lar effects were observed after the administration of intravenous doses of Δ^1THC at 2 mg/kg.

Hydrolysis in vitro with microsomal preparations showed that compound **2** is quickly hydrolyzed to Δ^1THC; K_m (Michaelis constant), $6.3 \times 10^{-4} M$; V_{max}, 3.8×10^{-8} mole/min per milligram of protein (*10*).

We had synthesized (*11*) another class of water-soluble esters of THC's (that is, the diethylaminobutyric ester) (*12*), which produced ataxia in unanesthetized dogs, similar to Δ^1THC, except that the onset of action was considerably delayed and the effective dosage was five to ten times higher. In compound **2** these drawbacks have been eliminated, and it has a pharmacological profile similar to that of Δ^1THC. It appears that the principal activity of compound **2** is due to hydrolysis in vivo to Δ^1THC.

Our method of producing a water-soluble ester derivative of Δ^1THC has also been applied to other compounds.

B. A. ZITKO, J. F. HOWES
R. K. RAZDAN*
B. C. DALZELL, H. C. DALZELL
JOHN C. SHEEHAN
H. G. PARS
Sheehan Institute and
Sharps Associates,
767-B Concord Avenue,
Cambridge, Massachusetts 02138
W. L. DEWEY, L. S. HARRIS
University of North Carolina,
Chapel Hill 27514

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10. Mice liver microsomes were prepared by homogenization of whole livers with isotonic potassium chloride followed by centrifugation at 9000*g* [J. F. Howes and W. H. Hunter, *J. Pharm. Pharmacol.* **20**, 107 (1968)]. The supernatant fraction was used, and the incubations were carried out at 37°C for various periods of time. The Δ^1THC formed during hydrolysis was isolated and quantitated by gas liquid chromatography (column, 1.8 m by 0.3 cm; 2 percent OV-17 on 100 to 200 mesh gas chrom Q at 240°C; Varian 4000; flame ionization detector). Kinetic data were calculated by the Lineweaver-Burk equation.
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* Address correspondence to R.K.R.

27 March 1972

Superior Colliculus of the Tree Shrew: A Structural and Functional Subdivision into Superficial and Deep Layers

Abstract. *Superficial lesions of the superior colliculus produced deficits in form discrimination, while deeper lesions produced, in addition, an inability to track objects. These two syndromes were related to an anatomical subdivision: Superficial lesions resulted in anterograde degeneration in the visual thalamus, whereas lesions confined to the deeper layers produced degeneration in the nonvisual thalamus and in brainstem motor areas.*

The tectum appears to be the dominant visual center in nonmammals, but in mammals it is overshadowed by the geniculostriate system. These phylogenetic facts, along with data from the neurological clinic, led to the traditional view that the tectum serves only reflex functions, primarily in the coordination of head and eye movements (*1*). Sherrington's ideas provided an underlying support for this traditional view of the superior colliculus since, according to Sherrington, essentially all the central nervous system except for the cerebral cortex was involved in reflex functions (*2*). However, more recent studies, in which the ablation technique was used, suggest a more complex role for the tectum (*3–5*).

Our own interest in the tectum grew out of a comparative inquiry into the mammalian striate cortex, which revealed a surprising degree of preservation of form vision after removal of area 17 (*6*). These results turned our attention to the extrastriate visual system: the tectum and its cortical target, which is reached by a relay in the pulvinar (*7*). As a result of our comparative inquiry, we realized that it is an oversimplification to say that the tectum is progressively overshadowed by the geniculostriate system. Even among closely related species the size of the superior colliculus can vary considerably, in correlation with different ecological requirements.

