THE UNIVERSITY OF CALGARY

THE EFFECTS OF DIFFUSE LIGHT AND PATTERN STIMULATION ON THE METABOLIC ACTIVITY OF THE RAT VISUAL SYSTEM

BY

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF PSYCHOLOGY

CALGARY, ALBERTA

SEPTEMBER, 1986

ISBN Ø-315-32714-6

THE UNIVERSITY OF CALGARY

FACULTY OF GRADUATE STUDIES

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ABSTRACT

The 2-deoxy-D-glucose (2-DG) autoradiographic technique was used to assess visual system metabolic activity in alert, hooded rats, during exposure to black and white stripes, diffuse light, and darkness. Assessments were made by imposing one visual condition on one eye and another on the remaining eye. The rat visual system is largely crossed, therefore interhemispheric differences in 2-DG uptake provided a measure of the relative effectiveness of the three visual conditions.

The study consisted of four experiments, the first two of which were preliminary. Experiment 1 revealed that 24 hours of light deprivation results in less 2-DG uptake than 20 minutes of light deprivation. While the difference was small, Experiment 1 indicated that this effect should be taken into account when interpreting the results for two of the groups in Experiment 3 which had one eyelid sutured shut for 24 hours and the other eye exposed to either a stripes display or to diffuse light. In Experiment 2 atropine was topically applied to one eye and then the rats were contained for the 2-DG uptake period in a back-lit box with horizontal and vertical stripes covering the walls, floor, and ceiling. The results suggested that atropine

hinders perception of the stripes display, so that use of the drug is contraindicated in studies of the present type.

Experiments 3 and 4 showed that the stripes display increased metabolic activity in all major structures of the visual system. In contrast, diffuse light increased activity in the ventral lateral geniculate, increased it only slightly in the dorsal lateral geniculate, produced no effect in the lateral posterior nucleus or cortex, and decreased activity in the superior colliculus. These findings are consistent with the classical role attributed to the thalamocortical system in the perception of spatial arrays of light. They also support the view that the ventral lateral geniculate nucleus is importantly involved in reactions which are governed by light intensity or luminous flux. The significance of the suppression of activity in the superior colliculus in response to diffuse light is less evident. It may reflect a tectal mechanism whereby shifts in the retinal image of the environment are prevented from inducing competing responses during an orienting movement, and thereby separate real from self-induced movement.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my supervisor, Dr. Rod Cooper, for his valuable guidance and generous assistance throughout all stages of this thesis.

Gratitude is also expressed to my Mother, for her consistent encouragement and faith, and to my good friends and classmates, Charlene Senn, Jo-Anne Barnard, and Marlys Reynar, for their ongoing support during my graduate career.

Thanks are given to Gen Thurlow for her friendship and helpful ideas and to John Ewing for some of the computer programs used in this thesis.

Special gratitude is extended in particular to my dear friend, Ingrid Tenor, for her encouragement, sound advice, and supportive pep-talks.

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INTRODUCTION

1. Scope of Introduction

Statement of Problem. The major objective of this thesis was to determine whether the carbon-14, 2-deoxyglucose autoradiographic technique is useful for examining functional activity of the rat visual system. The pioneering, but perhaps flawed, study of Toga and Collins (1981) suggests that it is insensitive. As a further test of the adequacy of the technique rats were exposed to pattern and diffuse light, two distinct stimulating conditions which would be expected to differentially affect regional visual system metabolic activity.

Cellular Response to Pattern and Diffuse Light. Section 2 of the Introduction contains a brief discussion of electrophysiological research on the visual system. The focus of interest is on how various visual structures respond to pattern and diffuse light stimulation.

Luxotonic Units. It is generally held that visual cortex does not respond to diffuse light. Nevertheless there is one group of researchers who claim to have isolated cortical cells in the monkey which respond in a sustained manner to steady diffuse light. The cells have

been termed "luxotonic" units, and they are discussed in section 3.

Technique. In this investigation of the response of the rat visual system to pattern and diffuse light the carbon-14 deoxyglucose (2-DG) autoradiographic technique was employed. Section 4 of the Introduction describes the technique and its application.

2-DG Studies of the Rat Visual System. Few studies have investigated the rat visual system using the 2-DG technique, and only one, by Toga and Collins (1981), has dealt specifically with its response to pattern stimulation. This study and its methodological weaknesses are discussed in the fifth and last section of the Introduction.

2. Cellular Response to Pattern and Diffuse Light

Retina. Electrophysiological studies have shown that diffuse light is much less effective as a stimulus for retinal cells than is a spatial array of light. Kuffler (1953) recorded from single ganglion cells in the cat retina and found that they have concentrically organized receptive fields, with the center and periphery being mutually antagonistic. An on-center cell fires vigorously to a spot of light presented to the center of its receptive field, and activity is suppressed when light is presented

to the surrounding area. Off-center cells react in an opposite manner; that is, light presented to the center suppresses the maintained discharges of the cell, while light presented to the surrounding area produces an increase in firing rate. As a result of the center producing a response opposite to the periphery, when light falls uniformly across the entire receptive field (as is the case with diffuse illumination) the center and surround summate to produce a relatively weak center response (Barlow, FitzHugh, & Kuffler, 1957). Therefore, as early as the output stage of the retina, many cells behave as "contrast" detectors, and fire vigorously to discrete stimuli or contrast and respond poorly to diffuse, full-field, illumination.

Kuffler's (1953) findings have been confirmed by Cleland, Dubin, and Levick (1971) who found that out of 137 cat retinal ganglion cells, only one lacked a concentric receptive field. These receptive field properties have been found not only in anesthetized animals but also in freely moving, unanesthetized ones (Hubel, 1960).

While much research has been performed on the cat and monkey, very few studies have investigated the receptive field properties of retinal ganglion cells in the rat. Brown and Rojas (1965), and Partridge and Brown (1970) studied them and found that some 20-50% of the units lack

the center-surround antagonistic receptive field properties described by Kuffler (1953). This may indicate that rat ganglion cells are more effectively stimulated by diffuse light than are cat.

Dorsal Lateral Geniculate Nucleus. Cells in the cat dorsal lateral geniculate nucleus (LGNd) have been found to have the same concentric receptive field properties as retinal ganglion cells (Cleland, Dubin, & Levick, 1971; Hubel, 1960, 1963). Hubel and Wiesel (1961) recorded from LGNd cells in cats and found that while an on-center cell would fire vigorously to a spot of light presented to the center of its receptive field, firing was suppressed when the stimulus size was increased to include the area surrounding the center, as would occur in diffuse light. Moreover, they report that in some cells diffuse light was a completely ineffective stimulus, and concluded that although the LGN and retinal ganglion cells have similiar receptive field properties, the LGN cells are even less responsive to diffuse light than are the retinal ganglion cells.

On the other hand, Jacobs and Yolton (1970), in their study of 370 LGNd cells in the squirrel monkey, found that 20% gave no evidence of a surround and moreover, that the majority of cells responded to diffuse light almost as

vigorously as to contrast stimulation. Whether their findings reflect a true species difference or a difference in methodology is unclear. Both groups paralyzed their animals but Hubel and Wiesel (1961) maintained their cats on thiopental sodium while Jacobs and Yolton (1970) discontinued anesthetic prior to recording.

Studies of the rat dorsal lateral geniculate nucleus are also marked by inconsistent results. Montero and Brugge (1969) found that increasing the size of a stimulus spot beyond the receptive field center had no effect on the responding of the LGNd cells they studied. The cells responded as well to diffuse light as to contrast. Consistent with these findings, Fukuda, Sumitomo, Sugitani, and Iwama (1979) found that only a small proportion of LGNd cells possessed a clear antagonistic surround. On the other hand, Molotchnikoff, Tremblay, and Lepore (1984) reported that 47% and Lennie and Perry (1981) that 88% of the rat LGN cells possessed center-surround antagonism. Hale, Sefton, and Dreher (1979) found that many (67%) rat LGNd cells showed center-surround antagonism and that the majority of the remaining cells possessed a surround that was antagonistic to the center but was itself silent. That is, the surround would not produce a response when stimulated in isolation, yet it was capable of suppressing the response of the center when a spot of light was

enlarged to include both. These divergent results make it difficult to predict how diffuse and pattern light might affect LGNd metabolic activity but they do point to the need for further study.

Ventral Lateral Geniculate Nucleus. Unlike the LGNd, electrophysiological research on the ventral lateral geniculate nucleus (LGNv) indicates that it may be responsive to diffuse light. In the cat, Spear, Smith, and Williams (1977) recorded from the LGNv and found that of 154 units, 77% were visually responsive; of these, 25% possessed center-surround antagonism and 35% did not. A small minority responded to movement. They also found that 21% responded only to whole field illumination and an additional 10% had a maintained discharge that varied with light intensity. Mathers and Mascetti (1975) recorded from the rabbit LGNv and also found cells with (40%) and without (14%) center-surround antagonism. They report that no cells, however, varied their maintained discharge rate as a function of light intensity.

Hale and Sefton (1978) recorded from the rat LGNv and found that 73% of the visually responsive cells produced a sustained increase in firing to a spot of light, and that just under 50% showed no antagonistic surround. Sumitomo, Sugitani, Fukuda, and Iwama (1979) confirmed that the

majority of rat LGNv cells (88%) produced a sustained response for the duration of the stimulus, however, they report that very few cells possess antagonistic surrounds. Both groups of researchers found that a large proportion of cells increased their firing rates with increases in light intensity. These studies suggest that the LGNv does respond to diffuse light. However, given the discrepancy between the reported incidence of cells in the rat LGNv that have antagonistic surrounds, it is difficult to determine whether diffuse light is as effective as a patterned stimulus.

Superior Colliculus. According to Sterling and Wickelgren (1969) superior colliculus (SC) cells of the cat show little or no spontaneous activity and give either no response to diffuse light or react with a brief on, off, or on-off discharge (Straschill & Taghavy, 1967; McIlwain & Buser, 1968). Like the LGNd, the SC responds better to contrast than to diffuse light, but unlike the LGNd, cells in the SC have been found to respond best to moving stimuli (Sterling & Wickelgren, 1969, 1970; Dreher & Hoffmann, 1973; Mason, 1979). Results similar to those found in the cat have also been reported for the mouse (Drager & Hubel, 1975) and the hamster (Tiao & Blakemore, 1976; Chalupa & Rhoades, 1977; Stein & Dixon, 1979).

Humphrey (1968), studied the receptive field properties of the SC in the rat. He found, as for the cat, that cells reacted poorly to diffuse light flashes and showed little spontaneous activity. In addition, while stationary objects were ineffective in driving cells, moving dark objects on a light background evoked a vigorous response. Fukuda and Iwama (1978) have reported similiar results for the albino rat. For the SC, then, there is good consensus among researchers studying different animals that diffuse light is an ineffective stimulus, and that the SC cells respond best to stimuli in motion.

Lateral Posterior Nucleus. The lateral posterior nucleus (LPN) of the rat thalamus, the homologue of the pulvinar in primates, unlike the LGN and the SC, is not a primary terminal station for optic nerve fibers. It nevertheless receives major inputs from both the SC and visual cortex (Mason and Groos, 1981) and does show changes in metabolic activity following optic nerve section (Cooper & Thurlow, 1985), hence it was included in the structures surveyed in the present study. To date there have been no studies of the visual receptive field properties of cells in this nucleus in the rat. In the cat, however, Wright (1971) found that of the 242 cells studied in the pulvinar complex, 60% were visually responsive. Of these, 58%

responded in short bursts to moving stimuli when they changed direction or velocity, and produced a weak, inconsistent response to flashing stationary stimuli. Thirty percent possessed concentric receptive fields with weakly antagonistic surrounds and 12% would respond only to flashed diffuse light. All the units produced very phasic responses.

Mason (1978) also studied the cat and found that 60% of the 192 units isolated were visually responsive, and of these, 66% responded either to movement in any direction or were direction selective. He also found that 14% responded optimally to diffuse light flashes. While these results agree very well with those of Wright (1971), Mason found that only 7% possessed concentric receptive fields. From the cat evidence, it appears that the majority of cells in the LPN respond optimally to moving stimuli and that diffuse light would only be effective for a small percentage of cells.

The findings are different for the rabbit. Stewart, Towns, and Birt (1973) made recordings from the LPN of 42 rabbits. Of 50 visually responsive cells, 78% responded optimally to diffuse whole eye illumination and no cells were found to have antagonistic surrounds. They also found no cells that were movement or direction sensitive. This study indicates that, unlike the cat, the majority of cells

in the rabbit LPN respond well to diffuse light stimulation and may respond poorly to pattern stimulation.

Given the absence of research on the receptive field properties of the rat LPN, and the differences between the cat and rabbit, it is difficult to predict how the rat LPN would respond to pattern and diffuse light. However, if the units in the rat LPN respond with only a burst of spikes at the onset and offset of a stimulus, then it is likely that steady diffuse light would be a less effective stimulus than pattern. Alternatively, if the rat LPN contains many cells of the type described by Stewart et al. which respond only to whole eye diffuse illumination, pattern may not be as effective a stimulus as diffuse light.

Visual Cortex. The receptive field properties of visual cortical cells have been studied in the unanesthetized (Hubel, 1959) and lightly anesthetized cat (Hubel and Wiesel, 1959). Under both conditions the cells responded most vigorously to stationary or moving dark bars, slits of light, or edges, and these stimuli had to be oriented at a specific angle to be effective. When light encompassed the entire receptive field, the majority of cells showed little or no response- diffuse light was an ineffective stimulus for cortical neurons.

It has been reported that visual cortical cells of the rat will respond to diffuse light flashes, but they are much more responsive to discrete stimuli such as objects or edges in motion (Shaw, Yinon, & Auerbach, 1974; 1975; Diao, Wang, & Pu, 1983). There is, however, some controversy with respect to receptive field specificity. Shaw, Yinon, and Auerbach (1975) found that 41% of the visual cortical cells sampled had "indefinite" receptive field properties. That is, although they were visually responsive, they appeared to lack a preference for any particular stimulus. The majority of the remaining cells responded best to moving stimuli and only 11% were orientation selective. Wiesenfeld and Kornel (1975) sampled 107 visual cortical cells. They found that 53% were responsive to stationary flashing stimuli or objects in motion, while 47% required moving stimuli. About 31% of the cells were orientation selective. More recently, Burne, Parnavelas, and Lin (1984) found that of 296 visually responsive units, 79% were orientation selective. It was their contention that the visual cortex of the rat was not qualitatively different from that of more "visual" animals such as the cat and monkey.

The discrepancies in the results of the three studies cited above may be partly attributable to the notion each group of researchers had of the refractive state of the rat

eye. Shaw et al (1975) found the rat eye to be about +7 to +11 diopters hypermetropic (farsighted) and employed lenses to correct for this. However, Glickstein and Millodot (1970) found that refracting a small eye poses difficulties and can lead to spurious measurements of hypermetropia. Indeed, Hughes (1977, 1979) reported that with a small pupil, the rat eye is nearly emmetropic (normal). Therefore, when Shaw et al. "corrected" their rats' vision they were, in effect, reducing acuity. Although Wiesenfeld and Kornel (1975) did not place lenses on their rats, both they and Shaw et al. administered atropine to their rats. Atropine not only dilates the pupil but also paralyzes accommodation, thus making it unlikely that a sharp image was formed on the retina. It was only Burne et al. who neither used lenses nor applied atropine, who reported that the rat visual system responds similarly to more "visual" animals. It is possible that the other studies failed to reach this conclusion simply because retinal images were not in proper focus.

3. Luxotonic Units

Not all investigators share the conviction that cortical cells, even in visually advanced animals, are unresponsive to diffuse light. One group has identified cells that respond to diffuse light. These cells, termed

"luxotonic units" by Bartlett and Doty (1974) are defined as those which respond to diffuse, featureless, wide angle illumination with an increase of their firing rate by a factor of two or more over spontaneous rates, or conversly, decrease their firing rate by at least 50%. Furthermore, to be so classified these cells must maintain an altered level of firing for at least 60 seconds. Therefore, units that respond to diffuse light with a short burst of activity would not be considered luxotonic units.

Bartlett and Doty (1974) recorded from 239 units in the visual cortex of unanesthetized, paralyzed squirrel monkeys and classified 40% of the units as luxotonic. Of these, the ratio of cells increasing their firing rate to diffuse light to those decreasing their firing, was 60:40. Bartlett and Doty found that the responsiveness of luxotonic units was disrupted or abolished with the administration of a light anesthetic (nitrous oxide), and suggested that others have failed to isolate such units due to anesthetic effects or simply because steady diffuse light was not employed as a stimulus. While 44% of the luxotonic units they found had no definite receptive field properties, others had small fields and would respond to pattern stimuli. It was their belief that due to the high light levels used, and the rapid rate of light adaptation, luxotonic units were likely fed by cones. Finally, while

their criterion for the classification of a unit as luxotonic only requires that the cell maintain responding for longer than 60 seconds, they observed several units that maintained a response for an hour.

Kayama, Riso, Bartlett, and Doty (1979) found that, of the 244 visual cortical units studied in alert macaques, 30% were luxotonic. The ratio of units that responded with an increase in firing to those that decreased their firing was 80:20. Again, in the macaque, as in the squirrel monkey, luxotonic units were found to be very sensitive to anesthetics (Riso, Brust-Carmona, Bartlett, & Doty, 1979).

DeYoe and Bartlett (1980) recorded from the visual cortex of alert, paralyzed cats. In contrast to the monkey studies, only 1.5% of the 528 units isolated could be classified as luxotonic. Furthermore, Kahrilas, Doty, and Bartlett (1980) studied 158 visually responsive units in alert rabbits and found only 2% that were luxotonic. To account for the species difference it was suggested by Kahrilas et al. that the primate may be unique in that all the retinal ganglion cells project to the lateral geniculate nucleus while this is not the case for either the cat or the rabbit and further that the luxotonic cortical units may be fed by LGN cells which receive collaterals from optic fibers whose principal function in nonprimates is to subserve pupillomotor control. Central

to this idea, is that luxotonic units play a critical role in governing pupil size and that the units have become "corticalized" in the monkey but remain subcortical in cats and rabbits.

