Mutants have been described previously that are deficient in messenger activity, notably hemoglobin mRNA in some thalassemias [for discussion, see (*13*)]. In some cases the deficiency reflects a deletion of the structural gene (*14*), in another case mRNA is made but no protein is synthesized (*15*), and in still other cases the cause is unknown. *Gur* represents the first eukaryotic locus whose normal function is identified as the regulation of a specific mRNA. As such, it offers an approach to the study of regulatory mechanisms fundamental to gene induction by steroid hormones.

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Thalamic Projections to Layer I of Striate Cortex Shown by Retrograde Transport of Horseradish Peroxidase

Abstract. *The diffusion of horseradish peroxidase was restricted to layers I and II of the striate cortex in* Tupaia glis *and* Galago senegalensis. *In the lateral geniculate body of* Tupaia *labeled cells were found only in layer 3; some labeled cells were also found in the lateral nucleus. In* Galago *labeled cells were found only in layers 4 and 5 of the lateral geniculate body; a band of cells was also found in the pulvinar nucleus. These results support the distinction between two overlapping thalamic systems, a layer I and a layer IV system.*

That some projections from the thalamus may reach layer I of the cortex in mammals has been known for some time and it comes as no surprise that Cajal noted some fibers, presumably of thalamic origin, ascending to the cortical surface (*1*). Lorente de No advanced the inquiry by defining several characteristics that distinguish the projections to layer I from the projections to layer IV. He called the layer I fibers "nonspecific," mainly because they appeared to give off collaterals into different cortical subdivisions (*2*).

Thalamic projections to layer I were not demonstrated experimentally until Nauta had developed a method for tracing degenerating axons and their terminals (*3*). Chiefly by the use of this method it was concluded that there are two overlapping but distinct thalamic systems: the specific projection system, which includes the main sensory relay nuclei and their projections mainly to layer IV, and the nonspecific system, which includes the intralaminar nuclei and their projections mainly to layer I (*4*).

In previous studies we have used the method of anterograde degeneration to trace projections from the various laminae of the lateral geniculate body (GL) (*5*) to the striate cortex in two species, the tree shrew (*Tupaia glis*) and the prosimian bush baby (*Galago senegalensis*) (*6, 7*). After large lesions of all six geniculate layers, cortical layer IV was black with degenerated axons and terminals but a few degenerating axons could also be seen ascending to layer I. The importance of trying to complement the anterograde method with that of retrograde transport of horseradish peroxidase (HRP) is that the cells in the thalamus which project to layer I can be identified.

The results presented here show that only one layer of GL in *Tupaia* and only two layers of GL in *Galago* project to layer I of striate cortex. These findings may contribute to our understanding not only of the relationship between the so-called specific and nonspecific systems, but also of the lamination of the GL.

The special feature of our method was that we did not inject HRP deeply into the cortex. Instead, the pia mater was pricked a number of times with a small glass pipette (less that 1 μm in diameter at the tip) and crystals of HRP (Boehringer Mannheim) were laid on the moist pial surface and allowed to dissolve in the cerebral spinal fluid for 10 to 30 minutes. Optimal results were obtained with the addition of 1 to 2 μl of 5 percent dimethyl sulfoxide into the HRP solution. The animals survived this procedure for 48 hours, when they were perfused and the brains prepared for histological study (*8*).

The effect of applying HRP to the striate cortex in five tree shrews is illustrated by two experiments shown in Fig. 1. The sites of application are shown on a 45° view of the cortex (Fig. 1a); a photomicrograph of one of the two sites is shown in Fig. 1b. In these experiments labeled cells were found concentrated in a restricted part of layer 3 of GL. A number of labeled cells were also found in nuclei medial to GL. Most of these cells were in the lateral nucleus (Li); a few, however, were found scattered among the fibers that pass between the borders of the pulvinar and the lateral nuclei.

