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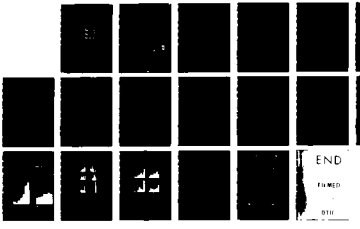

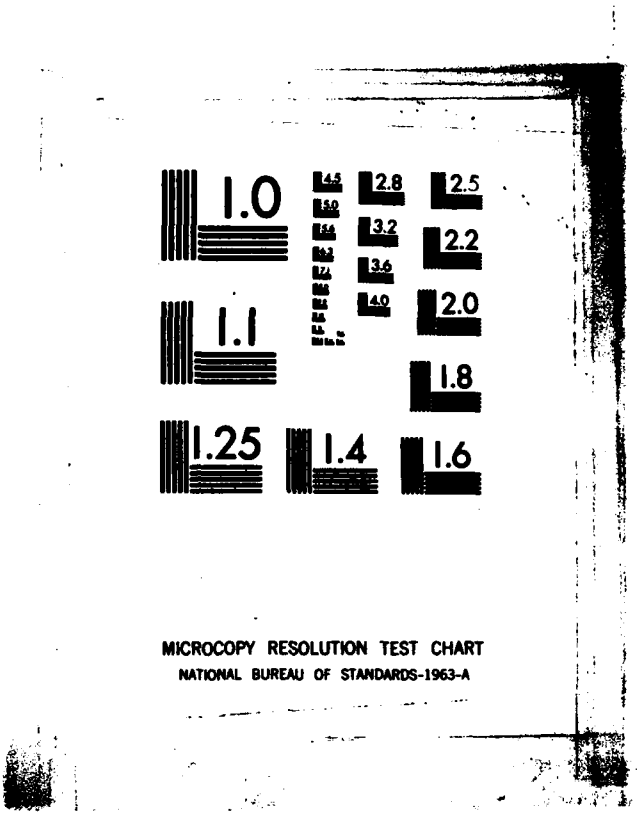
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In order to test the hypothesis that noradrenergic transmission modulates ocular dominance plasticity in kitten visual cortex, we monocularly deprived kittens while administering the α-2 adrenergic agonist clonidine (CLON). To avoid bias in testing hypothesis, we included, with a single blind technique, saline-treated control kittens in the series. First, using high pressure liquid chromatography, we demonstrated that CLON treatments resulted in an average decline in cere-brospinal fluid levels of the norepinephrine metabolite, 3-methoxy-4-hydroxy phenylethlene glyolol of 44%. Then, single unit recording

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in area 17 revealed the expected ocular dominance (OD) shift in monocularly deprived saline controls, but recording failed to find any shift in CLON treated kittens. Our results support the notion that CLON treatment interferes with ocular dominance plasticity by inhibiting noradrenergic transmission in visual cortex. We discuss side effects of CLON, concluding that CLON's sedative effect may contribute to the lack of OD shift.

Clonidine and Cortical Plasticity:

Possible Evidence for Noradrenergic Involvement

by

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SUMMARY

In order to test the hypothesis that noradrenergic transmission modulates ocular dominance plasticity in kitten visual cortex, we monocularly deprived kittens while administering the α–2 adrenergic agonist clonidine (CLON). To avoid bias in testing the hypothesis, we included, with a single blind technique, saline-treated control kittens in the series. First, using high pressure liquid chromatography, we demonstrated that CLON treatments resulted in an average decline in cerebrospinal fluid levels of the norepinephrine metabolite, 3-methoxy-4-hydroxy phenylethylene glycol of 44%. Then, single unit recording in area 17 revealed the expected ocular dominance (OD) shift in monocularly deprived saline controls, but recording failed to find any shift in CLON treated kittens. Our results support the notion that CLON treatment interferes with ocular dominance plasticity by inhibiting noradrenergic transmission in visual cortex. We discuss side effects of CLON, concluding that CLON's *sedative* effect may contribute to the lack of OD shift.

KEY WORDS

Clonidine, Visual Cortex, Monocular Deprivation, Single Unit Recording, MHPG, Development of CNS, Plasticity.

INTRODUCTION

Temporary closure of one eye for even a short duration during a three week to three month critical period results in a drastic reduction in the percentage of cells in a kitten's primary visual cortex that respond preferentially to stimulation through the deprived eye (56, review in 40). This monocular deprivation (MD) paradigm has become a model system, not only for studying the resulting changes in cortical organization, but also for evaluating the intrinsic mechanisms which modulate CNS plasticity. Biochemical, anatomical, and physiological evidence suggest that cortical plasticity is controlled in part by release of norepinephrine (NE) from fibers originating in the locus coeruleus (LC). Development of this fiber system has been studied extensively in rats where it appears that LC neurons are among the first extrinsic efferents to reach the telencephalon (46) and that the majority of synapses in cortex at postnatal day 6 are monoaminergic and probably noradrenergic (39).

In rats and monkeys, LC activity is related to sensory stimulation (18). Noradrenergic transmission has been implicated in numerous memory and plasticity functions in humans (38) and animals (17). Motivated in part by Kety's suggestion of a link between NE and learning (29), and cortical plasticity associated with learning, Kasamatsu and Pettigrew hypothesized that cortical catecholamines are essential to the plasticity of kitten visual cortex. They thought NE might be responsible for both the ocular dominance (OD) shift which demonstrates *susceptibility* to MD (25) and for the *recovery* of binocularity after MD is terminated (27). Kasamatsu and Pettigrew used the neurotoxin 6-hydroxydopamine (6-OHDA) delivered intraventricularly (25, 26), or by local continuous osmotic minipump perfusion (24, 28, 43), to deplete cortical NE. Our group (42) subsequently confirmed the results with minipump administration. Kasamatsu and Pettigrew further showed that *replacement* of depleted NE could restore plasticity (27, 43). Replacement was effective at concentrations less than or equal to normal endogenous levels (24). Further experiments suggested that effects of NE on plasticity are mediated by cortical *β-receptors* since microperfusion of *β-blocker* propranolol also blocks the plastic response to monocular deprivation (see review in 23). Of interest with respect to the NE hypothesis is Wilkinson's

finding that *β–receptor* binding in cat visual cortex increases during early development and levels off at the end of third postnatal month, a time which corresponds to the critical period for binocular vision (57).

Despite Kasamatsu's success in demonstrating support for the NE hypothesis, questions have arisen due to the results of further tests. We showed that depletion of cortical NE by neonatal injection of 6-OHDA did *not* prevent the later OD shift after MD (5, 6). Daw et al. (14) depleted cortical NE by section of the LC fiber bundle near lateral hypothalmus and, again, found no diminution of the OD shift after MD. Furthermore, Videen et al. (54) recorded *no difference* in the reaction kitten and adult cat visual cortex neurons to iontophoretically applied NE. Adrien et al. (2) obtained a similar result (no lack of shift) after lesion of the LC itself, *and* that group was unable to reproduce Kasamatsu and Pettigrew's original finding (26) that intra-ventricular injection of 6 OHDA prevents OD shift.

In light of these controversies, we sought a less destructive way to interfere with noradrenergic transmission in cortex. Clonidine (2-[(2, 6-dichlorophenyl)amino]-2-imidazoline hydrochloride) is a potent agonist at adrenergic α–2 receptors. Its administration decreases NE release from central (44) and peripheral (49) noradrenergic neurons. Clonidine (CLON), but not α–1 agonist phentolamine, inhibits release of NE from cortical synaptosomes (15A) supporting other studies that have demonstrated modulation of NE release in cortex by α–2 receptors located on the noradrenergic axon varicosities themselves (55). Alpha-2 receptors are also located on LC cell bodies (58) where they appear to mediate collateral inhibition (3). CLON applied iontophoretically (8) or administered i.v. (9) drastically reduces or completely inhibits the firing rate of LC neurons, producing a hyperpolarization by direct action at α–2 receptors (4) which appears to involve increased potassium conductance (16).

