Loss of Y-Cells in the Lateral Geniculate Nucleus of Monocularly Deprived Tree Shrews
Author(s): Thomas T. Norton, Vivien A. Casagrande and S. Murray Sherman
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8. Adults (3 to 6 months old) weighing 36 to 48 g were used. They were bilaterally castrated under ether anesthesia. After treatment they were decapitated and the wet weights of their paired prostates, ampullae (vasal glands positionally similar to rodent seminal vesicles), and epididymides were obtained.
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10. Shrews were ovariectomized 1 month before the tissues were incubated with [^{3}H]estradiol. For comparison, the corresponding tissues from immature (21 to 23 days old) Purdue-Wistar rats (Fig. 1) and adult rats ovariectomized 1 month previously were incubated. The [6,7-^{3}H]estradiol (specific radioactivity, 48 c/mmole) was prepared in ethanol and dried under nitrogen in 10-ml glass vials. Vials each containing [^{3}H]estradiol ($2 \times 10^{-8} M$) and 10 to 30 mg of tissue in 1 ml of Eagle's medium were incubated at 37°C under air for 1 hour. After incubation, the nuclear fractions were isolated and the [^{3}H]estradiol content was determined [J. N. Anderson, E. J. Peck, J. H. Clark, *J. Steroid Biochem.* **5**, 103 (1974)]. The protein content was determined with bovine serum albumin being used as a standard [O. H. Lowry, J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951)].
11. The amounts of [^{3}H]estradiol bound by the tissues of the adult ovariectomized rat, in terms of disintegrations per minute per milligram of nuclear protein, were: uterus, $2.0 \pm 0.2 \times 10^4$; vagina, $1.4 \pm 0.3 \times 10^4$; diaphragm, $0.3 \pm 0.02 \times 10^4$; and kidney, $0.3 \pm 0.05 \times 10^4$. These values are similar to those shown in Fig. 1 for the corresponding tissues of the adult ovariectomized shrew.
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14. G. L. Dryden observed mating by two female shrews that were sexually experienced before they were ovariectomized. M. J. Hasler (personal communication) also observed behavioral receptivity in ovariectomized females from a separate colony. To test more rigorously the ability of females to mate after ovariectomy, we bilaterally ovariectomized three mature (6 weeks old) but sexually inexperienced shrews. Beginning 1 month after ovariectomy, they were exposed for 1 hour at weekly intervals to studs. All three females exhibited sexual receptivity and all copulated, allowing intromission and ejaculation [as judged from male behavior; see (*12*)]. Three age-matched control females all copulated the second time they were exposed to studs. The ovariectomized shrews first copulated on test days 1, 14, and 28.
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Loss of Y-Cells in the Lateral Geniculate Nucleus of Monocularly Deprived Tree Shrews

Abstract. *In tree shrews* (Tupaia glis) *reared with one eye closed, Y-cells were almost entirely absent in the binocular segment of the lateral geniculate laminae receiving input from the deprived eye. Y-cells were found in the monocular segment of these laminae, and in the binocular segment of the laminae with input from the normal eye. X-cells were present in both the deprived and normal laminae and appeared unaffected by the deprivation. A number of abnormal cells were also found, and these were located primarily in the binocular segment where Y-cells were absent.*

Tree shrews share with primates a well-developed geniculostriate visual pathway and may bear strong resemblance to the common ancestor of the primate line (*1*). They differ from the cat, on which so much of our knowledge of visual functioning is based, not only in evolutionary history, but in a number of features of their visual system. For example, compared with cats, tree shrews have more retinal cones, a larger, more differentiated superior colliculus, and a lateral geniculate nucleus which projects solely to the striate cortex (*1–3*). Despite these and other important differences, we previously found that tree shrews have X- and Y-cells in their lateral geniculate nucleus (*4*). Our suggestion that other more disparate species might also possess X- and Y-cells has recently been confirmed (*5*). In this report, we extend the analogy between the X- and Y-cells of cat and tree shrew to include the effects of visual deprivation on the postnatal development of these cells.

