male and female human individuals. The rabbit may therefore provide a useful animal model in which differences in the extra-adrenal formation of DOC during pregnancy can be studied.

HERMANN H. DIETER*
URSULA MULLER-EBERHARD†
ERIC F. JOHNSON
Department of Biochemistry, Scripps Clinic and Research Foundation, La Jolla, California 92037

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* Present address: Institute of Toxicology, University of Dusseldorf, West Germany.
† Present address: Department of Pediatrics, New York Hospital–Cornell Medical School, New York 10021.

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Abnormal Development of Kitten Retino-Geniculate Connectivity in the Absence of Action Potentials

Abstract. *Action potentials were silenced in one eye of neonatal kittens by repeated intraocular injections of tetrodotoxin for 5 to 8 weeks. After tetrodotoxin blockade was allowed to wear off, receptive field properties of individual relay cells in the lateral geniculate nucleus were examined. The many* ON-OFF *and binocular fields found in the layers that receive input from the treated eye suggest that these cells had extremely abnormal retino-geniculate synaptic connections. These effects were different in kind from those seen after deprivation rearing that does not silence action potentials. Lack of action potential activity was concluded to lead to abnormal development in the central nervous system.*

Action potential activity per se has been suggested, mainly on theoretical grounds, as affecting development of central nervous system (CNS) organization (*1*, *2*). Some experiments in the peripheral nervous system support this idea. For example, abolition of impulse activity retards reduction from polyneuronal to mononeuronal innervation of developing rat muscle fibers (*3*). Studies in the CNS show that environmentally induced distortion of neural activity results in abnormal connections in the cat visual system (*4*). None of these experiments abolished action potentials, however, which continue for visual neurons even in the dark. Lack of a role for action potentials is suggested by the result that an axolotl eye sensitive to tetrodotoxin (TTX) and silenced by transplantation to a newt with endogenous TTX can develop an anatomically normal retino-tectal projection (*5*). We now report severe defects in the lateral geniculate nucleus (LGN) of kittens reared with the action potentials from one eye totally abolished by intraocular TTX injections. These defects are different from any reported in other types of deprivation rearing experiments on the kitten visual system and are thus presumed to be caused by the abolition of impulse activity.

Tetrodotoxin, a sodium channel blocking agent, injected into the vitreous humor of an eye reversibly blocks the firing of action potentials in retinal ganglion cells. One intraocular TTX injection in a dose tolerated by the animal produces complete blockade for more than 48 hours. It is possible to maintain blockade for weeks by reinjections at 2-day intervals. Total recovery of ganglion cell activity occurs 5 to 7 days after the final TTX injection (*6*).

Each of seven kittens received unilateral, intraocular TTX injections on alternate days, beginning 1 to 5 days after birth and continuing for 5 to 8 weeks. Animals were set up for single-unit recording 1 to 3 weeks after the final injection, according to standard techniques (*7*, *8*). Units were recorded from both the left and right LGN and on occasion in the retina and optic tract. Receptive fields were characterized with hand-held and flashing spot targets (*8*, *9*), and were all within 25° of the area centralis. No units in the LGN C layers are reported here, although binocular units at the Al-C boundary are included. We studied 70 cells in deprived laminae, 11 binocular cells at laminar boundaries, and 53 cells in laminae innervated by the uninjected eye.

