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.

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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

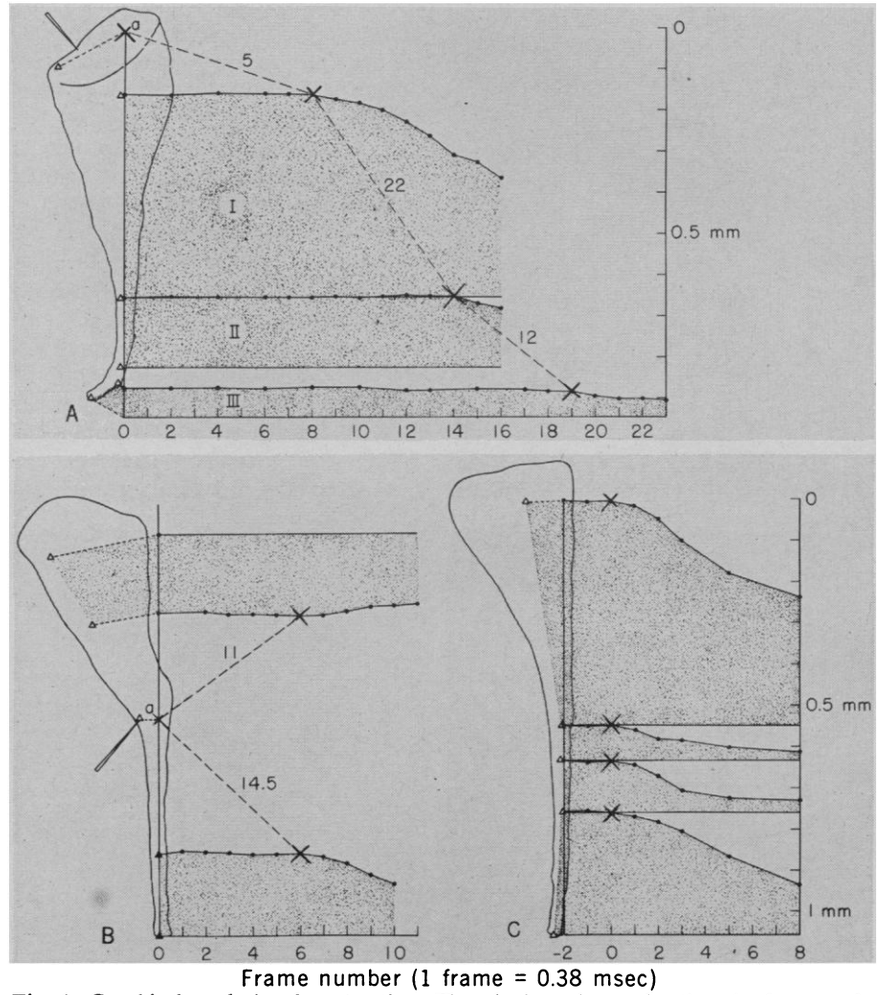

Fig. 1. Graphical analysis of contraction. The abscissa shows the time in frames of movie film. The first contractile movement occurs between frames 0 and 1. Sketches of frame 0 are shown to the left. *Stentor* are divided into segments defined by internal landmarks (Δ). The length of each segment is 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.

been implicated as an active extension system (*4*). The M bands lie beneath the km fibers and are composed of bundles of microfilaments.

Bannister and Tatchell (*2*) have described the behavior of the M bands in live *Stentor coeruleus*. The M bands are considerably thicker in contracted animals than in extended ones. They remain straight upon contraction but initially reextend at a faster rate than the rest of the body and are thrown into sinuous folds. These convoluted bands straighten (that is, shorten) upon a second contraction, an indication that they are contractile. In a recent electron microscopic study Huang (*4*) showed that there is a change in the filaments of the M bands associated with contraction. The filaments are 40 Å in diameter in relaxed *Stentor* whereas they are 120 Å in diameter in contracted animals. These observations strongly suggest that the M bands are responsible for contraction.

