duced elevations of corticosterone are accentuated by synthetic opiate agonists and enkephalin administration and blocked by opiate antagonists (*17, 18*). Chronic morphine administration, as in cross-tolerance experiments, seems to decrease pituitary-adrenal function (*17*). Thus, opiate agonists and antagonists may exert some of their influence on opioid stress-induced analgesia by ultimately regulating corticosterone through a direct effect on enkephalin-sensitive opiate receptors, as well as through action on midbrain pain pathways.

A. JOHN MACLENNAN
ROBERT C. DRUGAN
RICHARD L. HYSON
STEVEN F. MAIER
Department of Psychology, University of Colorado, Boulder 80309
JOHN MADDEN IV
JACK D. BARCHAS
Nancy Pritzker Laboratory of Behavioral Neurochemisty, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, California 94305

References and Notes

1. S. Amir, Z. Brown, Z. Amit, *Neurosci. Biobehav. Rev.* **4**, 77 (1980); R. J. Bodnar, D. D. Kelly, M. Brutus, M. Glusman, *ibid.*, p. 87; W. T. Chance, *ibid.*, p. 55.
2. J. E. Sherman and J. C. Liebeskind, in *Pain*, J. J. Bonica, Ed. (Raven, New York, 1980); A. I. Basbaum and H. L. Fields, *Ann. Neurol.* **4**, 451 (1978).
3. J. Madden IV, H. Akil, R. L. Patrick, J. D. Barchas, *Nature (London)* **265**, 358 (1977).
4. G. B. Chesher and B. Chan, *Life Sci.* **21**, 1569 (1977); R. C. Drugan, J. W. Grau, S. F. Maier, J. Madden IV, J. D. Barchas, *Pharmacol. Biochem. Behav.* **14**, 677 (1981); S. F. Maier, S. Davies, J. W. Grau, R. L. Jackson, D. H. Morrison, T. Moye, J. Madden IV, J. D. Barchas, *J. Comp. Physiol. Psychol.* **94**, 1172 (1980).
5. J. W. Lewis, J. T. Cannon, J. C. Liebeskind, *Science* **208**, 623 (1980); J. W. Lewis, J. E. Sherman, J. C. Liebeskind, *J. Neurosci.* **1**, 358 (1981).
6. S. J. Watson, H. Akil, C. N. Richard, J. D. Barchas, *Nature (London)* **275**, 226 (1978).
7. J. Rossier, E. P. French, C. Rivier, T. Shibasaki, R. Guillemin, F. E. Bloom, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 666 (1980).
8. E. R. Baisman, B. M. Cox, O. H. Osman, A. Goldstein, *Neuroendocrinology* **28**, 402 (1979).
9. J. Rossier, E. D. French, C. Rivier, N. Ling, R. Guillemin, F. E. Bloom, *Nature (London)* **270**, 618 (1977).
10. E. C. Azmitia, S. Algeria, E. Costa, *Science* **169**, 201 (1970); E. C. Azmitia and B. S. McEwen, *Brain Res.* **78**, 291 (1974); W. E. Foote, J. P. Lieb, R. L. Martz, M. W. Gordon, *ibid.* **41**, 242 (1972); G. L. Kovacs, G. Telegdy, K. Lissak, *Psychoneuroendocrinology* **1**, 219 (1976).
11. G. Telegdy and I. Vermes, *Neuroendocrinology* **18**, 16 (1975); G. Telegdy and G. L. Kovacs, in *Brain Mechanisms in Memory and Learning: From the Single Neuron to Man*, M. A. B. Brazier, Ed. (Raven, New York, 1979).
12. Animals were surgically prepared by Hormone Assay, Chicago, Ill., and were allowed either 2 weeks (adrenalectomy) or 3 weeks (hypophysectomy) to recover before testing. Completeness of surgeries was verified by two methods. (i) Trunk blood was collected 15 minutes after ether stress, and plasma was immediately separated and frozen. Corticosterone concentrations were determined by radioimmunoassay and expressed in micrograms per 100 ml of plasma (control, 31.3 ± 2.8; hypophysectomy, 1.2 ± 0.2; adrenalectomy, 1.1 ± 0.3). (ii) Visual inspection of the sella turcica confirmed the absence of pituitary fragments in all hypophysectomized animals; inspection of the retroperitoneal adipose tissue at the cranial pole of each kidney confirmed the absence of adrenal fragments in all adrenalectomized animals.
13. J. C. Buckingham and J. R. Hodges, *J. Endocrinol.* **63**, 213 (1974); J. C. Lissitzky, D. Giraud, B. Conte-Devoux, P. Gillioz, F. Bovelouresque, R. Eskay, C. Oliver, *Neurosci. Lett.* **19**, 191 (1980).
14. L. F. Tseng, H. H. Loh, C. H. Li, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4178 (1976).
15. R. B. Messing and L. D. Lytle, *Pain* **4**, 1 (1977).
16. S. K. Bhattacharya, P. R. Keshary, A. K. Sanyal, *Eur. J. Pharmacol.* **50**, 83 (1978); S. K. Kulkarni, *Life Sci.* **27**, 185 (1980).
17. J. C. Buckingham, *Pharmacol. Rev.* **31**, 253 (1981).
18. A. Gibson, M. Ginsburg, M. Hall, S. L. Hart, *Br. J. Pharmacol.* **65**, 139 (1979); *ibid.* **66**, 164 (1979).
19. We thank J. Rodgers, J. W. Grau, S. Davies, and S. Poage. Supported by NSF grant BNS 78-00508 to S.F.M. and ONR grant N00014-75-C-132 and NIMH grant MH 23861 to J.D.B.