CLON-induced decreases in brain NE transmission have been confirmed by measuring decreases in MHPG the primary metabolite of NE in the CNS (7, 35, 51). Although metabolite levels can be measured in blood (36), urine (35, 37), cerebrospinal fluid (1, 10, 45), or brain tissue (7, 51, 53); only CSF provides a *realiable* measurement that does not require sacrifice of the

animal.

Our aim in these studies was to use a minimum dose of CLON effective in reducing MHPG levels in kitten CSF, then test whether that dose of CLON would block the ocular dominance shift plasticity expected after critical period MD.

METHODS

Twenty kittens, born to Tabby (wild-type) queens in our quarantined colony, were used in this study. The kittens stayed in the colony, on a 12 hour light/12 hour dark cycle. Nineteen of the twenty kittens are shown in Table I, "Data Base". One other kitten died at 54 days of age, after four days of clonidine treatment.

Each kitten received a series of i.p. injections over a course of seven days. The injections were administered blind. Those receiving clonidine (CLON) got 375 $\mu g/kg$ at each injection. This dose was found in a pilot series of tests not reported here to provide a significant reduction in MHPG without grossly impairing the motor functions of the animal. The CLON was dissolved in saline at a concentration of 400 $\mu g/ml$, so each CLON injection was a volume of about 1/2 cc. The injections were given every four hours (except at midnight, when a double dose was given, during the "dark" cycle). See Figure 1 for details of a typical schedule. Kittens receiving saline took 1/2 cc i.p. every four hours. During drug or saline treatment, kittens' weights were monitored, and animals were hand-fed if necessary to prevent weight loss.

Monocular Occlusion

Under ketamine (25 mg/kg) and acepromazine (3mg/kg) i.m. anesthesia, supplemented by topical cloroptic (Allergan), we sutured closed the left eyelid of each kitten. See (5, 42) for further details. Unilateral lid closure lasted for the last five days of CLON or saline treatment. Five days is sufficient to cause a substantial ocular dominance shift in otherwise normal kittens (40).

Biochemical Procedures

Three CSF samples were taken from each kitten in the first two groups. Samples were withdrawn at 5 p.m. on the day preceding the first injection, on the day of lid suture, and on the last day of injections prior to single unit recording (See Figure 1). For CSF withdrawl, kittens were first anesthetized with ketamine and acepromazine, and then a 26 gauge needle was used to pull 0.2-0.4 cc of CSF from the foramen magnum. In some cases we had to separate blood from CSF by centrifugation.

HPLC methods were based on those described in (32). Liquified samples were first centrifuged at 3,000 r.p.m., then monoamine metabolites were separated with reverse phase HPLC on a Waters' micro bondapack C^{18} column using a phosphate buffer (pH 4.8, 4% methanol). A guard column packed with "bondapack C^{18} /coracil" was used. Metabolite levels were quantified using a BAS LC-4 A electrochemical detector set to an oxidative potential of 0.75 V. Resulting peak heights and areas were compared to those produced by external standards. The standards were run before and after the samples. Each sample was run at least twice, and samples which yielded ambiguous or incompletely separated peaks were run several more times until a clear reading was possible.

Cortical norepinephrine and dopamine were assayed by HPLC technique, for the last group of kittens, by methods described in (5, 42). Tissue and CSF samples were frozen at 70° C until HPLC analysis.

Physiological Recording

After one week of drug or saline injections, we prepared kittens in the first two groups for paralyzed single unit recording. Ketamine and acepromazine anesthesia was used. We did a tracheotomy and cannulation of the femoral vein. We paralyzed each kitten by continual infusion of Flaxedil (12 mg/kg/hr) in 5% dextrose/saline solution. The animals were maintained on an

anesthetic mixture of 70% nitrous oxide, 28% oxygen, and 2% carbon dioxide, supplemented by 2 mg/kg Nembutal, i.v., when necessary. End tidal CO_2 was monitored with an Infrared Industries gas analyzer. By adjustment of respirator stroke volume and inhaled CO_2, we kept the exhaled CO_2 at about 3.5%. The animal's temperature was kept at 38° C by a heating blanket in a feed-back loop with a thermometer. ECG and EEG were monitored throughout each experiment. If the EEG showed synchronous activity with paw pinch, Nembutal was administered. Further details of the preparation for single unit recording are in (5, 42).

Area centralae were marked on the tangent screen using the scale developed by Olson and Freeman (41). We used a specially designed *dual* microdrive advanced to record from both hemispheres simultaneously. Two tungsten-in-glass microelectrodes (34) were lowered down the banks of the postlateral gyri in Area 17 of both hemispheres using a 2.5 micron-per-step, microprocessor controlled, stepping motor system. The electrodes had 1/2 to 3 $M\Omega$ impedance at 500 Hz. After local amplification of both electrode signals, one could be selected for passage into further amplification and filtering system. We listened to the spikes on an audio monitor, viewed them on an oscilloscope, and made them available to a computer (MINC) for generating histograms correlated with stimuli on the screen.

Visual Stimuli and Classification of Responses

To test the visual responsiveness of the units, we moved various hand-held stimuli--prints by M. C. Escher, a magican's wand, etc.--near the screen, but after a crude estimate of a unit's preferences was determined, we began systematic study of the receptive field (RF) with a dual projector system for displaying oriented bars on the tangent screen. The projector system could be controlled either manually, or by the computer, to vary stimulus orientation, position, speed and direction of movement, and stimulus length and width. Shutters allowed us to test ON/OFF responses of units.

Each unit was evaluated for the following response features:

(1) RF size, shape, and position. (Receptive fields were plotted for responses through both eyes where possible). Preferred stimulus speed and size. ON/OFF responses.

(2) Ocular dominance--the seven category system devised by Hubel and Wiesel (22) was used.

(3) Selectivity--We used a three category scheme based roughly on (19) to classify cells according to their preference for stimulus orientation or direction of stimulus movement. *Aspecific* units responded equally well to movement in all directions; *Immature* units responded to all directions of movement, but showed a clear preference (factor of 2) for a given orientation or direction; *Selective* units possessed an axis of movement which produced *no* response; usually this axis was orthogonal to the preferred direction.

Recording sites were spaced at intervals of 75-150 μ . About half an hour was spent on each unit, and each experiment lasted between 30 and 36 hours. People who plotted RF's for a kitten were unaware of whether the kitten received CLON or saline injections.

In ten of the animals, we also studied the acute affects of CLON on visual responses of the last unit in the penetration. A single unit was isolated, and its responses were studied before, during, and after *i.v.* administration of up to 750 $\mu g/kg$ CLON.

After the final unit was studied, the kitten was killed with an i.v. injection of 2M *potassium* tartrate, then perfused through the left ventricle with saline. In some case, we examined the occipital cortex to verify that the penetrations were in area 17; in other cases, we removed both area 17's and froze them at -70° *C* for later tissue NE analysis.

RESULTS

General Observations

Beginning about 5-10 minutes after the first injection and continuing throughout the treatment, kittens receiving CLON were less physically active than littermate controls. CLON, in fact, is noted for its sedative effects (44), its ability to suppress exploratory behavior (21), and its increase of acoustic startle thresholds (12). Although our CLON-treated kittens were not as active as the controls, the CLON kittens appeared to sleep *less than* the controls. We would often go into the animal colony to give injections and find a CLON kitten sitting still, staring ahead, while its littermate control slept soundly.

CLON-treated kittens did not gain weight as well as controls, possibly because CLON had an appetite-suppressing quality. If arithmetic is performed on the Table I data, it will reveal that CLON-treated kittens weighed 460 gm at time of recording, vs. 560 gm for controls. Both groups were about 53 days of age at time of last weighing. As noted in Methods, we attempted to counter this by hand-feeding some CLON kittens.