I have conducted a high-speed cinematic analysis of *Stentor coeruleus* to investigate the response of the M bands during the initial part of the contractile phase and to study the general time course of contraction. A Hycam high-speed movie camera was used in conjunction with a Zeiss Nomarski microscope. Stroboscopic illumination (*7*), synchronized with the camera, gave an exposure time of 1 to 2 μsec per flash with a total flux level low enough so that there was no noticeable effect on the light-sensitive *Stentor*. With this arrangement, film speeds of up to 3000 frames per second were achieved. *Stentor* swam freely in pond water in a 140-μm space between the glass slide and the cover slip. The water was at room temperature, 24°C. Test animals were stimulated mechanically with glass microneedles (diameter of the tip, 0.5 to 5 μm) positioned between the slide and the cover slip, and electrically with pulses (1 msec, 10 volts, 0.1 ma) delivered through platinum wires 1.8 cm apart.

Movies were taken of the M bands at 2640 frames per second during the period when they show large convolutions. Electrical stimulation was used to initiate contraction. In every case observed so far, a definite straightening of the M bands was seen to precede contraction of the surrounding area of ectoplasm. Straightening occurs within 1 msec, whereas contraction continues for more than 10 msec.

If contraction were caused by the action of the M bands, they would straighten before the body begins to contract (slack bands cannot exert tension). Conversely, if another mechanism causes contraction and acts before the M bands have shortened, the convolutions would grow larger during the initial phase of contraction. Not only do the M bands shorten at the beginning of the contractile phase, but they are seen to straighten before any contraction of the animal is observed. The results support the theory that the M bands are responsible for contraction.

Cinematic analysis has allowed a detailed study of the time course of contraction. When a mechanical stimulus is used, contraction is first seen at the point of stimulation and is then observed to spread over the animal. The spread of the wave which initiates contraction is monitored by measurements of the distance between landmarks (Δ) inside the animal. Vacuoles are transformed from spherical to highly flattened shapes by the contraction, an indication that they are held in a viscous cytoplasmic matrix. Thus they are suitable reference markers. A distinct shortening between two markers is taken to indicate the invasion of the contractile wave into that segment. A "propagation velocity" for the contraction can then be calculated if one knows the distance between two segments and the time interval ΔT to the onset of contraction in those segments.

The time interval between stimulation (both mechanical and electrical) and the onset of contraction varies with stimulus strength. All time measurements have been made with reference to the initiation of contraction, not to stimulation.

The stalk region of *Stentor* is highly contractile. The onset of contraction in segments in this area is easily determined from an analysis of segment lengths and from the use of a flicker technique in which one alternately views two frames. Wave velocity values calculated from contracting stalk segments (segments II to III in Fig. 1A) are reliable and reproducible. These velocities range from 10 to 20 cm/sec in different trials. Their accuracy is limited by the difficulty associated with determining distances between indistinct