12 June 1981; revised 5 October 1981

Widespread Periodic Intrinsic Connections in the Tree Shrew Visual Cortex

Abstract. *Intrinsic connections within the tree shrew* (Tupaia glis) *visual cortex (area 17) are organized in periodic stripelike patterns within layers I, II, and III. This anatomical network resembles the regularly organized stripes of 2-deoxyglucose accumulation seen after stimulation of alert animals with uniformly oriented lines. Such connections imply that widespread lateral interactions are superimposed on the retinotopic organization of area 17 and suggest alternative interpretations of cortical columns.*

A vertical or columnar organization of neocortex (*1*) has been suggested to underlie analysis of separate aspects of sensation (*2, 3*). In the primate visual cortex, this vertical organization is exemplified by two systems: ocular dominance and orientation specificity (*3*). Physiological recording (*4*) and 2-deoxyglucose (*5*) experiments appear to demonstrate columns, but there are still many questions about their structure and function. In particular, there is no well-defined anatomical basis for a cortical column extending through layers I to VI. Only in the instance of ocular dominance has an anatomical substrate been detected. The segregation of thalamic fibers according to ocularity has been clearly visualized in layer IV of the macaque (*6*), and it is then presumed that this segregation is reflected onto the adjoining layers, thus leading to a column influenced largely by one eye (*3, 6*). No anatomical basis has yet been delineated for the columnar organization of orientation specificity.

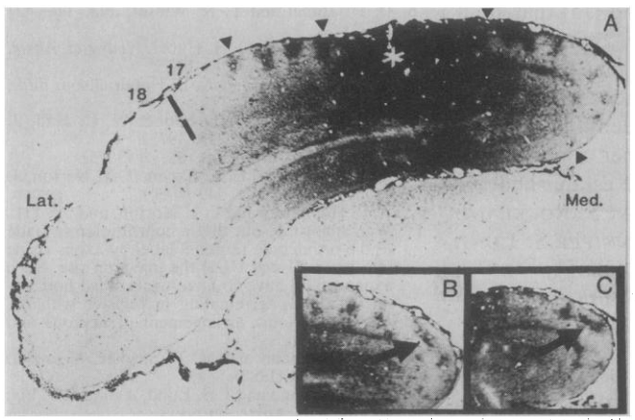
Reports of regularly arranged peaks of cytochrome oxidase or glutamic acid decarboxylase activity within layers II and III suggest even greater complexities in cortical organization (*7, 8*). The metabolically labeled patches appear to coincide with one another and can be related to patterns of ocular dominance and orientation specificity, as shown by accumulations of deoxyglucose. These findings suggest that the supragranular layers (II and III) have an intrinsic periodicity which must be taken into account in discussing the vertical organization of the cortex.

We recently demonstrated that the tree shrew (*Tupaia glis*) has a system of periodic intracortical connections within the supragranular layers which links widespread regions of primary visual cortex. Tree shrews, like monkeys, have a highly developed sense of vision and a complexly laminated visual cortex. Unlike the macaque, there is no physiological or anatomical evidence of ocular dominance domains in tree shrews (*9, 10*). However, deoxyglucose experiments reveal regularly spaced columns of high glucose uptake following visual stimulation of alert animals with lines of uniform orientation (*11*). The labeled columns form parallel slablike arrays that are aligned in a pattern resembling the physiologically mapped bands of orientation-specific neurons (*12*).