The effect of applying HRP to the surface of the striate cortex in five bush babies is illustrated by one experiment shown in Fig. 2. The locus of the application of HRP to layer I is shown in a diagrammatic 45° view of the cerebral hemisphere (Fig. 2a). A photomicrograph of a parasagittal section through the lateral geniculate body (Fig. 2b) shows the labeled cells confined to layers 4 and 5 (*9*). The pattern of labeled cells in the pulvinar nucleus is shown in a micrograph (Fig. 2d) and drawing of a sagittal section (Fig. 2c). The labeled cells form two limbs of an arc, the upper limb in the superior division of the pulvinar nucleus (Pul S) and the lower in the inferior division (Pul I). The orientation of most cells is especially clear because the HRP entered dendritic processes; Fig. 2d shows that the cell orientation is aligned with the tangent to the curved band.

These results complement our earlier studies of the cortical projections of the various geniculate layers in *Tupaia* and *Galago*. In the tree shrew experiments, anterograde degeneration was traced from GL layers 1 and 2 to the superficial

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tier of cortical layer IV; and from GL layers 5 and 6 to the deep tier of cortical layer IV, and to a lesser extent to cortical layer IIIb (*6*). No lesion was restricted to GL layer 3, but after a large lesion involving all six layers, an occasional degenerating axon was seen to ascend to the cortical surface. The present evidence suggests that these fibers originate exclusively in GL layer 3. This result cannot be attributed to a diffusion of HRP into cortical layers III and IV, since we would then expect to find some labeled cells in GL layers 1, 2, 5, and 6. Indeed a few faintly labeled cells in layers other than layer 3 were seen when HRP encroached on layers III and IV. Given the evidence that the cells of layer 3 project to cortical layer I, we conclude that the method of tracing anterograde degeneration was not a sensitive way to detect these fibers.

The earlier bush baby experiments showed that GL layers 1 and 2, the magnocellular layers, project to the superficial tier of cortical layer IV (*7*). In lesions restricted to layers 3, 4, and 5, terminal degeneration was identified in the deeper tier of cortical layer IV. Putting together the results of the present and the earlier studies, we conclude that layers 4 and 5 of GL send projections both to cortical layer I and to the deeper tier of layer IV (*10*).

Before these experiments we knew that cells in the pulvinar nucleus of *Galago* project to the striate cortex, but the cortical layer in which the projections terminated had not been determined (*11*). Also, we did not realize that all or most of the cells in a narrow and long curved band project to one restricted area of the striate cortex.

The pulvinar nucleus in the tree shrew does not project to the striate cortex, but the adjacent lateral nucleus projects to the striate cortex as well as to the adjacent extrastriate belt and the temporal lobe (*12*). Thus the projections of the lateral nucleus are extensive and overlap with those of both the lateral geniculate and the pulvinar nuclei; as judged from its extensive projections the lateral nucleus could qualify as a part of the intralaminar group. Whether or not the pulvinar nucleus in *Galago* can also be considered as part of the intralaminar group will be discussed below.

The chief significance of the results reported here is the bearing they may have on the larger issue of the functional significance of the "layer I" and "layer IV" systems. In trying to understand the difference between the two systems the first step is to classify thalamic nuclei as belonging to one or the other system. But the main sensory nuclei appear to contribute fibers to layer I as well as layer IV and the intralaminar nuclei project to layers IV and VI as well as to layer I (*4*).