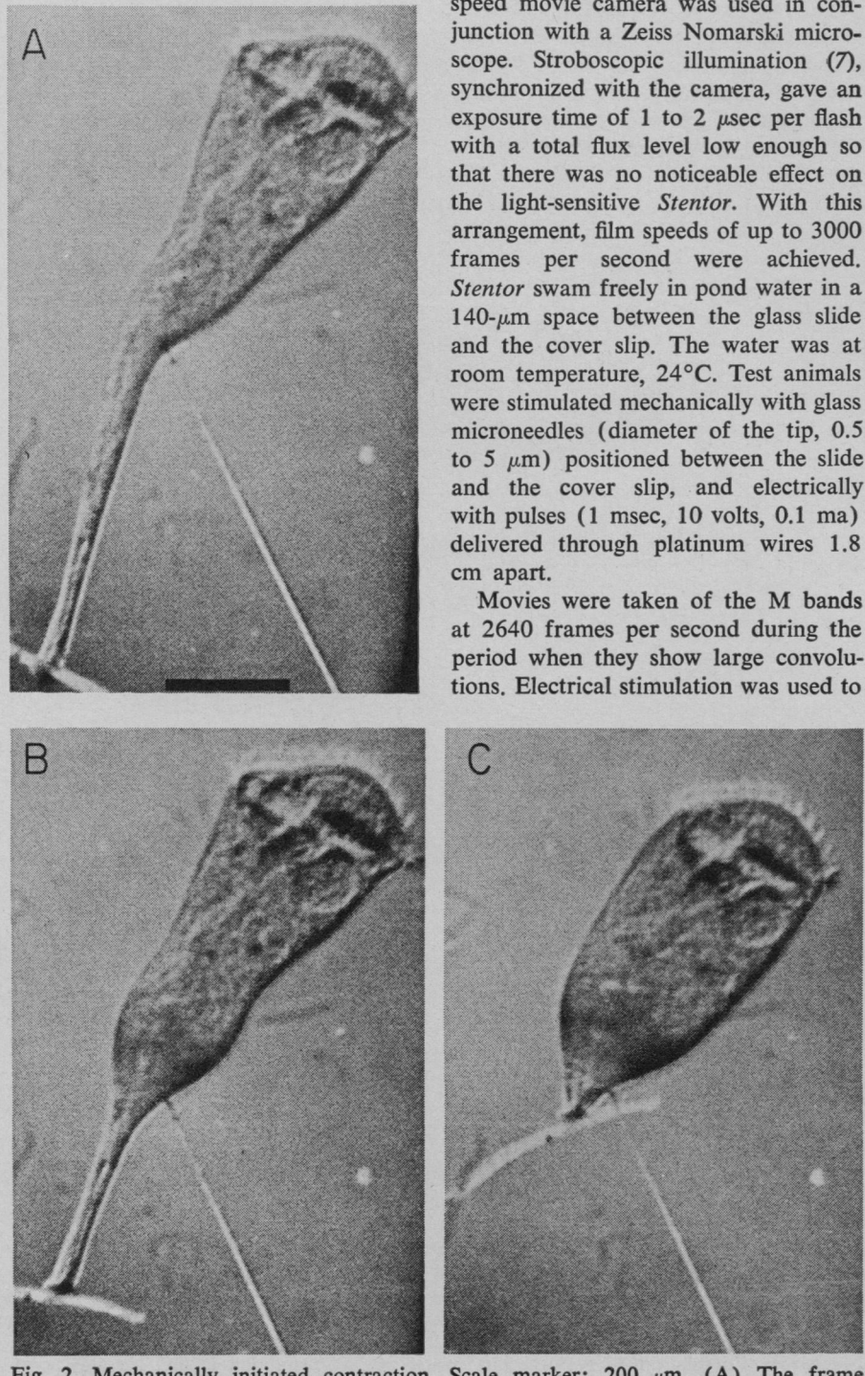

Fig. 2. Mechanically initiated contraction. Scale marker: 200 μm. (A) The frame preceding the first contractile movement; $T = 0$. (B) $T = 1.9$ msec. (C) $T = 5.3$ msec.

markers and by the relatively few frames of film spanned by the contraction. The anterior region of *Stentor* contracts little, and it is harder to determine the arrival of the contractile wave in this area. Measurements of propagation velocity in anterior segments (segments a to I and I to II in Fig. 1A) have higher uncertainties than those in the stalk. With these values included, the calculated velocities in nine different animals have ranged from 5 to 25 cm/sec.

It is possible to initiate contraction locally in the stalk region with a mechanical stimulus, as is illustrated in Fig. 2. A graphical analysis of this contraction (Fig. 1B) shows that the contractile wave is capable of spreading in both anterior and posterior directions. The mechanism responsible for such propagation, at least in the longitudinal direction, is not polarized.

A distinctly different picture of contraction emerges when electrical stimulation is used. Figure 1C displays an example of electrically induced contraction in which all segments begin to contract within the same 0.38-msec interval. This interval is too short to be due to a propagated contractile wave originating from a distinct initiation point as shown by examples of mechanically induced contraction that take several milliseconds to spread across the animal. Electrical stimulation is capable of initiating contraction in many areas of *Stentor* simultaneously.