Previous work has shown that in cats reared with sutured eyelids there is a selective reduction in the population of lateral geniculate Y-cells sampled with microelectrode techniques. In cats reared with one eye closed this inability to record from Y-cells is limited to the binocular segment of the lateral geniculate laminae receiving afferents from the deprived eye (that is, the deprived laminae); Y-cells are apparently unaffected in the deprived monocular segment, as are X-cells throughout the nucleus (*6*). We have found that the same pattern of Y-cell "loss" occurs in the lateral geniculate nucleus of monocularly deprived tree shrews, while X-cells seem to be unaffected.

In seven tree shrews (*Tupaia glis*) we sutured the lids of one eye at about 7 days after birth, well before the normal eye opening which occurs at about 20 days of age. The animals were hand-reared until weaning, and then cage-reared normally until 4 to 24 months of age, when they were anesthetized and prepared for single-unit recording. All experimental techniques, recording procedures, and criteria for classification of X-and Y-cells were identical to those we used previously in normal tree shrews and cats (*4, 6–8*).

In each animal we placed our recording electrode in the lateral geniculate nucleus contralateral to the deprived eye. This allowed sampling of neurons both in the binocular and monocular portions of the visual field in the four deprived laminae (*3*). The two laminae which receive ipsilateral nondeprived eye input provided control data from the binocular portion of the visual field.

Our primary finding is that we obtained recordings from very few Y-cells in the binocular portion of the visual field in the deprived laminae, while we found many Y-cells in the monocular portion. Figure 1 shows the distribution in the visual field of the cells sampled in the deprived laminae. In the binocular segment, only two of the 46 relay cells sampled were Y-cells, a significantly smaller proportion than were present in the normal laminae in these same animals (six Y-cells out of 18) or in the comparable laminae of normal tree shrews (15 Y-cells out of 33) (*4, 9*). Ten of the 20 cells sampled in the monocular segment of the deprived laminae were Y-cells, and this ratio is normal (*4*). The receptive-field center diameters, conduction latencies from optic chiasm and from cortex, and responses to visual stimuli of the Y-cells we sampled in these deprived laminae were not significantly different from the values established in our earlier experiment on normal tree shrews (*4, 7, 10*). The X-cells, and a few cells with mixed X- and Y-properties (that is, "mixed" cells) (*4*), were also not significantly different from the cells in normal animals in their response properties, and these cells were present in normal proportions in the deprived and nondeprived laminae of the lid-sutured tree shrews. Our data thus indicate an inability to record Y-cells, and suggest that this "loss" is restricted to the binocular segment of the deprived laminae. Furthermore, we found no evidence for an effect of the deprivation on X-cells (*11*).

In addition to the selective effect on Y-cells in the deprived tree shrews, we found 17 cells with properties that we never observed in normal animals. Sixteen of these 17 abnormal cells were located in the deprived laminae, 11 in the binocular, and five in the monocular segment. These cells were so sluggish in their response to visual stimuli that we could not determine the receptive-field

diameter, but could only find their approximate location in the visual field and the eye through which visual stimuli affected their firing. However, we obtained conduction latencies in most of these cells from stimulation of the optic chiasm (orthodromic OX latency) or the visual cortex (antidromic VC latency), or both. The OX latencies of the abnormal cells were quite long (average, 2.0 msec), significantly longer than the OX latencies of even normal X-cells (that is, 1.7 msec) (t-test, $P < .001$) (*4*). The VC latencies obtained in ten of these abnormal cells were quite variable. Three in the binocular visual field had very short latencies (0.75 to 1.0 msec) usually seen only in normal Y-cells, while the others had VC latencies (1.4 to 2.1 msec), as long or longer than most X-cells (*4*). Thus the abnormal cells generally had very long OX and VC latencies, with the exception of the three cells with VC latencies in the Y-cells' range.

Since abnormal cells were not found in normal tree shrews (*4*), we presume they resulted from the effects of the monocular deprivation on either developing X- or Y-cells, or both. Although we could not determine their origin in this study, several factors suggest to us that at least some, and perhaps all, of them may represent "missing" Y-cells in the deprived laminae. First, the very short VC latencies in some of the abnormal cells were consistent with a Y-cell origin. Second, if the number of Y-cells and abnormal cells in the deprived binocular segment are combined, the proportion of these two groups (28 percent) is not significantly different from the level of Y-cells (45 percent) in normal tree shrews (χ^2, $P > .1$) (*4*). Thus the appearance of the abnormal cells can account statistically for the disappearance of the Y-cells in the deprived binocular segment.