In this report we provide evidence that the large and well-developed superior colliculus of the tree shrew (*Tupaia glis*) plays an important part in pattern vision: In particular, no animal with bilateral ablation of the superior colliculus learned to discriminate between an inverted and an upright triangle. In contrast, this task is easy for tree shrews after total removal of area 17 (*6*). A second finding was unrelated to the original intent of the experiment. We found that after shallow lesions of the superior colliculus the tree shrews exhibited normal cage behavior, while after deep lesions the tree shrews sat motionless in their home cages and appeared to be blind; they did not even withdraw from a threatening gesture. This difference in syndromes might be trivial if the milder one was simply the result of an incomplete lesion. However, we will also offer anatomical evidence which suggests that there are two structural subdivisions of the superior colliculus as defined by their connections: a superficial one and a deeper one.

In all, we have complete behavioral data for eight tree shrews and histological data for four of these cases. The remaining four are alive and are subjects of experiments in progress. There is every reason to believe that the completed cases are representative, so we will devote the remainder of this report to presenting the results for these cases.

In all four cases the superior colliculus was removed in anesthetized neonates by aspiration; aseptic techniques were used. After a suitable period of maturation a striking behavioral difference appeared, dividing the four cases into two classes. In two of the four animals visually guided behavior, as revealed by tests in the home cage, such as tracking of a food reward, appeared normal. The other two usually remained motionless in their home cage, and this absence of behavioral response was especially striking when the animals were provoked by a threatening gesture. In

keeping with this appearance of blindness they failed to orient to moving or stationary objects. The four animals were tested for their capacity to locate and track a visual stimulus by introducing a piece of food, held on the end of a pair of forceps, and moving the morsel through the visual field. These procedures have become fairly standard practice in our laboratory as well as in several other laboratories (*4*), since they provide a means by which we can identify any abnormal behavior by comparing the performance of normal and brain damaged animals.

All four animals were also given objective tests of pattern discrimination in a conventional two-choice apparatus (*6*). We used simple tests: first, orientation of stripes, and second, orientation of triangles. When the animals selected horizontal stripes and upright triangles 19 out of 20 times in two consecutive ten-trial sessions, we considered that they had mastered the tasks. None of the four animals, even the two which showed apparently normal cage behavior, could master the triangle task to criterion within 80 sessions (see Fig. 1). This failure cannot be attributed to some general dementia since all animals easily learned to distinguish horizontal from vertical stripes. We place great weight on the fact that the two with the smaller lesion failed on the triangle task. First, if these animals had mastered this task, the most parsimonious explanation of the results would be that the two smaller lesions spared portions of a single structural unit. A second reason for our interest in their failure to distinguish the orientation of triangles is that, by contrast, tree shrews with complete removal of the striate cortex are capable of making this discrimination (see Fig. 1).

After behavioral testing was completed one eye was removed and the four animals were permitted to survive an additional 4 days. They were then given a lethal dose of anesthetic, and the brains were removed and sectioned. The tectal lesions were reconstructed, and the optic tract degeneration was traced by the Fink-Heimer method (*8*).

The main histological result was that the two smaller lesions were limited to superficial laminae (mainly layers 1 and 2), whereas the two deep lesions removed nearly all of the superior colliculus and invaded, to some extent, the pretectum and the central gray. This histological examination led us to consider the possibility that the behavioral results might be explained if the superior colliculus consists of two divisions, an upper one concerned with visual perception and a lower one responsible for organizing appropriate movements of head and eyes in locating and tracking objects. However, a number of anatomical questions are raised by this hypothesis. For example, if the deep layers of the superior colliculus