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Single Cell Activity in the Auditory Cortex of Rhesus Monkeys: Behavioral Dependency

Abstract. *The response to repetitive stimulation of single cells in the auditory cortex of the monkey is dependent upon behavioral performance and training of the subject in a simple auditory discrimination task. In the trained, performing animal, single cells are more responsive than in the animal that is trained but not performing in the task. In the naive monkey, evoked responses are labile and are maintained only with nonrepetitive auditory stimuli.*

The central auditory system, from the eighth nerve to the cortex, has been examined extensively by means of acute (*1*) extracellular recordings from single units (*2*), and patterns of neural response to a variety of acoustic stimuli throughout the auditory pathway have thus been specified. However, certain limitations must be placed on data obtained with this approach. For example, anesthetics greatly alter single cell responsiveness (*3*). Moreover, chronic (*1*) electrophysiological techniques yield data that indicate a dependence of single cell activity within the auditory system upon behavior or "attentiveness" of the animal (*4*). Thus, the contribution of data obtained in acute studies to the understanding of neural functioning in awake, behaving animals is to some degree restricted. Chronic recording techniques for investigation of neural activity provide a more nearly normal physiological preparation than that offered by the anesthetized subject. Coupled with behavioral procedures for training nonverbal animals, these techniques allow investigation of single cell activity in unanesthetized, behaving animals, and in animals trained to report the occurrence of stimuli presented to them.

We report observations of single cell responsiveness in the auditory cortex of rhesus monkeys (*Macaca mulatta*) with emphasis on the effects of three behavioral conditions: (i) trained animals performing an auditory discrimination task; (ii) animals trained to the auditory task, but not performing; and (iii) awake, untrained animals.

Training and both electrophysiological and behavioral testing took place in sound-attenuated experimental chambers (Industrial Acoustics 400A or 1200A), with subjects restrained in primate chairs. Head movement was eliminated during testing sessions by rigidly fixing the head at three anchor points (*5*). During aseptic surgery a stainless steel chamber was anchored permanently to the skull over the middle-posterior extent of the Sylvian fissure.

For electrophysiological testing, tungsten microelectrodes were driven through the intact dura into cortex by a remotely controlled micromanipulator (Trent Wells), which was attached to the steel chamber on the monkey's head. With this preparation, isolated cells may routinely be studied for 1 to 2 hours. At termination of some of the penetrations into the cortex, small marking lesions were made to aid in later identification of recording sites. Standard electronic equipment was used in amplification and recording of the electrophysiological activity and in generation and control of acoustic stimuli. Auditory stimuli included clicks, bursts of white noise, bursts of pure tone, and verbal utterances. These auditory stimuli were delivered through a PDR-600 ear speaker (Permaflux) adjacent to the ear contralateral to the cortical recording site. Calibration of pure tone and white noise acoustic signals was performed with a Brüel and Kjaer ½-inch condenser microphone and a calibrated probe tube. Probe tube measurements were made at the entrance to the external auditory meatus. Intensities are given in decibels referred to 0.0002 dyne/cm^2.

Subjects were trained, by techniques described (*6*), to perform in a simple auditory reaction time (RT) task. This task involved depressing a telegraph key at the onset of a light stimulus, maintaining the key depressed for a variable duration (1- to 4-second foreperiod), and releasing the key rapidly at onset of the acoustic stimulus. Key release responses of brief latency were positively reinforced by delivering a dollop of applesauce to the animal's mouth. Final performance of monkeys in this task is similar to that of humans in a simple auditory RT task.

Results in this report are based on responses of approximately 150 single cells examined in seven unanesthetized monkeys. In animals trained and performing in an auditory RT task, single cells were readily isolated and studied which exhibited a consistent maintained response to repetitive auditory stimuli. Some cells were excited by onset and offset of the auditory stimulus, and