While it is possible that the abnormal cells resulted from the effects of deprivation on X-cells, it seems more parsimonious to suggest that they may be derived from the effects of the deprivation on developing Y-cells. Recent experiments in monocularly deprived cats have revealed similar abnormal cells with long OX latencies in the deprived portion of the medial interlaminar nucleus (*12*). Since this nucleus in the cat receives only Y-cell input (*13*), these data in cat also suggest that one effect of visual deprivation on Y-cells may be to produce abnormal cells. It is interesting that some abnormal cells were seen in the monocular segment of the deprived tree shrews, indicating that cells in this portion of the visual field are not immune from the effects of monocular deprivation.

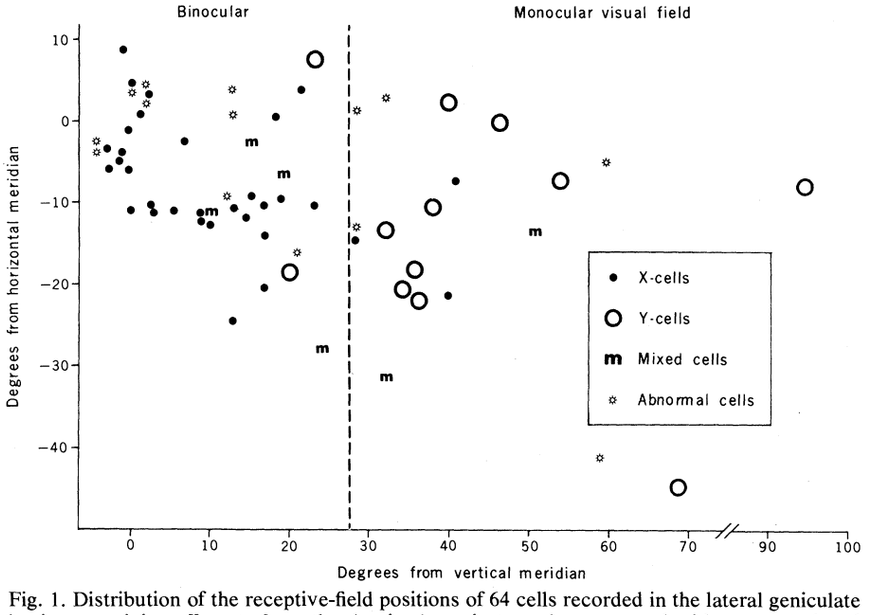

Fig. 1. Distribution of the receptive-field positions of 64 cells recorded in the lateral geniculate laminae receiving afferents from the deprived eye in tree shrews reared with one eye closed (*17*). The binocular and monocular segments of visual field are shown. While normal proportions of Y-cells were present in the monocular visual field of the deprived laminae, only two Y-cells were found in the binocular field, and these were located near the boundary of the monocular field. We also found a few "mixed" cells and some abnormal cells which are described in the text.

The pattern of Y-cell loss in monocularly deprived tree shrews is accompanied by anatomical and behavioral results which also indicate differences between the binocular and monocular segments. A proportional loss of large cells, which is restricted to the binocular segment of the deprived laminae, has been reported in monocularly deprived tree shrews tested after reverse suturing (*14*). In preliminary behavioral tests in monocularly deprived tree shrews (*15*) we found that the animals failed to respond to visual stimuli presented to the binocular visual field of the deprived eye, but did respond when the stimuli entered the monocular field. Thus the absence of normal Y-cells in the binocular segment is accompanied by a morphological loss of large cells and a loss of behavioral responsiveness, while the presence of normal Y-cells in the monocular segment is associated with relatively normal cell size and the presence of behavioral responsiveness. This same pattern has been reported in the cat (*16*).

In extending the analogy between the X- and Y-cells of the cat and tree shrew to include the effects of visual deprivation on the postnatal development of these cells, this study leads to three suggestions. First, the similarity of the effects suggests that similar mechanisms may be involved in the development of the X- and Y-cell systems in both species. Second, the presence of closely similar cell types and deprivation effects in two quite disparate species suggests that other species, particularly those recently shown to have X- and Y-cells (*5*), may show similar deprivation effects. Finally, the analogous behavioral effects of the deprivation in cat and tree shrew suggests that the Y-cell system may play a similar, although yet undefined, role in the visual behavior of these and possibly other species.