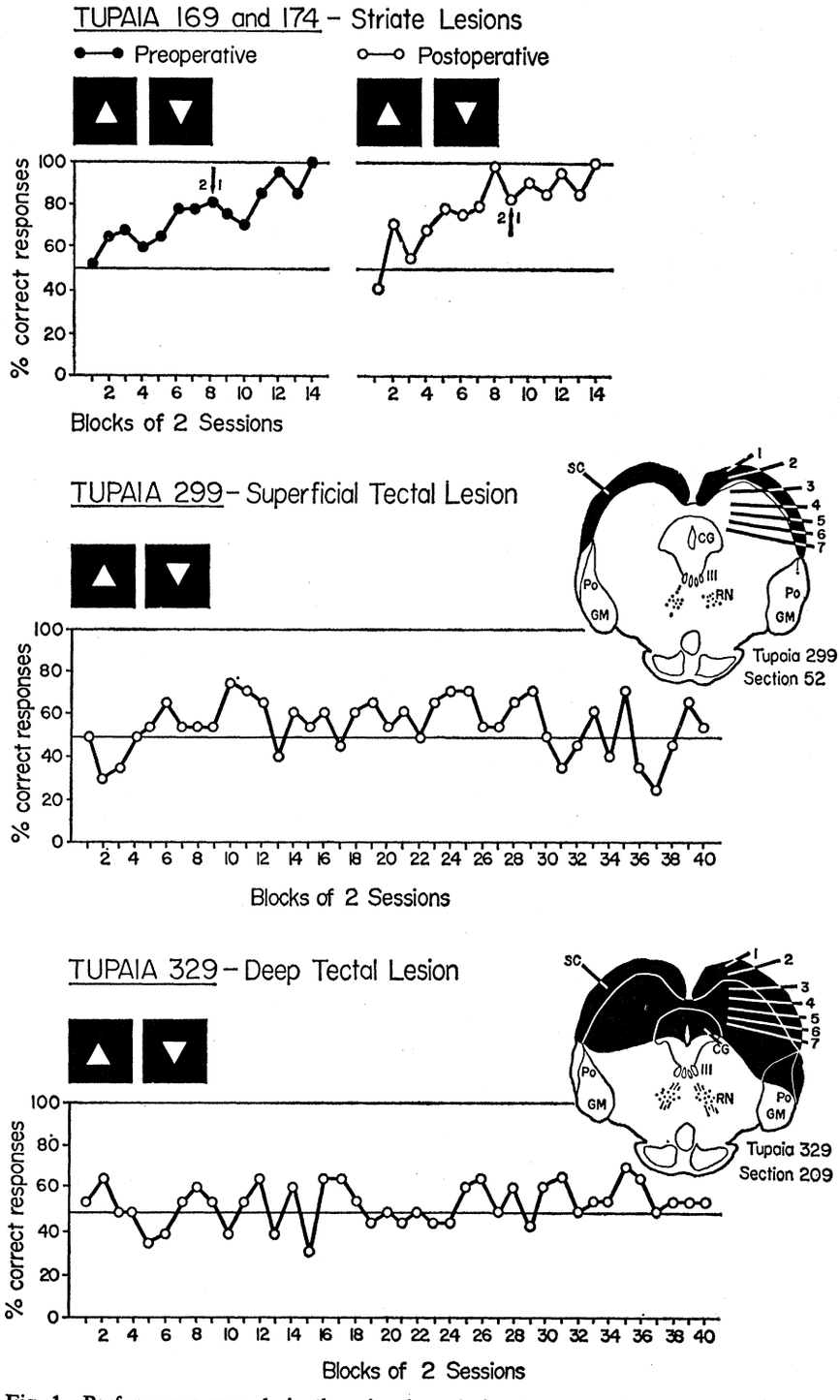
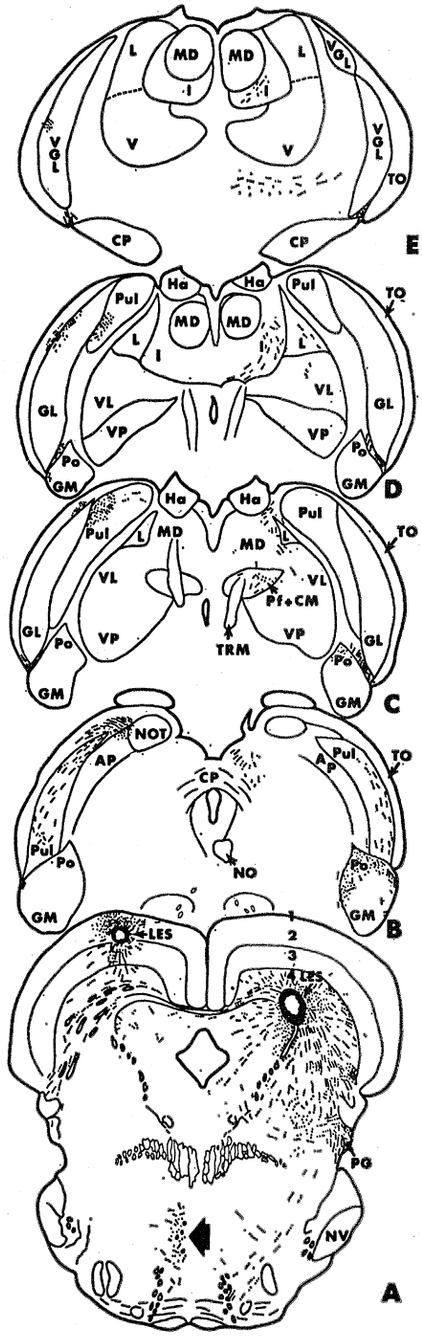