Several hundred recordings of ganglion cells and optic tract fibers from injected eyes failed to show any gross abnormality. This is not too surprising, since the region studied is fairly mature at birth and also because all retinal neurons distal to ganglion cells utilize slow potentials that are unaffected by TTX. Ganglion cell receptive fields were either ON-center or OFF-center, and they had other properties appropriate for kittens of the ages studies (*7*). In contrast, relay cells in the deprived LGN layers were strikingly abnormal. Normally, and also after monocular, binocular, or dark-rearing deprivation (*9*–*11*), LGN cells have either ON-center or OFF-center receptive fields that result from excitatory inputs from either ON-center or OFF-center retinal ganglion cells, but never from both types (*9*). Nearly 40 percent of cells recorded from in the deprived layers of TTX-treated animals received both ON and OFF excitatory input (ON-OFF cells in Fig. 1) (*12*). The LGN layers innervated by the uninjected eye served as controls, as did the LGN of an additional animal that had received a full series of sham injections (*13*); no ON-OFF cells were found in any of these cases. The fact that nondeprived LGN layers were normal rules out any systemic, nonspecific TTX effects. Further, it is unlikely that TTX has disruptive effects other than action potential blockade; its action is specific, and it neither destroys cells nor blocks axonal transport (*14*). The ON-OFF cells were not an artifact of stimulation characteristics. The ON-OFF responses occurred to a wide variety of stimulus spot sizes and intensities. Stimulus centering in the receptive field was carefully controlled. Despite deliberate stimulus decentering, ON-OFF histograms similar to Fig. 1 could not be generated in normal ON or OFF units.

Retinal ganglion cells can be classified as X and Y types, as well as ON-center and OFF-center types (*15*). In normal animals, inputs to LGN cells are segregated with respect to X and Y types,

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although about 5 to 10 percent of relay cells receive both X and Y input (*9, 11, 16*). Nine deprived layer cells were tested for X and Y input by noting their response latency to electrical stimulation at the optic disk. Of these nine cells, five received both X and Y latency input, strongly suggestive of a much higher than normal proportion of cells with mixed input.

Global retinotopic organization was normal in all LGN layers of the TTX-injected animals. However, 17 percent of the deprived LGN cells had spatially abnormal receptive fields, including significant elongation, multiple regions of high responsiveness, and separately distinguishable ON and OFF regions displaced by as much as 0.5°. About 10 percent of cells in the deprived layers could not be visually driven, and many others had weak responses. Most units had abnormally large receptive fields, some being both large and amorphous. Histological sections of the lateral geniculate nuclei in the TTX-treated animals showed reduced thickness of deprived A and C layers and cell body shrinkage in those layers.

In normal animals, each eye projects to separate layers of the LGN, with only an occasional binocular cell found in the interlaminar zones (*17, 18*). In the TTX-injected kittens 11 binocular cells were recorded at 30 layer boundaries crossed by an electrode tract, suggesting that a significant fraction of the relay cells near the laminar borders receive binocular excitatory input.

The time course of sensitivity to the effects of TTX was studied in two animals in which injections were started late: one was injected from 11 days to 10 weeks of age, the other from 15 days until 7 weeks of age. Both were studied 7 days after their last injection. They showed a smaller percentage of ON-OFF cells in the deprived layers than the seven animals that were treated soon after birth (Fig. 2). The percentages of spatially abnormal and binocular cells were also reduced. Hence, the susceptibility to TTX-induced effects is greatly diminished by about 2 weeks of age, before the onset of the cortical critical period (*19*). Two other animals had injections begun soon after birth and maintained for many weeks. One recovered for 4 weeks and the other for 11 weeks before recording. These animals ("Recovery") also had a smaller percentage of ON-OFF cells (Fig. 2), suggesting that the effects of TTX exposure are at least somewhat reversible.

Significant morphological development of the kitten LGN occurs postnatally (*20*). Our major finding is a developmental disruption; retinal ganglion cells deprived postnatally of action potential activity make connections with their LGN target cells that are abnormally segregated. These effects are different in kind from those seen after deprivation rearing that does not silence action potentials. To our knowledge, this is the first demonstration in the CNS that abolishing action potential activity can affect development of the connections of the silenced cells. However, we note that binocular injections of TTX in kittens, starting at 2 weeks of age, prevents the formation of ocular dominance columns in the visual cortex, a site at least one synapse removed from the silenced cells (*21*).