Alternatively, they have proposed that the variation in the number of luxotonic units found in different species is an indicator of the relative strength of the center-surround response. That is, the majority of cells in the cat and rabbit have antagonistic receptive field surrounds that effectively cancel the response of the center; these units respond exclusively to pattern. However, it has been found in the lateral geniculate of the squirrel monkey that the strength of the surround can vary widely from cell to cell (Jacobs & Yolton, 1970). Given full field illumination, the surround of certain cells completely suppressed the response of the center, while for other cells the surround was strong enough to actually dominate the response. For yet a third type of cell the surround was very weak and consequently the center response predominated under full field illumination. Therefore, it is possible that luxotonic units may simply reflect the balance between a cell's receptive field center and surround. However, it must be noted that Jacobs and Yolton (1970), in their investigation of the center-surround

balance, used full field flashes of light and not the steady diffuse light used to identify luxotonic units.

Conclusion

The evidence presented from electrophysiological research indicates that, in general, the visual system is more responsive to spatial arrays of light than featureless, diffuse light. The center-surround antagonistic receptive fields of retinal ganglion, and geniculate cells, and the complex receptive field organization of visual cortical cells, all increasingly select against activation by diffuse light. In the rat, it seems clear that visual cortex and superior colliculus cells respond best to pattern stimulation. However, studies of the receptive field properties of dorsal and ventral lateral geniculate cells have produced varied results and as yet examination of the lateral posterior nucleus has been neglected. Therefore, for these structures, the effectiveness of diffuse and pattern stimulation have yet to be determined.

The majority of electrophysiological studies which have investigated the receptive field properties of visual cells have not tested responsiveness to diffuse light or at best test only with light flashes. An exception, are those studies of luxotonic units. However, since the existence

of luxotonic units seems at best only established for the monkey, and have not been observed in the cat or rabbit, their existence in the rat would be surprising. Nevertheless, the allusion to luxotonic units does underscore the fact that the characterization of the properties of visual stimuli which drive visual system cells has by no means been exhaustive. At least in the monkey, simply prolonging stimulus presentation admits of new ways of describing the light conditions which affect visual cells.

Anesthesia, muscle paralysis, artificial maintenance of temperature and respiration are characteristic features of electrophysiological recording studies and perhaps a less than normal physiological state limits the observation of truly ecological receptive field properties. This, and the inherent selectivity of the recording electrode, can be circumvented by the 2-DG technique. This technique, which is described below, does not provide the resolution afforded by electrophysiological techniques, but it does enable the investigation of the entire brain of alert, behaving animals under more natural conditions.

4. The 2-Deoxy-D-[^{14}C]Glucose technique

The 2-deoxy-D-[^{14}C]glucose technique (Kennedy, DesRosiers, Jehle, Reivich, Sharp, & Sokoloff, 1975;

Sokoloff, Reivich, Kennedy, DesRosiers, Patlak, Pettigrew, Sakurada, & Shinohara, 1977; Hand, 1981) is a method of assessing regional neural activity throughout the brain over a 45 minute experimental period. The technique involves an intravenous injection of a trace amount of 2-deoxy-D-glucose (2-DG) labelled with the radioisotope carbon-14. The 2-DG is a glucose analogue which, along with glucose, is transported into the cell where hexokinase phosphorylates 2-DG into 2-DG-6-phosphate, and glucose into glucose-6-phosphate. Glucose-6-phosphate continues in the glycolytic cycle to eventually become CO_2 and H_2O. However, the 2-DG metabolism ceases at this point and 2-DG-6-phosphate remains trapped in the cell. Given that neural activity and neural metabolism are closely linked, more active cells will accumulate greater amounts of 2-DG-6-phosphate than less active cells, and thus the tissue radioactivity can be used as an indicator of neural activity. Forty-five minutes after the time of injection most of the free 2-DG is removed from the blood, and the animal is sacrificed. Its brain is removed immediately and frozen to prevent possible diffusion of the isotope in the tissue. The frozen brain is sectioned and dry mounted on slides, which are then apposed to X-ray film. After a sufficient exposure time has elapsed the film is developed and the resulting autoradiograph provides a graphic

representation of the metabolic activity that occurred during the 45 minute period following the 2-DG injection.

Although the 2-DG technique has a resolution that is many factors less than that of unit recording, it has the advantage of providing a picture of neural activity throughout the entire brain of a single animal. Therefore, this technique allows one to investigate the metabolic activity of the entire visual system of an animal under a given stimulus condition. Thus far the technique has proven itself useful in demonstrating orientation columns in the visual cortex of the macaque (Hubel, Wiesel, & Stryker, 1978), and the tree shrew (Humphrey, Skeen, & Norton, 1980), and spatial frequency columns in the visual cortex of cats (Tootell, Silverman, & DeValois, 1981). The technique has also been useful in showing ocular dominance columns in the visual cortex of the rhesus monkey (Kennedy, DesRosiers, Sakurada, Shinohara, Reivich, Jehle, & Sokoloff, 1976), the lack of ocular dominance columns in the squirrel monkey (Hendrickson & Wilson, 1979), and retinotopic organization (Tootell, Silverman, Switkes, & DeValois, 1982) in the macaque visual cortex.

The rat is a convenient animal to use in 2-DG visual system studies because it is small and requires only a fraction of the expensive 2-DG a larger animal would need. In addition, it possesses a visual system that is

approximately 90-97% crossed (Polyak, 1957; Cowey & Perry, 1979; Jeffery, 1984). Such a strongly crossed visual system allows the direct comparison of the effects of two stimulus conditions within the same animal. Simply by presenting a different stimulus to each eye, the effects in visual structures can be compared across hemispheres. Each animal then, can serve as its own control.

5. 2-DG Studies of the Rat Visual System

Miyaoka, Shinohara, Batipps, Pettigrew, Kennedy, & Sokoloff (1979) employed the 2-DG technique to study the effect of a flashing light on the metabolic activity of the rat visual system. They found that the metabolic activity within the stratum griseum superficiale (SGS) of the superior colliculus (SC) and both the ventral and dorsal lateral geniculate nuclei increased linearly as the intensity of the light was increased logarithmically. On the other hand, they found visual cortex to be essentially insensitive to a flashing light, showing little increase in metabolic rate from 0 to 7000 lux.

Toga and Collins (1981) studied 2-DG uptake in the visual system of freely moving albino rats and employed more diverse stimulus conditions. In addition, they removed one eye and then compared 2-DG uptake in the hemisphere fed by the enucleated eye to the hemisphere fed

by the intact eye. Their stimulus conditions included flashed light of 1, 4, 16.5, or 33 Hz. Total light flux was kept constant across conditions. Two checkerboard patterns were also used; one was flashed and the other was reversed at 16.5 Hz.

In the flashed light conditions, Toga and Collins found that 2-DG uptake in the SGS of the SC increased with flash frequency. So too in the dorsal lateral geniculate nucleus and cortex, but only up to 4 flashes per second. In their flashed pattern condition they found the 2-DG uptake both in the SGS of the SC and the LGNd to be greater in the stimulated hemisphere than in the side fed by the enucleated eye. However, there was no difference between the hemispheres at the cortical level. Finally, in their pattern reversal condition they found a small increase in the LGNd, but no effect in the SC or cortex. Toga and Collins, were surprised by the negative findings for cortex since they expected that it would be maximally activated by pattern stimulation. This prompted them to perform two additional experiments to further investigate the effect of pattern stimulation on cortex. They placed the rats in a glass cylinder and a pattern of vertical stripes of various widths was rotated around it. In one group the animals were free to move in the cylinder, in the other they were lightly anesthetized and immobilized. As in the other

pattern conditions there was no effect in cortex. They concluded that the "rat [visual cortex] does not possess a functional organization highly developed for pattern discrimination and analysis" (p.461).

Given an electrophysiological literature suggesting that rat cortex is not fundamentally different from the cat or the monkey, Toga and Collins' conclusions are difficult to accept. Moreover, their claims are all the more surprising when it is considered that they used eye enucleation, instituted 18-24 hours before the 2-DG injection, as a standard against which they compared the effects of different stimulus conditions. Cooper and Thurlow (1985), in their examination of the sequelae of denervation, demonstrated that 24 hours after eye enucleation 2-DG uptake is reduced below levels resulting from the same duration of eyelid closure. Since Cooper and Thurlow found this difference between eye removal and eye closure, it is difficult to understand how Toga and Collins were unable to observe a difference between eye enucleation and their pattern stimulation. Perhaps equally important, Toga and Collins dilated their rats' eyes with atropine. Presumably they equated an increase in pupil diameter with more effective stimulation. While this may be true for luminous flux, it seems a dubious procedure when the investigation is designed to yield information on pattern

vision, when visual acuity enters as a critical factor, a problem they completely ignore.

6. Foreword

The objective of this thesis was to evaluate the effects of pattern and diffuse light stimulation on the metabolic activity of the rat visual system. The 2-DG technique was used to measure activity throughout the entire brain of alert, freely moving rats. Major structures of the visual system were examined including both dorsal and ventral lateral geniculate nuclei, superior colliculus, lateral posterior nucleus, and visual cortex. The pretectal and accessory optic nuclei were left for future study. Experiment 1 was conducted to determine whether 24 hours of visual deprivation produced by eyelid suturing has an effect on the metabolic activity of the rat visual system. This information was necessary for interpreting the data of Experiment 3 where 24 hour lid suture was used as a condition against which the effects of pattern and diffuse light were compared. The effect of atropine, topically applied to the eye, was investigated in Experiment 2 to ascertain whether this procedure could have been a factor in Toga and Collins' (1981) failure to observe increased metabolic activity in visual cortex during their pattern stimulation. The fourth experiment

provided a better test of the effects of diffuse light than Experiment 3.

EXPERIMENT 1

INTRODUCTION

In 2-DG studies of strongly crossed visual systems it is convenient to present one stimulus condition to one eye and to compare the effects produced in the contralateral hemisphere with the effects produced by some standard visual condition in the other hemisphere. Both eye removal (Toga & Collins, 1981), and eyelid suture (Cooper & Thurlow, 1985) have been adopted as a standard in the past. However, recent research indicates that eye removal alone has complicated effects: the denervated visual system shows a sizeable depression in metabolic activity in the first several days following enucleation with some return of activity over ensuing weeks and months (Cooper & Thurlow, 1985). Thus, at best, eye removal is probably most useful as a "standard" after long postoperative recovery periods when effects have stabilized and even then the wisdom of adopting a condition which itself is less than well understood seems questionable. The less invasive lid-suture procedure seems more attractive but the effects of short-term light deprivation on the metabolic activity of the adult visual system are unknown. Therefore, for 24 hour lid suture to be properly used as a standard condition in determining the effects of pattern and diffuse light,

Experiment 1 was undertaken to find out more about the metabolic effects of this procedure.

METHOD

Subjects

The subjects were four, male, Long-Evans hooded rats between 52 and 79 days old at the time of 2-DG injection. The rats weighed between 200 and 300 grams and were supplied by the University of Calgary animal care services. The animals were housed individually in clear plastic cages and were reared and maintained on a 12 hour light-dark cycle. Food deprivation was used to keep the rats at a constant weight for the duration of the experiment. Water was available ad libitum.

Procedure

Experimental Preparation. Control of visual stimulation was in part achieved by opaque goggles modelled after those used by Miller and Cooper (1974) and which were held in place by a Teflon post. Under sodium pentobarbital anesthetic (Somnotol, 65 mg/kg, i.p.), the Teflon post was fixed to the skull with two stainless steel screws placed in the frontal bone. Following one week of recovery, the animals were adapted to wearing clear lensed goggles (Figure 1). Adaptation, as indicated by a cessation

Figure 1. Clear lens goggles.

of scratching, was usually achieved after three, one hour periods of wearing the goggles.

2-DG Injection. On the morning prior to the day of 2-DG injection each rat was anesthetized with sodium pentobarbital and a catheter filled with a heparin solution (100 USP/ml) was placed in the right external jugular vein (Yoburn, Morales, and Inturrisi, 1984), and brought under the skin to the back between the shoulders, where approximately 8 cm of tubing was left coiled under the skin. The incision on the neck was closed with silk sutures and the end of the catheter plugged. The incision on the back was closed with wound clips and the end of the catheter was anchored to these with suture thread. In addition one eye was sutured shut: animals 325 and 329 had their right eye shut and animals 247 and 326 had their left eye shut.

On the next day the rat was weighed and a 100 uCi/kg dose of 2-[^{14}C(u)]-deoxy-d-glucose (325 mCi/mmol) was withdrawn from its vial with a Hamilton syringe and placed in a centrifuge tube. A stream of nitrogen gas was used to evaporate the alcohol/water vehicle from the 2-DG. Once all the liquid had evaporated the 2-DG was reconstituted with approximately 0.7 cc of normal saline and loaded into a syringe. During the evaporation procedure the rat was

fitted with opaque goggles which covered both the open and lid-sutured eyes and was returned to its home cage. After 20 minutes had elapsed the animal was transferred to a holding cage, where the anchor threads were cut releasing the catheter that was coiled under the skin. This procedure produced no discomfort to the alert, unrestrained animal. The 2-DG injection was delivered through the catheter and was followed with approximately 0.5 cc of saline to flush the tubing. The catheter was then cut and plugged close to the rat's body, and the holding cage was placed immediately into a dark Grason-Stadler animal chest.

Histology and Autoradiography. Forty-five minutes after the 2-DG injection the rat was given a lethal dose of sodium pentobarbital through the catheter, the abdomen was incised and the descending aorta and posterior vena cava were clamped. The right atrium was cut and the animal was perfused through the left ventricle of the heart with 50 ml of normal saline followed by 100 ml of a modified Hand's fixative (Hand, 1981). The brain was then removed and frozen in 2-methyl butane cooled to -50^{o}C. Frontal sections 30μm thick were cut in a cryostat (American Optical, Cryo-cut II) at -20^{o}C, and dried onto warmed microscope slides. The mounted brain sections, together with seven Amersham [^{14}C]methacrylate standards, were apposed to Lo-dose mammography film (E.I. duPont de Nemours

& Co. Inc.), which was then exposed for 14 days and developed.

Analysis of Autoradiographs. To assess carbon-14 uptake quantitatively the autoradiographs were placed against an illuminated panel and measurements were made of the light transmitted through the images produced by the radioactive brain sections. A Spectra brightness spot meter (UB 1/4) mounted with a Canon 50 mm macro lens measured the light intensity within a 260 μm area delineated by a reticle centered within the instrument's viewing field and the meter readings were digitized and stored by a Cromemco System Three micro computer. Prior and subsequent to the measurement of a brain area, light meter readings were made of the images of the seven standards of known carbon-14 content. These values were later used to convert light meter readings of the brain areas to carbon-14 values. Furthermore, for each region measured, a corresponding number of readings were made through the lateral aspect of the ventroposterior thalamic nucleus. These were later used to express 2-DG uptake in a visual structure as a ratio of the uptake in this non-visual structure, thus controlling for possible variations in effective isotope across animals. Measurements of each brain region were made in a

predetermined pattern so that each point measured from the left hemisphere would correspond to the same point from the right hemisphere. All readings of subcortical brain structures were averaged across at least five sections.

In visual cortex, tentative conclusions drawn from visual inspection of the autoradiographs were substantiated by quantitative assessments of one to three sections. To ensure comparability of data across animals, sections were selected according to the position of visual cortex relative to the corpus callosum. In addition, a template was used to place marks around the periphery of the cortical image, which facilitated the task of maintaining a consistent pattern of measurements from the two hemispheres and from section to section. As shown in Figure 4, a total of 33 measurements from each hemisphere were made in supragranular cortex (layers II and III), in layer IV, and in infragranular cortex (layers V and VI).

In order to convert the light meter readings of the X-ray film to tissue carbon-14 content, it is first necessary to know the relationship between these two variables. While some researchers suppose the relation is linear (Sharp, Kilduff, Bzorgchami, Heller, & Ryan, 1983; Mitchell, & Crossman, 1984), others argue that it is non-linear (Kelly & McCullock, 1983; Kuhar & Unnerstal,

1985). These problems were avoided entirely by exposing the seven methacrylate standards of known carbon-14 content along with the brain sections and with the aid of a fortran program which used a spline subroutine (International Mathematics and Statistics Library), a function was found that described the relation between the light meter readings and tissue carbon-14 concentration. The program then used this function to determine carbon-14 values from light meter readings through the various brain areas. Following this procedure a ratio was formed by dividing each visual area carbon-14 value by the carbon-14 value obtained for the lateral ventroposterior thalamic nucleus. Finally, the ratio from one hemisphere was subtracted from the ratio of the corresponding point from the other hemisphere, resulting in the difference scores, (i.e. difference score= [(C14 value for side 1 - C14 value for side 2)/ C14 value for VPN] x 100).

In Experiment 1 and all succeeding experiments (2, 3, and 4) of this thesis the statistical assessment of cortical effects was based on averages of the sampling loci 12-18 in the granular layer of area 17 (Figure 4). This choice was indicated by two major considerations: (1) the granular layer is best defined in the autoradiographs and hence yielded the most reliable light meter readings, and (2) other work from this laboratory (Cooper & Thurlow,

1985) as well as new studies prompted by this thesis indicate that the effects of different visual conditions are most strongly registered in the granular layer of area 17.

While data for the supragranular and infragranular layers, as well as for areas 18 and 18a have been included in the results section of each experiment, these results should be treated with great caution. This caveat is given since (1) the supragranular layers were often subject to sectioning artifacts including rolling and tearing of the tissue, (2) the infragranular layers are poorly defined and result in poor reliablity of light meter readings, and (3) the smaller size of areas 18 and 18a make them more difficult to assess reliably.

The greater variability in the scores for the supra- and infragranular layers and for areas 18 and 18a, likely reflect the difficulties enumerated above, and provide further grounds for minimizing the results for these cortical regions. Fortunately, laboratory technique has now improved so that these problems are less likely to be of concern in future studies.