To demonstrate these intrinsic cortical connections we injected small volumes of horseradish peroxidase (HRP) (0.02 μl of 20 percent aqueous Boehringer HRP) in the primary visual cortex of seven tree shrews; in most cases the injection did not involve the underlying white matter. Bilateral injections were made in four animals. After 42 to 48 hours, the animals were anesthetized and perfused with mixed aldehydes followed by a sucrose buffer wash. The brain sections (30 μm) were reacted with tetramethyl benzidine (TMB) or diaminobenzidine chromogens. Effective injection sites—areas where dense reaction product obscured both neurons and neuropil—were estimated to be 0.5 to 1.2 mm in diameter.

Figure 1A shows an HRP injection in striate cortex; periodic densities of transported label extend for 2 to 3 mm on all sides of the injection site, and patches can be mapped over a distance of about 8

0036-8075/82/0319-1532$01.00/0



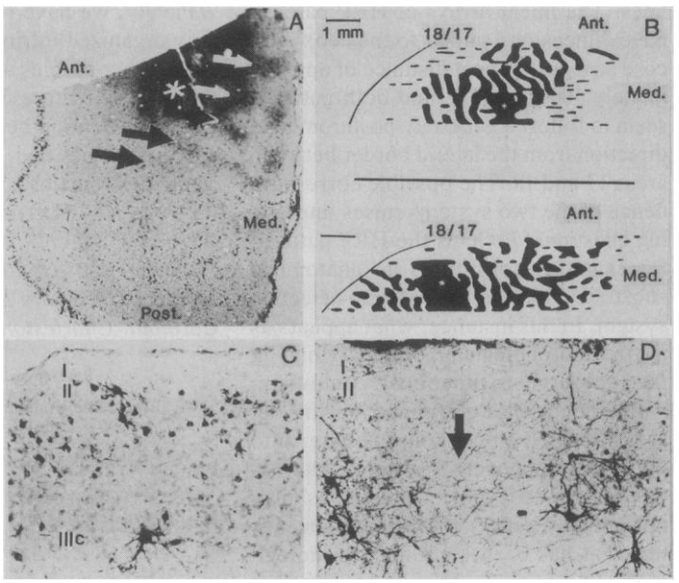


Fig. 1 (left). (A) Coronal section through the striate cortex of a tree shrew shows a large HRP injection (*) and HRP-labeled patches extending both laterally and around the medial surface. Arrowheads delimit groups of four lateral and eight medial patches (×16). (B and C) Consecutive sections through medial striate cortex, where longer segments (arrows) of HRP label are often found; this is the same brain as in (A) (×12). Fig. 2 (right). (A) Tangential section through striate cortex shows five stripelike arrays (arrows) of HRP-labeled intrinsic connections and the injection site (*); the fifth stripe appears faint (×15). (B) Computer reconstructions of two coronally sectioned brains show HRP injection sites (*) and overall pattern of intrinsic connections. (C) HRP-filled neurons in area 17 which project to prestriate cortex (×200). (D) Two HRP-filled patches with interconnecting fibers (arrow) (×200). Abbreviations: *Ant.*, anterior; *Lat.*, lateral; *Med.*, medial; and *Post.*, posterior.

mm^2. In coronal sections, the patches are limited to the supragranular layers and are most intense in layers II and IIIA. They average about 230 μm in cross section and are separated by nonlabeled spaces of slightly smaller dimension, with a center-to-center repeat distance of 450 to 500 μm. The patches were visible in all the injected hemispheres, but the intensity of the label and the area over which patches could be detected varied. They were most visible in cases with good orthograde transport, which was achieved best when two small injections were placed close together and the tissue reacted with TMB.

Patches of HRP activity consist of both orthogradely labeled axon terminations and a small number of retrogradely filled neurons (about six per patch per 30-μm section), many of which could be identified as small pyramidal neurons. Numerous long axons course horizontally in layers II and III from the injection site and between individual patches (Fig. 2D). In contrast, only a few axons can be traced from the deeper layers, suggesting that this system derives largely from intrinsic connections originating from the retrogradely labeled neurons in layers II and III (*13*). A likely correlate of the intrinsically projecting supragranular neurons is found in Golgi-stained preparations, which show small to medium-sized pyramidal neurons, with dendritic spreads of approximately 200 to 250 μm, located very superficially in the cortex. The axons of these neurons have particularly extensive laterally spreading collaterals, which can be traced for more than 1 mm in a single 90-μm section. (Their descending axon trunks have been followed as far as layer V, where they fail to impregnate with silver and presumably become myelinated.)