During the seven-day course of CLON treatment, we noted some development of tolerance, or at least a change in the range of behaviors shown by the treated animals. After three or four days, kittens were less sedated by the CLON and sometimes showed sham rage when picked up to be fed.

During treatment, we inspected kittens' eyes in a bright light, comparing CLON-treated and controls for mydriasis (31). CLON-treated kittens usually had slightly more dialated pupils, but constriction of their pupils in bright light was still evident. Corneal reflexes were normal for CLON-treated kittens.

Ocular Dominance and Selectivity of Single Units in Area 17.

The main result of this study is shown in Figure 2, where it can be seen that control animals had a substantial OD shift toward the open right eye, while the CLON-treated kittens had virtu-

ally no shift.

For quantitative comparison of OD shift, we computed a "shift index" based on the seven category system used in the OD histograms. The index is a measure of the average shift per cell away from binocularity; a completely binocular (group 4) cell has a shift of 0, a cell completely dominated by the experienced eye has a shift of 3, and a cell completely dominated by the deprived eye has a shift of -3 (see caption for Table I). The seventh column of Table I lists the shift index for all ten kittens used for the OD shift study. The average shift for controls was 1.6 units, for CLON-treated animals, it was 0.4 units.

To illustrate the range of variability in OD shift from one penetration to another, six histograms from CLON-treated kittens are shown in Figure 3. Histograms range from a "negative shift" of 0.9 units for the left hemisphere of K188 to a nearly complete shift of 2.38 in the left hemisphere of K191. In the latter case, it is possible that the small population of cells recorded (N=13) was a poor sample of the ocular dominance of the hemisphere as a whole. Despite this variability, however, Figure 2 makes it clear that CLON-treated animals had, overall, less OD shift than littermate controls.

Did seven days treatment with CLON affect RF properties other than ocular dominance? We looked at *direction selectivity* in particular and found no significant differences between control and CLON-treated cell populations--both groups had about 50% truly selective units and about 20% aspecific units, as based on the classification scheme described in Methods. Other single-unit properties, such as spontaneous firing rate, responses to flashed stimuli, and RF size, did not seem significantly different between the two groups either.

Did *acute* injection of CLON affect RF properties of kitten cortical neurons?* We studied the visual responses of 10 neurons in 10 different animals (CLON-treated and control) before, during, and after i.v. administration of up to 800 $\mu g/kg$ CLON. This large dose has a dramatic effect on *heart* rate, lowering it by 20-40% within minutes.

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* animals received their last i.p. dose of CLON 16 hours before single unit recording began.

In a few cases, there was a slight transient increase in spontaneous neural activity of the cell being recorded from. In no cases, however, were any sustained changes in visual response properties observed. In particular, ocular dominance and selectivity judgements remained the same before and after the i.v. injections of CLON. We conclude that the lack of ocular dominance shift observed in monocularly deprived CLON-treated kittens was *not* due to direct immediate action of CLON on visual responses of cortical neurons.

Were ocular dominance and selectivity of single units correlated in either the CLON or control cells? Figure 4 shows that in the control kittens, both selective and aspecific units had substantial shifts, while in the CLON-treated kittens, neither category showed much shift. Thus, direction selectivity could not be used as predictor of ocular dominance shift in either category.

Biochemical Analyses

By the HPLC techniques outlined in Methods, we were able to determine CSF levels of MHPG, the primary central metabolite of NE, 5-HIAA (a metabolite of dopamine) and HVA (a serotonin metabolite). Figure 5 shows the pooled data from controls, from kittens two days into CLON treatment, and from kittens after seven days of treatment. Note the decline of nearly 50% in MHPG concentration after seven days of CLON. 5-HIAA also declined substantially, while HVA levels were essentially unchanged. Our values of CSF metabolites in kittens are in the same range as those reported for adult cats (15). Although CLON effects on MHPG have not heretofore been reported in cats, in rats CLON has been shown to produce an MHPG decline of 37% (7, 53) and over 50% in monkey (36).

Did decrease in MHPG correlate with lack of ocular dominance shift? Table I suggests a weak correlation. The one control animal with the *least* shift (K187) also had a slight *decrease* in MHPG concentration over the course of seven days, while the one CLON-treated kitten with the *greatest* shift (K191) had the *least* change in MHPG of any of the CLON-treated animals. However, both K187 and K191 had shift indices of 0.91, while K187 had a MHPG decline of 10% versus 31% decline for K191; so not much should be made of their OD shift/MHPG-decline rank-

ings.

Figure 6 shows that levels of the catecholamines norepinephrine (NE) and dopamine (DA) in area 17 tissue were not significantly different in CLON-treated and control kittens. Note from Table I that a separate series of kittens was used for this study to reduce the possible influence of surgical stress on catecholamine levels. The results in figure 6 mean that our CLON-treated kittens had *normal* levels of NE in area 17, *yet failed to show the expected OD shift*, a point to be taken up in the discussion.

DISCUSSION

To recapitulate our results: Seven days of treatment with clonidine (CLON), overlapping five days of monocular deprivation (MD), prevents the usual area 17 ocular dominance (OD) shift we observed in littermate control kittens. The CLON treatment reduced MHPG concentration in CSF by nearly 50%, while not altering at all the cortical concentration of MHPG's precursor, the transmitter norepinephrine (NE). Both single unit recording and biochemical analyses were done without knowledge of whether a kitten received CLON or saline injections. Clonidine, administered acutely to kittens, did not directly alter receptive field (RF) properties, including OD.

Did clonidine prevent OD shift by interfering with NE transmission?

Others have shown that CLON dramatically suppresses the firing of locus coeruleus (LC) neurons (3, 4, 9, 52) and that CLON thus prevents release of NE (15A, 50). As an α–2 agonist, clonidine presumably works by overwhelming feedback receptors on LC terminals in cortex– receptors normally activated release of NE. Once activated, these α–2 receptors cause the LC neurons to hyperpolarize and stop firing. Our results that CLON reduces MHPG, *but not NE*, levels are consistent with this explanation of CLON's action.

That we found CSF levels of 5-HIAA an average of 35% lower after CLON treatment suggests that central serotonergic transmission was also impaired. This result is in keeping with electrophysiological studies showing that CLON depresses the firing rates not only of LC neurons, but also of serotonin-containing neurons in the Raphe nuclei (52). The effect of Raphe neurons was shown to be *secondary* to the effects on LC neurons since direct iontophoresis of CLON onto Raphe neurons did not cause inhibition, and since prior treatment with 6-OHDA abolished the effect of systemically administered CLON on Raphe neurons (52). Although we cannot rule out the possibility that CLON's effect on ocular dominance plasticity is mediated through serotonergic systems, the finding that cortical infusion of 5, 7 dihydroxy tryptamine depletes cortical serotonin without blocking plasticity (26) argues against this interpretation.

Physiological side effects of CLON.

CLON causes a decrease in blood pressure and a slight dialation of the pupils. We have no reason to believe CLON's affect on blood pressure had an influence on ocular dominance shift, especially after recording no significant changes in RF properties after i.v. injections of CLON much larger than any used during our drug conditioning. With regard to pupil dialation, we have recorded complete OD shifts in kittens with very dialated pupils (11A) albeit in dim light. Again, we feel this slight side effect had little or no influence on the lack of OD shift we observed.

Clonidine and physical activity of kittens.