RESULTS AND DISCUSSION

In Figures 2 B, 3 B, and 5 an upward bar or positive number denotes that the hemisphere receiving input

primarily from the 24-hour lid sutured eye had less 2-DG uptake than the hemisphere receiving input from the eye that was covered for a short period of time. Figure 2 A is a line drawing showing the sampling loci from which the light meter readings were made in the dorsal lateral geniculate nucleus (LGNd), ventral lateral geniculate nucleus (LGNv), and the lateral posterior nucleus (LPN). The difference scores for these structures are shown in Figure 2 B. The LGNd showed the most consistent effect; 2-DG uptake was greater in the hemisphere fed by the eye covered for a few minutes than in the hemisphere contralateral to the lid-sutured eye ($\underline{M}$= 2.58, $\underline{t}(3)$= 9.77, $\underline{p}$<.05). No consistent effect was found for the LGNv, as two animals had positive difference scores and two had negative scores ($\underline{M}$= -.53, $\underline{t}(3)$= -.36, $\underline{p}$>.20). Three rats showed greater 2-DG uptake in the LPN of the hemisphere fed by the eye covered for a short duration than in the hemisphere fed by the lid-sutured eye and in one animal there was no discernible effect ($\underline{M}$= 2.38, $\underline{t}(3)$= 2.59, $\underline{p}$<.05). Figure 3 A shows the sampling loci for stratum griseum superficiale (SGS), stratum opticum (SO), and stratum griseum mediale (SGM) of the superior colliculus and Figure 3 B shows the difference scores for these layers. While there were some inconsistencies, the overall tendency across subjects and layers is one of

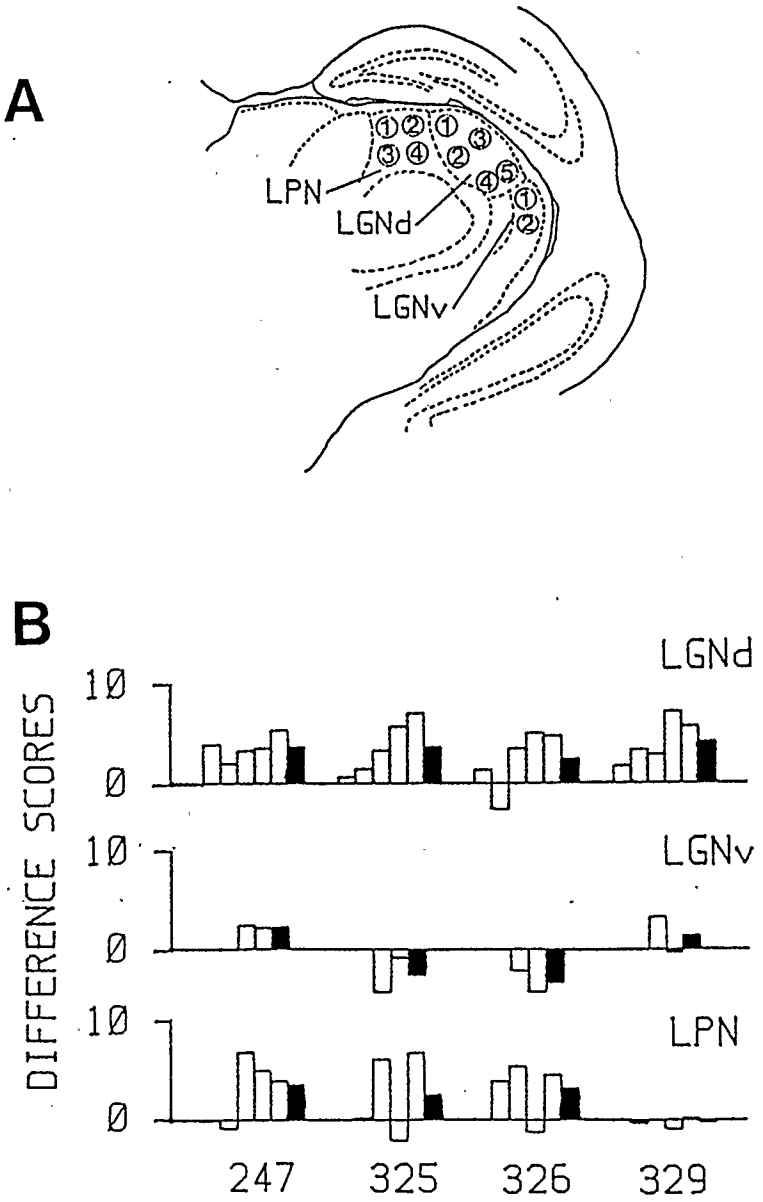


Figure 2. A. Line drawing showing the sampling loci for the LPN, LGNd, and LGNv.
B. Difference scores for the LGNd, LGNv, and LPN for each animal. Hollow bars from left to right correspond to the numbers for each area shown in A, and the solid bars are the mean difference scores for each area. Positive scores indicate decreased 2-DG uptake (lower metabolic activity) contralateral to the lid-sutured eye.

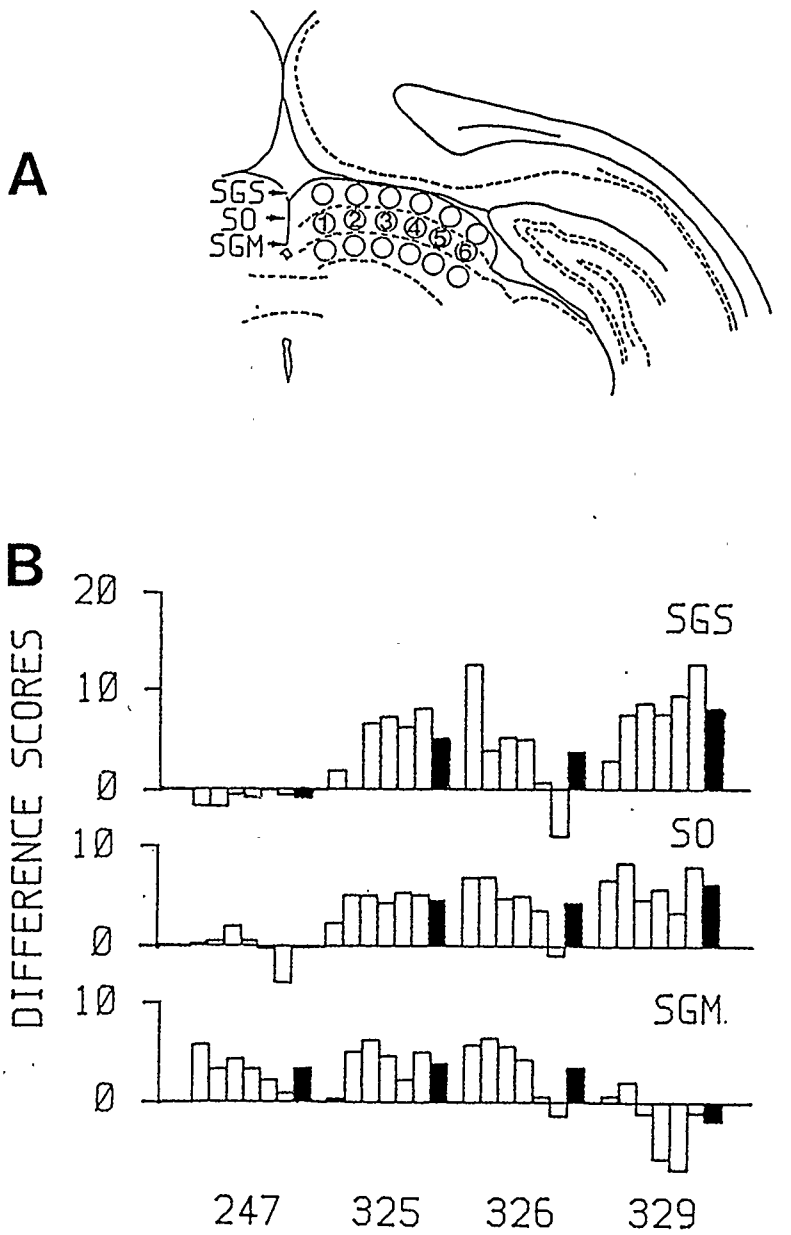


Figure 3. A. Line drawing showing the sampling loci for the SGS, SO, and SGM of the superior colliculus. B. Difference scores for the SGS, SO, and SGM for each animal. Hollow bars from left to right correspond to the numbers for each area shown in A, and the solid bars are the mean difference scores for the total layer. Positive scores indicate decreased 2-DG uptake (lower metabolic activity) contralateral to the lid sutured eye.

depression in the superior colliculus fed by the eye that was lid-sutured 24 hours earlier (SGS, M= 4.10, $t(3)$= 2.15, $p<.10$; SO, M= 3.87, $t(3)$= 2.82, $p<.05$; SGM, M= 2.40, $t(3)$= 1.66, $p<.10$).

Figure 4 is a line drawing showing the sampling loci from which the cortical readings were taken. Figure 5 shows the depression that was produced in visual cortex. There are three lines for each animal in Figure 5; the line made with X's represents the difference scores for the supragranular layers, the line with the square points, layer IV and the line with the filled circles, represents the infragranular layers. Damage during histology precluded the measurement of the supragranular layers for animal 247. The difference scores, from left to right, correspond to points 1 to 33 in Figure 4. All the animals showed less 2-DG uptake in the hemisphere receiving input from the 24-hour lid sutured eye, than the hemisphere fed by the eye that was deprived of light for only 20 minutes prior to the 2-DG incubation period (M= 13.02, $t(3)$= 8.71, $p<.005$).

In conclusion, the results of Experiment 1 indicate that 24 hours of light deprivation by eyelid suturing reduces metabolic activity below that level produced by a short period of darkness. The depression was most evident in the LGNd and visual cortex but was probably not confined

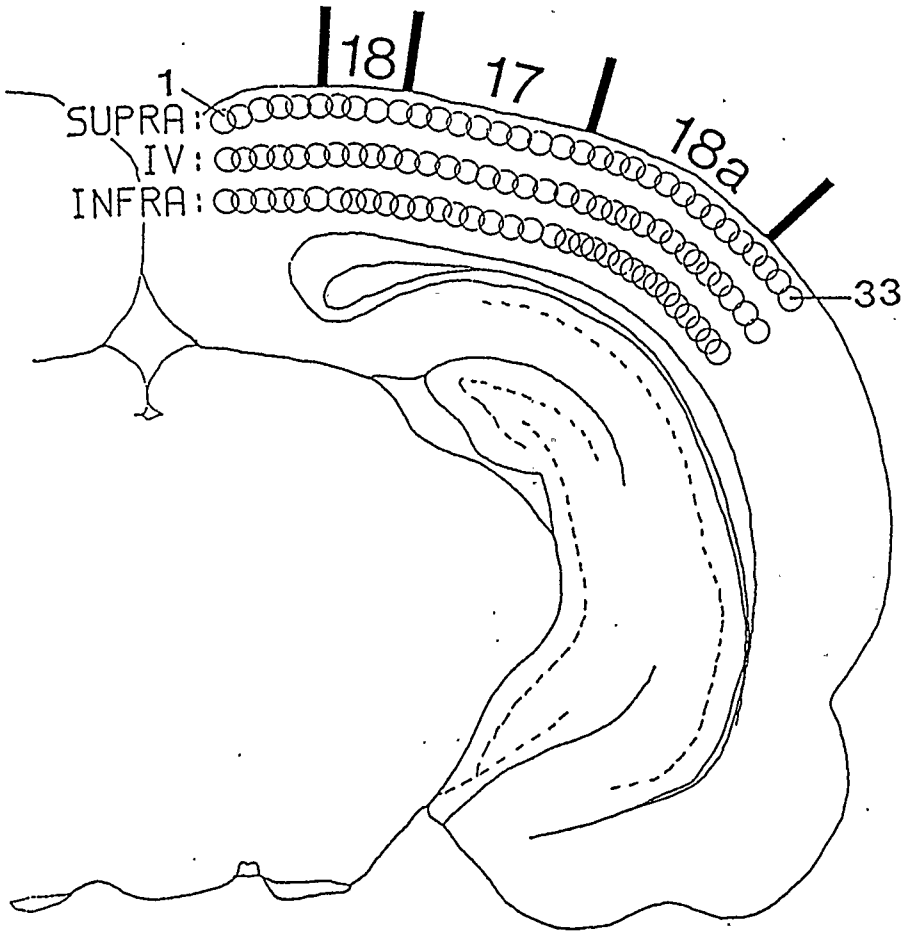


Figure 4. Line drawing showing the sampling loci for the supragranular layers, layer IV, and the infragranular layers of cortex. Shown are the approximate borders of visual areas 18, 17, and 18a.

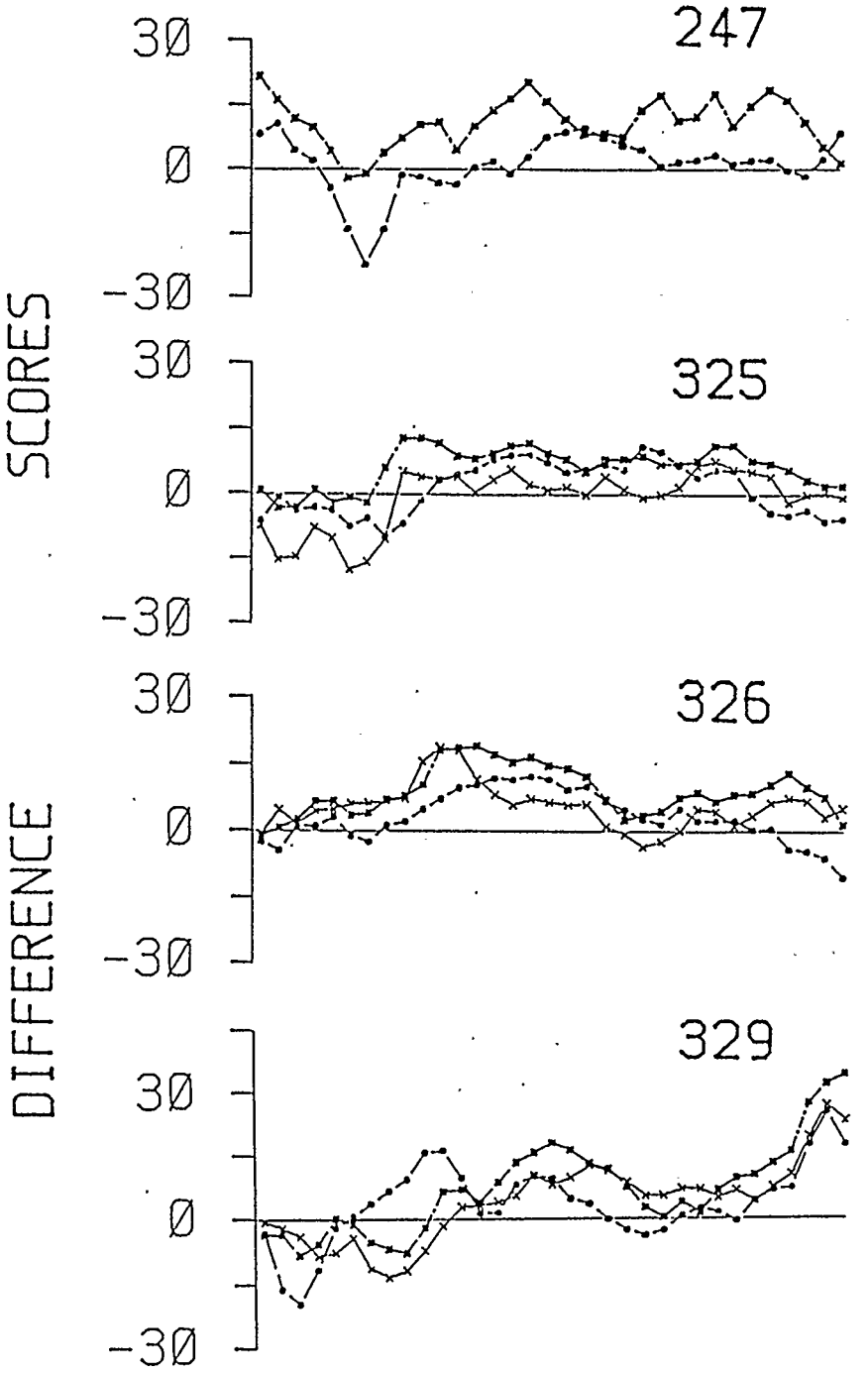


Figure 5. Difference scores for the three layers of cortex for each animal. The supragranular layers are marked with X's, layer IV with squares, and the infragranular layers with circles. Points from left to right correspond to points 1 to 33 shown in Figure 4. Positive scores indicate decreased 2-DG uptake contralateral to the lid sutured eye.

to these areas given the similar trends in the data for the LPN and the SC. Thus interhemispheric comparisons involving a lid-sutured eye, like eye enucleation, are subject to a confound. This should be kept in mind when considering the results of Experiment 3 where some animals had one eye sutured shut and the other eye exposed to either pattern or to diffuse light.

EXPERIMENT 2

INTRODUCTION

Many researchers who have studied the visual system have made it a common practise to dilate their subjects' pupils prior to recording, usually by the topical application of atropine sulfate, homatropine hydrobromide, tropicamide, or phenylephrine hydrochloride to the eye. All these drugs, except phenylephrine hydrochloride, not only dilate the pupil but also paralyze the ciliary musculature, thus preventing accommodation. Since the lens is then fixed for distant objects, stimuli presented to the animal, if not at the correct distance, will be out of focus on the retina. Furthermore, with a dilated pupil, spherical aberration also reduces the sharpness of the retinal image.

In studies of the visual system it is important that sharp images be formed on the retina. This point was made by Ikeda and Wright (1972). They found that the responses of cat retinal ganglion cells were severely reduced when a lens with as little power as two diopters was placed in front of the cat's eye and that an eight diopter lens eliminated the visual response. Fortunately most investigators refract the animal's eye to ensure that the stimulus is in good focus. Some researchers also employ

artificial pupils to limit the spherical aberration which results from pupil dilation.