Finding that only certain layers of GL

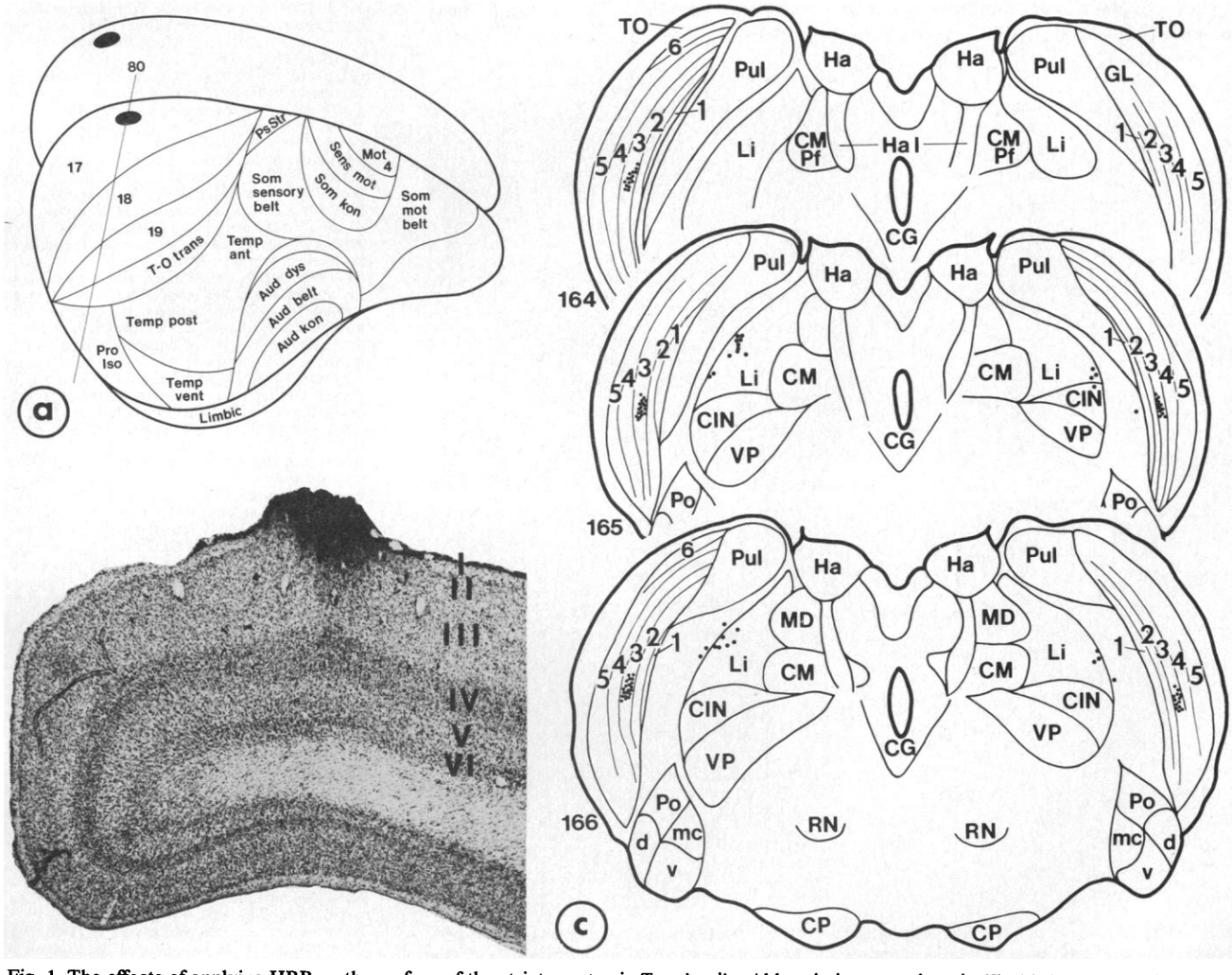

Fig. 1. The effects of applying HRP on the surface of the striate cortex in *Tupaia glis*. Abbreviations are given in (*5*). (a) A standard view of the cerebral hemispheres in which the loci of the two sites of application are depicted in black. The left hemisphere is visible in this 45° view of the hemispheres. (b) A photomicrograph of striate cortex from section 80 which shows the application site on the right hemisphere. (c) Three drawings through the thalamus in which every labeled cell is designated by a dot. All of the labeled cells in GL are in layer 3. Labeled cells are also found in the lateral nucleus (Li) and in the fibers lying between GL and Li.