CLON possesses sedative properties, although these appear to vary somewhat from species to species. In cats, CLON actually *decreases* sleep time while increasing the time spent in a drowsy waking state (33). We observed this motionless, but awake, state in our CLON-treated kittens. Is it possible that reduced activity of CLON-treated kittens may have contributed to their lack of OD shift in response to MD? In other words, is the experience of "optical flow" necessary for critical period plasticity? We mention two unpublished observations which bear on this question. In one experiment, we sedated a MD kitten with ethanol to a point where the kitten was not physically active, but was still awake. This did *not* prevent the expected OD shift. In another experiment, we attempted to limit optical flow for a MD kitten by allowing it visual experience of only distant objects. This kitten *did* show a lack of shift, and we are persuing this latter result. We think that, of CLON's various side effects, its suppression of normal running and playing is the most troublesome.

Comparison with catecholamine depletion studies.

The lack of ocular dominance shift observed in CLON-treated kittens is comparable to that seen in kittens administered 6-OHDA by cortical minipump infusion (42, 13). It is possible to conclude from our present results that decreasing NE transmission *without destroying NE-containing neurons or terminals, or reducing levels of NE in cortex*, is sufficient to block the plastic response to monocular deprivation.

However, as noted in the introduction, several reports have indicated that destruction of NE-containing neurons is not always sufficient to block OD plasticity (2, 5, 14). An important issue in these studies is the *timing* of the treatment with respect to deprivation. It is possible that if NE is depleted at least a week before MD, then compensation mechanisms arise which prevent the loss of plasticity observed in kittens depleted concurrently with MD.

An example of such a compensation would be receptor supersensitivity (48). We could test this notion by administering CLON for a week or more *before* beginning monocular deprivation, because CLON's suppression of MHPG production may last beyond its presence in the brain. Another issue, however, has been raised by Shaw & Cynader (47). *Many* sorts of interference with cortical function, even administration of glutamate, concurrent with MD, may be able to inhibit plasticity. The whole issue of whether NE should be singled out as *the* responsible agent for gating plasticity needs to be carefully thought out. Our results with CLON can be seen to support the NE hypothesis of Kasamatsu, or they can be seen as another example of general interference with normal cortical activity during critical period MD.

FIGURE CAPTIONS

Figure 1.– Timing diagrams of the experimental protocol, at three levels of resolution. Top line shows that kittens were given normal binocular experience until about six or seven weeks of age, when one week of clonidine (CLON) or saline treatment was begun. The middle line shows the intervals of CSF taps, and indicates that five days of monocular deprivation were began at the time of the second CSF tap. The bottom line shows the daily schedule of injections along with the cycle of general illumination in the colony.

Figure 2.– Composite ocular dominance histograms of six CLON-treated kittens and four control kittens. Experimental kittens were given i.p. injections of clonidine according to the schedule in Figure 1. Control kittens received equivalent injections of saline. Injections were done blind. Bars indicate the percentage of cells in each of the seven ocular dominance groups of Hubel & Weisel (22). The deprived and open eyes are indicated by filled and open circles, respectively. Category "B" represents cells driven equally well by both eyes. Results from both ipsi and contra hemispheres are combined in each histogram. Controls have the expected OD shift to the open eye, given that MD lasted only five days. A penetration from one hemisphere in K187 is shown in white. *This one penetration*, out of eight control penetrations, had no shift. Note that the CLON-treated animals show virtually no evidence of an OD shift.

Figure 3.– Individual OD histograms for six penetrations in the CLON treated kittens. Conventions are the same as in Figure 2. Note the variability in ocular dominance from hemisphere to hemisphere. However, even a pentration like the left hemisphere of K191, which showed considerable shift, is accompanied by the right hemisphere from the same animal, with virtually no shift.

Figure 4.– Matrix of OD histograms from three CLON-treated kittens and three littermate controls. The top row shows OD histograms for units classified as selective or *specific* for direction of stimulus movement. The bottom row shows histograms for those units classed as aspecific or not direction selective. As can be seen, *within* the control and CLON-treated groups, there was

little difference between ocular dominance histograms of selective and aspecific cells.

Figure 5.– Cerebrospinal fluid (CSF) concentrations of three neurotransmitter metabolites from controls (left), kittens two days into treatment with CLON (middle) and kitten after seven days of CLON treatment (right). The metabolites are 3-methoxy-4-hydroxy phenylethylene glycolol (MHPG), the principal CNS metabolite of NE; 5-hydroxy indole acetic acid (5-HIAA) a serotonin metabolite; homovanillic acid (HVA), a dopamine metabolite. The scales on the far left show concentration values for each metabolite in terms of ng/100 ml of CSF. For each HPLC run, results from about 0.3 cc of clear CSF were compared to known standard concentrations to obtain true values for each sample. In the CONTROL category, we include CSF samples taken from CLON-treated kitten *before* treatment started. Our values here are comparable to those published for adult cat (15). Note that both MHPG and 5-HIAA concentrations decline about 40% during the course of seven days CLON treatment. HVA concentration seem unaffected by CLON.

Figure 6.– Levels of norepinephrine (NE) and dopamine (DA) in cortical tissue. These HPLC assays were done in order directly to compare our present results to previous reports from our lab (5, 6, 42) and to reports of others (13, 14, 24). Clonidine, while it *does* affect NE *metabolite* concentrations (see Figure 5) does not seem to affect the concentrations of the catecholamine neurotransmitters themselves; both CLON-treated and saline-treated kittens had about the same tissue levels of NE and DA.

REFERENCES

1. ADER, J. P., AIZENSTEIN, M. L., POSTEMA, F. and KORF, J., Origin of free 3-methoxy-4-hydroxy phenylethylene glycol in rat cerebrospinal fluid, *J. Neural Trans.*, 46 (1979) 279-290.

2. ADRIEN, J., BUISSERET, P., FREGNAC, Y., GARY-BOBO, E., IMBERT, M. TASSIN, J., and TROTTER, Y., Noradrenaline et plasticite du cortex visuel du chaton: un reexamen, *C. R. Acad. Sci. Paris F. Serie III*, 295 (1982) 745-750.

3. AGHAJANIAN, G. K., CEDARBAUM, J. M., and WANG, R. Y., Evidence for norepinephrine-mediated collateral inhibition of locus coeruleus neurons, *Brain Research*, 136 (1977) 570-577.

4. AGHAJANIAN, G. K., and VANDERMAELEN, C. P., α-2- (adrenoceptor-mediated) hyperpolarization of locus coeruleus neurons: intracellular studies in vivo, *Science*, 215 (1982) 1394-1396.

5. BEAR, M. F., and DANIELS, J. D., The plastic response to monocular deprivation persists in kitten visual cortex after chronic depletion of norepinephrine, *J. Neuroscience*, 3 (1983) 407-416.

6. BEAR, M. F., PARADISO, M. A., SCHWARTZ, M., NELSON, S. B., CARNES, K. M., and DANIELS, J. D., Two methods of catecholamine depletion in kitten visual cortex yield different effects on plasticity, *Nature*, 302 (1983) 245-247.

7. BRAESTRUP, C., Effects of phenoxybenzamine, aceperone, and clonidine on the level of 3-methoxy-4-hydroxy phenylglycol (MOPEG) in rat brain, *J. Pharm. Pharmacol.*, 26 (1974) 139-141.

8. CEDARBAUM, J. M., and AGHAJANIAN, G. K., Catecholamine receptors on locus coeruleus neurons: pharmacological characterization, *Eur. J. Pharmacol.*, 44 (1977) 375-385.

9. CEDARBAUM, J. M., and AGHAJANIAN, G. K., Noradrenergic neurons of the locus coeruleus: inhibition by epinephrine and activation by the *α-antagonist* piperoxane, *Brain Research*, 112 (1976) 413-419.

10. CHASE, T. N., GORDON, E. K., and NG, L. K. Y., Norepinephrine metabolism in the central nervous system of man: studies using 3-methoxy-4-hydroxyphenyl-ethylene glycol levels in cerebrospinal fluid, *J. Neurochem.*, 21 (1973) 581-587.