A number of researchers, however, have applied atropine or similar acting drugs to the rat eye and taken no steps to correct for loss of accommodation or spherical aberration (Fukuda & Iwama, 1978; Fukuda et al., 1979; Diao et al., 1983; Molotchnikoff et al., 1984). Some have refracted their rats prior to stimulus presentation (Brown & Rojas, 1965; Montero et al., 1968; Shaw et al., 1975). Nevertheless, while the refraction procedure may be satisfactory for animals with large eyes, Glickstein and Millodot (1970) have found that the technique can produce spurious indications of hypermetropia (farsightedness) in animals with small eyes. Therefore, applying lenses based on incorrect refraction in an attempt to correct for the loss of accommodation produced by atropine may lead to an even more degraded retinal image.

Hughes (1977) has investigated the refractive state of the rat eye by recording from the optic nerve and noting the effect that lenses of various powers have on the receptive field properties of retinal ganglion cells. He found that with a small pupil the response was best with no lens in front of the eye, but following the application of atropine the best response was achieved with a lens that corrected for a refractive error of 6 diopters

hypermetropic. He concluded that with a small pupil the rat is nearly emmetropic (normal) but once the pupil is dilated the eye shows clear hypermetropia. Consistent with Hughes' findings, Hale, Sefton, and Dreher (1979), in a similiar experiment but recording from dorsal lateral geniculate cells, found that the rat is eight to ten diopters hypermetropic following the application of atropine.

In contrast to the above studies, Powers and Green (1978) recorded from the rat optic tract and found that the response of retinal ganglion cells to vertical grating patterns was reduced no more than 20% by lenses of +14 to -14 diopters and that pupil size had no effect on acuity. It should be noted however, that their rats were visually deprived from birth and conceivably this may have had a bearing on their results. Meyer and Salinsky (1977) presented a grating pattern and recorded cortical evoked potentials. They dilated their rats' pupils prior to recording and found that the refractive error of the eye, based on response attenuation with lenses of various powers, was +0.33 diopters or nearly emmetropic with a fully dilated pupil. In conclusion, the literature is confusing with the lack of consensus as to the effect of atropine on the rat's visual acuity.

In their study of the rat visual system, Toga and Collins (1981) dilated their subjects' pupils prior to the 2-DG injection and took no steps to check or correct the rat's visual acuity. Before investigating the effects of various stimulus conditions on 2-DG uptake it seemed important to determine what, if any effect atropine might have. In the present study atropine sulfate was topically applied to one eye, and the animal was then placed in a strongly patterned box. Interhemispheric differences in 2-DG uptake between visual structures were used as an indicator of the effect of atropine on acuity.

METHOD

Subjects

The subjects were six male, Long-Evans hooded rats between 60 and 88 days old at the time of 2-DG injection. The rats were supplied by the University of Calgary animal care services and weighed between 200 and 300 grams. The animals were housed individually in clear plastic cages, and were reared and maintained on a 12 hour light-dark cycle. Food deprivation was used to maintain weights constant for the duration of the experiment. Water was available ad libitum.

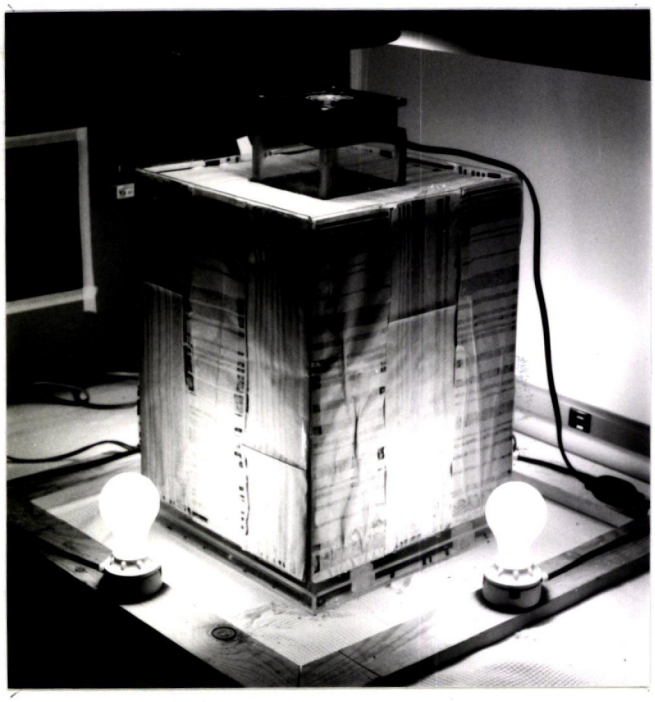
Procedure

Experimental Preparation. The stimulus situation consisted of a clear plastic box which was 29 x 29 x 38 cm high (Figure 6). The walls, floor, and ceiling were covered with high contrast black and white, horizontal and vertical, intersecting stripes of various widths (1-17 mm, 0.6-9.7 degrees visual angle). The walls and ceiling were back-lit with five 60 watt incandescent lamps so that the brightness of the light stripes were 329.7 cd/m^2 (1.98 log foot lamberts) and the dark stripes were 60.9 cd/m^2 (1.25 log foot lamberts) as measured with a SEI exposure photometer (Salford Electrical Instruments Ltd.). Ventilation was provided by a ceiling-mounted fan, which also supplied a constant background noise. The rats were placed on a 4 x 3.5 cm pedestal in the center of the chamber, elevated 19.5 cm above the floor. The floor was covered with cold water to a depth of 3 cm to discourage the rat from jumping off the pedestal.

The subjects were each given three, one-hour sessions in the stimulus chamber, to accustom them to the chamber and to encourage them to remain on the pedestal.

2-DG Injection.

The morning prior to the experiment each rat was anesthetized with sodium pentobarbital and a catheter was



A

B

Figure 6. A. Exterior of the stimulus chamber.
B. Interior of the stimulus chamber.

placed in its right external jugular vein as in Experiment 1.

On the morning of the 2-DG injection the 2-DG was reconstituted in saline as described in Experiment 1, and the rat was given 1% Isopto Atropine drops (Alcon Laboratories Inc.) in one eye. Subjects 245, 252, and 429 had their right pupil dilated and subjects 225, 428, and 430 had their left pupil dilated. To ensure complete pupil dilation 20 minutes were allowed to elapse between the time the subject received the atropine and the 2-DG injection. After the atropine application the animal was then transferred from its home cage to a holding cage where the 2-DG was injected through the catheter as previously described in Experiment 1. Immediately following the injection the animal was placed in the stimulus chamber.

Histology and Autoradiography. After 45 minutes had elapsed the animal was removed from the stimulus chamber, given a lethal dose of sodium pentobarbital through the catheter and perfused as in Experiment 1. Preparation and quantification of autoradiographs were carried out exactly as described in the previous experiment.

RESULTS

In Figure 7 the difference scores for the dorsal lateral geniculate nucleus (LGNd), ventral lateral geniculate nucleus (LGNv) and lateral posterior nucleus (LPN) are presented for the six animals. For this and Figures 8 and 9 a positive number signifies that the 2-DG uptake or metabolic activity was greater for the hemisphere fed by the untreated eye.

The 2-DG uptake for the dorsal lateral geniculate nucleus was clearly less for the side contralateral to the dilated pupil than for the side receiving input from the untreated eye (M= 11.37, $t(5)$= 14.52, $p<.0005$), (Figure 7). (This was particularly so for positions 1, 3, and 5 which represent the more monocular regions of the rat LGNd.) The differences between hemispheres in the LGNv and LPN were less and not so consistent although there was a trend that was similar to the results observed for the LGNd (LGNv, M= 1.92, $t(5)$= 1.02, $p<.20$; LPN, M= 2.84, $t(5)$= 1.76, $p<.10$).

Figure 8 shows the difference scores for the three superficial layers of the superior colliculus (SC). For stratum griseum superficiale the hemisphere fed by the dilated pupil showed less 2-DG uptake than the hemisphere fed by the untreated eye in all animals but 245 (M= 12.06, $t(5)$= 4.36, $p<.005$). The two deeper layers, stratum

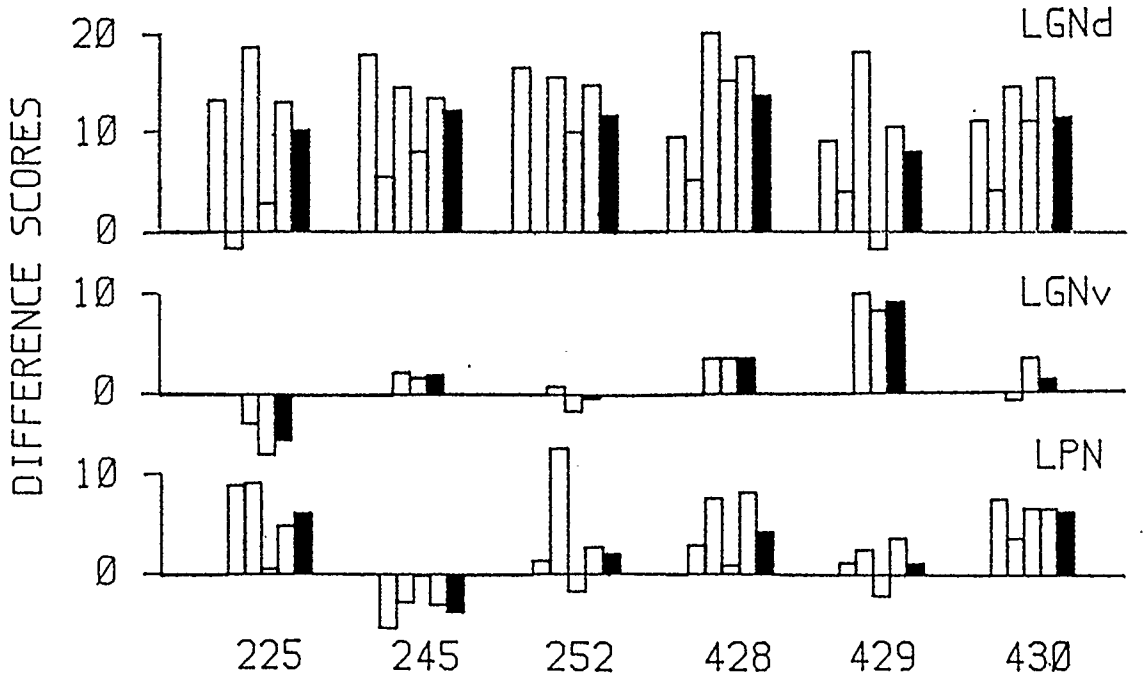


Figure 7. Difference scores for the LGNd, LGNv, and LPN for each animal. Hollow bars from left to right correspond to the numbers for each area shown in Figure 2 A, and the solid bars are the mean difference scores for each area. Positive scores indicate decreased 2-DG uptake (lower metabolic acitivity) contralateral to the atropine treated eye.

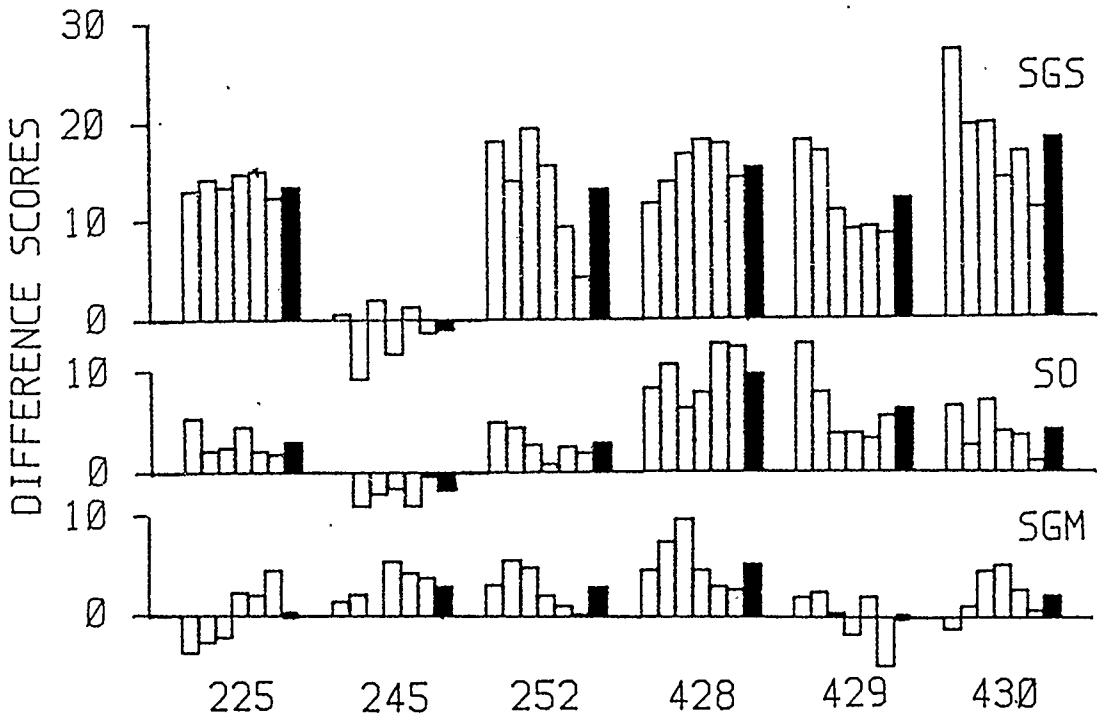


Figure 8. Difference scores for the SGS, SO, and SGM for each animal. Hollow bars from left to right correspond to the numbers for each layer shown in Figure 3 A, and the solid bars are the mean difference scores for each layer. Positive scores indicate decreased 2-DG uptake (lower metabolic activity) contralateral to the atropine treated eye.

opticum (SO) and stratum griseum mediale (SGM), showed a reduced but similar effect for most subjects (SO, $\underline{M}$= 4.15, $\underline{t}(5)$= 2.59, $\underline{p}$<.025; SGM, $\underline{M}$= 2.32, $\underline{t}(5)$= 2.76, $\underline{p}$<.025). It is unlikely that the aberrant results for animal 245 were due to measurement error since this subject also showed an effect opposite to the other animals in the LPN, an area receiving major projections from the superior colliculus.

The difference scores for visual cortex are shown in Figure 9. Readings from medial to lateral visual cortex are depicted as difference scores from left to right. Again, a positive number indicates that the hemisphere fed by the dilated pupil had less 2-DG uptake than the untreated hemisphere. Although, on average, there was less 2-DG uptake in layer IV of area 17 contralateral to the atropine treated eye, the difference was only marginally significant ($\underline{M}$= 6.87, $\underline{t}(5)$= 2.02, $\underline{p}$<.05). Two of the subjects were clearly exceptional and had mean scores in layer IV of area 17 that were in the opposite direction and had a two-tailed test been used, statistical significance would not have been reached.

DISCUSSION

Two-DG uptake in the LGNd and SC contralateral to the atropinized eye was reduced relative to the ipsilateral side. These results do not agree with the findings of

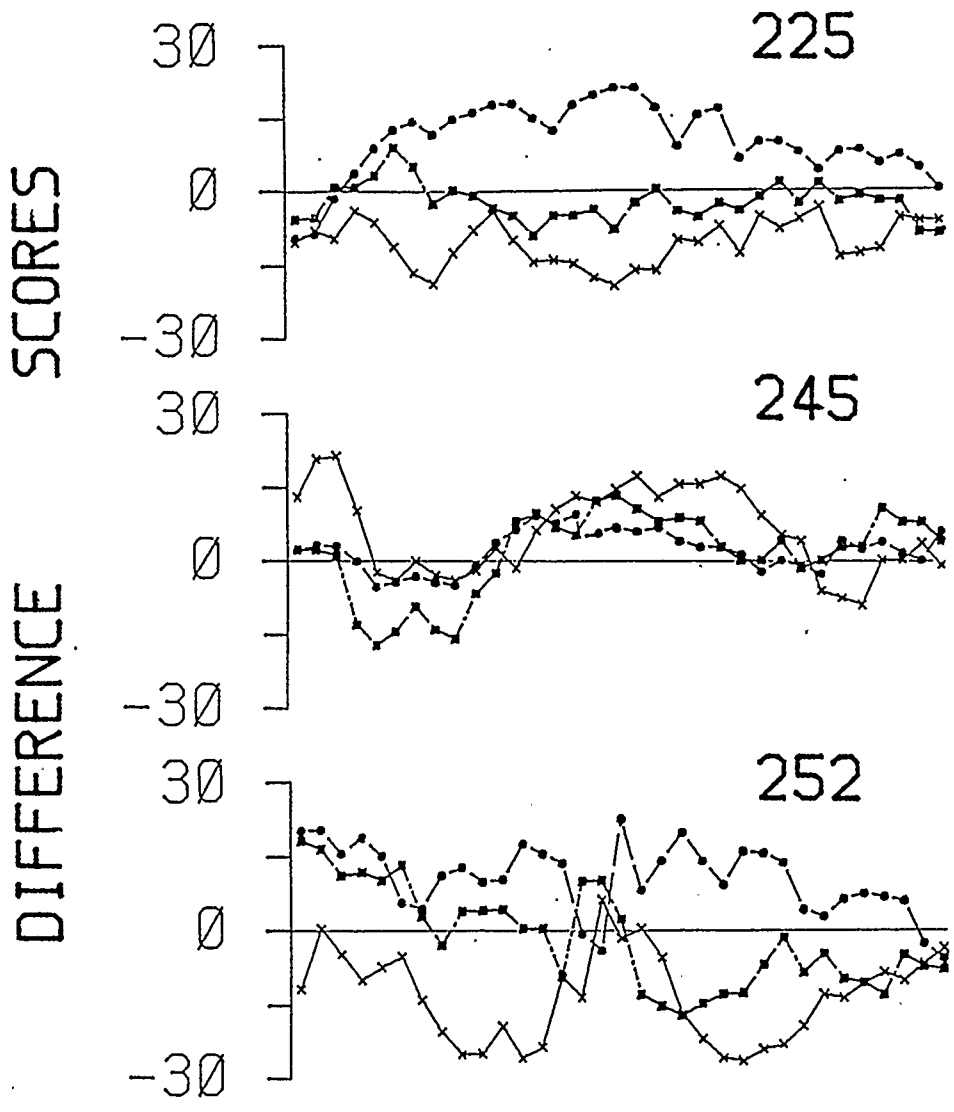


Figure 9. Difference scores for the three layers of cortex for each animal. The supragranular layers are marked with X's, layer IV with squares, and the infragranular layers with circles. Points from left to right correspond to points 1 to 33 shown in Figure 4. Positive scores indicate decreased 2-DG uptake contralateral to the atropine treated eye.