and, in particular, the layers with the smallest cells, project to layer I sharpens the separation between the two systems and supports the idea that the layer I system may reflect an earlier stage in phyletic history and may even be functionally more primitive. The cells in GL layer 3 of *Tupaia* are distinctly paler and smaller; further, layer 3 was singled out by Glickstein on the grounds that the terminal degeneration from the optic tract was not as intense in this layer (*13*). He even wondered whether the optic tract projects to layer 3 at all; there is now a consensus that it does receive projections from the optic tract but that this projection is sparser (*14*). Finally, the exceptional nature of layer 3 was recently uncovered in quite a different way. When Casagrande *et al.* sutured closed one eyelid of infant tree shrews, three of the four contralateral layers underwent a shrinking of cells (*15*). Layer 3 was the exception. This finding is probably explained by the fact that layer 3 projects to cortical layer I and therefore is not in competition with the ipsilateral layers for cortical sites. Thus the present results support the idea that it is competition for sites in the cortex that produces the cellular changes in GL.

In *Galago*, the cells of layers 4 and 5 are strikingly smaller and paler than the cells of layers 3 and 6, which in turn are smaller than the cells of layers 1 and 2 (*16*). Perhaps layers 4 and 5 are the exclusive targets of W cells which project also to the tectum. In any case, GL layers 4 and 5 may be closer to the tectal pathway both in function and phyletic history.

We cannot divide the cells of the pulvinar nucleus in *Galago* as we divided the cells of GL into those which project to layer I and those which project to layer IV. Quite the contrary, it seems likely as judged from the density of the labeled cells after HRP applications to striate cortex that the fiber which projects to layer I of striate cortex is a collateral of an axon which terminates in the temporal lobe. If such an organization proves to be true, the layer IV and layer I projections may still be segregated, not in terms of the neuron of origin but in terms of the destination of the collateral. In any case, it seems certain that only in the striate cortex where the pulvinar and GL projections overlap are the pulvinar fibers restricted to layer I. Whether this organization provides a model for the intralaminar nuclei—indeed whether the primate pulvinar can be regarded as a descendant of the nuclei of the intralaminar class—remains to be seen.

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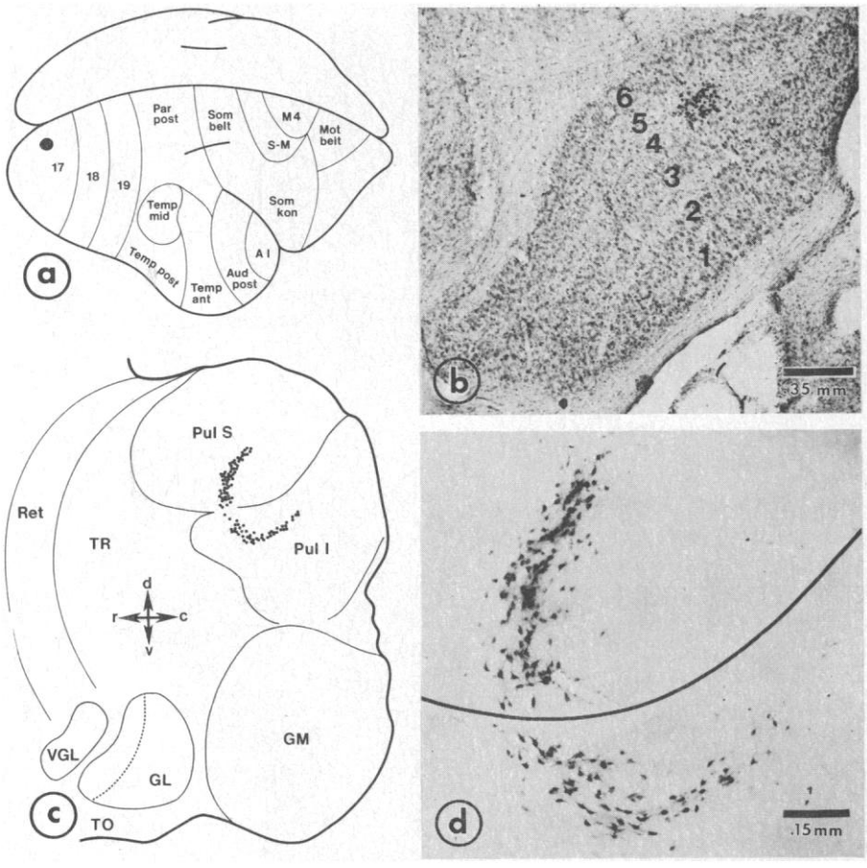