11. CRAWLEY, J. N., LAVERT, R. and RUTH, R. H., Clonidine reversal of increased norepinephrine metabolite levels during morphine withdrawal, *Eur. J. Pharmacol.*, 57 (1979) 247-250.

11A. DANIELS, J. D., PRESSMAN, E., SCHWARTZ, M., NELSON, S. B., and KRAUS, D. J., Effects of luminance and flicker on ocular dominance shift in kitten visual cortex, *Exp. Brain Res.*, 54 (1984) 186-190.

12. DAVIS, M., REDMOND, D. E. Jr., and BARABAN, J. M., Noradrenergic angonists and antagonists: effects on conditioned fear as measured by the potentiated startle paradigm, *Psychopharmacol.*, 65 (1979) 111-118.

13. DAW, N. W., RADER, R. K., ROBERTSON, T. W., and ARIEL, M., Effects of 6-hydroxydopamine on visual deprivation in the kitten striate cortex, *J. Neurosci.*, 3 (1983) 907-914.

14. DAW, N. W., ROBERTSON, T. W., RADER, R. K., VIDEEN, T. O., and COSCIA, C. J., Substantial reduction of cortical noradrenaline by lesions of adrenergic pathway does not prevent effects of monocular deprivation, *J. Neurosci.*, 4 (1984) 1354-1360.

15. DEGRELL, I., VENNER, K., KUMMER, P., and STOCK, G., Monoamine metabolites in the CSF of conscious unrestrained cats, *Brain Res.*, 277 (1983) 283-287.

15A. DELANGEN, C. D. J., HOGENBOORN, F., and MULDEN, A. H., Presynaptic noradrenergic *α-receptors* and modulation of H-Noradrenaline release from rat brain synaptosomes, *Eur. J. Pharmacol.*, 60 (1979) 79-89.

16. EGAN, T. M., MENDERSON, G., NORTH, R. A., and WILLIAMS, J. T., Electrophysiological analysis of 2 adrenoceptor activation in the locus coeruleus, *Br. J. Pharmacol.*, 78 (1983) 3P supplement.

17. FLICKER, C., MCCARLEY, R. W., and HOBSON, J. A., Aminergic Neurons: state control and plasticity in three model systems, *Cellular and Molecular Neurobiology*, 1 (1981) 123-166.

18. FOOTE, S. L., ASTON-JONES, G., and BLOOM, F. E., Impulse activity of locus coeruleus neurons in awake rats and monkeys is a function of sensory stimulation and arousal, *Proc. Nat. Acad. Sci.*, USA, 77 (1980) 3033-3037.

19. FREGNEC, Y. and IMBERT, M., Early development of visual cortical cells in normal and dark-reared kittens: relationship between orientation, selectivity, and ocular dominance, *J. Physiol.*, 278 (1978) 27-44.

20. GRANT, S. J. and REDMOND, D. E., The neuroanatomy and pharmacology of the nucleus locus coeruleus, In *Psychopharmacology of Clonidine*, H. Lal & S. Fielding (Eds.), 2-28, Alan R. Liss Publishing Co., Inc., New York.

21. HOEFKE, W., and JENNEWEIN, H. M., Mechanisms of antihypertensive action of clonidine in relation to its psychotrophic effects. In *Psychopharmacology of Clonidine*, Lal and S. Fielding (Eds). (1981) pp. 75-98, Alan R. Liss Publishing Co., Inc., New York.

22. HUBEL, D. H., and WIESEL, T. N., Receptive fields, binocular interaction and functional architecture in the cat's visual cortex, *J. Physiol.*, 160 (1962) 106-154.

23. KASAMATSU, T., Neuronal plasticity maintained by the central norepinephrine system in the cat visual cortex, *Prog. in Psychobiol. Physiol. Psyc.*, 10 (1983) 1-112.

24. KASAMATSU, T., ITAKURA, T., and JONSSON, G., Intracortical spread of exogenous catecholamines: effective concentration for modifying cortical plasticity, *J. Pharmacol. Exp. Ther.*, 217 (1981) 841-850.

25. KASAMATSU, T., and PETTIGREW, J. D., Depletion of brain catecholamines: failure of ocular dominance shift after monocular occulsion in kittens, *Science*, 194 (1976) 206-209.

26. KASAMATSU, T., and PETTIGREW, J. D., Preservation of binocularity after monocular deprivation in the striate cortex of kittens treated with 6-hydroxydopamine, *J. Comp. Neurol.*, 185 (1979) 139-162.

27. KASAMATSU, T., PETTIGREW, J. D., and ARY, M., Cortical recovery from effects of monocular deprivation: acceleration with norepinephrine and suppression with 6-hydroxydopamine, *J. Neurophysiol.*, 45 (1981) 254-266.

28. KASAMATSU, T. J., PETTIGREW, D., and ARY, M., Restoration of visual cortical plasticity by local microperfusion of norepinephrine, *J. Comp. Neurol.*, 185 (1979) 163-182.

29. KETY, S. S., The biogenic amines in the central nervous system; their possible roles in arousal, emotion and learning. In F. O. Schmitt (Ed.), *The Neurosciences, Second Study Program*, Rockefeller Univ. Press, New York (1970) 324-335.

30. KORF, J., AGHAJANIAN, G. K., and ROTH, R. H., Stimulation and destruction of the locus coeruleus: opposite effects on 3-methoxy-4-hydroxyphenylglycol sulfate levels in the rat cerebral cortex, *Eur. J. Pharmacol.*, 21 (1973) 305-310.

31. KOSS, M. C., Clonidine mydriasis in the cat, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 309 (1979) 235-239.

32. LANGLAIS, P. J., MCENTEE, W. J., and BIRD, E. D., 3-methoxy-4-hydroxyphenylethyleneglycol and other monoamine metabolites in human cerebrospinal fluid, *Clin. Chem.*, 26 (1980) 786-788.

33. LEPPAVUORI, A., and PUTKONEN, P. T. S., Alpha-adrenoceptor influences on the control of the sleep-waking cycle in the cat, *Brain Res.*, 193 (1980) 95-115.

34. LEVICK, W. R., Another tungsten microelectrode, *Med. Biol. Engng.*, 10 (1972) 510-515.

35. MARTIN, P. R., EBERT, M. H., GORDON, E. K. and KOPIN, I. J., Urinary catecholamine metabolites and effects of clonidine in patients with alcohol amnestic disorder, *Clin. Pharmacol. Ther.*, 33 (1983) 19-27.

36. MASS, J. W., and HATTOX, S. E., LANDIS, D. H., and ROTH, R. H., A direct method for studying 3-methoxy-4-hydroxy phenylethyleneglycol (MHPG) production by brain in awake animals, *Eur. J. Pharmacol.*, 46 (1977) 221-228.

37. MASS, J. W., and LANDIS, D. H., In vivo studies of the metabolism of norepinephrine in the central nervous system, *J. Pharmacol. Exp. Ther.*, 163 (1968) 147-162.

38. MCENTEE, W. J., and MAIR, R. G., Memory impairment in Korsakoff's psychosis: A correlation with brain noradrenergic activity, *Science*, 202 (1978) 905-907.

39. MOLLIVER, M. E., Role of monoamines in the development of the neocortex, *Neurosciences Res. Prog. Bull.*, 20 (1982) 492-507.

40. MOVSHON, J. A., and VAN SLUYTERS, R. C., Visual neural development, *Ann. Rev. Psychol.*, 32 (1981) 477-522.

41. OLSON, C. R., and FREEMAN, R. D., Rescaling of the retinal map of visual space during the growth of the kitten's eye, *Brain Res.*, 186 (1980) 55-65.

42. PARADISO, M. A., BEAR M. F., and DANIELS, J. D., Effects of intracortical infusion of 6-hydroxydopamine on the response of kitten visual cortex to monocular deprivation, *Exp. Brain Res.*, 51 (1983) 413-422.