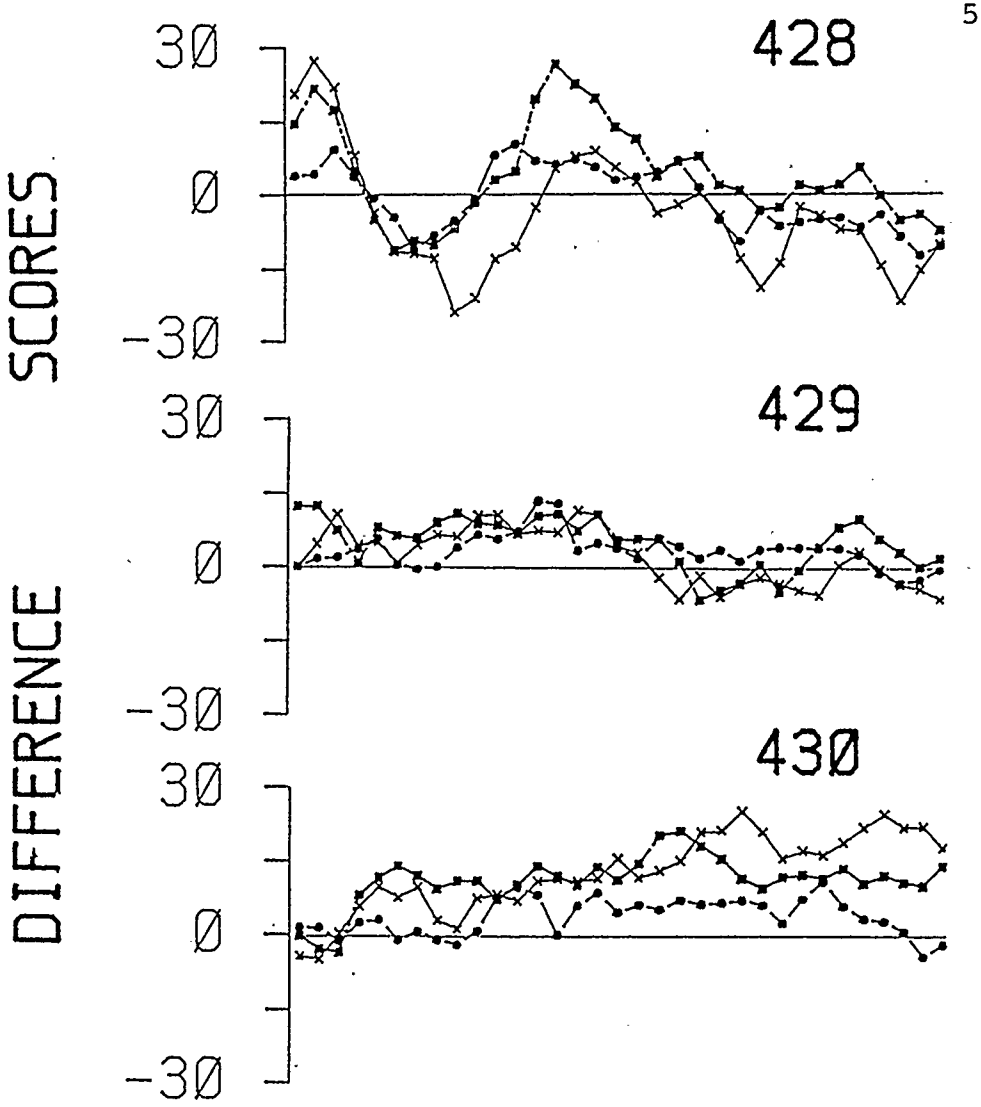


Figure 9. (continued)

Powers and Green (1978), who found that pupil size has no effect on acuity. However, their study may have been seriously confounded by the use of rats that had been visually deprived. The results do concur, however, with the position of Hughes (1977) and Hale et al. (1979), that atropine does have an effect on the operations of the rat visual system. It is reasonable to suppose that the atropine reduced the efficacy of the pattern stimulus by preventing the retinal image from being properly focussed.

This position requires further substantiation, however, given the lack of a truly convincing effect in visual cortex. The LGNd projects strongly to the areas assessed and it seems reasonable to expect that the depression registered in the LGNd would carry over to cortex. However, the cortical findings do agree to a certain extent with the findings of Meyer and Salinsky (1977). Measuring pattern evoked potentials from cortex they found that the rat eye is nearly emmetropic with a fully dilated pupil. This may indicate that cortex is somehow insensitive to this manipulation. Although the cortical results are puzzling, the strong effects observed in subcortical structures indicates that atropine is having a detrimental effect on the visual system and given that the LGNd projects to cortex, it is hard to accept that cortex is unaffected.

It should be noted that a clear pattern was found in the difference scores within the LGNd (Figure 7). The difference scores at points one, three, and five were higher than those at points two and four. This pattern has also been observed following eye removal (Cooper & Thurlow, 1985) and corresponds with the retinal projections to the structure. The lateral portion of the LGNd contains few ipsilateral retinal projections, while the medial part contains both crossed and uncrossed retinal projections (Hayhow, Sefton, and Webb, 1962; Hickey & Spear, 1976; Reese & Jeffery, 1983). Nevertheless, this pattern of difference scores was absent in some experiments of this study where it might be expected. Just what conditions are necessary for the appearance of this phenomenon could be made the special objective of a future investigation. In this regard, the greater resolution provided by the tritiated 2-DG technique may be worthy of consideration.

Although it is impossible to determine from this experiment how much of the effect of atropine was due to pupil dilation and how much was due to the paralysis of accommodation, it has been often held that the rat is not capable of accommodation due to its large lens and rather primitive ciliary musculature (Lashley, 1932; Walls, 1942). However, it has been suggested that the rat may accommodate not by deforming the lens but by moving the lens away from

the retina (Hughes, 1977). This possibility might be tested by simply placing the visual stimulus at a distance from the rat that would not require accommodation. Any effect that atropine might have on the visual system under these conditions would then be due to pupil dilation and not to lack of accommodation. For studies of the present type it seems best not to use atropine, at least until a valid measure of the refractive state of the rat eye can be developed and thereby make correction possible. Experiment 2 raises some doubts about past studies where atropine has been used.

EXPERIMENT 3

INTRODUCTION

The 2-deoxyglucose technique was used in Experiment 3 to determine the effects of diffuse and spatial arrays of light on metabolic activity in the rat's lateral geniculate nucleus (LGN), superior colliculus (SC), lateral posterior nucleus (LPN), and visual cortex (VC). Experiment 3 was prompted by the surprising failure of Toga and Collins (1981) to find elevations in VC 2-DG uptake when their rats were exposed to striped and reversing checkerboard displays. Cortical ablation (Lashley, 1931; Horel, Bettinger, Royce, & Meyer, 1966; Bland & Cooper, 1970; Dean, 1981) and single unit electrophysiological research (Shaw, Yinon, & Auerbach, 1974, 1975; Diao, Wang, & Pu, 1983; Burne, Parnavelas, & Lin, 1984) testify to the importance of rat VC in mediating pattern discriminations, and changes in metabolic activity are to be expected. In addition, Toga and Collins put atropine into the eyes of their rats. Judging from the results of Experiment 2 this procedure impaired rather than improved the pattern vision of their subjects and may have led to their negative findings. If the results obtained by Toga and Collins are truly representative, then serious reservations must be

raised about the sensitivity of the 2-DG method for analysis of rat visual system physiology.

Inclusion of the diffuse light condition in this experiment provided a means for determining whether the effects arising from exposure to the patterned display were in fact attributable to the spatial arrangement of light and not simply to luminous flux. Moreover, the effects of diffuse light are of interest in their own right. Most investigators would predict that diffuse light would be a poor stimulus for activation of the visual system, particularly for cortex (Hubel, 1959, 1963; Hubel & Wiesel, 1959) and the superior colliculus (Straschill & Taghavy, 1967; Humphrey, 1968; McIlwain & Buser, 1968). Nevertheless, a small group of investigators have argued that monkey cortex is more responsive to diffuse light than is usually supposed and that it is even legitimate to refer to "luxotonic units" (Bartlett & Doty, 1974).

The present investigation was conducted on alert rats. The stimulus conditions were achieved both by putting the animals into a patterned box and by placing goggles over their eyes during the 45 minute 2-DG incubation period.

The effects of pattern and diffuse light were evaluated by interhemispheric comparisons in three groups of rats. The first group wore goggles which allowed the rat to view the patterned box with one eye while the other

eye was covered with an opaque lens. The second group wore goggles which permitted equal luminous flux to reach each eye but restricted pattern stimulation to one eye and diffuse light to the other. The goggles of the third group permitted diffuse light to one eye while the other eye was covered with an opaque lens. To ensure that no light was available to the eye covered with the opaque lens, and that as consistent an effect as possible would result from this "standard" condition of occlusion, the eye was sutured shut 24 hours prior to the 2-DG injection. Twenty-four hours of light deprivation has been shown to produce a slight depression of visual system metabolism (Experiment 1), however, so this should be kept in mind when interpreting the results.

METHOD

Subjects

The subjects were 12 male, Long-Evans black hooded rats between 63 and 101 days old and weighed between 210 and 265 grams at the time of 2-DG injection. They were supplied by the University of Calgary animal care services, and housed individually in clear plastic cages. Water was available ad libitum, but the rats were food deprived to maintain a constant weight throughout the experiment.

Procedure

Experimental Preparation. The stimulus chamber described in Experiment 2 was used. The animals were first adapted to the chamber over three, 1 hour sessions spread over several days. A Teflon post was then attached to their skulls as described in Experiment 1, and the rats were allowed a week to recover. Adapting the animals to wearing the goggles was achieved by having the rats wear clear lensed goggles for one, 1-hour session while in their home cage followed by two to three, 1-hour sessions in the stimulus chamber.

2-DG Injection. On the morning preceding the 2-DG injection day, each rat was anesthetized with sodium pentobarbital and had a chronic catheter placed in the right external jugular vein using the procedure described in Experiment 1. For animals in the groups that required it, one eye was also sutured closed at the same time. On the next morning the rat had the goggles attached and it was placed in a holding cage where it was injected through the catheter with freshly reconstituted 2-DG in saline. The animal was then immediatley placed into the stimulus chamber.

Animals in the pattern-lid suture group wore goggles that had a clear lens on one side and to ensure complete occlusion, an opaque lens covered the lid sutured eye

(Figure 10 A). Rats in the pattern-diffuse group wore goggles with one light diffusing lens and one transparent lens (Figure 10 B). The diffusing lens reduced light transmission by about 26% and to make the transparent lens equivalent in this respect it was tinted. The correct degree of light attenuation was achieved by first dipping the lens in a gelatin subbing solution to ensure adhesion (Pappas, 1971), and then after it had dried, it was coated with photographic emulsion in a darkroom. The lens was then briefly exposed to light and developed. The same tinted lens was also used on the "pattern eye" of the animals in the pattern-lid suture group. The rats in the diffuse-lid suture group wore goggles which had a diffusing lens and an opaque lens covered the lid sutured eye (Figure 10 C).

Following the 45 minute 2-DG incubation and visual stimulation period the rats were removed from the stimulus chamber, given a lethal dose of sodium pentobarbital through the catheter, perfused, and processed for autoradiography as described in Experiment 1. Quantification of the autoradiographs was also the same as in Experiment 1. The difference scores for the three groups in Experiment 3, labeled as pattern-lid suture, pattern-diffuse, and diffuse-lid suture, were derived by the ratio scores of the hemisphere receiving pattern minus

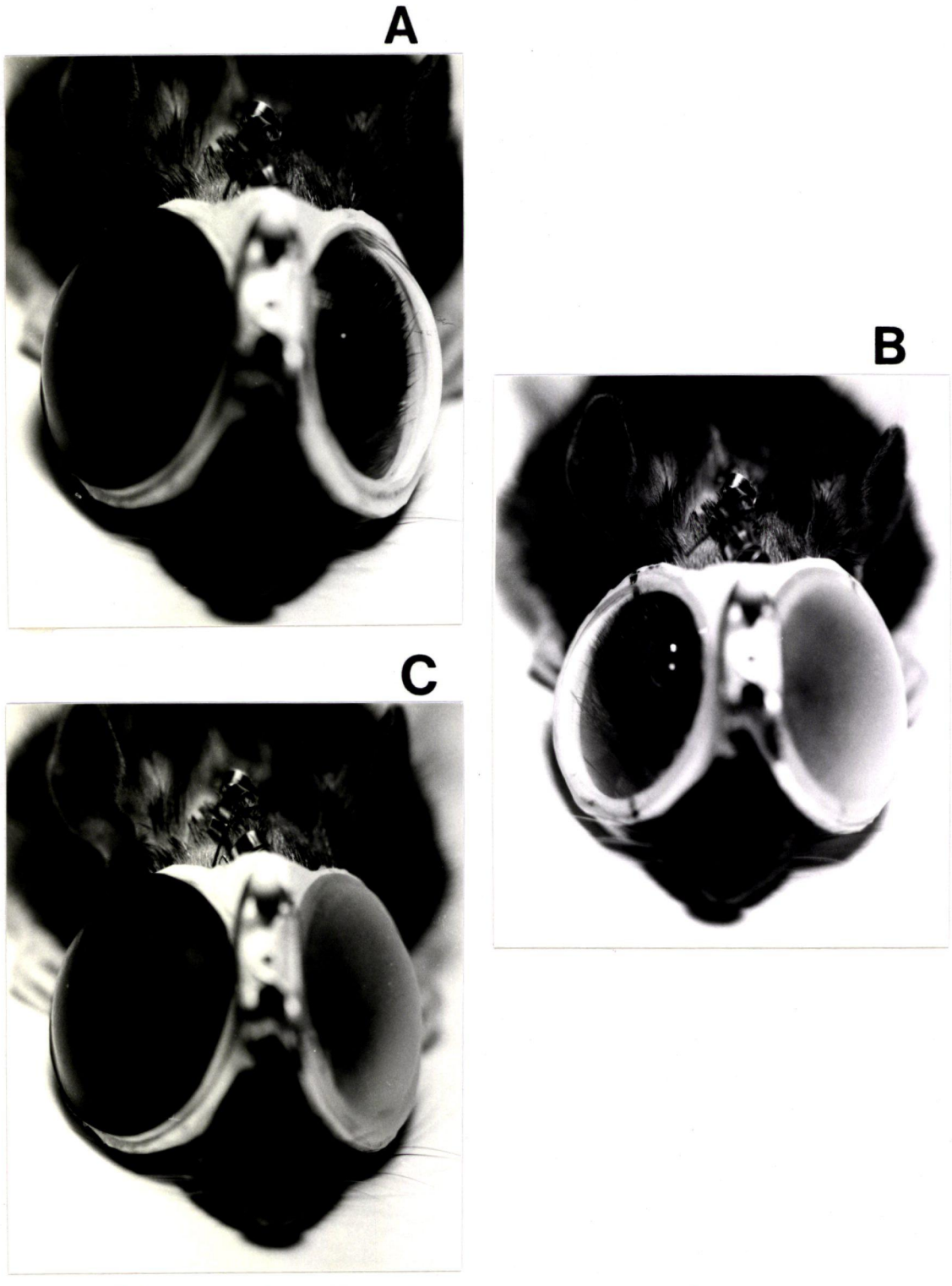


Figure 10. Goggles for the A. pattern-lid suture group,
B. pattern-diffuse group, and
C. diffuse-lid suture group.

the ratio scores of the hemisphere fed by the lid sutured eye, pattern minus diffuse, and diffuse minus lid suture.

RESULTS AND DISCUSSION

Lateral Geniculate Nucleus

Figure 11 summarizes the results for the dorsal and ventral parts of the lateral geniculate nucleus. Pattern proved to be most effective for the LGNd. The difference scores for the pattern-lid suture group were sizeable and the slight inflationary effect from the depression produced by 24 hours of lid suture, a mere three points (Experiment 1), does not significantly detract from the robust results for this group (M= 32.26, $t(3)$= 9.73, $p<.005$). Similiar remarks apply to the diffuse-lid suture group (M= 10.89, $t(4)$= 15.31, $p<.005$), although it is clear that diffuse light was less effective than pattern as the scores are smaller for these subjects (Pattern-Lid vs Diffuse-Lid, $t(7)$= 7.44, $p<.0001$). The scores for the pattern-diffuse group, which are not affected by an inflationary factor, also point to the superiority of pattern over diffuse light (M= 24.38, $t(2)$= 12.44, $p<.005$). Moreover, this group makes the important point that it was not merely luminous flux, but the spatial array of light which was the salient feature of the pattern display.