Theoretically, action potential blockade could disrupt normal development of appropriate connections in two main ways. (i) Ganglion cells could be indiscriminately connected to LGN target cells at birth and then reorganize during the first few postnatal weeks; the lack of action potentials could inhibit this reorganization. (ii) Ganglion cells could be connected to LGN target cells at birth in their adultlike pattern and then sprout inappropriate contacts in the absence of action potentials.

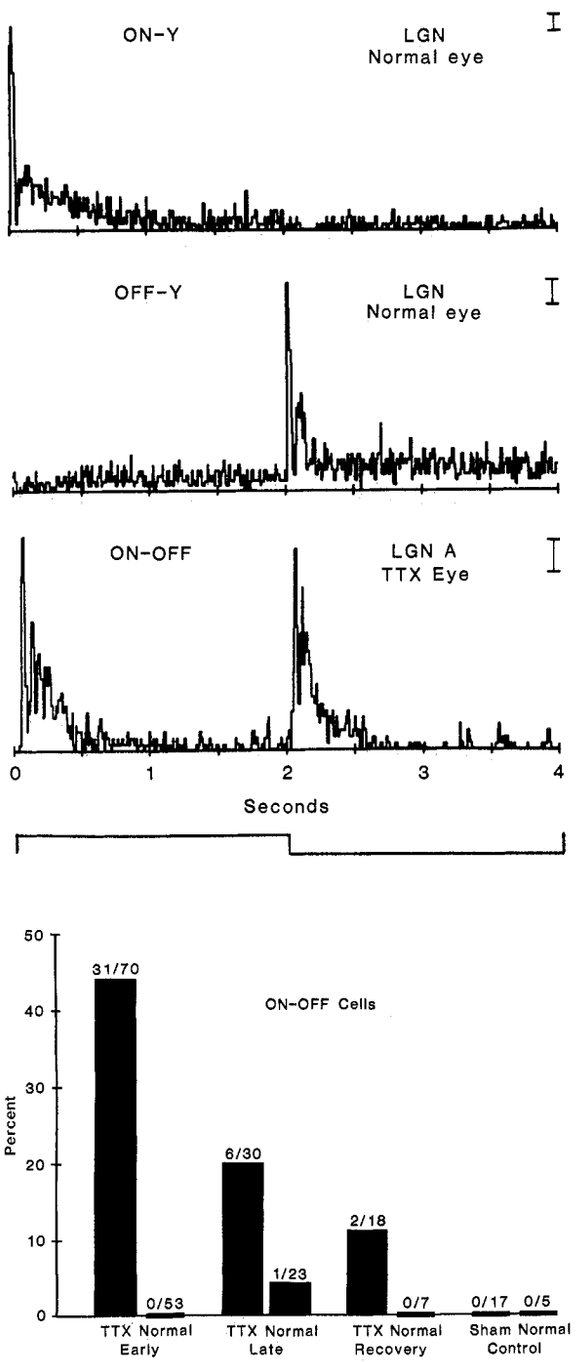

Fig. 1 (top). Typical peristimulus time histograms of responses of three LGN units to a flashing spot centered in the receptive field. Upper two histograms are different ON-Y and OFF-Y units driven by a normal eye. Lower histogram is an ON-OFF type unit driven by a previously TTX-deprived eye. The ON and OFF latencies of approximately 30 msec are equal in the ON-OFF unit and are equivalent to the corresponding excitatory latencies of the normal units. Similar records were obtained for X units. Stimuli were 300-cd/m^2 spots on an 8-cd/m^2 background. Spot sizes were 1°, 1.5°, and 1.3°, respectively. Upward deflection in the marker line below the histograms indicates when the stimulus spot was on. Calibration bars at the upper right of the histograms indicate 25 spikes per second. Fig. 2 (bottom). Percentages of ON-OFF cells found in various groups of animals. For each group the bar on the left (*TTX*) indicates cells in the LGN layers driven by the eye injected with TTX; the bar on the right (*Normal*) indicates cells sampled in the LGN layers driven by the normal eye. Above each bar, the figures indicate the actual number of cells from which the percentage is derived. The groups of animals represented are: *Early*, seven animals injected from soon after birth; *Late*, two animals with injections started at 11 and 15 days after birth; *Recovery*, two animals given more than 4 weeks of recovery before recording; *Sham*, one animal injected on a schedule similar to the "Early" animals but with a solution not containing TTX.