THOMAS T. NORTON
Department of Psychology,
Duke University,
Durham, North Carolina 27706
VIVIEN A. CASAGRANDE
Department of Anatomy,
Vanderbilt University,
Nashville, Tennessee 37232
S. MURRAY SHERMAN
Department of Physiology, University of Virginia, Charlottesville 22901

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7. We measured five properties: hand-plotted receptive-field center diameter ($X < 1° < Y$), latency to optic chiasm shock ($Y < 1.4$ msec $< X$), antidromic latency to striate cortex shock ($Y < 1.2$ msec $< X$), duration of response to presentation of standing contrast

(Y < 15 seconds < X), and presence (Y) or absence (X) or a response to rapidly moving stimuli of appropriate contrast (dark for OFF-surround, light for ON-surround cells). As in normal tree shrews, most cells (53 percent) consistently responded on these tests with either X- or Y-cell properties without an exception. An exception on one test occurred in 37 percent of the classified X- and Y-cells, and in a few instances (10 percent) two exceptions in the five tests were tolerated when the other responses were well within either the X or Y range. Thus, for instance, a cell might fail to respond to rapid movement but still be classified as a Y-cell because it had a large receptive-field center and short latencies and gave a very transient response to standing contrast. We did not use a test for receptive-field linearity in the tree shrews. However, recent experiments in one of our laboratories have shown that when a test for receptive-field linearity [see p. 538 in C. Enroth-Cugell and J. G. Robson, *J. Physiol. (London)* **187**, 517 (1966)] is used in the cat lateral geniculate nucleus, the X- and Y-cells classified by methods used in this study have, respectively, linear and nonlinear receptive-field properties (K. E. Kratz, S. V. Webb, S. M. Sherman, in preparation).

8. When focusing the animals on the tangent screen with contact lenses, we consistently found that the deprived eye was strongly myopic (about 20 diopters) relative to the normal eye [S. M. Sherman, T. T. Norton, V. A. Casagrande, *Brain Res.* **124**, 154 (1977)]. As a result we took great care in focusing both the deprived and normal eye on the tangent screen with retinoscopic tests. In addition, we tested the responses and receptive-field size of several cells with a range of spectacle lenses to find the optimal correction, which always agreed with our retinoscopic determination. Many X-cells driven by the deprived eye had small receptive fields and responded to fine gratings. We are thus confident that our results do not reflect incorrect focus during the recording sessions.
9. By a χ^2 test the proportion of binocular-segment Y-cells was lower than the proportion in the nondeprived laminae ($P < .01$) and lower than the proportion in laminae innervated by the contralateral eye in normal animals ($P < .001$).
10. We used *t*-tests for receptive-field diameter, chiasm latencies, and cortex latencies, and χ^2 tests for responses to standing contrast and moving stimuli.
11. Although only three X-cells were recorded in the deprived monocular segment, we have no evidence for a loss of X-cells there since this was not significantly lower than the proportion in the monocular segment of normal tree shrews by an χ^2 test (*4*).
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15. We used two additional monocularly deprived tree shrews from another experiment and tested their visual behavior 1 or 2 days after opening the deprived eye and suturing the normal eye closed to force the animals to use the deprived eye.
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17. Sixty-six cells were sampled, but two abnormal cells could only be generally located in the binocular field and are not plotted. When plotting cells on this graph, we used the estimate (A. L. Humphrey, J. E. Albano, T. T. Norton, *Brain Res.*, in press) that the vertical meridian is 37.5° medial to the optic disk and 5° above the optic disk. We originally plotted all cells relative to the optic disk, so relative cell locations are unchanged by any errors in estimation of the area centralis location. We found the edge of the boundary between the binocular and monocular visual fields to be approximately 10° medial to the optic disk, or 27.5° from the area centralis.
18. Supported by NIH grant EY 01085 and an Alfred P. Sloan Foundation research fellowship (T.T.N.), NIH grants EY 01565 and RCDA EY 00020 (S.M.S.), and NIH grant EY 01778 (V.A.C.). We thank T. Ferguson, B. Jeffers, and C. Pelham for histological assistance.