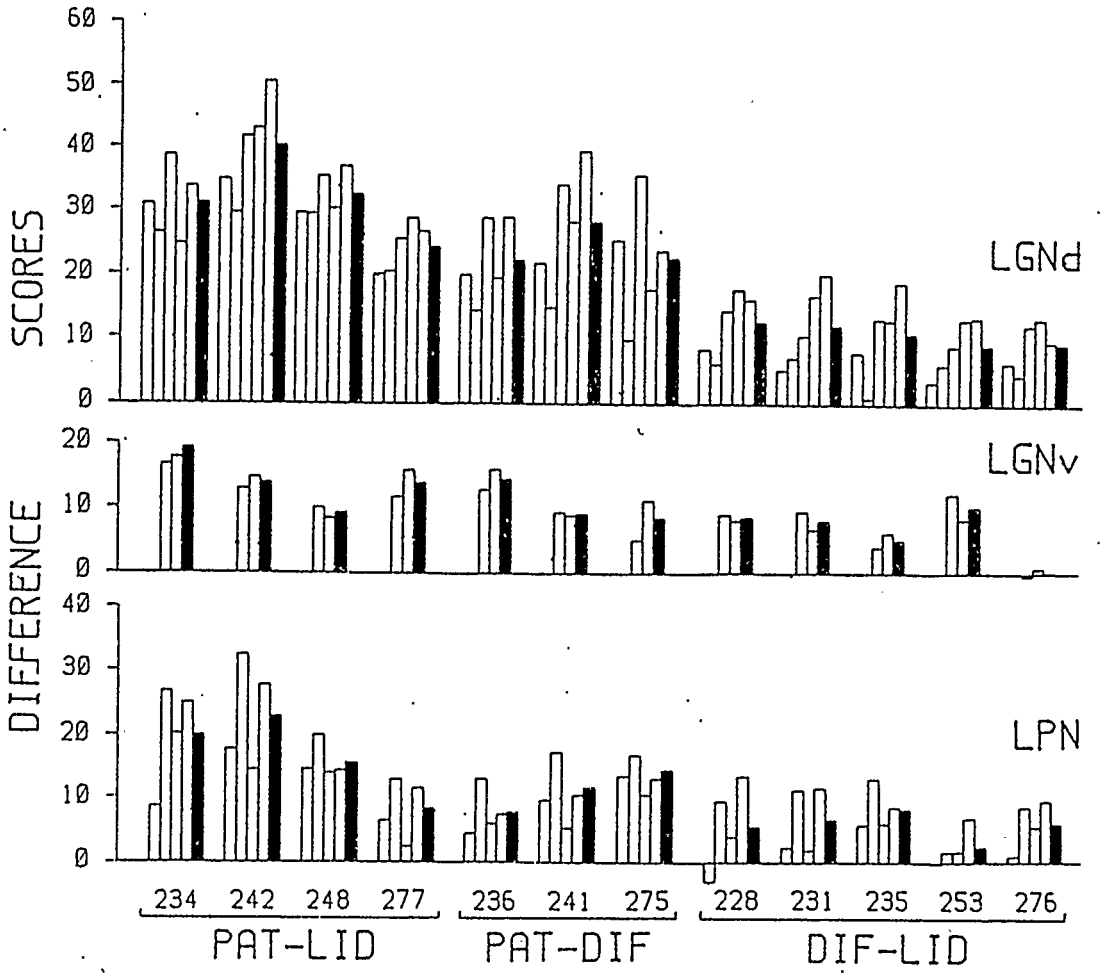


Figure 11. Difference scores for the LGNd, LGNv, and LPN for each animal. Hollow bars from left to right correspond to the numbers for each area shown in Figure 2 A, and the solid bars are the mean difference scores for each area.

These results would be predicted from the electrophysiological evidence based on the cat (Hubel & Wiesel, 1961) and rat (Hale, Sefton, & Dreher, 1979; Lennie & Perry, 1981; Molotchnikoff, Tremblay, & Lepore; 1984). Most cells in the dorsal lateral geniculate possess center-surround antagonistic receptive fields and thus would be expected to respond better to pattern stimulation than to diffuse light. However, they are not consistent with the early work of Montero and Brugge (1969) who found no cells in the rat dorsal lateral geniculate that have antagonistic receptive fields.

The results for the ventral part of the lateral geniculate were vaguely reminiscent of those for the dorsal part (Figure 11), but the effects were much reduced and the between group differences less obvious (Pattern-Lid, M= 13.74, $t(3)$= 8.42, p<.005; Pattern-Diffuse, M= 10.90, $t(2)$= 5.76,p<.025; Diffuse-Lid, M= 6.59, $t(4)$= 3.68, p<.025; Pattern-Lid vs Diffuse-Lid, $t(7)$= 2.95, p<.025). Nevertheless, as for the dorsal LGN, pattern and diffuse light seemed to be more effective than lid suture and pattern seemed to be more effective than diffuse light. These results are consistent with Hale and Sefton's (1978) electrophysiological study which showed that while a number of cells possessed no antagonistic surround, over half did. However, they disagree with the report that few cells

possess surround antagonism (Sumitomo, Sugitani, Fukuda, & Iwama, 1979), since if this were the case pattern should not have been more effective than diffuse light. On the other hand, such slight effects may be more indicative of the employment of less than optimal stimulus parameters for this poorly understood structure.

Superior Colliculus

Figure 12 summarizes the difference scores for stratum griseum superficiale (SGS), stratum opticum (SO), and stratum griseum mediale (SGM) of the superior colliculus (SC). Consistent with the electrophysiological work on the rat (Humphrey, 1968; Fukuda & Iwama, 1978), pattern was again found to be an effective activating stimulus. Conversely, there was some evidence for supposing that diffuse light was actually reducing metabolic activity below the level produced by 24 hours of lid suture.

It is clear from the difference scores of the pattern-lid suture group that pattern was an effective condition for increasing metabolic activity in the SC (SGS, $M = 17.81$, $t(3) = 18.82$, $p < .005$). Though the 24 hour lid suture may have inflated these scores, its contribution would have been small (Experiment 1). In the SGS, the effectiveness of pattern over diffuse light in the pattern-diffuse group ($M = 26.92$, $t(2) = 25.85$, $p < .005$), and

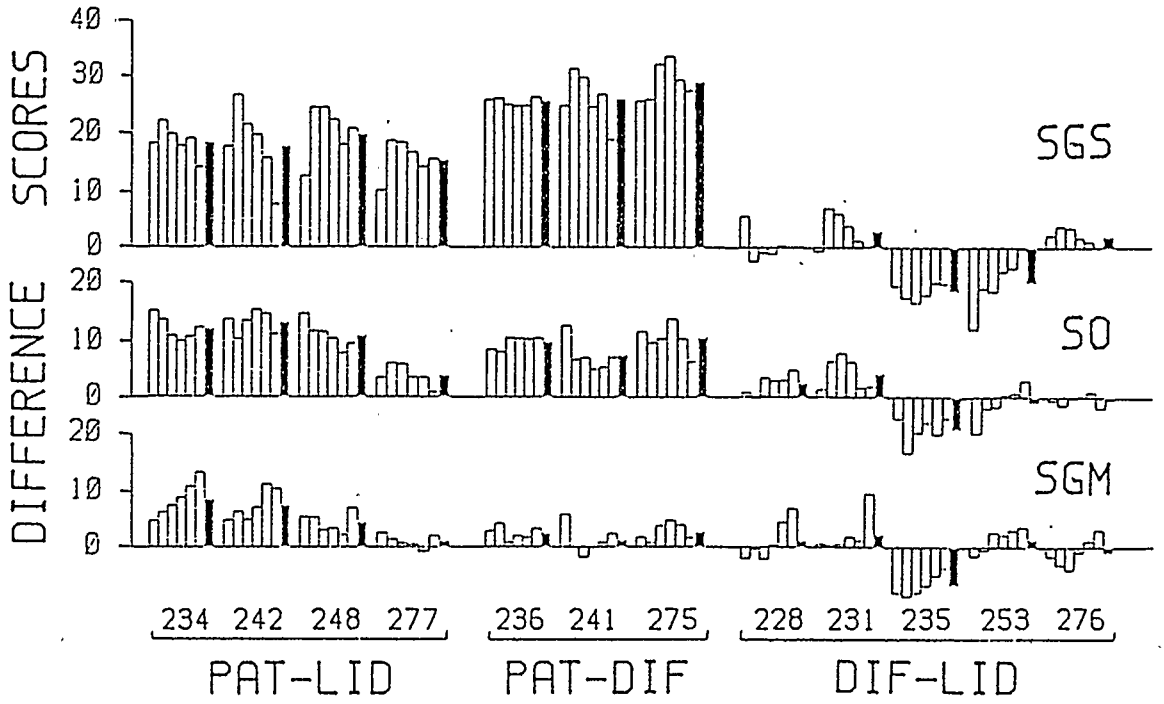


Figure 12. Difference scores for the SGS, SO, and SGM for each animal. Hollow bars from left to right correspond to the numbers for each layer shown in Figure 3 A, and the solid bars are the mean difference scores for each layer.

the difference between the pattern-lid and the diffuse-lid suture groups ($t(7)= 8.27$, $p<.0005$), indicates that it was the spatial, contrast, dimensions of the pattern stimulation and not the intensity of the light that was responsible for the increase in metabolic activity. It can also be seen from Figure 12 that the difference scores were greater for the pattern-diffuse group than for the pattern-lid suture group ($t(5)= 3.40$, $p<.01$). This suggests that diffuse light suppresses metabolic activity in this structure. Indeed, the greater difference scores of the pattern-diffuse group as compared to the pattern-lid suture group are all the more noteworthy when it is recalled that the 24 hours of lid suture is inflating the scores of the latter group. The results of the diffuse-lid group do not negate the possibility that diffuse light acts to inhibit SGS activity ($M= -1.66$, $t(4)= -.76$, $p>.20$). Although only two subjects showed negative scores the remaining three were only slightly positive and at least in part these scores could reflect depression resulting from the 24 hours of eyelid suture.

In the SO pattern was again found to be most effective and diffuse light was found to be no better or worse than 24-hour lid suture (Pattern-Lid, $M= 10.06$, $t(3)= 4.77$, $p<.01$; Pattern-Diffuse, $M= 9.26$, $t(2)= 10.12$, $p<.005$; Diffuse-Lid, $M= .00$, $t(4)= .00$, $p>.30$; Pattern-lid vs

Diffuse-Lid, $t(7)= 4.17$, $p<.005$). In the SGM difference scores were very small and the only detectable effect was found in the pattern-lid suture group ($M= 5.38$, $t(3)= 3.25$, $p<.025$; Diffuse-Lid, $M= -.57$, $t(4)= -.35$, $p>.30$), although the small difference in the pattern-diffuse group reached statistical significance ($M= 2.16$, $t(2)= 4.74$, $p<.025$), still showing pattern to be an effective stimulus (Pattern-Lid vs Diffuse-Lid, $t(7)= 2.82$, $p<.025$). The possibility that diffuse light acts as an inhibitory stimulus is not as strong for these two layers Contrary to the results for SGS, the pattern-diffuse scores were not greater than the pattern-lid scores. This leaves only the scores for the diffuse-lid suture group which, at best, provide equivocal support for a suppression effect.

The diminution in difference scores from the SGS to SO and again to SGM parallels the decrease in optic fibers terminating in these layers (Lund, 1969), and is consistent with the electrophysiological work that has found few visually responsive cells ventral to SO (in the cat, McIlwain & Buser, 1968; in the hamster, Stein & Dixon, 1979). Furthermore, it has been shown that following eye removal depression of metabolic activity is greatest for the upper layers of the SC (Cooper & Thurlow, 1985).

Lateral Posterior Nucleus

The lateral posterior nucleus receives few, if any, direct retinal projections, however, it does receive major afferents from the superior colliculus and visual cortex (Mason & Groos, 1981; Takahashi, 1985). As was the case for the LGN, pattern was the most effective stimulus for the lateral posterior nucleus. Diffuse light was less effective than pattern, and lid suture was least effective. The difference scores for the three groups are illustrated in Figure 11. The pattern-lid suture group showed the greatest difference in 2-DG uptake (M= 16.80, $t(3)$= 5.30, $p<.01$), followed by the pattern-diffuse group (M= 11.45, $t(2)$= 5.95, $p<.025$), and the diffuse-lid suture group showed the least (M= 5.87, $t(4)$= 6.07, $p<.005$; Pattern-Lid vs Diffuse-Lid, $t(7)$= 3.85, $p<.005$).

Although the difference scores in the diffuse-lid suture group were relatively small, on average, they were double that found when 24-hour eyelid suture is compared to a condition of darkness (Experiment 1). Therefore, the differences observed in the present experiment were not merely due to a depression resulting from 24-hour light deprivation of the lid sutured eye, but instead suggest activity produced by the diffuse light condition. This was surprising given that the superior colliculus seemed to be under inhibition during diffuse light stimulation and

indicates that this activity is produced by disinhibition from the superior colliculus or from activity in the pretectum or visual cortex.

Visual Cortex

Consistent with the electrophysiological research, pattern stimulation produced the greatest activity in visual cortex. The difference scores for the three experimental groups are shown in Figures 13, 14, and 15. Scores from left to right correspond to cortical readings from medial to lateral. Each subject is represented by three superimposed graphs: the supragranular layer denoted by X's, granular layer by squares, and infragranular by circles.

The difference scores were not uniform across the medial to lateral extent of visual cortex. However, a definite shape can be seen in the pattern-lid suture group; and though less consistent, also in the pattern-diffuse and diffuse-lid suture groups. The biggest difference scores occurred in the monocular regions of areas 17 and 18a (Zilles, 1985), while scores at the 17-18a border, which receives callosal fibers originating from contralateral visual cortex (Miller & Vogt, 1984; Olavarria & Montero, 1984), were minimal. In Cooper and Thurlow's (1985) demonstration of the depression of 2-DG uptake resulting

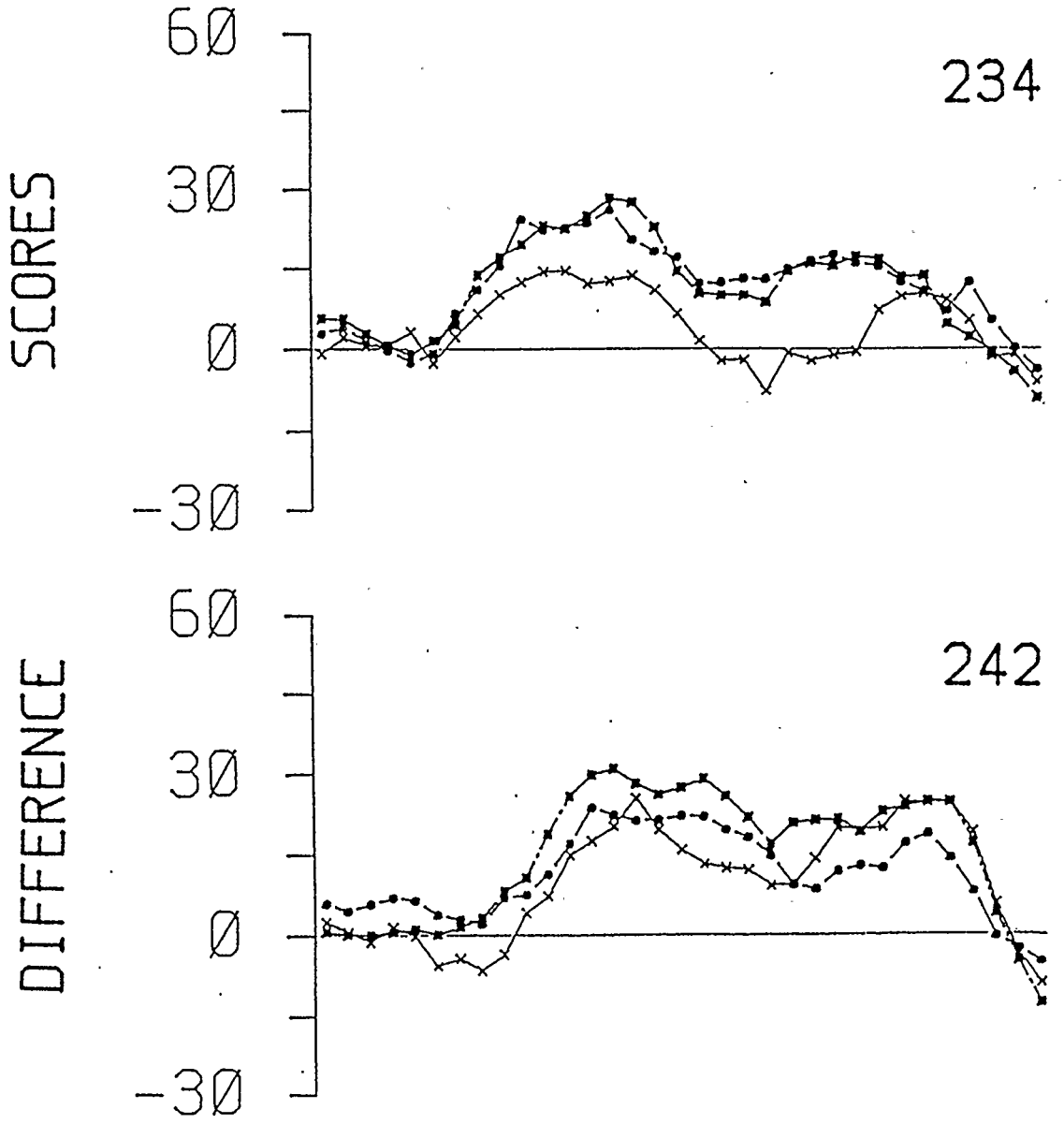


Figure 13. Pattern-lid suture difference scores for the three layers of cortex for each animal. The Supragranular layers are marked with X's, layer IV with squares, and the infragranular layers with circles. Points from left to right correspond to points 1 to 33 shown in Figure 4.