43. PETTIGREW, J. D., and KASAMATSU, T., Local perfusion of noradrenaline maintains visual cortical plasticity, *Nature*, 271 (1978) 761-763.

44. PICKWORTH, W. B., SHARPE, L. G., and GUPTA, V. N., Morphine-like effects of clonidine on the EEG, slow wave sleep and behaviour in the dog, *Eur. J. Pharmacol.*, 81 (1982) 551-557.

45. SARAN, R. K., SAHUJA, R. C., GUPTA, N. N., HASAN, M., BHARGAVA, K. P., SHANKER, K., and KISHOR, K., 3-methoxy-4-hydroxphenylglycol in cerebrospinal fluid and vanillylmandelic acid in urine of humans with hypertension, *Science*, 200 (1978) 317.

46. SCHLUMPF, M., SHOEMAKER, W. J., and BLOOM, F. E., Innervation of embryonic rat cerebral cortex by catecholamine-containing fibers, *J. Comp. Neurol.*, 192 (1980) 361-376.

47. SHAW, C. and CYNADER, M., Disruption of cortical activity prevents alterations of ocular dominance in monocularly deprived kittens, *Nature*, (1984) in press.

48. SPORN, J. R., HARDEN, T. K., WOLFE, B. B., and MOLINOFF, P. B., β -Adrenergic receptor involvement in 6-hydroxydopamine induced super sensitivity in rat cerebral cortex, *Science*, 194 (1976) 624-626.

49. STARKE, K., and ALTMAN, K. P., Inhibition of adrenergic neurotransmission by clonidine: an action on prejunctional α -receptors, *Neuropharm.*, 12 (1973) 339-347.

50. STARKE, K., and MONTEL, M., Involvement of *α-receptors* in clonidine-induced inhibition of transmitter release from central monoamine neurons, *Neuropharm.*, 12 (1973) 1073-1080.

51. SURGOVE, M. F., Effects of acutely and chronically administered antidepressants on the clonidine induced decrease in rat brain MHPG contents, *Life Sci.*, 29 (1981) 377-384.

52. SVENSSON, T. H., BUNNEY, B. S., and AGHAJANIAN, G. K., Inhibition of both noradrenergic and serotonergic neurons in brain by the α -adrenergic agonist clonidine, *Brain Res.*, 92 (1975) 291-306.

53. TANG, S. W., HELMESTE, D. M., and STANCER, H. C., The effect of acute and chronic desipramine and amitriptyline treatment on rat brain total 3-methoxy-4-hydroxyphenylglycol, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 305 (1978) 207-211.

54. VIDEEN, T. O., DAW, N. W., and RADER, R. K., The effect of norepinephrine on visual cortical neurons in kitten and adult cats, *J. Neurosci.*, 4 (1984) 1607-1617.

55. WEINER, J. J., VANDERLUGT, C., LAUGEN, C. D. J., and MULDEN, A. H., On the capacity of presynaptic alpha receptors to modulate norepinephrine release from slices of rat neocortex and the affinity of some agonists and antagonists for these receptors, *J. Pharmacol. Exp. Ther.*, 211 (1979) 445-451.

56. WIESEL, T. N., and HUBEL, D. H., Single cell responses in striate cortex of kittens deprived of vision in one eye, *J. Neurophysiol.*, 26 (1963) 1003-1017.

57. WILKINSON, M., SHAW, C., KHAN, T., and CYNADER, M., Ontogenesis of α -adrenergic binding sites in kitten visual cortex and the effects of visual deprivation, *Dev. Brain Res.*, 7 (1983) 349-352.

58. YOUNG, W. S., and KUHAR, M. J., Anatomical mapping of clonidine (*alpha* -2 noradrenergic) receptors in rat brain: relationship to function. In *Psychopharmacology of Clonidine*, Lal and Fielding, eds. pp. 41-52, (1981) Alan R. Liss, Inc. New York.

TABLE I

Data Base

ANIMAL	SEX	DRUG	AGE	WEIGHT	UNITS	SHIFT	% CHANGE MGPG	CORTICAL NE
K146	F	Saline	56 days	675 gm	57	1.93	0	-
K180	F	Saline	51	575	48	1.92	+6%	-
K187	F	Saline	44	530	56	0.91	-10%	-
K190	M	Saline	48	550	47	1.90	+19%	-
K174	F	CLON	58	620	46	0.63	-56%	-
K175	M	CLON	51	460	56	0.27	-32%	-
K176	F	CLON	53	570	59	-0.50	-33%	-
K188	F	CLON	48	500	51	0.36	-38%	-
K189	F	CLON	41	400	58	-0.78	-58%	-
K191	M	CLON	61	500	44	0.91	-31%	-
The following two kittens were used exclusively to add to the data base on MHPG changes after CLO								
K181	F	CLON	53	475	-	-	-53%	
K186	M	CLON	41	440	-	-	-53%	
The following seven kittens were used exclusively to determine cortical NE levels after CLON treatment, compared to littermate controls;								
K195	M	Saline	63	728	-	-	-	149 ng/gm
K199	F	Saline	50	454	-	-	-	134
K200	M	Saline	71	420	-	-	-	167
K257	M	Saline	44	560	-	-	-	133
K194	F	CLON	54	448	-	-	-	168
K255	F	CLON	36	280	-	-	-	131
K258	F	CLON	63	420	-	-	-	124

NOTES FOR TABLE I

CLON = 375 µgm/kg/4 hr CLON for seven days

Saline = ½cc Saline every 4 hr for seven days

Age and weight are taken at time of recording, or at the time of sacrifice for biochemistry.

The two kittens in the second group (K181, K186) had cloudy left corneas at the time of recording, and were thus excluded from the study of OD shift.

PTO = The seven kittens in the third group, used for cortical NE & DA assay, were not subjected to the stresses of lid suture and CSF taps.

Shift: weights are given to ocular dominance categories as follows:

3 open eye driven

2 only very weak response from closed eye

1 dominated by open eye

0 gp 4 binoc

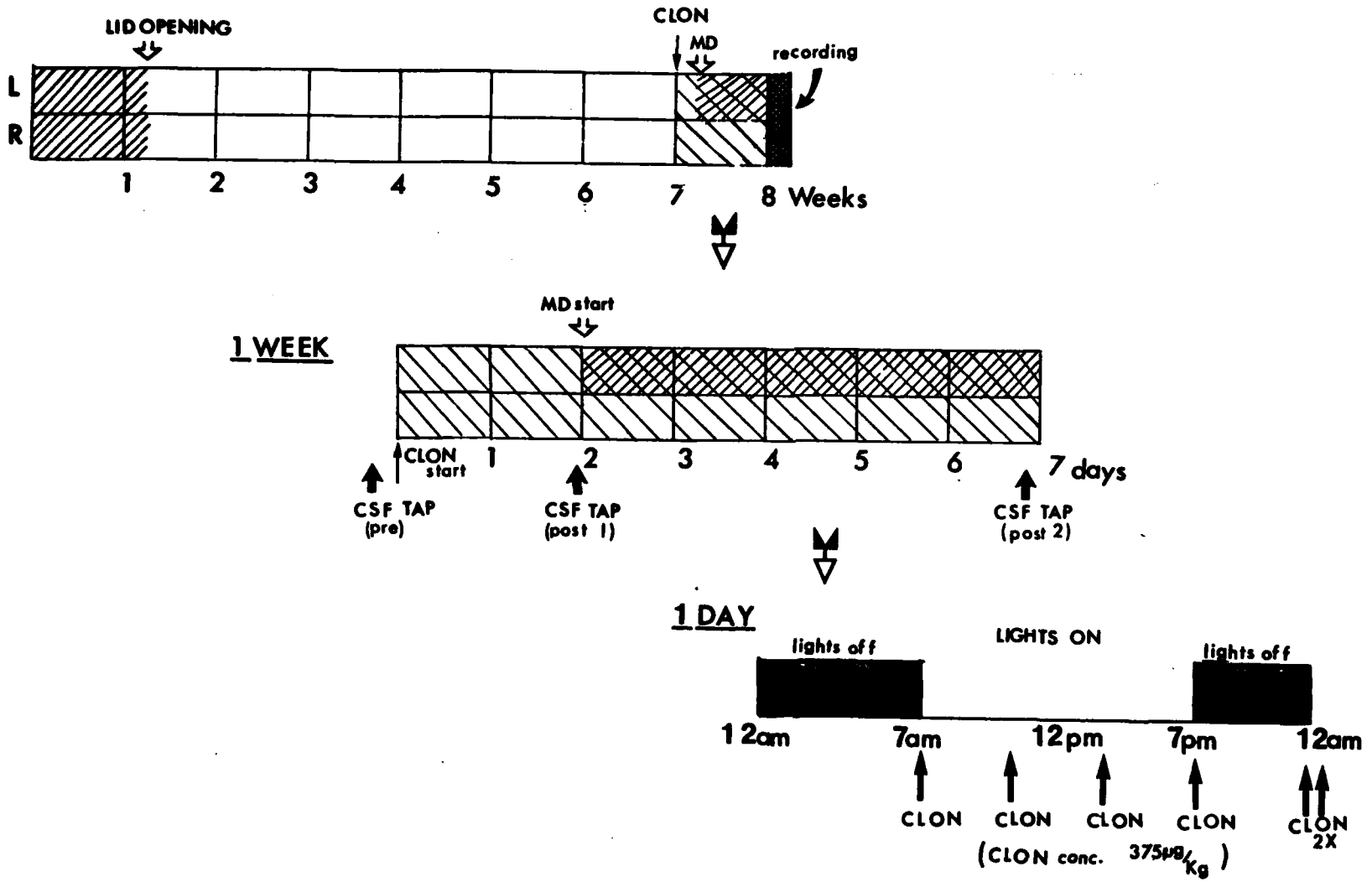
-1 dominated by closed eye

-2 only very weak response from open eye.

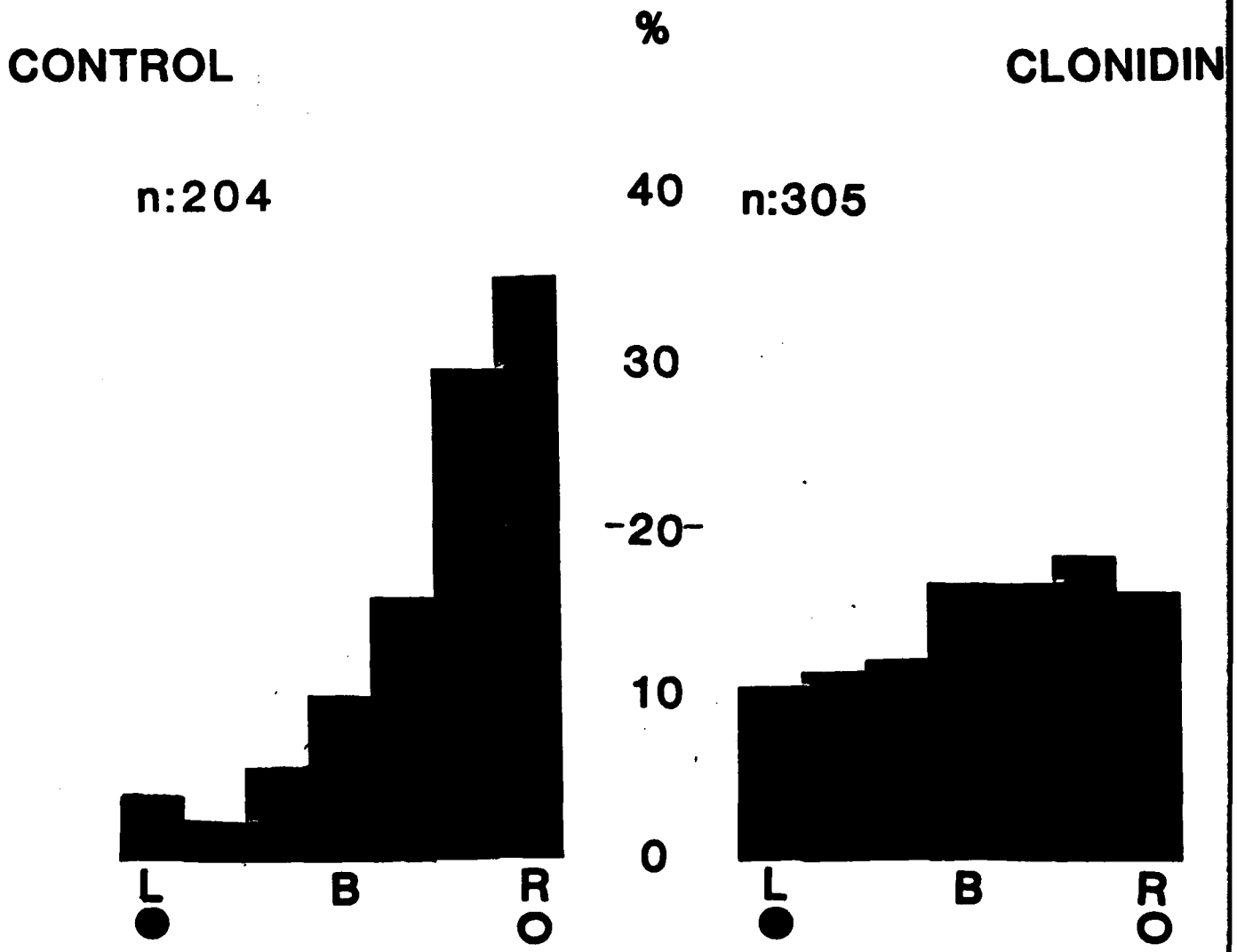
-3 closed eye driven

and an average was computed for all units from a kitten. ±3.00 would represent a complete shift. 0.0 no shift at all.

"% change MHPG" represents the change from CSF tap <u>before</u> CLON or saline treatment, to the CSF tap results after seven days treatment. See text for details of absolute values.


LID OPENING
CLON
MD
recording
L
R
1
2
3
4
5
6
7
8 Weeks
1 WEEK
MD start
CLON start
1
2
3
4
5
6
7 days
CSF TAP (pre)
CSF TAP (post 1)
CSF TAP (post 2)
1 DAY
lights off
LIGHTS ON
lights off
12am
7am
12pm
7pm
12am
CLON
CLON
CLON
CLON
CLON 2X
(CLON conc. 375µg/Kg)

OCULAR DOMINANCE



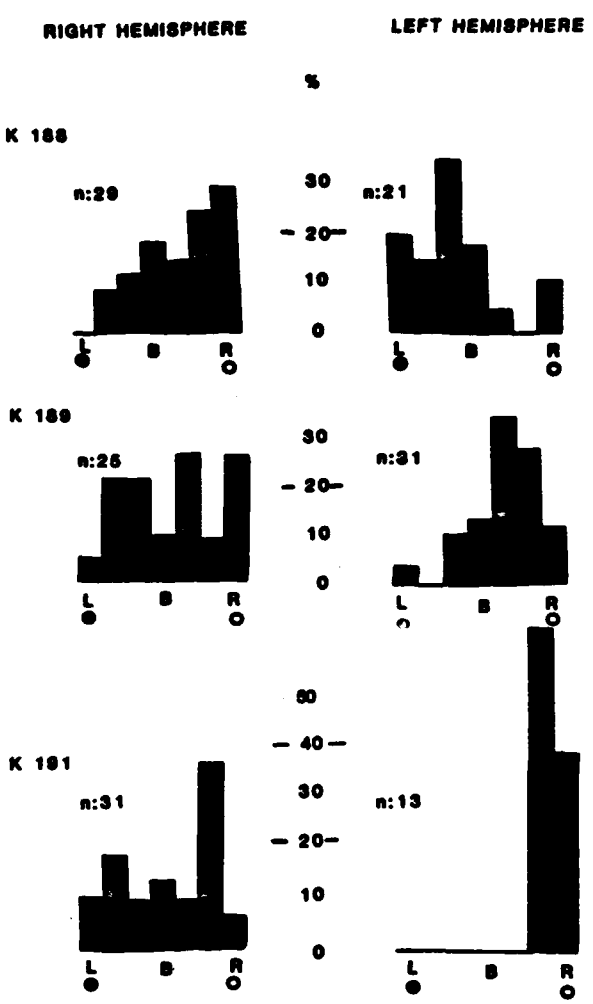

OCULAR DOMINANCE
RIGHT HEMISPHERE
LEFT HEMISPHERE
%
K 188
n:29
n:21
K 189
n:25
n:31
K 191
n:31
n:13
30
20
10
0
40
50
L
B
R