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Ultrasound Emission in Infant Rats as an Indicant of Arousal During Appetitive Learning and Extinction

Abstract. *Infant rats rewarded for crawling by being allowed to suckle on the dry nipple of an anesthetized dam showed a decreasing rate of ultrasound production during acquisition and an increasing rate during extinction. These results suggest that infant rats can be stressed and are aroused as a result of successive nonrewards just as adult rats are. In addition, these results do not support the hypothesis that infant rats lack inhibitory mechanisms related to poorly developed neural centers.*

Infants of most rodent species emit sounds at frequencies above the range of human hearing. These ultrasonic vocalizations, investigated in a number of species, typically occur when the infants are exposed to conditions of environmental stress (*1, 2*). Thermal and tactual stressors, unusual odors, pain, and hunger evoke the ultrasonic calls of infant rats (*1, 3, 4*). Developmentally, the rate of ultrasound production decreases as homoiothermy is attained (*4*).

Ultrasounds appear to be important signals for altricial infant rodents. They seem, for example, to initiate infant retrieval by the dam in rats (*5*) and to coordinate maternal behaviors in mice (*1*). Bell (*6*) has emphasized that ultrasounds reflect high arousal in infants and induce arousal in the dam. At a time when the infant is unable to thermoregulate, to see, or to ambulate well, the infant ultrasound seems to serve as a distress signal to the dam. In the rat, ultrasounds diminish at the age at which the pup gains the ability to thermoregulate, opens its eyes, and begins to leave the nest, at which time a chemical signaling system involving a maternal pheromone (*7*) is said to predominate.

Some of our previous work (*8, 9*) has demonstrated (i) that 10- and 11-day-old rat pups can learn to approach an anesthetized dam in a heated alley with dry suckling as a reward and (ii) that the approach behavior is extinguished with successive nonrewarded trials. Extinction in these experiments indicates that at least one kind of response-suppressive mechanism is operating at an age at which centers responsible for "inhibition" are said to be not well developed in rat pups (*10*). It could be argued that the extinction in our experiments reflected decreased arousal resulting from any increasing period of no contact with the dam. Many studies of adult animals show that extinction following appetitive learning *increases* arousal, presumably because of the frustrating effects of nonreward following a history of reward (*11*). If the mechanisms of appetitive extinction in infant rats are similar to those in older rats—being characterized by active (frustrative) processes and conflict—and if ultrasounds indicate arousal in the pup (*6*), one would expect ultrasounds to increase during extinction of a learned appetitive response. This experiment is the first to our knowledge that measures ultrasound production during infant learning, and it provides evidence for an active conception of appetitive extinction in rats, even at 11 days of age.

The training apparatus is described in detail elsewhere (*9*). It consisted of a Plexiglas alley (38 cm long, 7.5 cm wide, and 10 cm high) and a goalbox (17 by 25 by 10 cm). A Plexiglas gate bisected the goalbox and could prevent the pup from reaching the rear half of the goalbox. Another gate at the end of the alley prevented retracing after the pup had reached the goalbox. Both alley and goalbox were maintained at 37°C by commercial heating pads located beneath the Plexiglas floor. Two photobeams positioned along the sides of the alley at 13 and 38 cm from the start position activated two clocks which recorded start and total times. Ultrasounds were detected by a condenser microphone (Bruel and Kjaer 4135) with a cathode follower (Bruel and Kjaer 2615), a microphone amplifier (Bruel and Kjaer 2604), and a bandpass filter (Krohn-Hite 310-C). The filtered amplifier output was monitored visually on an oscilloscope (Tektronix 465), and any ultrasound pulses in the range of 20 to 50 khz (louder than 30 db) were counted by a specially constructed digital counter. The microphone was positioned over the intersection of the alley with the goalbox in such a way that ultrasounds anywhere in the apparatus were detected.

The subjects, eight albino rat pups bred in our laboratory but originally of Holtzman stock, were 11 days of age and weighed between 25 and 30 g. Each pup was separated from its dam 10 hours before runway training began and was kept in a covered plastic breeding cage maintained at 37°C. Approximately 20 minutes before runway training began, the dam was anesthetized with EquiThesin (2 ml per kilogram of body weight, injected intraperitoneally). Additional amounts of anesthetic were used as