Fig. 1. Performance records in the triangle task for four tree shrews, two before and after removal of the striate cortex (the records are averaged and arrows indicate where one animal completed training) and two following damage to the superior colliculus. Lesions of the superior colliculus are indicated by representative sections. The numbers 1 to 7 refer to the layers of the superior colliculus. Posterior nuclear group (*Po*); central gray (*CG*); red nucleus (*RN*); medial geniculate nucleus, principal divisions (*GM*); and superior colliculus (*SC*).

are entirely dependent on the superficial layers for their visual input (*9*), then we can wonder why the removal of the deep layers adds to the deficit in behavior dependent on visual guidance. The answer to this must be that the deeper laminae of the superior colliculus receive visual input from other sources. One possible additional source was suggested by an earlier finding (*10*) that recovery of the ability to orient and track following superficial lesions in neonatal hamsters might be attributed to a change in the usual pathway from the retina to the superior colliculus. However, we did not observe any aberrant retinal input to deep layers after lesions confined to the superficial layers. This failure to find terminal degeneration in the deep layers of the superior colliculus after removal of the eye suggests that there must be other pathways by which visual information can reach the deep layers. A number of sources come to mind, such as the pretectum, accessory optic nuclei, ventral lateral geniculate nucleus, reticular formation, extrastriate visual cortex, and, finally, whatever remains of layer 3 of the superior colliculus after removal of the two superficial laminae.

While several anatomical questions, therefore, still remain unanswered, support for the view of a functional subdivision of the superior colliculus was provided by a second experiment in which we traced the efferent projections of different laminae of the superior colliculus. For this purpose stereotaxic lesions were carefully placed in individual laminae. The resulting anterograde degeneration was traced by the Fink-Heimer technique after survival periods of 3 days to 2 weeks. The most significant findings are illustrated in Fig. 2. The superficial layers (stratum zonale, stratum griseum superficiale, and stratum opticum), which receive input from the retina and striate cortex (*9*), project mainly to the pulvinar, the pretectum, and the dorsal and ventral lateral geniculate. The significance of this distribution of terminal degeneration in diencephalic targets is that the nuclei are just those which are usually regarded as visual centers. In contrast, the deeper laminae project to dorsal thalamic areas which are not considered to be visual centers, including the posterior nuclear group and certain intralaminar cell groups. In addition, following lesions in the deeper layers, dense degeneration was seen in zones of the subthalamus and in motor areas of the brainstem, which in other forms have been shown to give rise to reticulospinal pathways (*11*). Thus, we conclude on both anatomical and behavioral grounds that the superior colliculus of the tree shrew consists of two divisions.