The overall topography of the intrinsic periodicities is difficult to assess from the small portions (about 8 mm^2) of the network that have been visualized. Several lines of evidence suggest that the periodicities may constitute a stripelike array, running in a generally posteromedial direction from the lateral border between areas 17 and 18. Computer reconstructions of two coronally sectioned brains (aligned by following blood vessels) show that individual patches line up in a stripelike array running anterolaterally to posteromedially (Fig. 2B). In addition, there are occasionally longer segments, where the microtome knife apparently cut obliquely through a stripe (Fig. 1, B and C). Finally, sections cut in a plane tangential to the pia mater show an overall stripelike pattern of label (Fig. 2A), with a suggestion of periodic densities within the stripes. In these tangential sections, there are axons that run obliquely between nonadjacent portions of the stripelike arrays, suggesting that connections occur lengthwise down the stripes as well as between them. Thus, the intrinsic connections seem to achieve considerable lateral interactions across the retinotopic map of striate cortex.

The persistence of regularly arranged labeled and unlabeled bands or patches even close to large injection sites was unexpected with such large injections (up to 2.5 mm^2, a distance which includes at least two labeled and unlabeled patches). Such results imply two distinct intercalated systems. One, illustrated by the HRP label, evidently has wide interconnections, but between the widely connected bands there is an interdigitated system with different and presumably local connections. The occurrence of these periodic, alternating regions of widespread and local connections indicates that there may be an anatomical mosaic within the supragranular layers of the tree shrew and raises the question of whether other connections may be organized in a periodic fashion to fit into this mosaic. Neurons in layers II and IIIA of area 17 (Fig. 2C), for example, send projections to prestriate cortex, as we ascertained by injecting HRP-[^{3}H]proline into prestriate cortex. (A few HRP-filled neurons also occur in layers IIIB and IIIC.) No obvious periodicity of these striate-efferent neurons can be detected. But since both systems occupy the same cortical depth, some overlap between them is likely, and some neurons may give rise to both intrinsic and extrinsic connections. Prestriate to striate projections, however, show terminals concentrated in layer I; that is, there is not significant overlap with the terminals of the intrinsic patch system.

The function of the periodic intrinsic connections is not known; it is interesting, however, that the intrinsic periodicities in the tree shrew striate cortex are reminiscent of the regularly organized stripes or columnar slabs visualized by 2-deoxyglucose following stimulation of alert animals with uniformly oriented

lines. The linear arrays of HRP patches have dimensions similar to the deoxyglucose stripes (a repeat distance of approximately 500 μm) (*11*), and both patterns seem to follow a generally posteromedial direction from the lateral border between areas 17 and 18. The possible correspondence of the two systems raises interesting questions. Perhaps the HRP pattern, for example, constitutes an anatomical substrate for the orientation-selective system. In this instance, what appears as a strict morphological periodicity may in fact subserve a functional continuum, with orientation determined by the degree to which single neurons enter into the widely connected system. Alternatively, the deoxyglucose columns may not actually reflect the orientation system that has been identified physiologically. In particular, can we be sure that during vertical stripe stimulation, the columns correspond to groups of vertical-selective neurons, and the nonlabeled interspaces correspond to horizontally selective neurons? In the same tissue, therefore, stimulation with horizontal stripes should result in an exact mirror reversal of the labeled and nonlabeled spaces. In combined physiological and deoxyglucose experiments in cats, lesions marking the locus of vertical selectivity apparently corresponded to peaks of verticality induced deoxyglucose patterns (*14*), but no direct evidence is yet available to confirm the phase shifting of the deoxyglucose pattern after stimulation with horizontal as opposed to vertical lines. As the HRP pattern in the tree shrew appears to illustrate a fixed anatomical locus, its correspondence with the pattern produced by deoxyglucose orientation suggests that the deoxyglucose pattern is also fixed and thus might not be directly related to the orientation aspect of the stimulus.

How literally should we take the image of a cortical column as a functional entity, designed to segregate out different submodalities? We might rather entertain the view of multiple repetitive structures intrinsic to the cortex, which may be adapted to fulfill a variety of functions. It may be useful to consider the vertical radial component of the cortex as enmeshed in an elaborate horizontal laminar organization, possibly in a multiple lattice-like design. Although the influence of each lattice could be translated through the cortical depth, this concept stresses the primacy of a particular lamina in elaborating a given function and underlines the importance of dynamic interconnections throughout the horizontal as well as the vertical extent of the cortex.

Finally, we have found (*15*) that similarly organized intrinsic connections occur in other species and may thus constitute a basic feature of neocortical structure. The relation between this anatomical framework and other reported periodicities remains to be established.