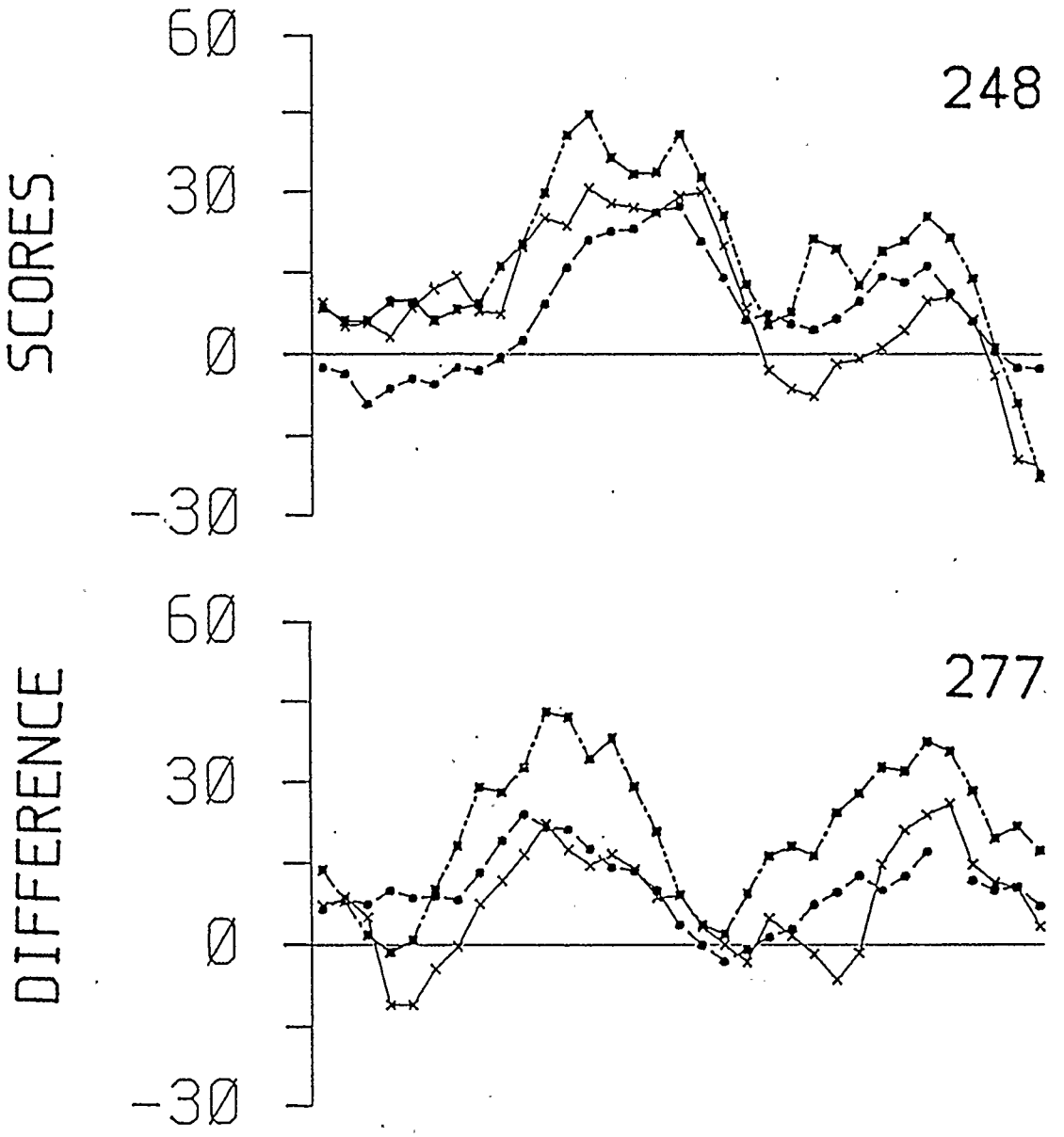


Figure 13. (continued)

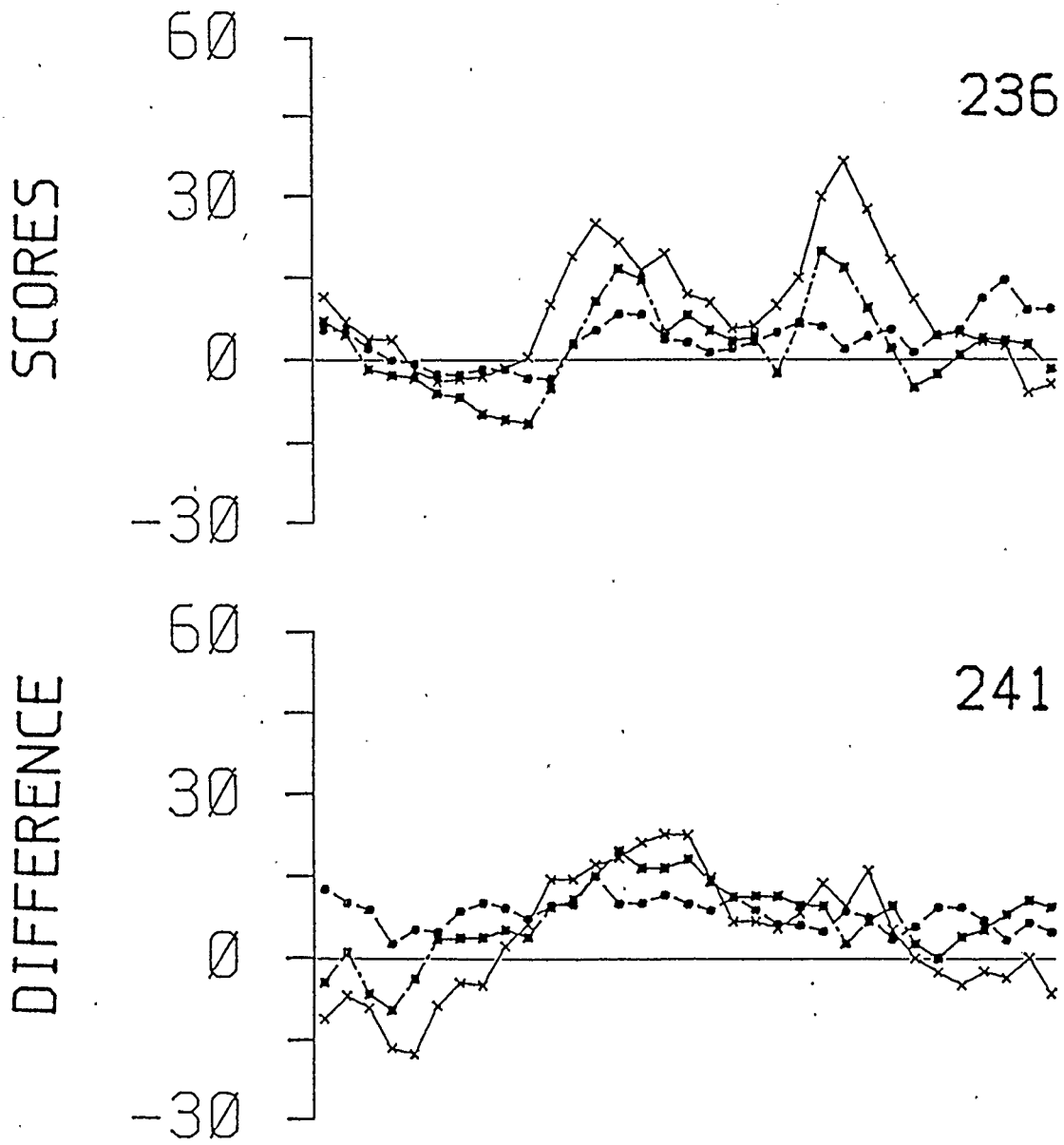


Figure 14. Pattern-diffuse difference scores for the three layers of cortex for each animal. The Supragranular layers are marked with X's, layer IV with squares, and the infragranular layers with circles. Points from left to right correspond to points 1 to 33 shown in Figure 4.

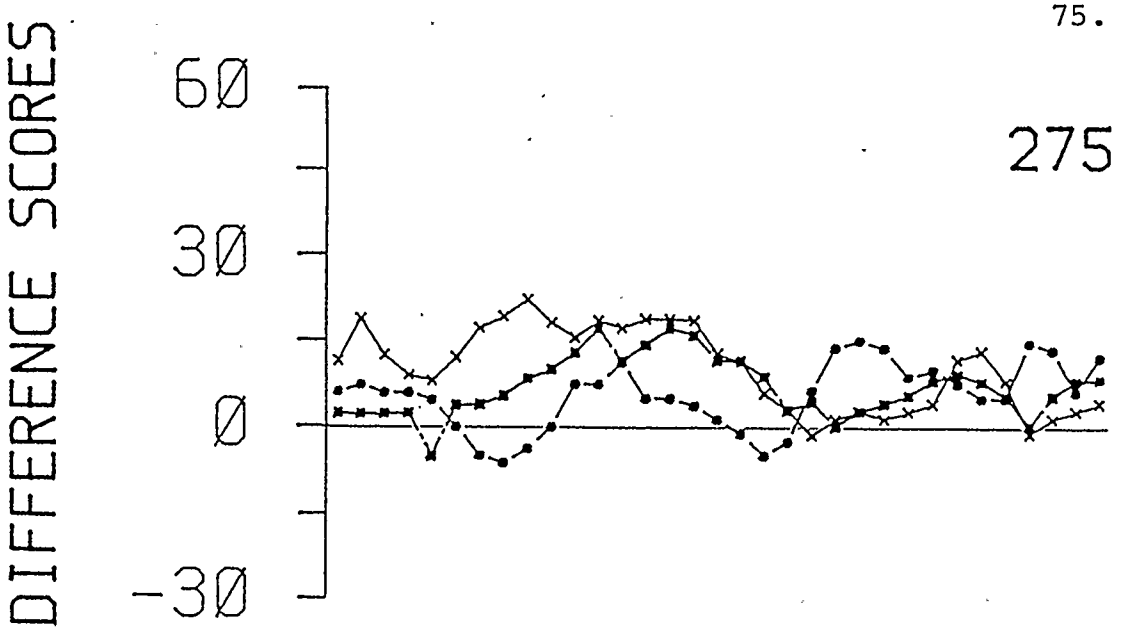


Figure 14. (continued)

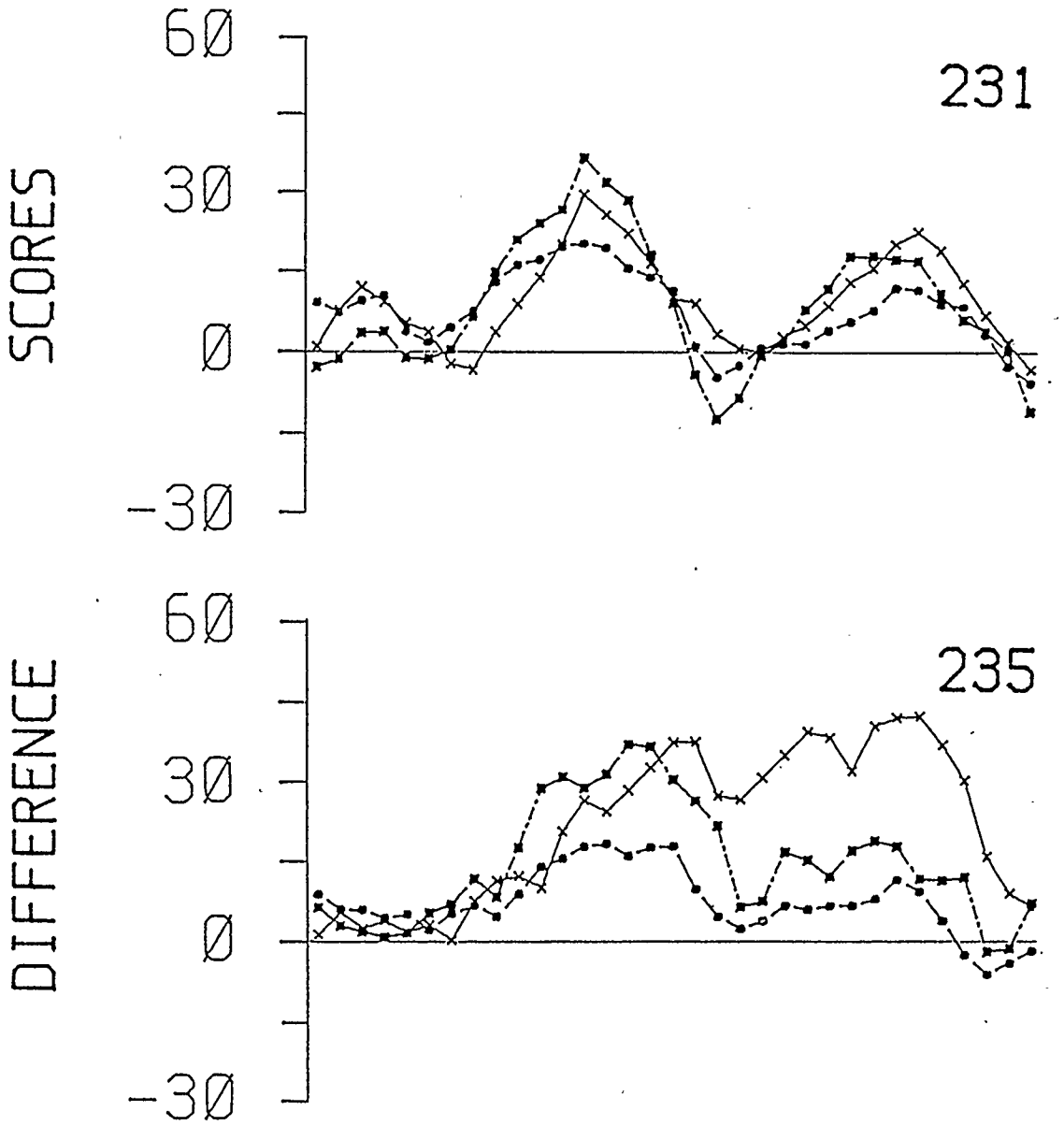


Figure 15. Diffuse-lid suture difference scores for the three layers of cortex for each animal. The Supragranular layers are marked with X's, layer IV with squares, and the infragranular layers with circles. Points from left to right correspond to points 1 to 33 shown in Figure 4.

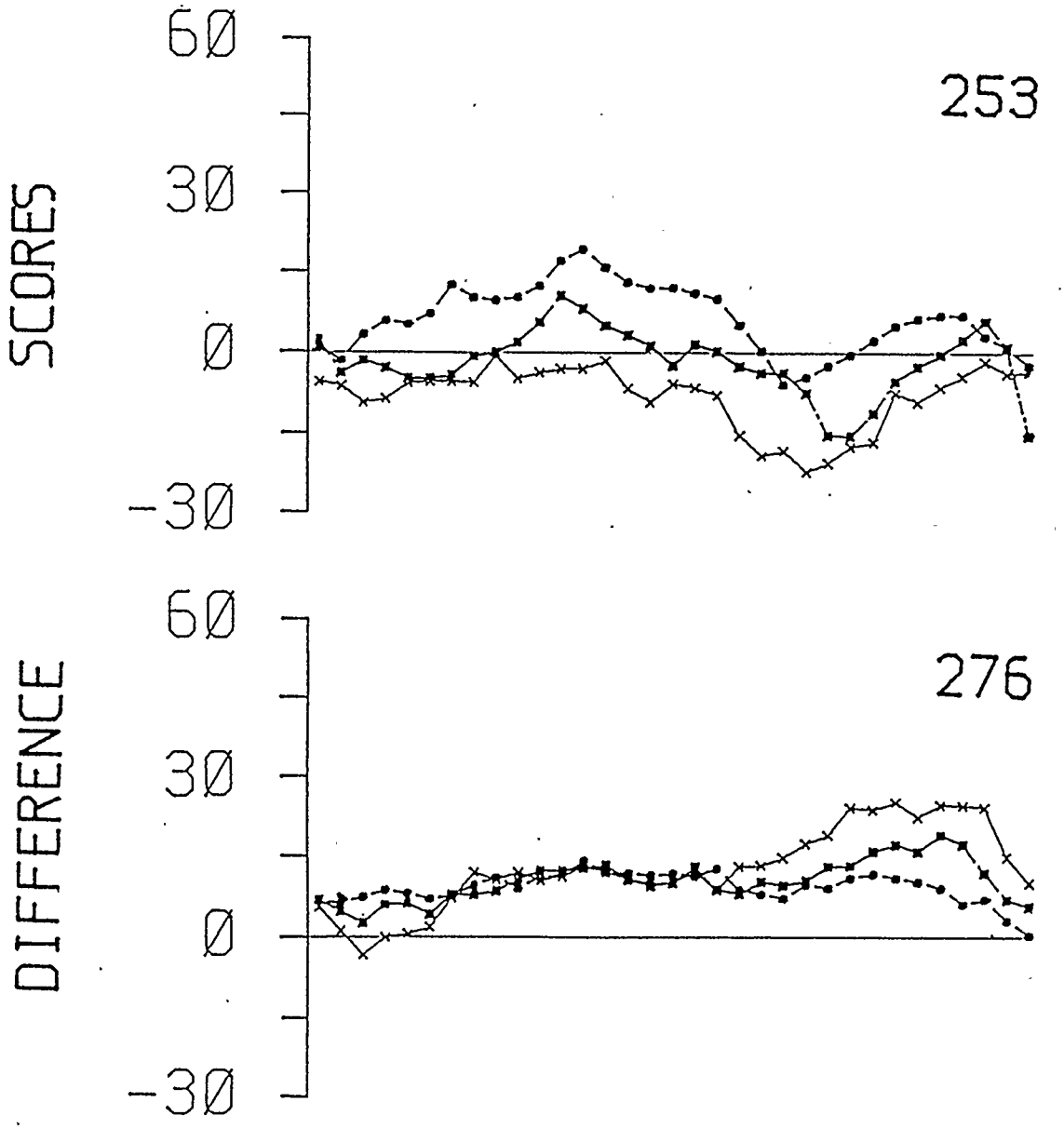


Figure 15. (continued)

from eye removal, they actually observed a reversal effect at the 17-18a border for some subjects. The results of the present experiment suggest that this phenomenon can also occur when stimulus conditions differ between the two eyes.

In Figures 13 and 14, a postive number indicates greater 2-DG uptake from pattern stimulation over lid-suture and diffuse light, respectively. In Figure 15 a postive number indicates that 2-DG uptake was greater for diffuse light stimulation than for lid suture. While the difference scores for the pattern-lid suture group may be inflated by the depressing effect produced by 24-hour lid suture (Experiment 1), this effect is not large enough to account for the entire difference observed in this group (M= 28.36, $t(3)$= 8.55, p<.005). Moreover, the difference scores for the pattern-diffuse group shown in Figure 14 were not confounded by 24-hour lid suture effects, and showed difference scores only slightly less than those for the pattern-lid suture group once the 13 points from the lid suture effect are removed (Experiment 1). Furthermore, because pattern was more effective than diffuse light (M= 13.20, $t(2)$= 6.12, p<.025), it can be concluded that the increased 2-DG uptake produced by the pattern stimulation was a result of the spatial, contrast, dimension of the stimulation and not merely due to luminous flux.