TUPAIA 266

Fig. 2. A series of frontal sections through the mesencephalon and diencephalon of tupaia 266 with a lesion (*LES*) limited to the stratum griseum superficiale (left) and the stratum griseum and album profundum (right). Axonal degeneration is represented in two ways: fibers of passage are shown by aligned dots and terminal degeneration by scattered dots. The deep lesion produced axonal degeneration within the crossed predorsal bundle (large, closed black arrow, A) and the ipsilateral tectobulbar pathway, both of which project to motor areas of the brainstem reticular formation. Additional terminal degeneration was present within the ipsilateral parabigeminal nucleus (*PG*), the posterior nuclear group (*Po*), the intralaminar nuclei (*I*), the centromedian-parafascicular complex (*Pf* + *CM*), and within the subthalamus. Some sparse fiber degeneration also was present within the ipsilateral pulvinar (*Pul*). In contrast, the superficial lesion produced terminal degeneration within the pretectum (*AP*), the dorsal (*GL*) and ventral (*VGL*) lateral geniculate nuclei, and the pulvinar. Layers of the superior colliculus are labeled after Tigges and Shantha (*15*). Cerebral peduncle (*CP*); medial geniculate nucleus, principle division (*GM*); habenular nucleus (*Ha*); lateral nucleus (*L*); mediodorsal nucleus (*MD*); oculomotor nucleus (*NO*); nucleus of the optic tract (*NOT*); trigeminal nerve (*NV*); optic tract (*TO*); tractus retroflexus of Meynert (*TRM*); ventral nucleus (*V*); ventrolateral nucleus (*VL*); and ventroposterior nucleus (*VP*).

There are several experiments with other mammals which suggest that this subdivision of the tree shrew's superior colliculus may not be unique. Reports of studies of the opossum and cat suggest that the upper and lower laminae may project to different thalamic zones (*12*). Some behavioral studies also lend support to our argument. Limitation in space does not permit a full review of the literature here, but we cite briefly some experiments in the cat (*13*). When one of the major tectal pathways to the brainstem (the predorsal bundle) is transected by a midline lesion, cats rapidly relearn to discriminate patterns (a cross versus a triangle), but show no response to threatening gestures. The lesion may have transected pathways leaving the deeper tectal laminae, and therefore it is noteworthy that the symptoms reproduce a part of the behavioral syndrome seen in our animals with deep lesions. On the other hand, deficits in pattern discrimination and in the capacity to follow moving objects have been demonstrated in cats following removal of the entire superior colliculus (*5, 14*). In these studies of the cat, damage to structures other than the superior colliculus, in the strict sense, may have contributed to the deficit. These structures, the pretectum and the periaquaductal gray, were also damaged in our tree shrews with deep lesions and, more important, it would be almost impossible to remove entirely the deep layers of the superior colliculus without at the same time injuring the superficial layers.

This point shows a limitation of the ablation method. Indeed, even if it proves to be the case that the two syndromes reported here can be replicated by lesions entirely restricted to superficial and deep layers of the superior colliculus, this could only set the stage for further functional analysis, for every subdivision is highly interrelated with other parts of the visual system.

V. A. CASAGRANDE
J. K. HARTING
W. C. HALL
I. T. DIAMOND
Departments of Anatomy, Physiology, and Psychology, Duke University, Durham, North Carolina 27706
G. F. MARTIN
Department of Anatomy, Ohio State University, Columbus 43210

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10 January 1972; revised 25 May 1972

Contraction in Stentor coeruleus: A Cinematic Analysis

Abstract. *The convoluted M bands of the protozoan* Stentor coeruleus *straighten before the animal contracts. Mechanical stimulation initiates contraction locally, and then contraction spreads over the animal with a propagation velocity of 5 to 25 centimeters per second. The contractile wave may spread in both anterior and posterior directions. Electrical stimulation initiates contraction in all areas of* Stentor *simultaneously.*