KATHLEEN S. ROCKLAND
JENNIFER S. LUND
Department of Ophthalmology, Medical University of South Carolina, Charleston 29425

References and Notes

1. R. Lorenté de No, in *Physiology of the Nervous System*, J. F. Fulton, Ed. (Oxford Univ. Press, New York, 1938), p. 291.
2. V. B. Mountcastle, *J. Neurophysiol.* **20**, 408 (1957).
3. D. H. Hubel and T. N. Wiesel, *Proc. R. Soc. London Ser. B.* **198**, 1 (1977).
4. ———, *J. Physiol. (London)* **195**, 215 (1968).
5. ———, M. P. Stryker, *J. Comp. Neurol.* **177**, 361 (1978).
6. D. H. Hubel and T. N. Wiesel, *ibid.* **146**, 421 (1972).
7. J. C. Horton and D. H. Hubel, *Neurosci. Abstr.* **6**, 315 (1980).
8. A. L. Humphrey and A. E. Hendrickson, *ibid.*, p. 315.
9. J. K. Harting, I. T. Diamond, W. C. Hall, *J. Comp. Neurol.* **150**, 393 (1973).
10. D. H. Hubel, *Brain Res.* **96**, 41 (1975).
11. A. L. Humphrey, L. C. Skeen, T. T. Norton, *J. Comp. Neurol.* **192**, 549 (1980).
12. A. L. Humphrey and T. T. Norton, *ibid.*, p. 531.
13. We cannot rule out that a contribution is made to the orthograde terminal label by axons rising into layers II and III at the injection site. Such axons would have to have widespread horizontal collaterals terminating in register with the intrinsic periodic arrangement of neurons and their collaterals.
14. A. Schoppmann and M. P. Stryker, *Neurosci. Abstr.* **6**, 314 (1980).
15. K. S. Rockland and J. S. Lund, *Assoc. Res. Vis. Ophthalmol. Abstr.*, in press.
16. We thank I. T. Diamond for kindly supplying some of the tree shrews used in this study and G. G. Blasdel and R. D. Lund for helpful criticism. This work was supported by NIH grant EY03321 and a postdoctoral fellowship to K.S.R. awarded from South Carolina State appropriations.

8 July 1981; revised 8 October 1981.

Behaviorally Conditioned Immunosuppression and Murine Systemic Lupus Erythematosus

Abstract. *Development of autoimmune disease in female New Zealand hybrid mice was dramatically modified by classical conditioning of immunosuppression. Groups of animals received each week a solution of sodium saccharin (conditioned stimulus). One group of conditioned animals received an injection of cyclophosphamide (the unconditioned stimulus) after half of the weekly occasions when they received the saccharin solution. The rate of development of proteinuria and mortality were significantly retarded in these conditioned mice relative to untreated controls and nonconditioned animals that received unpaired treatment with saccharin and cyclophosphamide.*

Several converging lines of evidence implicate the central nervous system in the regulation of immune processes (*1*). Of relevance here is that behavioral conditioning procedures can be used to suppress humoral and cell-mediated immune responses (*2*). Conditioned immunosuppression is accomplished by pairing consumption of a novel drinking solution—the conditioned stimulus (CS)—with an immunosuppressive drug, for example, cyclophosphamide—the unconditioned stimulus (US). When subsequently treated with antigen, conditioned animals that are reexposed to the CS show attenuated immune responses. The present study was designed to examine the impact of conditioned immunosuppression on the development of systemic lupus erythematosus (SLE) in New Zealand mice, an autoimmune disease for which the female (NZB × NZW)F_1 (NZF_1) mouse has become a standard experimental model (*3*). Treatment of NZF_1 mice with cyclophosphamide prolongs survival of animals that would otherwise develop a lethal glomerulonephritis between approximately 8 and 14 months of age (*4*). On the basis of observations that immune responses can be suppressed by conditioning procedures, we hypothesized that, in conditioned mice, the substitution of conditioned stimuli (placebo treatment) for the immunosuppressive drug would delay the development of proteinuria and mortality relative to nonconditioned animals treated with the same dose of drug.

Individually caged female NZF_1 mice were maintained under a 12-hour light-dark cycle and given free access to food and water. A chemotherapeutic regimen was initiated when the animals were 4 months of age. Once each week for 8 weeks all the animals were administered a 0.15 percent solution of sodium saccharin (SAC) by pipette (up to 1.0 ml). Cyclophosphamide (*5*) was injected according to the following schedule.

The standard treatment group (C100) received, once weekly, an intraperitoneal injection of cyclophosphamide (30 mg/kg) immediately after they received the SAC solution. These stimuli were presented at the same time on the same day of each week. Results derived from this

0036-8075/82/0319-1534$01.00/0