at 38 percent efficiency. Stereospecific [^{3}H]naloxone binding was defined as binding in the presence of 1 μ*M* dextrallorphan minus binding in the presence of 1 μ*M* levallorphan. Triplicate determinations showed a standard error of 2 to 5 percent of the mean, and binding in the presence of levallorphan was usually 10 to 15 percent of binding in the presence of dextrallorphan.

To measure specific enkephalin binding, 100 μl of the homogenate (stored for less than 1 week at −85°C) was added to tubes containing (in 0.05*M* tris HCl, *p*H 7.4 at 25°C) 0.2 percent bovine serum albumin, 0.08 mg of bacitracin per milliliter, 2 μ*M* guanosine triphosphate, 30 m*M* NaCl, 3 m*M* manganese acetate, and 1 n*M* D-Ala^2-[tyrosyl-3,5-^{3}H]enkephalin (5-D-leucine) (Amersham; 52 Ci/mmole) in a final volume of 500 μl. After the tubes were incubated at 25°C for 30 minutes, the reactions were stopped as before. Specific binding of the labeled enkephalin ([^{3}H]D-Ala) was defined as total binding in the above assay minus binding obtained in identical reaction mixtures also containing 1 μ*M* β-endorphin. Triplicate determinations showed a standard error of 2 to 5 percent of the mean, and binding in the presence of 1 μ*M* β-endorphin was usually 5 to 10 percent of the total binding. Specific binding of both ligands was linear with respect to the volume of homogenate added (from 25 to 100 μl).

In the visual system, sensory input seems to be processed sequentially through a series of cortical loci corresponding to the cytoarchitecturally defined areas OC, OB, OA, TEO, TE, and ventral TG (see Table 1 for cytoarchitectonic designations) (*8*, *9*). The density of stereospecific opiate receptors binding [^{3}H]naloxone (Table 1) increased in a gradient (Fig. 1A) along this system from the primary sensory cortex (OC) to the ventral temporal pole (TG), which projects most densely to the amygdala (*9–11*). A similar gradient (Fig. 1A) was detected in the auditory cortical system, from primary sensory cortex (TC/TB)

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