The mechanism underlying the ability of the ciliate *Stentor* to contract has long been an intriguing problem (*1*). Advances in the investigation of this question have been made through light microscopic observations (*2*) and electron micrographic studies (*2–4*). In an earlier cinematic study of *Stentor* Jones *et al.* (*5*) have measured the rate of contraction, showing that the process requires 10 to 20 msec. Early investigators attributed contraction to fibrillar systems running underneath *Stentor*'s longitudinal rows of body cilia (*6*). The nature of these fibers remained uncertain until studies with the electron microscope revealed two distinct systems, named the km fibers and the M bands by Randall and Jackson (*3*). The km fibers are composed of stacks of microtubules, each fiber running the length of the animal. The structure has

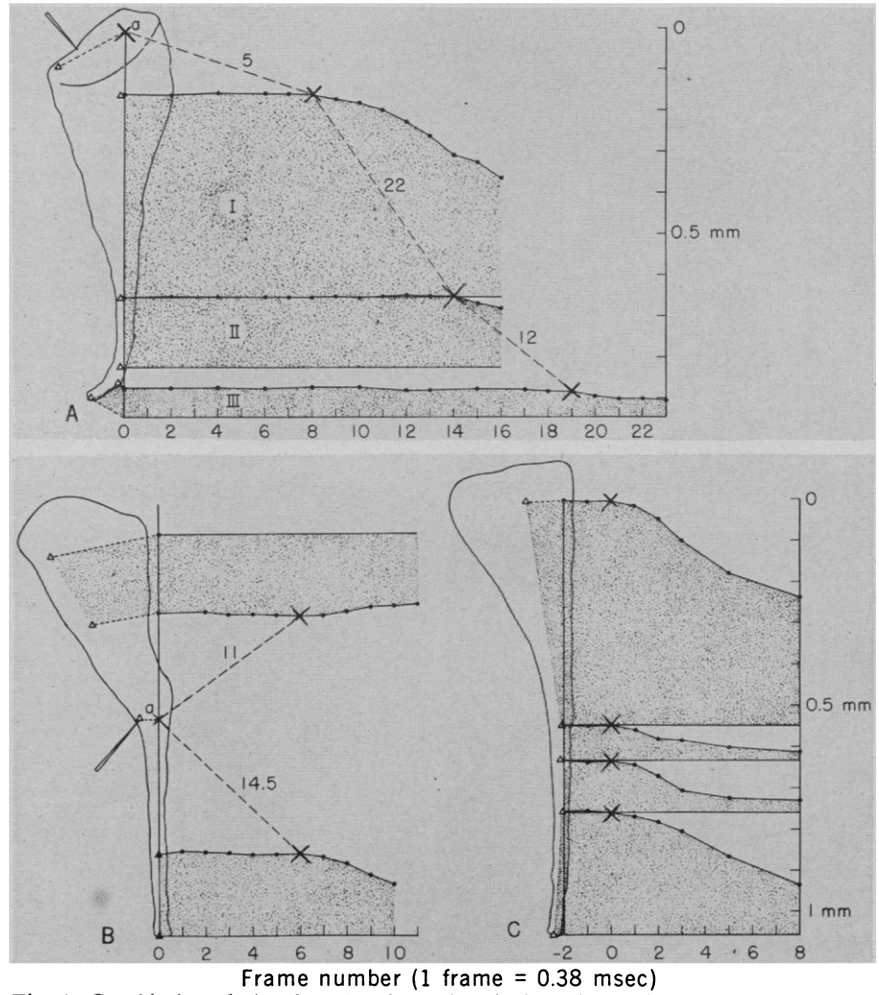

Fig. 1. Graphical analysis of contraction. The abscissa shows the time in frames of movie film. The first contractile movement occurs between frames 0 and 1. Sketches of frame 0 are shown to the left. *Stentor* are divided into segments defined by internal landmarks (Δ). The length of each segment is plotted independently as a function of time. The first sign of contraction in a segment or at a point is marked by ×. The absolute value of the slope of the line connecting ×'s equals the propagation velocity of the contractile wave. Values are given in centimeters per second. (A) Mechanical stimulation of the anterior frontal field. Contraction is first seen at point a. (B) Mechanical stimulation of the stalk. Point a contracts first. (C) Electrical stimulation; all segments contract simultaneously.