Some ON-OFF receptive fields that could be homologous to those found here have been reported in the LGN of kittens 1 to 2 weeks old (*22*). Failure of such cells to eliminate conflicting inputs could account for the results reported here. Models in which impulse activity brings about selective elimination or inactivation of synapses have been proposed (*2, 23*); elimination of initially excessive inputs has been suggested as a fairly general property of the developing nervous system (*24*).

Translaminar sprouting of remaining optic tract afferents occurs in the LGN of cats unilaterally enucleated during the first week of life (*25*). Action potential blockade may produce a functional enucleation that brings about the same effect, thus accounting for the binocular cells reported here. A different explanation for the binocular cells is suggested by the fact that adult LGN relay cells normally have dendrites that cross laminar boundaries (*26*), which are thought to receive only inhibitory input. Excitatory synapses onto these dendritic regions may be caused or preserved by the action potential blockade.

Action potential activity may contribute to specifying synaptic connections in a variety of ways. For example, blockade could reduce the availability of specific cell-cell recognition markers stored in synaptic vesicles and normally released by action potentials. Another possibility is that utilizable information exists in the pattern of background activity of postnatal ganglion cells. Neighboring ganglion cells of like receptive field type normally tend to fire together (*27*). In the LGN, competitive mechanisms (*2, 23*) may limit relay cell inputs to those having significant coincident activity. This could bring about segregation of ON and OFF and of X and Y pathways, as well as sharpen the retinotopic map.

STEVEN M. ARCHER
MARK W. DUBIN
LOUISA A. STARK
Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder 80309

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Prolactin and Growth Hormone Release by Morphine in the Rat: Different Receptor Mechanisms

Abstract. *Concentrations of prolactin and growth hormone in the serum of rats were significantly increased by morphine. Dose response studies demonstrated that maximum prolactin release required lower doses of morphine than those needed for the maximum growth hormone response. Selective blockade of μ_1 (high affinity) opiate receptors with the irreversible antagonist naloxazone reduced morphine-induced peak concentrations of prolactin by 80 percent while increasing peak growth hormone levels by 250 percent. These results suggest different receptor mechanisms for the opiate modulation of the two hormones. The μ_1 (high affinity) receptor sites appear to mediate the morphine-induced release of prolactin but not growth hormone.*

Opiates and opioid peptides increase the concentrations of prolactin and growth hormone in the serum of rats (*1*). This effect is specific and can be blocked by the opiate antagonist naloxone (*2*). That this opioid action occurs in the hypothalamus (*3*) is interesting in view of the presence of a number of opioid peptides in this region (*4*). Also present in this region are opiate receptors through which these agents produce their effects (*5*). Recent studies suggest that there are distinct subpopulations of opiate binding sites (*6*) which vary in their pharmacological profiles, regional distribution, ontogeny, phylogeny, and sensitivity to proteolytic enzymes and reagents (*7*). We now present evidence that the morphine-induced release of prolactin and growth hormone are mediated through separate and pharmacologically distinct opiate receptors.

Indwelling jugular cannulas were placed in male Sprague-Dawley rats (180 to 220 g; Charles River Breeding Laboratories) anesthetized with ethane and oxygen. Cannulas were routinely filled with a dilute solution of heparin in saline (25 U/ml) to prevent clotting. After implantation of the cannulas, the animals received either nothing, naloxone (50 mg/kg), or naloxazone (50 mg/kg) intravenously. Naloxazone solutions were made by dissolving free base in saline

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