Fig. 2. The effects of applying HRP on the surface of the striate cortex in *Galago senegalensis*. Abbreviations are given in (*5*). (a) A standard 45° view of the cerebral hemispheres, with the locus of the application site depicted in black. (b) A photomicrograph of a sagittal section through the lateral geniculate body. The labeled cells that now appear black can be seen restricted to layers 4 and 5. (c) Drawing of a sagittal section through the pulvinar nucleus in which each labeled cell is depicted by a dot. The direction arrows refer to dorsal, ventral, rostral, and caudal. (d) Photomicrograph of the labeled cells drawn in (c).

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5. Abbreviations are as follows: AI, auditory koniocortex; Aud, auditory; Aud dys, auditory dysgranular area; CG, central gray; CIN, central intralaminar nucleus; CM, center median; CP, cerebral peduncles; d, dorsal division of the medial geniculate body; GL, lateral geniculate body; GM, medial geniculate body; Ha, habenula; Ha I, habenulainterpeduncular tract; Kon, koniocortex; Li, lateral nucleus, intermediate division; mc, magnocellular division of the medial geniculate body; MD, medial dorsal nucleus; M4 and Mot 4, motor area 4 of Brodmann; Par, parietal area; Pf, parafasicular complex; Po, posterior nucleus; Pro Iso, proisocortex; PsStr, pseudostriate; Pul, pulvinar nucleus; Pul I, inferior division of the pulvinar nucleus; Pul S, superior division of the pulvinar nucleus; Ret, reticular nucleus; RN, red nucleus; S-M and Sens mot, sensory motor belt; Som, somatic; Temp, temporal cortical field; TO, optic tract; TR, thalamic radiations; v, ventral division of the medial geniculate body; VGL, ventral lateral geniculate body; and VP, ventral posterior nucleus.
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chem. Cytochem. **24**, 1273 (1976); *ibid.* **26**, 106 (1978)].
9. The present experiments were not the first to suggest that the cortical projections of geniculate layers 4 and 5 were "eccentric." In an earlier study, HRP was injected into layers III, IV, and V of the striate cortex; labeled cells were found in a column through all six layers of the GL [D. Raczkowski and I. T. Diamond, *Brain Res.* **144**, 383 (1978)]. But the width of the column was markedly less in layers 4 and 5. This earlier finding probably reflects the fact that the HRP entered the projections from GL layers 4 and 5 before these projections had arborized or spread to the limits they eventually attain in cortical layer I.
10. There is also evidence from anterograde degeneration studies in the monkey that the parvocellular layers of GL project to cortical layer IV with a much sparser projection to cortical layer I [D. H. Hubel and T. N. Wiesel, *J. Comp. Neurol.* **146**, 421 (1972); J. S. Lund, *ibid.* **147**, 455 (1973)]. We cannot tell from the available results whether the projections to layer I arise from one or more than one of the layers of GL; perhaps the projections to layer I originate from the smallest cells in all of the parvocellular layers. In any case our experience suggests that the intensity of the projection to layer I has probably been underestimated.
11. While we had no evidence that layer I of the striate cortex was the target of the pulvinar projections in *Galago*, the method of anterograde transport shows such a projection in the monkey [M. P. Ogren and A. E. Hendrickson, *Brain Res.* **137**, 343 (1977)].
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Sex Pheromone in the Dog

Abstract. *Methyl* p*-hydroxybenzoate has been identified in the vaginal secretions of female dogs in estrus. When small amounts of this compound were applied to the vulvas of anestrous or spayed females, males placed with these females became sexually aroused and attempted to mount them.*

It is commonly assumed that female dogs give off a special odor or odors when they go into heat. This odor apparently attracts male dogs to the vicinity of the female and initiates courtship. While there is considerable ethological and veterinary data on *Canid* sex behavior and its hormonal regulation, the strict specification of the odor cues given off by a female dog during proestrus and estrus has not been achieved (*1*). In fact the source (or sources) of the odorants involved in male attraction and excitation has not been well defined. It is not clear from the work of others whether the odors derive from the vagina, the urine, both, or from some other less likely anatomical region. The purpose of this report is to describe studies indicating that the odor of a specific compound found in the vaginas of female dogs during proestrus and estrus has the capacity to incite male dogs to sexually approach and attempt to mount totally anestrous females whose vulvas have been treated with synthetic pheromone. This behavior persists despite resistance on the part of the females.