FIGURE 3

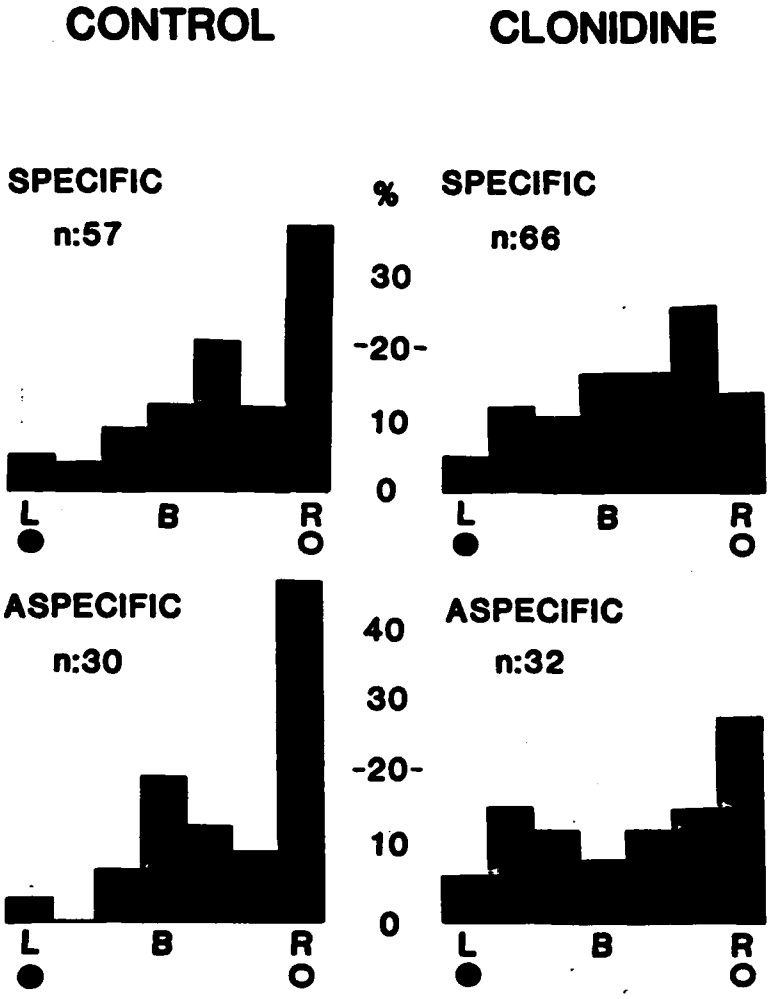
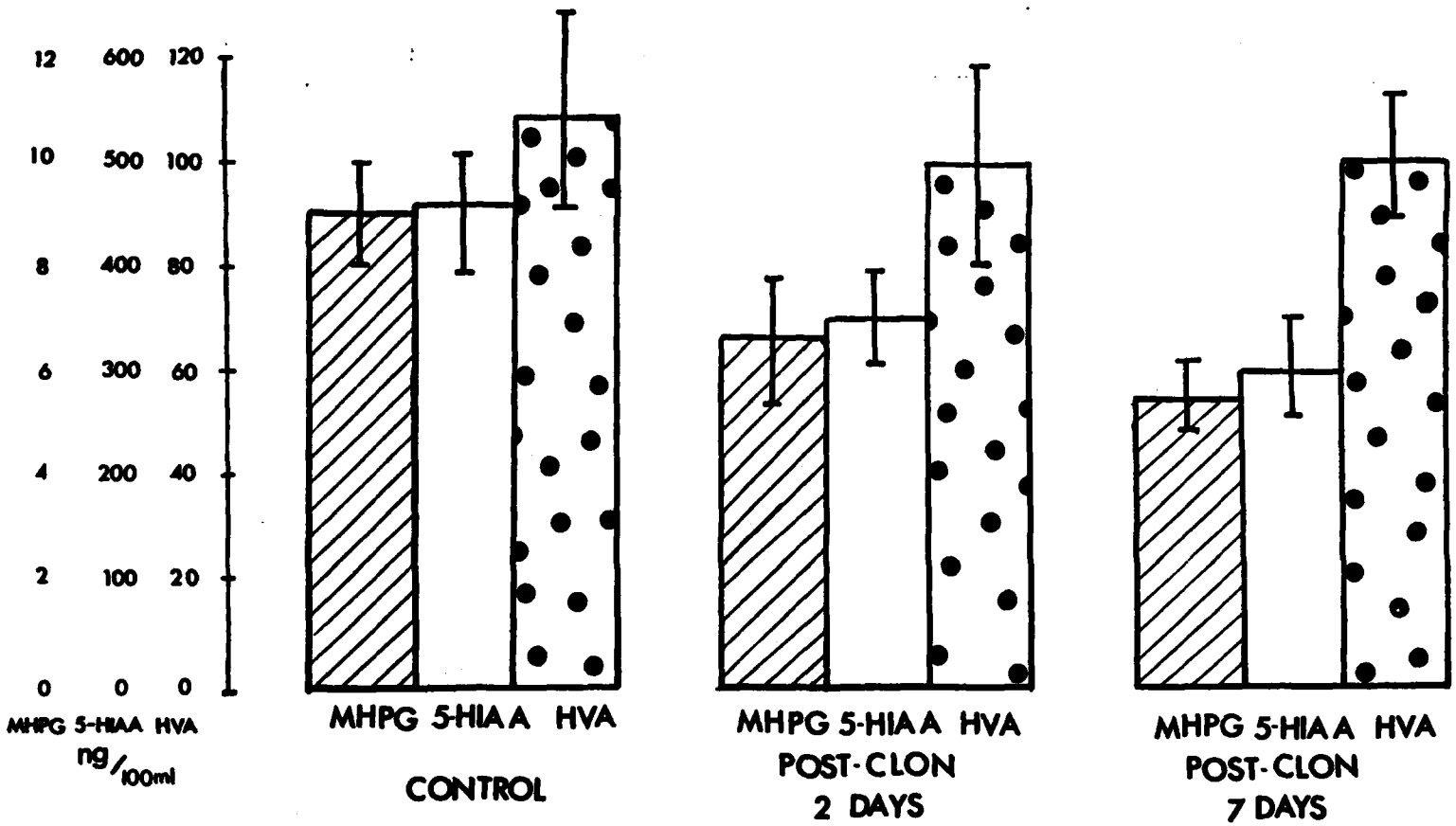

CONTROL
CLONIDINE
SPECIFIC
n:57
%
30
-20-
10
0
L
B
R
SPECIFIC
n:66
ASPECIFIC
n:30
40
30
-20-
10
0
ASPECIFIC
n:32

FIGURE 4

CSF METABOLITES



TISSUE CATECHOLAMINES