Given that the difference scores for the pattern-diffuse group were only slightly less than those for the pattern-lid suture group once the inflation of scores in the pattern-lid suture group are taken into account, it was expected that the scores for the diffuse-lid suture group would be small and probably no greater than those resulting from 24 hour lid suture alone. Surprisingly, this was not the case (Figure 15, $\underline{M}$= 17.09, $\underline{t}(3)$= 2.85, $\underline{p}<.05$). While two subjects had scores no larger than what might result from 24 hour lid suture, two subjects (231 and 235) had scores as large as those in the pattern-lid suture group. The unexpectedly strong cortical activity in rats 231 and 235 raised the possibility that they had been able to see the stripes in spite of the light diffusing mask covering their eyes. Although support for this conjecture is unsubstantial - the subcortical scores for 231 and 235 do not stand out from the other subjects of their group - a more rigorous test of the effects of diffuse light seemed needed if only to buttress the suspicion that it acts to inhibit the metabolic activity of the colliculus.

CONCLUSIONS

The results of the present experiment support the view that the visual system is most activated by spatial arrays

of light. In all the visual structures studied, pattern stimulation produced the greatest uptake of 2-DG, and it was clear from the diffuse light conditions that it was not simply the intensity of the light that was producing the effect. This redeems the technique and indicates that Toga and Collins' (1981) failure to observe an elevation of 2-DG uptake in visual cortex may have been due to inappropriate stimulus conditions, or, alternatively, their practise of dilating the rats' pupils with atropine (Experiment 2), using anesthetics (Sokoloff et al., 1977), or eye removal as a condition for comparison (Cooper & Thurlow, 1985), may have prevented their stimulus conditions from being effective.

EXPERIMENT 4

INTRODUCTION

Experiment 4 provided a more rigorous test of the effects of diffuse light on the rat visual system. To compensate for possible deficiencies in the diffusing lens, a white, featureless chamber was substituted for the striped one employed in Experiment 3. In addition, one eye was covered with a diffusing lens as before, while the other eye was covered with an opaque lens but was not sutured shut. This was done to eliminate the depression which results from 24 hours of visual deprivation (Experiment 1), and thereby provide a better comparison condition against which the possible inhibitory effect of diffuse light on the colliculus could be detected.

METHOD

Subjects

The subjects were four male Long-Evans black hooded rats ranging in age from 75 to 168 days at the time of 2-DG injection and in weight from 215 to 304 grams. They were housed individually in clear plastic cages and food deprived to control their weight. Water was available ad libitum.

Procedure

The rats had Teflon posts attached to the skull as described in Experiment 1, and following a recovery period wore the goggles for at least one, 1-hour session before the 2-DG injection. The catheter implantation, 2-DG injection, and autoradiographic analysis were carried out as in the previous experiments.

Before the injections the animals were fitted with goggles which had an opaque lens and a light diffusing lens. These were the same goggles used for the diffuse-lid suture group in Experiment 3. Immediately following the 2-DG injection the animals were placed in a stimulus chamber similar to the one employed in Experiments 2 and 3 but having blank white walls.

RESULTS AND DISCUSSION

The results are summarized in Figures 16, 17, and 18. The scores, as before, were derived by subtracting the carbon-14 ratio values of the hemisphere fed by the eye covered with the opaque lens from the values of the hemisphere fed by the eye receiving diffuse light. Therefore, a positive score indicates higher metabolic activity arising from steady diffuse light.

Both portions of the LGN showed greater activity from steady diffuse light than from the covered eye (LGNd,

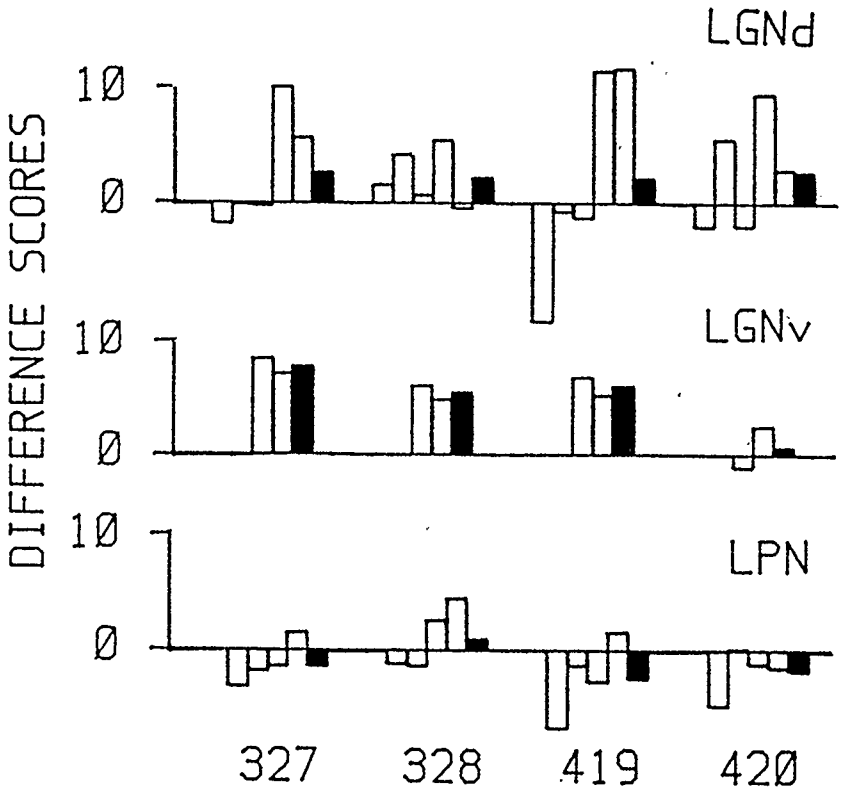


Figure 16. Difference scores for the LGNd, LGNv, and LPN for each animal. Hollow bars from left to right correspond to the numbers for each area shown in Figure 2 A, and the solid bars are the mean difference scores for each area. Positive scores indicate increased 2-DG uptake (higher metabolic acitivity) contralateral to the eye receiving diffuse light.

$\underline{M}$= 2.60, $\underline{t}(3)$= 17.89, $\underline{p}$<.0005; LGNv, $\underline{M}$= 5.17, $\underline{t}(3)$= 3.33, $\underline{p}$<.025). However, the effect in the LGNd was substantially smaller than was observed for this structure in the diffuse-lid suture group of Experiment 3. Although the scores from Experiment 3 were likely inflated by the 24-hour deprivation effect, that effect is not large enough to account for the difference between Experiments 3 and 4. Rather, the smaller effects observed in Experiment 4 are likely attributable to the improved diffuse light condition. This point was particularly important in the interpretation of the visual cortical data. As shown in Figure 17, steady diffuse light produced no consistent effect in visual cortex in Experiment 4. (The discrepant results observed in the upper layers of cortex for animal 420 are attributable to the damage that cortex sustained during perfusion.) Although diffuse light may have produced a small depression of 2-DG uptake in layer IV of area 17, the statistical significance of the difference was not high ($\underline{M}$= -11.68, $\underline{t}(3)$= -2.02, $\underline{p}$<.10).

As Hubel and Wiesel (1961) found for the cat, it also appears for the rat that diffuse light is a poor, if not a completely ineffective stimulus for visual cortex. It can also be concluded that either the rat does not possess luxotonic units, or it it does, that there are as many that

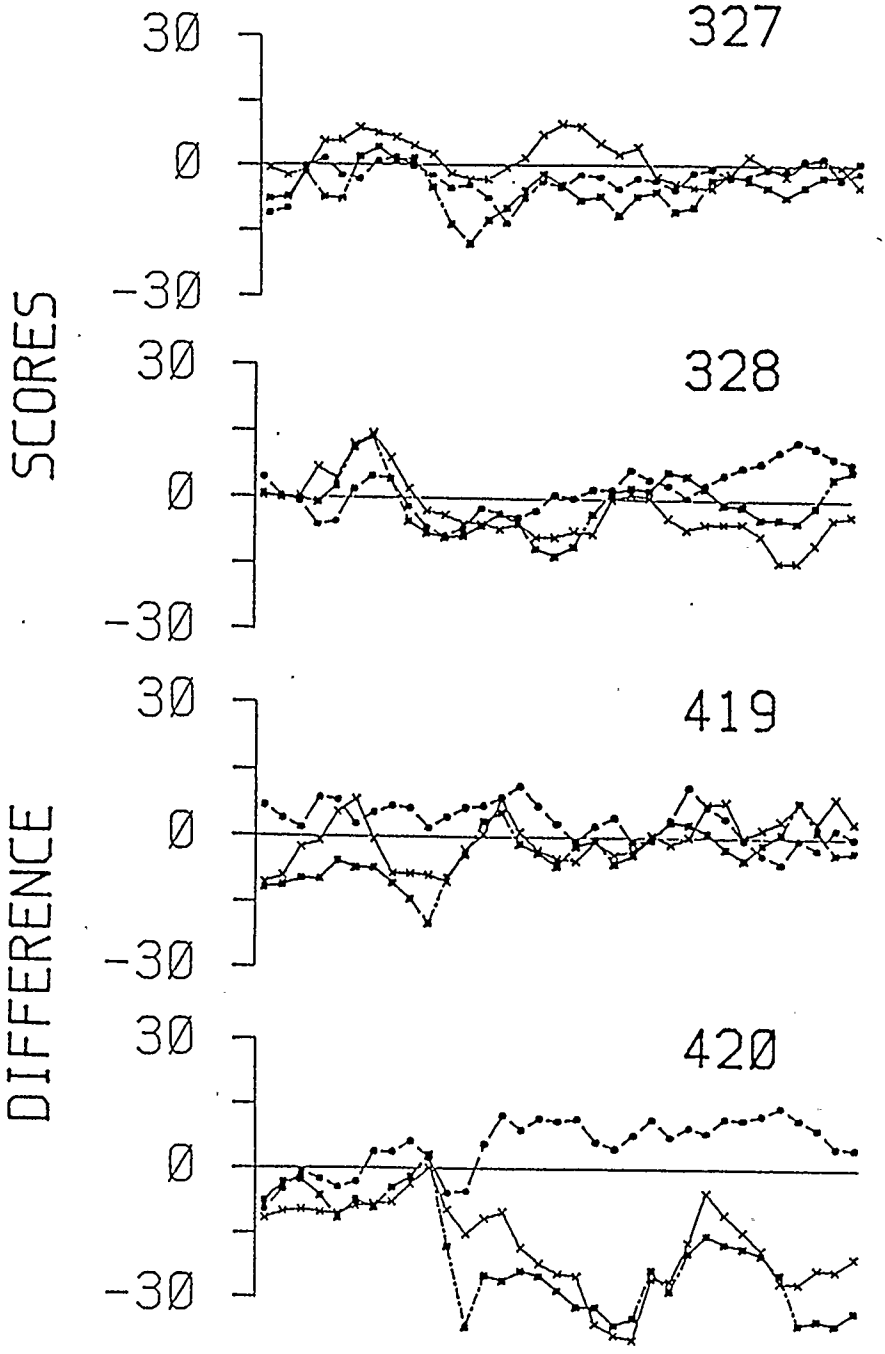


Figure 17. Diffuse-opaque difference scores for the three layers of cortex for each animal. The Supragranular layers are marked with X's, layer IV with squares, and the infragranular layers with circles. Points from left to right correspond to points 1 to 33 shown in Figure 4. Positive scores indicate increased 2-DG uptake (higher metabolic activity) contralateral to the eye receiving diffuse light.

increase responding in the dark as there are that increase responding in the light.

Like cortex, steady diffuse light was also ineffective for the LPN (M= -1.15, $t(3)$= -1.50, p>.10). The LGNv, however, showed an increase in 2-DG uptake which was only slightly less than that which was observed for the diffuse-lid suture group of Experiment 3. This confirms that this structure does indeed respond to diffuse light stimulation and much more so than the LGNd, for which diffuse light stimulation was less effective. In addition, the response of the LGNv to diffuse light is consistent with the view that this structure plays a role in luminous flux detection (Legg & Cowey, 1977).

Perhaps the most interesting finding of Experiment 4 was the widespread suppression of activity that diffuse light produced in the superior colliculus (SC). Figure 18 shows the difference scores for the stratum griseum superficiale (SGS), stratum opticum (SO), and stratum griseum mediale (SGM) of the SC. For each layer, 2-DG uptake was greater for the hemisphere contralateral to the eye which received no light than for the hemisphere contralateral to the eye receiving diffuse light (SGS, M= -18.37, $t(3)$= -7.95, p<.005; SO, M= -10.04, $t(3)$= -7.31, p<.005; SGM, M= -3.84, $t(3)$= -4.05, p<.025). These results confirm the possibility raised in Experiment

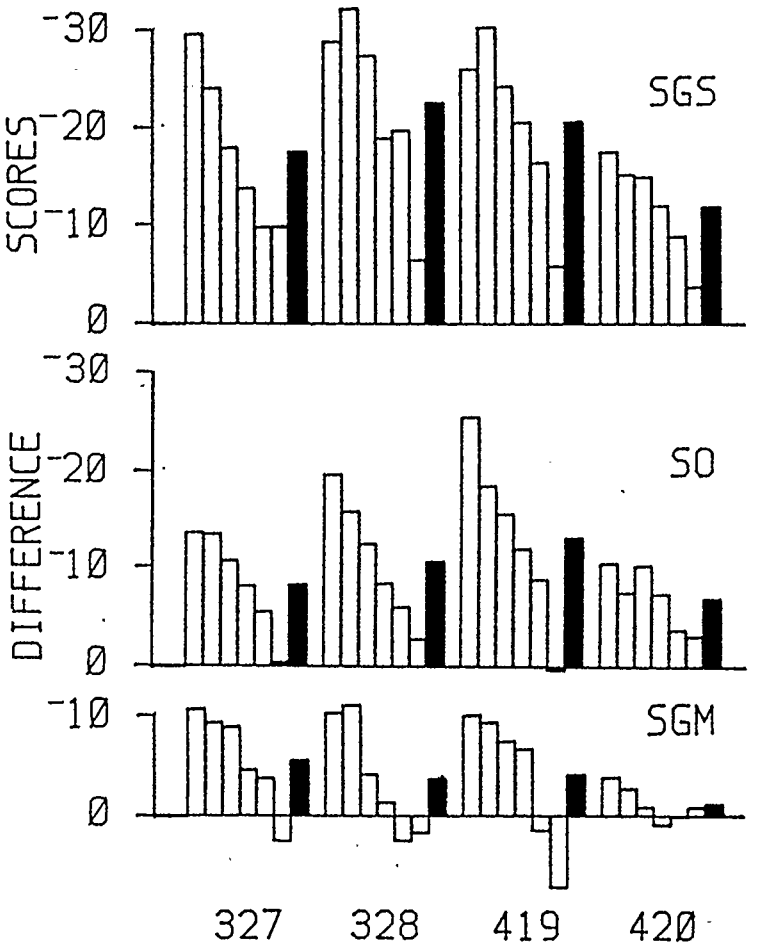


Figure 18. Difference scores for the SGS, SO, and SGM for each animal. Hollow bars from left to right correspond to the numbers for each layer shown in Figure 3 A, and the solid bars are the mean difference scores for each layer. These are negative difference scores indicating decreased 2-DG uptake (lower metabolic activity) contralateral to the eye receiving diffuse light.

3, that diffuse light actually dampens activity in the SC. The more robust effect observed in Experiment 4 seems attributable to the use of a better diffuse light condition and/or elimination of the depression produced by 24 hours of light deprivation.

Some electrophysiological researchers have claimed that spontaneous activity in the SC is very low (Humphrey, 1968; Sterling & Wickelgren, 1969; Chalupa & Rhoades, 1977; Fukuda & Iwama, 1978). The practise, however, of recording "spontaneous activity" while the animals face a diffusely lit screen seems questionable judging from the results of the present study which suggests that the colliculus is inhibited under such conditions. Spontaneous activity should probably be assessed in complete darkness. In addition, claims that SC cells prefer dark objects against a light background more than light objects against a dark background (Humphrey, 1968; Tiao & Blakemore, 1976) may have arisen from a failure to appreciate that the two types of background do not have identical effects on the colliculus.

Just why the superior colliculus should respond with a suppression of activity to diffuse light is open to speculation. One possibility is related to the role the superior colliculus may play in orienting an animal to a stimulus. Local activity in the retinotopic tectal map,

most effectively produced by a moving stimulus, is thought to be involved in the targetting response. When the eyes move in a complex visual environment, however, images from stationary objects would shift across the retina and could induce competing orienting responses. The widespread suppression in the superficial layers of the colliculus during exposure to diffuse light could be an expression of a mechanism whereby tectal activity is inhibited by a degraded retinal image of the environment, as would occur during eye movements. Whatever the functional implications of the present findings, it appears that diffuse light has a greater effect on visual system operations than is generally appreciated.

CONCLUDING SUMMARY

This thesis has resulted in several significant findings. First, Experiment 2 showed that the visual acuity of the rat is not unaffected by the topical application of atropine to the eye. The use of atropine and drugs of similar action remain a common practise and if used at all, should be very cautiously employed in studies of the present type.

Second, it was demonstrated in Experiment 3 that the rat visual system is responsive to spatial arrays of light, and by including a diffuse light condition it was possible to show that it was not simply the intensity of the light that produced the observed effects. That all the visual structures studied, including visual cortex, showed increased metabolic activity from pattern stimulation undermines Toga and Collins' (1981) position that rat visual cortex is unresponsive to such stimulation and leads one to suspect their research is misleading.

Third, Experiment 4 showed that steady diffuse light was an ineffective stimulus for the lateral posterior nucleus and visual cortex. However, it did increase activity in the ventral lateral geniculate nucleus and to a small degree also in the dorsal part. Most noteworthy, though, was the reduction in activity that was produced in the superior colliculus. Under conditions of steady

diffuse light this structure displayed a decrease in metabolic activity below spontaneous or maintained levels, indicating not that diffuse light was an ineffective stimulus but that the superior colliculus responds to it with widespread inhibition. This finding is important, if only from the standpoint that although depression in metabolic activity has been observed in brain structures following denervation or injury, to the best of my knowledge this is the first demonstration of suppressed metabolic activity in response to visual stimulation. Finally, although the effects of pattern and diffuse light on retinal activity were not directly assessed in the present study, it can be deduced from the results of the projection sites that retinal ganglion cells of the rat must convey information about both pattern and diffuse light.

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