The studies were conducted as follows. Three unspayed 3-year-old sexually experienced and multiparous beagles were monitored cytologically and behaviorally as they passed through their estrous cycles. Two dogs went into heat spontaneously. Another was hormonally induced with two 1-mg injections of estradiol cypionate (given intramuscularly in oil vehicle) spaced 48 hours apart. Two vaginal smears were taken daily from each dog, as well as a urine sample. The vaginal cells were collected with sterile cotton applicators that had been defatted with chloroform and methanol. After some of the vaginal material had been applied to a microscope slide, the cotton tip of the applicator was sealed in a small vial and kept frozen until it was used as a source of vaginal odorant during chromatographic analyses. On a daily basis, each female was paired with an adult male dog, and a record was kept of the behavioral activity during a 15-minute interaction with the male. In this way, it was possible to accumulate a chronological record of changes in vaginal cytology associated with estrus and correlate these with changes in frequency of specific behavioral acts exhibited by both the males and females as the females passed from anestrus to proestrus through estrus and into metestrus. At the same time gas chromatographic and mass spectrometric (GC-MS) analyses could be performed on the odors generated by the daily samples of vaginal secretion and urine collected from each female. Thus, we accumulated cytological, behavioral, and GC-MS data on each of the three dogs at all four stages of their estrous cycles. Since each dog went into proestrus at different times, the cycles of the three dogs were staggered over a total period of about 55 days. Vaginal smears, urine, and behavioral data were collected on all three dogs over the entire 55-day period.

Smears were obtained by gently inserting a defatted, sterile, saline-soaked cotton applicator through a glass speculum into the vagina. The use of the speculum allowed us to get a deep vaginal smear and to avoid contamination of the smear by the cellular debris that normally collects along the labia. Only cervical smears can be histologically interpreted with accuracy (*2*). The two swabs were rolled over a microscope slide and then placed in solvent-cleaned vials and frozen until the time of analysis. The slides were stained with Wright's stain by an automated stainer. They were examined under low and high power with a light microscope. Both the hormonally induced and naturally cycling dogs exhibited the classical pattern of cellular changes in vaginal smears associated with estrus. The smears of the two naturally cycling females remained highly cornified for about 8 days, once full cornification was observed. The smears of the hormonally induced female remained highly cornified for at least 17 days. This female remained behaviorally receptive for a concomitantly longer period of time.

The three females were tested with a male in outdoor runs (0.85 by 4 m). These were constructed of 2-m cyclone fence with smooth concrete floors and a metal sunroof. A set of eight fully adult male dogs varying in breed, size, age, and prior sexual experience were paired in a systematically rotating fashion with the females during the study. Three were purebred beagles, four were the product of beagle-spitz crosses, and one was a large German shepherd. Most tests were run between 8:00 a.m. and 3:00 p.m.

Each trial was conducted as follows. A female was placed randomly in one of seven clean runs. Almost invariably the female would urinate within 5 minutes of entry. One milliliter or more of urine was collected immediately with a pipette. If the female failed to urinate within 10 minutes, the test was initiated anyway. One of the eight males was brought to the testing area and introduced into the run containing the female. A timer was started and a written record of the male-female interaction was made for both the male and female; a standardized score sheet was used.

For the purposes of this report, a general summary of the pattern of behavioral changes observed is more appropriate than a detailed specification of the frequency data for each female and male in our study.

As a female passed from anestrus to proestrus, the frequency of her urination within the run during the interaction with the male rose sharply. Urination by the males also increased, as did ano-genital

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